University of Reading

Investigating mouthfeel perception of whey protein fortified products and the influence of age and associated individual differences

Thesis submitted for the degree of Doctor of Philosophy in Food and Nutritional Sciences

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Whey proteins are associated with numerous positive benefits and are commonly fortified into products. These products are often utilised in an ageing population to improve nutritional status. However, whey protein fortified products typically have negative mouthfeel attributes, likely to intensify with age, subsequently impacting compliance and acceptance. Accordingly, this thesis aimed to investigate mouthfeel perception (predominantly mouthdrying) in two whey models: liquid model (whey beverages) and solid model (cakes, biscuits, cupcakes and scones), with or without whey protein fortification, and the extent of modulation from individual differences.

Consumer studies were carried out using healthy volunteers (18-30 and/or 65+ years) to: (a) rate individual liking and perception of products; (b) collect saliva samples post beverage consumption to measure mucoadhesion (oral retention) in the oral cavity; and/or (c) measure individual differences (saliva flow, dental status, mouth behaviour, appetite and sensory thresholds). Additionally, a sensory panel (n = 12) evaluated whey models via quantitative descriptive analysis and determined a mouthdrying detection threshold in whey protein beverages (WPB).

Results demonstrated whey protein causes mouthdrying regardless of food model. WPB mouthdrying was detected at low protein levels and mucoadhesion was a suggested causal mechanism. Fortified solid models were also associated with reduced liking and modulated mouthfeel (moistness, chewiness and hardness). Age-related differences were present where older adults had reduced unstimulated saliva flow and increased mucoadhesion (WPB), mouthdrying sensitivity (WPB thresholds) and chewiness (fortified scones). However, measured individual differences (saliva flow, dental status, mouth behaviour and appetite) were not significantly related to mouthdrying perception. Two mitigating strategies successfully reduced mouthdrying: increasing lactose levels in

WPBs via cross-modal suppression and adding cream topping to scones by enhanced lubrication.

In summary, all tested whey protein fortified products were mouthdrying and mouthfeel sensitivity altered with age. These products can help to alleviate malnutrition and impede sarcopenia; however, they need an acceptable mouthfeel.

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Chapter 2

Victoria Norton (VN) produced all text, figures and tables with additional feedback and editing from Professor Lisa Methven (LM) and Dr Stella Lignou (SL). VN also drafted the response to reviewers' comments with subsequent guidance and editing by LM and SL.

Chapters 3 and 4

VN carried out all experimental work and statistical analysis with advice from LM and SL. VN produced all text, figures and tables with additional feedback and editing from LM, SL, Dr Stephanie Bull (SPB) and Professor Margot Gosney (MAG). VN also drafted the response to reviewers' comments with subsequent guidance and editing by LM and SL.

Chapter 5

VN carried out all experimental work and statistical analysis with advice from LM and SL. VN produced all text, figures and tables with additional feedback and editing from LM and SL. VN also drafted the response to reviewers' comments with subsequent guidance and editing by LM and SL.

Chapter 6

VN carried out all experimental work and statistical analysis with advice from LM and SL. VN produced all text, figures and tables with additional feedback and editing from LM, SL, Dr Marianthi Faka (Volac; MF) and Dr Julia Rodriguez-Garcia (JRG). VN also drafted the response to reviewers' comments with subsequent guidance and editing by LM and SL.

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List of abbreviations

AF: almond flour

2-AFC: two-alternative forced choice

3-AFC: three-alternative forced choice

ANOVA: analysis of variance

aPWP: acidic process whey protein

ASTM: American Society for Testing and Materials

aw: water activity

BAPEN: British Association for Parenteral and Enteral Nutrition

BCAA: branched chain amino acids

BDA: British Dietetic Association

β-LG: β-lactoglobulin

BNF: British National Formulary

BSA: bovine serum albumin

BT: baking time

CA: caseinates derived

CH: collagen hydrolysate

dE: colour difference

DLS: dynamic light scattering

DSP: descriptive sensory profiling

DS: dental status

EAA: essential amino acids

ESPEN: European Society for Clinical Nutrition and Metabolism

FLD: full lower denture

FUD: full upper denture

g/kg/d: grams per kilogram body weight per day

gLMS: generalised linear magnitude scale

HACCP: hazard analysis critical control point

HSD: honestly significant difference

HS-WPC: heat-stable whey protein concentrate

HWHP: hydrolysed wheat protein

HWPI: hydrolysed whey protein isolate

ISO: International Organization for Standardization

JAR: Just-About-Right

JND: just-noticeable difference

LSD: least significant difference

LSM: least square means

MB: mouth behaviour

MC: metal clip

MCR: Modular Compact Rheometer

MDT: mouthdrying detection threshold

MP: milk protein

MPC: milk protein concentrate

MPI: milk protein isolate

MUST: Malnutrition Universal Screening Tool

NHS: National Health Service

NICE: National Institute for Health and Care Excellence

NPN: non-protein nitrogen

OA: older adult

ONS: oral nutritional supplement

OT: oven temperature

PC: prospective consumption

PENG: Parenteral and Enteral Nutritional Group

PhP: physical properties

PIP: plastic plate

PLD: partial lower denture

PP: pea protein

PPI: pea protein isolate

PPs: proteose peptones

PUD: partial upper denture

PWP: process whey protein

QDA: quantitative descriptive analysis

RNI: Reference Nutrient Intake

RW: rennet whey

SCFP: School of Chemistry, Food and Pharmacy

SE: standard error

SF: saliva flow

SF-WPB: sugar-free whey protein beverage

SF-WPC: sugar-free whey protein concentrate

SMP: skimmed milk powder

SMS: Stable Micro System

SoF: soy flour

SP: soya protein

SPC: soya protein concentrate

SPI: soya protein isolate or isolate soya protein

SSF: stimulated saliva flow

SSPBC: saliva samples post beverage consumption

STD: single tooth denture

TPA: texture profile analysis

UREC: University Research Ethics Committee

USF: unstimulated saliva flow

VAS: visual analogue scale

WHO: World Health Organisation

WI: whey isolate

WP: whey protein or powder

WPB: whey protein beverage

WPBS: whey protein beverage sweetened

WPC: whey protein concentrate

WPCH: heated whey protein beverage

WPCU: unheated whey protein beverage

WPe: whey permeate

WPeB: whey permeate beverage

WPeBS: whey permeate beverage sweetened

WPH: whey protein hydrolysate

WPI: whey protein isolate

YA: younger adult

Chapter 1

Introduction

1.1. Thesis rationale

It is recognised that we have an ageing population in the UK, due at least in part to improvements in healthcare, lifestyle and technology compared with previous generations (Office for National Statistics, 2018). However, the ageing process can be influenced by physical, social and psychological factors which can contribute to increased risk of poor nutritional status (Armarya *et al*., 2015). Hence, this supports the importance of ensuring foods are developed to be suitable for older adults to help promote food intake, especially as nutritional provision can enhance functional and clinical outcomes (Stratton *et al*., 2018). Protein is of particular interest for an ageing population and intake requirements are considered to increase with age due to anabolic resistance (blunted protein synthesis response) and increased metabolism resulting from inflammatory conditions (Bauer *et al*., 2013; Deutz *et al*., 2014). Therefore, sufficient protein intake can help prevent age-related muscle mass, strength and functional losses (Deutz *et al*., 2014). Accordingly, protein is often fortified into products for older adults to help alleviate malnutrition and impede sarcopenia. Moreover, dietary proteins are frequently cited for their functionality benefits in developing foods, such as heat stability, foaming, water binding, solubility, gelation and emulsification (Harper, 2009). Whey protein was selected as a protein source to investigate for this thesis for the following reasons:

- Whey proteins provide positive nutritional benefits (being a complete protein source, readily digested and absorbed, as well as leading to greater muscle synthesis response) compared with other protein sources (Dangin *et al*., 2003; Hoffman & Falvo, 2004).

- Whey proteins have been proven to trigger muscle synthesis (Pennings *et al*., 2011) and protein gain in older adults, which could subsequently prevent muscle mass losses (Dangin *et al*., 2003; Kobayashi *et al*., 2016).
- Whey proteins can be readily fortified into products. For example, to create a high protein beverage or snack (34-90+% protein content depending on whey protein source) (Hoffman & Falvo, 2004; Croissant *et al*., 2009).
- Whey proteins are commonly fortified into a range of commercial products (e.g. oral nutritional supplements (ONS), protein bars, cereals, flapjack, brownies, cakes, chocolate and cookies).
- Whey proteins can, however, elicit negative sensory attributes (such as mouthdrying) which are considered limiting factors to its widespread application (Sano *et al*., 2005; Beecher *et al*., 2008; Lee & Vickers 2008; Vardhanabhuti *et al*., 2010; Kelly *et al*., 2010; Ye *et al*., 2011; 2012; Withers *et al*., 2014; Bull *et al*., 2017).

Whey protein fortified products are commonly used to improve nutritional status amongst older adults. However, such products are associated with mouthdrying (a textural defect) (fully defined in **Chapter 2** see Section 2.6) which is considered to increase with a consumer's age, repeated consumption and product heating time (Lemieux & Simard, 1994; Methven *et al*., 2010; Withers *et al*., 2013a; Bull *et al*., 2017). However, the exact causes of whey protein derived mouthdrying are yet to be resolved in the literature. Accordingly, understanding the proposed mechanisms could lead to product reformulation and result in improved consumer acceptance and subsequent intake. Currently, mucoadhesion (adhesion of protein to the oral cavity) (fully defined in **Chapter 2** see Section 2.6.2) is a suggested, but as a yet to be proven, cause of mouthdrying in a neutral pH whey protein beverage (WPB) (Withers *et al*., 2013b; Bull *et al*., 2020). This needs further investigation: (a) with an increased sample size (only previously

investigated with five volunteers); (b) involving an older adult population (not previously investigated in an ageing population); (c) with improved methodology (to address calculation and baseline value concerns); and (d) using a suitable non-protein control (previous studies have yet to use a non-protein whey control). Moreover, mouthdrying is well established in WPBs, yet the extent of mouthdrying sensations within a high protein solid food matrix is less clear. Furthermore, individual differences (such as age, food oral processing and saliva) are considered to have a role within sensory perception; however, the extent of such individual differences on mouthdrying and mucoadhesion remains uncertain.

1.2. Thesis hypotheses, aims and objectives

The overall aim of this thesis was to investigate the extent of perceived mouthfeel effects derived from whey protein fortified products and the influence of individual differences. Simple whey protein fortified beverages and snacks can help to alleviate malnutrition and impede sarcopenia; however, they need be palatable and acceptable. More specifically, this thesis hypothesises that: (a) whey protein fortified beverages and snacks will cause mouthdrying; (b) whey protein will adhere to the oral cavity post WPB consumption; (c) mucoadhesion will increase with age post WPB consumption; (d) mucoadhesion is a probable cause of whey protein derived mouthdrying; (e) individual differences (such as age, saliva flow, dental status, mouth behaviour, appetite and sensory thresholds) will influence perceived whey protein derived mouthdrying; and (f) mitigating strategies (such as varying in lactose or fat) will reduce whey protein derived mouthdrying. Accordingly, these hypotheses were tested through the following objectives as outlined in Figure 1.1.

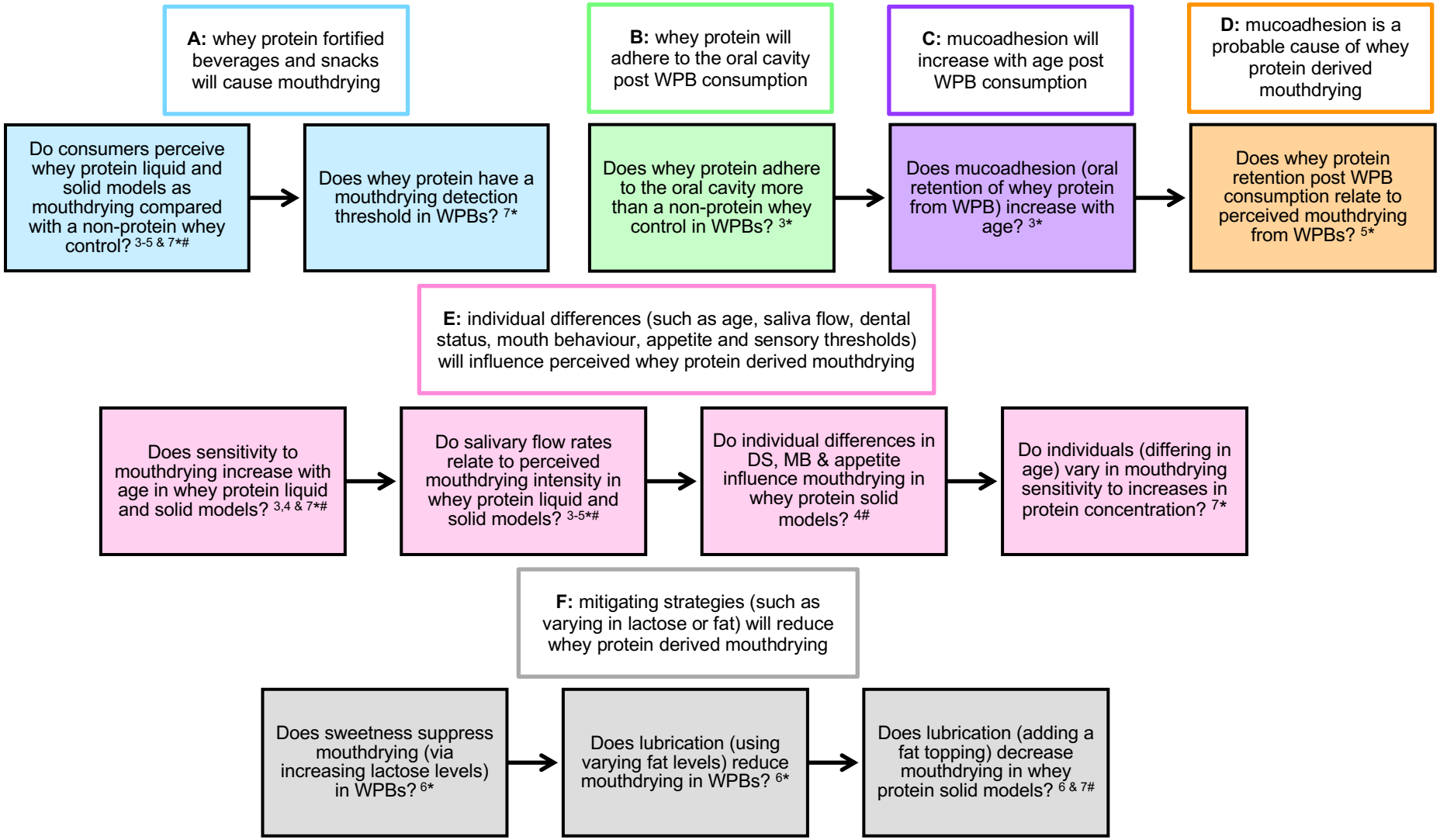


Figure 1.1. Overview of thesis hypotheses (non-shaded boxes; letters [A-F]) with corresponding objectives (shaded coloured boxes; numbers [3-7] represent thesis chapter tested in; * refers to liquid model objective; # indicates solid model objective). Acronyms: whey protein beverages (WPB); saliva flow (SF); dental status (DS); mouth behaviour (MB).

In order to test these objectives, appropriate methods and food models are required which also have to be suitable for older adults. Over the coming sections, rationale will be provided for the study populations, methodology, food models and individual differences selected.

1.3. Study populations

Within the literature, studies investigating older adults have often used differing age criteria (such as ranging from above 55, 60 or 65 years) which can make comparisons between studies challenging. However, this thesis defines older adults as individuals aged 65 years or over, as commonly defined in the UK (Office for National Statistics, 2018; Office for National Statistics, 2019; NHS[1] England, 2020). To investigate the effect of age, a younger adult population aged 18 to 30 years was selected to provide at least a 35 year gap between age groups. In addition, this thesis focuses on healthy community-based populations to understand the extent of differences between two healthy age groups, rather than frail or older adults in clinical settings.

1.4. Methodology

This thesis focuses mainly on using sensory related methods, combined with two oral physical measures (saliva flow and oral retention), to investigate whey protein derived mouthdrying. Table 1.1 provides an overview of methods used within this thesis and it should be noted there are other methods that could be used to investigate whey protein derived mouthdrying (as outlined in **Chapter 2**; Table 2.3). However, these are mostly considered to be outside the scope of this thesis as they are predominately instrumental based rather than consumer focused.

[1] NHS: National Health Service

Table 1.1. Overview of thesis methods.

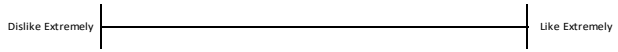
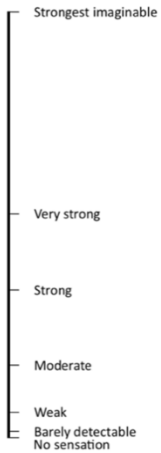
Method	Description	Thesis Rationale	Chapter
Saliva flow[1]	○ Saliva flow is a measure of saliva quantity and typically carried out in two ways: (1) at rest and without stimulation for 5-min (unstimulated saliva flow) and (2) with stimulation in response to a mechanical stimuli (i.e. chewing on a piece of parafilm (considered to replicate chewing behaviour and stimulate saliva) for 2-min (stimulated saliva flow)[(a-f)].	○ Saliva flow is modulated by age and could influence mouthdrying perception. Saliva flow collection is a non-invasive method[(a)] as such can be easily collected by all volunteers during a study visit.	3-5
Oral retention[1]	○ This involves collecting saliva samples post beverage consumption (at varying time points) and subsequent protein analysis (Bradford assay) to determine the amount of protein retained in the oral cavity[(g,h)].	○ This method provides a suitable in-mouth method to measure adhesion to the oral cavity for both younger and older adults.	3 & 5
Hedonic testing[2]	Hedonic scales can measure degree of liking[(i)] Examples include: ○ 9-point hedonic scale provides nine verbal categories ranging from 'dislike extremely to 'like extremely'.  ○ Visual analogue scale[#] (VAS) often anchored with 'dislike extremely' to 'like extremely'.  	○ Hedonic scales are considered a suitable test for older adults and are regularly used in sensory testing to evaluate liking[(j)]. ○ VAS[#] are widely cited within the literature due to being simple to use and can also utilise both liking and perception ratings.	3-5 & 7
Intensity testing[2]	Intensity testing provides perceived intensity of a specific attribute[(i)]. Examples include:  ○ generalised labelled magnitude scale (gLMS), a perceived intensity scale (0-100) with seven semantic descriptors ranging from 'no sensation' to 'strongest imaginable sensation of any kind'. ○ VAS[#] often anchored with 'not' to 'very'. ○ Two-Alternative Forced Choice^ (2-AFC) tests (a type of discrimination testing) can be used to select which sample is more intense for a particular attribute.	○ Descriptor scales can help consumers to interpret the scale better and are suitable for older adults[(j)]. ○ The 2-AFC^ test is suggested for its simplicity whilst focusing on a specific question and is considered suitable to detect small differences between samples[(i)] Sometimes such differences can be missed if presented monadically on rating scales[(k)].	3-5

Table 1.1. continued…

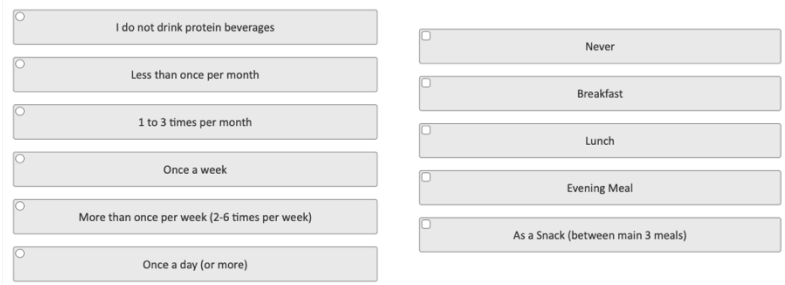
Method	Description	Thesis Rational	Chapter
Diagnostic testing[2]	○ Just-About-Right (JAR) scales measure appropriateness of attribute level to understand whether a specific attribute is close to optimum (JAR = 3 or 50 depending on the scale) using a five-point scale (or 0-100 scale) with five verbal categories ranging from 'much too weak' to 'much too strong'[(i)]. 	○ JAR scales can be useful to understand whether older adults require more or less of a particular attribute to be closer to optimum compared with younger adults. Like hedonic scales they are considered simple to use and suitable for older adults. JAR (category) and liking data can also be used for penalty analysis which provides feedback on whether an attribute not JAR influences consumers acceptability of the product[(i,j)].	3-5 & 7
Preference testing[2]	○ Preference testing determines which product is preferred. Consumers are required to make a choice using either a paired preference test (consumer receives two samples; selecting most preferred) or by ranking products in order of preference[(i)].	○ Similar rationale as a 2-AFC^ test. Preference testing can often pick up small differences that are sometimes missed on liking scales.	3-5
Comments boxes[2]	○ Provides consumers with opportunities to express their opinions relating to a product.	○ Comment boxes provide an unstructured option for consumers to provide feedback relating to the product.	3-5 & 7
Consumption questions[2]	○ Questions relating to frequency of consumption and when typically consumed are assessed by category scales. 	○ To check consumers familiarity with products.	3-5 & 7
Dental status[3]	○ Self-report questionnaire can provide a summary of an individual dental status without being invasive.	○ Dental status can alter with age and could have relevant to sensory perception.	4

Table 1.1. continued…

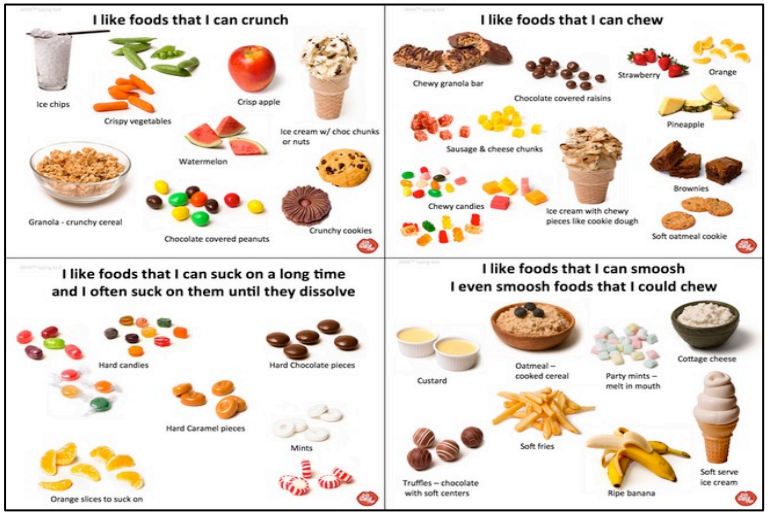
Method	Description	Thesis Rational	Chapter
Mouth behaviour[3]	○ Self-report texture related tool[l,m]. 	○ Mouth behaviour grouping could alter sensory perception.	4
Appetite ratings[3]	○ Subjective appetite ratings provide pre- and post- consumption measures relating to hunger (how hungry are you now?), thirst (how thirsty are you now?), fullness (how full are you now?), satiety (how satiated are you now?), desire to eat (how strong is your desire to eat now?) and prospective consumption (how much do you think you could (or would want to) eat right now?) typically using VAS# (0-100 mm) often anchored with 'not at all to extremely'[n,o].	○ Appetite ratings could provide useful insight into sensory perception and product compliance.	4
Threshold testing[4]	○ Threshold testing provides data on sensitivity of individuals and a concentration range at which an individual detects a perceived stimulus[i].	○ Sensory detection thresholds (minimum intensity of a stimulus required to cause a perceptual response)[i] are considered to alter with age and could explain differences in mouthdrying sensitivity. The three-alternative forced choice (3-AFC) test (two controls and one stimulus) is often used for threshold testing[i,p]. ○ Just-noticeable difference (JND) or discrimination thresholds denotes the intensity needed to elicit a perceptual change[i].	7

Table 1.1. continued…

Method	Description	Thesis Rational	Chapter
Descriptive sensory profiling (DSP)[5] (based on the trademarked quantitative descriptive analysis (QDA™))	○ DSP is a descriptive sensory analysis[(q,r)] and which provides an analytical tool to evaluate sensory attributes of products. Such analysis (ISO 13299:2016) is typically carried out using 8-12 trained sensory panellists (selection based on International Organisation for Standardisation (ISO) 8586:2012 and who undergo performance monitoring in accordance with ISO 11132:2012)[(s-v)]. In summary, panellists develop a consensus vocabulary with the help of a panel leader. For each attribute a reference or description is provided. Once the vocabulary is agreed and sufficient training has been completed on the attributes. Scoring (in duplicate during separate sessions) is carried out individually in isolated sensory booths† on visual analogue scale (VAS; 0-100) with appropriate anchors.	○ DSP provides a well-established method to understand analytically how products differ in specific sensory attributes relating to appearance, aroma, flavour, mouthfeel and aftertaste. ○ This thesis uses the commercial trained expert sensory panel based at University of Reading.	4-6
Apparent viscosity[6]	○ Describes whey beverages flow behaviour and subsequent thickness (typically reported as viscosity over shear rate) often defined as 50 s^{-1} to reflect the commonly reported oral shear rate[(w)]. All measurements of whey beverages were taken using an oscillatory rheometer	○ Differences in apparent viscosity could influence sensory perception or sample selection in alternative forced choice tests.	5-7
Colour, water activity, moisture content and height analysis[6]	○ Such analysis provides an overall description of products relating to their physical properties.	○ To provide supplementary information to support sensory results. In addition, to understand whether solid models (cakes, biscuits and scones) control and protein products differ in physical properties.	4 & 6
Texture profile analysis (TPA)[6]	○ TPA is an instrumental analysis and provides texture related measurements of products[(x,z)].	○ Enables instrumental data (i.e. hardness (maximum force at first compression), fracturability (force at first peak), cohesiveness (relative resistance), springiness (ability to spring back), chewiness (similar trend to hardness)) that can be subsequently related to the sensory results[(y,z)].	4 & 6

(a) Lucas *et al*., 2018; (b) Vandenberghe-Descamps *et al*., 2016; (c) Pushpass *et al*., 2019a; (d) Mackie & Pangborn, 1990 (e) Affoo *et al*., 2015; (f) Feron, 2019; (g) Cook *et al*., 2018; (h) Bull *et al*., 2020; (i) Lawless & Heymann 2010; (j) Methven *et al*., 2016; (k) Zhou *et al*., 2016; (l) Jeltema *et al*., 2015; (m) Jeltema *et al*., 2016; (n) Blundell *et al*., 2010; (o) Flint *et al*., 2000; (p) ISO, 2018; (q) Stone *et al.* 1974; (r) Stone & Sidel, 2004; (s) ISO, 2016; (t) Heymann *et al*., 2012; (u) ISO, 2012a; (v) ISO, 2012b; (w) National Dysphagia Diet Task Force, 2002; (x) Friedman *et al*., 1963; (y) Szczesniak, 2002; (z) Texture Technologies, 2019. # VAS was used in chapter 4 & 7 for simplicity as it enabled liking and intensity ratings on the same scale type for at home studies. 2-AFC^ (can also be referred to as paired comparison test) all in accordance with ISO 5495:2005 guidelines (ISO, 2005). †isolated booths (meeting ISO 8589:2007 guidelines (ISO, 2007)) were used prior to COVID-19 pandemic and to permit sensory testing during UK national lockdown all evaluation was conducted at individuals homes. Differing subscript numbers [(1-6)] denote different method type as follows: 1 = oral physical measures; 2 = sensory consumer methods; 3 = consumer individual differences related methods; 4 = sensory trained panel and consumer methods; 5 = sensory panel; 6 = physical properties analysis.

1.4.1. Sensory perception

Sensory evaluation can be defined as “a scientific discipline used to evoke, measure, analyse, and interpret reactions to those characteristics of foods and materials as they are perceived by the sense of sight, smell, taste, touch and hearing” (Stone & Sidel, 2004, p.13). Measurement and evaluation of the sensory characteristics of foods can enable us to understand better the sensory attributes of products, as well as consumers’ food preferences and liking (Stone & Sidel, 2004). ‘Perception’ is considered to occur when an individual perceives a sensation, in this case from food (Stone & Sidel, 2004). Within the context of this thesis the focus of sensory perception is primarily on mouthfeel; how this alters with whey protein fortification and is subsequently influenced by individual differences. As alluded to in Table 1.1, central to the methodology selection was ensuring suitability, since healthy older adults can often be described as a diverse group (Methven *et al*., 2016). Accordingly, ensuring methods are suitable to reflect the diversity within this age group is key; the commonly used methods include alternative forced choice, labelled scales, hedonic testing, line scales, sorting tasks and threshold testing (Methven *et al*., 2016).

1.4.2. Food oral processing

Food oral processing relates to a series of processes involved in eating, such as biting, chewing, transportation, bolus formation and swallowing, with these processes occurring in sequence or simultaneously (Chen, 2009). This can be described as a dynamic, complex and physiological process involving muscle activities, jaw and tongue movements, food breakdown and mixing with saliva, to ensure food can be safely swallowed (Engelen, 2018). Aroma, taste and texture can be evaluated as the result of chemicals travelling to receptors (olfactory and taste) and food particles interacting with oral surfaces (texture) (Engelen, 2018). Food oral processing behaviour is also influenced

by type of food (liquid, semi-solid and solid), age, gender and ethnicity (Ketel *et al*., 2019). Food consumption is also subject to oral movements from the tongue, teeth, lips, palate and cheeks which ensure the food bolus is safety swallowed and these movements are likely to influence sensory perception (de Wijk *et al*., 2003). This thesis only utilises self-reported food oral processing measures, such as dental status and mouth behaviour (**Chapter 4**) (discussed as limitations in **Chapter 8**).

1.4.3. Saliva

Saliva is a natural bodily fluid that is secreted from the major salivary glands (parotid, submandibular and sublingual glands) which produce approximately 92-95% of saliva, as well as from the minor salivary glands (Table 1.2) (Varga, 2015; Affoo *et al*., 2015; Munoz-Gonzalez *et al*., 2018a). Salivary glands are typically composed of three major cells, namely acinar, ductal and myoepithelial cells (Varga, 2015). Saliva is produced by the salivary glands in the acini (end pieces of glands) with serous cells producing watery seromucous secretions and mucous cells producing viscous mucin rich secretions (Whelton, 2012). Saliva secretion is also influenced by blood supply, for example, rapid saliva production requires a good blood supply (Whelton, 2012).

Table 1.2. Overview of salivary glands (Whelton, 2012; Varga, 2015; De Paula *et al*., 2017).

Gland	Size	Location	Secretion Type
Parotid Gland[1]	○ Largest major gland (~25-30 g)	○ Located behind the lower jar and in front of the ear	○ Watery saliva secretions
Submandibular Gland[1]	○ Second largest major gland (~7-15 g)	○ Located in the back of the floor of the mouth near to the lower jar	○ Moderately viscous saliva secretions
Sublingual Gland[1]	○ Smallest major gland (~3 g)	○ Located in the base of the mouth	○ Very viscous saliva secretions
Minor Salivary Glands	○ ~ between 600 and 1200 minor salivary glands	○ Located within the base of the tongue, buccal, labial, palatal and lingual regions	○ Predominantly mucous secretions

[1] denotes major salivary gland and ~ refers to approximately

A normal saliva flow is considered to be above 0.1 and 0.5 mL/min for unstimulated and stimulated saliva respectively (Humphrey & Williamson, 2001; Marton *et al*., 2008), with cited values often being between 0.3-0.4 mL/min (unstimulated saliva) and 1.5-2.0 mL/min (stimulated saliva) (Whelton, 2012). However, variability has been associated with saliva flow; therefore, controlling and standardising these variables (such as smoking, hydration status, previous stimulation, circadian rhythms, drugs, body position) is key to accurate measurement (Dawes, 1987). This thesis focused on the role of saliva flow on sensory perception in **Chapters 3-5** only (the COVID-19 pandemic prevented subsequent saliva flow related investigation). In addition, other saliva analysis (i.e. viscoelasticity) were considered; however, were not possible due to time constraints within a study day or were considered outside the scope of this thesis (discussed as limitations in **Chapter 8**).

1.5. Food models

Currently, there are two suggested nutritional approaches (namely supplementation and fortification) to enhance protein intake within the ageing population. Typically, either ONS (providing additional micro and macro nutrients) or fortified snacks (adding protein content without increasing portion size) are utilised between and/or after meals amongst older adults (BAPEN[2], 2016). More generally, a product needs at least 12% or 20% energy content from protein to be considered either a protein or high protein source (European Commission, 2019). Within clinical settings protein content in products is often variable (depending on exact purpose); however, ONS usually have 5.0 g (or more) (per 100 g) of protein (NICE[3], 2019). Similarly, the British Dietetic Association (BDA) suggest between meal snacks (at least two daily) should have 2.0 g and 4.0 g protein content for

[2] BAPEN: British Association for Parenteral and Enteral Nutrition
[3] NICE: National Institute for Health and Care Excellence

nutritionally well and vulnerable individuals respectively (BDA, 2017). Accordingly, this thesis focused on two different food models to investigate whey protein derived mouthdrying, namely a liquid model (beverages) and a solid model (snacks), which reflect the typical products used in clinical settings (Table 1.3) for older adults at risk of malnutrition. It is evident that a range of serving sizes (23-550 g and 100-500 mL) with differing protein content (0.9-50 g per 100 g/mL) are regularly utilised, but there is a lack of consistency in reporting, variability and/or mixture in protein sources (whey protein, milk and plant related or other), which can make comparisons challenging. Food models were used in this thesis since they can be easily manipulated and fortified with whey powders, whilst having minimal additional ingredients (i.e. commercially available products typically have an extensive ingredient list). Therefore, beverages fortified with different whey powders were chosen to represent models varying in: (a) heat treatment (suggested to intensify mouthdrying); (b) protein (suggested to cause mouthdrying) and protein level (suggested to intensify mouthdrying); (c) sweetness (suggested to improve palatability and suppress mouthdrying); or (d) fat (suggested to reduce mouthdrying). Snacks (such as cakes, biscuits, cupcakes and scones) fortified with different whey powders were selected to provide common and familiar foods as well as being products that vary in texture (soft and hard) or toppings (with and without). All thesis tested food models (WPBs, cakes, biscuits, cupcakes and scones) are summarised in Table 1.4 alongside commonly recommended ONS in Table 1.5.

Table 1.3. Examples of food matrices commonly fortified with protein within the literature, clinical settings and/or retail market.

Food Matrix	Protein Source	Serving Size	Protein (g)	
			Serving size	100 g or mL
Literature based studies				
Bread[a-e]	-†	27-35 g†	5.6-7.9	-
Yoghurt[a,b]	WPC	100-250 mL	8.0-20.0	8.0^
Fruit juices[c-e]	-	150-200 mL	10.0-10.6	-
Soups[c-e,f]	-MP^	75-79 g & 150mL	10.0-10.1	-
Mashed potato[c-e]	-	150 g	8.4-10.5	-
Dairy drinks([d,e]	-	150 mL	10.1	-
Cakes[d,e]	-	65 g	9.9	-
Ice cream[d,e]	-	100 mL	10.0	10.0^
Meat[d,e]	-	50-80 g	12.4-22.0	-
Milkshake (orange)[g]		150 g	16.0	-
Chocolate cake[g]		56 g	7.6	-
Pizza bun[g]	Combined approach of high	40 g	6.0	-
Fruit salad[g]	protein products & protein	65 g	8.4	-
Bun[g]	powder (using whey, gelatin or	40 g	4.9	-
Cheese crackers[g]	pea protein)	10 g	1.4	-
Sandwich (ham)[g]		40 g	5.3	-
Jelly (apple & cream)[g]		50 g	9.5	-
Enriched breakfast dishes[f]	MP	60-100 g	7.4-11.5	11.5^
Enriched fish dishes[f]	MP	55-70 g	7.6-8.9	-
Enriched meat dishes[f]	MP	55-75 g	6.5-7.9	-
Enriched side dishes[f]	MP	47-75 g	6.1-7.7	-
Enriched desserts[f]	MP	52-110 g	6.2-7.6	6.9^
High energy and/or protein snacks[h]	-	-	0.0-6.0	-
Protein rich ready meals[i]	-	500-550 g	30.5 (av)	-
Protein rich dairy products[i]	-	30 g & 150 mL	5.1-11.6	-
Biscuit[j]	WPI	40 g	5.0	12.4
Sauces[k]	WPI	50 g	0.5-2.3	0.9-4.5
Rye bread[l]	WPH & WPI	35 g	4.8-7.0	-
Cream cheese[l]	WPH & WPI	25 g	2.9-3.0	11.6-11.9
Muffins[m,n]	WP, AF & SF	100 g	9.1-14.1	9.1-14.1
Beverages[o,p]	WPC, WP	150-200 mL	20.7-24.0	-
Clinical settings (ONS based)				
ONS*	MP, MPC, SP, MPI, SPI & CA	125-200 mL	11.2-20.0	5.6-10.0
ONS soup*	MP, PP, SMP, MPC	150-200 mL	6.0-20.0	4.2-16.1
ONS juice*	WP, WPI, MP, SPI	150-220 mL	7.8-11.0	3.9-16.2
ONS yoghurt*	WP, MPI, SMP	125g & 200 mL	9.3-15.0	5.9-7.5
ONS dessert*	MP, MPC, MPI, SPI & CA	125 g	7.1-12.5	7.5-10.0
ONS other*	WI & CH	118 mL	20.0	16.9
Retail market-based products				
Protein milk*	WP, MP, MPC	330-500 mL	25.0	5.0-7.6
Protein yoghurt*	Quark (milk)	150-200 g	15.0-22.0	10.0-11.4
Protein cereals*	WPC, MPC, SMP & SPI	40-75 g	8.4-20.6	19.0-27.4
Protein bars*	WPC, WPI, WP, SPI, MP, CA, MPI, WPH & PPI	30-65 g	4.5-20.0	15.0-34.0
Protein flapjack*	WPC, WP, HWPI, SMP, MP, PP & HWHP	40-88 g	10.0-22.0	23.0-25.0^
Protein balls*	WPI, WP, SP & MP	35-50 g	9.8-15.0	20.5-43.0
Protein brownies*	WP, WPC, HWHP, CA, MP & SP	40-75 g	10.6-23.0	20.0-30.0
Protein cakes*	WPC, WP, MPI, SPI & MP	30-60 g	7.9-15.0	25.0-26.0
Protein pancakes*	WPC, SMP	45 g	16.0	35.5
Protein cookies*	WPI, MP, MPI, SP & HWHP	59-75 g	13.0-38.0	18.0-50.0^
Protein chocolate*	WPI	70 g	19.0-19.5	27.0-27.9
Protein chocolate bars*	WPC, WP, SMP, MP & MPI	47-51 g	10.1-15.0	20.2-30.0
Protein crisps*	WPI, WP, CA, MPI & SP	32-50 g	18.0-20.0	-
Protein other*	WPI, WP, SMP, SPI & SPC	23-36 g	7.7-9.0	25.0-33.4

Dash (-) denotes not recorded within study. ^ represents reported by one study only or not reported by all studies within subset. Av outlines average and ONS represents oral nutritional supplement. Protein sources defined as follows: **whey protein** (WPC: whey protein concentrate; WPI: whey protein isolate; WPH: whey protein hydrolysate: WP: whey protein or powder; WI: whey isolate; HWPI: hydrolysed whey protein isolate); **milk related** (MP: milk protein; MPC: milk protein

concentrate; MPI: milk protein isolate; SMP: skimmed milk powder; CA: caseinates derived); **plant related** (SP: soya protein; SPI: soya protein isolate or isolate soya protein; SPC: soya protein concentrate; SoF: soy flour; PP: pea protein; PPI: pea protein isolate; HWHP: hydrolysed wheat protein; AF: almond flour); and **other** (CH: collagen hydrolysate). †denotes small differences between studies: Beelen *et al*. (2017a) noted protein source as soy and dairy based whereas other studies([a,b,d]) protein source was not reported within studies. Van Til *et al*. (2015) study only reported serving size as slice rather than in grams. Literature based studies references: (a) Van Til *et al*., 2015; (b) Stelten *et al*., 2015; (c) Beelen *et al*., 2017a; (d) Beelen *et al*., 2017b; (e) Beelen *et al.,* 2018; (f) Munk *et al*., 2014; (g) Mortensen *et al*., 2019; (h) Campbell *et al*., 2014; (i) Borkent *et al*., 2019; (j) Tsikritzi *et al*., 2014; (k) Tsikritzi *et al*., 2015; (l) Song *et al*., 2018; (m) Wendin *et al*, 2017; (n) Hoglund *et al*., 2017; (o) Ridge *et al*., 2018; (p) Bauer *et al*., 2015. ONS and retail market products data* obtained from brands website. **ONS** describes six ONSs: 1 = Fortisip compact (Nutricia); 2 = Resource energy (Nestle); 3 = Fresubin protein energy drink; 4 = Altraplen compact (Nualtra); 5 = Ensure plus milkshake style; 6 = Aymes complete. **ONS soup** reflects six soups: 1 = Actasolve Savoury (Aymes); 2 = Vitasavoury (Vitaflo); 3 = Energis soup (Meritene®); 4 = Resource Soup (Nestle); 5 = Fortified soups (Apetito); 6 = Fresubin 2kcal savoury. **ONS juice** notes six juices: 1 = Altrajuice (Nualtra); 2 = Ensure Plus Juice; 3 = Fresubin Jucy drink; 4 = Fortijuice (Nutricia); 5 = Aymes ActaJuce; 6 = Aymes ActSolve Smoothie. **ONS yoghurt** represents four yoghurts: 1 =. Ensure plus yoghurt; 2 = Fortisip yoghurt; 3 = Fresubin YoDrink; 4 = Fresubin YOcreme. **ONS dessert** highlights six dessert: 1 = Forticreme Complete (Nutricia); 2 = Fresubin® 2 kcal Crème; 3 = Ensure plus crème; 4 = Resource Dessert 2.0 (nestle); 5 = Nutricrem (Nualtra); 6 = Aymes Actacal crème. **ONS other** signifies jelly (Prosource Jelly; Nutrinovo). **Protein milk** denotes four milks: 1 = Protein chocolate milk (Arla); 2 = Protein chocolate milk (Dale Farm); 3 = Protein chocolate milk (Maximuscle); 4 = High protein chocolate (For Goodness Shakes). **Protein yoghurt** reflects three yoghurts: 1 = protein yoghurt (Arla); 2 = Lindahl Kvarg (Nestle); 3 = Protein 22 (Graham's). **Protein cereals** notes four cereals: 1 = Protein granola (MyProtein); 2 = Protein granola (Lizi's); 3 = Protein oats (oomf); 4 = Protein porridge (Fuel). **Protein bars** represents four bars: 1 = Cereal bar (MyProtein); 2 = Granola bar (MyProtein); 3 = Carb killa (Grenade); 4 = Diet whey bar (PhD). **Protein flapjack** highlights four flapjacks: 1 = Snickers protein flapjack (Mars); 2 = Oats & whey protein flapjack (MyProtein); 3 = Protein flapjack (Oatein); 4 = Protein flapjack (Bulk). **Protein balls** signifies four balls: 1 = Choc protein balls (MyProtein); 2 = Energy bites (MyProtein); 3 = Protein ball (The Protein Ball Company); 4 = Chocolate balls (Bulk). **Protein brownies** denotes four brownies: 1 = Protein brownie (MyProtein); 2 = Protein brownie (Mountain Joe's); 3 = Protein brownie (The Protein Works); 4 = Oatein brownie. **Protein cakes** indicates two cakes: 1 = Pop Roll (MyProtein); 2 = Protein cake (PhD Smart). **Protein pancakes** expresses protein pancake (Nano ä). **Protein cookies** highlights four cookies: 1 = Protein cookie (MyProtein); 2 = Baked cookie (MyProtein); 3 = Protein cookie (Quest); 4 = Protein cookie (Oatein). **Protein chocolate** reflects two chocolates: 1 = Protein chocolate (MyProtein); 2 = Protein chocolate (Cocoa+). **Protein chocolate bars** describes three chocolate bars: 1 = Mars hi-protein chocolate bar; 2 = M&M's hi-protein bar; 3 = Snickers protein bar. **Protein crisps** notes two crisps: 1 = Protein crisps (Quest); 2 = Protein crisps (GOT7). **Protein other** represents two crispies: 1 = Protein choc crispies (MyProtein); 2 = Protein crispies (The Skinny Food Company).

Table 1.4. Overview of thesis tested food models and associated thesis chapter.

Thesis Model	Acronyms	Description	Experiment	Chapter
Whey permeate beverage[1]	WPeB	Standard WPeB	Oral retention, DSP & preference testing	3 & 5
Whey permeate beverage sweetened[1]	WPeBS	Standard WPeB with added vanilla and sucrose	DSP, liking, perception & oral retention	5
Whey permeate beverage[1]	WPeB	Standard WPeB with added hydrocolloid	MDT	7
Whey protein beverage[1]	WPB	Standard WPB	Oral retention, DSP & preference testing	3,5 & 6
Unheated whey protein beverage[1]	WPCU	Standard WPB	Liking, perception & oral retention	3
Heated whey protein beverage[1]	WPCH	Standard WPB and heated for 20-min at 70 °C	Liking, perception & oral retention	3
Whey protein beverage sweetened[1]	WPBS	Standard WPB with added vanilla and sucrose	DSP, liking, perception & oral retention	5
Sugar-free whey protein beverage[1]	SF-WPB	Standard SF-WPB (control)	DSP lactose & fat subset	6
Sugar-free whey protein beverage[1]	SF-WPB	Standard SF-WPB with added lactose	DSP lactose subset	6
Sugar-free whey protein beverage[1]	SF-WPB	Standard SF-WPB with added hydrocolloid	DSP fat subset	6
Sugar-free whey protein beverage[1]	SF-WPB	Standard SF-WPB with added hydrocolloid and fat	DSP fat subset	6
Sugar-free whey protein beverage[1]	SF-WPB	Standard SF-WPB with added hydrocolloid, protein and lactose	MDT & JND	7
Control cake[2]	-	Control cake	DSP, PhP, liking & perception	4
Protein cake[2]	-	Protein cake fortified with whey protein isolate (WPI)	DSP, PhP, liking & perception	4
Control biscuit[2]	-	Control biscuit	DSP, PhP, liking & perception	4
Protein biscuit[2]	-	Protein biscuit fortified with whey permeate	DSP, PhP, liking & perception	4
Control cupcake (optimised)[2]	-	Control cupcake fortified with whey permeate	DSP, PhP, liking & perception	4
Protein cupcake (optimised)[2]	WPC	Protein cupcake fortified with whey protein concentrate (WPC)	DSP, PhP, liking & perception	4
Protein cupcake (heat-stable)[2]	HS-WPC	Protein cupcake fortified with heat-stable WPC	DSP & PhP	6
Control scone[2]	-	Control scone fortified with whey permeate	DSP & PhP	6
Protein scone[2]	-	Protein scone fortified with whey protein concentrate	DSP & PhP	6
Protein scone + no topping[2]	-	Protein scone fortified with whey protein concentrate	DSP, PhP, liking & perception	6 & 7
Protein scone + cream topping[2]	-	Protein scone fortified with WPC and added cream topping	DSP, PhP, liking & perception	6 & 7

[1] Denotes whey liquid model and [2] represents whey solid model. Dash (-) notes not applicable. Standard whey protein beverage (WPB) uses whey protein concentrate whereas standard SF-WPB utilises uses sugar-free whey protein concentrate (SF-WPC). WPBs utilised in this thesis are considered near neutral pH with all models are fully defined in each corresponding chapters and in brief: (a) whey permeate models provided a non-protein whey control to enable comparisons with whey protein models; (b) use of sugar-free whey protein allowed controlled evaluation of sweetness; and (c) WPB heat treatment and heat-stable whey powders permitted investigation into the effect of different additional processes on subsequent perception or oral retention. Acronyms: descriptive sensory profiling (DSP); mouthdrying detection threshold (MDT); just-noticeable difference (JND) and physical properties (PhP).

Table 1.5. Macronutrient composition of thesis tested food models (whey protein beverages (WPB), cakes, biscuits, cupcakes and scones) compared with typical oral nutritional supplements (ONS) available on-the-market (mL per 100 mL; g per 100 g).

	Whey protein liquid and solid models[a]					**Oral Nutritional Supplements[b]**					
	WPB per 100 mL	Cakes per 100 g	Biscuits per 100 g	Cupcakes per 100 g	Scones per 100 g	ONS 1[†] per 100 mL	ONS 2[†] per 100 mL	ONS 3[†] per 100 mL	ONS 4[†] per 100 mL	ONS 5[†] per 100 mL	ONS 6[†] per 100 mL
Energy (kcal)	39.7	411	588	445	353	240	151	150	240	150	150
Fat (g)	0.7	22.0	34.0	24.0	13.0	9.3	5.0	6.7	9.6	4.9	6
of which saturates (g)	0.3	13.0	2.5	14.0	7.2	0.9	0.7	0.6	0.9	0.4	0.6
Carbohydrates (g)	0.4	41.0	56.0	45.0	44.0	29.7	21.0	12.1	28.8	20.2	18.0
of which sugars (g)	0.4	24.0	24.0	26.0	7.5	15.0	5.7	7.4	11.6	6.5	6.8
Protein (g)	8.2	12.0	14.0	12.0	15.0	9.6	5.6	10.0	9.6	6.3	6.0

[a] Reflects whey protein fortified models (protein version only) and data is obtained from Nutritics (v5.096, Dublin, Ireland) or ingredients technical sheets; [b] denotes six ONS (data acquired from brands websites) (ONS 1: Fortisip compact (Nutricia); ONS 2: Resource energy (Nestle); ONS 3: Fresubin protein energy drink; ONS 4: Altraplen compact (Nualtra); ONS 5: Ensure plus milkshake style; ONS 6: Aymes Complete) commonly used in clinical settings. [†] ONS protein source: ONS 1,2,3,6: milk proteins; ONS 4: milk protein concentrate, milk protein, soya protein; ONS 5: milk protein isolate, caseinates and soya protein isolate.

Accordingly, in summary protein content (per 100 mL or g) for: (a) WPBs was within the range of typical ONS products and (b) snacks were also in range of the ONS; however, lower in protein than some retail market products listed in Table 1.3. As evident from Table 1.4, this thesis utilised various whey powders. These powders often have differing protein content (Figure 1.2) and have undergone different processes which may subsequently impact the final powders. Whey[4] is a co-product resulting from cheese making (Bansal & Bhandari, 2016). In brief, sweet liquid whey (using rennet to coagulate) is subject to a series of filtration membrane processes which results in concentrating and separating protein from other components (i.e. salts, minerals, lactose, fats and water) (Singh & Ye, 2009; Fox; 2009; Lucey, 2009; Bansal & Bhandari, 2016). This is followed by spray-drying (water evaporation via hot air) resulting in a spray-dried whey powder (Schuck, 2009; Bansal & Bhandari, 2016). These processes can impact the whey constituents within powders. For example, whey protein concentrate (WPC) typically has less protein and more lactose, fat and minerals than whey protein isolate (WPI) (Hoffman & Falvo, 2004). Moreover, whey proteins are exposed to relatively low temperatures during spray-drying; therefore, such proteins are considered to remain in the native form[5] rather than denatured (Schuck, 2009). In addition, heat treatment (i.e. during processing or subsequent heating once incorporated into products) can alter whey protein functionality, consequently potentially impacting sensory properties (Bansal & Bhandari, 2016; Bull *et al*., 2017; Ispen, 2017).

In summary, this thesis focuses on three commercial whey-based ingredients: (1) whey protein concentrate (WPC); (2) whey protein isolate (WPI); and (3) whey permeate (WPe). Such ingredients were predominantly fortified into beverages and snacks without

[4] further discussed in **Chapter 2** (see Section 2.5)
[5] native proteins have a three-dimensional structure (provide a key role in protein stability and functionality) and heating can result in proteins losing its three-dimensional structure (i.e. denatured protein) (Patel & Creamer, 2009)

further heat treatment (other than baking solid models). However, two examples of additional heat treatment were tested: heating WPB at a temperature considered to cause protein denaturation and using WPC which had undergone further processing to provide a more heat-stable material to fortify into solid models.

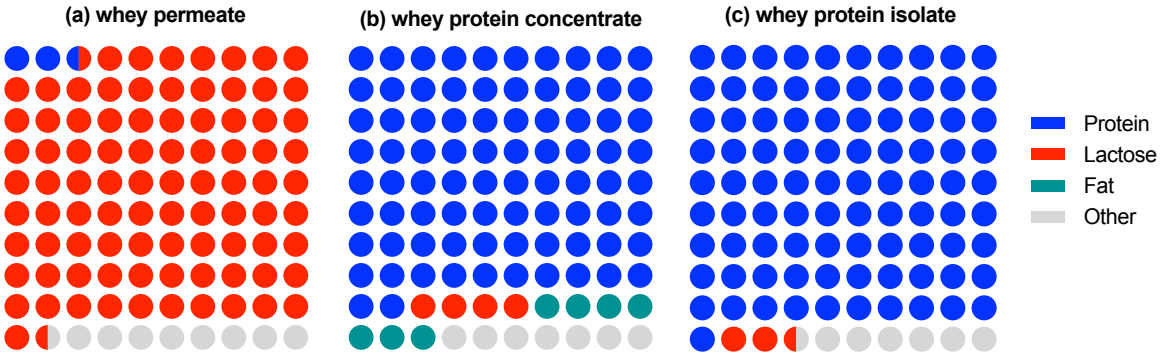


Figure 1.2. Summary of key whey powders (whey permeate, concentrate[6] and isolate) used within this thesis with resulting approximate protein, lactose, fat and other (i.e. moisture and ash (including minerals)) content (expressed as % with each coloured circle representing 1.0%). Data obtained from ingredients technical sheets.

Furthermore, it should also be noted that within an older adult population concerns over added sugar and high fat within products are less of an issue compared with the general population (Kokkinidou *et al*., 2018). All study portion sizes chosen were considered appropriate for older adults and varied depending on the product and purpose. For example, a 5 or 10 mL beverages sample was provided as this is commonly used for sensory evaluation to represent a single bolus size (Steele *et al*., 2019) and on-campus studies used a 45.0 g cake slice and 20.0 g biscuit, whereas at-home studies (to enable a whole product) used a 35.0 g cupcake and 30.0 g scone.

[6] other whey protein concentrates were used in this thesis: (1) sugar-free whey protein concentrate (~ 86% protein; 9.5% fat; 0.05% sugar; 7.0% other) and (2) heat-stable whey protein concentrate (~ 70% protein; 15% fat; 5.0% sugar; 10% other).

1.6. Individual differences

Individual differences may influence perception of whey protein fortified products; therefore, these should be considered when designing foods for older adults. However, previous research into mouthdrying typically has been evaluated using a trained sensory panel; accordingly, little is known about the extent of individual differences and how this influences subsequent perception. Hence, this thesis selected individual differences with particular relevance for older adults and likely impact on mouthdrying perception as outlined below:

- **Saliva flow** is considered to be modulated by age (Vandenberghe-Descamps *et al*., 2016) and could potentially increase perceived mouthdrying sensations from lack of saliva flow causing poor food clearance.
- Older adults often have poor **dental status** (Razak *et al*., 2014) and this could lead to difficulty in consuming foods, increased consumption time and subsequently alter mouthdrying perception.
- **Mouth behaviour** preferences could impact texture perception (Jeltema *et al*., 2015; 2016), whether this could be influenced by age and consequently alter mouthdrying is currently unclear.
- **Appetite** can decline with age (Giezenaar *et al*., 2016) and this could affect product compliance and perceived mouthdrying sensations.
- **Sensory thresholds** can change with age (Methven *et al*., 2012) and this could explain differences in individual mouthdrying sensitivity.

1.7. Thesis Outline

Figure 1.2 provides a thesis overview. The above introduction (**Chapter 1**) provided initial context coupled with the fundamental objectives and methodology rationales. The

literature review (**Chapter 2**) takes this further by providing the research that underpinned this thesis and highlights key gaps within the literature. Subsequent chapters (**Chapters 3-7**) focus on investigating mouthfeel perception in two whey protein food models and the extent of modulation from individual differences. The liquid model (whey beverages) is investigated in **Chapters 3 and 5-7** and the solid model (cakes, biscuits, cupcakes and scones) is progressed in **Chapters 4, 6 and 7**. The thesis concludes with a general discussion (**Chapter 8**) providing key findings, limitations, implications, suggested future work and conclusions.

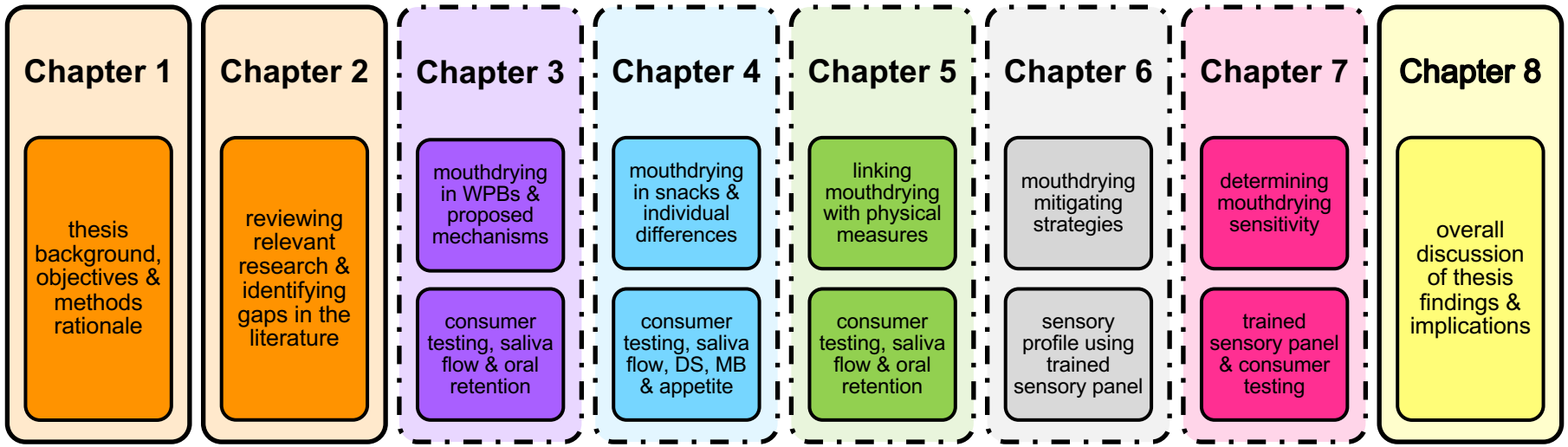


Figure 1.3. Thesis overview with a summary of the key focus for each chapter. Solid line (——) denotes introduction (**Chapter 1**), literature review (**Chapter 2**) and general discussion (**Chapter 8**) whereas dashed line (– · -) represents experimental based chapter (**Chapters 3-7**). Acronyms: whey protein beverages (WPB); dental status (DS); mouth behaviour (MB).

Chapter 2

Literature review

Influence of age and individual differences on mouthfeel perception of whey protein fortified products: a review

2.1. Context to chapter

Having introduced key themes in **Chapter 1**; this chapter provides an overview of fundamental research which underpins this thesis. More specifically, in order to ensure whey protein fortified products can be optimised to suit the needs of older adults, an extensive understanding of the factors likely to influence whey protein derived mouthdrying is needed. Surprisingly, research into such mouthdrying has so far typically focused on the product rather than individual differences, hence the need for this review. Accordingly, this review presented[7] in **Chapter 2** focuses on the following key areas:

- Brief introduction to relevant age-related factors
- Protein fortified products for older adults (including use of whey protein)
- Mouthfeel perception of whey protein fortified products
- Potential mechanisms underpinning whey protein derived mouthdrying
- Individual differences likely to influence mouthfeel perception
- Suggested future work

This chapter is published as Norton, V., Lignou, S. & Methven, L. (2021). Influence of age and individual differences on mouthfeel perception of whey protein fortified products: a review. *Foods,* **10**(2), 433.

[7] some published findings from this thesis (i.e. **Chapters 3 and 4**) are reported in this review

2.2. Abstract

Protein needs are considered to increase with age, with protein consumption being associated with many positive outcomes. Protein fortified products are often used to improve nutritional status and prevent age-related muscle mass loss in older adults. Accordingly, older adults are commonly provided with products fortified with whey protein; however, such products can cause mouthdrying, limiting consumption and product enjoyment. Currently, the extent to which age and individual differences (e.g. saliva, oral health, food oral processing) influence the perception of whey protein derived mouthdrying is relatively unclear. Previous research in this area has mainly focused on investigating mouthdrying, without taking into account individual differences that could influence this perception within the target population. Therefore, the main focus of this review is to provide an overview of the relevant individual differences likely to influence mouthfeel perception (specifically mouthdrying) from whey protein fortified products, thereby enabling the future design of such products to incorporate better the needs of older adults and improve their nutritional status. This review concludes that age and individual differences are likely to influence mouthdrying sensations from whey protein fortified products. Future research should focus more on the target population and individual differences to maximise the benefits from whey protein fortification.

Keywords: older adults; individual differences; whey protein; mouthdrying; protein fortified products

2.3. Introduction to malnutrition in older adults

In recent decades, there has been a worldwide increase in ageing populations and in 2019, globally, there were 703 million individuals aged 65 years or over; this is predicted to increase to 1.5 billion by 2050 (United Nations, 2019). Older adults are typically

described as people aged 65 years or over (Office for National Statistics, 2018; United Nations, 2019), and within this review, older adults will be referred to as individuals aged 65 years or over. However, this description reflects a broad range of individuals with differing needs and abilities. Ageing can be described simply as getting older and more specifically, from a biological viewpoint, as the accumulation of molecular and cellular damage over a lifespan contributing to a decline in function and increased disease risk (WHO, 2015). The health needs of an ageing population can, however, be described as complex and associated with physiological changes, disease and multimorbidity (WHO, 2015). The World Health Organisation (WHO) have used the term 'healthy ageing' to promote functional ability within an ageing population and more recently introduced 2020-2030 as the 'Decade of Healthy Ageing' to provide a focus on improving the lives of older adults (WHO, 2020). In addition, simple health behaviours, such as good nutrition and physical activity, can provide health and well-being benefits, as well as promoting longevity (WHO, 2015).

Good nutrition is associated with numerous positive benefits, such as improved health and well-being. Energy, protein, vitamin C, vitamin D, folate, iron, zinc and fibre are considered important nutrients for older adults (Pout, 2014). Energy requirements are considered to decline with age due to body composition changes and reduced physical activity (Ahmed & Haboubi, 2010). However, protein needs are considered to increase with age (as outlined in Section 2.4), but, as with most other nutrients, recommendations typically remain the same as those suggested for adults generally (Department of Health, 1992; Bauer *et al*., 2013; Deutz *et al*., 2014) (for a recent review on nutritional recommendations in older adults see Dorrington *et al*. (2020)). Additionally, there are adverse effects associated with the ageing process, which is considered to be a

multidimensional process, including physical, psychological and social changes, all of which are potential risk factors for malnutrition (Table 2.1) (Armarya *et al*., 2015).

Table 2.1. Suggested risk factors for malnutrition (adapted from Hickson, 2006; BDA, 2017; BAPEN, 2018).

Social	Physical	Medical	Psychological
→ Living and eating alone	→ Physical disabilities	→ Swallowing difficulties*	→ Anxiety
→ Poverty	→ Reduced appetite	→ Eating disorders	→ Depression
→ Difficulty in shopping or preparing food	→ Poor dentition	→ Medication	→ Dementia
→ Limited nutrition knowledge and cooking skills		→ Conditions leading to reduced appetite and absorption/utilisation of nutrients	→ Bereavement

* dysphagia

Varying definitions of malnutrition are reported within the literature (Elia, 2017). One of the most commonly used describes malnutrition as a "deficiency or excess (or imbalance) of energy, protein and other nutrients" resulting in negative consequences "on tissue / body form (body shape, size and composition) and function and clinical outcome" (BAPEN, 2018, p.1). Typically, the focus within older adults is largely on undernutrition (a deficiency in both macronutrients and micronutrients) (Maleta, 2006; BAPEN, 2018). Malnutrition is prevalent amongst older adults with increased risks associated with age, gender (female), disease status and clinical settings (hospitals, care homes and mental health units) (BAPEN, 2018; Leiji-Halfwerk *et al*., 2019). Twenty-three percent of European older adults are considered at risk of malnutrition and over one million older adults are affected in the UK (BAPEN, 2018; Leiji-Halfwerk *et al*., 2019). Malnutrition is commonly linked with reduced functional status, muscle function, bone mass and cognitive function, poor wound healing, delayed recovery from surgery, mortality and higher hospital readmission rates (Armarya *et al*., 2015). A five-step screening process 'Malnutrition Universal Screening Tool' (MUST) is regularly used in the UK to categorise patients for risk of malnutrition in a range of clinical settings (Todorovic *et al*., 2003). Typically, an individual can be described as 'malnourished' if they have a body mass

index (BMI) lower than 18.5 kg/m^2 or an unplanned weight loss (> 10% within the last 3-6 months) or a combination of a BMI lower than 20 kg/m^2 and an unplanned weight loss (> 5% within the last 3-6 months) (NICE, 2017).

Malnutrition can contribute to sarcopenia, which is an age-related loss of skeletal muscle mass and function that is exacerbated by low protein intake alongside poor conversion of protein to muscle mass (Fielding *et al*., 2011). Sarcopenia has been reported to affect 5-13% of adults in their sixties and 11-50% of those in their eighties (von Haehling *et al*., 2010). Despite it being considered a preventable and treatable condition, it is a contributor to increasing health costs; muscle weakness conditions in the UK cost £2.5 billion per annum (Fielding *et al*., 2011; Stevenson *et al*., 2019). Sarcopenia has been identified as a potential precursor to frailty, both conditions being multi-dimensional, reversible and with inflammatory links (Wilson *et al*., 2017). Frailty can lead to reduced strength, endurance and physiological function, thereby increasing vulnerability to external stressors (Morley *et al*., 2013).

Reduced appetite can lead to increased risk of malnutrition in older adults. Factors such as loss of smell, oral and taste impairments, medication, anorexia of ageing[8], physiological, psychological and social factors can all contribute to a decline in appetite (Schiffman & Graham, 2000; Morley, 2001; Gura & Ciccone, 2010; Malafarina *et al*., 2013; Vandenberghe-Descamps *et al*., 2017). Additionally, this decline is considered to result partially from delayed gastric emptying (increased time food spends in the stomach), thereby increasing satiation and reducing appetite (Nieuwenhuizen *et al*., 2010). The type of foods (liquid or solid) consumed can influence food intake. For example, in a study involving healthy older adults, consuming a liquid beverage resulted

[8] anorexia of ageing: a physiological decline in food intake with age

in increased subsequent intake (13.4% increase in oatmeal) compared with a solid energy bar (Stull *et al*., 2008). Furthermore, the texture of foods (such as chewy, hard and viscous) influence appetite regulation, where increased processing within the mouth has been shown to lead to increased feelings of satiety (Chambers, 2016). A meta-analysis by Giezenaar *et al*. (2016) highlighted reduction in energy intake, hunger and increased fullness were all associated with age; therefore, promoting foods that encourage food intake is key to counterbalancing this.

Poor oral health can have a detrimental impact on an individual's nutritional status, health and well-being (Razak *et al*., 2014). Oral impairments can impact biting, chewing and swallowing of foods (Rathee & Hooda, 2009). Older adults typically suffer from teeth loss, dental caries, reduced saliva flow, changes in oral mucous membrane and chewing efficiency, mouth dryness and increased periodontal diseases[9] and use of dentures, all likely to influence food habits and intake (Razak *et al*., 2014). For example, data from the UK National Diet and Nutrition Survey 2008-2014 identified that dental status impacted food selection, and nutrient intake in older adults with compromised dental status (such as edentate and/or dentate with denture wearers[10]) had a negative effect on intake (Watson *et al*., 2019). Kremer *et al*. (2007a) demonstrated that older adults, who were denture wearers, perceived custards to be less creamy and less easy to swallow compared with those with natural teeth. Dentures can result in changes in mouth movements, chewing efficiency and sensory thresholds (Kremer *et al*., 2007a; Rathee & Hooda, 2009; Methven *et al*., 2016). Saliva lubrication can influence comfort of wearing dentures (Rathee & Hooda, 2009) and decline in oral health can also contribute to taste disorders within older adults (Imoscopi *et al*., 2012). For example, poor oral hygiene, dry

[9] periodontal diseases: an inflammatory gum related condition (Kinane *et al*., 2017)
[10] edentate: no natural teeth and dentate: natural teeth

mouth, caries and high growth of oral bacteria have been shown to decrease taste ability in acutely hospitalised older adults (70-103 years) (Solemadal *et al*., 2012). Therefore, maintaining good oral health can increase appetite, food intake and improve taste perception (Solemadal *et al*., 2012). The impact of medication must also be considered when investigating nutritional status and age. A recent Health Survey for England (2016) identified that whereas only 19% of young adults (16-24 years) used at least one prescription medication per week, this increased to 80% of older adults (65-74 years) and was 96% for those over 85 years (Moody *et al*., 2017). Medication commonly has side effects such as affecting oral health, appetite and taste (Ciancio, 2004; Gura & Ciccone, 2010), thereby contributing to an increased risk of poor nutritional status.

Nutritional support can provide a cost-effective treatment to improve functional and clinical outcomes for individuals at risk of malnutrition (Stratton *et al*., 2018). Accordingly, the British Dietetic Association (BDA) promotes a food first approach to enhance nutritional intake; recommended strategies are outlined in Figure 2.1 (BDA, 2017).

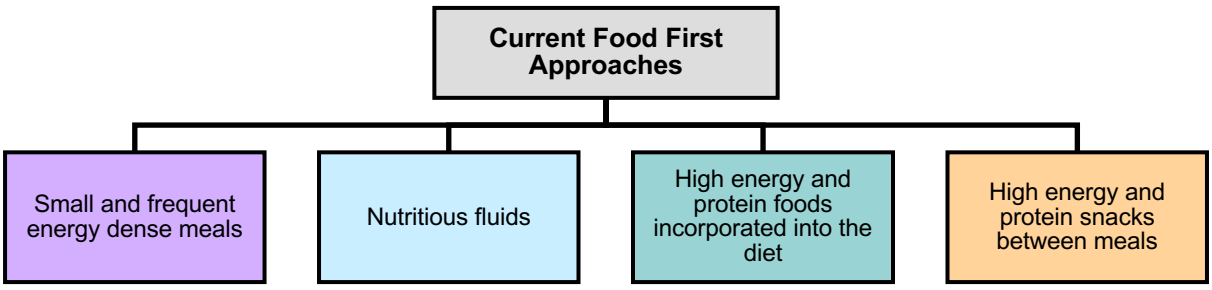


Figure 2.1. Commonly used strategies to improve nutritional intake in older adults at risk of malnutrition (adapted from BDA, 2017).

In summary, the health and nutritional needs of an ageing population are considered complex and involve numerous age-related changes, which subsequently influence food intake and quality of life, all contributory factors to poor nutritional status amongst older adults. Protein fortified products provide a key role in promoting a food-first approach to

enhance nutritional intake and support the recognised increased protein needs with age. Such products often contain animal derived proteins, for example whey proteins, which are considered complete sources of protein (including all essential amino acids), whereas plant derived proteins are typically incomplete sources of protein (lacking one or two essential amino acids) (Hoffman & Falvo, 2004). Furthermore, animal derived proteins are more readily digestible and effective in muscle protein synthesis than plant derived proteins (Hoffman & Falvo, 2004). It is, therefore, important that whey protein fortified products have consumer acceptance, which relies on a good sensory profile. However, recent reviews (Pires *et al*., 2020; Carter *et al*., 2020) have shown such products are associated with astringency or mouthdrying attributes. To date, such reviews have not considered the additional dimension of individual differences within the target population. Establishing the impact that age and individual differences may have on whey protein perception would have particular relevance for older adults so as to mitigate characteristics linked to poor consumer acceptance. Accordingly, the main aims of this review are to summarise the latest research relating to: (a) protein fortified products for older adults; (b) exploration of the mechanisms underpinning whey protein derived mouthdrying; (c) the influence that age and individual differences could have on the perception of whey protein derived mouthdrying; and (d) provide suggestions for future research.

2.4. Protein requirements and the importance of protein fortified products in the diet of older adults

Proteins are polymers of amino acids and provide key roles in tissue growth and repair (Department of Health, 1991). The ‘Protein for Life’ research team has recently identified that many individuals within the UK have inadequate protein intake to maintain muscle strength and function in older age (Stevenson *et al*., 2019). In addition, an improvement

in protein intake during the life course could potentially reduce the onset of certain health conditions and also slow the rate of muscle decline (Stevenson *et al*., 2018). The 'Protein for Life' focus groups, which involved healthy adults, also identified a lack of certainty over optimal protein intakes during the life course (Stevenson *et al*., 2019). The UK current reference nutrient intake (RNI) for adults is 0.75 g/kg/d[11] (Department of Health, 1991), yet protein needs are considered to increase with age. For example, recently, the PROT-AGE study group and the European Society for Clinical Nutrition and Metabolism (ESPEN) expert group have both reviewed protein intake in the older population (Bauer *et al*., 2013; Deutz *et al*., 2014). Both studies have recommended a protein intake of 1.0-1.2 g/kg/d for older adults and higher protein intakes (1.2-1.5 g/kg/d) for older adults suffering from acute and chronic disease (Bauer *et al*., 2013; Deutz *et al*., 2014). These findings are also supported by recent Parenteral and Enteral Nutritional Group (PENG) and BDA guidelines for nutritionally vulnerable adults in clinical settings, which recommended protein intake of 1.1 g/kg/d (PENG, 2011; BDA, 2017). These increased protein intakes are considered necessary to maintain good health, encourage recovery from illness and preserve functionality as a result of age-related changes in protein metabolism (Bauer *et al*., 2013). In addition, factors such as sarcopenia, anabolic resistance, disease related protein catabolism, low postprandial amino acid availability and decreased muscle perfusion can also result in increased protein needs for an older adult (Deutz *et al*., 2014). The ESPEN Expert Group identified various possible causes for reduced protein intake in older adults, including socioeconomic status, medical conditions, physiological changes, genetic predisposition and physical disability (Deutz *et al*., 2014).

[11] g/kd/d: grams per kilogram body weight per day

Protein is considered a satiating macronutrient which can lead to reduced intake at subsequent meals compared with fat and carbohydrates (Veldhorst *et al*., 2008). The proposed mechanisms include increased diet induced energy expenditure, satiety hormones and amino acids, as well as modulation of gluconeogenesis[12] (Veldhorst *et al*., 2008). However, such studies have generally been in younger adults and the response may be modulated by age (Giezenaar *et al*., 2015; 2017). For example, in two studies by Giezenaar *et al*. (2015; 2017) whey protein drinks were not found to be satiating in older adults compared with younger adults. They identified older male volunteers showing an increase in appetite, slower gastric emptying and increased overall energy intake (Giezenaar *et al*., 2015). However, appetite decreased following consumption of whey protein drinks in younger male volunteers (Giezenaar *et al*., 2015). Additionally, with older male and female volunteers, *ad libitum* energy intake was not affected 3-h post whey protein drink consumption (Giezenaar *et al*., 2017). Appetite is considered to decrease with age *per se* and these findings provide support for protein supplementation as an effective nutritional intervention to increase protein intake.

In order to enhance nutritional intake in older adults, a product needs to be palatable, appetising, of suitable portion size and energy dense (Nieuwenhuizen *et al*., 2010). Typically, oral nutritional supplements (ONS) and protein fortified products are used to improve protein intake in older adults. ONS are commonly consumed by older adults and those at risk of malnutrition, where they are unable to meet nutritional needs from their diet (BAPEN, 2016). They consist of products which provide macro and micronutrients in semi-solid, powder or liquid form (BAPEN, 2016). Protein powders have varied applications and uses within food processing (Wang *et al*., 2018a) and many high energy drinks contain whey protein (further outlined in Section 2.5) due to its high nutritional and

[12] gluconeogenesis: conversion of non-carbohydrate substrates into glucose (Zhang *et al*., 2019)

functional values (Croissant *et al*., 2009; Giezenaar *et al*., 2015) often as whey protein isolate (WPI) or whey protein concentrate (WPC) (Evans *et al*., 2010). Protein fortified meals and snacks can provide a simple alternative to ONS and provide familiar foods to older adults which can encourage consumption by increasing energy and protein intake (Morilla-Herrera *et al*., 2016; Mills *et al*., 2018). Variety is required to avoid taste fatigue and improve compliance and intake amongst older adults, including different flavours, textures and appearance (Nieuwenhuizen *et al*., 2010).

Multiple studies have demonstrated benefits from protein supplementation and/or protein fortification. For example, Cawood *et al*. (2012) carried out a systematic review of 36 studies highlighting benefits of high protein ONS (20-54% energy from protein) and concluded there was a 19% reduction in complications (healing of surgical wounds, pressure ulcers and infections rates) following consumption across various settings (hospital and community settings). A recent randomised control trial was carried out with 104 malnourished care-home residents comparing ONS outcomes (n = 53) with dietary advice (n = 51) for 12 weeks (Parsons *et al*., 2017). The ONS had energy density between 1.3-4.5 kcal/mL with voluntary intake measured against a target of 600 kcal and 16 g protein per day (Parsons *et al*., 2017). This study supported that nutritional intake and quality of life were significantly improved in the ONS group compared with the conventional dietary advice group (Parsons *et al*., 2017). Bauer *et al*. (2015) demonstrated a significant improvement in muscle mass following a three-month period of ONS consumption, containing vitamin D and leucine-enriched whey protein, compared with the control group, amongst older adults with sarcopenia.

Food fortification using familiar foods could also be considered as a viable route to increase protein intake within an ageing population. A study involving a hospital setting compared the provision of protein fortified meals (23 dishes fortified with milk protein 6.1

g to 11.5 g of protein per dish; breakfast, soups, fish, meat, side dishes and desserts) with the standard hospital menu (three main meals with two to three in-between meals) (Munk *et al*., 2014). The fortified food service resulted in significant improvement in protein intake amongst patients at nutritional risk (Munk *et al*., 2014). Appleton and Smith (2015) noted that using improved visual cues, recognisable foods and/or identification labels can enhance liking for flavours of drinks in older adults. Beelen *et al*. (2017a) carried out a pilot study using familiar products (bread, soups, fruit juices and instant mashed potato) enriched with 5.6 g to 10.0 g protein (dairy and soy) per portion. This pilot study highlighted the benefits of such products in increasing protein intake within an older population (*n* = 22) in a clinical setting (Beelen *et al*., 2017a). The same research group carried out a subsequent randomised controlled trial using similar protein enriched familiar products (bread, cakes, soups, porridge, meat, mashed potatoes, ice cream, fruit juice and dairy products; protein content per portion varying from 5.8 g to 21.6 g) (Beelen *et al*. 2017b). They demonstrated an increase in protein intake over a 12-week period in older adults (*n* = 75), resulting in 72% of the individuals meeting the recommended intake of 1.2 g/kg/d, whereas only 31% of those in the control group met those recommendations (Beelen *et al*., 2017b).

Negative outcomes have been associated with a high protein intake. For example, its effects on kidney function, though a recent meta-analysis demonstrated that in healthy adults this was not the case (Devries *et al*., 2018). Similarly, it has also been suggested it could have a negative outcome on the gut microbiota. However, a recent randomised control trial in older men demonstrated that despite consuming 1.6 g/kg/d for 10 weeks, the gut microbiota composition and microbiota derived volatile organic compounds production remained unaltered (Mitchell *et al*., 2020). In addition, Stratton and Elia (2007) noted that minimal gastrointestinal symptoms (such as nausea, bloating and diarrhoea)

could arise from ONS consumption; however, they also highlighted that there are limited studies which evaluate fully gastrointestinal tolerance. Moreover, such side effects from protein supplementation typically result from the non-protein components (e.g. lactose intolerance) (Parker & Watson, 2007).

In summary, protein needs are considered to increase with age, with increased protein intake associated with many positive functional outcomes. Hence, ONS and protein fortified products prove beneficial to nutritional status.

2.5. The use of whey protein to fortify foods for older adults

Bovine milk is commonly incorporated into human diets with its associated nutritional and functional benefits (Anema, 2014). Milk typically comprises water, lipids, lactose (sugar) and protein, as well as minor components (such as minerals (notably calcium), vitamins (both water- and fat-soluble vitamins), hormones, enzymes and miscellaneous compounds) (O'Mahony, 2014). Milk protein mainly derives from casein (phosphoproteins; 80% of milk proteins and insoluble at pH 4.6) and whey (globular proteins; 20% of milk proteins and soluble at pH 4.6), as well as proteinaceous materials (proteose peptone (PPs) and non-protein nitrogen (NPN)) (Madureira *et al*., 2007; O'Mahony, 2014). Whey is a by-product of cheese making; it is the liquid remaining once the milk has been coagulated (curdled) (Bansal & Bhandari, 2016). Liquid whey can be dried to produce different whey powders (see Bansal and Bhandari (2016) for an extensive overview). In summary, WPI (> 90% protein) is typically subjected to further processing compared with WPC (34-89% protein), resulting in its higher protein concentration and lower fat, ash (mineral) and lactose content (Hoffman & Falvo, 2004; Croissant *et al*., 2009). Demineralised whey powder is a reduced minerals whey powder, associated with reduced corresponding tastes such as salty and bitter (Bansal & Bhandari, 2016). Whey permeate is a by-product of whey production and is a

deproteinised whey powder comprising predominantly of lactose and minerals (Frankowski *et al*., 2014).

Whey proteins consist of β-lactoglobulin, α-lactalbumin, glycomacropeptide, bovine serum albumin (BSA), immunoglobulins, lactoferrin and lactoperoxidase in varying amounts (Etzel, 2004; Smithers, 2008), as summarised in Figure 2.2. Whey proteins provide a source of essential amino acids (EAAs) and branched chain amino acids (BCAAs; leucine, isoleucine and valine) (Hoffman & Falvo, 2004). In addition, whey protein is a rapidly digestible protein that is considered to provide greater nutritional benefits to older adults compared with other protein sources (such as casein), which leads to its frequent use in clinical nutritional products (Dangin *et al*., 2003; Sahathevan *et al*., 2018). For example, the benefits of whey protein have been identified in an acute study with an older male population where postprandial muscle protein accretion was found to be more effectively stimulated by whey protein, compared with casein and casein hydrolysate (Pennings *et al*., 2011). Whey protein ingestion can result in an improved muscle protein synthetic response, which is considered to be due to its higher leucine content and quicker digestion and absorption kinetics compared with other protein sources (Pennings *et al*., 2011). Review papers have identified a number of additional potential health benefits associated with whey protein consumption, such as its antimicrobial, antiviral and anticarcinogenic effects, as well as improved immune, bone and cardiovascular health (Madureira *et al*., 2007; Solak & Akin, 2012).

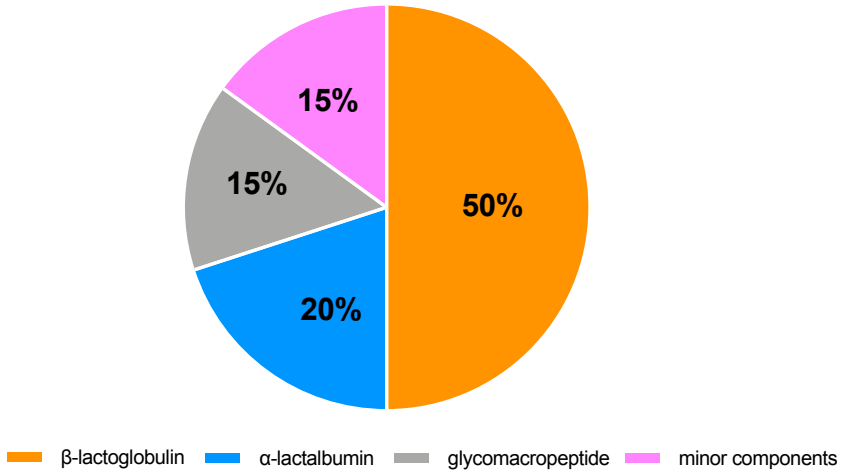


Figure 2.2. Overview of whey protein typical percentage composition (Smithers, 2008) (minor components of whey protein: bovine serum albumin (BSA), immunoglobulins, lactoferrin and lactoperoxidase).

In order for ONS and protein fortified products to lead to beneficial nutritional and health outcomes, enough product should be consumed to meet an individual's daily nutritional requirements. However, compliance is reported to be variable, reducing the nutritional impact, in addition to cost and waste implications (Gosney, 2003). For example, a systematic review from 46 studies identified ONS compliance levels varying between 37% and 100% (with average compliance at 78%) within different settings (hospital setting: 67% and community setting: 81%) (Hubbard *et al.*, 2012).

Whey protein fortified products typically have poor consumer acceptance and this has been linked to both undesirable taste and aroma attributes, as well as negative mouthfeel attributes, such as a build-up of mouthdrying, mouthcoating, chalky, metallic and filming, associated with repeated consumption (Gosney 2001; Childs & Drake, 2010; Kennedy *et al.*, 2010; Methven *et al.*, 2010; Thomas *et al.*, 2016; 2018; Bull *et al.*, 2017). Mouthdrying has been perceived by consumers in two different whey protein fortified food matrices (Norton *et al.*, 2020a, **Chapter 3**; 2020b, **Chapter 4**). For example, within a liquid model, beverages fortified with whey protein were associated with mouthdrying, low liking scores and presence of off-flavours (Childs & Drake, 2010; Oltman *et al.*, 2015; Zhang et al.,

2020; Norton *et al*., 2020a, **Chapter 3**). Within a solid model, snacks (such as cakes, muffins, biscuits and rye bread) fortified with whey protein were perceived as mouthdrying and/or had a dry texture and reduced liking (Wendin *et al*., 2017; Song *et al*., 2018; Norton *et al*., 2020b, **Chapter 4**). These studies demonstrate consumers can perceive negative sensory attributes associated with whey protein fortified products and mitigating such attributes may be the key to promoting compliance and suitability for older adults.

The sensory profile (measured using trained sensory panels) of whey proteins typically includes attributes such as aroma intensity, sweet aromatic, musty, cooked/milky, doughy/fatty (described as "aroma associated with canned biscuit dough"[13]), metallic, cucumber, cabbage, brothy, cardboard/wet paper, animal/wet dog, pasta water, soapy, faecal, catty, grainy, opacity, bitter, astringent, chalky, thick, mouthdrying, mouthcoating, furring and body (Karagul-Yuceer *et al*., 2003; Whetstine *et al*., 2005; Wright *et al*., 2006; Russell *et al*., 2006; Bull *et al*., 2017). WPI and WPC are considered to have relatively similar sensory profiles (despite processing differences), with the following key differences: WPI has been shown to elicit attributes such as soapy, animal/wet dog, cucumber and bitter, which are typically not present in WPC, whereas WPC has attributes such as sweet aromatic and cooked/milky, which are not present in WPI (Whetstine *et al*., 2005).

Whey proteins are commonly fortified into a range of food matrices, with differing effects on the sensory profile. For example, trained sensory panels identified mouthfeel attributes such as chalky, drying, mouthcoating, astringency, furring and body following whey protein beverage (WPB) consumption and heat treatment of WPB is considered to intensify further these sensory properties (Bull *et al*., 2017). The addition of WPI to sauces

[13] (Whetstine *et al*., 2005, p.3828)

has been found to contribute additional flavour attributes (fishy, vegetable soup, chemical, savoury, bitter) as well as mouthfeel (grainy) (Tsikritzi *et al*., 2015). Similarly, fortification of biscuits with WPI has been shown to alter appearance (roughness, density), flavour (bitter, savoury, burnt sugar, off flavours) and mouthfeel (teeth packing and slower melt rate) (Tsikritzi *et al*., 2014; Norton *et al*., 2020b, **Chapter 4**). Cakes have also been fortified with WPI and WPC, which led to an increase in negative mouthfeel attributes such as mouthdrying, chewy, increased crumb size and firmness of bite (Norton *et al*., 2020b, **Chapter 4**).

In summary, whey protein fortification is a commonly used to help prevent age-related muscle mass losses. However, negative sensory attributes leading to poor consumer acceptance and compliance are commonly associated with ONS and protein fortified products. Whey proteins are frequently cited as being a source of mouthdrying in a range of different whey protein fortified food matrices. We consider that this needs further investigation, given that older adults are noted to suffer commonly from dry mouth (Thomson, 2016) and/or reduced saliva flow (Vandenberghe-Descamps *et al*., 2016).

2.6. Mouthfeel and mouthdrying perception of whey protein fortified products

Texture is considered a dynamic process as foods are continuously being manipulated within the mouth (Guinard & Mazzucchelli, 1996) and is more specifically defined as “the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the sense of vision, hearing, touch and kinesthetics” (Szczesniak, 2002, p.215). Szczesniak and Kahn (1971) proposed consumers’ awareness of texture is increased if expectations are not met; therefore, suggesting texture provides a key role in food preference. Szczesniak (2002) described texture as a ‘sensory property’ and is considered best described and perceived by humans mainly via touch and pressure senses within the mouth during food evaluation. Mouthfeel can be

described as “the tactile (feel) properties perceived from the time at which solid, semi-solid or liquid foods or beverages are placed in the mouth until they are swallowed” (Guinard & Mazzucchelli, 1996, p.213).

Astringency, oral drying and mouthdrying are commonly used terms (and are often used interchangeably) to describe this considered ‘textural defect’ associated with dairy products (Lemieux & Simard, 1994). The term astringency has been defined as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (ASTM[14] E253-20). Such a perceived texture change within the oral cavity usually results from the consumption of plant derived products rich in polyphenols, such as tea, wine, nuts and fruit (Green, 1993; Breslin *et al*., 1993). As highlighted in recent reviews, astringency is considered a ‘complex sensation’ and potentially derived from multiple mechanisms and often builds and persists post consumption (Bajec & Pickering, 2008a; Gibbins & Carpenter, 2013; Pires *et al*., 2020)[15]. Plant derived protein beverages (fortified with pea and soy protein) have been shown to impart astringency sensations; however, this is proposed to result from their polyphenol content rather than as a direct result of the protein composition (Russell *et al*., 2006; Damodaran & Arora, 2013; Cosson *et al*., 2020). Polyphenols are considered to interact with salivary proteins causing aggregation and precipitation, thereby reducing lubrication of saliva, increasing friction and potentially exposing mechanoreceptors, resulting in an astringent sensation (Lyman & Green, 1990;

[14] ASTM: American Society for Testing and Materials

[15] Mouthfeel is typically perceived as a tactile sensation within the oral cavity and saliva is considered to have a contributing role (Gibbins & Carpenter, 2013). More specifically, the salivary film (which coats oral surfaces) and salivary mucosal pellicle (a proteinaceous coating) can both impact oral lubrication and subsequently mouthfeel (Gibbins & Carpenter, 2013). Astringency can arise from the activation of mechanoreceptors located within the oral cavity (Gibbins & Carpenter, 2013). Gibbins and Carpenter (2013) suggest the key astringency mechanisms involve: (1) salivary protein aggregation; (2) altered salivary film; (3) decreased salivary lubrication; (4) receptor exposure; and (5) mechanoreceptors/nociceptors or nerve innervation. A recent review noted that whey proteins can exhibit astringency at low pH by interacting with salivary proteins and removing the lubricating saliva layer in the oral cavity (Carter *et al*., 2020). However, mouthdrying can occur in WPBs at neutral pH where the mechanism may differ as discussed in Section 2.6.1.

Thorngate & Noble, 1995; Jobstl *et al*., 2004; Gibbins & Carpenter, 2013). However, as polyphenolic compounds are not present in whey protein sources, the term mouthdrying (a drying sensation in the mouth during or after consumption of a product) is considered more suitable in the context of dairy products. Accordingly, this review uses the term mouthdrying to describe whey protein derived mouthdrying. However, astringency related oral drying from food models has been researched widely (see reviews Bajec & Pickering, 2008a; Gibbins & Carpenter, 2013; Pires *et al*., 2020) compared with whey protein derived mouthdrying and could, therefore, provide suggestions in terms of mechanisms, mitigating strategies and testing methods.

A recent review by Pires *et al*. (2020) highlighted factors such as pH, temperature, saliva, viscosity and polysaccharides as being likely to influence astringency perception. Furthermore, the detection thresholds for individuals vary for astringent stimuli, which are perhaps influenced by differences in the number of receptors (Bajec & Pickering, 2008a; 2008b; Linne & Simons, 2017). This has been related to indirect markers (such as 6-n-propylthiouracil (PROP status) and fungiform papillae density), as well as to direct measures of variation in oral tactile sensitivity and saliva flow (Bajec & Pickering, 2008a; 2008b; Linne & Simons, 2017). A link has also been proposed between individual salivary protein content (pre- and post-stimulation) and astringency ratings in liquid food models (juices with added tannic acid and aqueous solutions with tannic acid and alum) (Dinnella *et al*., 2011; Fleming *et al*., 2016). Individuals grouped as ‘high responders’ (showing reduced replenishment of salivary proteins) perceived astringency as more intense (Dinnella *et al*., 2011; Fleming *et al*., 2016). However, in a solid chocolate model, differences in salivary protein were not related to perception of astringency (Fleming *et al*., 2016). The high fat level in the chocolate may have increased lubricity, which perhaps

negated the effect of differences in salivary protein content on the perception of astringency (Fleming *et al*., 2016).

Understanding oral movements[16], and where in the oral cavity volunteers perceive drying sensations, could also provide useful insights. Breslin *et al*. (1993) proposed astringency sensations could occur from altered and increased mechanoreceptor activity and demonstrated astringency perception was more apparent with oral movements. A lack of tongue movements minimised perceived astringency in one study, suggesting astringency perception requires at least some oral movement (Schobel *et al*., 2014). Astringency can also be perceived on the upper lip and gum (Breslin *et al*., 1993), suggesting a whole mouth approach is best to understand perceived astringency sensations. A key limitation within this area is the inability to measure astringency effectively. Currently, no method has been developed to achieve this, though typically, a combined approach of direct and indirect methods is used, as highlighted in a recent review (Pires *et al*., 2020).

2.6.1. Whey protein derived mouthdrying

Consuming dairy products can also result in a perceived texture change within the oral cavity similar to that with plant-derived products (Green, 1993). More specifically, whey proteins have been shown to be a source of mouthdrying in fortified products and ONS; hence, addressing the potential causes of whey protein derived mouthdrying is a key priority (Withers *et al*., 2014). Proposed causes are summarised in Table 2.2. Initial research suggested that the low pH associated with some WPBs can cause mouthdrying

[16] Carter *et al*. (2020) suggested that astringency may occur from different mechanisms depending on the oral movement. For example, they proposed that where astringent molecules interact directly with salivary proteins, then oral movement may not be necessary for the detection of astringency (Carter *et al*., 2020). Therefore, this would be dependent on the binding and not directly on friction (Carter *et al*., 2020). However, it may also be possible that surface binding and subsequent friction, which are dependent on oral friction, can also result in astringency (Carter *et al*., 2020).

due to protein precipitation in the mouth and subsequent saliva protein interactions (Beecher *et al*., 2008; Kelly *et al*., 2010; Vardhanabhuti *et al*., 2010; Ye *et al*., 2011; Andrewes *et al*., 2011). The resulting mouthdrying could be related to increased particle size and turbidity (Beecher *et al*., 2008; Ye *et al*., 2011). Particle size also increases with heating time (Bull *et al*., 2017) and elicits a mouthdrying response at a neutral pH WPB (Withers *et al*., 2014; Bull *et al*., 2017). Mouthdrying could also be influenced by disruption of the salivary structure causing reduced lubrication from saliva and resulting in increased friction and perceived mouthdrying (Vardhanabhuti *et al*., 2011). There is evidence that whey proteins, a natural polymer, demonstrate tissue adhesion (Wang *et al*., 2018b) and mucoadhesion properties (Hsein *et al*., 2015). For example, a previous *in vitro* study has shown that, despite being washed with artificial saliva, proteins remained on the buccal mucosa or tongue apex (with proteins bound to the oral mucosa) (Withers *et al*., 2013b). Indeed, more recently, our research group confirmed in a human oral retention study that protein does adhere to the oral cavity post WPB consumption to a greater extent (Norton *et al*., 2020a, **Chapter 3**). Although whey proteins have a high nutritional value, they become unstable when heated, resulting in protein denaturation and aggregation, which influences the structure and stability of the protein (Wijayanti *et al*., 2014). Heat treatment of whey proteins can result in increased mouthdrying (Josephson *et al*., 1967; Bull *et al*., 2017). Bull *et al*. (2020) demonstrated increased oral retention of whey protein following a heated WPB compared with an unheated WPB; therefore, suggesting oral retention could have a role in mouthdrying. The increased mucoadhesion strength associated with whey protein denaturation is considered to derive from interactions associated with hydrogen bonding and disulphide bridges (Hsein *et al*., 2015). Furthermore, a recent review investigated interactions between saliva and food proteins (focusing both on whey proteins and non-whey proteins) and suggested electrostatic interactions between

positively charged food proteins and negatively charged regions of mucin as a likely mechanism (Celebioglu *et al*., 2020). However, as noted above, there could be other relevant mechanisms involved, as proteins (including β-lactoglobulin) would remain positively charged at the neutral pH within the oral cavity (Withers *et al*., 2013b). More broadly, Celebioglu *et al*. (2020) concluded that both hydrophobic and hydrophilic interactions may be responsible for mucin interactions with various types of food protein in varied food matrix conditions (e.g. pH dependency).

Table 2.2. Commonly proposed causes of whey protein beverage (WPB) derived mouthdrying (adapted from Norton *et al*., 2020a, **Chapter 3**) and associated limitations.

Proposed Cause	WPB Model[1]	Description	Limitations
pH of WPB	WPC[a,b], WPI[c,d], WPI, β-LG & LF[e,f]	○ Low pH can cause precipitation of the protein	○ There is evidence of mouthdrying from WPB at both low and neutral pH
Saliva and protein interactions	β-LG[g], WPI[c,h], WPI, β-LG & LF[e,f]	○ Perception of mouthdrying has links to saliva and protein interactions	○ Studies have used *in vivo* analysis mixing human or artificial saliva with whey proteins, but this requires sensory analysis to correlate instrumental data with mouthdrying
Reduced lubrication from saliva	β-LG[i]	○ Increased friction within the oral cavity from reduced lubrication	○ Using instrumental analysis (such as tribology) to predict in-mouth experiences, but this requires sensory analysis to correlate instrumental data with mouthdrying
Adhesion and binding properties	WPC[j,k], β-LG and LF[l], WPI[m], β-LG[n]	○ Whey proteins binding to oral epithelial cells, proteins remaining on surfaces, mucoadhesive properties, increased oral retention and whey protein adhering to the oral cavity	○ *In vivo*, animal models, small subject size, without a non-protein source control, but this requires sensory analysis to correlate instrumental data with mouthdrying
Heating time	WPC[a], RW[o]	○ Mouthdrying is considered to increase with product heating time, potentially due to protein denaturation	○ Mouthdrying is present in samples without heat treatment, albeit at lower levels, so this cannot be the sole cause

[1] Whey protein beverage (WPB) model: whey protein concentration (WPC), whey protein isolate (WPI), β-lactoglobulin (β-LG), lactoferrin (LF) and rennet whey (RW). (a) Bull *et al*., 2017; (b) Withers *et al*., 2014; (c) Beecher *et al*., 2008; (d) Sano *et al*., 2005; (e) Vardhanabhuti *et al*., 2010; (f) Ye *et al*., 2011; (g) Kelly *et al*., 2010; (h) Andrewes *et al*., 2011; (i) Vardhanabhuti *et al*., 2011; (j) Norton *et al*., 2020a, **Chapter 3**; (k) Bull *et al*., 2020; (l) Ye *et al*., 2012; (m) Hsein *et al*., 2015; (n) Withers *et al*., 2013b; (o) Josephson *et al*., 1967.

The investigation of these potential causes of whey protein derived mouthdrying requires appropriate methods and ideally should be tested within the target population. Currently, the majority of the literature, as outlined in Table 2.3, has focused on using *in vivo* and physiochemical analysis to understand the proposed mechanisms of whey protein derived mouthdrying alongside collecting sensory data. Key limitations are, however, associated with these methods: (1) researchers are only able to provide correlations between potential underpinning mechanisms and sensory data, and therefore, are unable to prove relationships; (2) a lack of research involving the human mouth, apart from the oral retention method developed by our research group (Cook *et al*., 2018; Bull *et al*., 2020; Norton *et al*., 2020a, **Chapter 3**); (3) the ongoing challenge of quantifying mouthdrying using a 'physical measure' at the same time as scoring mouthdrying perception within products; (4) there is no defined mouthdrying threshold test to quantify individual sensitivity; and (5) few studies have explored the role of individual differences on mouthdrying using consumers. Despite mouthdrying sensations being present in different whey protein fortified food matrices, the majority of cited studies which have investigated mouthdrying in the solid food matrices have only used sensory methods (Table 2.3) (Wendin *et al*., 2017; Song *et al*., 2018; Norton *et al*., 2020b, **Chapter 4**). Therefore, less is known about potential mechanisms involved compared with a WPB. It is likely that within a dry low moisture system, such as a solid food, particles could aggregate or adhere to the oral cavity, causing friction (Lucas *et al*., 2004) resulting in subsequent mouthdrying sensations. Furthermore, the strength of the interaction could be influenced by saliva, with adhesion, friction, surface tension and salivary viscosity being considered contributing factors (Lucas *et al*., 2004). In addition, a previous review of mucoadhesion in food systems suggested mucoadhesion strength could potentially be increased within a solid model from food product absorbing water from the oral cavity,

promoting interactions, leading to swelling and spreading, as well as strengthened mucoadhesion (Cook *et al*., 2017). Similarly, as alluded to by Celebioglu *et al*. (2020), hydrophobic and hydrophilic interactions could also be relevant within a solid model, for example causing mouthdrying from poor dispersion between whey protein and saliva.

Table 2.3a. Sensory methods commonly used to investigate whey protein derived mouthdrying. Key limitation: unable to explain the cause of mouthdrying.

Method	Food Matrix	Description	Limitations
Descriptive analysis using a trained sensory panel[1,2]	Cakes & biscuits[(a)], WPB[(b-l)], rye bread & cream cheese[(m)]	○ Provides an objective measure of mouthdrying	○ Studies have used different methods (such as Spectrum™ and DSP/QDA™), scored differing number of attributes (2 to 36) and there are potential issues with providing a standard mouthdrying reference to ensure consistency across studies
Threshold using a trained sensory panel[1]	WPB[(f,n,o)]	○ Evaluates mouthdrying intensity strength compared with protein concentration	○ Studies have rated mouthdrying intensity using different methods (for example: 0-5 and 0-7 scales, Spectrum™ and scalar scoring), different types of whey protein beverages and studies have used varying number of panellists (7-12 panellists)
Sequential profiling and time intensity methods using trained sensory panels[1]	WPB[(c,f,g,p)]	○ Sequential profiling measures changes in sensory attributes with repeated consumption and time intensity provides data on time, duration and intensity of mouthdrying	○ Typically, sequential profiling methods have not solely focused on mouthdrying and there are also potential issues with providing a standard mouthdrying reference to ensure consistency across studies
Sensory methods using consumers[1,2]	WPB[(b,d,e,q,r)], cakes & biscuits[(a)], muffins[(s)], rye bread & cream cheese[(m)]	○ Provides feedback on products using the target consumer population. Common methods to evaluate mouthdrying include focus group sessions, 9-point hedonic liking, Just-About-Right (JAR), generalised linear magnitude scale (gLMS), visual analogue scale (VAS) and two-alternative forced choice test (2-AFC)	○ Limited studies have tested mouthdrying using consumers and there are potential issues with test sensitivity of methods used. Carter *et al*. (2020) noted consumers are untrained and potentially less able to quantify mouthdrying objectively

Table 2.3b. Physiochemical analysis commonly used to investigate whey protein derived mouthdrying. Key limitation: requires sensory data to provide correlations.

Method	WPB Model	Description	Limitations
Taste sensor[1]*	WPI, PWP & aPWP[(n))]	○ Measures the change in membrane potential as a result of adsorption	○ Analysis has been carried out in low pH WPBs; therefore, this method may not be suitable for neutral pH WPBs
Turbidity[1]*#	β-LG[(f)], WPI[(g)], β-LG & LF[(i)]	○ Measures aggregation of protein and saliva	○ Saliva has been mixed artificially with whey protein and this may differ to saliva samples collected post beverage consumption ○ Saliva samples in the referenced studies were only collected from 2-5 volunteers; however, saliva is considered to vary between individuals ○ Turbidity in isolation is unlikely to explain the cause of mouthdrying
Electrophoresis analysis[1]*#	β-LG & LF[(h,o)]	○ Determines protein composition using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)	○ As for turbidity: saliva has been mixed artificially with whey protein; saliva samples only collected from 2-5 volunteers
Dynamic light scattering[1]*#	WPC[(c)], β-LG & LF[(i)]	○ Measures the size and distribution of protein and/or with saliva	○ As for turbidity: saliva has been mixed artificially with whey protein ○ Particle size in WPB increases with heating time, however, mouthdrying is also present in unheated WPBs. Therefore, particle size in isolation is unlikely to explain the cause of mouthdrying
Zeta potential[1]*#	WPC[(c)], β-LG & LF[(i,j)]	○ Measures electrostatic interactions of protein, with or without saliva	○ Bull *et al*. (2020) identified within a neutral pH that WPBs (samples varying in levels of heat treatment) had similar zeta potential scores, therefore proposed mouthdrying in this study was not related to electrostatic interactions and proposed other mechanisms could be involved. However, saliva was not collected in this study
Portable infrared spectrometer[1]*	WPI, WPC & WPH[(k)]	○ Predicts mouthdrying in low pH WPB	○ This method was only tested in low pH WPBs; therefore, this method may not relate to mouthdrying from neutral pH WPBs
Tribology[1]*	β-LG[(t)]	○ Measures friction and lubrication	○ In some conditions (i.e. increasing protein concentration from 0.5 to 4%) sensory results were unable to correlate with tribology data

Table 2.3c. *In vivo* analysis commonly used to investigate whey protein derived mouthdrying. Key limitation: requires sensory data to provide correlations.

Method	WPB Model	Description	Limitations
Saliva flow[1]*	β-LG[(f)]	○ Evaluates saliva flow following different stimulants and relating this to whey protein-derived mouthdrying	○ Studies have been limited by the number of saliva samples which can be collected within one session and this referenced study was limited by a relatively small sample size (10 volunteers) with a gender imbalance (2 males and 8 females)
Animal models[1]#	β-LG[(u)]	○ Measures the adhesion of proteins to porcine oral mucosa tissue	○ Methods needs to be adapted to enable human investigation
Oral retention[1]#	WPC[(q,v)]	○ Measures the protein remaining in saliva samples post beverage consumption	○ Previous limitations were small subject size and no non-protein control. More recent limitations include the link between mucoadhesion and mouthdrying within the same method have not been investigated
Dynamic *in vivo* models[1]*	WPI[(w)]	○ Aims to replicate in-mouth beverage consumption by measuring whey protein and saliva interactions	○ Models were estimated based on limited data from the literature; therefore, may not fully reflect individual variability

[1] Refers to studies using a whey protein beverage (WPB) model (whey protein isolate (WPI), process whey protein (PWP), acidic process whey protein (aPWP), whey protein concentration (WPC), whey protein hydrolysate (WPH), β-lactoglobulin (β-LG) and lactoferrin (LF); [2]refers to studies using a whey protein solid model; *denotes studies using a low pH WPB model; #denotes studies using a neutral pH WPB model. Acronyms: quantitative descriptive analysis (QDA) and descriptive sensory profiling (DSP). (a) Norton *et al*., 2020b, **Chapter 4**; (b) Childs & Drake, 2010; (c) Bull *et al*., 2017; (d) Oltman *et al*., 2015; (e) Zhang *et al*., 2020; (f) Kelly *et al*., 2010; (g) Beecher *et al*., 2008; (h) Vardhanabhuti *et al*., 2010; (i) Ye *et al*., 2011; (j) Ye *et al*., 2012; (k) Wang *et al*., 2016; (l) Lee & Vickers, 2008; (m) Song *et al*., 2018; (n) Sano *et al*., 2005; (o) Ye *et al*., 2012; (p) Withers *et al*., 2014; (q) Norton *et al.*, 2020a, **Chapter 3**; (r) Withers *et al*., 2013a; (s) Wendin *et al*., 2017; (t) Vardhanabhuti *et al*., 2011; (u) Withers *et al*., 2013b; (v) Bull *et al*., 2020; (w) Andrewes *et al*., 2011.

Strategies to reduce mouthdrying have been previously investigated with limited success. For example, Withers *et al*. (2014) tested different mouthdrying mitigation strategies using a sensory trained panel by adding sucrose (3.0% wt/wt), modulating viscosity by adding a starch thickener (1.8% wt/wt) and increasing fat levels by using both sunflower oil and milk fat (2.0% wt/wt), and concluded that all these strategies had minimal effect on mouthdrying in dairy beverages at the tested levels. This highlights the challenges associated with suppressing mouthdrying and a need to understand better the potential

mechanism involved in mouthdrying to enable improved mitigation strategies to be developed (Withers *et al*., 2014).

In summary, addressing and understanding the proposed causes of mouthdrying is important to increase the enjoyment derived from products and subsequent compliance. Texture has a key role in food preferences and learning from astringency related oral drying can provide useful insights into whey protein derived mouthdrying.

2.6.2. Mucoadhesion and mouthfeel perception

There is a growing interest in the mucoadhesion phenomenon and its associated prolonged 'oral exposure', which may influence sensory perception (Cook *et al*., 2017). Our research group has proposed mucoadhesion to be the probable cause of whey protein derived mouthdrying particularly in beverages at near-neutral pH. A proposed WPB mucoadhesion mechanism is outlined in Figure 2.3.

Mucoadhesion is a concept that has been well utilised in drug delivery systems due to its ability to enhance retention at mucosal membranes (Smart, 2005; Andrews *et al*., 2009; Khutoryanskiy, 2011) and has more recently been considered in a food context (Cook *et al*., 2017). Mucoadhesion can be simply described as the adhesion of materials to mucosal membranes (moist surfaces lining the walls of different body cavies) (Khutoryanskiy, 2011).

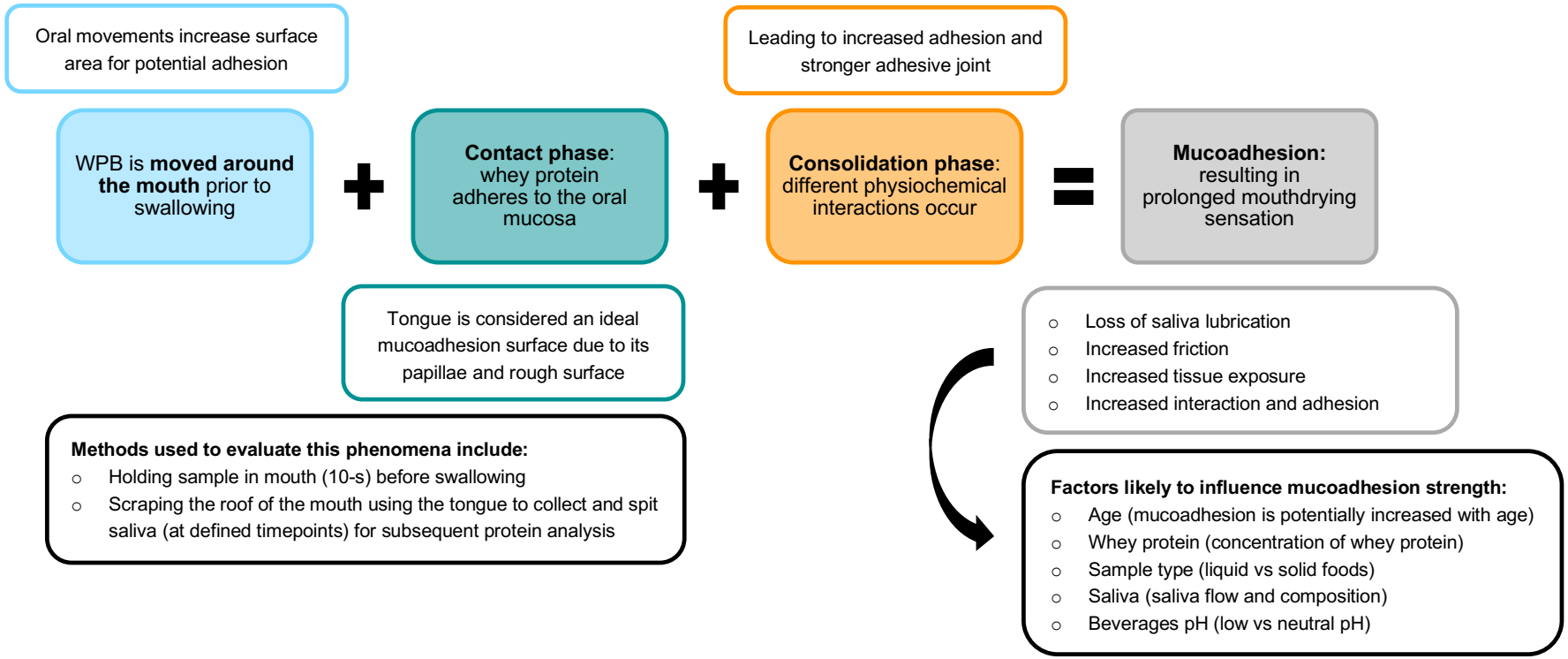


Figure 2.3. Proposed mucoadhesion mechanism of neutral pH whey protein beverages (WPB) (Smart, 2005; Khutoryanskiy, 2011; Vardhanabhuti *et al*., 2011; Cook *et al*., 2017; 2018; Bull *et al*., 2020; Norton *et al*., 2020a, **Chapter 3**).

Mucoadhesion can result from different physicochemical interactions, such as hydrogen bonding, hydrophobic interactions, electrostatic interactions, Van der Waals forces and disulphide bridges (Smart, 2005; Bernkop-Schnurch & Thiomers, 2005; Bassi da Silva *et al*., 2018). Mucoadhesion can be explained based on different theories, for example, wetting, mechanical, electronic, diffusion, dehydration and adsorption (Khutoryanskiy, 2011). Two different stages have been cited in establishing mucoadhesion (Smart, 2005; Khutoryanskiy, 2011). The first is a contact phase which can occur from the adhesion of a material (whey protein) to the mucosal membrane (oral mucosa), resulting in spreading and swelling (Smart, 2005; Khutoryanskiy, 2011). The second is a consolidation phase resulting from physicochemical interactions, which lead to stronger adhesion (Smart, 2005; Khutoryanskiy, 2011). Mucoadhesion has often been measured using physical techniques (rheological, optical and spectroscopic) and *in vivo* methods (tensile, rotating disc, flow-through, tribology and oral retention) (Khutoryanskiy, 2011; Vardhanabhuti *et al*., 2011; Cook *et al*., 2018; Bull *et al*., 2020; Norton *et al*., 2020a, **Chapter 3**).

Mucoadhesion is considered in the context of this review to be the binding or sticking of whey proteins to the oral cavity (cheeks, gums and tongue) (Bull *et al*., 2017). In order to measure such adhesion to the oral cavity within humans, our research group developed an oral retention method (Cook *et al*., 2018; Bull *et al*., 2020). This method enables researchers to measure the amount of protein retained in the mouth over time by measuring protein concentration in saliva samples (Bull *et al*., 2020). However, the key limitation of this method has related to a very small subject sample size and the absence of a non-protein whey source control. More recently, Norton *et al*. (2020a, **Chapter 3**) validated the oral retention method by establishing that WPB consumption significantly increased protein content in saliva samples post beverage, compared with a non-protein control (whey permeate beverage) using a group of younger consumers. Furthermore,

factors such as saliva flow, composition and viscosity are considered to influence retention of samples (Cook *et al*., 2018). Accordingly, it is proposed that a reduced saliva flow could lead to greater mucoadhesion as a result of increased tissue exposure, adhesion and interactions from proteins within the oral cavity (Bull *et al*., 2020). Recent work by our research group highlighted that reduced salivary flow rate correlated with increased mucoadhesion; however, differences in saliva flow had no significant influence on mouthdrying perception (Norton *et al*., 2020a, **Chapter 3**). Therefore, we conclude the need for further research in this area involving more sensitive salivary flow rate methods, as well as including the rating of mouthdrying perception within such methods (a key limitation as alluded to in Table 2.3) to enable better correlations with mucoadhesion.

The extent of mucoadhesion within older adults is relatively unknown. However, it is proposed that mucoadhesion is likely to be strengthened within an ageing population as (a) sensitivity to mouthdrying can increase with age (Withers *et al*., 2013a) and (b) salivary flow rates can decrease with age (Vandenberghe-Descamps *et al*., 2016). Recently, we investigated this phenomenon in 84 consumers (42 younger adults aged 18-30 years and 42 older adults aged 65 years or over) (Norton *et al*., 2020a, **Chapter 3**). Older adults had significantly increased protein concentration in saliva samples post WPB consumption, regardless of the extent of whey protein heat treatment, compared with younger adults (Norton *et al*., 2020a, **Chapter 3**). This suggests mucoadhesion increases with age and could result in a prolonged drying sensation; however, this latter point needs further proof. Understanding the potential mechanisms involved in whey protein derived mouthdrying will be key to ensure products are optimised so as to ensure the benefits associated with consumption of whey protein fortified products are achieved by older adults.

In summary, mucoadhesion is a relatively new area within mouthfeel perception and early indications suggest mucoadhesion has a role as a potential cause of mouthdrying. However, this is yet to be proven, and therefore, future work should address this phenomenon, as well as identifying whether mucoadhesion is present in different food models and considering the role that individual differences may have on mucoadhesion.

2.7. Age and individual differences likely to influence mouthfeel perception

Sensory perception is considered to alter with age. The most obvious age-associated changes relate to vision and hearing, although touch and pain thresholds also increase with age (Wickremarachi & Llewelyn, 2006; Tortora & Nielsen, 2009). It is well documented that taste impairments and loss of smell are commonly associated with ageing. For example, older adults have increased taste detection thresholds across all taste modalities and accordingly perceived taste perception declines with age (Methven *et al*., 2012). Olfactory function also reduces with age and the combination of taste and olfactory decline can result in older adults often perceiving foods to lack flavour (Schiffman & Graham, 2000; Schiffman & Zervakis, 2002; Methven *et al*., 2012; Doty & Kamath, 2014) (ageing and taste has been reviewed previously see Methven *et al*. (2012)). Age has been shown to have varying effects on texture and mouthfeel perception. For example, studies have shown that older adults perceived soups as less creamy, sweet waffles as less fatty and elastic and dairy beverages as more mouthdrying compared with younger adults (Kremer *et al*., 2005; 2007b; Withers *et al*., 2013a). However, in other studies the effects of age have been less apparent, such as perceived thickness and mouthcoating of dairy beverages remaining consistent between younger and older age groups (Withers *et al*., 2013a). Again, in a study comparing different nut types using temporal dominance of sensations, the overall progression of dominant attributes during chewing was consistent between age groups (Hutchings *et al.*, 2014).

Older adults did, however, select hardness as a more dominant attribute compared with younger adults (Hutchings *et al*., 2014). This suggests some aspects of texture perception are potentially preserved with age; however, this could be attribute and product dependent. Accordingly, these changes can influence food choice, potentially making food less interesting and enjoyable, and therefore may increase the risk of poor nutritional status. Currently, less is known about how mouthfeel perception changes with age. Moreover, it has been suggested by previous authors that a greater emphasis could be placed on mouthfeel sensations to compensate for taste and smell loss in older adults (Forde & Delahunty, 2004).

2.7.1. Whey protein derived mouthdrying and changes in perception with age

Surprisingly, despite multiple high protein products being available on the market and whey protein fortified products being commonly used to improve nutritional status, these products are typically not designed with, or for, older adults. Withers *et al*. (2013a) suggested some aspects of texture perception are influenced by age, as older adults reported greater sensitivity to mouthdrying compared with younger adults following consumption of dairy beverages. This study investigated mouthdrying by comparing a heated rennet whey sample with a skimmed milk sample using a paired comparison test (Withers *et al*., 2013a). Only the older adults were able to distinguish the rennet sample as more mouthdrying (Withers *et al*., 2013a); rennet whey was proven previously to be a source of mouthdrying (Josephson *et al*., 1967). However, two more recent studies by our research group have been unable to demonstrate an overall effect of age on mouthdrying in different whey protein food models using a gLMS (generalised linear magnitude scale) and VAS (visual analogue scale) (Norton *et al*., 2020a, **Chapter 3**; Norton *et al*., 2020b, **Chapter 4**). They concluded that within a liquid model using WPBs, the potential cause of the minimal effect between age groups related to the lack of

sensitivity of the gLMS compared with a paired comparison test (Norton *et al*., 2020a, **Chapter 3**). Whereas, in the solid model, which used two different methods (a single point in time and a full portion size at home), the older adults were able to perceive the protein cakes and biscuits as more mouthdrying compared with the control versions (Norton *et al*., 2020b, **Chapter 4**). This supported Withers *et al*. (2013a) findings but did not reach overall significance and highlighted the challenges with measuring mouthdrying within an older population and ensuring a suitable test is selected to measure such mouthdrying.

Sensory testing needs to replicate normal eating behaviour and measure changes in consumption over repeated consumption, rather than just a single sip or bite. This is especially relevant to products such as ONS, which are associated with changes in liking and mouthfeel with multiple sips (Methven *et al*., 2010; Thomas *et al*., 2016; 2018). This demonstrates the challenges within older adults of balancing the appropriate volume to replicate normal consumption versus sample fatigue from too many samples (Methven *et al*., 2016) (for a review of sensory and consumer methodology in older adults, see Methven *et al*. (2016)). Moreover, to measure effectively changes in the perception of mouthdrying with age, it is important to ensure the methods to be used are suitable for a broad range of older adults within a test group, so as to secure useful and meaningful results.

2.7.2. Individual differences that could influence perception of whey protein fortified products

Individuals are defined by differences that distinguish them from others and such differences can influence sensory perception. For example, consumers typically differ in physiology (such as age, biological sex, health status and associated medications, appetite, dental status, saliva flow, muscle strength, sensory acuity - including differences in taste, olfaction and oral tactile sensitivity), social factors (such as cultural and

demographic groups) and preferences (such as food preferences, mouth behaviour and food neophobia) (Engelen & van der Bilt, 2008; Jeltema *et al*., 2015; 2016; Engelen, 2018; van den Heuvel *et al*., 2019; Ketel *et al*., 2019; 2020; Laureati *et al*., 2020). When designing products for older adults, individual differences are likely to influence perception. Accordingly, these differences will be explored in the following sections with a specific focus on their relevance for older adults. Table 2.4 highlights that individual differences, such as age, oral health, saliva and food oral processing are considered to have a role within sensory perception. However, currently the extent of the effect of such individual differences on the perception of whey protein derived mouthdrying is relatively uncertain.

Table 2.4. Relevant individual differences likely to influence perception of whey protein derived mouthdrying in older adults (↑ increases with age; ↓ decreases with age; n/a not applicable).

Category	Factors	Effect of Age	Effect on Mouthdrying	Food Matrix	Methodology Limitations
Physiology	Age[1]*	n/a	○ Whey protein fortified products cause mouthdrying which may be influenced by age	WPB[(a,b),] cakes & biscuits[(c)]	○ Inconsistent results between studies could result from differences in test sensitivity used (for example, paired comparison test versus generalised labelled magnitude scale)
	Appetite[1,2]*#†	↓	○ ONS and whey protein fortified products can increase perceived thirst, reduce hunger and prospective consumption	Cupcakes[(c),] ONS[(e,f)]	○ Self-report using visual analogue scale. Appetite was not measured at subsequent meals
	Dental status[1,2]*†	↓	○ Poor dental status could make consumption of solid foods more difficult and therefore negatively impact product liking	Cakes & biscuits[(c)], meat & cereal[(f)]	○ Self-report questionnaire or limited oral parameters measured
	Saliva flow[1,2]*#	↓	○ Saliva flow can decrease with age; however, whether this influences subsequent perception is relatively unclear	WPB[(a,g)], cakes & biscuits[(c)], meat & cereal[(f)]	○ Volunteers may have been too healthy to demonstrate an effect of saliva flow
	Detection thresholds to sensory stimuli[1]#	↑	○ Detection thresholds for many stimuli (such as tastants and volatile compounds) increase with age and perception increases (at different rates depending on the stimuli) with stimuli intensity. Studies to date suggest that perceived mouthdrying initially increases with protein concentration until a plateau is reached	WPB[(g,h,i,j)]	○ No defined mouthdrying threshold method has been developed
Social	Culture[2]*	n/a	○ Cultural groups have different food oral processing behaviour and this could influence food choice and mouthfeel perception	18 different food products varying in physical properties[(k)], carrot, cheese & sausage[(l)]	○ Only limited populations have been studied (for example Dutch nationality and Caucasian ethnicity compared with Chinese nationality and Asian ethnicity)
Preferences	Food preference and neophobia[2]‡	No set direction	○ Food preferences and neophobia could influence compliance with ONS and whey protein fortified products	n/a[(m,n)]	○ Self-report questionnaire
	Mouth behaviour[1]*	Not known	○ Mouth behaviour could influence texture perception of whey protein fortified products and may alter with age	Cakes & biscuits[(c)]	○ Self-report questionnaire

[1]Refers to studies using whey protein food matrices; [2]refers to factors that may influence whey protein derived mouthdrying but have currently not been investigated within a whey protein food matrix. Study type: *younger adults (18-35 years) and older adult (over 65 years) study; #younger adults only (20-60 years); †older adults only (60-75 years[(d)]; over 65 years[(f)]; ‡ other: (children aged 9-12 years and parents[(m)]; n/a review paper[(n)]). All volunteers considered healthy unless otherwise stated. Abbreviations: whey protein beverage (WPB); oral nutritional supplements (ONS). (a) Norton *et al*., 2020a, **Chapter 3**; (b) Withers *et al*., 2013b; (c) Norton *et al*., 2020b, **Chapter 4**; (d) Thomas *et al*., 2016; (e) Thomas *et al*., 2018; (f) Vandenberghe-Descamps *et al*., 2017; (g) Kelly *et al*., 2010; (h) Childs & Drake, 2010; (i) Sano *et al*., 2005; (j) Ye *et al*., 2012; (k) Ketal *et al*., 2019; (l) Ketal *et al*., 2020; (m) Laureati *et al*., 2020; (n) van den Heuvel *et al*., 2019.

2.7.3. Food oral processing and mouthfeel perception

The oral cavity consists primarily of lips, gums, cheeks, hard and soft palates, teeth, tongue, salivary glands, orofacial muscles and mucous membranes (Tortora & Nielsen, 2009; Pereira, 2012). The oral mucosa (three types within the oral cavity, namely lining, masticatory and specialised mucosa) is a moist soft tissue membrane lining the oral cavity providing key functions such as protection, lubrication and moistening (Tortora & Nielsen, 2009; Hand & Frank, 2014). Oral receptors respond to food digestion and processing, thereby leading to taste, odour, irritation and texture perceptions (Engelen & van der Bilt, 2008). The mouth is considered a sensitive organ and receptors such as mechanoreceptors (touch and proprioception), which respond to tactile stimuli, are considered the most relevant for texture perception (Engelen & van der Bilt, 2008). Although there is no specific texture receptor, texture is considered to be perceived by the tongue, palate and other soft tissues within the mouth (Engelen & van der Bilt, 2008). For further details on oral cavity anatomy and physiology and relevant oral receptors within a food context, see references (Engelen & van der Bilt, 2008; Chen, 2009; Tortora & Nielsen, 2009; Engelen, 2012; Hand & Frank, 2014).

Individuals differ in their masticatory function, bite force, swallowing threshold, saliva volume and composition, oral receptors and sensitivity (Engelen, 2018). Therefore, it is combination of differences in food structure and individual oral physiology that cause variation in food oral processing and subsequently in sensory perception (Chen, 2009). Differences in food oral processing influence perception not only of texture and mouthfeel, but also flavour, thereby affecting food choice and acceptability (Chen, 2009; Stokes *et al*., 2013). Mouth behaviour can be described as the way an individual manipulates food in their mouth and is considered to influence food choice, texture preference and satisfaction (Jeltema *et al*., 2015; 2016). There are four major mouth behaviour groups:

crunchers (individuals that like foods that break on biting) and chewers (those that prefer to chew foods), being considered the more predominant groups compared with suckers (those that prefer harder foods which can be sucked on) and smooshers (likers of soft foods and less mouth activity) (Jeltema *et al*., 2015; 2016). Mouth behaviour (as outlined in Table 2.4) can also have implications for older adults. For example, a decline in dental status can influence food choice, resulting in a preference for softer foods rather than hard crunchy foods (Jeltema *et al*., 2015; 2016).

Food lubrication within the mouth is considered to be influenced by size and concentration of oil droplets, viscosity of saliva, protein content of saliva and properties of the particles (size, shape and hardness) within the oral fluid and surface properties of the oral mucosa and teeth (de Wijk & Prinz, 2006). The role of oral lubrication in food intake is also a consideration; therefore, manipulating oral lubrication could be particularly relevant within older adults who are at increased risk of malnutrition and their saliva flow often being reduced (Krop *et al*., 2019a).

Understanding changes in food oral processing with age is key to improving food intake, particularly in an older adult population. For example, older adults are considered to consume foods more slowly, have increased chewing duration and reduced tongue strength compared with younger adults (Crow & Ship, 1996; Mioche *et al*., 2004a; Ketal *et al*., 2019). Teeth loss is also associated with ageing, data from the 'Adult Dental Health Survey 2009 - England' demonstrated edentate increasing with age from 1% at 45-54 years, 5% at 55-64 years, 15% at 65-74 years, 29% at 75-84 years and 45% at 85 years and over (Health and Social Care Information Centre, 2011). Teeth loss is also associated with reduced masticatory abilities (Ikebe *et al*., 2011). Steele *et al*. (1997) noted from a study involving 1211 adults aged 60 years or over that having 21 or more natural teeth resulted in less eating problems. Mastication behaviour can influence mouth behaviour

preferences, texture perception of foods and food choice and intake, thereby impacting an individual's nutritional status (Mioche *et al*., 2004b; Wilson *et al*., 2018). For example, lower mucosal moisture has been associated with reduced and poor chewing capacity in older adults (Shinkawa *et al*., 2009).

In summary, food oral processing is considered to play an important role in determining food choice and acceptance, with age-related changes likely to impact this further. Changes in food oral processing are likely to impact perception and acceptance of protein fortified foods. Overall, an understanding of the differences between age groups and their sensory sensitivity will assist in the provision of more suitable food products to match the needs of older adults.

2.7.4. Differences in saliva flow with age and their potential effect on mouthfeel perception

Saliva is a viscoelastic solution, consisting of approximately 99.5% water, with the remainder (~0.5%) being proteins, enzymes, electrolytes and nitrogenous products (Humphrey & Williamson, 2001; de Almeida *et al*., 2008; Tortora & Nielsen, 2009; Carpenter, 2013). Saliva performs a key role in the maintenance of oral health, as well as enabling taste, providing a buffer capacity and mineralisation, aiding digestion and preventing tooth decay, as well as being a lubricant and having antimicrobial functions (Humphrey & Williamson, 2001; de Almeida *et al*., 2008; Carpenter, 2013).

Saliva-related diseases can negatively impact oral health, quality of life, dietary habits and nutritional status (Gupta *et al*., 2006). For example, xerostomia (dry mouth) is a syndrome involving an absence of saliva and results in eating difficulties, tooth decay and oral candida infection and its prevalence is considered to increase with age (May *et al*., 2015). Hyposalivation (reduced saliva flow) is typically cited as < 0.1 and 0.5 mL/min for

unstimulated saliva and stimulated saliva flow, respectively (Gupta *et al*., 2006; Marton *et al*., 2008; Munoz-Gonzalez *et al*., 2018b). It correlates with adverse health outcomes, as well as reduced taste perception, chewing and swallowing difficulties (Gupta *et al*., 2006; Marton *et al*., 2008; Munoz-Gonzalez *et al*., 2018b). Common causes of hyposalivation include medication, dehydration and disease (Gupta *et al*., 2006). Prevalence of dry mouth within an older population is considered between 12-39% and increases with age (Thomson, 2015).

There is evidence of age-related changes in saliva. For example, a review by Xu *et al*. (2019) highlighted salivary changes with age, supporting reduced saliva flow, changes in calcium and mucin content and increased ionic concentration influencing the quantity and quality of saliva. A meta-analysis involving 47 studies concluded significantly reduced salivary flow rates in older adults, and this reduction was not considered to be related to use of medication (Affoo *et al*., 2015). Vandenberghe-Descamps *et al*. (2016) demonstrated that healthy older adults had 38.5% and 38% lower unstimulated and stimulated salivary flows respectively, compared with younger adults, and the results were independent of medication and dental status. The acinar cells are considered to degenerate with age and can influence salivary flow rates (Whelton, 2012). Affoo *et al*. (2015) indicated a gland specific reduction in salivary flow rates in older adults and highlighted that the parotid gland and the minor glands are potentially less influenced by age. An overview of saliva flow contributions from salivary glands (Whelton, 2012) is outlined in Figure 2.4. The submandibular gland, which contributes 60% of unstimulated saliva production, has an increased sensitivity to metabolic and physiological changes, which is a proposed cause of greater changes seen in unstimulated saliva flow with age compared with stimulated saliva (Whelton, 2012). Accordingly, a reduced saliva flow is considered an issue and is commonly associated with decreased lubrication, protection,

oral clearance, mucosal surfaces hydration and coating abilities within the oral cavity (Lee *et al*., 2002, Nagler & Hershkovich, 2005; Turner & Ship, 2007; Chaudhury *et al*., 2015). It is, therefore, likely to contribute to changes in food habits, further negatively impact nutritional status and alter sensory perception (Table 2.5). However, as stimulated saliva flow is potentially less influenced by age (Affoo *et al*., 2015), this may minimise changes from saliva flow in response to food consumption and subsequent sensory perception.

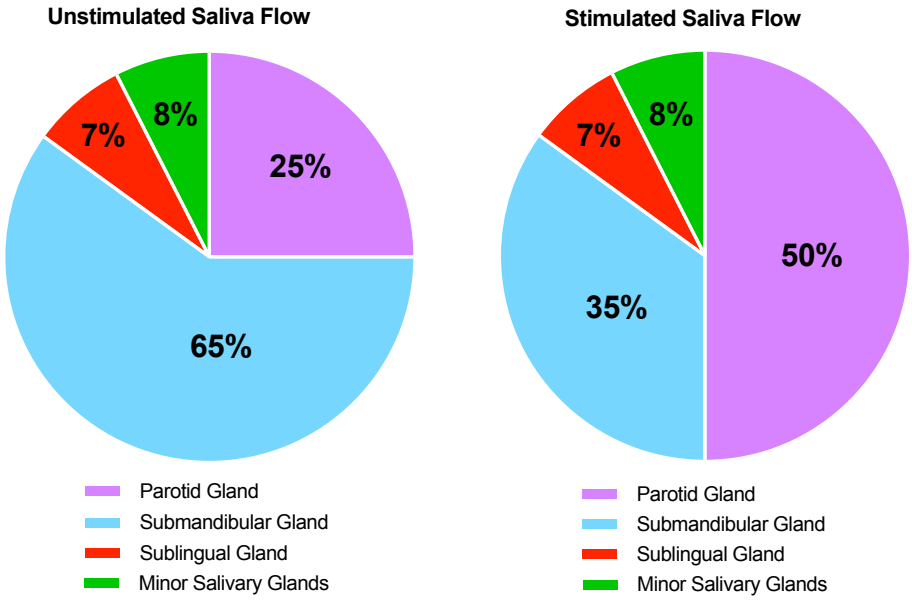


Figure 2.4. Saliva flow contribution from salivary glands (Whelton, 2012).

Food breakdown and perception of taste, flavour and texture of foods are all influenced by saliva, which affects the eating process and food intake (Mioche *et al*., 2004b; Munoz-Gonzalez *et al*., 2018a). Saliva provides a key role in our eating experience, with food oral processing and perception both being influenced by a number of food and saliva interactions as outlined in Table 2.5 (Mosca & Chen, 2017). Without saliva, food deformation, breakdown and destabilisation would be negatively influenced, along with food perception and swallowing (Mosca & Chen, 2017). In a food bolus, food particles are incorporated with saliva into something safe to swallow, and this process is in most cases considered automatic (Chen, 2012). However, bolus formation and swallowing can

provide additional risks in an older adult, thereby affecting an individual's food choice and intake (Chen, 2012). Additionally, these processes are considered to be influenced by the surface coating of food particles, particle size distribution and saliva incorporation, with moisture content and type of food structure also influencing the volume of saliva required (Chen, 2012; Mosca & Chen, 2017).

Table 2.5. Summary of proposed food and saliva interactions and effect on sensory perception, as suggested by Mosca and Chen (2017).

Proposed Mechanism	Description	Sensory Perception
Surface coating and wetting	○ This ensures lubrication, food breakdown, bolus formation and safe swallowing	○ Insufficient saliva can result in drying sensations
Colloidal interactions	○ Colloidal food products such as beverages and emulsions can interact with saliva causing destabilisation	○ Texture and mouthfeel attributes
Complexation	○ Reduction in saliva lubrication and increased friction	○ Mouthdrying and astringency sensations
Enzymatic breakdown	○ Rheological properties changes from amylase activity and macromolecules partial hydrolysis	○ Texture and flavour perception
Binding of aroma compounds	○ Saliva dissolves tastants and binds aroma	○ Flavour perception

In terms of sensory perception, unstimulated saliva provides background taste, whilst stimulated saliva is part of the mechanical process during eating and can increase salivary flow rates by 5-50 times, with more than 50% secreted from parotid glands (Affoo *et al*., 2015; Feron, 2019). As highlighted in Table 2.5, saliva is likely to contribute negatively to mouthfeel perception and could impact the perception of whey protein derived mouthdrying. The spinnbarkeit test relates to the stringiness of saliva and its adhesion properties within the mouth; saliva provides lubrication and protection, both of which are considered important for sensory perception (Vijay *et al*., 2015; Pushpass *et al*., 2019b). Altered or reduced viscoelasticity can impact mouthfeel perception, with viscoelasticity being noted to reduce with age (Vijay *et al*., 2015; Pushpass *et al*., 2019b). Furthermore, it has been suggested that an altered aroma perception in older adults could

be caused by reduced stimulated saliva flow (Munoz-Gonzalez *et al*., 2019). There are challenges associated with understanding the role of saliva on subsequent perception, and these are partly due to methodology limitations (as highlighted in a recent review by Munoz-Gonzalez *et al*. (2018b)). Typically, studies have grouped volunteers into low or high saliva flow, often resulting in minimal effects on sensory perception (Vandenberghe-Descamps *et al*., 2017; Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**).

In summary, there is a clear need to understand how saliva can impact sensory perception and consumption of foods in older adults. The influence of saliva and age-related changes in saliva on the sensory perception of foods, and specifically protein fortified products, needs further investigation.

2.8. Conclusion

This review highlighted that individual differences (such as age, appetite, dental status, saliva flow, detection thresholds to sensory stimuli, cultural differences and preferences) could influence whey protein derived mouthdrying, which in turn impacts the eating experience. Protein needs are considered to increase with age and protein consumption is associated with numerous benefits. More specifically, whey protein is commonly fortified into products due to its associated functional benefits. However, such products can elicit mouthdrying, which is considered to hinder consumption and acceptance. Therefore, improvements in such products are key to increasing liking and reducing wastage. Furthermore, mouthdrying is considered to increase with age, and despite previous investigations, the causes of whey protein derived mouthdrying are currently not fully understood. Further research is needed to understand these, with mucoadhesion currently being a proposed, but as yet to be proven, cause. In addition, more research is needed into potential mitigation strategies (such as using fat, sucrose or adjusting

viscosity) to modulate mouthdrying and their subsequent influence on consumer acceptance. Despite mouthdrying being present in both a liquid and solid food model, research has mainly focused on WPB mechanisms rather than solid model mechanisms; therefore, future research should look to address this gap within the literature. Individual differences, such as age, oral health, saliva and food oral processing, are considered to have a role within mouthfeel perception. However, currently, the effect of such individual differences on mouthdrying and mucoadhesion is relatively uncertain. If taking account of age and individual differences could lead to increased protein consumption by tailoring whey protein fortified products to meet individual needs, then this could significantly improve nutritional status in older adults and help to reduce their susceptibility to malnutrition and sarcopenia.

Chapter 3

An investigation of the influence of age and saliva flow on the oral retention of whey protein and its potential effect on the perception and acceptance of whey protein beverages

3.1. Context to chapter

Previous research had indicated that whey proteins could adhere to the oral cavity; however, this needed to be proven compared with a non-protein whey control, as well as using a robust method and a larger sample size. In addition, the influence of age and saliva flow on such adhesion is unclear. Whey beverages provide a simple model to test such adhesion and whey protein beverage (WPB) heat treatment could intensify mouthdrying mechanisms. Therefore, this chapter aims to investigate four overall thesis hypotheses: (a) whey protein fortified beverages will cause mouthdrying; (b) whey protein will adhere to the oral cavity post WPB consumption; (c) mucoadhesion will increase with age post WPB consumption; and (e) individual differences (such as age and saliva flow) will influence perceived whey protein derived mouthdrying. Accordingly, these hypotheses were tested via the following objectives:

- Do consumers perceive whey protein fortified liquid models as mouthdrying compared with non-protein whey control? More specifically in this chapter: does WPB heat treatment intensify consumers perceived mouthdrying?
- Does whey protein adhere to the oral cavity more than a non-protein whey control in WPBs?
- Does mucoadhesion (oral retention of whey protein from WPB) increase with age?
- Does sensitivity to whey protein derived mouthdrying increase with age in whey protein liquid models?
- Do salivary flow rates relate to perceived mouthdrying intensity in whey protein liquid models?

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3.2. Abstract

Protein fortified products are regularly recommended to older adults to improve nutritional status and limit sarcopenia. However, protein fortification can elicit negative sensory attributes such as mouthdrying. Sensitivity to mouthdrying can increase with age, yet the influence of saliva flow and mucoadhesion remain uncertain. Here, two studies tested different whey protein beverages (WPB); 22 healthy younger volunteers completed a pilot study and 84 healthy volunteers from two age groups (18-30; 65+) completed the main study. In both studies salivary flow rates (mL/min) were measured and saliva samples were collected at time intervals post beverage consumption to measure mucoadhesion to the oral cavity, where protein concentration was analysed by Bradford Assay. Volunteers rated perception and acceptability of WPB in the main study. WPB consumption resulted in significantly increased protein concentration ($p < 0.0001$) in saliva samples compared with a control whey permeate. Older adults had significantly lower unstimulated saliva flow ($p = 0.003$) and significantly increased protein concentration ($p = 0.02$) in saliva samples, compared with younger adults. Heating of WPB significantly ($p < 0.05$) increased mouthdrying and thickness and reduced sweetness compared with unheated WPB. Mucoadhesion is concluded to be a true phenomenon in WPBs and increases with age.

Keywords: mucoadhesion; mouthdrying; older adults; whey protein; saliva flow

3.3. Introduction

Malnutrition is prevalent within the UK, with over one million older adults affected, and the risk of malnutrition is considered to increase in clinical settings (hospitals, care homes and mental health units) (BAPEN, 2018). Such malnutrition has multiple contributing factors and can refer to an overall insufficient intake of all nutrients or specific macro- or

micronutrients (BAPEN, 2018). Protein is of specific interest as protein needs are considered to increase with age, for example, the PROT-AGE study group recommend a protein intake of 1.0-1.2 g/kg/d for older adults (Bauer *et al*., 2013). They suggested that this higher requirement was to maintain good health, encourage recovery from illness and preserve functionality and that increased needs of older adults compared with younger adults resulted from age-related changes in protein metabolism (Bauer *et al.*, 2013). The intake of food generally, and of protein rich foods specifically, can be reduced in older age due to chemosensory impairments, such as loss of taste and smell, which are commonly associated with older adults and considered to relate to ageing, medication, disease, malnutrition, environment and surgical interventions (Schiffman & Graham, 2000). However, the influence of saliva, and age-related changes in saliva, on the sensory perception of foods and protein fortified foods, has received little attention.

Saliva is a viscoelastic solution, consisting of 99% water, with the remainder being protein and ion components, which enable taste, aid digestion and prevent tooth decay, as well as acting as a lubricant and having antimicrobial properties (Carpenter, 2013). Saliva flow is considered to decrease with age (Vandenberghe-Descamps *et al*., 2016). Accordingly, a reduced saliva flow is considered a problem and is commonly associated with decreased lubrication, protection, oral clearance, mucosal surface hydration and coating abilities within the oral cavity (Lee *et al*., 2002; Nagler & Hershkovich, 2005; Turner & Ship, 2007; Chaudhury *et al*., 2015). Furthermore, food breakdown and perception of taste, flavour and texture of foods are all influenced by saliva, impacting upon the eating process and food intake (Mioche *et al*., 2004b; Munoz-Gonzalez *et al*., 2018a). This emphasises the need to understand how saliva can impact sensory perception and consumption of foods in older adults.

Foods for older adults are often fortified by whey protein due to the high bioavailability of this protein source (Pennings *et al*., 2011). To increase energy, protein and micronutrient intake, oral nutritional supplements (ONS) are often prescribed to older adults, and these are commonly fortified with whey protein and other dairy proteins sources. However, ONS typically have poor consumer acceptance, which has been linked to both undesirable aroma and taste and a build-up of perceived mouthdrying following repeated consumption (Gosney, 2003; Childs & Drake, 2010; Kennedy *et al*., 2010; Methven *et al*., 2010). Previous research has shown that older adults have greater sensitivity to mouthdrying compared with younger adults following consumption of dairy beverages (Withers *et al*., 2013a). Sensory profiling has identified negative mouthfeel attributes to be perceived after consumption of whey protein (e.g. chalky, drying, mouthcoating, astringent) and heat treatment of whey protein is considered to intensify further these sensory properties (Bull *et al*., 2017). Such findings have clinical significance, particularly as individuals are commonly recommended to consume up to 600 mL of ONS per day (Methven *et al*., 2010).

Astringency, drying and mouthdrying are terms commonly used to describe the ‘textural defect’ associated with dairy products (Lemieux & Simard, 1994); these terms are often used within the literature as interchangeable. Astringency typically refers to a mouth puckering like sensation caused by precipitation of salivary proteins on binding to polyphenols which reduces salivary lubrication (Gibbins & Carpenter, 2013); however, polyphenols (a group of secondary plant metabolites) (Draijier *et al*., 2016) are not present in whey protein. In this paper the term mouthdrying is used to refer to a drying sensation in the mouth during or after the consumption of a product. The causes of whey protein derived mouthdrying are currently not fully understood, despite previous investigation and are summarised in Table 3.1.

Table 3.1. Commonly proposed causes of whey protein beverage (WPB) derived mouthdrying.

Proposed Cause	Description
The pH of WPB	Low pH can cause precipitation of the protein; however, there is evidence of mouthdrying from WPB at both low and neutral pH[(a-f)]
Saliva and protein interactions	Perception of mouthdrying has links to saliva and protein interactions[(a,b,d,g,h)]
Reduced and lubrication of saliva	Increased friction within the oral cavity from reduced lubrication[(i)]
Adhesion and binding properties	Whey proteins binding to oral epithelial cells, proteins remaining on surfaces, mucoadhesive properties and increased oral retention[(j-l)]
Heating time	Mouthdrying is considered to increase with product heating time, potentially due to protein denaturation[(f,n)]

(a) Vardhanabhuti *et al*., 2010; (b) Ye *et al*., 2011; (c) Sano *et al*., 2005; (d) Beecher *et al*., 2008; (e) Withers *et al*., 2014; (f) Bull *et al*., 2017; (g) Andrewes *et al*., 2011; (h) Kelly *et al*., 2010; (i) Vardhanabhuti *et al*., 2011; (j) Ye *et al*., 2012; (k) Withers *et al*., 2013b; (l) Hsein *et al*., 2015; (m) Bull *et al*., 2020; (n) Josephson *et al.*,1967.

Our research group consider the adhesion of whey protein to be a highly probable cause of whey protein derived mouthdrying. Mucoadhesion, a concept that has been utilised in drug delivery systems (Smart, 2005; Andrews *et al*., 2009; Carvalho *et al*., 2010; Khutoryanskiy, 2011) and more recently considered in a food context (Cook *et al*., 2017). Mucoadhesion can be described as physicochemical interactions between a polymetric material and mucosal environment (Khutoryanskiy, 2011) and is considered in the context of this paper to be the binding or sticking of whey proteins to the oral cavity (Bull *et al*., 2017). An oral retention method has been developed to measure the amount of protein retained in the mouth over time by measuring protein in saliva samples with factors such as salivary flow, composition and viscosity considered to influence retention of samples (Cook *et al*., 2018; Bull *et al*., 2020). One limitation of the oral retention method of measuring protein mucoadhesion to date has been small subject sample sizes (Bull *et al*., 2020). Currently, the extent to which mucoadhesion and mouthdrying are influenced by saliva flow in older adults remains uncertain. This paper hypothesises whey protein beverages (WPB) will cause mucoadhesion of protein to the oral cavity following consumption and that older adults will have reduced salivary flow, greater adhesion of

protein to the oral cavity, and increased mouthdrying perception of WPB, compared with younger adults. This hypothesis was tested through the following objectives:

(1) A pilot study was carried out with the objective of establishing whether the protein measured in the oral cavity post WPB consumption resulted from mucoadhesion of the WPB (rather than resulting from consumption-induced release of salivary protein). The pilot study was conducted in younger adults and measured protein concentration of saliva samples post beverage consumption (WPB and whey permeate beverage (WPeB)) at four different timepoints (15-s, 30-s, 60-s and 120-s), in order to validate the oral retention method.

(2) The main study had the following objectives to: (a) measure salivary flow rates from unstimulated and stimulated saliva; (b) determine if protein adheres to the oral cavity of older adults to a greater extent than younger adults; (c) determine if salivary flow rates influence mucoadhesion and perception of WPBs; and (d) evaluate whether heat treatment of protein in WPB causes mouthdrying and reduced acceptance within each volunteer group. This study recruited younger and older adults to test these objectives.

3.4. Materials and methods

3.4.1. Overview of pilot and main study

The pilot study was a single blinded randomised crossover trial with one study visit, involving 22 healthy male and female younger volunteers (18-30 years; 25.7 ± 3.0 years). The main study consisted of 84 healthy male and female volunteers from two age groups (42 younger adults; 18-30 years, 24.3 ± 3.6 years and 42 older adults; over 65 years, 73.6 ± 6.2 years) who completed a single blinded randomised crossover trial involving three study visits (volunteer overview, Table S.3.1). In both studies the subject size was determined by power calculations (alpha risk = 0.05 and 80% power) based on previous

study data (Bull *et al*., 2020) using protein retention in the oral cavity as the primary outcome measure. In the pilot study, comparing WPB with WPeB, we estimated a difference in protein concentration of 1.5 mg/mL saliva and standard deviation of 1.5, which concluded a minimum sample size of 15. In the main study to compare oral retention for WPB in younger versus older adults we anticipated a smaller difference of 0.7 mg/mL (standard deviation 1.5), inferring a minimum sample size of 72. Volunteers were recruited from the local Reading area. The studies were conducted in accordance with the Declaration of Helsinki. All volunteers had the study fully explained to them and provided informed written consent before taking part. They were informed that all data would be anonymous and kept fully confidential and that there was a right to withdraw. The studies received a favourable opinion for conduct from the University of Reading Research Ethics Committee[17] (pilot study: SCFP 28/19 and main study: UREC 18/46) and the study was registered on the clinical trials database (www.clinicaltrials.gov as NCT03798730).

All volunteers were screened to ensure suitability (minimal medication, non-smoker, no food allergies or intolerances, non-diabetic and not having had either cancer, oral surgery or a stroke). Volunteers who met the inclusion criteria and were willing to take part were invited to attend study visits held at the Sensory Science Centre, University of Reading; the study overview is summarised in Figure 3.1**.** In order to control extraneous variables, volunteers refrained on the day of each study visit from tea and coffee and drank a glass of water one hour before the visit. Each individual volunteer completed all their study visits at the same time of day in a temperature-controlled room (22 °C) under artificial daylight.

[17] additional ethics related information outlined in **Appendix A**

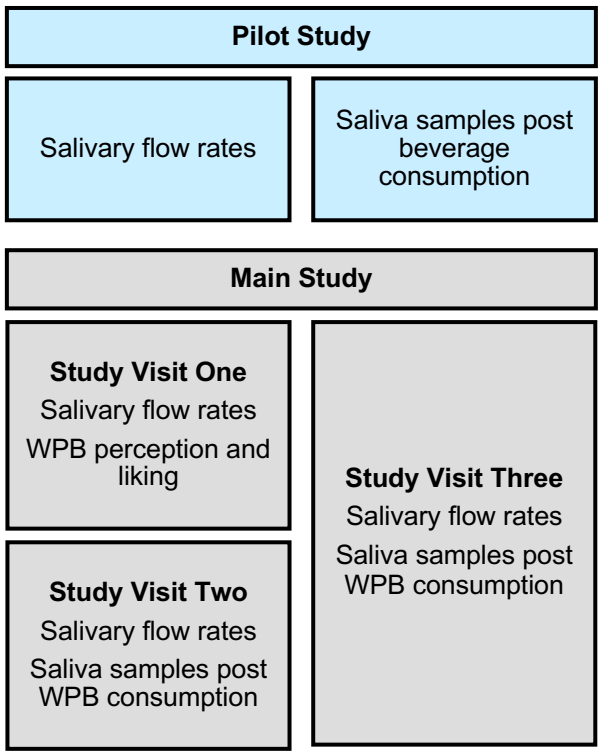

Figure 3.1. Overview of pilot and main studies (WPB: whey protein beverage).

3.4.2. Materials

Whey powders were provided by Volac (Volac International Ltd., Royston, UK) as whey protein concentrate (WPC, Volactive Ultra-Whey 80, providing a minimum protein content of 80% and the remaining 20% being lactose, fat, moisture and ash), as well as whey permeate (WPe, Volactose Taw Whey Permeate, providing a minimum lactose content of 89% and the remaining 11% being ash, moisture, protein and fat). Parafilm®, Bradford reagent (500 mL, 0.1-1.4 mg/mL) and protein standard 2.0 mg/mL (Bovine Serum Albumin, BSA) were supplied by Sigma-Aldrich (Dorset, UK).

3.4.3. Liquid model preparation

The pilot study tested two different beverages, first, a WPB (10.0% w/v, WPC powder in deionised water) was used as a protein beverage. A 10.0% concentration is considered sufficient to stimulate a postprandial muscle protein synthesis response in older adults and has previously been used in WPB testing (Luiking *et al*., 2014; Bull *et al*., 2017).

Second, a WPeB (4.0% w/v, whey permeate powder in deionised water) was used as the control beverage. The WPeB concentration was selected as being below the lactose sweet recognition threshold (4.19%); therefore, unlikely to cause sweetness-stimulated additional saliva flow and having a relatively similar mineral profile to the protein beverage (Belitz *et al*., 2004; Buzalaf *et al*., 2012).

The main study tested two different WPBs (10.0% w/v, WPC powder in deionised water, as outlined above) for the influence of heat treatment; using unheated WPB (WPCU) and heated WPB (WPCH), an overview of both studies beverages is outlined in Table 3.2. Sample heating temperature (70 °C) was chosen as beta-lactoglobulin, the most abundant protein in WPC, has a critical temperature for denaturation of 70 °C (De Wit & Swinkels, 1980; Boye & Alli, 2000; Bull *et al*., 2017). The sample heating time of 20-min was selected as the maximum time the product could be maintained at 70 °C without aggregation and remain acceptable to serve to consumers (Bull *et al*., 2017). In both the pilot and the main study, the method was adapted from previous work (Bull *et al*., 2017) with both samples being prepared simultaneously, as summarised in Figure 3.2.

Table 3.2. Overview[18] of whey protein beverage (WPB, 10.0% w/v) and whey permeate beverage (WPeB, 4.0% w/v).

	Whey Protein Beverage			Whey Permeate Beverage	
	per 5 mL[1]	per 10 mL[2]	per 100 mL	per 10 mL[2]	per 100 mL
Nutritional Composition					
Energy (kcal)	2.0	4.0	39.7	1.5	14.7
Fat (g)	0.04	0.07	0.7	0.0008	0.008
of which saturates (g)	0.01	0.03	0.3	-	-
Carbohydrate (g)	0.02	0.04	0.4	0.4	3.6
of which sugars (g)	0.02	0.04	0.4	-	-
Protein (g)	0.4	0.8	8.2	0.01	0.1
Typical Mineral Profile					
Calcium (mg)	-	5.5	55.0	2.2	21.6
Magnesium (mg)	-	0.5	5.0	0.4	4.4
Phosphorus (mg)	-	3.5	35.0	2.4	24.4
Potassium (mg)	-	5.0	50.0	5.7	57.2
Sodium (mg)	-	1.5	15.0	1.8	18.4
Chloride (mg)	-	0.5	5.0	1.8	18.4
Chemical Properties					
Protein (%)	-	0.8	8.2	0.01	0.1
Moisture (%)	-	0.05	0.5	0.004	0.04
Ash (%)	-	0.04	0.4	0.02	0.2
Lactose (%)	-	0.04	0.4	0.4	3.6
Fat (%)	-	0.07	0.7	0.0008	0.008
pH	-	6.5	6.5	5.6	5.6

[1] 5 mL sip size was used for WPB perception and liking in the main study only; [2] 10 mL sip size was used in the oral retention method in both studies.

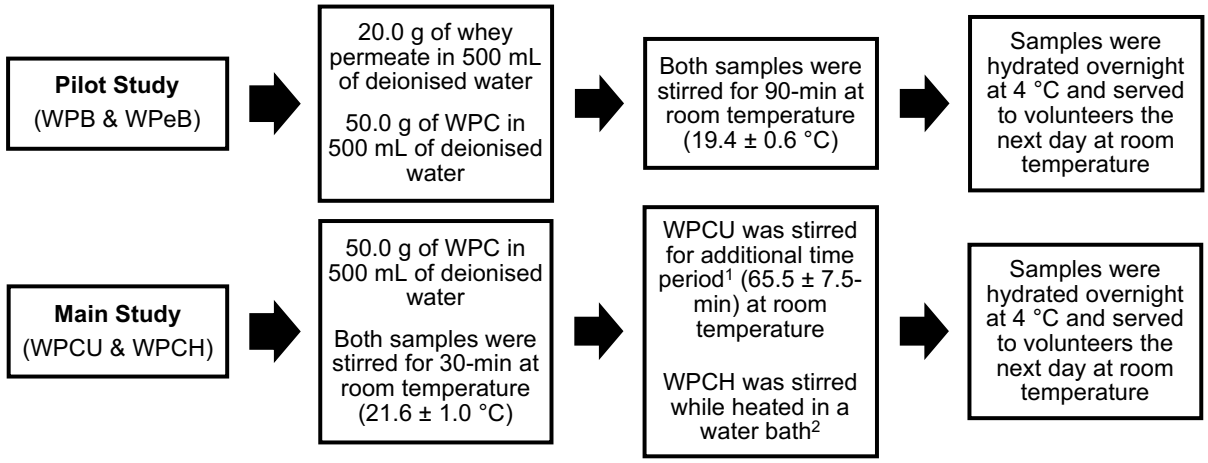

Figure 3.2. Overview of beverage preparation[19] in both studies (WPB: whey protein beverage; WPeB: whey permeate beverage; WPC: whey protein concentrate; WPCU: unheated WPB; WPCH: heated WPB). [1] additional time period was based on the time it took to heat and cool WPCH; [2] the time to 70 °C was recorded (20.9 ± 4.7-min) and maintained at 70 °C for a further 20-min and cooled to room temperature.

[18] data was calculated from technical data sheets of ingredients used

[19] stirred: magnetic stirrers (Stuart™ SM5, Cole-Parmer Stuart, Staffordshire, UK) at a medium speed and fully submerged magnetic stirrers (Thermo Scientific™ Cimarec™ i, Thermo Scientific, Loughborough, UK) were used in the water bath (Grant Instruments Optima GD120, Essex, UK); a temperature probe (Tenma TP101, Leeds, UK) was used in all samples to monitor sample temperature to ensure consistency between batches.

3.4.4. Salivary flow rates

Saliva collection methods were adapted from previous studies (Vandenberghe-Descamps *et al*., 2016; Pushpass *et al*., 2019a). In the pilot study saliva collection (unstimulated and stimulated saliva) was carried out at the beginning of the study visit with approximately 10 to 15-min break between collection methods. During the main study unstimulated saliva was collected by volunteers at the beginning of all three study visits and two replicates of stimulated saliva were collected from volunteers during study visit one, with approximately 10 to 15-min break between each collection. The rationale for the saliva collection was based on unstimulated saliva being considered a baseline measure and potentially more influenced by age than stimulated saliva (Affoo *et al*., 2015; Feron, 2019). In addition, it was unrealistic to expect older volunteers to provide a total of 10 saliva samples during a single study visit; therefore, it was considered impossible to collect both unstimulated and stimulated saliva at study visits two and three. However, stimulated saliva was used as a baseline value for saliva samples post beverage consumption (see Section 3.4.5) as stimulated saliva is produced during food mastication and has supported better correlations with study outcomes compared with unstimulated saliva (Feron *et al*., 2014; Affoo *et al*., 2015) and accordingly has been used in other saliva studies for analytical saliva analysis (Munoz-Gonzalez *et al*., 2019).

Unstimulated saliva was collected by asking volunteers to collect saliva in their mouths and to spit out saliva every time they felt the urge to swallow during a 5-min time period; saliva was collected in a wide lid collection tube (60 mL). Stimulated saliva was collected by asking volunteers to spit out saliva every time they felt the urge to swallow during a 2-min time period, while chewing on parafilm® (5.0 × 5.0 cm), again into a wide lid collection tube. Saliva weights for all volunteers were monitored by weighing collection tubes before and after collection. Using the weights collected, salivary flow rates were calculated as

mL/min, using the assumption 1.0 g of saliva equates to 1.0 mL. All saliva samples were stored on ice pending analysis.

3.4.5. Saliva samples post beverage consumption

An adapted oral retention method (Figure 3.3) (Cook *et al*., 2018; Bull *et al*., 2020) was used in both studies to measure the protein remaining in saliva samples post beverage consumption. Stimulated saliva samples were collected (as described above) and used as a baseline measurement. All volunteers were provided with eight 10 mL beverage samples (pilot study: 4 × WPeB and 4 × WPB; main study: 4 × WPCU and 4 × WPCH) in a balanced order[20]; all samples were presented in opaque black plastic cups (25 mL) (BB Plastics, West Yorkshire, UK) (to mask minor visual differences between samples) coded with a random three-digit number. Volunteers also gave eight saliva samples post beverage consumption at defined randomised time points (15-s, 30-s, 60-s and 120-s). The procedure was carried out in duplicate for all volunteers (visits two and three) during the main study. In order to prevent crossover effects, volunteers had an enforced 5-min break between samples where they consumed warm filtered water; this is considered more effective than cold water at removing fatty dairy residues from the mouth (Withers *et al*., 2013a). The rationale for choosing a 5-min break was based on protein in saliva samples being considered to have plateaued within 3-min of WPB consumption (regardless of heating time) (Bull *et al*., 2020). Saliva weight of all samples was measured by recording tube weight pre- and post-collection; all saliva samples were stored on ice pending analysis. The oral retention method development stages are outlined in Section S.3.1.

[20] monadic balanced order, with sample sets randomly allocated to volunteers

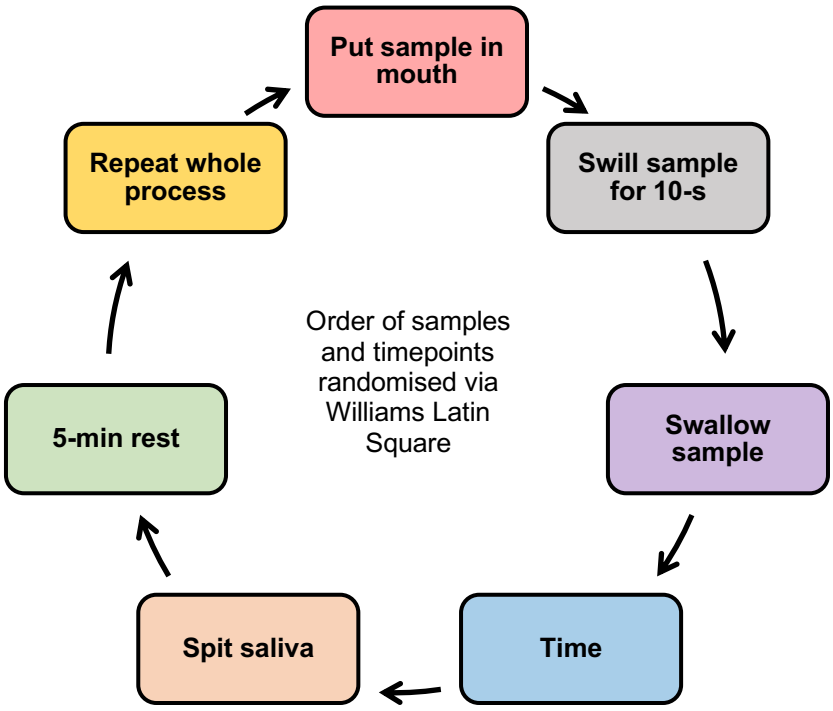

Figure 3.3. Brief overview of saliva samples post beverage consumption protocol. Volunteers were provided with verbal and written instructions as to the protocol and given the opportunity to ask questions. Volunteers were provided with one 10 mL sample and asked to swill the sample around in their mouth for 10-s before swallowing. After this a randomised countdown clock (time; either 15-s, 30-s, 60-s or 120-s) was started and once it reached zero, volunteers gave a saliva sample into wide lid collection tube (60 mL). A 5-min rest period followed, with the procedure being repeated for the seven remaining samples and timepoints.

3.4.6. Protein analysis of saliva samples

In both studies, protein concentration (mg/mL) in saliva samples was analysed using Bradford Assay (Bradford, 1976; Zor & Selinger, 1996). Samples were measured in triplicate with biological and analytical replicates using a 96 Well Plate Assay Protocol (Tecan Spark Control v2.1, Maneodorf, Switzerland). BSA was used as the protein standard, providing six decreasing dilutions mixed with purified water (SUEZ, Bristol, UK), ranging from 2.0 mg/mL to 0.125 mg/mL in triplicate, as well as a blank consisting of purified water on each individual 96 well plate. All saliva samples collected were analysed as a 1:2 dilution combining saliva and purified water with 5.0 µL pipetted into each well. Bradford Reagent (250 µL) was added to each well and each plate was placed on a

shaker for 30-s and read within a 5 to 60-min period. All analysis was carried out immediately following a volunteer's study visit. Each volunteer's baseline saliva protein measurement (stimulated saliva protein concentration) was subtracted from their sample saliva measurements at each time point to calculate the concentration of protein[21] remaining in saliva samples post beverage consumption[22].

3.4.7. WPB individual perception and liking

During the main study volunteers rated liking, effort to consume (easiness to drink and swallow), attribute perception and appropriateness of attribute level (Just-About-Right, JAR) of WPBs (WPCU and WPCH) (Figure 3.4) individually on an iPad (Apple, London, UK), either in isolated booths (younger adults) or at a table (older adults) using Compusense Cloud software[23] (Compusense, Ontario, Canada). Samples, coded with three-digit random numbers, were provided in a monadic sequential balanced order, with sample sets randomly allocated to volunteers. Volunteers received 5 mL of WPB in opaque black plastic cups (25 mL) and all volunteers were trained by a short video as to how to use the generalised Labelled Magnitude Scale (gLMS), a scale ranging from no sensation (0) to strongest imaginable sensation of any kind (100) (Bartoshuk *et al*., 2004). Volunteers had an enforced rest period of 45-s between samples and consumed warm filtered water (rationale as outlined in Section 3.4.5) before completing the same series of questions on the second sample.

[21] the standard curve was established by plotting protein standard (0.0-2.0 mg/mL) versus absorbance (nm; 590 divided by 490 nm to provide an overall value). The resulting graph provided a linear equation to obtain protein concentration that was used in the subsequent oral retention calculations.
[22] see Figure S.3.3 for overview of oral retention calculation
[23] version 21.0.7713.26683

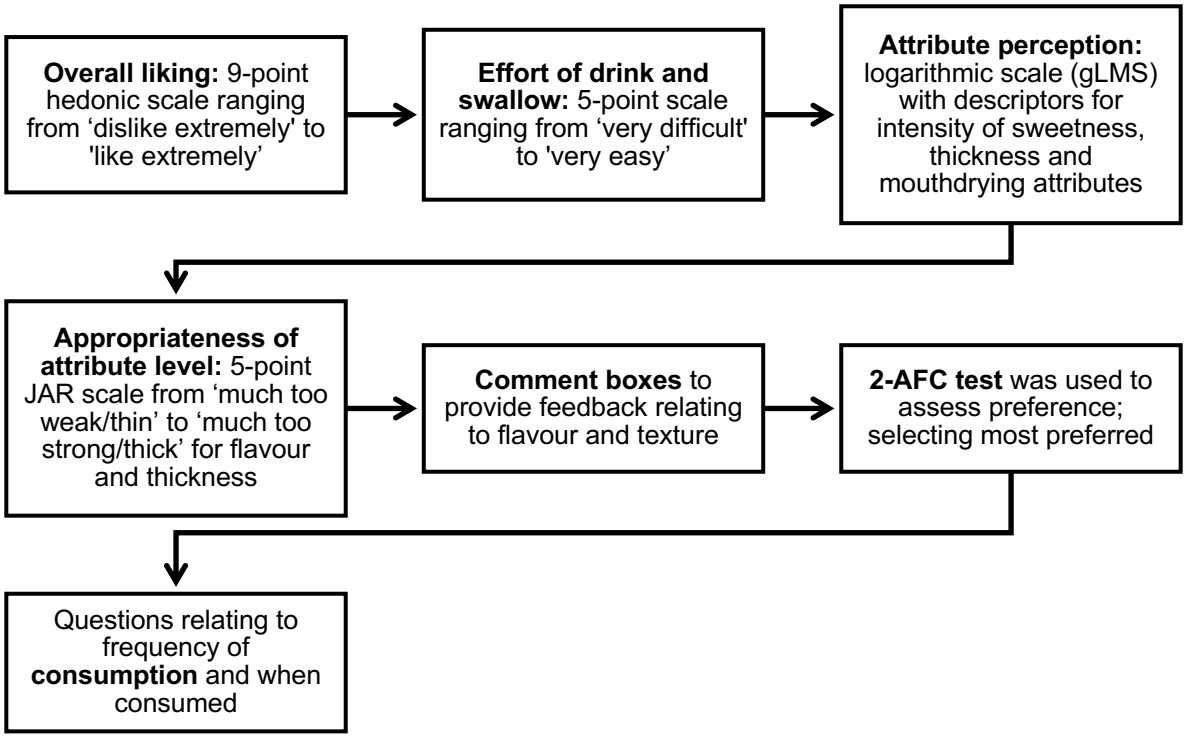

Figure 3.4. Overview of whey protein beverage (WPB) individual perception[24] and liking (gLMS: generalised Labelled Magnitude Scale; JAR: Just-About-Right; 2-AFC: two-alternative forced choice).

3.4.8. Statistical analysis

3.4.8.1. Pilot study

Statistical analysis was all carried out in SAS® software (SAS Institute Inc., Version 9.4, North Carolina, NC, USA) using a linear mixed model considered robust enough for unbalanced data (Torrico *et al*., 2018) and adjusted for multiplicity using Bonferroni. Saliva samples post beverage consumption were analysed using explanatory variables such as beverage type, time, gender and with volunteer code as a random effect and the dependent variable was protein concentration.

[24] sweetness: refers to the sweet taste of the sample stimulated by sucrose; thickness: refers to the feeling of thickness of the sample within your mouth; mouthdrying: refers to the drying sensation in the mouth during or after consumption of a sample.

3.4.8.2. Main study

Tertiary analysis was used to categorise volunteers into low, medium and high groups, based on average salivary flow rates using XLSTAT (version 2019.2.2, Addinsoft, Boston, MA, USA); these groupings were also used for statistical analysis for unstimulated salivary flow rates. In order to test associations between age and categorical data (saliva flow rate grouping and medication) a chi-square test was carried out on contingency tables using XLSTAT.

Statistical analysis was also carried out in SAS® software using a linear mixed model and adjusted for multiplicity using Bonferroni. Analysis of saliva samples post beverage consumption used explanatory variables such as visit, age, beverage type, time, gender, saliva flow, medication and with volunteer code as a random effect and the dependent variable was protein concentration. Baseline saliva samples and salivary flow rates were analysed using explanatory variables of age, gender, visit and with volunteer code as a random effect and the dependent variables were protein concentration and saliva flow respectively. The data relating to volunteer WPB perception and liking was analysed using explanatory variables of age, gender, beverage type, saliva flow, medication and with volunteer code as a random effect and the dependent variables were gLMS data, liking and JAR scores. All attribute data which was collected on the gLMS log-scale and was transformed to linear data (anti-logged). Values are expressed as least square means (LSM) estimates, as these values best reflect the statistical model.

Penalty analysis was carried out by XLSTAT with WPB JAR and liking scores, with 20% selected as the threshold for population size. Penalty analysis evaluated the influence of volunteer perception of appropriateness of attribute level rating (JAR) on volunteer liking by calculating the mean drop in liking rating (scale 1-9) compared with mean liking of volunteers that rated the attribute as JAR (JAR 3 on 1-5 scale), determining whether this

drop in liking score is significant. Analysis of significant preferences between WPB samples was calculated using Binomial expansion in V-Power (Ennis & Jesionka, 2011). In all analyses $p < 0.05$ was used as the value for significant difference.

3.5. Results

3.5.1. Pilot study

Salivary flow rates were 0.89 ± 0.33 mL/min and 2.56 ± 0.94 mL/min for unstimulated and stimulated saliva respectively. Beverage type (WPB or WPeB) had a significant effect ($p < 0.0001$), with the WPB leading to substantially and significantly more protein being collected in the saliva samples at all time points post beverage consumption (Figure 3.5). Time also had a significant effect ($p = 0.0004$), with saliva samples post WPB consumption showing a higher protein content at 15-s which decline over time (30-s, 60-s and 120-s), whereas WPeB had a lower saliva protein content throughout which remained relatively constant.

Figure 3.5. Protein concentration in saliva samples post beverage consumption ($n = 22$) by timepoints. Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) were reported between beverages at all timepoints with relevant p value above each timepoint.

3.5.2. Main study

3.5.2.1. Salivary flow rates

Older adults demonstrated significantly lower unstimulated saliva flow (p = 0.003) when compared with younger adults (LSM estimates ± SE: younger adults 0.90 ± 0.07 and older adults 0.62 ± 0.06 mL/min). However, age had no significant effect (p = 0.53) on stimulated saliva flow (younger adults 2.53 ± 0.19 and older adults 2.37 ± 0.18 mL/min). Volunteers were grouped by tertiary analysis into low, medium and high salivary flow rate, based on average salivary flow rates (Table 3.3). There was a significant association (p = 0.04) between age and saliva flow grouping for unstimulated saliva; however, stimulated saliva flow grouping was shown to be not significantly (p = 0.20) related to age. Age was significantly associated (p < 0.0001) with medication, indicating increasing use of medication with age (Table S.3.1); however, medication had no significant effect on saliva flow in older adults (unstimulated saliva flow (USF): p = 0.70 and stimulated saliva flow (SSF): p = 0.26) (data not shown[25]). Gender had a significant effect (USF: p = 0.02 and SSF: p = 0.02) on saliva flow regardless of collection method; males having significantly higher salivary flow compared with females (USF: males 0.88 ± 0.07 and females 0.66 ± 0.05 mL/min and SSF: males 2.73 ± 0.20 and females 2.17 ± 0.15 mL/min).

Table 3.3. Summary of volunteers salivary flow rates categories[1] (mL/min).

	Unstimulated Saliva Flow			**Stimulated Saliva Flow**		
	Low (0.04-0.53)	Medium (0.53-0.77)	High (0.77-2.18)	Low (0.23-1.63)	Medium (1.63-2.76)	High (2.77-6.13)
Total (n = 84)	28	27	29	25	30	29
Younger Adults (n = 42)	9	14	19	9	18	15
Older Adults (n = 42)	19	13	10	16	12	14

[1]Categories are defined by tertiles with mL/min range for the category.

[25] see Figure S3.4

3.5.2.2. Saliva samples post WPB consumption

Older adults had significantly higher protein concentration (p = 0.02) in their saliva samples compared with younger adults post WPB consumption at all timepoints (Figure 3.6). Time had a significant effect (p < 0.0001) with most saliva samples post WPB consumption demonstrating a higher protein content at 15-s compared with 30-s, 60-s and 120-s. Although there was an overall significant difference (p = 0.046) between samples, with unheated WPB samples leading to a slightly higher protein concentration in saliva samples compared with a heated sample. This difference was not consistent at each time point and there were no significant differences between the samples at the timepoints (p = 0.14). There was significant variability between individual visits (p < 0.0001), but the overall trends remained the same (Figure S.3.5). Although there was no overall significant effect of saliva flow (p = 0.06) on adhered protein concentration, the tendency was for the adhered protein content to decrease with increasing unstimulated saliva flow rate and this was significant at the 60-s collection time (p = 0.02) (Figure 3.7). There was no significant effect on protein concentration relating to gender and medication (Figure S.3.6).

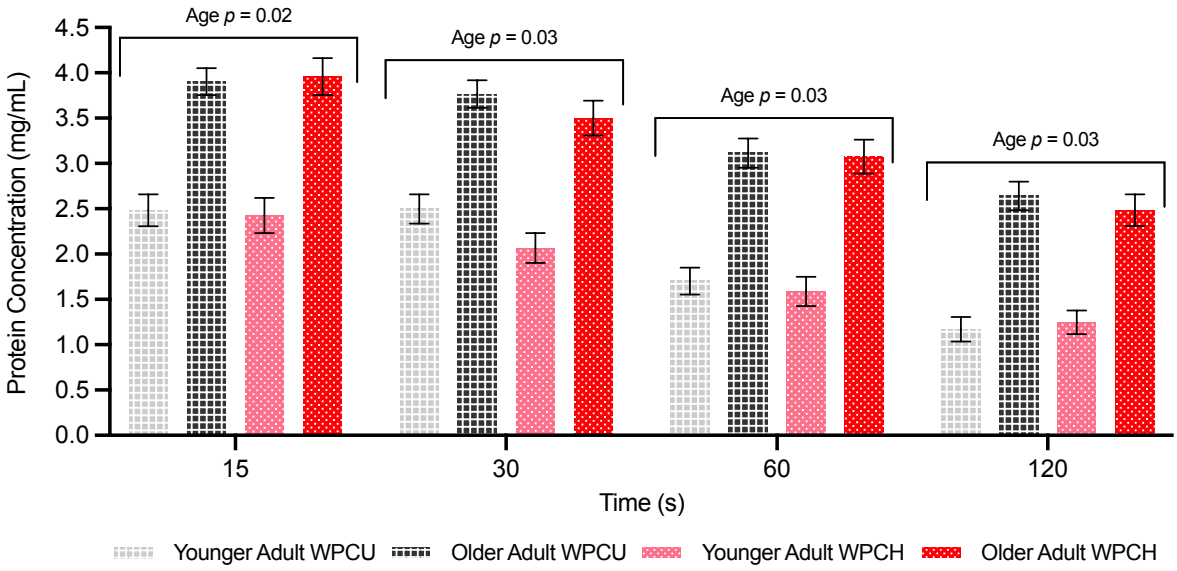

Figure 3.6. Protein concentration in saliva samples post whey protein beverage (WPB) consumption by age and timepoints (WPCU: unheated WPB; WPCH: heated WPB). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) were reported between age groups at all timepoints with relevant p value above each timepoint. Data from visit two (n = 84; YA (younger adults) n = 42 and OA (older adults) n = 42) and visit three (n = 82; YA n = 40 (two YA dropped out after visit two) and OA n = 42) combined. Baseline saliva protein concentration values are outlined in Table S.3.4.

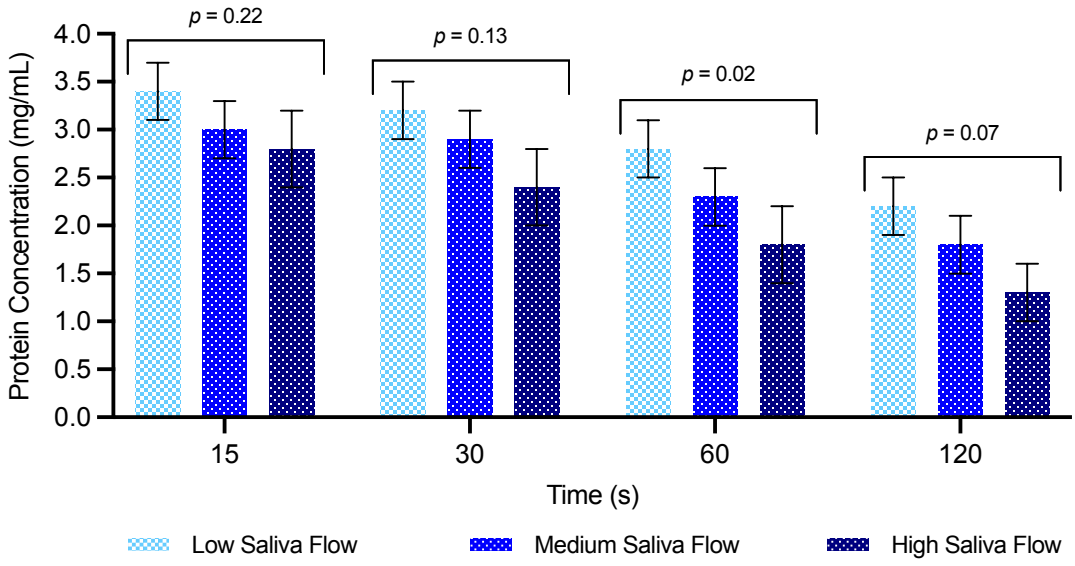

Figure 3.7. Protein concentration in saliva samples post whey protein beverage consumption by timepoints and saliva flow groupings. Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) were reported only at 60-s with relevant p value above each timepoint. Data from visit two (n = 84) and visit three (n = 82) combined. Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertiary analysis.

3.5.2.3. WPB individual perception and liking

The heated WPB was perceived as significantly ($p < 0.05$) thicker, less sweet and easy to swallow and resulted in more mouthdrying compared with the unheated WPB (Table 3.4). The increased thickness resulted in the beverage being significantly closer to 'Just-About-Right' thickness as opposed to too thin (Table 3.5). There were no significant differences between the age groups; however, older adults did score attributes mouthdrying and thickness lower than the younger adults for the heated WPB (Table 3.4). There was a significant effect ($p = 0.03$) of liking where older adults had significantly higher liking scores following the heated WPB compared with younger adults; however, there was no significant effect of liking on the unheated WPB. There was no significant effect of age on effort to consume and JAR attributes, though younger adults scored unheated WPB notable thicker than older adults (Tables 3.4 and 3.5).

There was no overall significant effect of saliva flow on WPB liking and perception; however, by categorising the volunteers into low, medium and high saliva flow groupings by tertiary analysis using unstimulated saliva flow, we found there were some interesting trends. In particular there was trends at a lower saliva flow for mouthdrying to be lower (low versus medium and high SF: $p = 0.33$ and $p = 0.36$ respectively), sweetness to be higher (low versus medium and high SF: $p = 0.44$ and $p = 0.09$ respectively) and thickness to be lower (low versus medium and high SF: $p = 0.55$ and $p = 0.23$ respectively) (Table 3.4). Volunteers not taking medication had significantly higher overall liking scores ($p = 0.03$) compared with medication users and males reported significantly higher easiness to swallow scores ($p = 0.02$) compared with females; however, no further significant effects on perception and liking were reported relating to medication or gender (Table S.3.5).

Table 3.4. Volunteers' liking, effort to consume and attribute perception mean ratings of whey protein beverages (WPB); overall and by age and unstimulated saliva flow rate.

	Overall (*n* = 84)		**Age**		**Unstimulated Saliva Flow**		
		Significance of sample (*p* value)	Younger Adults (*n* = 42)	Older Adults (*n* = 42)	Low Saliva Flow (*n* = 27)	Medium Saliva Flow (*n* = 28)	High Saliva Flow (*n* = 29)
Overall Liking							
WPCU	3.7 ± 0.3	0.10	3.6 ± 0.4^{A}	3.7 ± 0.3	3.5 ± 0.4	3.8 ± 0.3^{A}	3.6 ± 0.4
WPCH	3.3 ± 0.3		2.8 ± 0.4^{aB}	3.9 ± 0.3^{b}	2.9 ± 0.4	3.3 ± 0.3^{B}	3.7 ± 0.4
Easiness to Drink							
WPCU	3.9 ± 0.1	0.11	4.0 ± 0.2	3.9 ± 0.2	4.2 ± 0.2^{A}	3.8 ± 0.2	3.8 ± 0.2
WPCH	3.7 ± 0.1		3.6 ± 0.3	3.8 ± 0.2	3.7 ± 0.2^{B}	3.8 ± 0.2	3.6 ± 0.2
Easiness to Swallow							
WPCU	4.2 ± 0.1	**0.0004**	4.4 ± 0.2^{A}	4.1 ± 0.2	4.4 ± 0.2^{A}	4.2 ± 0.1^{A}	3.9 ± 0.2
WPCH	3.9 ± 0.1		3.7 ± 0.2^{B}	4.0 ± 0.2	3.9 ± 0.2^{B}	3.9 ± 0.1^{B}	3.9 ± 0.2
Mouthdrying							
WPCU	16.9 ± 3.5	**< 0.0001**	18.1 ± 5.2^{A}	15.7 ± 3.8^{A}	15.5 ± 4.8^{A}	15.8 ± 4.6^{A}	19.3 ± 5.0^{A}
WPCH	28.0 ± 3.5		34.4 ± 5.2^{B}	21.8 ± 3.8^{B}	23.9 ± 4.8^{B}	30.3 ± 4.6^{B}	30.0 ± 5.0^{B}
Sweetness							
WPCU	7.6 ± 1.1	**0.04**	7.1 ± 1.7	7.9 ± 1.2	8.7 ± 1.6	9.1 ± 1.5	4.8 ± 1.6
WPCH	6.0 ± 1.1		6.4 ± 1.7	5.6 ± 1.2	8.2 ± 1.6	5.5 ± 1.5	4.3 ± 1.6
Thickness							
WPCU	9.7 ± 2.0	**< 0.0001**	11.5 ± 2.9^{A}	7.9 ± 2.1^{A}	9.5 ± 2.7^{A}	9.9 ± 2.6^{A}	9.7 ± 2.9^{A}
WPCH	17.3 ± 2.0		19.5 ± 2.9^{B}	15.2 ± 2.1^{B}	13.3 ± 2.7^{B}	18.2 ± 2.6^{B}	20.6 ± 2.9^{B}

Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) within a row (i.e. age YA vs OA and saliva flow pairwise comparisons) are denoted by differing small letters and within a column (i.e. within an age group between samples or within saliva flow groupings between samples) are denoted by differing capital letters. WPCU (unheated WPB) and WPCH (heated WPB). Liking and effort to consume were measured on a 9- and 5-point scale respectively, attribute perception was measured on a gLMS logarithmic scale (anti-logged values 0-100 scale presented). Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertiary analysis.

3.5.2.4. Preference, penalty analysis and qualitative feedback

There was no significant preference (p = 0.46) between WPB samples; however, preference was significantly influenced (p = 0.03) by age. Younger adults preferred the unheated WPB whereas older adults preferred the heated WPB (Table S.3.6). The volunteers' perception of appropriateness of attribute level (Just-About-Right, JAR, ratings) can influence their overall liking, as shown in the penalty analysis (Table 3.5). There was a significant influence of thickness where the older adults found the heated WPB to be too thin this led to a significant and substantial reduction in the liking rating.

Table 3.5. Volunteers' appropriateness of attribute level (Just-About-Right, JAR) mean ratings of whey protein beverages (WPB) and their influence on volunteer liking ratings; overall and by age (YA: younger adult and OA: older adult) and unstimulated saliva flow rate.

	Overall (*n* = 84)		**Age**		**Unstimulated Saliva Flow**			**Penalty Analysis** (mean liking drop where attribute deviates from Just-About-Right)			
		Significance of sample (*p* value)	Younger Adults (*n* = 42)	Older Adults (*n* = 42)	Low Saliva Flow (*n* = 27)	Medium Saliva Flow (*n* = 28)	High Saliva Flow (*n* = 29)	Too Little (YA)	Too Much (YA)	Too Little (OA)	Too Much (OA)
JAR Flavour											
WPCU	2.3 ± 0.1	0.29	2.4 ± 0.3	2.2 ± 0.2^{A}	2.7 ± 0.2	2.0 ± 0.2	2.2 ± 0.2	1.04*	2.18	1.21*	1.07
WPCH	2.5 ± 0.1		2.5 ± 0.3	2.5 ± 0.1^{B}	2.6 ± 0.2	2.1 ± 0.2	2.7 ± 0.2	0.59*	0.71	0.80*	0.63
JAR Thickness											
WPCU	2.2 ± 0.1	**< 0.0001**	2.4 ± 0.2^{A}	2.1 ± 0.1^{A}	2.4 ± 0.2	2.2 ± 0.1^{A}	2.0 ± 0.2^{A}	1.06*	0.80	0.56*	-0.58
WPCH	2.7 ± 0.1		2.9 ± 0.2^{B}	2.6 ± 0.1^{B}	2.6 ± 0.2	2.6 ± 0.1^{B}	2.9 ± 0.2^{B}	0.73*	0.29	1.17#	1.68

Values are expressed as LSM estimates ± standard error from SAS output. Significant differences within a column (i.e. within an age group between samples or within saliva flow grouping between samples) are denoted by differing capital letters. Significant differences *(p* < 0.05) within a row (i.e. between penalty analysis groups within a sample for older adults) are denoted by #. WPCU (unheated WPB) and WPCH (heated WPB). Within penalty analysis * represents where the size of the group is lower than 20% of the population. Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertiary analysis.

Volunteers' comments were categorised into emerging themes, such as, flavour, texture, descriptive feedback, positive and negative comments and no comments provided (Table S.3.7). Overall, there was general negative feedback provided for all WPB samples; volunteers provided comments relating to flavour and texture for both the unheated and heated WPB. In total 211 comments were provided with only 30 positive comments and the remaining 181 comments were all negative, some examples are summarised in Table 3.6.

Table 3.6. Examples of volunteers whey protein beverages (WPB) comments (WPCU: unheated WPB and WPCH: heated WPB).

Sample	Comments and Volunteers Details
WPCU	*Tasteless and mouthdrying (v3, female, younger adult, aged 22).*
	Bland flavour, unappealing colour, unsatisfying dry finish and aftertaste (v49, male, older adult, aged 65).
WPCH	*It felt strange. It was thick and made my mouth feel dry afterwards. Almost as if all the moisture in my mouth had been sucked from it (v9, male, younger adult, aged 19).*
	My mouth and teeth feel yucky. Like when you eat rhubarb, I would like to go and clean my teeth (v79, female, older adult, aged 75).

3.6. Discussion

3.6.1. Mucoadhesion and WPB

The pilot study demonstrated that whey protein does adhere to the oral cavity (mucoadhesion) as the protein measured in the saliva samples following the consumption of a WPB was significantly and substantially higher than the protein content in saliva samples following consumption of a control whey permeate beverage (WPeB). These findings are supported by previous work which suggested proteins have adhesion and binding properties, for example: milk proteins can remain on oral surfaces (Withers *et al*., 2013b), WPB can bind to oral epithelial cells (Ye *et al*., 2012), have mucoadhesive properties (Hsein *et al*., 2015) and increased oral retention following a heated WPB compared with an unheated WPB (Bull *et al*., 2020). However, these studies were carried

out using animal models *in vivo* (measuring adhesion of proteins to porcine oral mucosa), with small subject sample sizes in human studies (five volunteers) or without a non-protein whey source control. The pilot study demonstrated that our oral retention method is an effective and valid method to measure mucoadhesion in a WPB model.

3.6.2. Salivary flow rates

Older adults had, on average, a 27% lower unstimulated salivary flow rate when compared with younger adults. These findings are supported by Vandenberghe-Descamps *et al*. (2016) who found that healthy older adults had 38.5% lower resting salivary flow when compared with younger adults and a 38% lower stimulated saliva; their results were independent of dental and medication status. However, our study did not find any difference in stimulated salivary flow rate between younger and older adults. It should, however, be noted that Vandenberghe-Descamps *et al*. (2016) measured unstimulated and stimulated saliva over a 10 and 5-min time period compared with our study which used a 5 and 2-min time period which could have caused a greater difference between age groups. Age-related changes to saliva flow are considered to relate to the submandibular and sublingual glands, which provide 70% of unstimulated saliva but less than 50% of stimulated saliva, providing a rationale for the greater reduction in unstimulated saliva compared with stimulated saliva in older adults (Affoo *et al*., 2015).

Almost all of our study volunteers lacked experience in saliva collection; accordingly, stress and behavioural factors could have contributed to poor adherence (Bhattarai *et al*., 2018), for example, embarrassment about spitting, particularly in an unfamiliar setting. Our volunteers reported collecting stimulated saliva easier than unstimulated saliva; therefore, stimulated saliva could be considered a more robust and representative measure compared with unstimulated saliva. As some volunteers struggled to produce unstimulated saliva, despite being considered healthy, future studies should consider

familiarisation sessions before collecting such samples. Poor oral clearance is associated with reduced saliva function (Turner & Ship, 2007) and therefore could potentially explain the cause of food debris in unstimulated saliva samples, which was more prevalent in older adults in our study. However, there are age-related changes associated with saliva (reduced volume and altered composition) resulting in saliva being potentially less watery and more concentrated (Nagler & Hershkovich, 2005). A key challenge is understanding the causes of high variability in saliva flow associated within and between groups; however, both lifestyle and the ageing process are thought to be potential causes of reduced saliva flow (Vandenberghe-Descamps *et al*., 2016).

3.6.3. Saliva samples post WPB consumption

There was an age-related increase in protein concentration in saliva samples post WPB consumption, which supported increased adhesion to the oral cavity from mucoadhesion (Hsein *et al*., 2015; Bull *et al*., 2020). A link between increasing protein concentration and reduced salivary flow rates has been previously suggested (Lee *et al*., 2002). This was demonstrated by our volunteers where a low saliva flow correlated with increased protein concentration. Therefore, suggesting increased mucoadhesion of the whey protein; however, this needs further investigation. There is evidence of increased salivary albumin concentrations associated with frail older adults (Meurman *et al*., 2002). Salivary albumin has a role within the oral cavity as a serum ultrafiltrate and can potentially leak into saliva secretions (Ladgotra *et al*., 2016) and is therefore a further possible contributor to increased protein concentration found within this study. Therefore, although from the pilot study we can conclusively report that the protein content in the oral cavity post WPB consumption is due to adhesion of the protein from the beverage. We cannot rule out the possibility that the difference between younger and older adults could result from differences in salivary proteins rather than differences in mucoadhesion of the whey

protein. The role of saliva composition and the changes to its physical properties were not measured in our study; however, our study did find reduced salivary flow rates with age. A reduced saliva flow can be associated with decreased lubrication, protection, oral clearance, mucosal surfaces hydration and coating abilities within the oral cavity (Lee *et al*., 2002; Nagler & Hershkovich, 2005; Turner & Ship, 2007; Chaudhury *et al*., 2015) and could lead to strengthened mucoadhesion by increased tissue exposure to whey proteins and therefore more adherence and interactions from proteins within the oral cavity (Bull *et al*., 2020). In addition, there is evidence of saliva protein concentration being influenced by stress, inflammation, infection, hormonal changes and circadian variation (Rudney, 1995). These factors could potentially explain the differences in protein concentration between visits; however, it should be noted the overall trend was not affected.

There were minimal differences in protein adhesion related to whey protein heat treatment (unheated and heated WPB) which was unexpected. Previously, greater adhesion had been found to correlate with heated WPB samples compared with unheated WPB in a study using a small sample size of younger adults (Bull *et al*., 2020). It is unlikely that the minimal differences found were due to cross-over effects and build-up of protein in the mouth, as the pilot study demonstrated substantial and significant differences between WPB and WPeB adhesion levels. This suggests that crossover effects between samples were minimal and indeed volunteers were provided with warm filtered water as a palate cleanser between samples, as well as having a 5-min rest between samples to minimise such effects. We therefore conclude that whey protein does adhere to the oral cavity and that any difference in adhesion due to heat treatment of the protein is minimal. Consideration is also required into how the different collection timepoints (15-s, 30-s, 60-s and 120-s) influence saliva samples as a result of oral processing. For example, decreased muscle strength and swallowing difficulties are associated with ageing

(Hickson, 2006; Fielding *et al*., 2011). In the context of this work this could have influenced how quickly an individual could swallow a 10 mL sample (which could be particularly relevant at the 15-s timepoint) and affect their ability to hold a sample in the mouth during the 10-s swill time and the gathering of saliva in preparation for spitting, especially relevant at 120-s.

3.6.4. WPB individual perception and liking

Mouthdrying was reported in this study following both unheated and heated WPB consumption. The heated WPB resulted in significantly increased perception of mouthdrying and thickness and significantly reduced sweetness, which led to a reduction in easiness to swallow. These differences were potentially caused by increased particle size on protein denaturation (Bull *et al*., 2017). There was no difference in liking nor preference between the unheated and heated samples, which is explained by the overall low liking scores and potentially lack of familiarity amongst the volunteers with the taste and flavours associated with protein beverages.

3.6.5. Saliva and WPB individual perception and liking

Individual saliva flow rates did influence perception and liking of products. It was expected that a reduced saliva flow would result in increased perception of mouthdrying; however, the trend proved inconsistent, as unexpectedly, individuals with medium or high salivary flow rated mouthdrying higher compared with those with low salivary flow. This does support previous research which indicate an increased particle size detection threshold with increased saliva production in semi solid foods (Santagiuliana *et al*., 2019). This may suggest a hydration mechanism associated with mucoadhesion, where the lubrication ability of saliva will strengthen adhesion properties with a resulting perception of mouthdrying (Cook *et al*., 2017). It would therefore be assumed that within a liquid model, such as WPB, a low salivary flow will have reduced lubrication properties and therefore

reduced adhesion properties, with lower resulting mouthdrying intensity. Although current results provide only a trend, it should be noted that mouthdrying in this study was measured at a single point in time. Therefore, future work should focus on investigating mouthdrying over time to gain a better understanding into the role of saliva flow on perception.

3.6.6. Age-related changes in WPB individual perception and liking

Age-related trends were found within the age groups; however, in most cases these trends lacked significance between the age groups, apart from the heated WPB, where older adults had significantly higher liking scores compared with younger adults. It could be therefore suggested that both age groups perceived the differences within a similar range. It was hypothesised that younger adults would be less sensitive to mouthdrying (Withers *et al*., 2013a); however, younger adults reported greater intensity of mouthdrying compared with older adults. It could be suggested that the cause of the minimal difference between age groups may relate to how our study measured perception rather than lack of differences between age groups. For example, Withers *et al*. (2013a) measured mouthdrying using a paired discrimination test and our study measured mouthdrying using a gLMS scale, therefore potentially the differences in the results between the two studies could relate to the sensitivity of the tests used. Older adults tend to be less sensitive to taste and tactile sensations (Smith *et al*., 2006; Methven *et al*., 2012) which supports the conclusion in our study where older adults reported ‘too little thickness’ more frequently than younger adults. Older adults reported a significant preference for the heated WPB which was the thicker beverage which contrasted with the younger adults. Perception of fluid viscosity has been found to decline with age (Smith *et al*., 2006) potentially explaining why our older adults preferred a thicker beverage and providing a design pointer for products for older adults.

3.7. Conclusion

Protein does adhere to the oral cavity to a greater extent post WPB consumption compared with WPeB consumption, indicating mucoadhesion of whey protein to be a true phenomenon. Saliva samples post WPB consumption, regardless of the extent of whey protein heat treatment, demonstrated increased adhesion to the oral cavity in older adults compared with younger adults. Such increased mucoadhesion with age may contribute to dislike of WPB, potentially due to a prolonged mouthdrying sensation, leading to poor consumption. Therefore, by understanding the potential mechanisms involved in whey protein derived mouthdrying, products could be reformulated to be more acceptable. It was expected that a reduced salivary flow would strengthen mucoadhesion; this trend was present but inconclusive, indicating the need for further research in this area. Heating of WPB resulted in significantly increased mouthdrying and thickness and significantly reduced sweetness, compared with the unheated WPB. It would be necessary to carry out further investigation to determine conclusively whether perception and acceptance are influenced by age and individual differences in saliva flow, as the lack of clear age-related trends could have related to the sensory analysis being carried out at a single time point and not over repeated consumption. Further research is needed to fully establish whether mouthdrying changes with repeated consumption, whether it results from increased mucoadhesion, and to confirm the influence of age and saliva flow on mouthdrying and mucoadhesion. The overall aim of this work is to increase acceptance of protein fortified beverages for older adults at risk of malnutrition and sarcopenia. Simple beverages, such as WPB, can alleviate this problem; however, they need to be acceptable and palatable.

S.3. Supplementary

S.3.1. Volunteer details

Table S.3.1. Summary of gender and medication of volunteers in both studies.

	Pilot Study (*n* = 22)				**Main Study** (*n* = 84)							
	Gender				Gender				Medication			
	Male		Female		Male		Female		Yes		No	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Total	5	23	17	77	31	37	53	63	19	23	65	77
Younger Adults	5	23	17	77	12	29	30	71	0	0	42	100
Older Adults	0	0	0	0	19	45	23	55	19	45	23	55

n and % reflect number and percentage in each contributing group. Pilot study: all younger adults without any medication and main study (younger adults: *n* = 42; older adults: *n* = 42).

S.3.2. Oral retention method development

The following matters were taken into consideration in order to ensure the robustness of the calculations used in the studies. The volume of the saliva samples was recorded at all time points. However, this measure appeared to lack consistency, as for example, with time, protein concentration in saliva samples was being artificially increased due to a greater amount of saliva being produced at the later time points (60-s and 120-s) compared with the earlier time points (15-s and 30-s). As expected, time had a significant effect ($p < 0.0001$) on saliva volume, with saliva volume in both beverages gradually increasing over time and peaking at 120-s (Figure S.3.1). Importantly, there was no significant overall differences ($p = 0.48$) in saliva volume between samples at all timepoints (Figure S.3.1). This supports saliva production being broadly similar, regardless of the beverage consumed, without impacting the protein content remaining in saliva samples. This was further demonstrated by a whey permeate beverage (WPeB) producing a significantly lower protein concentration ($p < 0.0001$) in saliva samples at all timepoints compared with a whey protein beverage (WPB) (Figure 3.5 in Section 3.5.1). In summary, this demonstrates there would be no benefit in taking account of the volume of saliva samples at each timepoint, as there is unlikely to be a dilution effect associated

with the volume of saliva produced, indicating protein concentration alone is a suitable and valid measure.

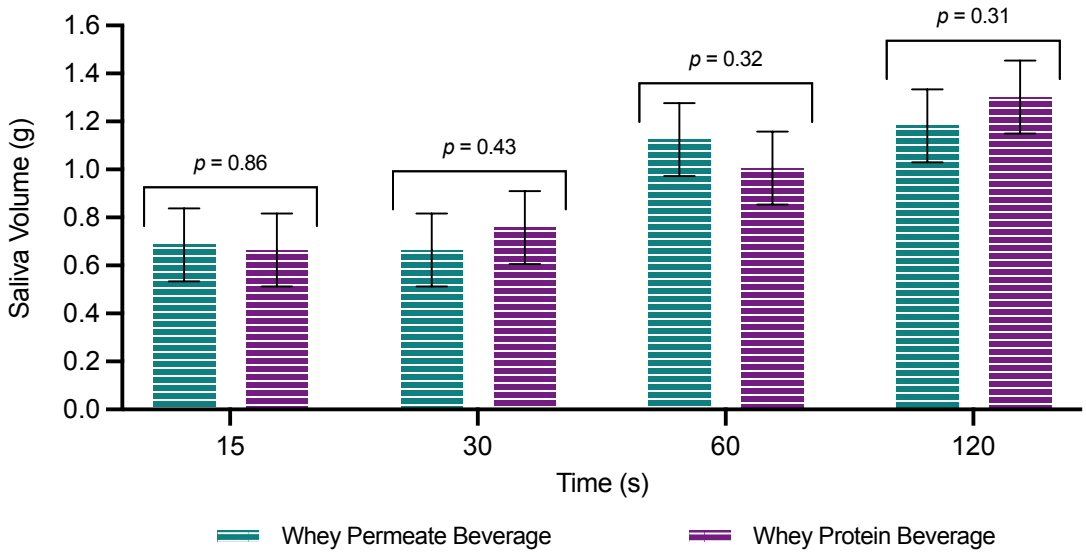

Figure S.3.1. Saliva volume collected post beverage consumption *(n* = 22*)* by timepoints. Values are expressed as LSM estimates ± standard error from SAS output. No significant differences ($p < 0.05$) were reported between samples at all timepoints with relevant *p* value above each timepoint.

Stimulated saliva was used as a baseline value as it is produced during food mastication and is considered to be less affected by age compared with unstimulated saliva (Affoo *et al*., 2015). Furthermore, our studies demonstrated better volunteer adherence to stimulated saliva collection methods compared with unstimulated saliva collection methods. Saliva flow variability has been previously noted within and between groups (Vandenberghe-Descamps *et al*., 2016). In order to address these concerns relating to saliva variability a coefficient of variance was calculated between individuals and the protein concentration in stimulated saliva appeared to be less variable compared with unstimulated saliva. By way of example, the coefficient of variance between individuals in the pilot study was 31.8% and 58.7% for stimulated saliva and unstimulated saliva respectively. It should be noted as per Figure S.3.2 that the same baseline stimulated saliva value was deducted at each timepoint; therefore, it is likely the potential effect of

mucoadhesion could be underestimated. For example, the protein concentration in saliva samples post WPeB was significantly lower ($p < 0.0001$) compared with the baseline stimulated saliva at all timepoints. However, to address this outcome confidence intervals have been calculated on both the final results and the different baseline values to assist in the understanding of the potential range of results (Tables S.3.2 and S.3.3). Future work should focus on developing a suitable baseline measure that takes into account changes over time (i.e. the influence at different timepoints) without adding more saliva samples. Accordingly, the calculation set out in Figure S.3.3 was used to establish each volunteer's protein concentration remaining in the saliva samples at each time point.

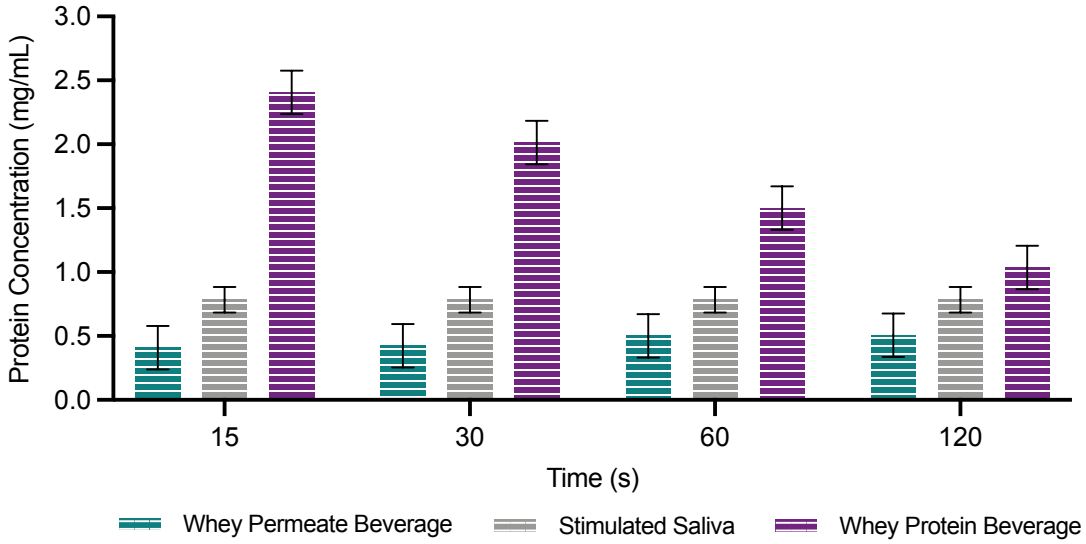

Figure S.3.2. Protein concentration in saliva samples *(n* = 22). Values are expressed as LSM estimates ± standard error from SAS output.

Table S.3.2. Volunteers' (*n* = 22) protein concentration (mg/mL) in saliva samples post whey protein beverage (WPB) consumption with corresponding 95% confidence intervals.

Timepoint	Protein Concentration	Confidence Interval	
		Lower	Upper
15-s	2.40 ± 0.18	1.86	2.93
30-s	2.01 ± 0.18	1.46	2.56
60-s	1.50 ± 0.18	1.06	1.94
120-s	1.03 ± 0.18	0.50	1.56

Values are expressed as LMS estimates ± standard error from SAS output.

Table S.3.3. Examples of protein concentration (mg/mL) of whey permeate beverage (WPeB) and baseline stimulated saliva values with corresponding 95% confidence intervals.

Timepoint	Whey Permeate Beverage			Stimulated Saliva			Significance of sample (*p* value)
	Protein Concentration	Confidence Interval		Protein Concentration	Confidence Interval		
		Lower	Upper		Lower	Upper	
15-s	0.40 ± 0.18	0.33	0.46	0.78 ± 0.20	0.67	0.89	**< 0.0001**
30-s	0.42 ± 0.18	0.35	0.49	0.78 ± 0.20	0.67	0.89	**< 0.0001**
60-s	0.50 ± 0.18	0.42	0.57	0.78 ± 0.20	0.67	0.89	**< 0.0001**
120-s	0.50 ± 0.18	0.39	0.60	0.78 ± 0.20	0.67	0.89	**< 0.0001**

Values are expressed as LMS estimates ± standard error from SAS output. *Data obtained from 22 volunteers.*

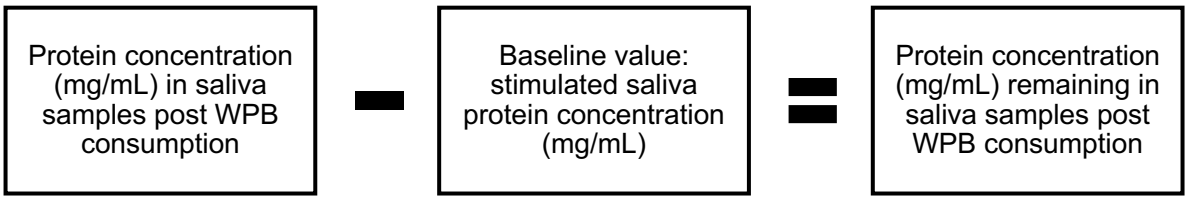

Figure S.3.3. Summary of oral retention method calculation (WPB: whey protein beverage).

S.3.3. Additional study data

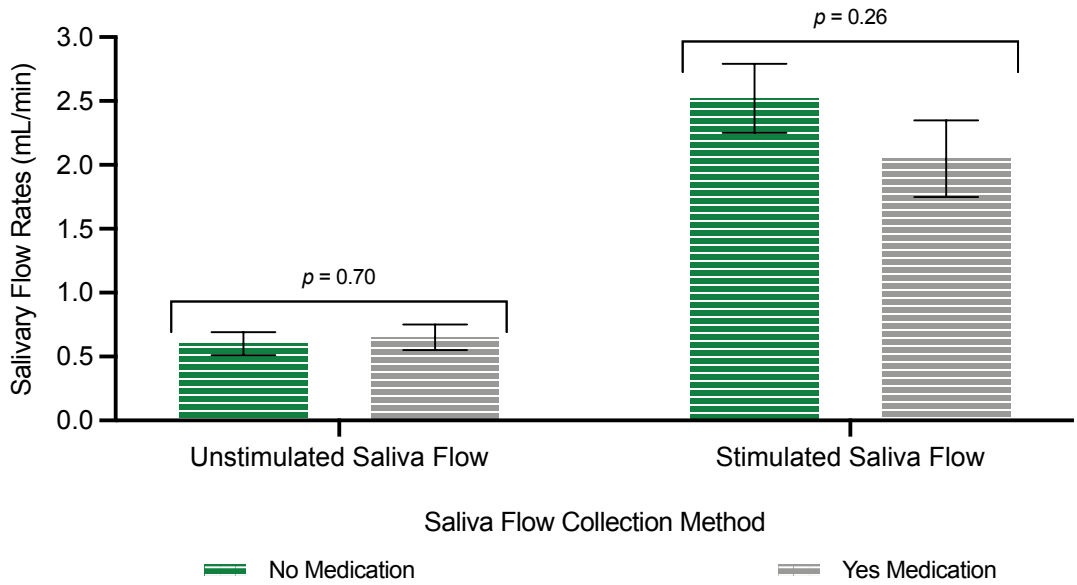

Figure S.3.4. Additional factors influencing older adults (*n* = 42) salivary flow rates (mL/min). Values are expressed as LSM estimates ± standard error from SAS output and no significant differences ($p < 0.05$) were reported between groups with relevant *p* value above each group. *(Note: this figure was 'data not shown' in published paper).*

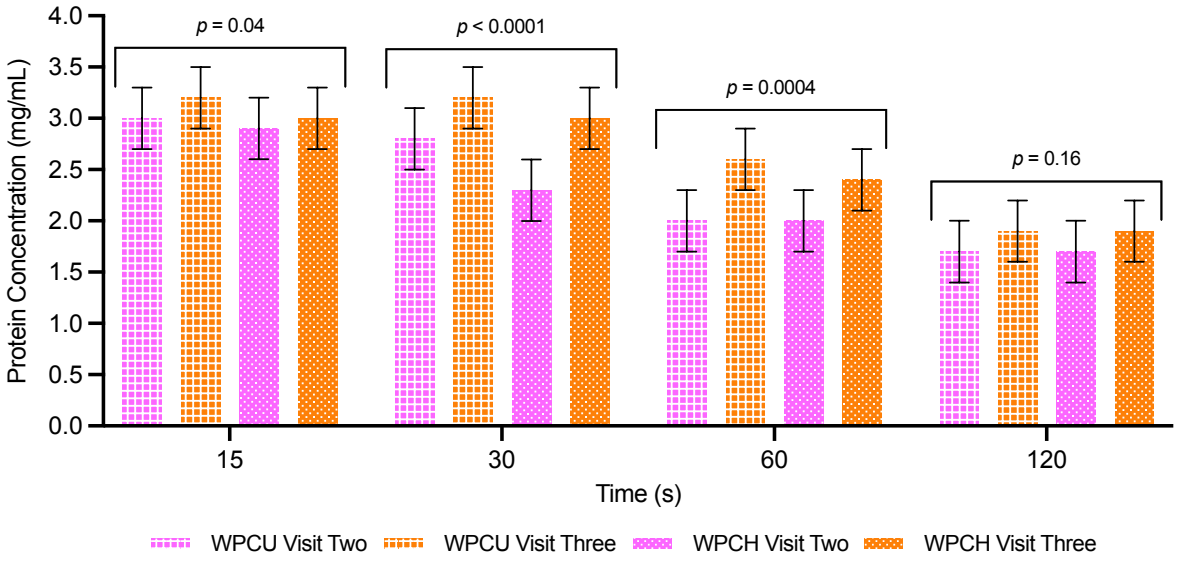

Figure S.3.5. Influence of visit on protein concentration in saliva samples post whey protein beverage (WPB) consumption by sample and timepoints (WPCU: unheated WPB; WPCH: heated WPB). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) were reported between sample*visit*timepoints with relevant p value above each timepoint (visit two: $n = 84$; *YA $n = 42$ and OA $n = 42$* and visit three: $n = 82$; *YA $n = 40$ (two YA dropped out after visit two) and OA $n = 42$*).

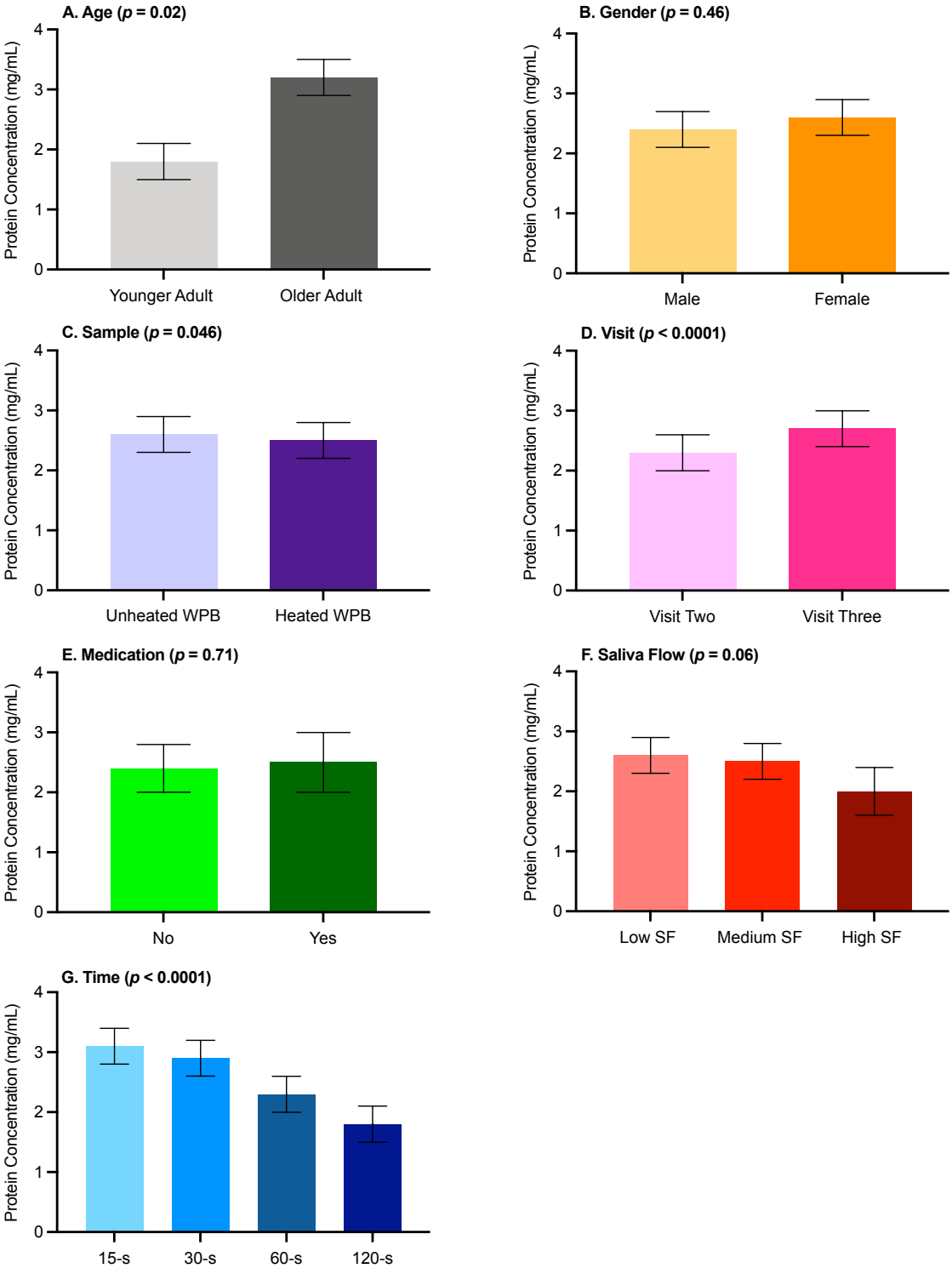

Figure S.3.6. Additional factors influencing protein concentration in saliva samples post whey protein beverage consumption *(n = 84; measured in duplicate at visit two and visit three) (WPB: whey protein beverage; SF: saliva flow)*. Values are expressed as LSM estimates ± standard error from SAS output and with relevant *p* value above each category and a higher value would suggest greater adhesion.

Table S.3.4. Summary of baseline protein concentration (mg/mL) in saliva samples in both studies.

	Pilot Study (*n* = 22)			**Main Study** (*n* = 84)						
	Overall	Gender		Age		Gender		Visit or Replicate		
		Male (*n* = 5)	Female (*n* = 17)	YA (*n* = 42)	OA (*n* = 42)	Male (*n* = 31)	Female (*n* = 53)	One (*n* = 84)	Two (*n* = 84)	Three (*n* = 82)
USF	0.78 ± 0.1	0.79 ± 0.03	0.78 ± 0.03	1.0 ± 0.1	1.6 ± 0.1*	1.3 ± 0.1	1.2 ± 0.09	1.2 ± 0.09	1.2 ± 0.09	1.3 ± 0.09
SSF	0.78 ± 0.1	0.80 ± 0.03	0.78 ± 0.03	1.0 ± 0.07	1.0 ± 0.07	1.0 ± 0.08	1.1 ± 0.06	1.0 ± 0.06	1.0 ± 0.06	-

Values are expressed as LSM estimates ± standard error from SAS output and significant differences ($p < 0.05$) between groups are denoted by *. USF: unstimulated saliva flow; SSF: stimulated saliva flow; YA: younger adult; OA: older adult. Visit only applied to unstimulated saliva flow and replicate only applied to stimulated saliva flow.

Table S.3.5. Additional factors influencing volunteers (*n* = 84) liking, effort to consume, attribute perception and appropriateness of attribute level (Just-About-Right, JAR) mean ratings of whey protein beverages.

	Medication		**Gender**	
	No (*n* = 65)	Yes (*n* = 19)	Male (*n* = 31)	Female (*n* = 53)
Overall Liking	4.0 ± 0.2	2.9 ± 0.4*	3.4 ± 0.3	3.5 ± 0.3
Easiness to Drink	4.0 ± 0.1	3.6 ± 0.2	3.9 ± 0.2	3.7 ± 0.1
Easiness to Swallow	4.1 ± 0.1	3.9 ± 0.2	4.2 ± 0.1	3.8 ± 0.1*
Mouthdrying	21.1 ± 3.0	23.8 ± 5.3	21.0 ± 4.2	23.0 ± 3.8
Sweetness	6.2 ± 0.9	7.2 ± 1.7	7.4 ± 1.3	6.1 ± 1.2
Thickness	10.8 ± 1.7	16.3 ± 2.9	15.3 ± 2.3	11.8 ± 2.0
JAR Flavour	2.3 ± 0.1	2.5 ± 0.2	2.5 ± 0.2	2.3 ± 0.1
JAR Thickness	2.4 ± 0.1	2.6 ± 0.2	2.7 ± 0.1	2.3 ± 0.1

Values are expressed as LSM estimates ± standard error from SAS output and significant differences ($p < 0.05$) between groups are denoted by *. *Liking and effort to consume were measured on a 9- and 5-point scale respectively, attribute perception was measured on a gLMS logarithmic scale (anti-logged values 0-100 scale presented) and JAR via 5-point JAR scale.*

Table S.3.6. Volunteers' counts of whey protein beverage (WPB) preference and consumption habits.

	Preference			**Consumption Habits**	
	WPCU	WPCH	Significance of sample (*p* value)	Yes	No
Total (*n* = 84)	41	43	0.46	10	74
Younger Adults (*n* = 42)	27	15	**0.03**	8	39
Older Adults (*n* = 42)	14	28	**0.03**	2	35

WPCU (unheated WPB) and WPCH (heated WPB). WPBs were most frequently consumed at breakfast (*n* = 9) and nutritional drinks consumption was also recorded (*n* = 10/84 YA: *n* = 8/42 and OA: *n* = 2/42). *Data was obtained from a 2-AFC test to assess most preferred and consumption habits: yes denotes 'I consume protein beverages' and no refers to 'I do not drink protein beverages'.*

Table S.3.7. Summary of volunteers' (*n* = 84) whey protein beverage (WPB) comments.

	Flavour Related Comments									**Texture Related Comments**								
	Positive			Negative			No Comments Provided			Positive			Negative			No Comments Provided		
	Total	YA	OA	Total	YA	OA	Total	YA	OA	Total	YA	OA	Total	YA	OA	Total	YA	OA
WPCU	2	0	2	53	24	29	29	18	11	15	9	6	33	13	20	36	20	16
WPCH	1	0	1	57	23	34	26	19	7	12	7	5	39	17	22	33	18	15

WPCU (unheated WPB) and WPCH (heated WPB). Main study (YA, younger adults: *n* = 42; OA, older adults: *n* = 42). Positive refers to refreshing, OK, Just-About-Right (JAR), smooth and negative refers to aftertaste, metallic, soapy, mouthdrying, bland, neutral, no flavour, horrible, watery and thickness.

Chapter 4

Consistent effects of whey protein fortification on consumer perception and liking of solid food matrices (cakes and biscuits) regardless of age and saliva flow

4.1. Context to chapter

Predominately research has focused on mouthdrying sensations from whey protein liquid model (such as whey protein beverages (WPB)) consequently less is known about perceived mouthdrying within solid food matrices. However, familiar snacks (such as cakes and biscuits) could be considered a viable route to provide tasty high energy and protein snacks for older adults at risk of malnutrition. Having tested WPBs in **Chapter 3**, the focus now switched to solid models (cakes, biscuits and cupcakes) correspondingly this chapter aims to investigate two overall thesis hypotheses: (a) whey protein fortified snacks will cause mouthdrying; and (e) individual differences (such as age, saliva flow, dental status, mouth behaviour and appetite) will influence perceived whey protein derived mouthdrying. Hence, these hypotheses were tested via the following objectives:

- Do consumers perceive whey protein fortified solid models as mouthdrying compared with non-protein whey control?
- Does sensitivity to whey protein derived mouthdrying increase with age in whey protein solid models?
- Do salivary flow rates relate to perceived mouthdrying intensity in whey protein solid models?
- Do individual differences in dental status, mouth behaviour and appetite influence perceived whey protein derived mouthdrying in whey protein solid models?

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4.2. Abstract

Although there are numerous high protein products on the market, they are typically not designed with, or for, older consumers. This is surprising considering that dietary guidelines recognise the need for higher protein intake in later life. Protein fortified products are, however, associated with negative sensory attributes and poor consumer acceptance. This paper investigates the extent of mouthdrying sensations within a high protein solid food matrix, along with the effect of age and saliva flow. Solid models using cakes and biscuits, with or without protein fortification, were investigated. The sensory profile and physical properties were analysed and two volunteer studies (n = 84; n = 70) were carried out using two age groups (18-30; 65+). Volunteers rated individual perception and liking of products, and salivary flow rates (mL/min) were measured. Unstimulated salivary flow rates were significantly lower ($p < 0.05$) in older adults, although this was not found to influence product perception. Protein fortification of cakes and biscuits significantly increased ($p < 0.05$) perceived mouthdrying, hardness and off-flavours, and significantly reduced ($p < 0.05$) melting rate, moistness and liking compared with the control versions. There is a clear need to address negative sensory attributes associated with protein fortification of cakes and biscuits to ensure product suitability for older adults.

Keywords: protein fortified foods; mouthdrying; older adults; whey protein; saliva flow

4.3. Introduction

Older adults are at risk of poor nutritional status due to age-related changes, such as anorexia of ageing (a physiological decline in food intake with age), changes in sensory sensitivity, and oral impairments (dysphagia, tooth loss and decreased salivary flow), all of which can influence an individual's food intake and increase the risk of malnutrition

(Morley, 2001; Vandenberghe-Descamps *et al*., 2017). Immune function is also considered to decline with age and is associated with an increased risk of infection (Wilson *et al*., 2017). Malnutrition covers both over- and under-nutrition; however, in older adults, undernutrition predominates, being a deficiency of both macronutrients and micronutrients (Maleta, 2006; BAPEN, 2018). Food intake can also be reduced in older adults due to chemosensory impairments (such as loss of taste and smell) which influence food choices and consumption (Schiffman & Graham, 2000).

Protein requirements are considered to increase with age and are associated with many positive functional outcomes. Despite this, and the many high protein products available on the market, these are typically not designed with, or for, older adults. The current UK reference nutrient intake (RNI) is 0.75 g/kg/d (Department of Health, 1991). However, dietary guidelines recognise the need for higher protein-intake in later life (1.0-1.2 g/kg/d) with the aim of maintaining and regaining lean body mass and function, as well as aiding in recovery from illness and helping to overcome age-related changes in protein metabolism (Bauer *et al*., 2013). Accordingly, protein fortified meals and snacks (often fortified with whey protein) can provide familiar foods to older adults in order to encourage consumption and increase energy and protein intake (Morilla-Herrera *et al*., 2016; Mills *et al*., 2018). Older adults consuming familiar protein enriched products were demonstrated to have increased protein intake (1.5 ± 0.6 g/kg/d) compared with a control group (1.0 ± 0.4 g/kg/d) over a 12-week period (Beelen *et al*., 2017b). In addition, previous research has suggested that older adults have a higher liking for cakes compared with younger adults (Michon *et al*., 2010). Therefore, supporting the use of popular products (such as cakes) to potentially increase food intake within older adults.

Protein fortification can result in off-flavours. Whey proteins are rich in sulfur amino acids and when heated they can release sulfurous and eggy aromas which influence the

subsequent flavour (Higgs & Boland, 2009; Drake *et al*., 2009). When this occurs, it results in products tasting stale or 'cabbage-like' and will reduce acceptability of products (Morr & Ha, 1991; Wright *et al*., 2006). Mouthdrying sensations can also result from protein fortification, as previously demonstrated in high protein liquid model systems, a sensation shown to increase with consumers' age and with repeated consumption of dairy beverages and oral nutritional supplements (ONS) (Methven *et al*., 2010; Withers *et al*., 2013a). Although terms such as astringency, drying and mouthdrying are commonly used interchangeably within the literature, within this paper mouthdrying specifically refers to a drying sensation in the mouthdrying or after consumption of a product. Currently the exact causes of whey protein derived mouthdrying are not fully understood and are part of our ongoing investigation (Norton *et al*., 2020a, **Chapter 3**).

Individual differences in oral physiology can potentially influence food oral processing and sensory perception (Chen, 2009). Accordingly, the way an individual manipulates food in their mouth, usually described as mouth behaviour, is considered to influence food choice, texture preference and satisfaction (Jeltema *et al*., 2015; 2016). Food oral processing and saliva perform key roles in the breakdown of food and sensory perception (Munoz-Gonzalez *et al*., 2018a). Older adults are considered to consume foods more slowly and have reduced salivary flow rates compared with younger adults (Vandenberghe-Descamps *et al*., 2016; Ketel *et al*., 2019). Appetite is also considered to decline with age; therefore, understanding its role in sensory perception is key. For example, ONS can consumption increase thirst from drying sensations (Thomas *et al*., 2018). All these issues highlight the need to understand how differences in individual perception can impact sensory perception and consumption of foods in older adults.

Currently, the influence of mouthdrying on the overall perception and liking of protein fortified solid foods and the effect of saliva flow and age on this phenomenon are unclear.

It is recognised that protein fortified foods can help to alleviate malnutrition; however, they need to be acceptable and palatable. Fundamental investigation of the perception of protein in foods and of individual factors influencing this perception may result in knowledge that assists in product optimisation as well as subsequent health benefits from increased consumption of protein fortified foods by older adults. This paper hypothesises that (a) protein fortification of cakes and biscuits will cause mouthdrying and reduce acceptance of products; and (b) individual differences (such as age, mouth behaviour, dental status, saliva flow and appetite) will influence perception and liking of products. Accordingly, hypotheses were tested as follows:

(1) A pilot study was carried out to establish whether protein fortification of cakes and biscuits causes mouthdrying, thereby reducing acceptance, and whether individual differences influence perception and liking of products. The specific objectives were to: (a) analyse the sensory profile and physical properties of cakes and biscuits; (b) evaluate perception and acceptance of cakes and biscuits, with and without protein fortification and relate these to age, dental status, mouth behaviour and salivary flow rates; and (c) use the results to optimise products for the main study.

(2) The main study aimed to further investigate protein derived mouthdrying and its relationship with product acceptance. The specific objectives were to: (a) analyse the sensory profile and physical properties of the optimised cakes; (b) evaluate the perception and acceptance of cakes with and without protein fortification after consumption of a full portion, and the influence of this on rated appetite; and (c) relate these measures to individual differences including age, dental status, mouth behaviour and salivary flow rates.

4.4. Materials and methods

4.4.1. Overview of pilot and main study

The pilot study consisted of 84 healthy male and female volunteers from two age groups (42 younger adults; 18-30 years, 24.3 ± 3.6 years and 42 older adults; over 65 years, 73.6 ± 6.2 years) who completed a single blinded randomised crossover trial involving two study visits. The main study was a single blinded randomised crossover trial, with two study visits and two tasting sessions at home, involving 70 healthy male and female volunteers from two age groups (38 younger adults; 18-30 years, 25.8 ± 3.2 and 32 older adults; over 65 years, 74.6 ± 5.7 years) (volunteer overview[26]). In both studies the subject size was determined by power calculations (alpha risk = 0.05 and 80% power) based on the primary outcome measures (liking and mouthdrying). In the pilot study we estimated a difference, on a 9-point hedonic scale, of 0.8, assuming a standard deviation of 1.2. In the main study we based the calculations on the pilot study intensity ratings (0-100) and we predicted a larger difference of 12, assuming a standard deviation of 16.5. This concluded, a minimum sample size of 35 and 31, respectively were required to demonstrate a significant difference between samples. However, we doubled the sample size in order to compare both between and within age groups, as there was no additional data on which to base the power calculations. Volunteers were recruited from the local Reading area (UK). The studies were conducted in accordance with the Declaration of Helsinki. All volunteers had the study fully explained, provided informed written consent, and were informed that all data would be anonymous, fully confidential, and of their right to withdraw. The studies received a favourable opinion for conduct from the University of Reading Research Ethics Committee and were registered on the clinical trials database

[26] Table 4.5 in the results section

(www.clinicaltrials.gov) (pilot study: UREC 18/46 and NCT03798730 and main study: UREC 19/67 and NCT04302779).

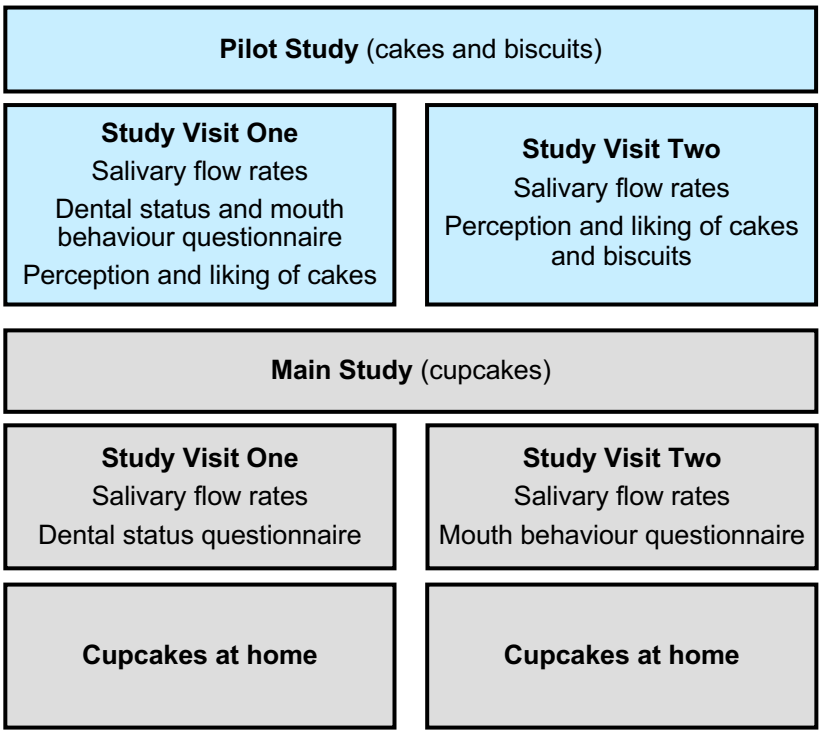

Figure 4.1. Overview of pilot and main studies.

All volunteers were screened for suitability (minimal medication, non-smoker, no food allergies or intolerances, non-diabetic and not having had either cancer, oral surgery or a stroke). Volunteers who met the inclusion criteria and were willing to take part were invited to attend study visits held at the Sensory Science Centre, University of Reading; the study overview is summarised in Figure 4.1. In order to control extraneous variables, volunteers refrained on the day of each study visit from tea and coffee and drank a glass of water one hour before the visit. Each individual volunteer completed all their study visits at the same time of day in a temperature-controlled room (22 °C) under artificial daylight.

4.4.2. Materials

Baking ingredients were obtained from Sainsbury's (Reading, UK). Sil Cream 64, a 100% vegetable oil, was supplied by Silbury (Silbury, Banbury, UK) and parafilm® was supplied

by Sigma-Aldrich (Dorset, UK). Whey powders were provided by Volac (Volac International Ltd., Royston, UK). These consisted of whey protein concentrate (WPC, Volactive Ultra-Whey 80 Instant, providing a minimum protein content of 80%, the remaining 20% being lactose, fat, moisture and ash), whey protein isolate (WPI, Volactive Ultra-Whey 90 Instant, providing a minimum protein content of 90%, the remaining 10% being lactose, fat, moisture and ash) and whey permeate (WPe, Volactose Taw Whey Permeate, providing a minimum lactose content of 89% the remaining 11% being ash, moisture, protein and fat).

4.4.3. Solid model preparation

The rationale for the solid model was that high energy and protein snacks can help to alleviate malnutrition, and cakes and biscuits are suitable familiar and tasty products for this purpose. The pilot study tested cake and biscuit products, each with a control, and a protein fortified version using WPI. In the main study, the control and protein cakes were optimised in a number of ways: (a) to provide a better match of ingredients between the two versions, the control incorporated whey permeate as a minimal protein alternative in place of the whey protein powder; (b) to match the final moisture content of the cakes within 1.0%; (c) to replace WPI with WPC because, although WPC is lower in protein, its minor constituents (e.g. lactose and fat) may contribute positively to sensory attributes (Whetstine *et al*., 2005); (d) to add lemon zest to potentially improve both flavour and acceptability; and (e) the cakes were formed as individual cupcakes to ensure uniformity and suitability for consumption at home. All product formulations are outlined in Table S.4.1.

The recipes had been developed previously by Tsikritzi *et al*. (2014) and the University of Reading Food Research Group and were adapted for this study. In summary, for the

cake dough (pilot study), the butter and sugar were creamed until smooth[27], the remaining ingredients were added and mixed[28] and batter (450g) was weighed out into 600 mL loaf tins and baked until golden brown (30-min at 170 °C[29]). For the biscuit dough (pilot study), the fat and sugar were creamed until smooth[30], the remaining ingredients were added and mixed[31], dough was rolled out and sheeted (thickness: 1.0 cm; diameter cutter: 4.5 cm) and subsequently baked (9-min at 190 °C). During the main study, cupcakes were mixed using an all-in-one method[32] and the batter (38.2 g) was weighed out into individual paper cases (80.0 mm × 62.5 mm) and baked until golden brown (20-min at 170 °C, final weight 35.0 g). Nutritional composition was analysed (Nutritics v5.096, Dublin, Ireland) taking account of heat loss (Table 4.1). All samples were packaged in heat-sealed pouches (polypropylene for cakes, aluminium for biscuits), frozen at -18 °C and defrosted at room temperature before consumption. A sample (150 g) from each batch was sent for microbiological testing at an accredited laboratory (SYNLAB, Northumberland, UK)[33].

[27] medium speed (1 to 2-min, Kenwood Titanium Major KMM020, Hampshire, UK)
[28] low speed (2-min)
[29] pre-heated Atlas Salva Oven, London, UK
[30] medium speed (3 to 5-min)
[31] low speed (2 to 4-min)
[32] all ingredients were creamed until smooth (low speed, 5 to 8-min)
[33] additional baking related information are outlined in **Appendix B**

Table 4.1. Nutritional composition per portion size[1] and 100 g[2] with % of reference intake for cakes, biscuits and cupcakes.

	Cakes						**Biscuits**						**Cupcakes**					
	Control			Protein			Control			Protein			Control			Protein		
	45g[1]	100g[2]	%	45g[1]	100g[2]	%	20g[1]	100g[2]	%	20g[1]	100g[2]	%	35g[1]	100g[2]	%	35g[1]	100g[2]	%
Energy (kcal)	184	410	21	185	411	21	119	597	30	118	588	29	155	442	22	156	445	22
Fat (g)	10	23	33	9.8	22	31	7.2	36	51	6.7	34	49	8.2	24	34	8.4	24	34
of which saturates (g)	6.2	14	70	5.8	13	65	0.5	2.7	14	0.5	2.5	13	4.9	14	70	5	14	70
Carbohydrate (g)	20	44	17	19	41	16	12	61	23	11	56	22	18	51	20	16	45	17
of which sugars (g)	12	26	29	11	24	27	5.2	26	29	4.8	24	27	9.2	26	29	9.3	26	29
Fibre (g)	0.6	1.4	6	0.6	1.3	5	0.7	3.4	14	0.6	3.1	12	0.5	1.1	4	0.5	1.1	4
Protein (g)	2.7	6	12	5.3	12	24	1.1	5.6	11	2.7	14	28	2.1	6	12	4.1	12	24
Salt (g)	0.3	0.7	12	0.3	0.7	12	0.06	0.3	5	0.07	0.4	6	0.1	0.3	5	0.1	0.3	5

4.4.4. Sensory profile and physical properties of cakes and biscuits

Sensory profiling was carried out using descriptive sensory profiling (DSP) (a modified quantitative descriptive analysis (QDA™) (Stone *et al*., 1974; Stone & Sidel, 2004)) to determine the sensory differences between the control and protein products. A screened and trained sensory panel (*n* = 12; 11 female and 1 male) was used, each member with a minimum of one years' experience and with expertise in profiling techniques, having received at least four hours specific training on profiling protein and control products. All sensory evaluation was carried out in a temperature-controlled room (22 °C), in isolated booths, and under artificial daylight. Warm filtered water (~ 40 °C) was used as a palate cleanser between samples; this is considered more effective than cold water at removing fatty dairy residues from the mouth (Withers *et al*., 2013a). The trained panel were provided with the same products as the study volunteers (45.0 g cake slice, 20.0 g biscuit and 35.0 g cupcake; preparation as Section 4.4.3). The trained panel (cakes *n* = 12; biscuits *n* = 9; cupcakes *n* = 11) developed a consensus vocabulary identifying between 33 to 36 attributes per product across the different modalities (appearance, aroma, flavour, mouthfeel and aftertaste following a 1-min delay) as outlined in Tables 4.2 and 4.3 in the results section. All panellists scored in duplicate, for each sample, in separate sessions. Samples, coded with three-digit random numbers, were provided in a monadic sequential balanced order, with sample sets randomly allocated to panellists. Visual analogue scales (VAS) (0-100) with suitable anchors were used (Compusense Cloud Software, Guelph, ON, Canada).

The moisture content of cakes, biscuits and cupcake were measured using a moisture analyser (%) (Sartorius MA150, Goettingen, Germany) and water activity (a_w) (Hydrolab C1, West Sussex, UK) was also measured. Colour measurements, *L** (dark-light); *a** (red-green); *b** (yellow-blue), were taken from the crumb and crust of the cakes and cupcakes

and from the top and bottom surface of the biscuits by colorimeter (Chroma Meter CR-400, Osaka, Japan). The hue angle (arctan (*b**/*a**)) (McLellan *et al*., 1995) and total colour difference (dE) from the control sample were also calculated (Bodart *et al*., 2007):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (4.1)$$

Texture profile analysis (TPA) was carried out using a texture analyser (XTPlus, Stable Micro System (SMS), Godalming, UK) equipped with a load cell of 5.0 kg. For the cake crumb an adapted double compression test based on previous work (Rodriguez-Garcia *et al*., 2014) was carried out with a cylindrical probe (SMS rig code P/75) using a test speed of 5.0 mms/s with 5-s delay between compression tests (compression was to 25% of original height), on a 15.0 mm deep slice. Parameters[34] recorded were hardness, chewiness, springiness and cohesiveness, along with adhesiveness, resilience and gumminess for cupcakes. Biscuit analysis was carried out using a three-point bend test (SMS rig code HDP/3 PB) on the texture analyser with hardness and fracturability as parameters, at a test speed of 3.0 mm/s (Oksuz & Karakas, 2016). The height of the cakes and cupcakes and the thickness and diameter of the biscuits was measured by digital calipers[35]. All analysis was performed in triplicate on different days on all study batches consumed by the volunteers.

4.4.5. Dental status and mouth behaviour questionnaire[36]

Volunteers completed a dental status questionnaire adapted from the World Health Organisation's (WHO) Oral Health Questionnaire which focused on key areas including

[34] hardness: maximum force (first compression); cohesiveness: relative resistance (i.e. ability to withstand second deformation compared with first deformation) (area two divided by area one); springiness: product's spring back ability post first compression (distance two divided by distance one); gumminess: relates to semi-solid products (hardness × cohesiveness); chewiness: relates to solid products (hardness × cohesiveness × springiness) (similar trend to hardness); adhesiveness: negative work between compression cycles; resilience: product's 'fights to regain its original height' (area four divided by area three); fracturability: force at first peak (Szczesniak, 2002; Texture Technologies, 2019).

[35] Whitworth Tool Inc., Kentucky, USA

[36] **Chapter 4** denture use and questionnaire examples are outlined in **Appendix C**

number of teeth, dentures, functional unit counting, qualitative questions, and dental cleaning methods and frequency (WHO, 2013); pictures were added to improve clarity. Volunteers also completed a validated online test using the JBMB Typing Tool to categorise individual mouth behaviour preferences; this tool grouped volunteers into four types: chewers, crunchers, smooshers and suckers (Jeltema *et al*., 2015; 2016). In both studies, all volunteers independently completed both questionnaires during their study visits.

4.4.6. Salivary flow rates

During the pilot study unstimulated saliva was collected at the beginning of each visit and two replicates of stimulated saliva were collected during visit one (10 to 15-min break between collections). During the main study, saliva collection (unstimulated and stimulated) was carried out at the beginning of each study visit (10 to 15-min break between collections). Saliva collection methods were adapted from previous work (Vandenberghe-Descamps *et al*., 2016; Pushpass *et al*., 2019a). In brief, during unstimulated saliva collection, volunteers collected saliva in their mouths and spat out saliva every time they felt the urge to swallow during a 5-min time period. Stimulated saliva was collected by volunteers spitting out saliva every time they felt the urge to swallow during a 2-min time period while chewing on parafilm® (5.0 × 5.0 cm). Saliva was collected in wide lid collection tubes (60 mL). Saliva weights were monitored by weighing collection tubes before and after collection. Salivary flow rates were calculated as mL/min, using the assumption that 1.0 g of saliva equates to 1.0 mL. All saliva samples were stored on ice pending analysis.

4.4.7. Individual perception, appetite and liking ratings of products

During the pilot study, volunteers rated liking, easiness to eat and to swallow, attribute perception and appropriateness of attribute level (Just-About-Right, JAR) of cakes and

biscuits individually on an iPad[37], either in isolated booths (younger adults) or at a table (older adults), using Compusense Cloud Software. Samples, coded with three-digit random numbers, were provided in a monadic sequential balanced order, with sample sets randomly allocated to volunteers. Volunteers received a 45.0 g cake slice and a 20.0 g biscuit; these being considered appropriate portion sizes for older adults and volunteers evaluated the same cakes at both visits to check reliability. All volunteers were trained by a short video in how to use the generalised Labelled Magnitude Scale (gLMS), a scale ranging from no sensation (0) to strongest imaginable sensation of any kind (100) (Bartoshuk *et al*., 2004). Volunteers had an enforced rest period of 45-s between samples and consumed warm filtered water (rationale as outlined in Section 4.4.4) before completing the same series of questions on the second sample.

During the main study, appetite ratings (hunger, thirst, desire to eat, fullness, satiety and prospective food consumption) were recorded on 0 to 100 mm visual analogue scales (VAS), with appropriate anchors (FIint *et al*., 2000; Blundell *et al*., 2010) before and after consumption of each cupcake. Volunteers additionally rated liking, easiness to eat and swallow and attribute perception for each 35.0 g cupcake individually at home. To avoid using multiple scale types at home, VAS were again used. All scoring at home was done using paper booklets, and samples were coded with three-digit random numbers and provided monadically on two separate occasions in a sequential balanced order, randomly allocated to volunteers. All volunteers first received training on how to use the VAS via non-food related questions. An overview of individual perception and liking measures taken is outlined in Figure 4.2.

[37] Apple, London, UK

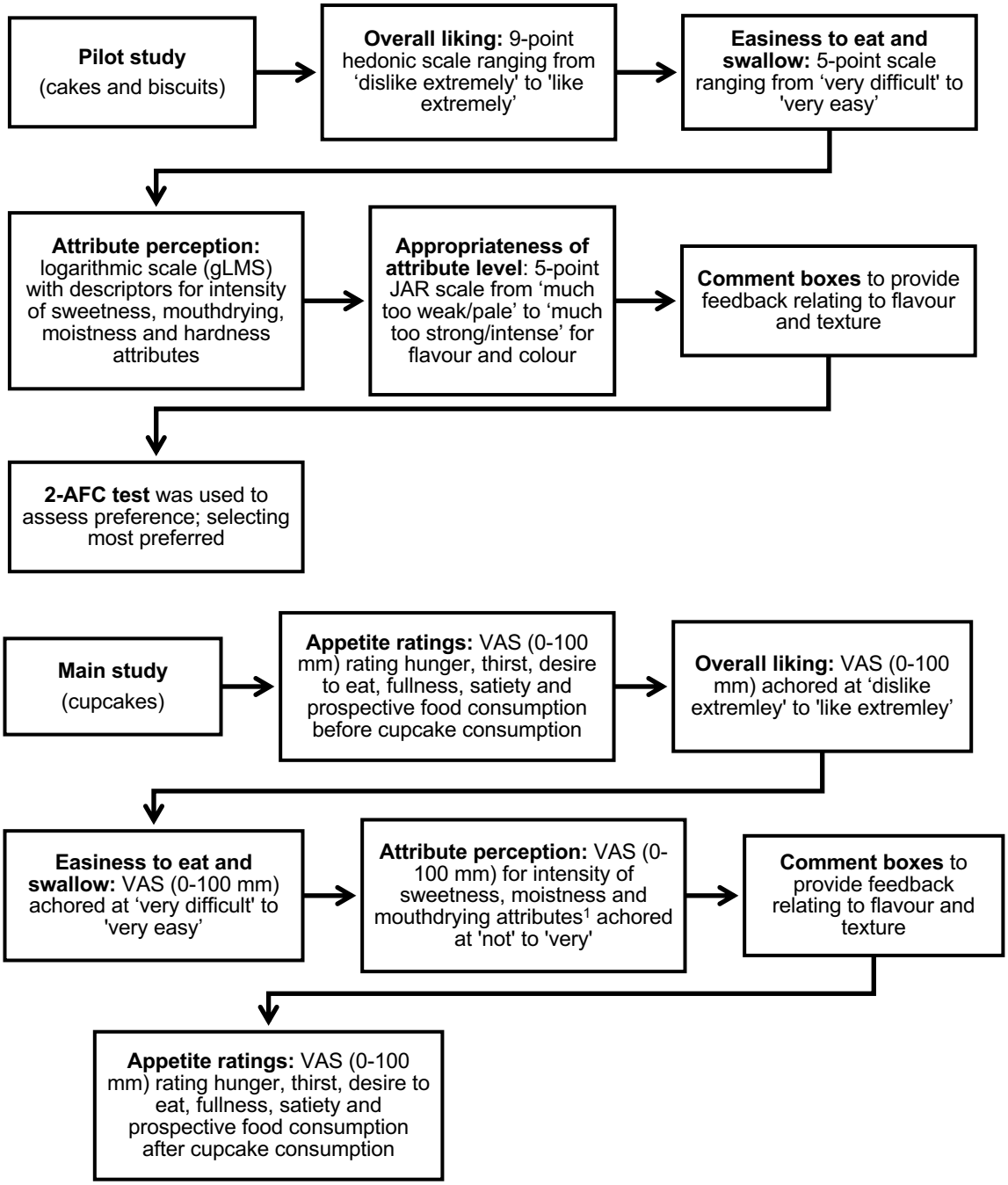

Figure 4.2. Overview of individual product perception[38] and liking measures taken during both studies (gLMS: generalised Labelled Magnitude Scale; JAR: Just-About-Right; 2-AFC: two-alternative forced choice; VAS: visual analogue scale).

[38] sweetness: refers to the sweet taste of the sample stimulated by sucrose; moistness; refers to degree of slightly damp sponge; hardness: refers to hardness on first bite of the biscuit; mouthdrying: refers to the drying sensation in the mouth during or after consumption of a sample.

4.4.8. Statistical analysis

4.4.8.1. Sensory profile and physical properties of cakes and biscuits

DSP data and trained panel performance[39] was analysed using analysis of variance (ANOVA; considered the most appropriate analysis for this type of data (Lawless & Heymann, 2010; Hasted, 2018)) in SenPAQ (version 5.01, Qi Statistics, Berkshire, UK), where the main effects (sample and assessor) were tested against the sample by assessor interaction, with sample as fixed effect and assessor as random effect.

Physical properties data were analysed in XLSTAT (version 2019.2.2, Addinsoft, Boston, MA, USA). Normally distributed data (based on normality of residuals (Shapiro-Wilk) $p > 0.05$) were analysed using *t*-tests, and data not normally distributed were treated as nonparametric and analysed using a Mann-Whitney test.

4.4.8.2. Pilot and main study

Volunteers were categorised into low, medium and high groups based on average unstimulated salivary flow rates, using tertile analysis in XLSTAT; these groupings were also used for subsequent statistical analysis. In order to test associations between age and categorical data (saliva flow rate grouping, mouth behaviour, dental status and medication), a chi-square test was carried out on contingency tables using XLSTAT. Linear mixed model analysis was carried out in SAS® software (Version 9.4, SAS Institute Inc., Cary, NC, USA) as this is considered to be sufficiently robust for unbalanced data (Torrico *et al*., 2018) and adjusted for multiplicity using Bonferroni. Salivary flow rates were analysed using explanatory variables of age, sex and with volunteer code as a random effect, and the dependent variable was saliva flow. The data relating to volunteers' individual perception, liking and appetite was analysed using explanatory

[39] see **Appendix D** for descriptive sensory profiling (DSP) panel performance summary

variables of age, sex, sample, saliva flow, mouth behaviour, dental status, medication, with volunteer code as a random effect. The dependent variables were in the (i) pilot study: attribute perception, liking and JAR rating scores; and (ii) main study: attribute perception, liking and appetite ratings. Attribute data collected in the pilot study on the gLMS log-scale was first transformed to linear data (anti-logged). Values were expressed as least square means (LSM) estimates, as these values best reflect the statistical model. Penalty analysis was carried out by XLSTAT using cake and biscuit JAR and liking scores, with 20% selected as the threshold for population size. Penalty analysis evaluated the influence of volunteer perception of appropriateness of attribute level rating (JAR) on volunteer liking by calculating the mean drop in liking rating (scale 1-9) compared with mean liking of volunteers that rated the attribute as JAR (JAR 3 on 1-5 scale), determining whether this drop in liking score was significant. Analysis of significant preferences between cake and biscuit samples was calculated using Binomial expansion in V-Power (Ennis & Jesionka, 2011). In all analyses $p < 0.05$ was used as the value for significant difference.

4.5. Results

4.5.1. Sensory profile and physical properties of cakes and biscuits

DSP evaluation identified that 26 of the 34 attributes were significantly different between the control and protein cake and 10 of the 33 attributes were significantly different between the control and protein biscuit. Therefore, demonstrating in both cases that protein fortification significantly increased off-flavours (i.e. rancid or sulfurous), coupled either with increased mouthdrying or with a slower melting rate compared with the control versions. There was a similar result for cupcakes where 19 of the 36 attributes were significantly different between products. Although the introduction of lemon zest led to a reduction in off-flavours compared with the pilot study cakes, the protein fortification still

resulted in significantly increased mouthdrying and firmness of bite. Key attributes are summarised in Figure 4.3 (all attributes are outlined in Tables 4.2 and 4.3). Protein fortification led to cakes and cupcakes that were perceived as substantially less dark in their yellow colour, whereas there was no significant difference in the perceived colour of the biscuits.

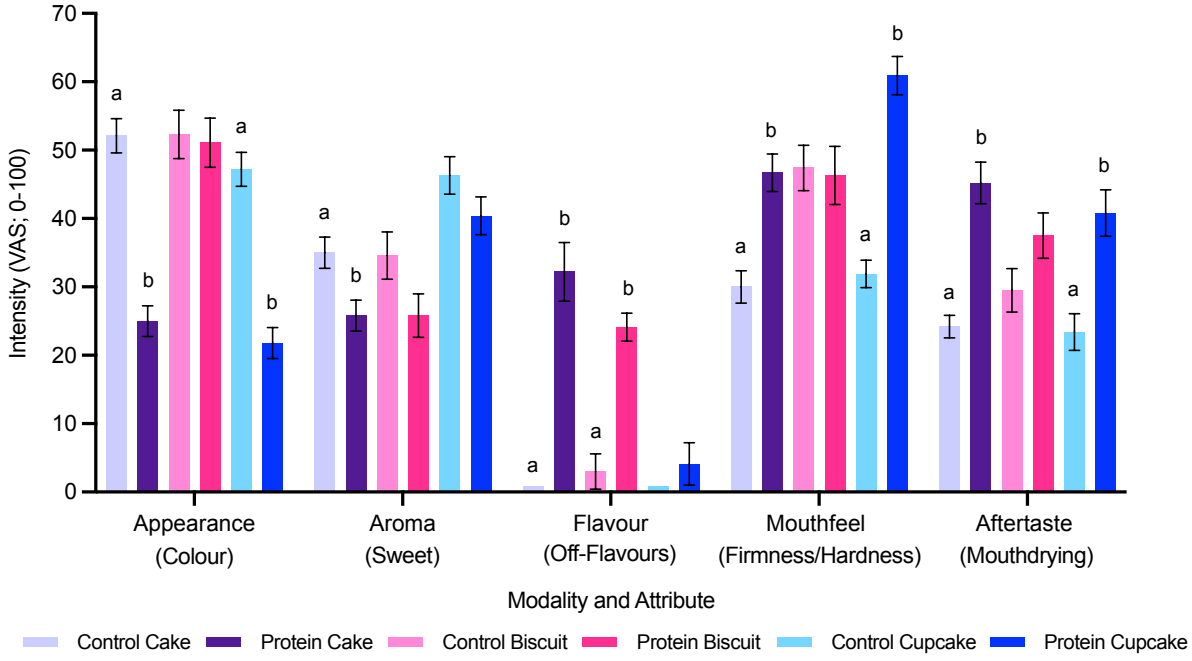

Figure 4.3. Summary of key sensory profile attributes for each modality and product (measured on a visual analogue scale (VAS, 0-100)). Values are expressed as mean[40] ± standard error. Significant differences ($p < 0.05$) between control and protein sample pairs are denoted by differing letters; no letter between sample pair reflects no significant difference. Colour (cake/cupcake: darkness of yellow colour of crumb; biscuit: darkness of colour (top)) and firmness refers to cakes/cupcakes and hardness refers to biscuits.

[40] means of two replicates from panellists (cakes $n = 12$; cupcakes $n = 11$; biscuits $n = 9$)

Table 4.2. Summary of sensory profile with corresponding reference/description and results for cakes (*n* = 12) and cupcakes (*n* = 11). Values are expressed as means[41] (measured on a visual analogue scale (VAS, 0-100)) (dash (-) represents not applicable).

Modality	Attribute	Reference and/or Description	Cakes		Significance of sample (*p* value)	Cupcakes		Significance of sample (*p* value)
			Control	Protein		Control	Protein	
Appearance	Moist appearance	Slightly or moderately wet to touch	50.4	23.6	**0.0002**	48.5	15.7	**< 0.0001**
	Dense appearance of sponge	Compact in structure	51.9	37.9	**0.006**	31.6	64.2	**< 0.0001**
	Appearance of large holes in sponge	Holes within crumb structure	12.5	22.9	**0.03**	25.3	48.4	**0.0009**
	Yellow colour of crumb (inside)	Intensity of yellow colour within crumb	52.1	25.0	**< 0.0001**	47.2	21.8	**< 0.0001**
Aroma	Overall aroma intensity	Intensity of aroma within cake	47.8	50.9	0.43	54.2	48.8	0.16
	Sweet	Sucrose (5.76 g/L)	35.0	25.8	**0.01**	46.3	40.4	0.17
	Vanilla/lemon	Vanilla extract/lemon zest	21.8	10.7	**0.009**	44.5	36.5	0.08
	Buttery	Cooked butter (melted unsalted butter)	20.8	8.9	**0.0001**	23.0	6.9	**0.002**
	Eggy	Intensity of eggy note	36.2	9.5	**< 0.0001**	7.8	17.1	**0.005**
	Rancid/off-flavours	Curded buttermilk (cooked buttermilk)	0.0	32.9	**< 0.0001**	0.0	6.2	0.11
Flavour	Overall flavour intensity	Intensity of flavour within cake	48.9	49.9	0.82	51.2	40.3	**0.03**
	Sweet	Sucrose (5.76 g/L)	40.7	27.0	**0.002**	46.8	40.0	0.19
	Metallic	Iron (II) Sulphate Heptahydrate (0.0036 g/L)	1.8	1.2	0.64	2.1	4.5	0.20
	Vanilla/lemon	Vanilla extract/lemon zest	25.0	11.1	**0.0009**	44.2	33.0	**0.02**
	Buttery	Cooked butter (melted unsalted butter)	22.0	8.0	**0.001**	20.8	6.3	**0.03**
	Eggy	Intensity of eggy note	31.3	8.9	**< 0.0001**	7.0	12.4	**0.03**
	Liquorice	Liquorice (liquorice twists)	-	-	-	0.9	5.7	0.053
	Rancid/off-flavours	Curded buttermilk (cooked buttermilk)	0.0	32.3	**< 0.0001**	0.0	4.1	0.22
Mouthfeel	Firmness of bite	Degree of force with first bite	30.0	46.7	**0.001**	31.9	60.9	**< 0.0001**
	Moist sponge	Slightly damp sponge	59.1	15.9	**< 0.0001**	55.1	16.5	**< 0.0001**
	Chewy	Ease of ability to chew	11.9	46.4	**0.0001**	29.0	59.6	**< 0.0001**
	Greasy lips	Degree of oiliness/greasiness on lips	15.7	7.2	**0.048**	14.5	2.3	**0.02**
	Crumbliness of sponge	Ease to break into small pieces	30.3	41.4	0.18	31.7	23.3	0.22
	Crumb size	Size of crumb inside of cake	27.7	52.2	**0.0004**	23.4	45.4	**0.0002**
	Pasty (cohesive)	Sticking to surfaces	27.0	9.1	**0.014**	46.4	17.2	**0.0001**
	Rate of breakdown & clearance	Clearing sample from mouth	52.8	31.5	**0.0014**	55.9	28.8	**0.0006**
	Cooling sensation (numbing)	A stimulation resulting in feeling of coolness	6.8	3.4	0.20	1.1	4.3	0.22
Aftertaste	Mouthdrying	Drying sensation in the mouth	24.2	45.2	**0.0001**	23.4	40.8	**0.001**
	Sweet	Sucrose (5.76 g/L)	33.6	21.6	**0.0002**	40.5	36.7	0.38
	Vanilla/lemon	Vanilla extract/lemon zest	17.9	6.1	**0.006**	33.3	21.2	**0.007**
	Buttery	Cooked butter (melted unsalted butter)	18.5	2.8	**0.0001**	11.3	3.6	0.09
	Rancid/off-flavours	Curded buttermilk (cooked buttermilk)	0.0	18.8	**0.0008**	0.0	2.3	0.17
	Salty	Sodium Chloride	1.3	2.5	0.10	2.6	1.7	0.42
	Salivating	Increased saliva within mouth	26.9	31.5	0.08	29.7	29.0	0.84
	Metallic	Iron (II) Sulphate Heptahydrate (0.0036 g/L)	6.8	7.5	0.86	2.7	8.1	0.07
	Liquorice	Liquorice (liquorice twists)	-	-	-	1.0	5.8	0.06

[41] of two replicates

Table 4.3. Summary of sensory profile with corresponding reference/description and results for biscuits (*n* = 9). Values are expressed as means[42] (measured on a visual analogue scale (VAS, 0-100)).

Modality	Attribute	Reference and/or Description	Biscuits		Significance of sample (*p* value)
			Control	Protein	
Appearance	Evenness of shape	Uniform shape of biscuit	51.4	58.0	0.08
	Smoothness of surface	Texture without roughness, top surface only	31.2	35.4	0.21
	Darkness of colour (top of biscuit)	Intensity of colour, top surface only	52.3	51.1	0.79
	Darkness of colour (inside of biscuit)	Intensity of colour, inside surface only	45.9	32.3	**0.0001**
	Darkness of colour (bottom of biscuit)	Intensity of colour, bottom surface only	73.5	79.5	**0.007**
	Thickness	Degree of thickness of biscuit	43.6	54.0	**0.006**
	Crumb/aeration	Size of crumb biscuit	38.4	42.3	0.27
Aroma	Lemon	Lemon (sliced lemon)	35.2	33.2	0.71
	Sweet	Sucrose (5.76 g/L)	34.6	25.8	0.06
	Oaty	Raw oats	18.4	15.2	0.32
	Fatty	Piece of lard	9.6	8.2	0.75
	Baked	Cooked in an oven	48.9	41.0	0.09
	Sulfate off note	Cooked cabbage	4.9	18.1	0.08
Flavour	Sweet	Sucrose (5.76 g/L)	43.7	28.8	**0.02**
	Oaty	Raw oats	25.6	23.6	0.46
	Fatty	Piece of lard	8.6	14.5	0.08
	Bitter	Quinine (0.04 g/L)	9.7	11.8	0.45
	Lemony	Lemon zest	33.1	23.8	**0.047**
	Sulfate off note	Cooked cabbage	3.0	24.1	**0.002**
	Metallic	Iron (II) Sulphate Heptahydrate (0.0036 g/L)	3.7	6.0	0.06
Mouthfeel	Hardness of first bite	Degree of force with first bite	47.4	46.3	0.72
	Crumbly	Ease of break into small pieces	59.6	50.4	0.06
	Melt rate/dissolving rate	Speed of dissolve and melt within mouth	56.7	48.3	**0.004**
	Mouthdrying	Drying sensation in the mouth	35.5	42.8	0.19
	Teeth packing	Biscuit sticking to the surface of teeth	58.3	50.7	**0.03**
	Grainy	Not smooth or fine, rough to touch	39.3	35.1	0.38
	Crunchy	Degree of force and sound with chewing	45.7	41.7	0.33
Aftertaste	Sweet	Sucrose (5.76 g/L)	28.9	18.3	**0.02**
	Teeth packing (residue)	Biscuit sticking to the surface of teeth	44.6	41.2	0.47
	Mouthdrying	Drying sensation in the mouth	29.5	37.5	0.14
	Lemony	Lemon zest	22.4	15.9	0.06
	Bitter	Quinine (0.04 g/L)	10.2	10.6	0.79
	Sulfate off note	Cooked cabbage	0.8	10.0	**0.003**

[42] of two replicates

There were significant differences in physical properties between the control and protein versions of cakes and biscuits, as outlined in Table 4.4. Protein fortification of cakes and biscuits resulted in significantly reduced moisture content and significantly increased hardness compared with the control versions. Protein fortification of cupcakes resulted in no significant differences in moisture content between the two cupcakes. This was considered to be as a result of the improved balance of ingredients and was in contrast to the pilot study cakes. However, the protein cupcake did result in significant increases in height, hardness, cohesiveness, resilience and chewiness, compared with the control cupcake. The protein fortified biscuits were found to be significantly darker, redder and more yellow in instrumental measurements. Colour differences between control and protein fortified cakes were less apparent; however, as with biscuits there was an overall colour difference of greater than three (the minimum expected to lead to a perceptual difference (Bodart *et al*., 2007)). These results do not completely parallel the sensory results (Figure 4.3) where protein fortification led to a considerably more noticeable colour difference (less intense yellow) in cakes and cupcakes. However, the cakes are aerated and translucent which makes accurate instrumental colour measurements more difficult in comparison with the opaque biscuits. In conclusion, protein fortification led to increased colour development; however, this is less well represented by the instrumental readings of the cakes, due to aeration.

Table 4.4. Physical properties of cakes, biscuits and cupcakes.

	Cakes		Biscuits		Cupcakes	
	Control	Protein	Control	Protein	Control	Protein
Height (mm)	60.6 ± 1.0[a]	70.5 ± 1.2[b]	-	-	28.0 ± 0.5[a]	41.3 ± 0.6[b]
Diameter (mm)	-	-	61.9 ± 0.4	62.7 ± 0.2	-	-
Moisture Content (%)	28.8 ± 0.4[a]	25.3 ± 0.5[b]	3.1 ± 0.04[a]	2.3 ± 0.05[b]	23.5 ± 0.4	24.2 ± 0.2
Water Activity (a_w)	0.87 ± 0.003	0.87 ± 0.003	0.36 ± 0.01[a]	0.27 ± 0.007[b]	0.81 ± 0.0005	0.85 ± 0.004
Colour *L** (dark-light)	74.0 ± 3.8	73.2 ± 2.1	61.1 ± 2.4[a]	58.3 ± 2.3[b]	78.7 ± 0.5	77.8 ± 1.0
Colour *a** (green-red)	-3.8 ± 0.2	-3.6 ± 0.2	3.9 ± 0.9[a]	10.4 ± 1.6[b]	-3.7 ± 0.1	-3.3 ± 0.03
Colour *b** (blue-yellow)	29.0 ± 1.9[a]	26.2 ± 0.3[b]	28.6 ± 1.2[a]	32.5 ± 1.7[b]	28.8 ± 0.1	25.6 ± 0.5
Hue Angle (arctan (*b**/*a**))	97.4 ± 0.2	97.8 ± 0.1	82.2 ± 0.4[a]	71.2 ± 1.1[b]	97.5 ± 0.4	97.5 ± 0.3
Colour Difference (dE)	3.8 ± 0.7		8.9 ± 0.6		3.6 ± 0.5	
Hardness (g)	181 ± 18[a]	372 ± 18[b]	2384 ± 140[a]	3201 ± 178[b]	784 ± 50[a]	1130 ± 83[b]
Chewiness (-)	135 ± 48[a]	294 ± 53[b]	-	-	504 ± 51[a]	814 ± 86[b]
Springiness (%)	0.96 ± 0.01	0.96 ± 0.01	-	-	89.5 ± 3.6	89.1 ± 1.8
Cohesiveness (-)	0.78 ± 0.01[a]	0.82 ± 0.009[b]	-	-	0.71 ± 0.03[a]	0.80 ± 0.02[b]
Adhesiveness (g.sec)	-	-	-	-	-2.2 ± 0.8[a]	-0.06 ± 0.2[b]
Resilience (%)	-	-	-	-	29.2 ± 1.5	31.9 ± 0.8
Gumminess (-)	-	-	-	-	559 ± 41[a]	910 ± 82[b]
Fracturability (mm)	-	-	43.0 ± 6.7[a]	84.2 ± 12[b]	-	-

Values are expressed as mean of three replicates ± standard error. Significant differences ($p < 0.05$) between control and protein sample pairs are denoted by differing letters. (-) represents no unit (dimensionless data). Some measures did not apply to all product types. The colour measurements in the table above relate to the crumb (cakes and cupcakes) and the top surface (biscuits).

Table 4.5. Summary of volunteers' sex, medication, dental status, mouth behaviour and unstimulated saliva flow groupings in both studies.

	Sex				**Medication**				**Dental Status**				**Mouth behaviour**						**Saliva Flow Categories**					
	Male		Female		Yes		No		Good		Reduced		Chewer		Cruncher		Other		Low		Medium		High	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Pilot Study																								
Total (*n* = 84)	31	37	53	63	19	23	65	77	64	76	20	24	42	50	33	39	9	11	27	32	28	33	29	35
Younger Adults (*n* = 42)	12	29	30	71	0	0	42	100	41	98	1	2	23	55	12	20	7	16	8	19	14	33	20	48
Older Adults (*n* = 42)	19	45	23	55	19	45	23	55	23	55	19	45	19	45	21	50	2	5	19	45	14	33	9	21
Main Study																								
Total (*n* = 70)	27	39	43	61	18	26	52	74	59	84	11	16	29	43	25	37	13	19	23	33	23	33	24	34
Younger Adults (*n* = 38)	13	34	25	66	0	0	38	100	38	100	0	0	16	42	15	39	7	18	8	21	15	39	15	39
Older Adults (*n* = 32)	14	44	18	56	18	56	14	44	21	66	11	34	13	45	10	34	6	21	15	47	8	25	9	28

'*n*' and '%' reflect number and percentage in each contributing group. Mouth behaviour 'other' reflects smooshers/sucker in the pilot study and smooshers in the main study. Missing data for mouth behaviour (main study *n* = 3). In both studies dental status is significantly associated with medication (pilot study: $p = 0.006$; main study: $p < 0.0001$) and is independent of mouth behaviour category (pilot study: $p = 0.95$; main study: $p = 0.97$). Saliva flow groupings are derived from unstimulated salivary flow and categories are defined by tertiles with mL/min range for each category (pilot study: low saliva flow: 0.04-0.53 mL/min; medium saliva flow: 0.53-0.77 mL/min; high saliva flow: 0.77-2.18 mL/min and main study: low saliva flow: 0.23-0.58 mL/min; medium saliva flow: 0.58-0.95 mL/min; high saliva flow: 0.95-1.52 mL/min).

4.5.2. Dental status and mouth behaviour questionnaire data

In both studies, the dental status of the volunteers was categorised into two groups: good dental status (20 or more teeth, no dentures and minimal missing teeth less than four) or reduced dental status (less than 20 teeth, dentures and missing teeth more than four). There was a significant association ($p < 0.0001$) between dental status and age in both studies, where predominately only older adults supported reduced dental status (Table 4.5). The mouth behaviour of the volunteers, as defined by Jeltema *et al*. (2015; 2016), was classified into three types: chewers, crunchers and other/smooshers (pilot study: smooshers and suckers grouped together due to limited numbers in each group; main study: no suckers were recorded). Mouth behaviour was shown to be marginally independent ($p = 0.06$) of age in the pilot study and independent ($p = 0.86$) of age in the main study, where volunteers categorised themselves as chewers and crunchers more commonly than smooshers/other. All data is summarised in Table 4.5.

4.5.3. Salivary flow rates

Older adults demonstrated significantly lower unstimulated saliva flow ($p < 0.05$) compared with younger adults in both studies. However, age had no significant effect on stimulated saliva flow (Figure 4.4). Volunteers were grouped by tertile analysis into low, medium and high salivary flow rates, based on average unstimulated salivary flow rates. In the pilot study, there was a significant association ($p = 0.01$) between age and saliva flow grouping for unstimulated saliva. However, in the main study unstimulated saliva flow groupings were shown to be marginally insignificantly ($p = 0.07$) related to age (Table 4.5). Age was significantly associated ($p < 0.0001$) with medication, where only older adults reported regular medication use (Table 4.5). The effect of medication status on unstimulated saliva flow in older adults varied between the studies; in the pilot study there was no significant effect ($p = 0.70$), whereas in the main study there was a significant

effect (p = 0.004) (Figure S.4.1). The difference between the pilot and main studies may have been due to increasing experience with the saliva collection method (Figure S.4.2) and an imbalance of the proportion of volunteers taking medication between the two studies. However, medication status had no significant effect on stimulated saliva flow in older adults (Figure S.4.1). Dental status had no significant effect on unstimulated saliva flow; however, those volunteers with a reduced dental status had significantly ($p < 0.05$) lower stimulated saliva flow in both studies (Figure S.4.1). Sex had a significant effect ($p < 0.05$) on saliva flow regardless of collection method, males having significantly higher salivary flow compared with females (Figure 4.4).

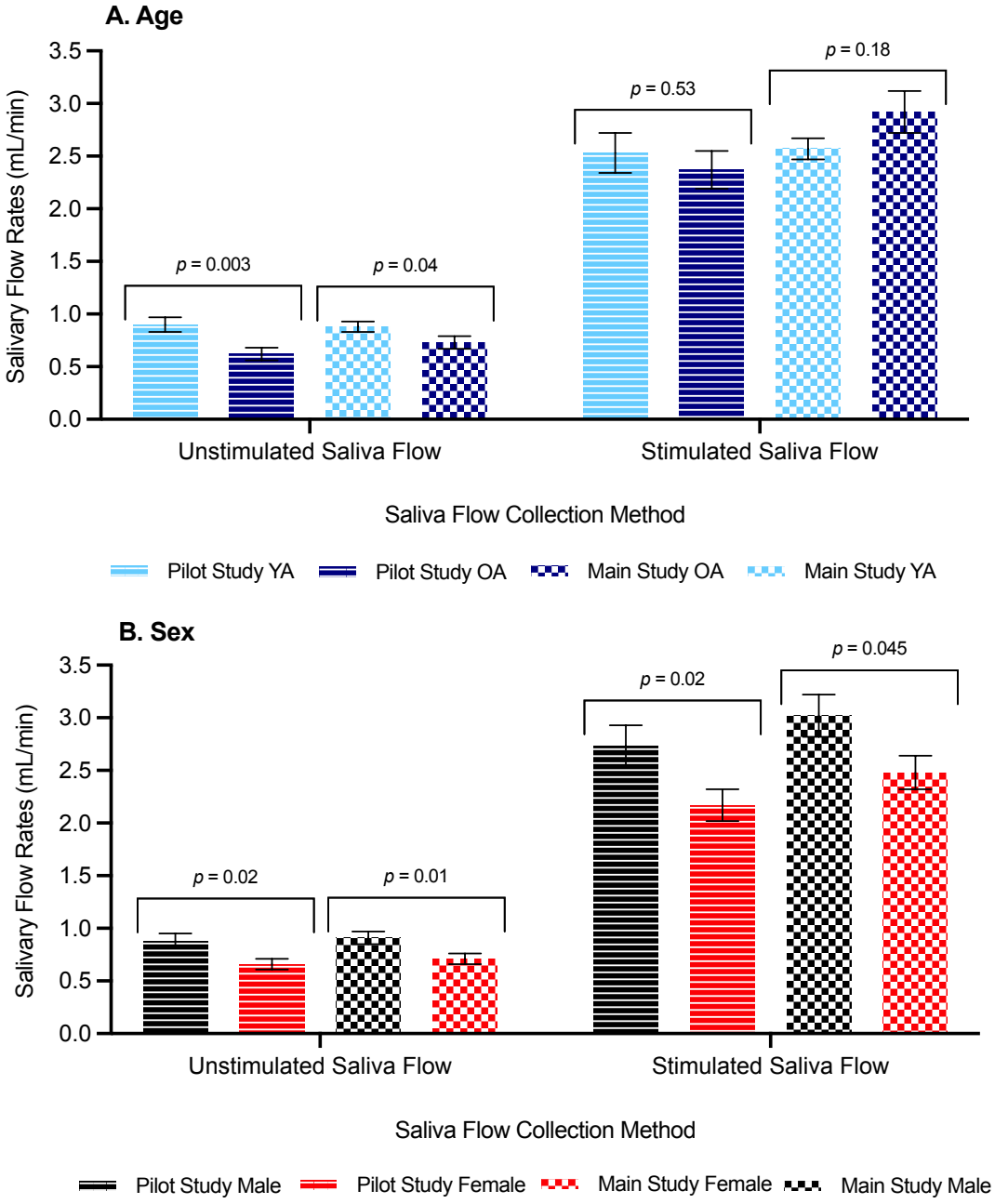

Figure 4.4. Summary of volunteers' salivary flow rates (mL/min) in both studies *(pilot study n = 84; YA: n = 42; OA: n = 42; main study n = 70; YA: n = 38; OA: n = 32)*. Values are expressed as LSM estimates ± standard error from SAS output. Significant differences *(p* < 0.05) are reported between groups with relevant *p* value above each group.

4.5.4. Individual product perception and liking

During the pilot study, volunteers consumed a single bite of cake or biscuit, whereas during the main study volunteers consumed a full portion (35.0 g cupcake) at home. As detailed in Table 4.6 and Figures 4.5a and 4.6, protein fortification of cakes and cupcakes

significantly reduced ($p < 0.05$) overall liking, easiness to eat and swallow, sweetness and moistness and significantly increased ($p < 0.05$) mouthdrying, compared with the control versions. Protein fortification of biscuits significantly reduced ($p < 0.05$) liking and moistness and significantly increased ($p < 0.05$) mouthdrying and hardness compared with the control biscuits (Table 4.6 and Figure 4.5b). Regarding volunteer optimum levels for attributes (JAR scales, Table 4.7), protein fortification of cakes significantly reduced ($p < 0.0001$) flavour intensity and colour to below the optimum (3 = JAR), but significantly increased ($p < 0.0001$) biscuit colour to optimum.

During the pilot study, age significantly influenced ($p < 0.05$) liking and appropriateness of attributes. Older adults reported the protein cakes to be too low in flavour and colour, and biscuits to have a colour closer to optimum, compared with the younger adults (Table 4.7). There were no further overall significant effects of age reported on liking, perception and easiness to consume. However, pairwise comparisons revealed that older adults found all protein fortified products (cakes, biscuits and cupcakes) to be significantly ($p < 0.05$) more mouthdrying compared with the control versions, whereas for younger adults this was only significant for cakes (Figures 4.5 and 4.6).

There was no overall significant effect of saliva flow on liking, perception, ease to eat and swallow and JAR attributes; however, by categorising volunteers by unstimulated saliva flow, some trends did emerge. For biscuits, perceived mouthdrying intensity decreased with increasing salivary flow rates (low versus medium and high SF: $p = 0.24$ and $p = 0.58$, respectively). Whereas for cupcakes, perceived mouthdrying intensity increased with increasing salivary flow rates, regardless of the cupcake consumed (low versus medium and high SF: $p = 0.30$ and $p = 0.37$, respectively) (Figures S.4.3 and S.4.4).

In the pilot study, mouth behaviour type significantly influenced liking of appearance scores for cakes (p = 0.05) and biscuits (p = 0.009), where chewers gave higher scores than crunchers. Volunteers evaluated the same cakes at both study visits and scoring remained consistent in most cases (no significant effect of visit on cake liking, perception, JAR and preference) apart from easiness to eat/swallow, which were both rated as significantly less easy to eat (p = 0.01) and swallow (p = 0.002) at visit two (difference between visits: 0.2 on 5-point hedonic scale; Table S.4.2). In the main study, dental status significantly influenced (p < 0.05) liking and easiness to eat and swallow scores, where those with reduced dental status reported significantly lower scores compared with those with good dental status. No further significant effects were reported relating to mouth behaviour, dental status, medication and sex (Table S.4.2)

Table 4.6. Volunteers' liking and easiness to eat and swallow mean ratings of cakes[43], biscuits and cupcakes in both studies; overall and by age and unstimulated saliva flow rate.

	Overall		Age		Unstimulated Saliva Flow		
		Significance of sample (*p* value)	Younger Adults	Older Adults	Low Saliva Flow[3]	Medium Saliva Flow[4]	High Saliva Flow[5]
Appearance Liking							
Control Cake[1]	6.7 ± 0.1	0.20	6.6 ± 0.2	6.8 ± 0.2	6.7 ± 0.2	6.8 ± 0.2	6.6 ± 0.2
Protein Cake[1]	6.9 ± 0.1		6.9 ± 0.3	6.9 ± 0.2	7.1 ± 0.2	6.8 ± 0.2	6.7 ± 0.2
Control Biscuit[1]	5.6 ± 0.2	0.08	4.8 ± 0.4^{aA}	6.4 ± 0.3^{b}	5.4 ± 0.3	5.4 ± 0.3	5.8 ± 0.4
Protein Biscuit[1]	5.9 ± 0.2		5.5 ± 0.4^{B}	6.3 ± 0.3	5.5 ± 0.3	6.0 ± 0.3	6.2 ± 0.4
Control Cupcake[2]	57.2 ± 3.8	0.18	59.8 ± 5.5	54.7 ± 4.5^{A}	56.3 ± 5.4	58.0 ± 5.1	57.3 ± 5.8
Protein Cupcake[2]	60.0 ± 3.8		56.2 ± 5.4	65.5 ± 4.4^{B}	56.6 ± 5.2	61.4 ± 5.0	64.5 ± 5.8
Overall Liking							
Control Cake[1]	6.6 ± 0.2	**< 0.0001**	6.5 ± 0.3^{A}	6.9 ± 0.3^{A}	6.5 ± 0.3^{A}	6.9 ± 0.3^{A}	6.7 ± 0.3^{A}
Protein Cake[1]	5.0 ± 0.2		5.0 ± 0.3^{B}	5.0 ± 0.2^{B}	5.2 ± 0.3^{B}	4.8 ± 0.3^{B}	5.0 ± 0.3^{B}
Control Biscuit[1]	6.2 ± 0.2	**0.002**	5.5 ± 0.4^{a}	7.0 ± 0.3^{bA}	5.8 ± 0.4	6.5 ± 0.3^{A}	6.4 ± 0.4
Protein Biscuit[1]	5.3 ± 0.2		4.8 ± 0.4	5.9 ± 0.3^{B}	5.0 ± 0.4	5.3 ± 0.3^{B}	5.6 ± 0.4
Control Cupcake[2]	65.4 ± 4.0	**< 0.0001**	68.2 ± 5.9^{A}	62.6 ± 4.8^{A}	62.2 ± 5.8	68.8 ± 5.5^{A}	65.0 ± 6.2^{A}
Protein Cupcake[2]	51.3 ± 4.0		56.7 ± 5.8^{B}	45.8 ± 4.7^{B}	53.8 ± 5.6	48.9 ± 5.4^{B}	51.0 ± 6.3^{B}
Easiness to Eat							
Control Cake[1]	4.3 ± 0.1	**< 0.0001**	4.2 ± 0.2^{A}	4.3 ± 0.1^{A}	4.2 ± 0.1^{A}	4.1 ± 0.1^{A}	4.3 ± 0.1^{A}
Protein Cake[1]	3.2 ± 0.1		3.2 ± 0.2^{B}	3.1 ± 0.1^{B}	3.3 ± 0.1^{B}	3.1 ± 0.1^{B}	3.1 ± 0.1^{B}
Control Biscuit[1]	3.5 ± 0.1	0.23	3.0 ± 0.2^{a}	4.0 ± 0.2^{bA}	3.3 ± 0.2	3.7 ± 0.2	3.5 ± 0.2
Protein Biscuit[1]	3.3 ± 0.1		3.5 ± 0.2	3.5 ± 0.2^{B}	3.4 ± 0.2	3.3 ± 0.2	3.3 ± 0.2
Control Cupcake[2]	67.3 ± 3.9	**< 0.0001**	66.8 ± 5.6^{A}	67.8 ± 4.7^{A}	68.7 ± 5.7^{A}	65.1 ± 5.4^{A}	68.0 ± 6.0^{A}
Protein Cupcake[2]	49.4 ± 3.9		50.0 ± 5.6^{B}	48.8 ± 4.6^{B}	57.9 ± 5.5^{B}	46.9 ± 5.4^{B}	43.5 ± 6.0^{B}
Easiness to Swallow							
Control Cake[1]	4.0 ± 0.1	**< 0.0001**	3.9 ± 0.2^{A}	4.1 ± 0.1^{A}	4.0 ± 0.1^{A}	3.9 ± 0.1^{A}	4.1 ± 0.2^{A}
Protein Cake[1]	3.0 ± 0.1		2.9 ± 0.2^{B}	3.1 ± 0.1^{B}	3.1 ± 0.1^{B}	2.9 ± 0.1^{B}	2.9 ± 0.2^{B}
Control Biscuit[1]	3.4 ± 0.1	0.95	3.0 ± 0.2^{a}	3.8 ± 0.2^{b}	3.2 ± 0.2	3.5 ± 0.1	3.6 ± 0.2
Protein Biscuit[1]	3.4 ± 0.1		3.3 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.1	3.5 ± 0.2
Control Cupcake[2]	64.5 ± 3.7	**< 0.0001**	62.4 ± 5.2^{A}	66.5 ± 4.5^{A}	65.4 ± 5.6	62.1 ± 5.3^{A}	65.9 ± 5.7^{A}
Protein Cupcake[2]	47.7 ± 3.7		48.8 ± 5.2^{B}	46.5 ± 4.5^{B}	56.0 ± 5.3^{a}	49.1 ± 5.2^{aB}	37.9 ± 5.7^{bB}

Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) within a row (i.e. age YA vs OA and saliva flow pairwise comparisons) are denoted by differing small letters; and within a column (i.e. within an age group between samples or within saliva flow groupings between samples) are denoted by differing capital letters. During the pilot study[1] (n = 84; YA: n = 42; OA: n = 42) all cakes and biscuits were measured on a 9- and 5-point scale, respectively and during the main study[2] (n = 70; YA: n = 38; OA: n = 32) all cupcakes were measured on a visual analogue scale (VAS) 0-100 mm. Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertile analysis (low saliva flow[3], pilot study n = 27; main study n = 23; medium saliva flow[4], pilot study n = 28; main study n = 23; high saliva flow[5], pilot study n = 29; main study n = 24).

[43] cakes were measured in duplicate at visit one and two

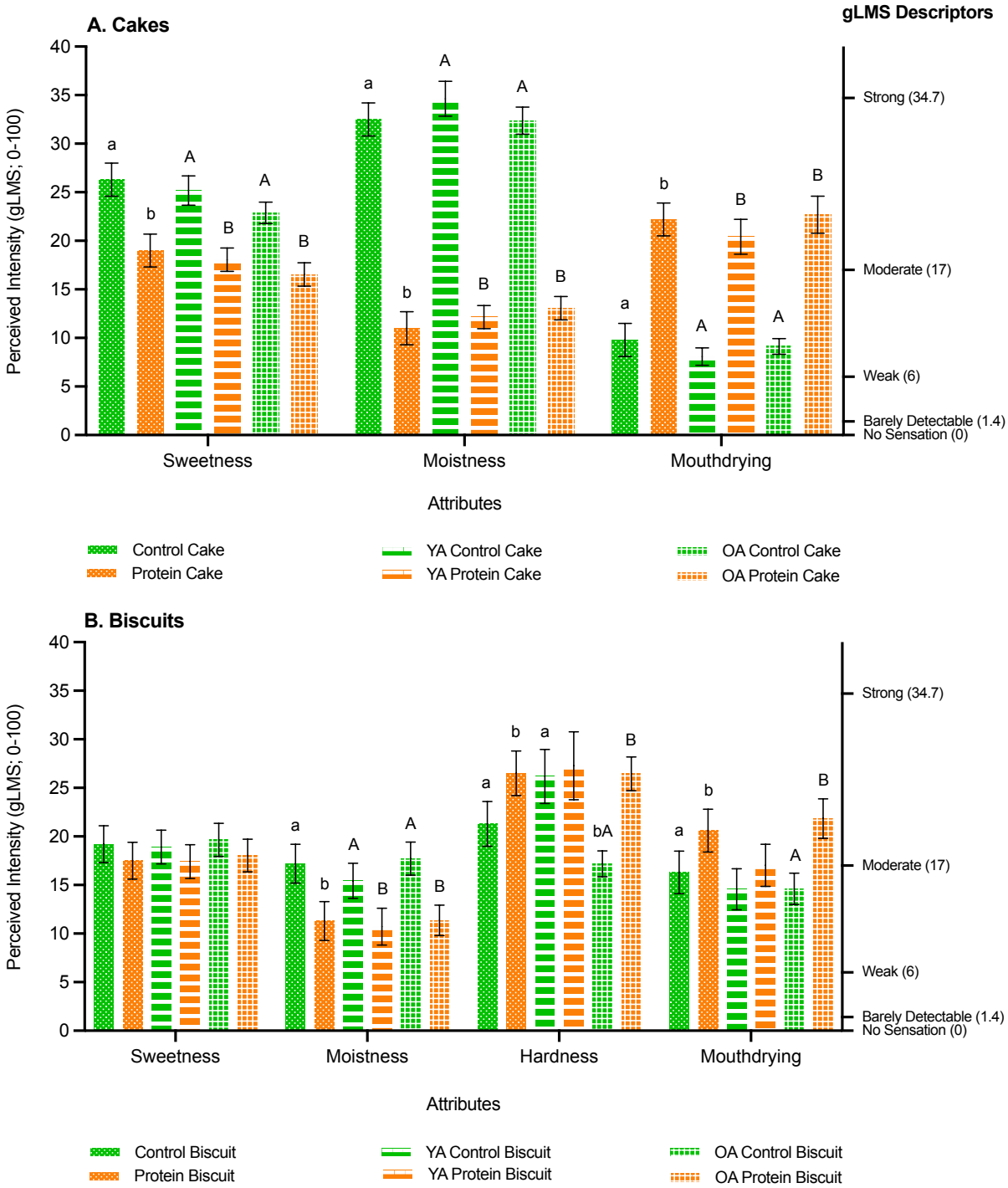

Figure 4.5. Volunteers attribute perception mean ratings of (**A**) cakes[44] and (**B**) biscuits by overall and age (pilot study: *n* = 84; generalised Labelled Magnitude Scale (gLMS) anti-logged data, scale 0-100 summarised on the right of the figure). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) between samples (by overall: control vs protein, and age: younger adults (YA: *n* = *42*) vs older adults (OA: *n* = *42*)) are denoted by differing small letters, and significant differences within age groups are denoted by differing capital letters; no letter reflects no significant difference.

[44] cakes were measured in duplicate at visit one and two

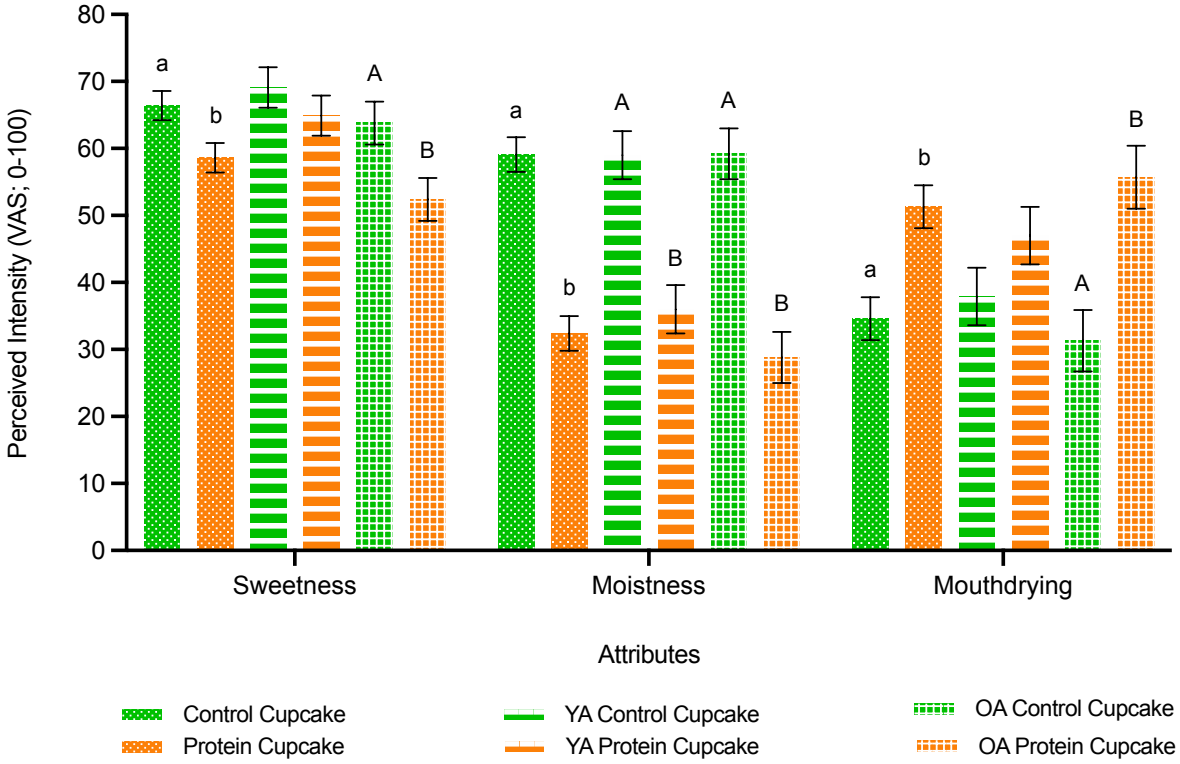

Figure 4.6. Volunteers attribute perception mean ratings of cupcakes by overall and age (main study: *n* = 70; VAS, 0-100 mm). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) between samples (by overall: control vs protein, and age: younger adults (YA: *n* = *38*) vs older adults (OA: *n* = *32*)) are denoted by differing small letters, and significant differences within age groups are denoted by differing capital letters; no letter reflects no significant difference.

4.5.5. Preference and penalty analysis (pilot study only)

There was a significant preference reported for the control cake ($p < 0.0001$) and control biscuit ($p = 0.02$) compared with the protein cake and biscuit (Table S.4.3). The volunteers' perception of appropriateness of attribute level (JAR ratings) influenced their overall liking, as shown in the penalty analysis (Table 4.7). There was a significant influence of flavour, with older adults reporting 'too little' flavour more commonly than younger adults, which significantly penalised the liking scores.

Table 4.7. Volunteers' appropriateness of attribute level[45] (Just-About-Right, JAR) mean ratings of cakes and biscuits and their influence on volunteer liking ratings in the pilot study; overall and by age (YA: younger adult and OA: older adult) and unstimulated saliva flow.

	Overall (*n* = 84)		**Age**		**Unstimulated Saliva Flow**			**Penalty Analysis** (mean liking drop where attribute deviate from Just-About-Right)			
		Significance of sample (*p* value)	Younger Adults (*n* = 42)	Older Adults (*n* = 42)	Low Saliva Flow (*n* = 27)	Medium Saliva Flow (*n* = 28)	High Saliva Flow (*n* = 29)	Too Little (YA)	Too Much (YA)	Too Little (OA)	Too Much (OA)
JAR Flavour											
Control Cake	2.9 ± 0.08	**< 0.0001**	2.9 ± 0.1	2.8 ± 0.1^{A}	2.7 ± 0.1^{a}	3.0 ± 0.1^{bA}	2.8 ± 0.1^{aA}	0.65	1.68	1.79$^{\#}$	-0.70
Protein Cake	2.6 ± 0.08		2.8 ± 0.1^{a}	2.3 ± 0.1^{bB}	2.4 ± 0.1	2.6 ± 0.1^{B}	2.5 ± 0.1^{B}	0.76†	2.05	1.83$^{\#}$	1.49
Control Biscuit	2.7 ± 0.1	0.37	2.5 ± 0.1	2.8 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	0.01†	-0.12	0.80†	-
Protein Biscuit	2.6 ± 0.1		2.5 ± 0.2	2.7 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.8 ± 0.1	0.50†	1.43	2.12$^{\#}$	0.85
JAR Colour											
Control Cake	3.0 ± 0.04	**< 0.0001**	3.1 ± 0.06^{aA}	2.9 ± 0.05^{b}	3.0 ± 0.07	3.0 ± 0.06^{A}	3.0 ± 0.07^{A}	0.91	0.16	-0.26	0.90
Protein Cake	2.8 ± 0.04		2.8 ± 0.06^{B}	2.8 ± 0.05	2.9 ± 0.07^{a}	2.7 ± 0.06^{bB}	2.8 ± 0.07^{aB}	0.57†	-	1.17	1.26
Control Biscuit	2.4 ± 0.1	**< 0.0001**	2.2 ± 0.1^{aA}	2.8 ± 0.1^{bA}	2.5 ± 0.1^{A}	2.4 ± 0.1^{A}	2.4 ± 0.1^{A}	-0.61	-	0.30†	0.40
Protein Biscuit	3.2 ± 0.1		3.3 ± 0.1^{B}	3.3 ± 0.1^{B}	3.3 ± 0.1^{B}	3.2 ± 0.1^{B}	3.2 ± 0.1^{B}	1.46	1.00	0.27	0.18†

Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) within a row (i.e. age YA vs OA and saliva flow pairwise comparisons) are denoted by differing small letters, and within a column (i.e. within an age group between samples or within saliva flow groupings between samples) are denoted by differing capital letters. # represents a significant difference ($p < 0.05$) between penalty analysis groups within a sample for older adults, † represents where the size of the group is lower than 20% of the population, and - represents where 0% of the population selected the category. Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertile analysis.

[45] all cakes and biscuits were measured on a 5-point JAR scale (and cakes were measured in duplicate at visit one and two)

4.5.6. Qualitative feedback

In both studies volunteers' comments were categorised into key themes, such as, flavour, texture, positive and negative comments and no comments provided (Table S.4.4). During the pilot study, there was general positive feedback provided for the control cake (186 positive comments out of 229 comments provided) and biscuits (72 positive comments out of 108 comments provided), demonstrating suitability for older adults. In contrast, negative comments were associated with the protein cake (175 negative comments out of 236 comments provided) and biscuits (79 negative comments out of 106 comments provided) relating to both flavour and texture, both of which were considered to be less appetising. During the main study, there was a general trend towards positive comments relating to the lemon flavour of both the control (59 positive comments out of 63 comments provided) and protein cupcakes (40 positive comments out of 63 comments provided). However, protein fortification of cupcakes (42 negative comments out of 62 comments provided) still resulted in a greater number of negative comments relating to texture compared with the control version (28 negative comments out of 62 comments provided). Examples of comments are summarised in Table 4.8.

Table 4.8. Examples of volunteer comments in both studies.

Sample	Comments and Volunteer Details
Control Cake[1]	*Pretty soft and with a nice edge easy to chew and swallow. Not as grainy as the last sample (v38, male, younger adult, aged 25). A nice moist cake with a good flavour. Very easy to eat (v58, male, older adult, aged 77)*
Protein Cake[1]	*It is missing fluffiness, it feels a lot like a sponge with too many air bubbles (v12, female, younger adult, aged 24). Dry, rubbery, tasteless (v63, female, older adult, aged 76)*
Control Biscuit[1]	*Really nice, I would buy this (v31, female, younger adult, aged 29). A very nice tasty biscuit, excellent and a very good flavour (v66, male, older adult, aged 81)*
Protein Biscuit[1]	*Disliked the flavour, too artificial, unpleasant aftertaste (v33, female, younger adult, aged 21). The combination of lack of distinctive flavour and texture is not appealing and makes it unattractive (v86, female, older adult, aged 68)*
Control Cupcake[2]	*Flavour is lovely and tasty (v31, female, younger adult, aged 28). Good texture and pleasant mouthfeel. Moist. Enjoyable to chew. I was surprised as I didn't particularly like the appearance of the sample when I first opened it (v62, female, older adult, aged 73)*
Protein Cupcake[2]	*Lovely flavour, just missing icing (v22, younger adults, aged 21). Very dry. Pleasantly chewy. Quite sweet but without much flavour (v73, male, older adult, aged 78)*

[1]pilot study; [2]main study.

4.5.7. Appetite ratings (main study only)

Consuming a 35.0 g protein cupcake significantly increased (p = 0.04) thirst compared with consumption of the same size control cupcake. Older adults reported that consumption of the protein cupcake significantly reduced (p = 0.02) prospective consumption, whereas younger adults did not. No further significant differences in appetite ratings were reported due to cupcake type or age. However, desire to eat ratings were significantly influenced by sex (p = 0.01), where females reported a greater reduction in desire to eat scores following cupcake consumption compared with males. There was also a significant effect of mouth behaviour (p = 0.03), where crunchers reported a lower reduction in desire to eat scores post consumption compared with smooshers and chewers. Two appetite ratings (desire to eat and prospective consumption) were significantly influenced (p < 0.05) by dental status, with a lower reduction in ratings following consumption from those with a reduced dental status, compared with those with good dental status. Unstimulated salivary flow rates were

grouped using tertile analysis; however, despite some significant effects between samples and saliva flow groupings there were no clear trends (Tables 4.9 and S.4.2).

Table 4.9. Volunteers' appetite mean ratings (change from baseline) of cupcakes in the main study; overall and by age and unstimulated saliva flow rate.

	Overall (*n* = 70)		**Age**		**Unstimulated Saliva Flow**		
		Significance of sample (*p* value)	Younger Adults (*n* = 38)	Older Adults (*n* = 32)	Low Saliva Flow (*n* = 23)	Medium Saliva Flow (*n* = 23)	High Saliva Flow (*n* = 24)
Hungry							
Control Cupcake	-10.8 ± 3.7	1.00	-14.4 ± 5.3	-7.1 ± 4.5	13.0 ± 5.5	7.1 ± 5.2	12.2 ± 5.7
Protein Cupcake	-13.1 ± 3.7		-9.5 ± 5.2	-16.8 ± 4.5	10.4 ± 5.4	15.0 ± 5.2	14.0 ± 5.7
Thirsty							
Control Cupcake	9.4 ± 4.0	**0.04**	15.1 ± 5.8	3.6 ± 4.8	6.3 ± 6.0^{aA}	15.1 ± 5.6^{b}	6.6 ± 6.1^{a}
Protein Cupcake	15.5 ± 4.0		16.1 ± 5.7	15.0 ± 4.8	18.2 ± 5.9^{B}	11.9 ± 5.5	16.4 ± 6.1
Desire to Eat							
Control Cupcake	-14.9 ± 3.7	0.10	-20.8 ± 5.2	-9.0 ± 4.4	12.0 ± 5.5	9.5 ± 5.1	23.2 ± 5.6
Protein Cupcake	-19.0 ± 3.7		-20.7 ± 5.2	-17.4 ± 4.4	20.8 ± 5.3	18.2 ± 5.1	18.2 ± 5.6
Satiety							
Control Cupcake	6.7 ± 4.1	0.27	7.5 ± 5.9	6.0 ± 4.9	1.7 ± 6.1	4.0 ± 5.7	14.6 ± 6.2
Protein Cupcake	7.5 ± 4.0		3.6 ± 5.8	11.4 ± 4.9	10.2 ± 6.0^{a}	3.5 ± 5.6^{b}	8.8 ± 6.2^{a}
Fullness							
Control Cupcake	9.8 ± 3.9	0.48	13.9 ± 5.6	5.7 ± 4.7	5.0 ± 5.9^{A}	12.5 ± 5.6	12.0 ± 6.0
Protein Cupcake	8.0 ± 3.9		6.2 ± 5.5	9.8 ± 4.7	9.6 ± 5.8^{aB}	1.4 ± 5.5^{b}	13.1 ± 6.0^{a}
Prospective Consumption							
Control Cupcake	-4.2 ± 3.3	0.45	-4.9 ± 4.8	-3.6 ± 4.0	-2.2 ± 5.0^{a}	7.0 ± 4.7^{b}	7.9 ± 5.0^{a}
Protein Cupcake	-5.8 ± 3.3		-0.4 ± 4.7^{a}	-11.0 ± 4.0^{b}	-3.7 ± 4.9	9.2 ± 4.7	11.7 ± 5.1

Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) within a row (i.e. age YA vs OA and saliva flow pairwise comparisons) are denoted by differing small letters, and within a column (i.e. within an age group between samples or within saliva flow groupings between samples) are denoted by differing capital letters. Appetite ratings were measured on a VAS (0-100 mm) and reflect a change from baseline (positive/negative values relate to the specific appetite rating being measured, for example, a negative hunger rating represents a decline in hunger). Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertile analysis.

4.6. Discussion

4.6.1. Sensory profile, physical properties, perception and liking of products

Protein fortification of cakes and biscuits was associated with two key sensorial issues, namely mouthdrying and flavour, as well as with reduced acceptability and liking of products, compared with the control versions. Milk proteins are considered to contribute to viscosity, structure and mouthfeel of dairy products and peptides and amino acids provide bases for volatile aroma active compounds (Drake *et al*., 2009). Taste can be negatively impacted by aromatic amino acids, particularly when using high quality protein

powders, and this provides additional challenges when increasing protein content in products (Munk *et al*., 2014). However, fortifying foods with whey protein has been successfully achieved with positive results, for example, fortifying tomato sauces with WPI resulted in increased liking, when compared with the control, in healthy older adults (Tsikritzi *et al*., 2015).

The negative impact of protein fortification is likely to be explained by the differences in physical properties following fortification, such differences having been previously identified (Gallagher *et al*., 2005; Diaz-Ramirez *et al*., 2016). In the pilot study protein fortification led to a significant reduction in product moisture content compared with control. This was addressed in the main study where the control and protein cupcake formulations were adjusted in order to achieve the same post-baking moisture content. It can, therefore, be assumed that any differences in perception and liking were not related to differences in final moisture content. In addition, water activity in the cakes was broadly similar, though the protein fortification of biscuits did significantly reduce water activity. Despite this, volunteers rated all protein fortified products to be significantly less moist and to cause more mouthdrying, in addition to the lingering mouthdrying evident from the sensory profile. Protein biscuits were also perceived to be significantly harder; this was supported by the texture analyser results which showed significantly increased hardness and fracturability scores. Mouthfeel attributes from the sensory profile, such as chewiness and firmness of bite, were significantly higher in the protein cakes, again supported by results from the texture analyser. Both the sensory profiling panel and the volunteers found the protein fortified products to be less sweet, the trained panel additionally concluding that vanilla and lemon flavours were reduced. Protein fortification led to cakes and cupcakes that were substantially less yellow in colour (as rated by the sensory panel) and biscuits that were significantly darker and redder (as measured instrumentally).

However, the colour differences between the control and protein versions had no effect on the mean liking of product appearance. These results are generally supported by previous studies; for example, Tsikritzi *et al*. (2014; 2015) noted that whey protein fortification can result in undesirable texture and flavours.

There was evidence of Maillard reactions which can influence the colour and flavour of products (Gallagher *et al*., 2005), as such protein fortification of biscuits (products low in moisture and water activity content) led to more colour development (darker and redder) from increased Maillard reactions due to the increase in available amino groups. In addition, there are differences in water absorption between flour and dairy powders, implying less water activity available, further contributing to more optimum conditions for the Maillard reaction (Gallagher *et al*., 2005). The rate of Maillard reaction is considered dependent on water activity, temperature and level of precursors (i.e. reducing sugars and available amino acid groups) (Higgs & Boland, 2009). Furthermore, within the protein cakes, it is likely that WPI/WPC contributed to more air bubbles being trapped within the batter, accordingly, increasing the cake volume (Diaz-Ramirez *et al*., 2016). Whey proteins can also become unstable when heated, resulting in protein denaturation and aggregation, influencing both the structure and the stability of the protein (Wijayanti *et al*., 2014). Denaturation of proteins during the baking process is considered to influence the protein's interactions, elasticity, binding sites and flavour, leading to changes in the final product (Kuhn *et al*., 2007; Drake *et al*., 2009; Diaz-Ramirez *et al*., 2016).

Proteins are reported to interact with flavour compounds by releasing and binding to them and thereby changing perception of the product flavour (Heng *et al*., 2004). This could explain the blunting and reduced intensity of flavour perceived by both the trained panel and the volunteers, as well as the number of comments made as to blandness and lack of flavour from the volunteers. In addition, whey proteins are rich in sulfur amino acids

and upon heating can release sulfurous and eggy aromas which influence overall flavour (Higgs & Boland, 2009; Drake *et al*., 2009). These effects could potentially explain the off-flavours (such as, eggy, rancid, fatty and sulfate flavours) identified by the trained panel after consumption of the protein products; therefore, are potential contributors to the reduced acceptability of products. It should be noted that these off-flavours were more evident in the cakes and biscuit in the pilot study; however, still evident in the protein cupcakes in the main study, but to a lesser extent. It is, therefore, likely that the addition of lemon zest had a positive impact on both flavour and acceptability in the main study compared with the cakes used in the pilot study. Future research needs to focus on understanding the causes of off-flavours, as well as texture differences between the control and protein versions, and how these influence the acceptability of the final product.

4.6.2. Individual differences in perception and liking of products

Changes in oral impairments and sensory sensitivity are commonly associated with ageing and are considered to influence food consumption (Vandenberghe-Descamps *et al*., 2017). Our study demonstrated that individuals with reduced dental status reported significantly lower liking and easiness to eat and swallow scores in the main study only, where predominately only older adults had reduced dental status. Accordingly, these findings are supportive of developing foods appropriate for older adults, for example those with reduced dental status. Jeltema *et al*. (2015; 2016) proposed that mouth behaviour can also influence food choice, texture preference and satisfaction. However, both studies demonstrated that individual differences in mouth behaviour had no effect on volunteers' ratings of products, apart from appearance scores for cakes and biscuits in the pilot study. Therefore, regardless of mouth behaviour type it can be assumed that volunteers rate perception and liking to the same extent. The influence of age was less

clear on individual perception and liking, for example, there was no overall effect of age. However, older adults perceived the protein versions, regardless of the food type, to cause more mouthdrying compared with the control versions. This was despite using two different methods to measure perception and liking: in the pilot study using a single point in time and in the main study using a full portion size at home. These findings did support previous work in a liquid model system, where older adults were shown to have a greater sensitivity to mouthdrying (Withers *et al*., 2013a).

Our study showed that older adults demonstrated a significantly lower unstimulated salivary flow rate, compared with younger adults, supporting the findings of Vandenberghe-Descamps *et al*. (2016) that salivary flow rates decrease with age. However, our study demonstrated no age-related differences in stimulated salivary flow rates, which was consistent with the findings in our previous work (Norton *et al*., 2020a, **Chapter 3**). Furthermore, it is proposed that age-related changes in salivary flow rates are gland specific, with the parotid and minor salivary glands potentially being less influenced by age (Affoo *et al*., 2015). Saliva plays a key role within our eating experience (Mosca & Chen, 2017); accordingly, it was expected that a reduced saliva flow could influence perception and liking of products. However, the trends proved inconsistent and varied depending on the product type, and typically volunteers perceived the differences to the same extent despite their differences in saliva flow. For example, as expected, perceived mouthdrying intensity decreased with increasing salivary flow rates with the protein biscuits, which are a harder, drier and a less moist product. It is recognised that saliva performs a key role in ensuring that the food bolus is moist and lubricated so that the product can be safety consumed (Engelen *et al*., 2005). Reduced saliva flow is associated with poor oral clearance (Turner & Ship, 2007) and accordingly food particles are more likely to linger within the mouth, thereby increasing perception of mouthdrying

in relation to the biscuits. However, perceived mouthdrying intensity increased with increasing salivary flow rates following the protein cupcake; this was a surprisingly trend, given that cupcakes are a softer and smoother product. This latter finding is, however, supported by our previous work in a whey protein beverage model (Norton *et al*., 2020a, **Chapter 3**) where we suggested a potential hydration mechanism associated with mucoadhesion, with the lubrication abilities of saliva strengthening adhesion properties and resulting in an increased perception of mouthdrying (Cook *et al*., 2017). The results of the current study, which were different between biscuits and cakes, might indicate that the effect of saliva flow on the perception of mouthdrying, and the underlying mechanism, will be dependent on factors in addition to protein content, such as the structure and moisture content of the food. For example, the role of food particle size needs to be considered and understood (van Vliet *et al*., 2009). Within a solid system, such as cakes and biscuits, saliva plays a role in determining whether a food particle will aggregate or adhere to the oral mucosa, with the latter increasing friction and influencing sensory perception (Lucas *et al*., 2004). It is proposed that mucoadhesion strength should be greater within a solid model, compared with a liquid system (Cook *et al*., 2017).

Given the derived benefits from ONS and protein fortified products, it is important to understand the causes of poor compliance and consumer acceptance, which have been associated with mouthdrying and off-flavours. Accordingly, appetite ratings, such as how hunger and thirst influence sensory perception and consumption of products, is of particular relevance (Thomas *et al*., 2018). Thomas *et al*. (2018) demonstrated that multiple sips of ONS can increase thirst during consumption and also correlated this with increased drying sensations. Our study also demonstrated that protein cupcakes increased perception of mouthdrying and thirst.

4.6.3. Limitations

The main limitations of this study relate to sex and health. First, the study identified sex related effects; however, it should be noted that the study had a sex imbalance, particularly in the younger adults. Second, studies with older adults should have sufficient sample size to allow for the diverse nature of older adults within a group described as 'healthy' and to ensure sufficient power between age groups (Methven *et al.*, 2016). Despite aiming for healthy older adults with minimal medication use, the studies presented in this paper include volunteers of differing age, medication use, level of impairment (physical, visual and hearing) and previous experience of sensory studies. All these additional factors are likely to have influenced individual perception of a product, in addition to any salivary flow changes associated with age.

4.7. Conclusion

Protein fortification of cakes and biscuits significantly increased perceived mouthdrying, hardness and off-flavours and significantly reduced melting rate, moistness and liking, compared with the control versions. Such intensity and direction of attributes are likely to have contributed to dislike of and poor compliance with products and indicate the need for reformulation of the products to ensure product suitability for older adults. Consumption of simple and familiar snacks, such as cakes and biscuits, can help to alleviate malnutrition and sarcopenia; however, they clearly need to be acceptable and palatable. Individual differences (such as age, mouth behaviour, dental status, saliva flow and appetite) were expected to play a greater role in perception and liking of products; trends were, however, present but inconclusive, indicating the need for further research into the impact of these individual differences. Further investigation remains necessary to understand the causes of mouthdrying resulting from solid food models and to establish

whether there is a link between mouthdrying and mucoadhesion. In addition, there would be a clear benefit for older adults at risk of malnutrition and sarcopenia were these effects (mouthdrying and off-flavours) to be mitigated.

S.4. Supplementary

S.4.1. Additional study data

Table S.4.1. Cake and biscuit (g per 100 g) ingredients formations in both studies.

Ingredients	Cakes		Biscuits		Cupcakes	
	Control	Protein	Control	Protein	Control	Protein
Sainsburys self raising flour	21.9	20.6	-	-	23.0	23.0
Dr Oetker baking powder	1.1	1.0	0.8	0.8	-	-
Sainsburys Woodland free-range eggs	21.9	20.6	-	-	18.6	18.6
Sainsburys British whole milk	9.8	9.2	-	-	5.0	5.0
Sainsburys English unsalted soft butter	21.9	20.6	-	-	23.0	23.0
Sainsburys white caster sugar	21.9	20.6	-	-	23.0	23.0
Dr Oetker Madagascan vanilla extract	1.1	1.0	-	-	-	-
Volac whey protein isolate	-	6.1	-	7.5	-	-
Volac whey permeate	-	-	-	-	6.6	-
Volac whey protein concentrate	-	-	-	-	-	6.6
Lemon zest (Sainsburys unwaxed lemons)	-	-	2.1	1.9	0.8	0.8
Silbury Cream 64	-	-	26.9	24.9	-	-
Sainsburys light soft brown sugar	-	-	21.3	19.6	-	-
Sainsburys British plain flour	-	-	19.9	18.4	-	-
Sainsburys Scottish porridge oats	-	-	19.9	18.4	-	-
Water	-	-	8.5	7.9	-	-
Dr Oetker glycerine	-	-	0.3	0.3	-	-

Age was significantly associated ($p < 0.0001$) with medication in both studies, where only older adults reported regular medication use (Table 4.5 in the results section). However, the influence of medication on saliva flow in older adults varied between the studies as outlined in Figure S.4.1. Accordingly, histogram analysis was carried out to understand better the distribution of the data and this revealed data in the pilot study was centered more towards the left-hand side and more spread over a greater range, whilst in the main study the data was more compact within a similar range, as demonstrated in Figure S.4.2. Therefore, it could be suggested that volunteers lacked experience in saliva collection hence the lower saliva flow rates, whereas in the main study volunteers were more familiar with saliva collection leading to higher salivary flow rates and a proposed rationale for a significant effect of medication on unstimulated saliva flow in the main study. As highlighted in our previous work (Norton *et al.*, 2020a, **Chapter 3**) if volunteers are unfamiliar with saliva collection, a familiarisation session could be beneficial. It should be

noted that each volunteer's medication was screened for potential side effects likely to influence saliva flow; therefore, it was considered medication was unlikely to have caused an increase in saliva flow in this case. In addition, there was an imbalance of numbers of volunteers taking medication in the main study compared with the pilot study. During the pilot study less than half (19 out of 42 older adults) of the volunteers were taking medication, whereas in the main study it was more than half (18 out of 32 older adults).

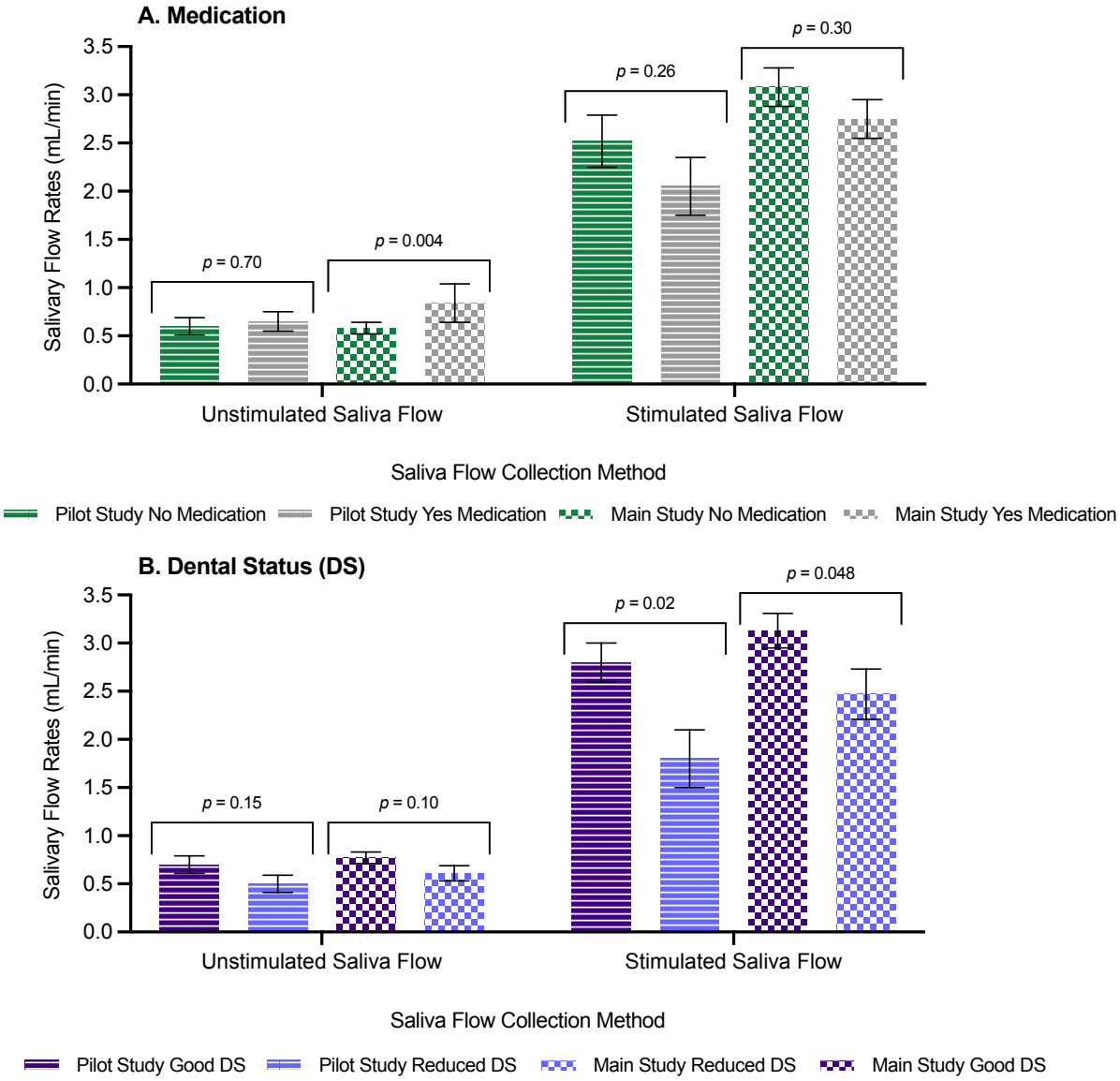

Figure S.4.1. Additional factors influencing older adults salivary flow rates (mL/min) in both studies (*pilot study n = 42; main study n = 32*). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) were reported between groups with relevant *p* value above each group.

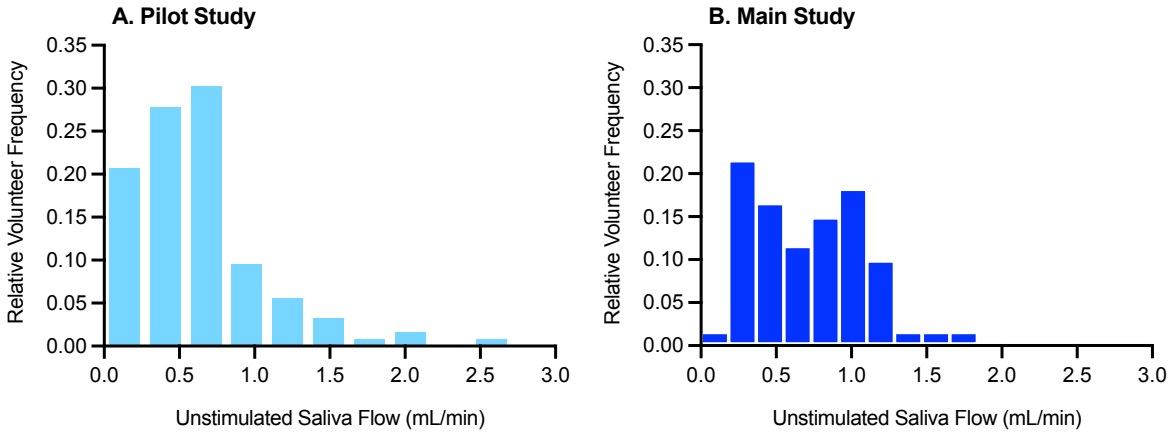

Figure S.4.2. Histograms of older adults unstimulated saliva flow (mL/min) in both studies *(pilot study n = 42; main study n = 32*).

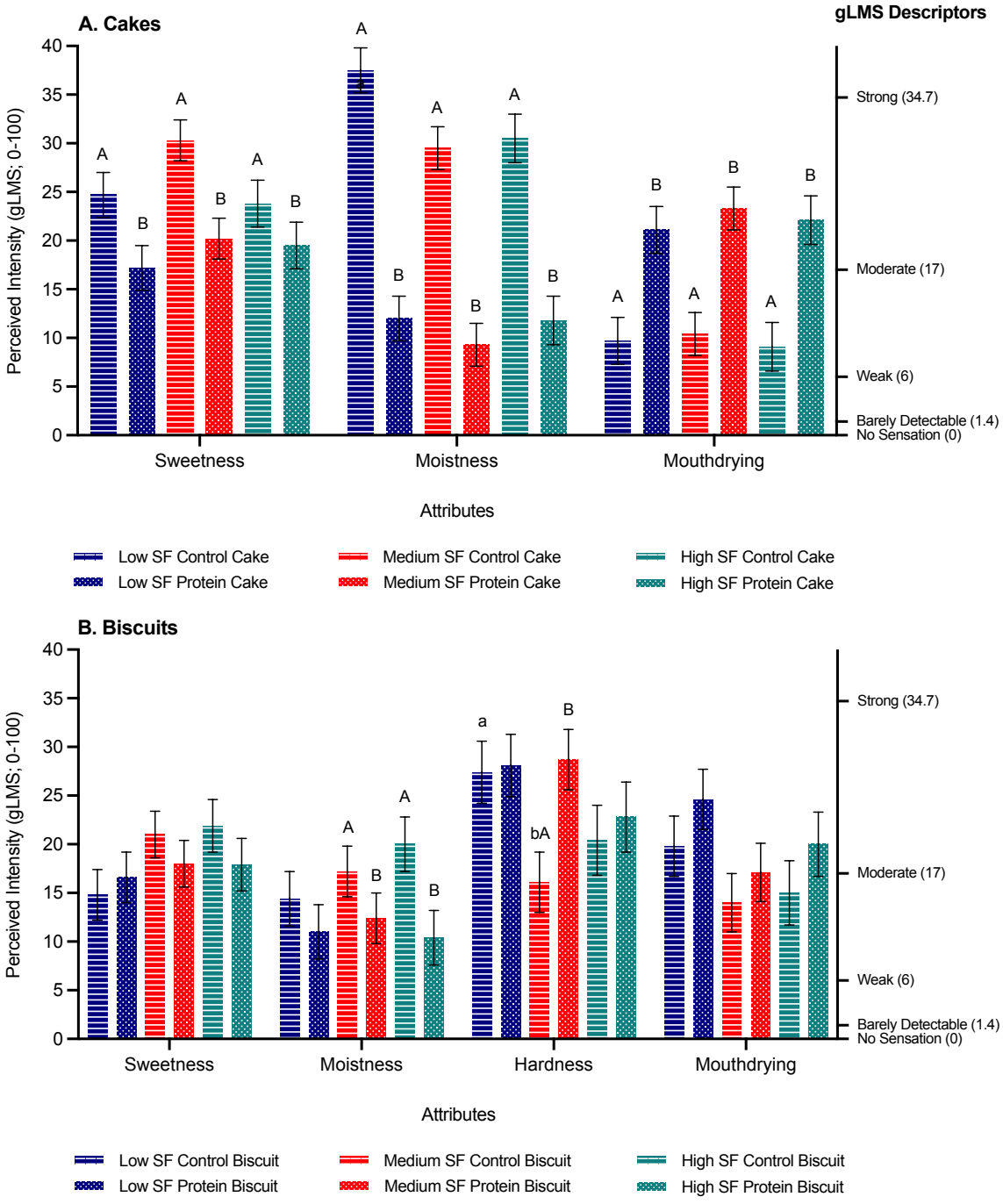

Figure S.4.3. Volunteers attribute perception mean ratings of (**A**) cakes[46] and (**B**) biscuits by saliva flow (SF) (pilot study: *n* = 84; gLMS antilogged data, scale 0-100 summarised on the right of the figure). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) between saliva flow groups within sample type are denoted by differing small letters and significant differences between samples within saliva flow groupings are denoted by differing capital letters; no letter reflects no significance difference. Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertiary analysis (*low saliva flow n = 27; medium saliva flow n = 28; high saliva flow n = 29*).

[46] cakes were measured in duplicate at visit one and two

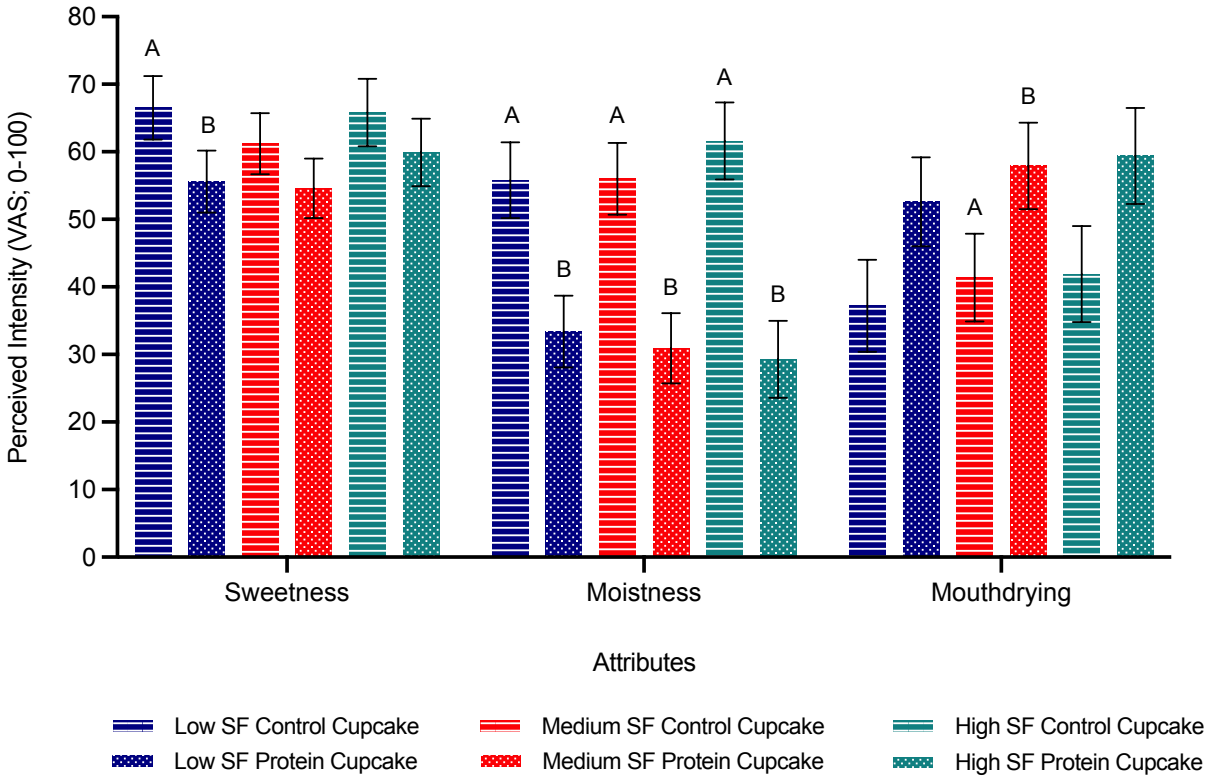

Figure S.4.4. Volunteers attribute perception mean ratings of cupcakes by saliva flow (SF) (main study: *n* = 70; VAS, 0-100 mm). Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) between saliva flow groups within sample type are denoted by differing small letters and significant differences between samples within saliva flow groupings are denoted by differing capital letters; no letter no significance difference. Individual saliva flow groupings are derived from unstimulated saliva flow only, through tertiary analysis (*low saliva flow n = 23; medium saliva flow n = 23; high saliva flow n = 24).*

Table S.4.2. Additional factors influencing volunteers liking, easiness to eat and swallow, attribute perception, appropriateness of attribute level (Just-About-Right, JAR) and appetite of products in both studies (PS[1]: pilot study: cakes & biscuits; MS[2]: main study: cupcakes).

	Medication		**Dental Status**		**Mouth behaviour**			**Sex**		**Visit**	
	No PS (*n* = 65) MS (*n* = 52)	Yes PS (*n* = 19) MS (*n* = 18)	Good PS (*n* = 64) MS (*n* = 59)	Reduced PS (*n* = 20) MS (*n* = 11)	Chewer PS (*n* = 42) MS (*n* = 29)	Cruncher PS (*n* = 33) MS (*n* = 25)	Other PS (*n* = 9) MS (n = 13)	Male PS (*n* = 31) MS (*n* = 27)	Female PS (*n* = 53) MS (*n* = 43)	One PS (*n* = 84) MS (n/a)	Two PS (*n* = 82) MS (n/a)
Appearance Liking[1,2]											
Cake[1]	6.7 ± 0.1	6.8 ± 0.2	6.8 ± 0.1	6.8 ± 0.2	6.9 ± 0.1	6.3 ± 0.2*	7.0 ± 0.3	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.1	6.8 ± 0.1
Biscuit[1]	5.8 ± 0.2	5.7 ± 0.4	5.9 ± 0.2	5.6 ± 0.3	6.0 ± 0.2	5.3 ± 0.2*	5.9 ± 0.4	5.3 ± 0.2	6.1 ± 0.2	-	-
Cupcake[2]	63.1 ± 5.2	54.9 ± 5.4	60.1 ± 3.5	57.9 ± 7.1	54.1 ± 4.1	62.0 ± 4.6	60.9 ± 5.5	61.3 ± 4.4	56.8 ± 3.9	-	-
Overall Liking[1,2]											
Cake[1]	6.2 ± 0.2	5.4 ± 0.3*	5.7 ± 0.2	5.9 ± 0.3	6.0 ± 0.2	5.7 ± 0.2	5.7 ± 0.4	5.9 ± 0.2	5.8 ± 0.2	5.8 ± 0.2	5.9 ± 0.2
Biscuit[1]	5.9 ± 0.2	5.7 ± 0.2	5.6 ± 0.2	5.9 ± 0.3	5.7 ± 0.2	5.6 ± 0.3	6.1 ± 0.5	5.7 ± 0.2	5.8 ± 0.3	-	-
Cupcake[2]	59.9 ± 5.7	56.7 ± 5.8	65.5 ± 3.8	51.1 ± 7.8*	50.4 ± 4.5	61.7 ± 5.0	62.8 ± 6.0	60.2 ± 4.9	56.3 ± 4.2	-	-
Easiness to Eat[1,2]											
Cake[1]	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.2	3.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.6 ± 0.1*
Biscuit[1]	3.6 ± 0.1	3.2 ± 0.2	3.5 ± 0.1	3.3 ± 0.2	3.4 ± 0.1	3.3 ± 0.1	3.5 ± 0.2	3.3 ± 0.1	3.4 ± 0.1	-	-
Cupcake[2]	56.5 ± 5.3	60.1 ± 5.4	73.0 ± 3.5	43.6 ± 7.1*	52.7 ± 4.1	60.4 ± 4.6	61.8 ± 5.5	55.6 ± 4.5	61.0 ± 3.9	-	-
Easiness to Swallow[1,2]											
Cake[1]	3.6 ± 0.1	3.4 ± 0.2	3.4 ± 0.1	3.5 ± 0.2	3.6 ± 0.1	3.4 ± 0.1	3.4 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.3 ± 0.1*
Biscuit[1]	3.6 ± 0.1	3.2 ± 0.2	3.4 ± 0.1	3.4 ± 0.2	3.4 ± 0.1	3.3 ± 0.1	3.5 ± 0.2	3.2 ± 0.1	3.6 ± 0.1	-	-
Cupcake[2]	54.2 ± 4.7	57.9 ± 4.9	68.6 ± 3.1	43.6 ± 6.4*	50.5 ± 3.7	57.5 ± 4.1	60.1 ± 4.9	52.3 ± 4.0	59.8 ± 3.4	-	-
Sweetness[1,2]											
Cake[1]	21.0 ± 1.4	24.3 ± 2.5	21.7 ± 1.6	23.5 ± 2.3	21.1 ± 1.7	22.3 ± 1.8	24.4 ± 3.1	22.0 ± 2.0	23.2 ± 1.8	22.6 ± 1.7	22.7 ± 1.7
Biscuit[1]	18.3 ± 1.5	18.4 ± 2.7	19.9 ± 1.7	16.9 ± 2.5	16.6 ± 1.8	19.9 ± 2.0	18.7 ± 3.3	18.2 ± 2.1	18.5 ± 1.9	-	-
Cupcake[2]	65.0 ± 4.4	56.2 ± 4.6	61.6 ± 3.0	59.7 ± 6.0	56.2 ± 3.5	62.0 ± 3.9	63.6 ± 4.7	62.0 ± 3.8	59.1 ± 3.3	-	-
Moistness[1,2]											
Cake[1]	22.4 ± 1.4	21.5 ± 2.5	22.8 ± 1.6	20.8 ± 2.3	23.3 ± 1.7	21.0 ± 1.8	20.9 ± 2.9	22.1 ± 2.0	21.4 ± 1.7	21.9 ± 1.7	21.7 ± 1.7
Biscuit[1]	14.6 ± 1.7	14.0 ± 2.9	14.9 ± 1.8	13.7 ± 2.7	11.6 ± 2.0	14.2 ± 2.1	17.0 ± 3.7	14.4 ± 2.2	14.0 ± 2.0	-	-
Cupcake[2]	49.6 ± 4.7	39.3 ± 4.9	43.3 ± 3.1	45.7 ± 6.4	44.4 ± 3.7	43.6 ± 4.1	45.6 ± 4.9	44.7 ± 4.0	44.3 ± 3.4	-	-
Mouthdrying[1,2]											
Cake[1]	13.2 ± 1.4	18.8 ± 2.5*	18.1 ± 1.6	13.9 ± 2.3	15.7 ± 1.7	15.7 ± 1.8	16.7 ± 3.1	17.3 ± 2.0	14.6 ± 1.8	15.7 ± 1.7	16.3 ± 1.7
Biscuit[1]	20.5 ± 2.0	16.4 ± 3.4	20.5 ± 2.1	16.8 ± 3.2	18.4 ± 2.3	17.5 ± 2.5	19.4 ± 4.2	18.7 ± 2.7	18.2 ± 2.4	-	-
Cupcake[2]	43.3 ± 6.0	53.4 ± 6.1	44.8 ± 4.0	52.0 ± 8.1	48.4 ± 4.7	46.8 ± 5.2	50.0 ± 6.2	54.4 ± 5.1	42.3 ± 4.4	-	-
Hardness[1]											
Biscuit[1]	22.8 ± 1.9	25.1 ± 3.1	25.6 ± 2.0	22.2 ± 3.0	25.3 ± 2.2	22.7 ± 2.3	23.8 ± 4.3	24.0 ± 2.6	23.8 ± 2.4	-	-
JAR Flavour[1]											
Cake[1]	2.7 ± 0.07	2.6 ± 0.1	2.6 ± 0.08	2.7 ± 0.1	2.7 ± 0.08	2.8 ± 0.08	2.7 ± 0.1	2.7 ± 0.09	2.7 ± 0.08	2.7 ± 0.08	2.7 ± 0.08
Biscuit[1]	2.6 ± 0.09	2.6 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	-	-

Table S.4.2. continued…

	Medication		Dental Status		Mouth behaviour			Sex		Visit	
	No PS (*n* = 65) MS (*n* = 52)	Yes PS (*n* = 19) MS (*n* = 18)	Good PS (*n* = 64) MS (*n* = 59)	Reduced PS (n = 20) MS (*n* = 11)	Chewer PS (*n* = 42) MS (*n* = 29)	Cruncher PS (*n* = 33) MS (*n* = 25)	Other PS (*n* = 9) MS (*n* = 13)	Male PS (*n* = 31) MS (*n* = 27)	Female PS (*n* = 53) MS (*n* = 43)	One PS (*n* = 84) MS (n/a)	Two PS (*n* = 82) MS (n/a)
JAR Colour[1]											
Cake[1]	2.9 ± 0.03	2.9 ± 0.06	2.9 ± 0.04	3.0 ± 0.06	3.0 ± 0.04	2.9 ± 0.04	2.8 ± 0.08	2.9 ± 0.04	2.9 ± 0.04	2.9 ± 0.04	2.9 ± 0.04
Biscuit[1]	2.9 ± 0.08	2.8 ± 0.1	2.9 ± 0.09	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	-	-
Appetite[2]											
Hungry[2]	-13.8 ± 4.7	-10.1 ± 5.0	-14.7 ± 3.1	-9.2 ± 6.4	-10.3 ± 3.7	-14.0 ± 4.2	-11.5 ± 4.9	-12.6 ± 4.1	-11.3 ± 3.4	-	-
Thirsty[2]	12.0 ± 5.2	13.0 ± 5.5	6.5 ± 3.4	18.4 ± 7.2	11.3 ± 4.1	11.0 ± 4.8	14.9 ± 5.4	13.0 ± 4.6	11.9 ± 3.9	-	-
Desire[2]	-12.8 ± 4.7	-21.1 ± 5.0	-22.1 ± 3.1	-11.9 ± 6.6*	-17.5 ± 3.7	-12.7 ± 4.2	-20.7 ± 4.9*	-13.1 ± 4.1	-20.8 ± 3.5*	-	-
Satiety[2]	8.6 ± 5.3	5.6 ± 5.6	13.4 ± 3.5	0.82 ± 7.3	6.8 ± 4.2	6.0 ± 4.8	8.6 ± 5.6	1.3 ± 4.7	12.9 ± 4.0	-	-
Fullness[2]	10.8 ± 4.9	7.0 ± 5.1	14.0 ± 3.2	3.9 ± 6.8	9.7 ± 3.9	9.8 ± 4.4	7.1 ± 5.1	5.5 ± 4.3	12.2 ± 3.6	-	-
PC[2]	-5.5 ± 4.1	-4.5 ± 4.3	-11.4 ± 2.8	-1.4 ± 5.7*	-4.6 ± 3.2	-3.6 ± 3.8	-6.8 ± 4.3	-0.29 ± 3.7	-10.2 ± 3.0	-	-

Values are expressed as LSM estimates ± standard error from SAS output. Significant differences ($p < 0.05$) between categories are denoted by *. Pilot study[147] (PS) $n = 84$; cake and biscuit liking, easiness to eat and swallow and JAR measured on a 9-point and 5-point scale and attribute perception was measured on a gLMS logarithmic scale (antilogged values 0-100 scale presented). Main study[2] (MS) $n = 70$; cupcakes were measured on a VAS 0-100 mm and appetite ratings reflect a change from baseline (positive/negative values relate to the specific appetite rating being measured, for example, a negative hunger rating represents a decline in hunger). PC: prospective consumption. Visit only applied to the pilot study cakes (n/a denotes not applicable) and appetite only applied to cupcakes during the main study. Mouth behaviour 'other' reflects smooshers/sucker in the pilot study and smooshers in the main study.

[47] pilot study cake data was measured in duplicate at visit one and two

Table S.4.3. Volunteer counts of cake and biscuit preference in the pilot study.

	Cake		Significance of sample (*p* value)	Biscuit		Significance of sample (*p* value)
	Control	Protein		Control	Protein	
Total (*n* = 84)	144	22	**< 0.0001**	52	32	**0.02**
Younger Adults (*n* = 42)	66	16	**< 0.0001**	22	30	0.44
Older Adults (*n* = 42)	78	6	**< 0.0001**	30	12	**0.004**

Cakes were measured in duplicate at visit one and two (*n* = 166; YA: *n* = 82; OA: *n* = 84 (where two YA dropped out after visit one)). *Data was obtained from a 2-AFC test to assess most preferred.*

Table S.4.4. Summary of volunteers comments in both studies.

	Flavour Related Comments									Texture Related Comments								
	Positive			Negative			No Comments Provided			Positive			Negative			No Comments Provided		
	Total	YA	OA	Total	YA	OA	Total	YA	OA	Total	YA	OA	Total	YA	OA	Total	YA	OA
Control Cake[1]	85	33	52	26	11	15	55	38	17	101	38	63	17	10	7	48	34	14
Protein Cake[1]	29	17	12	81	26	55	57	39	18	32	7	25	94	52	42	40	24	19
Control Biscuit[1]	34	10	24	22	14	8	28	10	18	38	14	24	14	7	7	32	21	11
Protein Biscuit[1]	9	2	7	46	22	23	29	12	17	18	9	9	33	14	19	33	19	14
Control Cupcake[2]	59	33	26	4	1	3	3	2	1	34	19	15	28	14	14	4	3	1
Protein Cupcake[2]	40	26	14	23	7	16	4	4	0	20	13	7	42	19	23	5	5	0

Positive refers to good, Just-About Right (JAR), nice, pleasant, tasty, fresh, soft, vanilla or lemony flavour and negative refers to weak flavour, dry, disliked, hard, rough, coarse, dense and sticky. Pilot study[1] (cakes and biscuits) *n* = 84; younger adults (YA): *n* = 42; older adults (OA): *n* = 42 and main study[2] (cupcakes) *n* = 70; YA: *n* = 38; OA: *n* = 32. During the pilot study[1] cakes were measured in duplicate at visit one and two (*n* = 166; OA: *n* = 84; YA: *n* = 82 (where two YA dropped out after visit one)).

Chapter 5

Whey protein derived mouthdrying found to relate directly to retention post consumption but not to induced differences in salivary flow rate

5.1. Context to chapter

Due to the ongoing COVID-19 pandemic, all future work had to be adapted as older adults were classified as high risk and accordingly, we were no longer able to carry out studies with older adults on campus. Therefore, all future studies with older adults had to be simplified and modified to ensure suitability to be conducted at home. However, a study with younger adults on campus was considered possible with careful planning and appropriate risk assessments during latter part of 2020. As such the focus of this chapter was to improve methods to investigate mouthdrying and it is hoped in the future these can be applied to older adults. More specifically, **Chapter 3** had established whey protein adheres to the oral cavity post whey protein beverage (WPB) consumption. Such a phenomenon had not previously been directly linked to perceived mouthdrying within the same experiment. In addition, WPBs were associated with low liking scores in **Chapter 3**; therefore, in this chapter sample palatability was improved and its subsequent role on reducing perceived mouthdrying was tested. Moreover, minimal effects relating to saliva flow and sensory perception were present in **Chapters 3 and 4**; accordingly, saliva flow was modulated via two conditions (decrease versus increase) to understand its effects on perceived mouthdrying post beverage consumption. Therefore, this chapter aims to investigate four overall thesis hypotheses: (a) whey protein fortified beverages will cause mouthdrying; (b) whey protein will adhere to the oral cavity post WPB consumption; (d) mucoadhesion is a probable cause of whey protein derived mouthdrying; and (e)

individual differences (such as saliva flow) will influence perceived whey protein derived mouthdrying. Accordingly, these hypotheses were tested via the following objectives:

- Do consumers perceive whey protein fortified liquid models as mouthdrying compared with non-protein whey control?
- Does whey protein adhere to the oral cavity more than a non-protein whey control in WPBs? More specifically in this chapter: does sweetness impact the ability of whey protein to adhere to the oral cavity post WPB consumption?
- Does whey protein retention post WPB consumption relate to perceived mouthdrying in WPBs?
- Do salivary flow rates relate to perceived mouthdrying intensity in whey protein liquid models? More specifically in this chapter: does modulating saliva flow alter perceived mouthdrying post WPB consumption?

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5.2. Abstract

Whey protein is fortified into beverages to provide functional benefits; however, these beverages are considered mouthdrying. To date whey protein derived mouthdrying has not been quantified using a 'physical measure' in parallel with rated perception. Saliva flow could also relate to whey protein derived mouthdrying; however, this has not been previously tested as an intervention. Accordingly, volunteers (*n* = 40) tested mouthdrying in different whey beverages and the sensory profile was evaluated by a trained sensory panel (*n* = 10). Volunteers also rated mouthdrying combined with collection of saliva samples post beverage consumption to measure retention to the oral cavity. To modulate saliva flow rate, volunteers both chewed on parafilm (to increase saliva flow) and used cotton wool (to remove saliva) before tasting beverages and rating mouthdrying. Both the volunteers and sensory panel rated whey protein beverages (WPB) as significantly more mouthdrying than the control beverage (whey permeate). The significantly higher rating of mouthdrying from the volunteers coincided with significantly higher protein concentration in saliva samples post WPB consumption, supporting mucoadhesion as the mechanism. Modulating saliva flow did not lead to any difference in rated mouthdrying and future work would be beneficial to evaluate further the influence of natural variation in salivary flow rate.

Keywords: protein fortified foods; mouthdrying; older adults; whey protein; saliva flow

5.3. Introduction

Protein needs are suggested to increase with age (1.0-1.2 g/kg/d) (Bauer *et al*., 2013) despite the current UK reference nutrient intake (RNI) for adults only being 0.75 g/kg/d (Department of Health, 1991). Accordingly, there is an increasing emphasis on improving protein intake across the lifespan to offset potentially associated health conditions, slow

the rate of muscle decline and promote healthy ageing (Stevenson *et al*., 2018; 2019). Protein consumption is also associated with a number of positive benefits, such as improved health outcomes, appetite regulation, weight management and enhanced sports performance (Bauer *et al*., 2013; Philips *et al*., 2016). Oral nutritional supplements (ONS) and protein fortified products are often used to improve energy and protein intake especially in older adults. Whey protein is commonly fortified into these products due its associated functional benefits, such as higher leucine content and quicker digestion and absorption kinetics (Pennings *et al*., 2011). Products need to be an appropriate portion size, energy dense, palatable, energy dense and appetising to increase successfully nutritional intake (Nieuwenhuizen *et al*., 2010).

Despite the widespread recognised benefits of ONS consumption, product compliance and consumption of adequate product to meet individual needs are considered limiting factors in maximising such benefits, together with related cost and waste ramifications (Gosney, 2003; Nieuwenhuizen *et al*., 2010; Hubbard *et al*., 2012). In addition, product palatability (for example, appearance, aroma, flavour and texture) can be a key driver of product acceptability by consumers (Stevenson *et al*., 2018). More specifically, texture is suggested to provide a key role in food preferences, where texture awareness can relate to product expectation (Szczesniak & Kahn, 1971). This is particularly relevant for dairy products as mouthfeel attributes are commonly associated with product dislike, typically build with repeated consumption and are challenging to define (Gosney, 2003; Methven *et al*., 2010; Withers *et al*., 2014; Thomas *et al*., 2016; 2018; Bull *et al*., 2017). Food and beverage matrices fortified with whey protein have also resulted in negative mouthfeel attributes such as mouthdrying, hardness, slower melt rate, teeth packing, increased crumb size, chalky, grainy, rough, mouthcoating and dense (Tsikritzi *et al*., 2014; 2015; Bull *et al*., 2017; Norton *et al*., 2020b, **Chapter 4**). Previously, our research group

proposed whey permeate (a deproteinised whey powder) as a suitable non-protein whey control to fortify cakes and beverages and to provide comparisons with whey protein fortification in order to investigate mouthdrying and mucoadhesion respectively (Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**). However, other previous studies into mouthdrying within whey protein beverage (WPB) models have typically been carried out without a non-protein control and are therefore limited in that they unable to prove if the protein within WPB is causing the mouthdrying. Understanding and addressing these proposed causes of poor compliance is key to maximising benefits from such products.

Dairy products have been associated with a 'textural defect' (Lemieux & Simard, 1994) often referred to as astringency, drying and mouthdrying. However, astringency is as "a result of exposure to substances such as alums or tannins" (ASTM E253-20) which are not usually present in whey protein. Accordingly, the term mouthdrying (a drying sensation in the mouth during or after consumption of a product) is considered more appropriate in the context of dairy products. The proposed causes of such whey protein derived mouthdrying remain unconfirmed and form part of our current investigation (Norton *et al*., 2020a, **Chapter 3**). Our research group proposed mucoadhesion as a probable cause of whey protein derived mouthdrying (especially from neutral pH WPB); however, further proof is required.

Mucoadhesion has been studied in drug delivery and food systems (Smart, 2005; Andrews *et al*., 2009; Carvalho *et al*., 2010; Khutoryanskiy, 2011; Cook *et al*., 2017) and is considered in the context of this paper as the binding or sticking (retention) of whey proteins to the oral cavity (Bull *et al*., 2017). Recently, our research group demonstrated that protein is considered to adhere to the oral cavity (mucoadhesion) to a greater extent post WPB consumption compared with a whey permeate beverage (WPeB), and mucoadhesion is considered to increase with age (Norton *et al*., 2020a, **Chapter 3**).

Despite establishing a valid 'physical measure' to measure mucoadhesion (Norton *et al*., 2020a, **Chapter 3**), a potential limitation of this previous study was that the link between mucoadhesion and mouthdrying within the same method was not investigated.

Saliva is associated with a number of key functions, such as lubrication, food clearance, taste, mouthfeel, digestion and oral health (Carpenter, 2013). In addition, salivary flow rates are considered to reduce with age (Vandenberghe-Descamps *et al*., 2016) and could alter sensory perception (Fischer *et al*., 1994; Engelen *et al*., 2003a; Nayak & Carpenter, 2008; Vandenberghe-Descamps *et al*., 2017; Mosca & Chen, 2017; Munoz-Gonzalez *et al*., 2018a). However, previous research into this has so far been relatively inconclusive as regards the effect on subsequent perception of protein products (Kelly *et al*., 2010; Vandenberghe-Descamps *et al*., 2017; Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**) and needs further investigation. Accordingly, understanding salivary flow changes, and its relevance to sensory perception and food acceptance, is of growing relevance.

Previous research indicates mouthdrying and mucoadhesion are present within dairy beverages and increase with consumers' age (Withers *et al*., 2013a; Norton *et al*., 2020a, **Chapter 3**). However, trying to prove that the perception of mouthdrying increases with age has produced mixed results, potentially due to the lack of sensitivity of rating scales (i.e. the generalised Labelled Magnitude Scale, gLMS) compared with discrimination testing in detecting mouthdrying in older adults (Withers *et al*., 2013a; Norton *et al*., 2020a, **Chapter 3**). Therefore, our study will evaluate mouthdrying using both tests to explore further these concerns. The link between whether greater WPB retention results in increased WPB mouthdrying perception and the influence of salivary flow on such perception, are both relatively unclear. Accordingly, further investigation is necessary to understand these phenomena for the benefit of older adults in the future. This study

hypothesises that (a) modulating salivary flow will alter mouthdrying perception and (b) oral retention is directly related to whey protein derived mouthdrying. In order to evaluate these hypotheses mouthdrying was evaluated via descriptive sensory profiling (DSP), two-alternative forced choice test (2-AFC) and gLMS. This study had the following objectives to: (a) provide more conclusive evidence that mucoadhesion and mouthdrying of WPBs are intrinsically linked; and (b) test whether modulating saliva flow can influence perceived mouthdrying of beverages.

5.4. Materials and methods

5.4.1. Study overview

Forty volunteers (24.9 ± 3.4 years, healthy) completed a single blinded randomised crossover trial over two study visits (Table 5.1). Power calculations (alpha risk = 0.05 and 80% power) were used to calculate the subject size based on previous work in WPBs (Norton *et al*., 2020a, **Chapter 3**) using mouthdrying intensity ratings (0-100) as the primary outcome measure and, assuming a difference of 16 and standard deviation of 23, indicating the lowest sample size of 32. The study was conducted in compliance with current COVID-19 guidelines at the time (August and September 2020; with appropriate risk assessments and social distancing). The study was fully explained to the volunteers and their informed written consent was obtained prior to their participation. In addition, it was made clear that all data would be anonymised and kept confidential, as well as there being a right to withdraw. The study received a favourable opinion for conduct from the University of Reading, School of Chemistry, Food and Pharmacy Research Ethics Committee (SCFP 32/20) and the study was registered on the clinical trials database (www.clinicaltrials.gov as NCT04507399). Volunteers were screened to ensure they met inclusion criteria (minimal medication, no COVID-19 symptoms or not having had COVID-

19 within the last four weeks, not smokers, with no known allergies or intolerances to food, not with diabetes nor cancer and not having had oral surgery or a stroke). The study visits (Figure 5.1) were held at the Sensory Science Centre, University of Reading.

Table 5.1. Summary of volunteers sex, medication and salivary flow rates categories (*n* and % indicate number and percentage). Saliva flow categories were defined as below (or equal to) or above the median (missing data *n* = 1).

Variable	**Total** (*n* = 40)	
	n	%
Sex		
Male	12	30
Female	28	70
Medication		
No	38	95
Yes	2	5
Unstimulated saliva flow (mL/min)		
Low (0.10-0.70)	19	49
High (0.70-1.35)	20	51
Stimulated saliva flow (mL/min)		
Low (0.78-2.23)	21	54
High (2.23-4.08)	18	46

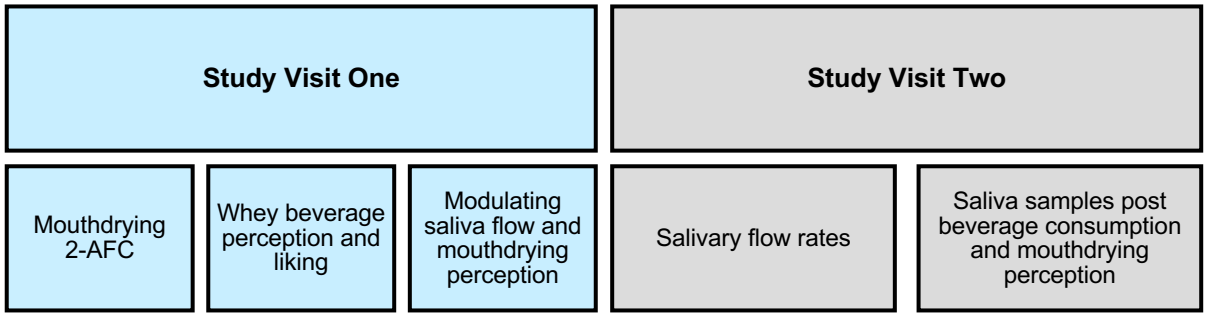

Figure 5.1. Study overview (2-AFC: two-alternative forced choice).

5.4.2. Materials

Two whey powders were used: whey protein concentrate (WPC) (Volactive Ultra-Whey 80; minimum protein content 80%, remainder as lactose, fat, moisture and ash) and whey permeate (WPe) (Volactose Taw Whey Permeate; minimum lactose content 89%, remainder ash, moisture, protein and fat) (Volac International Ltd., Royston, UK). Sucrose (Caster sugar, Tate & Lyle, London, UK) and vanilla extract (Nielsen-Massey,

Netherlands) were sourced from Sainsbury's (Reading, UK). Parafilm®, Bradford reagent (0.1-1.4 mg/mL) and protein standard (Bovine Serum Albumin, BSA, 2.0 mg/mL) were purchased from Sigma-Aldrich (Dorset, UK).

5.4.3. Model beverage preparation

Four different whey beverages were tested: (1) a control whey permeate beverage (WPeB; 4.0% w/v, WPe powder in deionised water) and (2) a whey protein beverage (WPB; 10.0% w/v, WPC powder in deionised water). The rationale was as outlined in our previous work (Norton *et al*., 2020a; **Chapter 3**). WPe provides a non-protein whey control at a concentration selected to keep the lactose content below sweet taste recognition and WPC concentration is relevant to commercial products as well as commonly utilised in WPB testing (Belitz *et al*., 2004; Buzalaf *et al*., 2012; Luiking *et al*., 2014; Bull *et al*., 2017). In addition, sample palatability was improved by adding sucrose and vanilla as previous work highlighted that unsweetened WPBs were rated as disliked moderately (mean 3 on 9-point hedonic scale) (Norton *et al*., 2020a, **Chapter 3**). This resulted in (3) a sweetened control whey permeate beverage (WPeBS; 4.0% w/v WPe, 1.49% w/v sucrose, 1.0% w/v vanilla extract) and (4) a sweetened whey protein beverage (WPBS; 10.0% w/v WPC powder, 2.0% w/v sucrose, 1.0% w/v vanilla extract). Less sucrose was added to the WPeBS compared with the WPBS due to the lactose content of the WPe; they were matched on relative sweetness. Formulations are summarised in Table 5.2. Samples were prepared simultaneously and stirred (magnetic stirrers at medium speed; Stuart™ SM5, Cole-Parmer, Staffordshire, UK) for 90-min at room temperature (21.8 ± 2.0 °C). Samples were left to hydrate overnight at 4 °C before being served to volunteers at room temperature.

Table 5.2. Composition of whey beverages (WPeB: whey permeate beverage; WPeBS: whey permeate beverage sweetened; WPB: whey protein beverage; WPBS: whey protein beverage sweetened) per 10 mL[1] (as tasted) and per 100 mL[2].

	Whey Permeate Beverages				**Whey Protein Beverages**			
	WPeB		WPeBS		WPB		WPBS	
	10 mL[1]	100 mL[2]	10 mL[1]	100 mL[2]	10 mL[1]	100 mL[2]	10 mL[1]	100 mL[2]
Energy (kcal)	1.5	14.7	2.4	23.7	4.0	39.7	5.1	50.7
Fat (g)	0.0008	0.008	0.0008	0.008	0.07	0.7	0.07	0.7
of which saturates (g)	-	-	-	-	0.03	0.3	0.03	0.3
Carbohydrate (g)	0.4	3.6	0.5	5.1	0.04	0.4	0.2	2.4
of which sugars (g)	0.4	3.6	0.5	5.1	0.04	0.4	0.2	2.4
Protein (g)	0.01	0.1	0.01	0.1	0.8	8.2	0.8	8.2
Moisture (g)	0.004	0.04	0.004	0.04	0.05	0.5	0.05	0.5
Ash (g)	0.02	0.2	0.02	0.2	0.04	0.4	0.04	0.4

Composition was calculated from technical data sheets of ingredients used. The apparent viscosity of beverages was measured and considered broadly similar, as outlined in Figure S.5.1.

5.4.4. Sensory methods

All sensory evaluation (trained sensory panel and volunteers) was carried out under red lights (to mask minor visual differences between samples) in isolated booths using Compusense Cloud Software (Version 21.0.7713.26683, Compusense, ON, Canada). Palate cleansing between samples used filtered warm water (Withers *et al*., 2013a). All samples were presented at the same time on different trays (due to COVID-19 serving restrictions) but tasted in a randomly allocated sequential balanced order and coded with a random three-digit number. Samples (10 mL) were presented in black plastic cups (25 mL; opaque) (BB Plastics, West Yorkshire, UK).

5.4.5. Sensory profile

Descriptive sensory profiling (a modified quantitative descriptive analysis (QDA™) (Stone *et al*., 1974; Stone & Sidel, 2004)) was used to determine the sensory differences between the whey beverages, as well as to quantify the attribute changes arising from the addition of sucrose and vanilla. All panellists (*n* = 10; 9 female and 1 male, screened and trained) had a minimum of one years' experience and at least six hours training involving whey beverages. Both trained panel and study volunteers had the same

samples (WPeB, WPeBS, WPB and WPBS). The trained panel developed a consensus vocabulary (adapted from Bull *et al*., 2017) identifying 21 attributes as outlined in Table 5.3. Appearance was not evaluated due to potential visual differences between samples which could lead to bias evaluation; accordingly, to address these concerns samples were presented in opaque black plastic cups and under red lights to minimise such differences between samples. Panellists evaluated the samples in duplicate (in different sessions) using unstructured line scales[48] (0-100) with appropriate anchors.

5.4.6. Mouthdrying two-alterative forced choice test (2-AFC)

Volunteers were provided with clear instructions, presented with two samples and asked which sample was more mouthdrying via a single paired comparison test[49] comparing WPeBS with WPBS (in accordance with ISO 5495:2005). The rationale for using a 2-AFC test was due to its simplicity and ability to detect small differences between samples; it had previously been used successfully to find such differences between products (Withers *et al*., 2013a; Adjei, 2017).

5.4.7. Whey beverage individual perception and liking

Volunteers rated liking (9-point hedonic scale), easiness to drink and swallow (5-point category scale), attribute perception (logarithmic scale (gLMS) with descriptors for intensity of sweetness, thickness and mouthdrying attributes), appropriateness of attribute level (Just-About-Right, JAR; 5-point JAR scale), preference and consumption of whey beverages (a series of 2-AFC tests to assess most preferred and frequency of consumption on 6-point category scale) and provided comments relating to flavour and texture. All volunteers completed a training exercise (Figure S.5.2; rating 15 remembered or imagined sensations adapted from Hayes *et al*., 2013) to become familiar with gLMS

[48] also referred to as visual analogue scales (VAS)
[49] terms paired comparison test and two-alternative forced choice test (2-AFC) are used interchangeably

(Bartoshuk *et al*., 2004). Volunteers had a break (45-s) between samples during which they cleansed their palate by drinking warm filtered water.

5.4.8. Modulating saliva flow and mouthdrying perception

To understand the role of saliva on mouthdrying perception (Figure 5.2); saliva flow was modulated for 2-min by either decreasing saliva flow via placing 4 × cotton wool rolls (40.0 mm × 10.0 mm) (two on each side split between the upper and lower jar) within the mouth or increasing saliva flow by chewing on parafilm® (5.0 × 5.0 cm) (adapted from Brunstrom *et al*., 1997; Nayak & Carpenter, 2008). Volunteers were given four 10 mL beverage samples (2 × WPeBS and 2 × WPBS) and immediately following consumption scored the sample for mouthdrying on a gLMS as well as scoring the aftereffects of mouthdrying at 15-s, 30-s, 60-s and 120-s time intervals post consumption. Volunteers also had an enforced 3-min break between samples (rationale based on initial testing within our labatory and protein concentration in saliva samples post WPB consumption being considered to have plateaued within 3-min) (Bull *et al*., 2020; Norton *et al*., 2020a, **Chapter 3**), where they swilled and consumed warm filtered water.

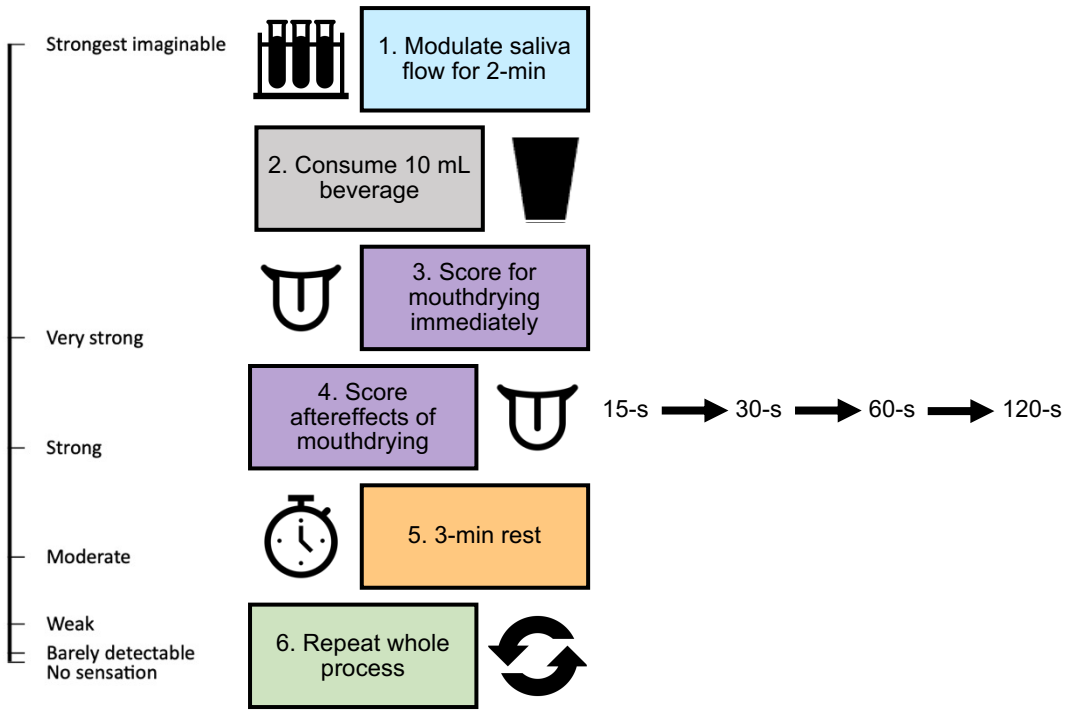

Figure 5.2. Brief overview of modulating saliva flow and mouthdrying perception protocol.

5.4.9. Salivary flow rates

Unstimulated and stimulated saliva were both collected at the beginning of study visit two with a sufficient rest (~10-min) in between. Saliva collection methods were as outlined in our previous work (Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**). In summary, unstimulated saliva was collected for 5-min whereas stimulated saliva was collected for 2-min whilst chewing parafilm® (5.0 × 5.0 cm). Saliva samples were collected in tubes (60 mL, wide) and flow rates calculated as mL/min. Samples were stored on ice pending analysis.

5.4.10. Saliva samples post beverage consumption and mouthdrying perception

An oral retention method from Norton *et al*. (2020a, **Chapter 3**) was developed to measure protein retained in saliva after swallowing, alongside rating of mouthdrying (Figure 5.3). Stimulated saliva samples were collected (as outlined in Section 5.4.9) and used as a baseline measurement (rationale based on Norton *et al*., 2020a, **Chapter 3**). Eight beverage samples (4 × WPeBS and 4 × WPBS; 10 mL) were provided at two time

points (15-s and 60-s, randomised). These were considered key time points based on previous work (Bull *et al*., 2017; Norton *et al*., 2020a, **Chapter 3**). Volunteers (on eight occasions) gave four saliva samples and rated four beverages for perceived mouthdrying on a gLMS post beverage consumption. A 5-min break was obligatory between samples to prevent crossover effects and ensure protein concentration in saliva samples had plateaued (Bull *et al*., 2020; Norton *et al*., 2020a, **Chapter 3**). Warm filtered water was consumed to palate cleanse during this break. Tubes were weighed before and after collection to measure saliva weight and all saliva samples were stored on ice pending analysis.

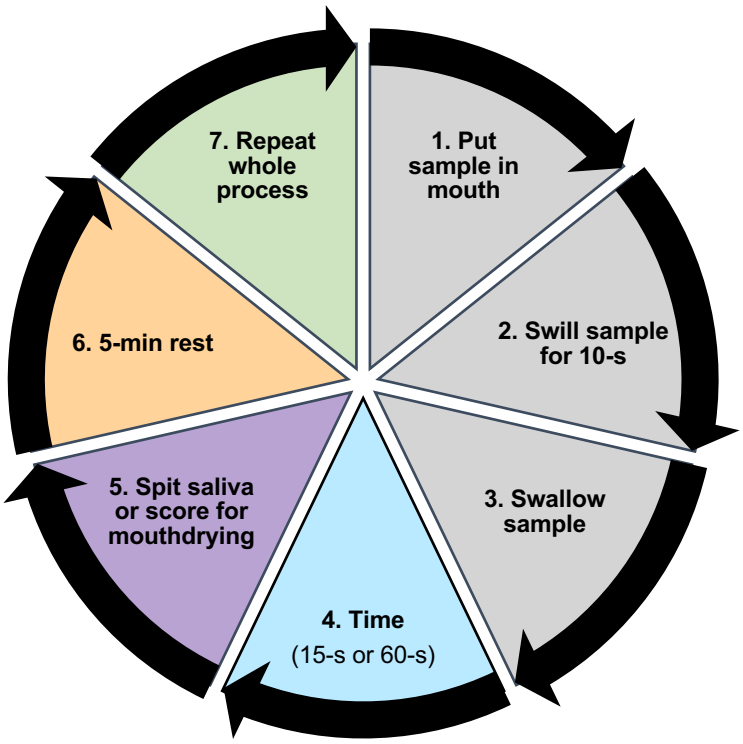

Figure 5.3. Summary of protocol for saliva sample collection and mouthdrying perception rating post beverage consumption.

5.4.11. Protein analysis of saliva samples

Bradford Assay was used to analyse the protein concentration (mg/mL) in saliva samples (Bradford, 1976; Zor & Selinger, 1996) as described in Norton *et al*. (2020a, **Chapter 3**).

In summary, all analysis was performed in triplicate with biological and analytical replicates. BSA was used as the protein standard (six dilutions: 0.125 to 2.0 mg/mL). Saliva samples diluted 1:2 (saliva: purified water) and analysis followed immediately after each volunteer's visit. Volunteers baseline values (i.e. protein concentration in stimulated saliva) were subtracted from sample measurements to calculate protein concentration remaining post WPBS consumption. WPeBS was used as a control beverage and as outlined in previous work (Norton *et al*., 2020a, **Chapter 3**); the protein concentration was already below the baseline value (i.e. stimulated saliva protein concentration) therefore no additional calculations were required.

5.4.12. Statistical analysis

Analysis of variance (ANOVA) was used to analyse sensory profile data (Lawless & Heymann, 2010; Hasted, 2018) with main effects tested against the sample by assessor interaction, sample fitted as a fixed effect and assessor as a random effect using SenPAQ software (version 5.01, Qi Statistics, Kent, UK). Fisher's least significant difference (LSD) was used to test sample pairs assuming a 5% significance level.

Mouthdrying 2-AFC data was analysed using Binomial expansion and Thurstonian modelling in V-power to calculate *p* values, power and d' value (Ennis & Jesionka, 2011). Quantile analysis (based on the median) grouped volunteers into low and high salivary flow rates (XLSTAT version 2020.1.3, Addinsoft, Paris, France).

Perception and liking data from volunteers were analysed via linear mixed models[50] using sample, sex and saliva flow as explanatory variables, volunteers fitted as a random effect, and attribute perception, liking and JAR rating scores as dependent variables (SAS® software, version 9.4, Cary, NC, USA, applying Bonferroni). Volunteers modulated saliva

[50] linear mixed models are considered robust enough for unbalanced data (Torrico *et al*., 2018)

flow and mouthdrying perception data were analysed with sample, time, condition, sex and saliva flow as explanatory variables, volunteers fitted as a random effect, and mouthdrying perception rating as the dependent variable. Salivary flow rates and baseline saliva samples were analysed using explanatory variable of sex, volunteers fitted as a random effect and saliva flow and protein concentration as dependent variables respectively. Volunteers saliva samples post beverage consumption and mouthdrying perception data was analysed with the explanatory variables of sample, time, sex, saliva flow, volunteers fitted as random effects and protein concentration and mouthdrying perception as the dependent variables. All attribute data was collected on the gLMS log-scale and was transformed to linear data (anti-logged). Data reflects least square means (LSM) estimates.

Penalty analysis of the JAR and liking data was carried out (as previously described in Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**) using XLSTAT. Paired preferences were analysed using Binomial expansion in V-Power (Ennis & Jesionka, 2011). It should be noted that only two volunteers were taking medication and therefore outlier analysis was conducted using a Dixon test in XLSTAT. Outlier analysis demonstrated that these volunteers were not considered outliers (except for one volunteer for one output measure (thickness)). Analysis was therefore carried out with and without this volunteer's data, with the overall result being the same and accordingly all data was included within the statistical analysis. Significant differences were defined in all analyses by $p < 0.05$.

5.5. Results

5.5.1. Sensory profile

The sensory profile demonstrated that 12 of the 21 attributes were significantly different ($p < 0.05$) between samples as outlined in Table 5.3.

Table 5.3. Sensory profile (means of two replicates ± standard error) of whey beverages (WPeB: whey permeate beverage; WPeBS: whey permeate beverage sweetened; WPB: whey protein beverage; WPBS: whey protein beverage sweetened).

Modality	Attribute	Reference and/or Description	Whey Beverages				Significance of sample (*p* value)
			WPeB	WPeBS	WPB	WPBS	
Aroma	Cooked milk	Heated pasteurised semi-skimmed milk	9.2 ± 2.7	8.1 ± 2.9	20.6 ± 4.4	18.4 ± 3.9	0.12
	Powdered milk (wet)	Skimmed milk powder (10.0% w/v, skimmed milk powder in deionised water)	7.7 ± 2.6	20.7 ± 3.9	11.9 ± 3.9	17.8 ± 3.8	0.07
	Whey isolate	Volactive Ultra-Whey 90 Instant (5.0% w/v, WPI powder in deionised water)	8.8 ± 2.4	6.3 ± 3.8	7.6 ± 2.8	10.1 ± 3.7	0.80
	Vanilla	Vanilla extract (Nielsen-Massey)	0.7 ± 1.9^{c}	42.1± 5.1^{a}	1.1 ± 1.9^{c}	31.8 ± 4.8^{b}	**< 0.0001**
Flavour	Sour	Citric acid (0.76 g/L)	17.5 ± 3.5^{ab}	8.0± 4.9^{b}	23.9 ± 4.0^{a}	17.5 ± 4.9^{ab}	**0.048**
	Metallic	Iron (II) sulphate heptahydrate (0.0036 g/L)	8.7 ± 3.3	8.2 ± 2.5	10.1 ± 3.7	5.9 ± 3.7	0.44
	Salty	Sodium chloride (1.19 g/L)	7.7 ± 2.2	5.0 ± 2.2	9.4 ± 2.6	6.3 ± 1.9	0.27
	Sweet	Sucrose (5.76 g/L)	19.6 ± 3.0^{b}	52.2 ± 6.4^{a}	12.1 ± 2.5^{b}	46.6 ± 5.8^{a}	**< 0.0001**
	Cooked butter	Melted unsalted butter	9.6 ± 3.0	3.3 ± 6.6	9.8 ± 2.6	9.7 ± 6.0	0.43
	Cooked milk	Heated pasteurised semi-skimmed milk	15.2 ± 3.3	12.1 ± 2.9	24.4 ± 4.0	24.3 ± 4.4	0.17
	Powdered milk (wet)	Skimmed milk powder (10.0% w/v, skimmed milk powder in deionised water)	6.1 ± 3.4	16.4 ± 3.8	14.3 ± 4.3	19.2 ± 4.1	0.12
	Whey isolate	Volactive Ultra-Whey 90 Instant (5.0% w/v, WPI powder in deionised water)	14.7 ± 2.8	8.6 ± 3.8	17.5 ± 3.4	14.2 ± 4.1	0.32
	Vanilla	Vanilla extract (Nielsen-Massey)	2.5 ± 2.6^{b}	41.3 ± 5.3^{a}	0.0 ± 2.9^{b}	33.5 ± 5.0^{a}	**< 0.0001**
Mouthfeel	Body	Fullness of sample	21.0 ± 3.3^{b}	21.4 ± 4.2^{b}	31.2 ± 4.6^{a}	31.4 ± 4.2^{a}	**0.006**
	Chalky	Dry fine insoluble powder	4.3 ± 3.4^{b}	3.9 ± 3.1^{b}	27.3 ± 5.1^{a}	16.8 ± 3.8^{a}	**0.0003**
	Mouthdrying	Drying sensation in the mouth	26.5 ± 4.1^{c}	30.3 ± 4.5^{c}	51.2 ± 6.3^{a}	42.7 ± 4.5^{b}	**< 0.0001**
Aftertaste	Aftertaste strength	The strength of the overall aftertaste	17.9 ± 3.3^{b}	38.1 ± 4.0^{a}	23.7 ± 5.1^{b}	38.2 ± 3.6^{a}	**< 0.0001**
	Mouthdrying	Drying sensation in the mouth	24.6 ± 2.8^{b}	30.2 ± 4.3^{b}	50.4 ± 4.6^{a}	44.0 ± 3.6^{a}	**< 0.0001**
	Metallic	Iron (II) sulphate heptahydrate (0.0036 g/L)	4.9 ± 3.3^{b}	3.3 ± 4.7^{b}	9.2 ± 5.8^{a}	5.7 ± 5.2^{ab}	**0.02**
	Vanilla	Vanilla extract (Nielsen-Massey)	1.7 ± 1.1^{b}	27.4 ± 4.1^{a}	0.0 ± 1.8^{b}	26.7 ± 4.8^{a}	**< 0.0001**
	Sweet	Sucrose (5.76 g/L)	12.7 ± 2.2^{b}	35.6 ± 3.8^{a}	7.5 ± 1.9^{b}	34.2 ± 5.0^{a}	**< 0.0001**

The trained panel (n = 10) scored all samples in duplicate in separate sessions and data was collected using unstructured line scales (0-100). Sample significant differences within a row are denoted by differing superscript letters.

In summary, it demonstrated whey protein beverages (WPB and WPBS) significantly increased mouthdrying, chalky and body compared with whey permeate beverages (WPeB and WPeBS). Adding sucrose and vanilla to beverages (WPeBS and WPBS) resulted in significantly increased sweet and vanilla notes compared with WPeB and WPB, as well as significantly reduced mouthdrying in WPBS compared with WPB, therefore improving sample palatability.

5.5.2. Mouthdrying two-alterative forced choice test (2-AFC)

The mouthdrying paired comparison test demonstrated that WPBS was significantly more mouthdrying ($p < 0.0001$; d' value: 1.19; power: 0.99) compared with WPeBS; 60% of the volunteers were able to distinguish that WPBS was more mouthdrying.

5.5.3. Whey beverage individual perception and liking

Volunteers perceived WPBS as significantly ($p < 0.05$) more mouthdrying, thicker, less sweet and less easy to consume compared with WPeBS (Figures 5.4 and 5.5). There was no significant difference ($p = 0.53$) in liking between whey beverages with both beverages perceived, on average, as neither like nor dislike on a 9-point hedonic scale. There was also no significant difference in Just-About-Right flavour and thickness between whey beverages, where both were perceived as closer to Just-About-Right (JAR = 3) compared with too weak/thin for flavour and thickness respectively (Table 5.4). Saliva flow had no significant effect on whey beverage liking, perception, easiness to consume or JAR attributes, whether it was tested as overall or by grouping volunteers into low and high saliva flow (Table S.5.1). There was also no significant effect of sex on whey beverage individual perception and liking (Table S.5.1).

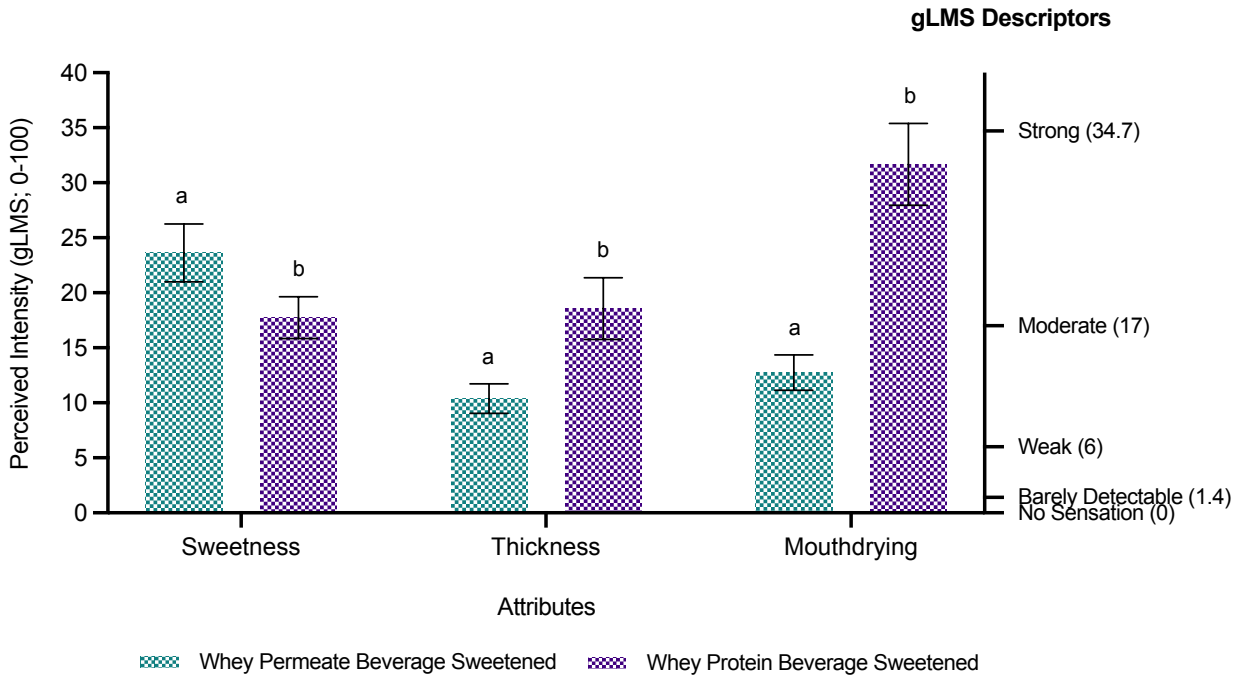

Figure 5.4. Volunteers' attribute perception mean ratings (± standard error) of whey beverages (*n* = 40; anti-logged data). Sample significant differences denoted by differing small letters.

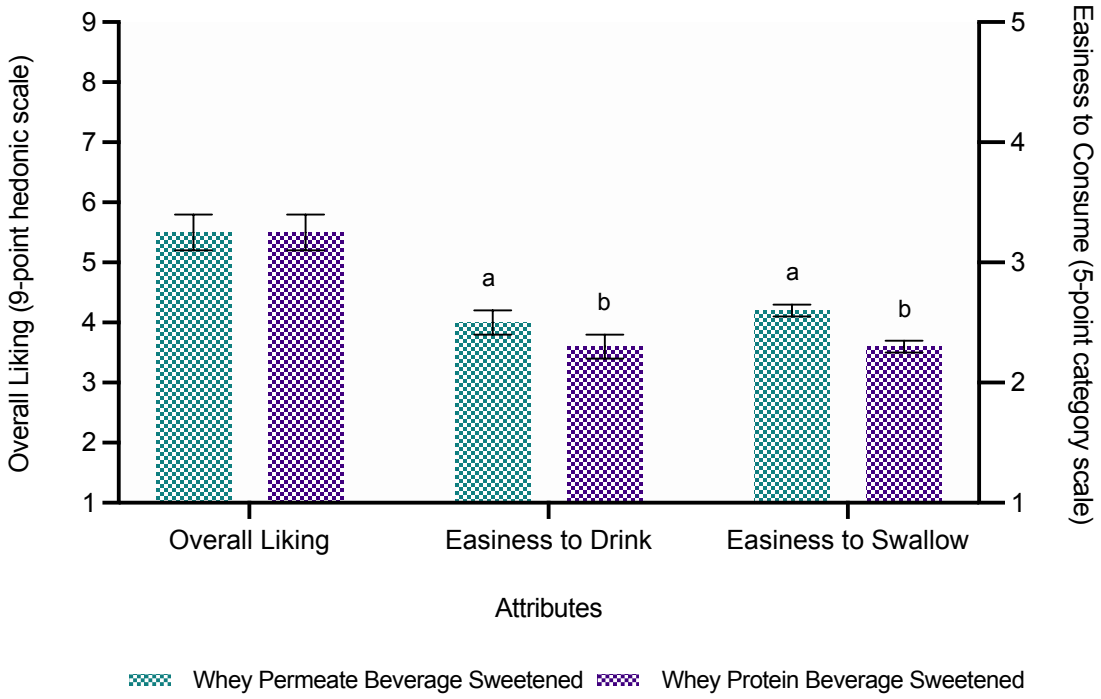

Figure 5.5. Volunteers' liking (left axis; measured on a 9-point hedonic scale) and easiness to consume (drink or swallow) (right axis; measured on a 5-point category scale) mean ratings (± standard error) of whey beverages (*n* = 40). Sample significant differences denoted by differing small letters.

Table 5.4. Mean Just-About-Right (JAR) ratings and subsequent influence on liking ratings (penalty analysis) (WPeBS: whey permeate beverage sweetened; WPBS: whey protein beverage sweetened).

	Overall (*n* = 40)		**Penalty Analysis**			
		Significance of sample (*p* value)	Too Little		Too Much	
			Mean Drop	Frequency (%)	Mean Drop	Frequency (%)
JAR Flavour						
WPeBS	2.8 ± 0.1		1.48#	25	1.21	15
WPBS	2.9 ± 0.1	0.82	1.34#	25	2.54	15
JAR Thickness						
WPeBS	2.6 ± 0.1		0.11	48	-1.18*	5
WPBS	2.8 ± 0.1	0.17	0.71	35	3.40*	13

represents a significant difference ($p < 0.05$) within a sample between mean liking compared with where the sample was considered Just-About-Right; * denotes size of the group lower than 20% of population. Frequency (%) is the % of volunteers within each group (too little or too much).

There was no significant difference ($p = 0.13$) in preference between whey beverages. However, this study successfully demonstrated improvements in sample palatability compared with previous samples, as WPeBS and WPBS were both significantly preferred ($p < 0.0001$) compared with WPeB and WPBS (Table 5.5). Where attributes are not at the optimum level for a volunteer (as reflected in Just-About-Right, JAR, ratings) this may impact liking. The penalty analysis (Table 5.4) concluded liking was negatively impacted where flavour was considered too low. Volunteers generally provided positive feedback for flavour and texture of both beverages; 86 comments were provided of which 53 were positive and 33 were negative (Table 5.6).

Table 5.5. Volunteers' counts[51] of whey beverage preference (WPeB: whey permeate beverage; WPeBS: whey permeate beverage sweetened; WPB: whey protein beverage; WPBS: whey protein beverage sweetened).

Pair Number	**Sample**	**Preference**	**Significance of sample (*p* value)**
1	WPeBS	24	0.13
1	WPBS	16	
2	WPB	5	**< 0.0001**
2	WPBS	35	
3	WPeB	5	**< 0.0001**
3	WPeBS	35	

[51] Data (*n* = 40) obtained from a series of 2-AFC tests to assess most preferred

Table 5.6. Examples of volunteers comments relating to whey beverages (WPeBS: whey permeate beverage sweetened; WPBS: whey protein beverage sweetened).

Sample	Comments and Volunteers Details
WPeBS	*Thin, almost like drinking water (v2, female, aged 28). Nice sweet taste, but not too strong (v4, male, aged 26). There wasn't much flavour to detect (v6, female, aged 25). Texture was OK (v9, female, aged 21)*
WPBS	*Very soothing (v1, male, aged 27). Smooth texture, bit mouthdrying (v4, male, aged 26). It is quite powdery (v7, female, aged 24). A bit too watery and thin (v30, male, aged 19)*

5.5.4. Modulating saliva flow and mouthdrying perception

Modulating saliva flow led to no significant change (p = 0.96) in perceived mouthdrying, as mouthdrying perception remained relatively consistent within each beverage type (Figure 5.6). In common with the results where saliva was not modulated, there was a significant effect of sample (p < 0.0001) where WPBS was more mouthdrying compared with WPeBS at all timepoints (0-s, 15-s, 30-s, 60-s and 120-s) (Figure 5.6). Time also had an overall significant effect (p = 0.0002) where perceived mouthdrying slightly increased over time (Figure 5.6). There was no significant effect of saliva flow and sex on mouthdrying perception following modulated saliva flow (Figure S.5.3).

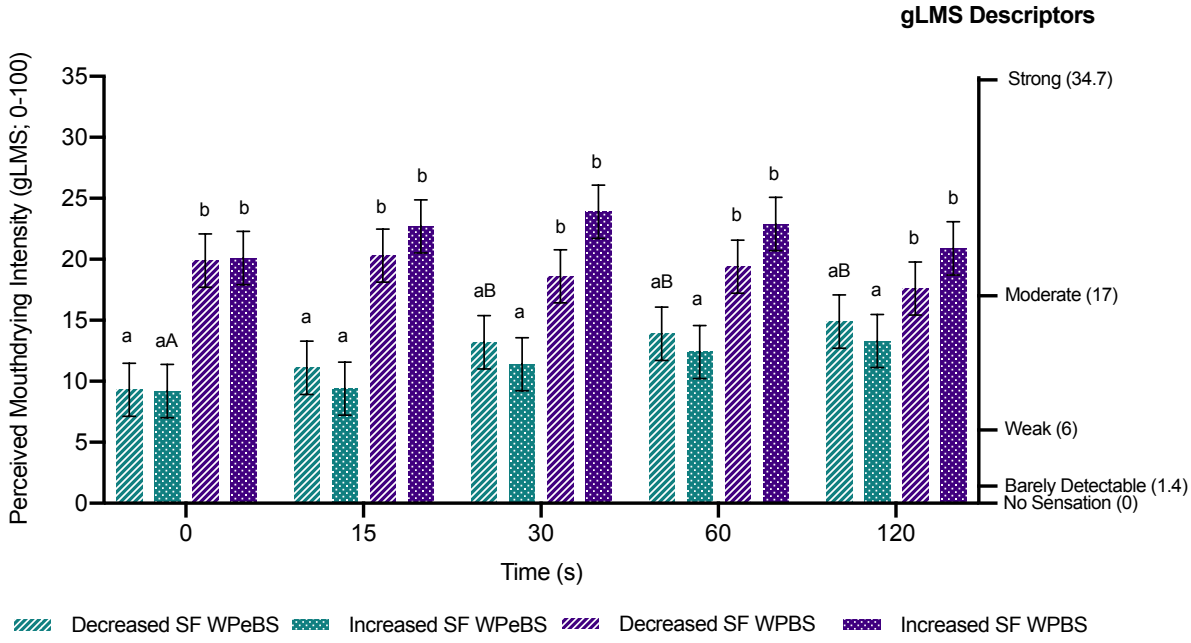

Figure 5.6. Volunteers' (n = 40) perceived mouthdrying (± standard error) post beverage (WPeBS: whey permeate beverage sweetened; WPBS: whey protein beverage sweetened) consumption over time following saliva flow (SF) being modulated (increased: chewing on

parafilm and decreased: by placing cotton wool rolls within the mouth). Sample significant differences are represented by differing small letters (between samples) and capital letters (within samples).

5.5.5. Salivary flow rates

Unstimulated salivary flow rates were 0.72 ± 0.04 mL/min, whereas stimulated flow was 2.29 ± 0.11 mL/min. Volunteers were also categorised by quantile analysis into low and high salivary flow rates (Table 5.1). There was no significant effect of sex (unstimulated saliva flow (USF): p = 0.15 and stimulated saliva flow (SSF): p = 0.053) on saliva flow regardless of collection method. However, there was a tendency for males to have a higher salivary flow compared with females (USF: males 0.81 ± 0.09 and females 0.68 ± 0.05 mL/min and SSF: males 2.61 ± 0.20 and females 2.15 ± 0.13 mL/min).

5.5.6. Saliva samples post beverage consumption and mouthdrying perception

WPBS led to a significantly higher protein concentration (p < 0.001) in saliva samples post swallow compared with WPeBS at both timepoints (15-s and 60-s) (Figure 5.7). There was no significant effect of time overall on protein concentration in saliva samples post beverage consumption (p = 0.052); however, there was a significant time by sample interaction (p = 0.03). Pairwise comparison highlighted that WPBS consumption resulted in saliva samples showing a significantly higher (p = 0.003) protein content at 15-s compared with 60-s, whereas WPeBS had a lower saliva protein content across all timepoints (p = 0.83) (Figure 5.7). Results from the saliva samples post beverage consumption supported the mouthdrying perception results, where WPBS resulted in significantly higher mouthdrying scores (p < 0.001) compared with WPeBS at both timepoints (Figure 5.7). However, there was no overall significant effect of time (p = 0.26) on perceived mouthdrying where WPBS decreased very slightly over time whereas WPeBS remained relatively consistent (Figure 5.7). There were no significant effects of

protein concentration in saliva samples and mouthdrying perception relating to sex or saliva flow (Figure S.5.4).

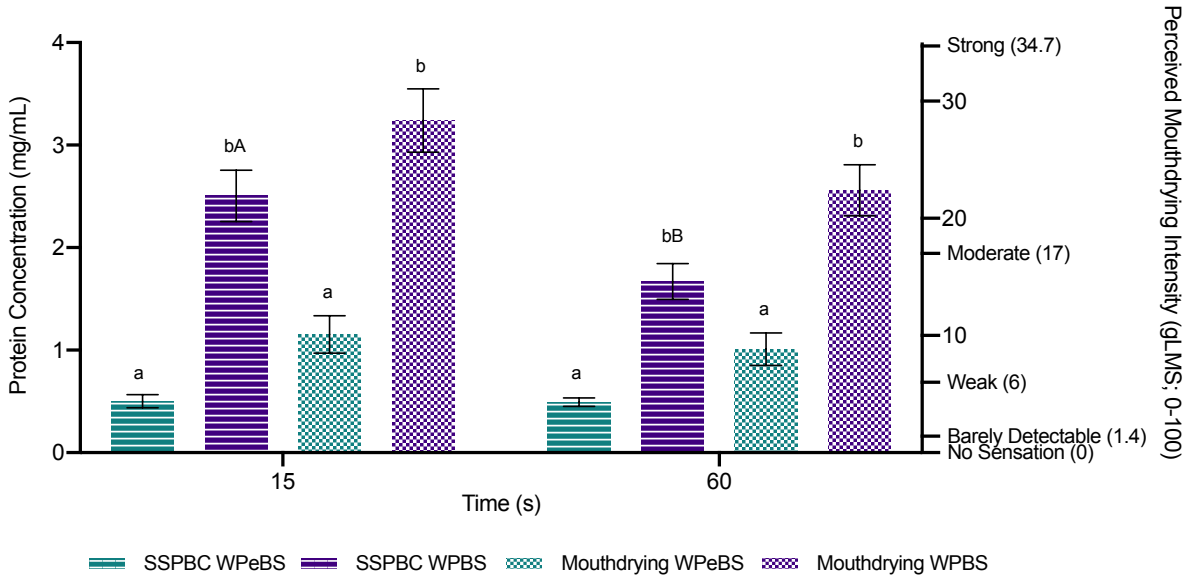

Figure 5.7. Protein concentration in saliva samples post beverage consumption (SSPBC) (left axis) and perceived mouthdrying (right axis; measured on gLMS (0-100)) (± standard error) (*n* = 40). WPeBS: whey permeate beverage sweetened and WPBS: whey protein beverage sweetened. Sample significant differences are represented by differing small letters (between samples) and capital letters (within samples)[52].

5.6. Discussion

5.6.1. Sensory profile and whey beverage individual perception and liking

Fortifying beverages with whey protein increased mouthdrying, chalky, thickness, body and reduced sweetness and easiness to consume compared with a non-protein control (in this case a whey permeate beverage (WPeB)). These findings support previous work in this area that WPBs are associated with mouthdrying, mouthcoating and chalky attributes (Withers *et al*., 2014; Bull *et al*., 2017). These studies were, however, carried out without a non-protein control; therefore, our study concluded that it is indeed the protein in WPBs, rather than other constituents of whey, that cause mouthdrying within WPBs. Previous research highlighted the lack of sensitivity of a gLMS (0-100) compared

[52] Baseline protein concentration in saliva samples outlined in Table S5.2.

with a 2-AFC in detecting mouthdrying in older adults (Withers *et al*., 2013a; Norton *et al*., 2020a, **Chapter 3**). Accordingly, to address these concerns, our study also measured mouthdrying using a paired comparison test to a ensure differences between samples were not missed on a gLMS (0-100), which can occur if samples are presented monadically (Zhou *et al*., 2016). The 2-AFC clearly demonstrated the majority of the volunteers (32 out of 40) supported WPBS as being more mouthdrying compared with WPeBS. Therefore, our study proved volunteers perceived WPBS as more mouthdrying compared with WPeBS (by both gLMS and 2-AFC), which was additionally supported by the trained panel findings. A limitation of our study was not being able to recruit older adults due to the ongoing COVID-19 pandemic. Accordingly, next steps should include future work with older adults to prove conclusively that sensitivity to mouthdrying increases with age, using a more sensitive discrimination test (i.e. 2-AFC) in different food matrices.

Previous work by Norton *et al*. (2020a, **Chapter 3**) demonstrated low liking scores by volunteers for model WPBs; therefore, this study added sucrose and vanilla to improve potentially flavour and acceptability. The sensory profile concluded that adding sucrose and vanilla increased sweet and vanilla notes, which subsequently reduced perception of mouthdrying. This did lead to an improvement in volunteers' liking ratings and a clear preference for the 'improved beverages'. The addition of sucrose and flavour led to increased product acceptance and reduced perceived mouthdrying such additions are commonly found in commercial oral nutritional supplements (ONS). However, the sweetened WPB (WPBS) was still mouthdrying and further mitigation may lead to increased palatability. This could maximise product benefits, especially as these products are most often consumed by older adults who may be more sensitive to the products oral adhesion (Norton *et al*., 2020a, **Chapter 3**) and mouthdrying (Withers *et al*., 2013a).

It was hypothesised that WPBs would cause mouthdrying, thereby reducing beverage acceptability. However, surprisingly, there was no difference in liking or preference between the two beverages (WPeBS and WPBS). This could be explained by the WPeBS where volunteers lack familiarity with the product and highlighted its minimal flavour, watery, thin and sweet nature, as demonstrated by volunteers consumption habits, penalty analysis and comments. These findings were also supported by the trained panel who identified sweet and vanilla taste, as well as being lower in cooked notes (such as cooked milk and butter) in the WPeBs compared with WPBs. Furthermore, sweetness and thickness are considered key drivers of acceptability in milk beverages (Villegas *et al*., 2010), which could explain the relatively low liking scores and no difference in liking or preference between the beverages demonstrated in our study.

The sensory profile demonstrated that the WPBS had more body compared with the WPeBS and this result was matched by the volunteers who also perceived WPBS to be thicker. Although the viscosity of the beverages was considered to be broadly similar (Figure S.5.1), there was a mean difference of 0.83 mPa·s at 50 s^{-1} (a commonly cited oral shear rate) (National Dysphagia Diet Task Force, 2002). A previous study has shown the Weber fraction (K) for oral thickness perception of model beverages to be 0.26 (Camacho *et al*., 2015), and therefore with the WPBS thickness at 1.78 mPa·s, the calculated just-noticeable difference (JND) would be 0.46 mPa·s. Hence the literature supports that there would be a perceptual difference in thickness between the WPeBS and WPBS. However, a previous study measuring astringency of low pH WPBs used maltodextrin to modify viscosity (1.6 to 7.7 mPa·s) and found it had no effect on perceived astringency (Beecher *et al*., 2008). This supports our current study in that the noticeable difference in thickness is unlikely to have influenced perception of mouthdrying; however, the previous study utilised a low pH whey model, where it is likely that the mechanism of

astringency was different to the mechanism of mouthdrying proposed in our neutral pH WPBs (mucoadhesion) (Bull *et al*., 2017; Norton *et al*., 2020a, **Chapter 3**). Therefore, it is advisable that future work aims to ensure viscosity is fully matched between beverages (potentially by using hydrocolloids). However, it may be challenging to match such low viscosities and in addition the use of hydrocolloids may potentially alter taste, flavour and mouthfeel properties (Cook *et al*., 2017) and lead to a different viscosity response to shear compared with the viscosity profile resulting from protein.

5.6.2. Modulating saliva flow and mouthdrying perception

There are numerous key functions associated with saliva (Carpenter, 2013) and saliva can influence sensory perception. Therefore, it was hypothesised that modulating salivary flow by either decreasing or increasing saliva flow would alter mouthdrying perception. However, no changes in mouthdrying perception were demonstrated immediately post beverage consumption nor over time (as evidenced from the aftereffects) as a result of modulating saliva flow. These findings support previous work which has demonstrated no, or only a minimal, effect of saliva flow on perception of other sensory attributes. For example, modifying salivary flow rates (unstimulated saliva flow and stimulated saliva flow using odour, parafilm and citric acid) had no effect on sensory ratings (eight attributes: flavour (vanilla, bitter/chemical), mouthfeel (temperature, thickness, melting, creaminess) and afterfeel (fat, astringent) of custard desserts (Engelen *et al*., 2003b)). In addition, artificially increasing saliva (by adding saliva related fluids to the product) had minimal effect on sensory perception (apart from increasing melting and decreasing thickness, creaminess and fatty afterfeel sensations) of custard desserts (Engelen *et al*., 2003a). Therefore, neither different salivary flow rates nor artificially increasing saliva volume had previously resulted in substantial differences in sensory perception in semi-solid foods. Salivary composition (total protein concentration and amylase activity) has

been shown to alter texture perception of custard desserts & mayonnaise (Engelen *et al*., 2007). However, more recently, Crawford and Running (2020) demonstrated changes in salivary proteins (proline-rich proteins and cystatins) had only minimal effects on the sensory perception of chocolate milks. Vandenberghe-Descamps *et al*. (2017) also demonstrated very few effects from differences in saliva flow on perception; they proposed that individuals may adapt their food oral processing to compensate for differences in saliva flow status and which may result in little impact on subsequent perception.

Within plant derived food models (such as tea and wine), saliva is considered to influence astringency perception. For example, volunteers with low salivary flow rates perceived wines to be more astringent over a longer duration compared with those with higher salivary flow rates (Fischer *et al*., 1994). Whereas after consuming black tea, perceived astringency has been shown to increase with decreasing saliva flow (by washing with water) and decrease with increasing saliva flow (by chewing on parafilm) (Nayak & Carpenter, 2008). However, these findings were not demonstrated in our study using whey beverages. This is likely to be as a result of the different mechanism involved in astringency (i.e. polyphenols binding to salivary proteins) compared with mouthdrying in neutral pH beverage (i.e. oral retention) and accordingly mechanisms may respond differently to salivary flow rate.

In addition, our study decreased saliva flow by using cotton wool rolls within the mouth (rather than washing with water) to replicate the ‘dry’ feeling within the mouth (a method successfully utilised previously by Brunstrom *et al*. (1997)). Such findings could suggest the role of saliva flow on sensory perception is potentially food model specific and dependent on the underlying mechanism responsible for the mouthdrying sensation. Accordingly, further research is necessary to understand the role of saliva flow on

mouthdrying perception in whey protein food models as current research has resulted in minimal differences so far. This could relate to how studies have measured or modulated saliva flow and is therefore potentially not a true reflection of natural variation in saliva.

5.6.3. Saliva samples post beverage consumption and mouthdrying perception

Whey protein adhered to the oral cavity (oral retention as a marker of mucoadhesion) post WPBS to a greater extent compared with WPeBS, supporting previous work in this area (Norton *et al*., 2020a, **Chapter 3**). Furthermore, our study demonstrated that perceived mouthdrying was significantly increased following WPBS consumption compared with WPeBS, which matched the oral retention results. Retention declined over time; however, this trend was not matched by perceived mouthdrying which did not reduce significantly over time. Previously, a build-up of whey protein derived mouthdrying was suggested to be as a result of a possible mucoadhesion mechanism (Withers *et al*., 2013b; Bull *et al*., 2017; 2020; Norton *et al*., 2020a, **Chapter 3**). Mucoadhesion within a WPB is considered to be as a result of the following potential mechanisms (Norton *et al*., 2021a, **Chapter 2**):

(1) movement of the sample in the mouth provides a greater surface area for whey protein to adhere to the oral cavity;

(2) spreading and swelling on the oral mucosa leads to increased adhesion and stronger adhesive joint via different physiochemical interactions (Smart, 2005; Khutoryanskiy, 2011);

(3) mucoadhesion is considered to result from a prolonged oral exposure and loss of saliva lubrication and increased friction, tissue exposure, adhesion and interaction (Vardhanabhuti *et al*., 2011; Bull *et al*., 2020) can result in perceived mouthdrying potentially caused by mucoadhesion.

Therefore, our study reinforces the suggestion that mucoadhesion could be a cause of whey protein derived mouthdrying, as this study measured for the first time both oral retention of protein and mouthdrying within the same protocol and demonstrated both increased retention and perceived mouthdrying following WPB consumption. This study aimed to quantify mouthdrying using a 'physical measure' (i.e. retention as a measure of mucoadhesion) at the same time as scoring mouthdrying perception within WPBs, as no previous study to our knowledge has investigated this. Typically, correlations are found in the literature between potential mechanisms and sensory data and this can result in an inability to prove relationships which should be a key priority for ongoing research. Future work however remains necessary to prove mucoadhesion is the cause of the oral retention and to demonstrate that a reduction in retention would lead to a subsequent decrease in perceived mouthdrying.

5.7. Conclusion

This study demonstrated by using three different methods (DSP, 2-AFC and gLMS) that WPBs were significantly more mouthdrying compared with WPeBs. In addition, increasing sweetness in WPBs significantly reduced perceived mouthdrying and increased consumer preference. Such results suggest improving mouthfeel attributes associated with WPBs could be a key strategy to improve compliance and product suitability for older adults. This study was unable to demonstrate a role of saliva flow on mouthdrying perception. However further research using improved methodology that captures the natural variation in saliva flow is needed to understand better the impact of salivary flow changes on mouthdrying perception in whey protein food models. Previously, mucoadhesion had been considered as a probable cause of whey protein derived mouthdrying and our study highlighted WPB consumption significantly increased

oral retention of the protein, which coincided with perceived mouthdrying. Hence, we conclude that whey protein is the cause of WPB retention and mouthdrying. Mucoadhesion is the probable cause of whey protein derived mouthdrying and oral retention provides a physical measure of perceived mouthdrying. However, it still needs further proof that modulating retention would result in changes in perceived mouthdrying. Understanding such mechanisms could result in improved products and increased consumption, this is important as protein consumption is associated with numerous benefits. There is a growing emphasis on improving protein intake across the lifespan to enhance health outcomes and given the potential importance of WPBs in achieving this, they must have high palatability to promote consumption and maximise the benefits from protein products.

S.5. Supplementary

S.5.1. Whey beverages viscosity

The apparent viscosity of the samples was measured using a rheometer (Modular Compact Rheometer (MCR) 102, Anton Paar, Graz, Austria) with RheoCompass™ software (Version 1.21, Anton Paar, Graz, Austria). The method was adapted from previous work (de Wijk *et al*., 2006; Prinz *et al*., 2006; Moret-Tatay *et al*., 2015) and all analysis was carried out with shear rates increasing logarithmically from 0.001 to 1000 s^{-1} providing 43 data points using a parallel plate (PP50; 50.0 mm) with a gap size of 1.0 mm and the temperature set at 22 °C. All samples were carefully loaded and allowed to rest for 5-min before any measurements were taken and all analysis was performed using six replicates for each beverage from different batches and whey beverages were considered broadly similar, as outlined in Figure S.5.1.

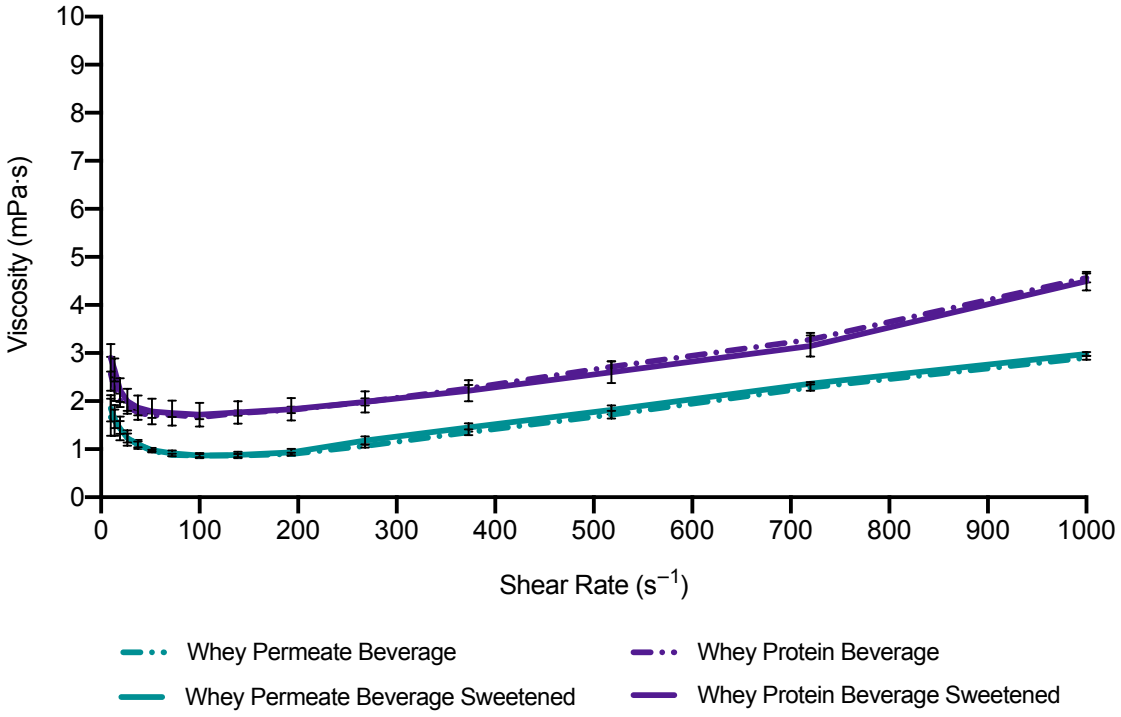

Figure S.5.1. Apparent viscosity (means of six replicates ± standard error) of whey beverages.

S.5.2. Additional study data

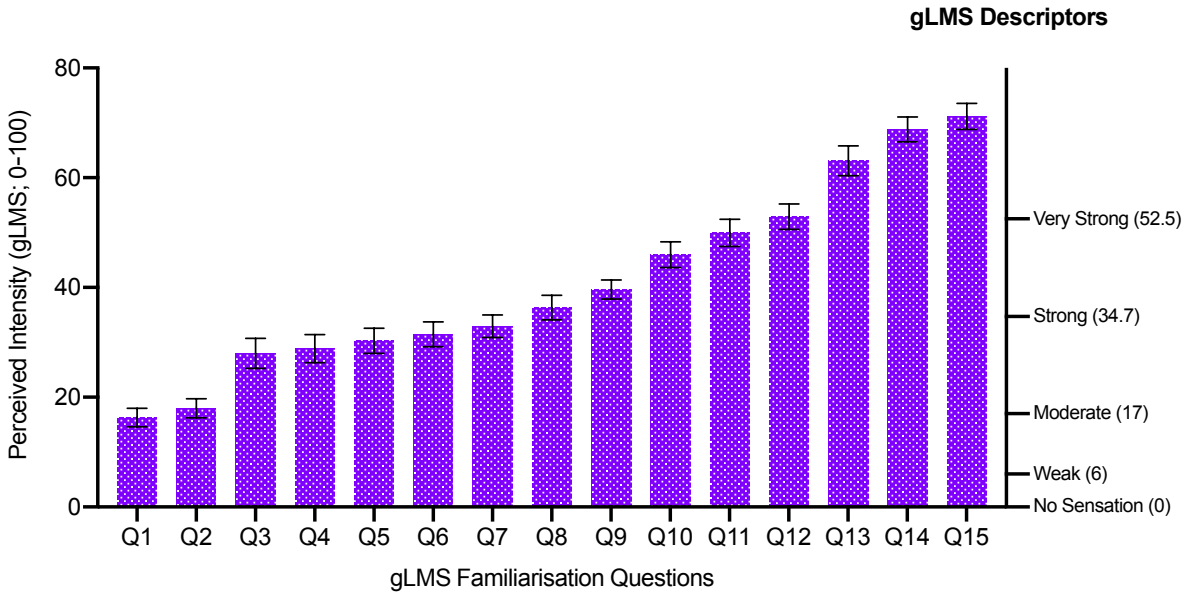

Figure S.5.2. Summary of volunteers (*n* = 40; means of two replicates (visit one and visit two) ± standard error) gLMS familiarisation questions (adapted from Hayes *et al*., 2013)). Q1. The brightness of a dimly lit room; Q2. The loudness of a whisper; Q3. The firmness of a handshake; Q4. The warmth of a summer breeze on your face; Q5. The loudness of a conversation; Q6. The cooling of a peppermint candy; Q7. The brightness of a well-lit room; Q8. The sweetness of candy floss; Q9. The bitterness of black coffee; Q10. The sourness of a lemon; Q11. The burn of a chili pepper; Q12. The pain of biting your tongue; Q13. The heat from putting hand in scalding water; Q14. The brightest light you have ever seen and Q15. The loudest sound you have ever heard.

Table S.5.1. Additional factors (such as sex and saliva flow) influencing volunteers (*n* = 40) liking, easiness to consume, attribute perception and appropriateness of attribute level (Just-About-Right, JAR) mean ratings (± standard error) of whey beverages.

	Sex		**Saliva Flow**	
	Male (*n* = 12)	Female (*n* = 28)	Low Saliva Flow (*n* = 19)	High Saliva Flow (*n* = 20)
Overall Liking	5.9 ± 0.4	5.1 ± 0.3	5.5 ± 0.3	5.4 ± 0.3
Easiness to Drink	3.8 ± 0.2	3.8 ± 0.2	3.9 ± 0.2	3.7 ± 0.2
Easiness to Swallow	3.7 ± 0.2	4.1 ± 0.1	4.0 ± 0.2	3.9 ± 0.2
Sweetness	21.9 ± 3.3	18.6 ± 2.2	19.9 ± 2.9	20.5 ± 2.5
Thickness	15.2 ± 3.1	14.4 ± 2.0	13.5 ± 2.7	16.1 ± 2.3
Mouthdrying	18.9 ± 3.8	22.2 ± 2.5	20.5 ± 3.3	20.6 ± 2.8
JAR Flavour	2.7 ± 0.2	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1
JAR Thickness	2.6 ± 0.2	2.7 ± 0.1	2.6 ± 0.2	2.7 ± 0.1

Individual saliva flow groupings are derived from unstimulated saliva flow only, through quantile 'median' analysis. *Liking and easiness to consume were measured on a 9- and 5-point scale respectively, attribute perception was measured on a gLMS logarithmic scale (anti-logged values 0-100 scale presented) and JAR via 5-point JAR scale.*

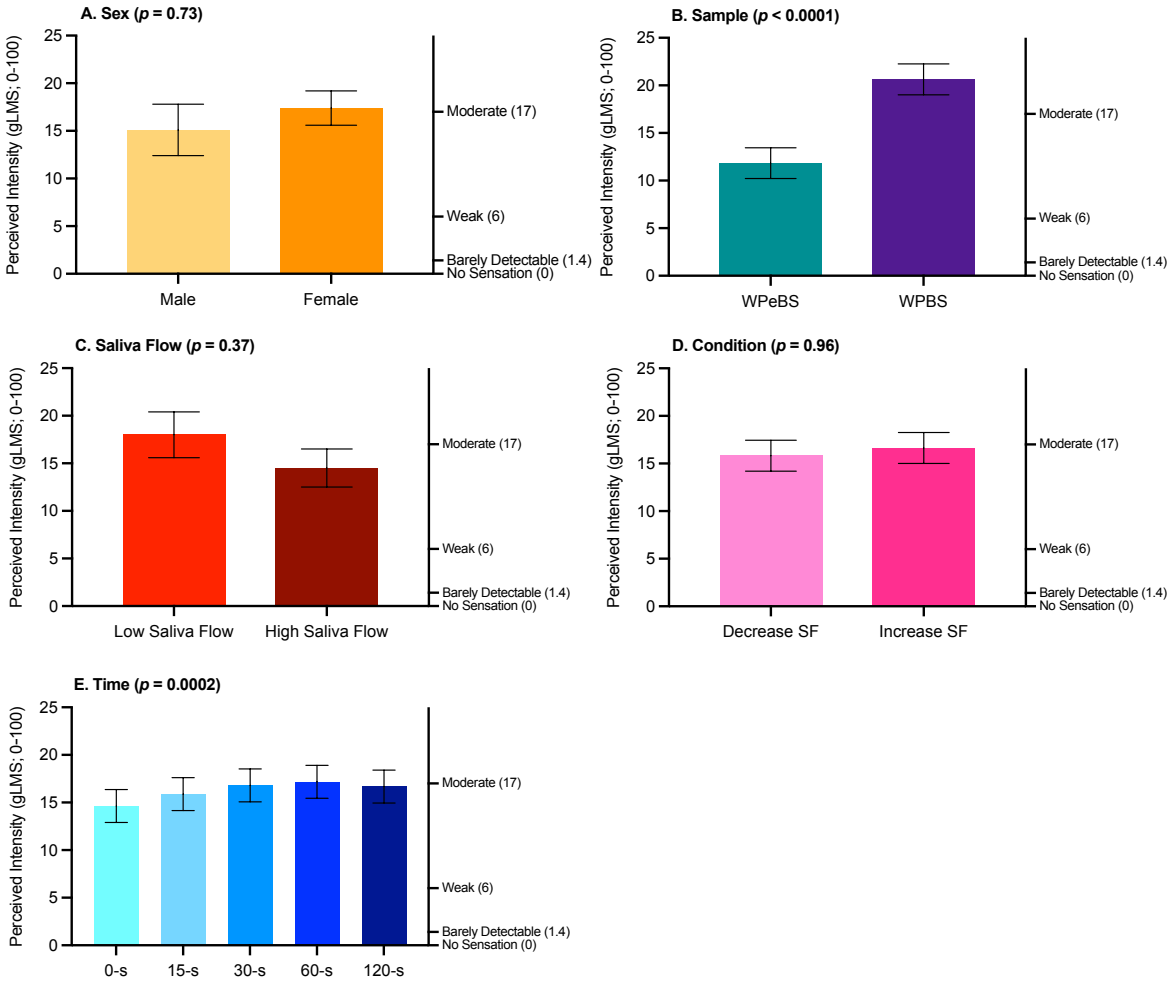

Figure S.5.3. Additional factors influencing volunteers (n = 40) modulating saliva flow perceived mouthdrying (± standard error) with relevant p value above each category (WPeBS: whey permeate beverage sweetened; WPBS: whey protein beverage sweetened; SF: saliva flow).

Table S.5.2. Summary of baseline protein concentration (mg/mL) in saliva samples.

	Overall (n = 40)	**Sex**	
		Male (n = 12)	Female (n = 28)
USF	0.94 ± 0.1	0.87 ± 0.2	0.97 ± 0.1
SSF	0.95 ± 0.07	0.84 ± 0.1	1.0 ± 0.1

No significant differences (p < 0.05) were reported between groups. USF: unstimulated saliva flow and SSF: stimulated saliva flow.

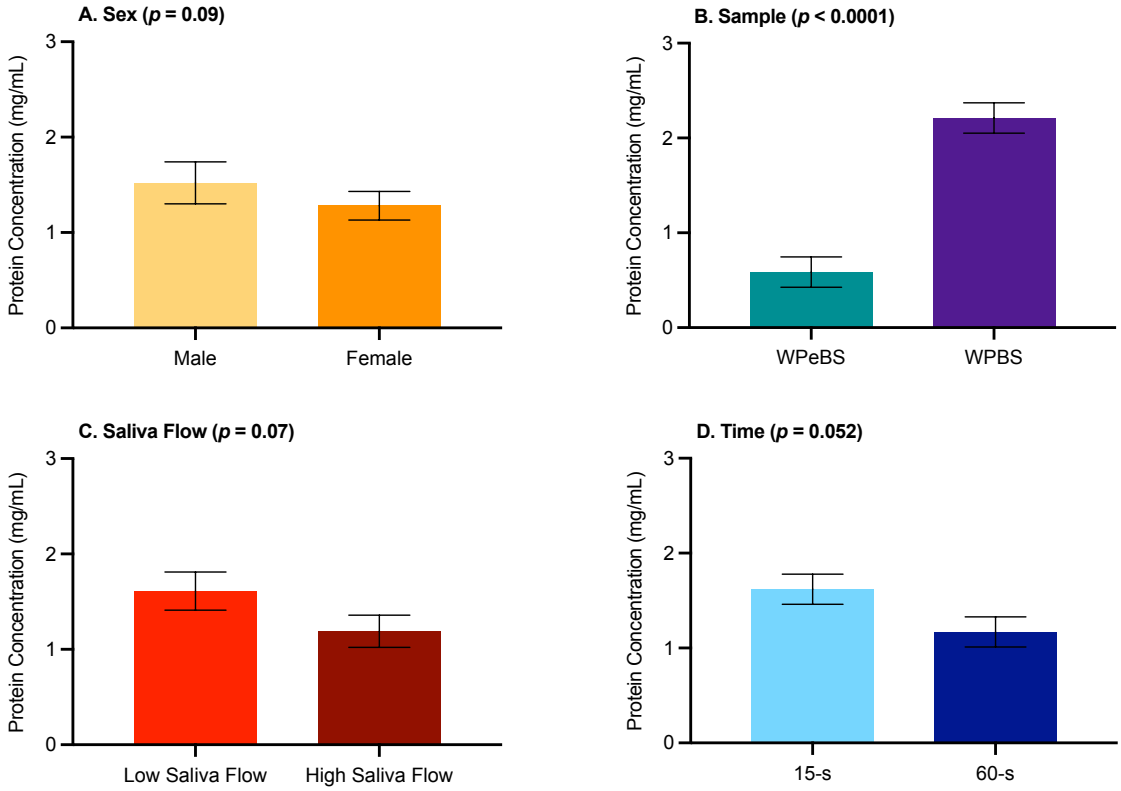

Figure S.5.4a. Additional factors influencing protein concentration (± standard error) in saliva samples post beverage (n = 40) (WPeBS: whey permeate beverage sweetened; WPBS: whey protein beverage sweetened) consumption with relevant p value above each category and a higher value would suggester greater adhesion.

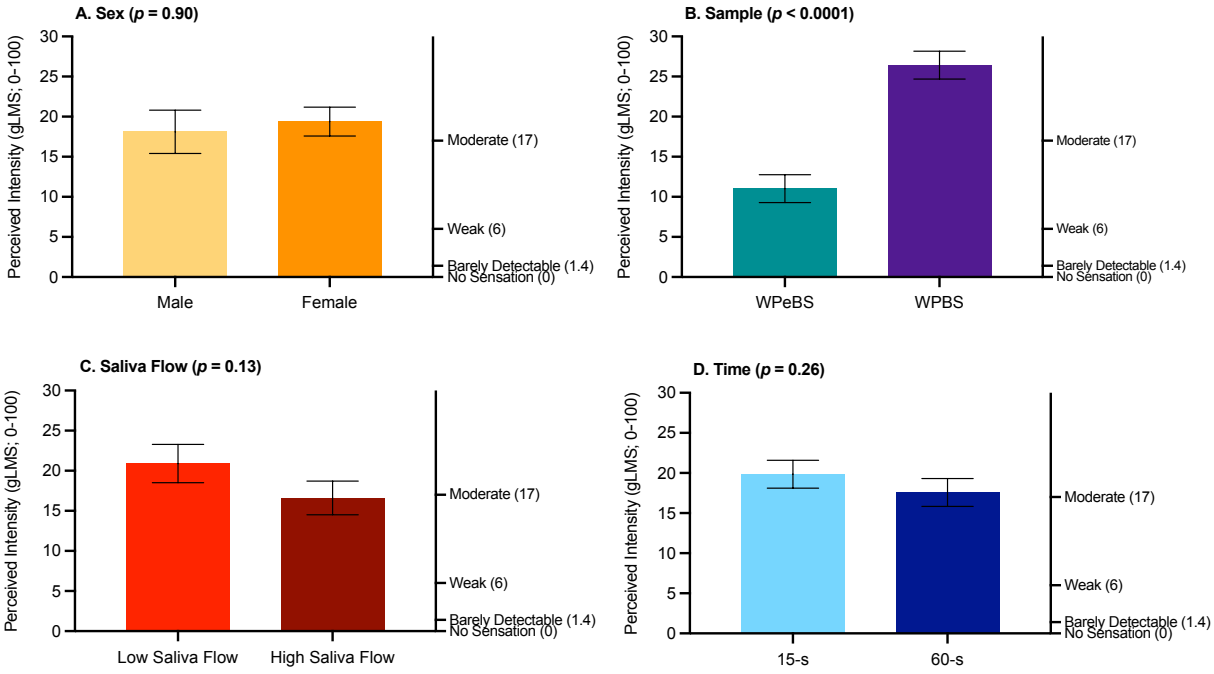

Figure S.5.4b. Additional factors influencing volunteers (n = 40) perceived mouthdrying (± standard error) with relevant p value above each category (WPeBS: whey permeate beverage sweetened; WPBS: whey protein beverage sweetened).

Chapter 6

Investigating methods to mitigate whey protein derived mouthdrying

6.1. Context to chapter

Unfortunately, the COVID-19 pandemic continued and so did the national lockdowns; this resulted in future work again being revised so as to ensure thesis progression.

Since mouthdrying is present regardless of the whey protein model (**Chapters 3-5**) mitigating strategies are key to suppress this effect. Therefore, this chapter studied potential mouthdrying mitigating strategies using the commercial trained sensory panel (based at University of Reading) to understand the effectiveness and extent of any reduction prior to any potential subsequent investigation with consumers. This chapter aims to investigate the following thesis hypothesis: (f) mitigating strategies (such as varying in lactose or fat) will reduce whey protein derived mouthdrying. Accordingly, this hypothesis was tested via the following objectives:

- Does sweetness suppress whey protein derived mouthdrying (via increasing lactose levels) in whey protein beverages (WPB)?
- Does lubrication (using varying fat levels) reduce whey protein derived mouthdrying in WPBs?
- Does increasing lubrication (adding a fat topping) decrease whey protein derived mouthdrying in whey protein solid models?

In addition, altering processing (via WPB heat treatment) impacted subsequent perception in **Chapter 3** and here a whey protein powder which undergone additional heat treatment during manufacturing was tested as a preliminary strategy in solid models.

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6.2. Abstract

Mouthdrying is commonly associated with whey protein fortified products. Therefore, mitigating strategies could be key to reducing mouthdrying and maximising the benefits from such products. Currently, few studies have successfully mitigated whey protein derived mouthdrying and this paper aims to investigate different strategies to reduce mouthdrying effects. Accordingly, a series of experiments were carried out with a trained sensory panel (*n* = 11). Two different whey protein food matrices were tested: (a) whey protein beverages (WPB) varying in lactose (0.05-12.4% w/v) and fat (0.9-7.2% w/v) levels and (b) whey protein fortified snacks: cupcakes with differing whey protein concentrate (WPC) powders (standard and heat-stable) and scones with varying fat content (with and without cream topping). Overall results suggested the tested strategies had limited significant effects on whey protein derived mouthdrying. Increasing lactose (9.4% w/v) in WPBs and fat levels (via cream topping) on scones significantly suppressed mouthdrying. However, all other tested strategies (increasing fat in WPBs and heat-stable WPC in cupcakes) had no significant effect on suppressing perceived mouthdrying. This work demonstrates the challenges with mitigating whey protein derived mouthdrying; however, cross-modal taste suppression and increasing lubrication warrant further investigation.

6.3. Introduction

Whey protein can be described as a value-added ingredient due to its well-cited nutritional and health benefits (Madureira *et al*., 2007; Solak & Akin, 2012). Accordingly, whey protein is often fortified into different food matrices to enhance protein intake; such applications typically include the older consumer (to help prevent malnutrition and sarcopenia) or the sport, health and lifestyle consumer (to enhance performance or

health) (Phillips *et al*., 2016). However, regardless of the application, the sensory profile of such products is key to consumer acceptance and subsequent consumption. As alluded to in our recent review, negative sensory attributes are associated with whey protein fortified products, more specifically, mouthdrying (Norton *et al*., 2021a, **Chapter 2**).

Whey protein derived mouthdrying can be described as the drying sensation in the mouth during or post consumption (Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**; 2021a, **Chapter 2**; 2021b, **Chapter 5**). In addition, mouthdrying and/or dry texture is present in both liquid and solid models fortified with whey protein, such as cakes, beverages, biscuits, muffins and rye breads (Wendin *et al*., 2017; Bull *et al*., 2017; Song *et al*., 2018; Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**; 2021b, **Chapter 5**). To date the causes of such mouthdrying are inconclusive; however, adhesion of protein to the oral cavity (mucoadhesion) has been found to correlate with mouthdrying perception in whey protein beverages (WPB) (Norton *et al*., 2021b, **Chapter 5**). This is highly relevant to older consumers as mouthdrying sensitivity and mucoadhesion are both considered to increase with age (Withers *et al*., 2013a; Norton *et al*., 2020a, **Chapter 3**). Therefore, strategies to mitigate mouthdrying are key to promoting consumer compliance and acceptance.

Despite mouthdrying being present in a range of whey protein fortified products, there are few studies which have successfully mitigated whey protein derived mouthdrying. For example, Withers *et al*. (2014) focused on three strategies, namely increasing sweetness (3.0% wt/wt sucrose), viscosity (1.8% wt/wt starch thickener) and fat (2.0% wt/wt sunflower oil and milk fat) based on previous astringency work. It was suggested that sweetness could suppress mouthdrying, viscosity could reduce interactions within the oral cavity and fat could improve lubrication; therefore, subsequently mask mouthdrying

(Withers *et al*., 2014). However, the results using a trained sensory panel suggested such strategies at the levels tested had a limited effect on perceived mouthdrying (Withers *et al*., 2014). In brief, increasing sweetness and fat significantly increased mouthdrying to a minor extent, whilst viscosity had a small significant effect in suppressing mouthdrying in a protein fortified milk matrix, but there was no significant reduction in the complete oral nutritional supplement (ONS) (Withers *et al*., 2014). Therefore, concluding more research would be beneficial to understand better strategies to effectively mitigate mouthdrying.

Various other studies have shown that sweetness could have a role in suppressing dairy or plant based mouthdrying. Methven *et al*. (2010) demonstrated in ONS (standard ONS versus sweetness suppressed ONS) that increased sweetness correlated with reduced mouthdrying. This finding was supported by two additional studies: (1) soymilks with increased sucrose reduced astringency (Courregelongue *et al*., 1999) and (2) adding sucrose and vanilla flavouring suppressed mouthdrying in WPBs (Norton *et al*., 2021b, **Chapter 5**). However, these studies were limited as they added a set amount of sugar to increase sweetness, rather than a progression to understand at what point sweetness could suppress mouthdrying.

Withers *et al*. (2014) used sunflower oil and milk fat to fortify dairy beverages, yet they found that these fats at the levels used (2.0% wt/wt) were unable to suppress mouthdrying in the liquid beverage. However, other fat sources or levels may have an effect. For example, where cream was added to skimmed milk, varying in fat content (0.2-5.0% wt/wt), the higher fat levels (2.0 or 5.0% wt/wt) were found to reduce perceived astringency (Li *et al*., 2018). Therefore, adding fat to WPBs could influence mouthdrying, but this is yet to be fully investigated. In addition, Engelen *et al*., (2005) noted that increasing fat levels (by adding a topping such as butter) to solid food models could reduce the number of chews, via increased lubrication. Similarly, utilising toppings (firm

cheese, cheese spread and mayonnaise) decreased dryness and firmness in bread and crackers (van Eck *et al*., 2019). Accordingly, adding toppings to whey protein fortified solid foods could be a potential strategy to suppress whey protein derived mouthdrying.

Previous research suggests that heat treatment of WPBs (unheated versus heated for 20-min) alters the mouthfeel attributes (increasing body, chalky, mouthdrying, mouthcoating, and furring) (Bull *et al*., 2017). In addition, differences in processing can impact the functional properties of whey proteins (Kew *et al*., 2020). This suggests whey protein powders (such as whey protein concentrate, WPC), which have undergone an additional heat treatment process during manufacturing, could influence subsequent texture perception of fortified products (Ipsen, 2017). For example, a heat-stable WPC could lead to a creamier mouthfeel in a product compared with a standard WPC, potentially by increasing flow and reducing friction (Cakir-Fuller, 2015; Aggarwal *et al*., 2016; Ipsen, 2017). Accordingly, this warrants investigation into its subsequent effects on perceived mouthdrying within a solid food matrix.

Currently, few studies have effectively suppressed whey protein derived mouthdrying in either a liquid or solid model and a more fundamental investigation is needed. Mitigating mouthdrying could create more acceptable products and promote product consumption. This paper hypothesises that mitigating strategies will reduce mouthdrying as follows: (a) lactose will suppress mouthdrying via cross-modal suppression; (b) increasing lubrication via fat will suppress mouthdrying; and (c) heat-stable WPC in cupcakes will reduce mouthdrying. This paper tests whether these strategies can reduce perceived mouthdrying in two different whey protein food matrices (liquid and solid model), using a sensory trained panel.

6.4. Materials and methods

6.4.1. Overview of experiments

A series of experiments (as outlined in Figure 6.1) were conducted using a trained screened experienced sensory panel (*n* = 11; 10 female and 1 male). The experiments were not subjected to a specific ethical review nor additional consent, as the trained sensory panel were tasting products made from standard commercial food ingredients. However, it should be noted that all panellists had consented to evaluate different food and beverage products as part of their employment contract.

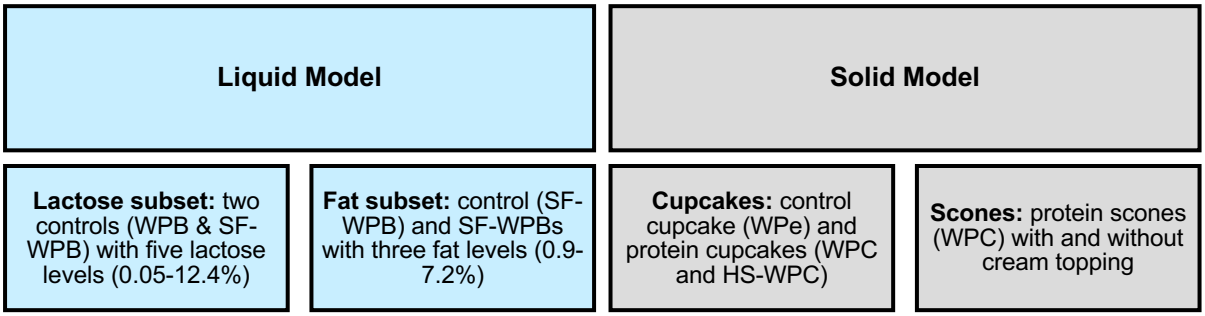

Figure 6.1. Overview of experiments (WPB: whey protein beverage; SF-WPB: sugar-free whey protein beverage; WPe: whey permeate; WPC: whey protein concentrate; HS-WPC: heat-stable whey protein concentrate). Brackets after each sample name denote specific lactose or fat content expressed as % w/v.

6.4.2. Materials

Volac (Volac International Ltd., Royston, UK) provided five different whey derived powders: (1) whey protein concentrate instantised with sunflower lecithin (WPC, Volactive® UltraWhey 80 Instant; 81% protein); (2) sugar-free whey protein concentrate instantised with sunflower lecithin (SF-WPC, Volactive® UltraWhey Sugar Free WPC Instant; 86% protein); (3) heat-stable whey protein concentrate (HS-WPC, Volactive® UltraWhey Velicious™; 70% protein); and (4) whey permeate (WPe, Volactose® Taw Whey Permeate; 89% lactose) and (5) lactose (Volactose® Edible Lactose; 99% lactose). Maltodextrin and xanthan gum-based thickener (Nestle Resource Thicken Up Clear) was

obtained from NutriDrinks (London, UK). Soya lecithin (Louis Francois, Lecithine De Soja En Poudre I.P. - E322) was acquired from Sous Chef (London, UK). Baking ingredients, double cream (British Double Fresh Cream, UK) and clotted cream (Rodda's Clotted Cream, Cornwall) were all purchased from Sainsburys (Reading, UK).

6.4.3. Whey protein liquid models

6.4.3.1. Lactose subset

Two control beverages were tested: (a) whey protein beverage (WPB, 10.0% w/v WPC powder in deionised water) and (b) sugar-free whey protein beverage (SF-WPB, 10.0% w/v SF-WPC powder in deionised water). SF-WPB was fortified with lactose at five different levels to represent a range from 0.4% to 12.4% w/v, based on a ×3.0 progression. The rationale for the lactose levels was that 0.4% w/v matches the control WPB lactose levels, 3.4% w/v is considered just below the lactose relative sweetness detection threshold (Belitz *et al*., 2004) and 12.4% w/v provides a similar relative sweetness level (~ 2.0% w/v sucrose) to our previous work (Norton *et al*., 2021b, **Chapter 5**).

6.4.3.2. Fat subset

The control beverage was a sugar-free whey protein beverage (SF-WPB, 10.0% w/v SF-WPC powder in deionised water). Double cream was added to SF-WPB at three different levels (1.8%, 3.6% and 7.2% w/v) to represent the mid-range fat levels found in ONS. A hydrocolloid (maltodextrin and xanthan gum-based thickener) was added (0.03-0.10% w/v) to minimise differences in viscosity between fat levels, without influencing flavour or mouthfeel attributes. Lecithin (0.1% w/v) was also added to ensure a stable dispersion of the fat phase in the beverage.

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Table 6.1. Overview of whey protein liquid model lactose and fat subset (WPB: whey protein beverage; SF-WPB: sugar-free whey protein beverage).

	Lactose Subset							**Fat Subset**			
	Controls		SF-WPBs (10.0% w/v) varying in lactose levels					Control	SF-WPBs (10.0% w/v) varying in fat levels		
	WPB (0.4%)	SF-WPB (0.05%)	SF-WPB (0.4%)	SF-WPB (3.4%)	SF-WPB (6.4%)	SF-WPB (9.4%)	SF-WPB (12.4%)	SF-WPB (0.9%)	SF-WPB (1.8%)	SF-WPB (3.6%)	SF-WPB (7.2%)
Formulations											
Deionised water (mL)	90.0	90.0	90.0	87.0	84.0	81.0	78.0	90.0	88.0	84.0	77.0
WPC (g)	10.0	-	-	-	-	-	-	-	-	-	-
SF-WPC (g)	-	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Lactose (g)	-	-	0.4	3.4	6.4	9.4	12.4	-	-	-	-
Double cream (g)	-	-	-	-	-	-	-	-	1.8	5.6	13.2
Lecithin (g)	-	-	-	-	-	-	-	-	0.1	0.1	0.1
Hydrocolloid (g)	-	-	-	-	-	-	-	0.1	0.05	0.03	-
Composition											
Energy (kcal)	40.1	41.6	41.8	43.0	44.1	45.3	46.5	41.6	49.5	66.1	99.3
Fat (g)	0.8	0.95	0.95	0.95	0.95	0.95	0.95	0.95	1.8	3.6	7.2
of which saturates (g)	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.35	0.9	2.0	4.3
Carbohydrate (g)	0.4	0.05	0.4	3.4	6.4	9.4	12.4	0.05	0.08	0.1	0.2
of which sugars (g)	0.4	0.05	0.4	3.4	6.4	9.4	12.4	0.05	0.08	0.1	0.2
Protein (g)	8.2	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.7	8.8

Composition data is obtained from ingredients technical sheets and dash (-) represents not applicable. Brackets after each sample name denote specific lactose or fat content expressed as % w/v. Acronyms: whey protein concentrate (WPC); sugar-free whey protein concentrate (SF-WPC). Viscosity of WPBs were measured to ensure similarity within subsets and summarised in Table S.6.1.

All beverages are summarised in Table 6.1. In both subsets, the preparation method utilised is as described in previous work (Norton *et al*., 2020a, **Chapter 3**; 2021b, **Chapter 5**), where all beverages were prepared and stirred (Stuart™ SM5 Bibby Fascia, Cole-Parmer, Staffordshire, UK) for 90-min at room temperature (19.3 ± 0.5 °C), hydrated overnight (4 °C) and assessed or consumed at room temperature the following day

6.4.4. Whey protein solid models

All solid model formulations and nutritional compositions (Nutritics v5.64, Dublin, Ireland) are outlined in Table 6.2.

Table 6.2. Overview of whey protein solid model cupcakes and scones (WPC: whey protein concentrate; HS-WPC: heat-stable whey protein concentrate) per 100 g.

	Cupcakes			**Scones**		
	Control	WPC	HS-WPC	Control	Protein	Protein + cream topping
Formulations						
Unsalted butter (g)	23.0	23.0	22.7	10.2	10.2	10.2
Self-raising flour (g)	23.0	23.0	22.7	46.0	46.0	46.0
Caster sugar (g)	23.0	23.0	22.7	5.1	5.1	5.1
Milk (whole) (g)	5.0	5.0	4.9	20.4	20.4	20.4
Eggs (free-range) (g)	18.6	18.6	18.4	10.2	10.2	10.2
Lemon zest (g)	0.76	0.76	0.75	-	-	-
Whey permeate (g)	6.64	-	-	8.0	-	-
WPC (g)	-	6.64	-	-	8.0	8.0
HS-WPC (g)	-	-	7.7	-	-	-
Clotted cream (g)	-	-	-	-	-	26.7
Composition						
Energy (kcal)	442	445	448	350	353	509
Fat (g)	23.0	24.0	25.0	12.0	13.0	30.0
of which saturates (g)	14.0	14.0	14.0	6.9	7.2	17.8
Carbohydrate (g)	51.0	45.0	44.0	52.0	44.0	44.6
of which sugars (g)	26.0	26.0	26.0	15.0	7.5	8.1
Fibre (g)	1.1	1.1	1.1	2.0	2.0	2.0
Protein (g)	6.0	12.0	12.0	7.6	15.0	15.4
Salt (g)	0.2	0.2	0.2	0.5	0.5	0.5

Composition data is obtained from Nutritics software and dash (-) represents not applicable. Acronyms: whey protein concentrate (WPC); heat-stable whey protein concentrate (HS-WPC).

6.4.4.1. Cupcakes

Three different lemon cupcakes were developed based on our previous work (Norton *et al*., 2020b, **Chapter 4**). The control cupcake was fortified with whey permeate and the

protein cupcakes were fortified with (a) whey protein concentrate (WPC cupcake) and (b) heat-stable whey protein concentrate (HS-WPC cupcake) to understand the influence of processing differences on subsequent perception. The recipes were prepared as previously described (Norton *et al*., 2020b, **Chapter 4**). In summary, an all-in-one method was utilised (low speed 5 to 8-min, Kenwood Titanium Major KMM020, Hampshire, UK) until well-mixed and the batter (38.2 g) was individually weighed into paper cases (80.0 mm × 62.5 mm). Cupcakes were baked at 170 °C for 20-min (in a pre-heated Altas Salva Oven, London, UK). Cupcakes were individually packaged in heat-sealed polypropylene pouches, frozen at -18 °C until time of consumption and a sample (150 g) from each batch was sent for microbiological testing (SYNLAB, Northumberland, UK).

6.4.4.2. Scones

6.4.4.2.1. Scones sensory profiling

Cupcakes were already considered high in fat content (23-25 g); hence, scones were formulated to investigate the effect of fat on mouthdrying perception. Two different scones were tested: (a) control scone fortified with whey permeate and (b) protein scone fortified with whey protein concentrate. In summary, self-raising flour, sugar, whey powders and butter were mixed until resembling fine breadcrumbs (low speed, 5 to 10-min). Eggs and milk were added and mixed (low speed, 2-min). Dough pieces were rolled (sheeted, 1.0 cm thickness), cut (using 4.5 cm cutter) and weighed (32.5 g). All tops of scones were brushed with eggs and milk mixture and baked at 200 °C for 12-min. Scones were baked and consumed fresh (within 4-h) for full sensory profiling. The rationale for baking scones fresh related particularly to the control scone being adversely affected by freezing due to starch retrogradation (Wang *et al*., 2015) and subsequent staling.

6.4.4.2.2. Scones with and without topping

Sensory profiling results (Table S.6.2) demonstrated the key differences between the control and protein scone were mainly related to mouthfeel. Thus, in order to evaluate the effect of fat on mouthfeel perception, only the protein scone was assessed with and without cream topping (8.0 g of clotted cream providing 5.0 g of fat). Scones were individually packaged (heat-sealed polypropylene pouches), frozen (-18 °C) until time of consumption and a sample (150 g) from each batch was sent for microbiological testing.

6.4.4.3. Physical properties of cupcakes and scones

The physical properties of the cupcakes and scones were analysed in triplicate from three different batches (n = 9). In brief, the following analysis was carried out based on our previous work (Norton *et al*., 2020b, **Chapter 4**): (a) moisture content (%) (moisture analyser, Sartorius MA37, Germany); (b) water activity (a_w) (Hydrolab C1, UK); (c) crumb colour was measured (colorimeter, Chroma Meter CR-400, Japan) and the results were expressed in accordance with the CIELAB system (illuminant C and 10° viewing angle) where L^* (lightness) was recorded and the a^* (red-green) and b^* (yellow-blue) colour coordinates were converted to the hue angle (arctan (b^*/a^*)) (McLellan *et al*., 1995); (d) height (mm) (digital calipers, Whitworth Tool Inc., USA); and (e) texture profile analysis (TPA) using a double compression test (cylindrical probe, P/75; 15.0 mm slice) on a texture analyser (XTPlus, Stable Micro System, Godalming, UK).

6.4.5. Sensory profile

The trained sensory panel (with extensive experience of profiling whey protein fortified products) used descriptive sensory profiling (DSP) (a modified quantitative descriptive analysis (QDA^{TM}) (Stone *et al*., 1974; Stone & Sidel, 2004)) (in accordance with ISO 8586:2012 and ISO 11132:2012) to determine the sensory profile (ISO, 2012a; 2012b;

Heymann *et al*., 2012). All experiments were carried out at each panellist's home due to COVID-19 restrictions, whilst adhering to COVID-19 guidelines at the time (January to April 2021) with suitable risk assessments. All sessions were conducted on Microsoft Teams (Version 1.3.00.28778, Washington, USA); scoring was completed individually using Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Guelph, Ontario, Canada). All samples were prepared at the University of Reading and provided to panellists each morning; testing was completed individually on an iPad (Apple, London, UK) in a quiet and aroma free location. All scoring was conducted in duplicate in separate sessions and on visual analogue scales (VAS; 0-100) with products (coded with a random three-digit number) consumed in a sequential balanced order with randomly allocated sample sets. In all experiments the panellists developed a consensus vocabulary (Table 6.3) adapted from our previous work (Norton *et al*., 2020b, **Chapter 4**, 2021b, **Chapter 5**) with modifications for each experiment are summarised in Table 6.4.

Table 6.3. Summary of descriptive sensory profiling (DSP) attributes with reference and/or description for all experiments (whey protein beverages with lactose[1], whey protein beverages with fat[2], cupcakes[3], scones[4] and scones with and without cream topping[5]).

Modality	Attribute	Reference and/or Description
Appearance	Moist appearance[3,4]	Slightly or moderately wet to touch
	Dense appearance of sponge/dough[3,4]	Compact in structure
	Appearance of large holes in sponge[3,4]	Holes within crumb/dough structure (none to lots)
	Yellow colour of crumb/dough (inside)[3,4]	Intensity of yellow colour within crumb/dough (pale to dark)
Aroma	Cooked milk[1]	Heated pasteurised semi-skimmed milk
	Powdered milk (wet)[1]	Skimmed milk powder (10.0% w/v, skimmed milk powder in deionised water)
	Whey isolate[1]	Volactive Ultra-Whey 90 Instant (5.0% w/v, WPI powder in deionised water)
	Overall aroma intensity[3,4]	Intensity of aroma within cupcake/scone
	Sweet[3,4]	Sucrose (5.76 g/L)
	Lemon[3]	Lemon zest (grated)
	Buttery[3,4]	Cooked butter (melted unsalted butter)
	Eggy[3]	Intensity of eggy notes
	Floury[4]	Intensity of floury notes (self-raising flour)
	Savoury/Cheesey[4]	Toasted cheddar cheese
	Off-Flavours[3,4]	Curded buttermilk (cooked buttermilk)
Flavour	Sour[1,2]	Citric acid (0.76 g/L)
	Metallic[1,2,3,4]	Iron (II) sulphate heptahydrate (0.0036 g/L)
	Salty[1,2]	Sodium chloride (1.19 g/L)
	Sweet[1,2,3,4]	Sucrose (5.76 g/L)
	Cooked butter[1,2]	Melted unsalted butter
	Cooked milk[1,2]	Heated pasteurised semi-skimmed milk
	Powdered milk (wet)[1,2]	Skimmed milk powder (10.0% w/v, skimmed milk powder in deionised water)
	Whey isolate[1,2]	Volactive Ultra-Whey 90 Instant (5.0% w/v, WPI powder in deionised water)
	Overall flavour intensity[3,4]	Intensity of flavour within cake
	Lemony[3]	Lemon zest (grated)
	Buttery[3,4]	Cooked butter (melted unsalted butter)
	Floury[4]	Intensity of floury notes (self-raising flour)
	Savoury/Cheesey[4]	Toasted cheddar cheese
	Eggy[3]	Intensity of eggy note
	Liquorice[3]	Liquorice (liquorice twists)
	Off-flavours[3,4]	Curded buttermilk (cooked buttermilk)
Mouthfeel	Body[1,2]	Fullness of sample (low to high)
	Powdery[1,2]	Dry fine insoluble powder
	Mouthdrying[1.2,3,4,5]	Drying sensation in the mouth
	Firmness of bite[3,4,5]	Degree of force with first bite (soft to firm)
	Moist sponge/dough[3,4,5]	Slightly damp sponge/dough (dry to moist)
	Chewy[3,4.5]	Ease of ability to chew
	Greasy lips[3,4]	Degree of oiliness/greasiness on lips
	Crumbliness of sponge/dough[3,4,5]	Ease to break into small pieces
	Crumb size[3]	Size of crumb inside of cake
	Pasty (cohesive)[3,4,5]	Sticking to surfaces
	Rate of breakdown & clearance[3,4,5]	Clearing sample from mouth (slow to fast)
	Cooling sensation[3]	A stimulation resulting in feeling of coolness
Aftertaste	Aftertaste strength[1,2]	The strength of the overall aftertaste
	Mouthdrying[1,2,3,4]	Drying sensation in the mouth
	Metallic[1,2,3,4]	Iron (II) sulphate heptahydrate (0.0036 g/L)
	Sweet[1,3,4]	Sucrose (5.76 g/L)
	Lemon[3]	Lemon zest (grated)
	Buttery[3,4]	Cooked butter (melted unsalted butter)
	Savoury/Cheesey[4]	Toasted cheddar cheese
	Off-flavours[3,4]	Curded buttermilk (cooked buttermilk)
	Salty[3,4]	Sodium chloride (1.19 g/L)
	Salivating[3,4]	Increased saliva within mouth
	Liquorice[3]	Liquorice (liquorice twists)

All anchors not to very unless otherwise stated

Table 6.4. Overview of sensory profile modifications for each experiment (WPB: whey protein beverage).

Experiment	Panellists[a]	Attributes[b]	Consumption Instructions	Additional Comments
WPBs with lactose[1]	10	18	○ Panellists assessed aroma, then consumed a sip to evaluate flavour followed by two further sips for mouthfeel and aftertaste	○ Panellists were provided with 10 mL of beverage in 25 mL plastic cups (opaque & black (BB Plastics, UK)) ○ To prevent bias evaluation, modality appearance was not assessed in case of potential visual differences
WPBs with fat[2]	11	14	○ Panellists consumed a sip to evaluate taste/flavour followed by two further sips for mouthfeel and aftertaste	○ Panellists were provided with 10 mL of beverage in 25 mL plastic cups ○ To prevent bias evaluation, modality appearance was not assessed in case of potential visual differences ○ All evaluation was carried out using nose clips; therefore, aroma was also not evaluated
Cupcakes[3]	10	37	○ Panellists were asked to break each cupcake in half and consume from the middle ○ Panellists assessed appearance and aroma then consumed a bite to evaluate flavour followed by two further bites for mouthfeel and aftertaste	○ Panellists were provided with a 35.0 g cupcake ○ All modalities were evaluated
Scones[4]	10	32	○ Panellists were asked to break each scone in half and consume from the middle ○ Panellists assessed appearance and aroma then consumed a bite to evaluate flavour followed by two further bites for mouthfeel and aftertaste	○ Panellists were provided with a 30.0 g scone ○ All modalities were evaluated
Scones with and without cream topping[5]	8	7	○ Panellists were asked to break each scone in half and consume from the middle	○ Panellists were provided with a 30.0 g protein scone with and without cream topping (8.0 g; clotted cream) ○ Only selected mouthfeel attributes were evaluated based on full sensory profiling results[4]

Subscript numbers[1-5] reflect experiment number. [a] refers to the differing number of panellists present in each experiment. [b] denotes the varying number of attributes identified within each experiment as fully defined in Table 6.3. In experiments [(1-4)] there was a 60-s delay before scoring aftertaste and warm filtered water (~40 °C) was used as the palate cleanser in all experiments.

6.4.6. Statistical analysis

In all experiments, DSP data was analysed using SenPAQ (version 6.3, Qi Statistics, UK) by analysis of variance (ANOVA; rationale as outlined in our previous work (Norton *et al*., 2020b, **Chapter 4**; 2021b, **Chapter 5**)). The main effects (product and panellist) were tested against the product by panellist interaction (with product and panellists as fixed and random effects respectively). Post hoc analysis (if ANOVA denoted significant value) was carried out using either Fishers least significant difference (LSD) (less than five samples) or Tukey-Kramer honestly significant difference (HSD) (five or more samples) to determine multiple comparisons (Hasted, 2018).

XLSTAT (version 2020.1.3, Addinsoft, New York, USA) was used to analyse cupcake and scone physical properties data; specific statistical tests were based on distribution of data (normally distributed data as defined by normality of residuals $p > 0.05$) and number of samples: (a) cupcakes via ANOVA (normally distributed data with multiple pairwise comparisons carried out using Fishers LSD) and Kruskal-Wallis test (non-normally distributed data); and (b) scones were analysed using *t*-tests (normally distributed data) and Mann-Whitney test (non-normally distributed data). In all experiments sample significance was defined as $p < 0.05$.

6.5. Results

6.5.1. Whey protein beverages with lactose

Fortifying WPBs with lactose resulted in nine out of 18 attributes being significantly different as demonstrated in Table 6.5. In brief, to varying extents, increasing lactose significantly reduces sourness, whey isolate, powdery, mouthdrying and metallic notes, as well as significantly increasing sweetness, cooked milk, aftertaste strength and sweet aftertaste. The sensory profile also demonstrated minimal differences between the two

controls (WPB and SF-WPB). However, it should be noted that lactose had only a small effect on significantly suppressing mouthdrying, and this was only significant at 9.4% w/v lactose which correlated with high sweetness intensity.

6.5.2. Whey protein beverages with fat

The sensory profile of WPBs, varying in fat, resulted in six significant differences (from 14 attributes) demonstrating fat significantly reduced metallic taste and whey isolate flavour, whilst significantly increasing cooked milk flavour, body, aftertaste strength and mouthdrying aftertaste (Figure 6.2). In summary, increasing fat (via double cream) had no significant effect on mouthdrying during consumption; however, post consumption (aftertaste) mouthdrying was significant but did not follow a consistent trend with increasing fat levels.

Table 6.5. Influence of lactose content on the sensory profile of whey protein liquid models (WPB: whey protein beverage; SF-WPB: sugar-free whey protein beverage).

Modality	Attribute	Controls		SF-WPB varying in lactose levels					Significance of sample (*p* value)
		WPB (0.4%)	SF-WPB (0.05%)	SF-WPB (0.4%)	SF-WPB (3.4%)	SF-WPB (6.4%)	SF-WPB (9.4%)	SF-WPB (12.4%)	
Aroma	Cooked milk	12.3 ± 2.8	16.5 ± 3.9	12.9 ± 3.5	10.4 ± 2.6	16.2 ± 3.4	16.8 ± 3.6	18.4 ± 3.5	0.48
	Powdered milk (wet)	21.0 ± 3.8	18.8 ± 4.1	21.6 ± 4.1	24.9 ± 4.1	18.7 ± 4.3	22.0 ± 4.1	18.6 ± 4.0	0.72
	Whey isolate	18.6 ± 3.7	12.9 ± 3.6	13.2 ± 3.6	15.4 ± 3.1	17.6 ± 3.4	14.0 ± 2.3	13.6 ± 3.1	0.57
Flavour	Sour	22.1± 3.5^{a}	24.4 ± 4.0^{a}	21.8 ± 4.1^{ab}	22.0 ± 3.8^{a}	18.5 ± 3.3^{abc}	13.7 ± 3.2^{c}	13.8 ± 3.3^{bc}	**0.0002**
	Metallic	12.4 ± 3.1	11.8 ± 2.8	11.6 ± 3.2	12.1 ± 2.7	8.8 ± 2.4	9.5 ± 2.1	9.1 ± 2.4	0.27
	Salty	9.5 ± 1.4	7.2 ± 2.0	7.7 ± 1.9	9.1 ± 1.8	10.2 ± 1.2	10.3 ± 1.3	7.7 ± 1.2	0.50
	Sweet	8.1 ± 2.1^{c}	5.3 ± 1.3^{c}	5.7 ± 2.2^{c}	15.0 ± 2.5^{c}	29.7 ± 3.3^{b}	42.0 ± 1.9^{a}	47.2 ± 2.0^{a}	**< 0.0001**
	Cooked butter	6.2 ±1.7	2.7 ±1.4	6.3 ± 2.1	2.6 ± 1.2	4.3 ± 1.4	5.5 ± 1.6	6.4 ± 1.5	0.47
	Cooked milk	7.5 ± 2.2^{b}	9.6 ± 2.8^{b}	9.0 ± 2.8^{b}	10.4 ± 2.6^{b}	19.7 ± 3.3^{ab}	24.5 ± 3.0^{a}	23.8 ± 3.6^{a}	**< 0.0001**
	Powdered milk (wet)	22.3 ± 4.1	17.9 ± 4.3	23.3 ± 3.8	22.0 ± 3.9	16.6 ± 4.6	21.1 ± 4.2	19.0 ± 4.3	0.67
	Whey isolate	27.9 ± 3.1^{a}	28.5 ± 3.9^{a}	22.2 ± 4.0^{ab}	25.0 ± 3.1^{ab}	21.8 ± 3.0^{ab}	17.7 ± 2.5^{ab}	15.3 ± 2.6^{b}	**0.003**
Mouthfeel	Body	30.4 ± 2.1	29.4 ± 1.9	31.6 ± 2.3	29.7 ± 2.1	26.8 ± 1.9	30.9 ± 1.9	28.4 ± 1.8	0.36
	Powdery	14.3 ± 4.2^{ab}	11.5 ± 4.0^{ab}	16.2 ± 4.8^{a}	12.5 ± 3.9^{ab}	7.5 ± 2.5^{b}	8.6 ± 3.4^{ab}	8.1 ± 3.1^{ab}	**0.02**
	Mouthdrying	47.2 ± 3.6^{ab}	49.1 ± 3.7^{a}	45.8 ± 4.0^{ab}	47.0 ± 3.9^{ab}	41.7 ± 3.4^{ab}	39.9 ± 3.1^{b}	41.2 ± 3.1^{ab}	**0.02**
Aftertaste	Aftertaste strength	26.9 ± 2.3^{ab}	22.2 ± 1.8^{b}	23.0 ± 1.9^{b}	24.0 ± 1.9^{b}	27.8 ± 1.5^{ab}	30.2 ± 1.5^{a}	27.9 ± 1.7^{ab}	**0.0004**
	Mouthdrying	43.0 ± 3.2	45.9 ± 4.0	46.5 ± 3.6	44.2 ± 2.6	38.6 ± 3.4	41.5 ± 2.8	41.7 ± 2.7	0.32
	Metallic	7.9 ± 2.7^{a}	6.4 ± 2.1^{a}	8.0 ± 2.9^{a}	5.0 ± 1.8^{a}	3.7 ± 1.5^{a}	3.0 ± 1.3^{a}	4.1 ± 1.8^{a}	**0.01**
	Sweet	4.7± 1.3^{c}	3.3 ± 1.5^{c}	5.3 ± 2.1^{c}	7.0 ± 1.9^{c}	18.6 ± 2.3^{b}	27.1 ± 2.0^{a}	30.3 ± 2.4^{a}	**< 0.0001**

Data represents means of two replicates ± standard error from trained sensory panel (n = 10) measured on visual analogue scales (VAS; 0-100). Differing small letters represent sample significance from multiple comparisons and brackets after sample name denote specific lactose content expressed as % w/v. All attributes are fully defined in Table 6.3.

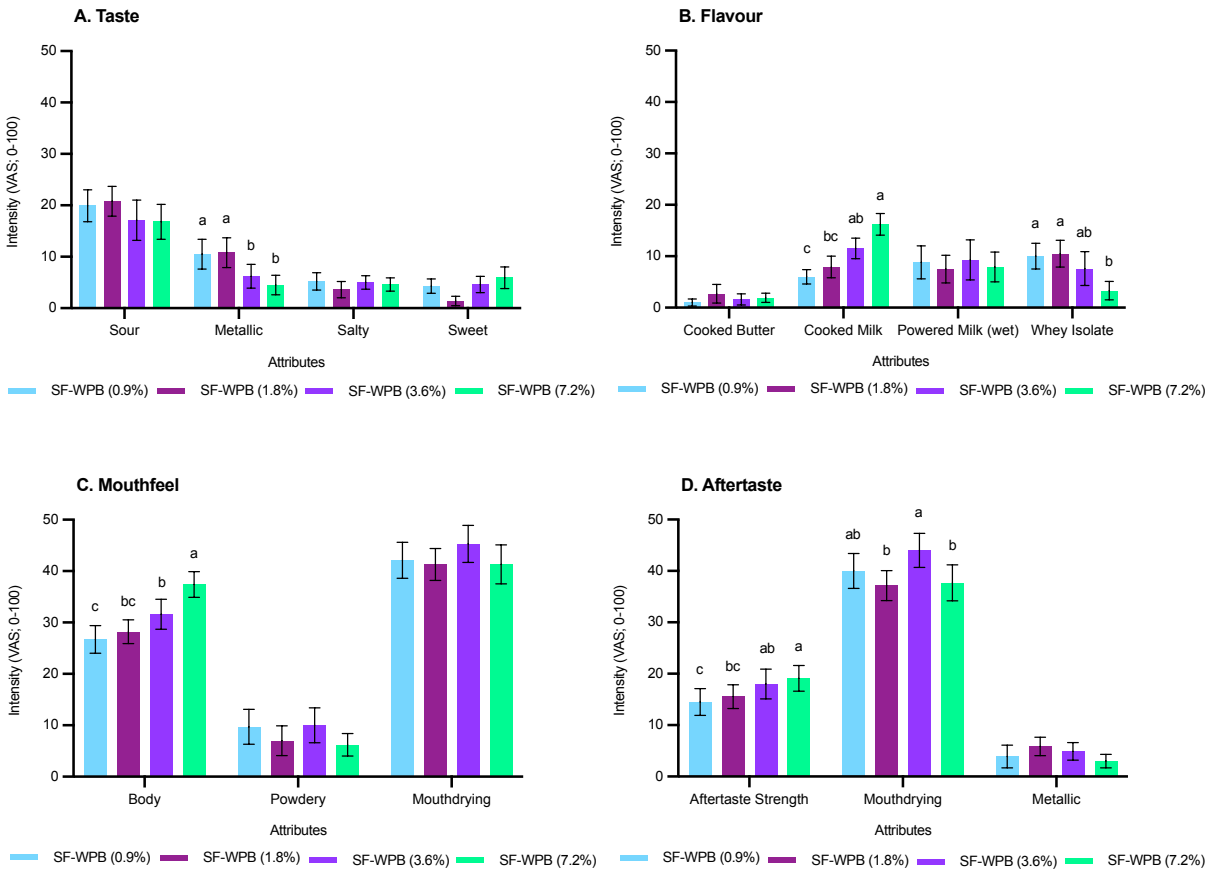


Figure 6.2. Influence of fat content on the sensory profile of whey protein liquid models (SF-WPB: sugar-free whey protein beverage). Data represents means of two replicates ± standard error from trained sensory panel (*n* = 11) measured on visual analogue scales (VAS; 0-100). Differing small letters represent sample significance from multiple comparisons and brackets after sample name denote specific fat content expressed as % w/v. All attributes are fully defined in Table 6.3.

6.5.3. Cupcakes

There were 15 significant differences reported from 37 attributes, as outlined in Table 6.6. In summary, protein fortification (WPC and HS-WPC cupcake) resulted in significantly increased firmness of bite, chewiness and mouthdrying, whilst significantly reducing moist sponge and rate of breakdown and clearance compared with the control cupcake. Overall, there were minimal differences in the sensory profile between the two protein versions (WPC and HS-WPC cupcakes) with mouthdrying reported to the same extent between the two protein versions. The physical properties of cupcakes are summarised in Figure S.6.1, where the heat-stable WPC had a greater effect on the physical

properties. For example, HS-WPC cupcakes had significantly lower hardness, cohesiveness, chewiness and had a more yellow crumb colour (higher *b** and lower hue angle) compared with WPC cupcakes.

Table 6.6. Influence of processing differences in whey protein powders on the sensory profile of whey protein solid models (WPC: whey protein concentrate; HS-WPC: heat-stable whey protein concentrate).

Modality	Attribute	Cupcakes			Significance of sample (*p* value)
		Control	WPC	HS-WPC	
Appearance	Moist appearance	52.3 ± 3.1^{a}	26.7 ± 2.6^{b}	19.1 ± 2.3^{b}	**< 0.0001**
	Dense appearance of sponge	39.9 ± 2.7^{b}	56.7 ± 3.2^{a}	64.0 ± 3.3^{a}	**0.0001**
	Appearance of large holes in sponge	19.8 ± 2.1^{b}	39.8 ± 4.0^{a}	48.1 ± 4.0^{a}	**< 0.0001**
	Yellow colour of crumb (inside)	52.6 ± 1.9^{a}	35.9 ± 2.2^{c}	46.8 ± 2.8^{b}	**< 0.0001**
Aroma	Overall aroma intensity	53.6 ± 2.1	51.3 ± 2.4	52.2 ± 1.7	0.73
	Sweet	38.2 ± 2.9	38.4 ± 1.8	38.3 ± 1.7	1.00
	Lemon	36.7 ± 3.1	37.7 ± 3.3	37.4 ± 3.2	0.97
	Buttery	22.2 ± 3.2^{a}	12.6 ± 2.6^{b}	14.3 ± 2.9^{b}	**0.003**
	Eggy	14.7 ± 2.8	13.9 ± 2.9	14.2 ± 3.1	0.98
	Off-flavours	0.0 ± 0.03	3.2 ± 1.5	4.1 ± 2.1	0.22
Flavour	Overall flavour intensity	51.6 ± 2.2	44.3 ± 2.3	48.5 ± 2.2	0.07
	Sweet	44.2 ± 3.2	38.2 ± 2.1	43.0 ± 1.9	0.22
	Metallic	0.6 ± 0.5^{b}	4.1 ± 1.8^{ab}	6.7 ± 2.2^{a}	**0.04**
	Lemony	37.9 ± 2.5	32.1 ± 2.7	32.1 ± 2.3	0.28
	Buttery	23.0 ± 2.8^{a}	8.7 ± 2.1^{b}	11.1 ± 2.7^{b}	**0.0005**
	Eggy	12.3 ± 2.5	9.2 ± 2.6	12.3 ± 2.8	0.55
	Liquorice	1.4 ± 1.1	5.3 ± 1.9	5.6 ± 2.7	0.23
	Off-flavours	0.0 ± 0.03	2.3 ± 1.3	3.1 ± 1.5	0.19
Mouthfeel	Firmness of bite	31.3 ± 1.7^{b}	60.1 ± 2.5^{a}	63.2 ± 2.8^{a}	**< 0.0001**
	Moist sponge	60.8 ± 2.2^{a}	18.9 ± 1.6^{b}	19.0 ± 2.6^{b}	**< 0.0001**
	Chewy	27.5 ± 2.3^{b}	48.8 ± 4.4^{a}	56.4 ± 3.0^{a}	**< 0.0001**
	Mouthdrying	24.5 ± 2.6^{b}	42.3 ± 3.6^{a}	46.3 ± 3.3^{a}	**< 0.0001**
	Greasy lips	13.7 ± 2.5^{a}	2.3 ± 1.1^{b}	3.2 ± 1.5^{b}	**0.0003**
	Crumbliness of sponge	36.5 ± 3.4	33.3 ± 3.8	32.0 ± 4.1	0.76
	Crumb size	35.0 ± 2.2	45.4 ± 3.6	43.7 ± 3.9	0.06
	Pasty (cohesive)	40.0 ± 4.1	36.5 ± 3.7	36.4 ± 4.6	0.84
	Rate of breakdown & clearance	52.6 ± 3.5^{a}	32.8 ± 1.7^{b}	35.1 ± 2.9^{b}	**0.0001**
	Cooling sensation	4.9 ± 2.2	3.7 ± ± 1.9	7.1 ± 2.3	0.33
Aftertaste	Mouthdrying	27.4 ± 2.7^{b}	38.8 ± 3.5^{a}	40.6 ± 3.8^{a}	**0.0001**
	Sweet	39.3 ± 3.1	35.9 ± 2.8	36.8 ± 2.7	0.45
	Lemon	27.3 ± 2.8	24.5 ± 2.7	25.1 ± 2.3	0.54
	Buttery	11.3 ± 2.2^{a}	4.9 ± 1.9^{b}	8.6 ± 2.1^{ab}	**0.01**
	Off-flavours	0.0 ± 0.02	1.8 ± 1.2	1.7 ± 1.0	0.36
	Salty	2.1 ± 0.9	5.8 ± 1.7	3.8 ± 1.6	0.18
	Salivating	29.4 ± 2.5	32.3 ± 3.5	34.4 ± 3.1	0.26
	Metallic	2.5 ± 1.4	6.5 ± 2.4	8.4 ± 2.2	0.06
	Liquorice	1.7 ± 1.2	2.6 ± 1.4	5.8 ± 2.6	0.11

Data represents means of two replicates ± standard error from trained sensory panel (n = 10) measured on visual analogue scales (VAS; 0-100). Differing small letters represent sample significance from multiple comparisons and all attributes are fully defined in Table 6.3.

6.5.4. Scones

Sensory profiling demonstrated four significant differences from 32 attributes between the control and protein scones, as described in Table S6.2. Scones fortified with whey protein (WPC) had a significantly more savoury/cheesey aroma and were mouthdrying, as well as having a significantly less moist appearance and moist dough mouthfeel, compared with the control scone. Whey protein fortification significantly altered the physical properties of the scones, where the protein scone was significantly harder and chewier (Figure S.6.2).

Key mouthfeel attributes (n = 7) were assessed for the protein scone with and without the cream topping. This demonstrated that fat (via clotted cream) significantly reduced mouthdrying and chewiness, as well as significantly increasing rate of breakdown and clearance (Figure 6.3). This concludes that increasing fat levels in scones can significantly suppress mouthdrying.

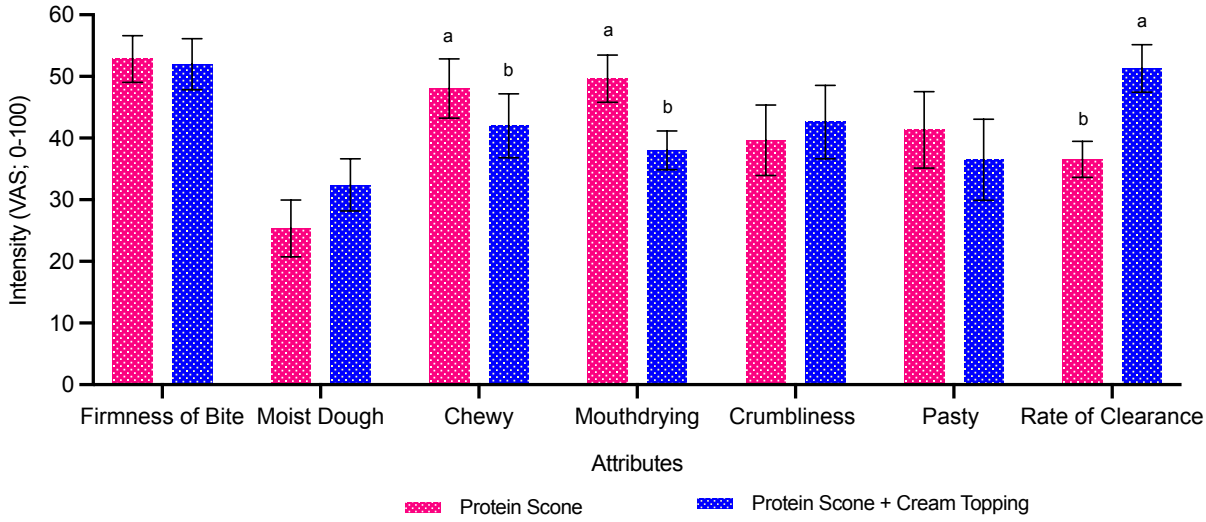


Figure 6.3. Mean mouthfeel attribute ratings of scones with and without cream topping. Data represents means of two replicates ± standard error from trained sensory panel (n = 8) measured on visual analogue scales (VAS; 0-100). Differing small letters represent sample significance from multiple comparisons. All attributes are fully defined in Table 6.3.

6.6. Discussion

6.6.1. Whey protein beverages with lactose

SF-WPBs were fortified with lactose at a spectrum of different sweetness levels. However, results suggest lactose was only able to significantly suppress mouthdrying at one of the higher lactose levels (9.4% w/v) and only to a minor extent. These results imply that a substantial amount of lactose is necessary to reduce mouthdrying and a plateau is reached beyond which further addition has no effect (i.e. at 12.4% w/v lactose the SF-WPB was not significantly sweeter and mouthdrying was not further reduced). This indicates a cross-modal effect related to the increase in sweetness. Sweetness suppressing mouthdrying is supported by previous work in this area (Methven *et al*., 2010; Courregelongue *et al*., 1999; Norton *et al*., 2021b, **Chapter 5**). Conversely, one study did not find mouthdrying to be reduced by increasing sweetness, this could relate to the beverage models utilised being more complex and involving multiple ingredients (milk protein concentrate, whey protein concentrate and skim milk) or the sensory method employed (sequential profiling) (Withers *et al*., 2014). The proposed mechanism for sweetness suppressing mouthdrying is via a cross-modal cognitive effect rather than a physical change, as a sweetened WPB still adheres to the oral cavity (Norton *et al*., 2021b, **Chapter 5**). In addition, the sensory profile results highlighted minimal differences between the two controls: (a) WPB (0.4% w/v lactose) and (b) SF-WPB (0.05% w/v lactose). There were no significant differences in sweetness and only slight differences in mouthdrying and aftertaste strength; this could have useful applications for the sport, health and lifestyle consumers interested in products with minimal sugar content.

6.6.2. Whey protein beverages with fat

Fat provides oral lubrication and alters roughness, friction and creaminess (de Wijk & Prinz, 2005), hence adding fat could help to suppress whey protein derived mouthdrying.

Accordingly, SF-WPBs were fortified with three different levels of fat (via double cream) and this had no significant effect on mouthdrying during consumption. However, increasing fat content had significant, but mixed effects, post consumption (aftertaste) on mouthdrying from: (a) 1.8% to 3.6% w/v fat, mouthdrying increased and (b) 3.6% to 7.2% w/v fat, mouthdrying reduced. Furthermore, none of the SF-WPBs with added fat were significantly different in mouthdrying aftertaste compared with the control SF-WPB (0.9% w/v fat). Similarly, Withers *et al*. (2014) also demonstrated that increasing fat (sunflower oil and milk fat at 2.0% wt/wt) could result in a significant, but minimal, increase in mouthdrying. However, a previous study which fortified skimmed milk with cream found that the higher fat levels (2.0 or 5.0% wt/wt) correlated with reduced astringency (Li *et al*., 2018). This suggests that the model beverage could be relevant where the different mechanisms associated with mouthdrying and astringency are potentially different (Norton *et al*., 2021b, **Chapter 5**) leading to variations in results. It is also noteworthy that fat was able to mask other negative sensory attributes (such as whey isolate and metallic notes), which could also have a positive effect on consumer acceptance. Therefore, altering the fat levels within products could be an alternative approach to improving mouthfeel of WPBs.

6.6.3. Cupcakes

Differing WPCs (standard and heat-stable) had a minimal effect on the sensory profile, where the perception of the two protein fortified versions was very similar in contrast with the control cupcake. Interestingly, there were significant differences in physical properties resulting in the heat-stable cupcake (HS-WPC) having lower hardness, chewiness and cohesiveness compared with the WPC cupcake. This resulted in a potentially more favourable texture compared with the WPC cupcake; however, these differences had limited effect on the sensory profile. Cake crumb is formed by a two gel-forming system:

starch swelling and gelatinisation, and a protein network denaturation and coagulation, both contributing to cake texture (firmness and cohesiveness) (Wilderjans *et al*., 2010; Lambrecht *et al*., 2018; van der Smann & Renzetti, 2020). Hence, it is hypothesised that the HS-WPC powder could have influenced the formation of the starch-filled protein network as a result of the HS-WPC protein particles aggregating with exposed thiol groups, as well as interactions with other sulfhydryl groups from the egg or gluten. This potentially disrupted the network formation during coagulation and resulted in a weaker crumb structure. In addition, the sensory profile and physical properties demonstrated slight colour differences between the cupcakes. The HS-WPC cupcakes generally supported a colour profile more similar to the control cupcakes (i.e. more yellow colour) compared with the WPC cupcakes, as noted particularly by the sensory panel results. It was expected that the additional processes, resulting in a heat-stable WPC powder, would impact positively the final product, leading to a creamier and smoother mouthfeel, potentially resulting from improved lubrication and/or reduced adhesion to the oral cavity (Liu *et al*., 2016a; 2016b; Ipsen, 2017; Norton *et al*., 2020a, **Chapter 3**; 2021b, **Chapter 5**). It is possible that the trained panellists found minimal differences in the sensory profile between the two protein versions due to the cupcake model being relatively high in fat; therefore, any difference in processing or heat treatment of the whey protein, could have a greater effect in other foods models. Previous research, using heat-treated whey protein in liquid and semi-solid models, has demonstrated a positive effect on product sensory profile (Liu *et al*., 2016b). Liu *et al*. (2016b) noted heat-treated whey protein can result in rough and dry perception if particles sizes are above the detection threshold. Therefore, future studies should consider not only the processing and heat stability of the whey protein powder, but also the particle size.

6.6.4. Scones

As expected, fortifying scones with whey protein (WPC) altered the sensory profile, demonstrating key sensorial issue namely mouthdrying, supporting previous work in this area (Norton *et al*., 2020b, **Chapter 4**). However, this present work hypothesised that the effect of fat could be greater in a solid model (such as scones) than in a liquid model. Engelen *et al*. (2005) noted that hard and dry products typically need more chewing and time in the mouth prior to swallowing. Furthermore, adding butter to cake and toast significantly decreased number of chews, presumably from increased lubrication (Engelen *et al*., 2005). In addition, fat is suggested to provide flavour, taste and mouthfeel (Aggarwal *et al*., 2016). Our work builds on the Engelen *et al*. (2005) findings by demonstrating that using a high fat topping can alter the mouthfeel attributes by reducing chewiness and mouthdrying, as well as increasing rate of breakdown and clearance. van Eck *et al*. (2019) also proved that toppings (such as firm cheese, cheese spread and mayonnaise) can reduce dryness and firmness and increase flavour perception of bread and crackers. It was suggested that this is due to saliva aiding bolus formation, whilst the nature of the topping and the product characteristics also influence the extent of change in perception (van Eck *et al*., 2019). More specifically in whey protein models incorporating ingredients such as butter into cream cheese improved flavour and liking (Song *et al*., 2018). Furthermore, this highlights that the use of toppings can make foods more acceptable and reduce negative mouthfeel attributes; accordingly, could be a viable route for improving the protein intake of older adults.

6.7. Conclusion

This paper demonstrated, despite using four different mitigating strategies in two different whey protein food models, that these strategies had limited effect on suppressing whey

protein derived mouthdrying. Fortifying WPBs with lactose significantly reduced mouthdrying to a small extent; however, this correlated with increased sweetness highlighting cross-modality, rather than physical modification, as the probable mechanism. Increasing fat levels in whey protein fortified scones (via clotted cream) significantly reduced mouthdrying. However, increasing fat levels in WPBs did not significantly reduce mouthdrying. Hence, these results suggest increasing lubrication could be more relevant in a solid model compared with the liquid model. Heat-stable WPC in cupcakes had no significant effect on reducing perceived mouthdrying but led to some improvements in the physical properties compared with WPC cupcake. This work highlights the challenges with mitigating mouthdrying; however, there is a clear need to explore methods of improving lubrication in the mouth. Developing our understanding of the proposed causes of whey protein derived mouthdrying remains key so that fortified products can be reformulated to improve the sensory profile and subsequently mitigate mouthdrying. This has relevance for the growing whey protein fortified products market for both older adults and the sport, health and lifestyle consumers.

S.6. Supplementary

S.6.1. Whey protein liquid models viscosity

Table S.6.1 summaries the apparent viscosity of WPBs with varying amounts of lactose and fat. The apparent viscosity of the WPB was measured following Norton *et al*. (2021b, **Chapter 5**) methodology. An oscillatory rheometer (MCR 302, Anton Paar Ltd., St Albans, UK) was used and parallel plate geometry (50.0 mm diameter) was employed. The gap size was 1.0 mm. All samples were allowed to rest for 5-min before the measurement. Apparent viscosity was measured as a function of shear rate over the 0.001 to 1000 s-1 range at 22 °C.

Table S.6.1. Apparent viscosity (mPa·s; at shear rate 50 s^{-1}) of whey protein liquid models.

Subset	Description	Beverage Type	Apparent Viscosity
Lactose Subset	Controls	WPB (0.4%)	1.7 ± 0.04
		SF-WPB (0.05%)	1.7 ± 0.08
	SF-WPBs (10.0%) varying in lactose levels	SF-WPB (0.4%)	1.8 ± 0.08
		SF-WPB (3.4%)	1.9 ± 0.2
		SF-WPB (6.4%)	2.1 ± 0.2
		SF-WPB (9.4%)	2.3 ± 0.02
		SF-WPB (12.4%)	2.4 ± 0.07
Fat Subset	Control	SF-WPB (0.9%)	7.6 ± 0.04
	SF-WPBs (10.0%) varying in fat levels	SF-WPB (1.8%)	7.7 ± 0.5
		SF-WPB (3.6%)	7.4 ± 0.8
		SF-WPB (7.2%)	7.6 ± 1.0

Data represents means of six replicates ± standard error. Brackets after each sample name denotes specific lactose or fat content expressed as % w/v. The grey shading demonstrates the control beverage for lactose and fat subset respectively.

S.6.2. Additional study data

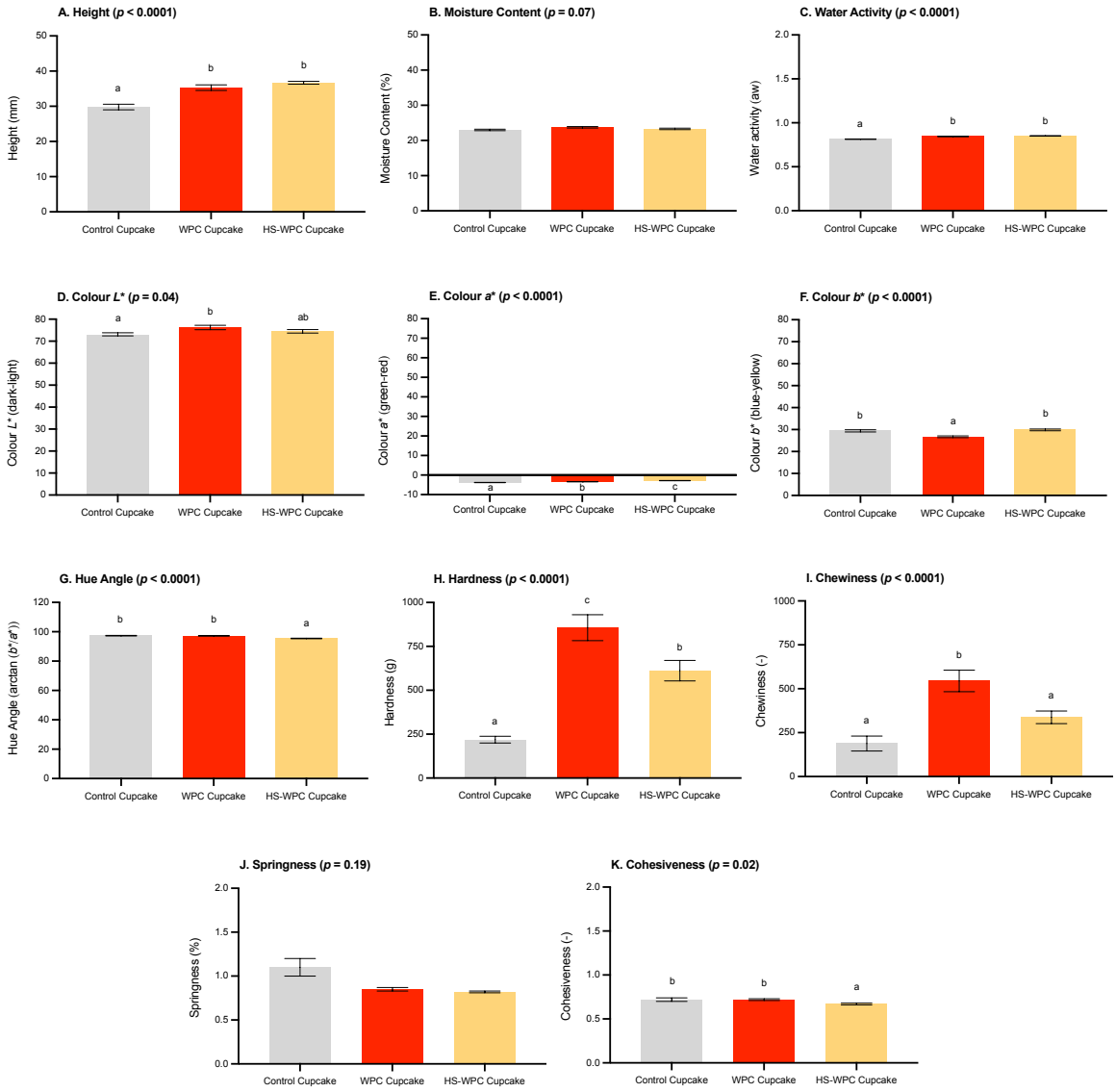


Figure S.6.1. Summary of cupcakes physical properties. (WPC: whey protein concentrate; HS-WPC: heat-stable whey protein concentrate). Data represents means of three replicates from three different batches (n = 9) ± standard error. Differing small letters represent sample significance from multiple comparisons and (-) denotes unitless data.

Table S.6.2. Sensory profile of scones.

Modality	Attribute	Scones		Significance of sample (*p* value)
		Control	Protein	
Appearance	Moist appearance	34.8 ± 4.7	22.9 ± 3.6	**0.005**
	Dense appearance of dough	43.1 ± 3.9	51.5 ± 4.0	0.16
	Appearance of large holes in dough	25.8 ± 3.6	20.6 ± 2.6	0.15
	Yellow colour of dough (inside)	36.2 ± 3.5	34.4 ± 4.3	0.55
Aroma	Overall aroma intensity	50.1 ± 2.4	50.7 ± 3.9	0.89
	Sweet	29.3 ± 3.4	28.6 ± 3.1	0.77
	Buttery	23.2 ± 2.0	18.9 ± 2.5	0.23
	Floury	18.4 ±3.1	22.3 ± 3.1	0.12
	Savoury/cheesey	3.4 ± 1.2	9.1 ± 2.5	**0.04**
	Off-flavours	1.1 ± 1.0	2.6 ± 1.2	0.50
Flavour	Overall flavour intensity	43.4 ± 2.3	38.0 ± 3.5	0.17
	Sweet	24.3 ± 2.7	20.4 ± 2.8	0.29
	Metallic	0.7 ± 0.6	0.4 ± 0.4	0.27
	Buttery	19.8 ± 1.6	12.1 ± 2.1	0.054
	Floury	20.7 ± 2.6	25.3 ± 2.4	0.12
	Savoury/cheesey	2.4 ± 1.0	5.5 ± 2.5	0.35
	Off-flavours	1.7 ± 1.2	0.9 ± 0.8	0.68
Mouthfeel	Firmness of bite	31.6 ± 2.2	40.3 ± 3.2	0.06
	Moist dough	37.3 ± 4.5	22.4 ± 3.1	**0.01**
	Chewy	31.0 ± 3.2	39.4 ± 3.4	0.07
	Mouthdrying	35.3 ± 3.1	43.9 ± 3.2	**0.002**
	Crumbliness of dough	30.1 ± 3.5	29.1 ± 2.5	0.80
	Pasty (cohesive)	35.4 ± 4.0	36.7 ± 4.2	0.69
	Rate of breakdown & clearance	40.7 ± 2.7	36.5 ± 3.8	0.34
Aftertaste	Mouthdrying	29.8 ± 2.3	35.8 ± 1.9	0.07
	Sweet	21.0 ± 2.6	17.9 ± 2.4	0.29
	Buttery	12.6 ± 1.9	6.7 ± 1.7	0.11
	Savoury/cheesey	0.5 ± 0.4	1.6 ± 0.9	0.16
	Off-flavours	1.1 ± 0.7	0.4 ± 0.4	0.33
	Salty	2.6 ± 1.0	1.9 ± 0.9	0.56
	Salivating	23.6 ± 2.7	23.4 ± 3.6	0.96
	Metallic	2.2 ± 1.0	0.0 ± 0.01	0.11

Data represents means of two replicates ± standard error from trained sensory panel (n = 10) measured on visual analogue scales (VAS; 0-100). All attributes are fully defined in Table 6.3.

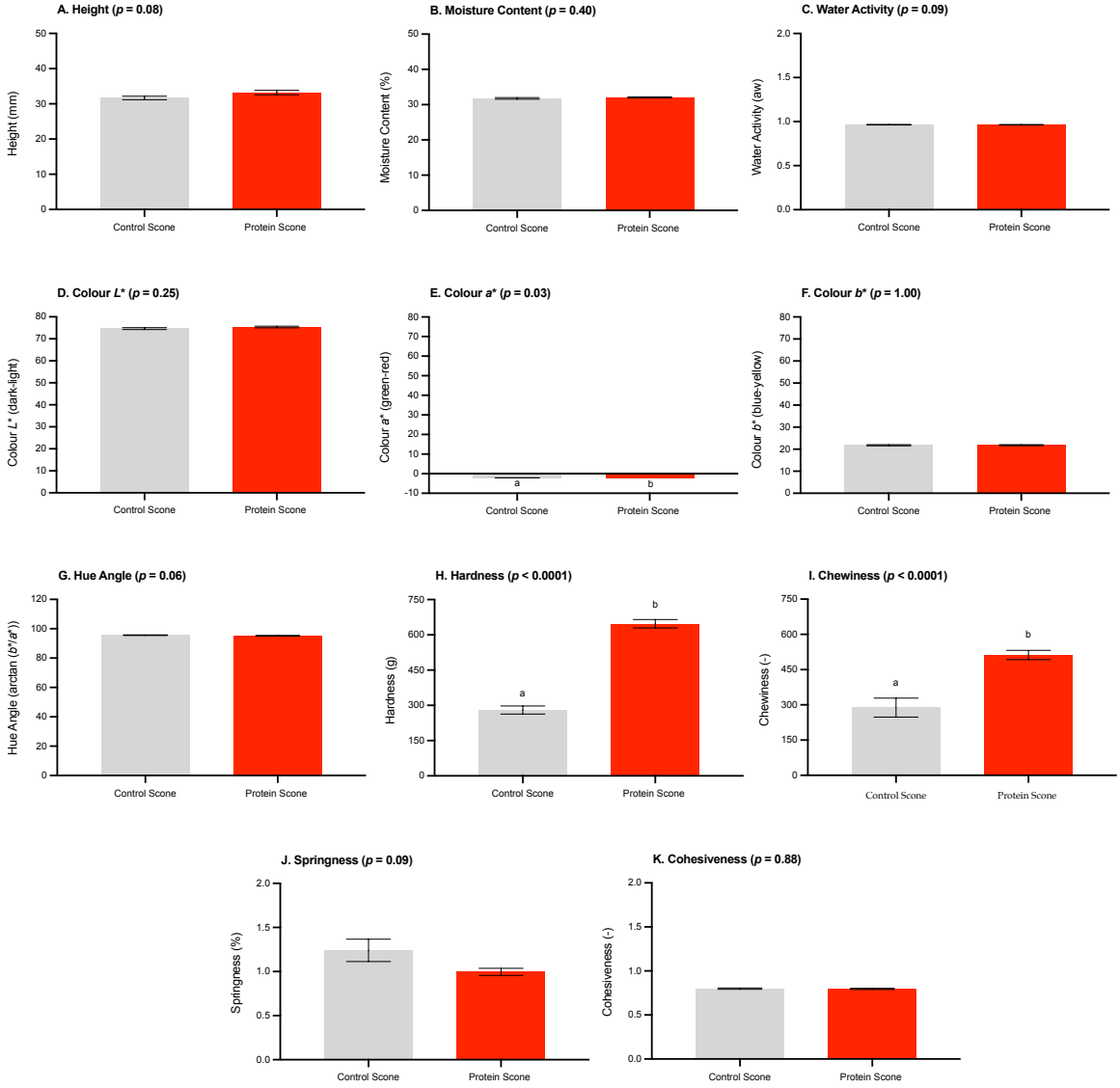


Figure S.6.2. Summary of scones physical properties. Data represents means of three replicates from three different batches (n = 9) ± standard error. Differing small letters represent sample significance from multiple comparisons.

Chapter 7

Mouthfeel sensitivity to whey protein: investigating influences of protein content, consumer age, food format and fat addition

7.1. Context to chapter

Similar to **Chapters 5 and 6**, the COVID-19 pandemic continued to impact future work and how research could be conducted. Therefore, a two-fold approach was utilised using a trained sensory panel to establish protein levels prior to an at home tasting study using consumers of varying age. Previously, mouthdrying related research has not established a mouthdrying threshold nor considered individual sensitivity; hence, this was the focus of this final experimental chapter. In addition, previously in **Chapters 3 and 4**, lack of test sensitivity had been a proposed rationale for minimal age-related mouthdrying effects; accordingly, tests like just-noticeable difference (JND) thresholds could potentially address this concern. **Chapter 6** demonstrated by a trained sensory panel that increasing lubrication (via fat) was a promising strategy to suppress mouthdrying in whey protein fortified scones; however, this needed to be re-investigated in this chapter with both younger and older adults. Hence, this chapter aims to test the following three thesis hypothesises: (a) whey protein fortified beverages and snacks will cause mouthdrying; (e) individual differences (such as age and sensory thresholds) will influence perceived whey protein derived mouthdrying; and (f) mitigating strategies (such as varying in fat) will reduce whey protein derived mouthdrying. Accordingly, these hypothesises were tested via the following objectives:

- Does whey protein have a mouthdrying detection threshold (MDT) in whey protein beverages (WPB)?
- Does sensitivity to whey protein derived mouthdrying increase with age in whey protein liquid and solid models? More specifically in this chapter: (a) could differences in age be

established by using more sensitive tests; (b) does increasing protein content increase mouthdrying in WPB; and (c) is mouthdrying and/or mouthfeel modulated in fortified scones by age?

- Do individuals (differing in ages) vary in mouthdrying sensitivity to increases in protein concentration in WPBs?
- Does increasing lubrication (adding a fat topping) decrease whey protein derived mouthdrying in whey protein solid models? More specifically in this chapter: tested using consumers of varying age rather than trained sensory panel

This chapter is prepared in paper publication format and intended to submit in *Food Quality and Preference* as Norton, V., Lignou, S., Faka, M. & Methven, L. Mouthfeel sensitivity to whey protein: investigating influences of protein content, consumer age, food format and fat addition.

7.2. Abstract

Whey protein can elicit mouthdrying sensations; however, the influence of protein level and age on sensitivity remains unclear. Additionally, previous research suggests that increasing fat in whey protein solid models can enhance lubrication and suppress mouthdrying, but this needs testing in older adults. Here, a trained sensory panel (*n* = 10) determined a mouthdrying detection threshold (MDT) in whey protein beverages (WPB). To compare sensitivity between younger and older adults (*n* = 116; 18-30; 65+): (1) WPB just-noticeable difference (JND) thresholds were established and (2) liking and perception of whey protein fortified beverages and scones were rated. The trained panel detected mouthdrying at all protein levels (0.14% to 10.0% w/v) with the MDT being established between 0.41% (50% discriminators) and 1.37% (Best Estimate Threshold, BET) w/v protein. The JND mouthdrying threshold was significantly lower (p = 0.02) in older adults compared with younger adults (0.75% versus 0.90% w/v protein; BET). Increasing protein levels in WPBs significantly increased mouthdrying and reduced liking and easiness to consume (utilising rating scales). Whey protein fortified scones with cream topping significantly increased liking, easiness to consume, sweetness, moistness and rate of clearance, and reduced mouthdrying and chewiness. Older adults perceived WPBs as significantly easier to consume and the scones significantly chewier than younger adults. Age-related mouthfeel effects and individual differences in mouthdrying sensitivity are key factors for product design.

Keywords: whey protein fortified products; mouthdrying; mouthfeel; sensitivity; ageing

7.3. Introduction

Ageing is commonly associated with negative consequences, such as changes in smell, taste, vision, appetite and oral health, which are relevant to sensory perception (SACN,

2021). However, balanced nutrition can help to alleviate and/or modulate these issues (Pout, 2014; SACN, 2021). More specifically, maintaining protein intake can help prevent age-related muscle and functional decline (Bauer *et al*., 2013; Deutz *et al*., 2014). In addition, there is growing evidence that older adults have increased protein needs (such as 1.0-1.2 g/kg/d) in order to counterbalance age-related protein metabolism changes compared with younger adults (Bauer *et al*., 2013; Deutz *et al*., 2014). To achieve such intake, products are often fortified with whey protein, due to its beneficial nutritional and functional properties (Madureira *et al*., 2017). Moreover, whey proteins are recognised as being key to enhancing protein intake within an ageing population, since they can modulate muscle synthesis and protein gain (Dangin *et al*., 2003; Pennings *et al*., 2011).

There are, however, sensorial issues linked with whey protein fortified products which can subsequently impact product consumption and compliance (Norton *et al*., 2021a, **Chapter 2**). Such issues typically relate to mouthdrying, a textural defect (Lemieux & Simard, 1994) associated with whey protein. Mouthdrying and/or dry/harder texture can typically be perceived by trained sensory panels and/or consumers across a range of whey fortified matrices and/or oral nutritional supplement (ONS) (Sano *et al*., 2005; Methven *et al*., 2010; Kelly *et al*., 2010; Childs & Drake, 2010; Ye *et al*., 2012; Withers *et al*., 2013a; 2014; Thomas *et al*., 2016; 2018; Bull *et al*., 2017; Wendin *et al*., 2017; Song *et al*, 2018; Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**; 2021b, **Chapter 5**; 2021c, **Chapter 6**). Mouthdrying also intensifies with repeated consumption, product heating time and/or age, subsequently negatively impacting liking (Methven *et al*., 2010; Withers *et al*., 2013a; Thomas *et al*., 2016; 2018; Bull *et al*., 2017). Potential mouthdrying mitigation strategies using trained sensory panels have had varying success in reducing perceived mouthdrying (Withers *et al*., 2014; Norton *et al*., 2021c, **Chapter 6**). Recently, increasing lubrication via fat (using a cream topping) significantly suppressed

mouthdrying in scones fortified with whey protein (Norton *et al*., 2021c, **Chapter 6**). However, this needs further investigation using naïve consumers of differing ages to understand conclusively the effectiveness of this proposed strategy. Accordingly, defining the causes of whey protein derived mouthdrying has been the focus of research in this field (Sano *et al*., 2005; Beecher *et al*., 2008; Lee & Vickers 2008; Vardhanabhuti *et al*., 2010; Kelly *et al*., 2010; Ye *et al*., 2011, 2012; Withers *et al*., 2013b; Bull *et al*., 2017; 2020, Norton *et al*., 2020a, **Chapter 3**; 2021b, **Chapter 5**), alongside investigating successful mitigation strategies (Withers *et al*., 2014; Norton *et al*., 2021b, **Chapter 5**; 2021c, **Chapter 6**). Most cited studies have, however, quantified whey protein derived mouthdrying using trained sensory panels and/or consumers, without considering differences in individual sensitivity.

As noted in our recent review, the extent of age-related changes in mouthfeel perception could be product and attribute related; however, this needs further proof (Norton *et al*., 2021a, **Chapter 2**). Individuals typically differ in sensitivity to sensory stimuli (Methven *et al*., 2012; Doty & Kamath, 2014; Engelen, 2018) and such differences could influence mouthdrying perception. Previously, determining whether mouthdrying sensitivity increases with age has resulted in differing results depending on the specific test used. For example, older adults were better at detecting mouthdrying than younger adults using discrimination testing (two-alternative forced choice, 2-AFC) in dairy beverages (Withers *et al*., 2013a). However, when utilising rating scales (0-100) (visual analogue scale, VAS or generalised Labelled Magnitude Scale, gLMS), no significant differences were found between age groups relating to mouthdrying from whey protein fortified beverages, cakes and biscuits (Norton *et al*., 2020a, **Chapter 3**; 2020b, **Chapter 4**). Accordingly, to address such inconsistencies, research using more sensitive discrimination tests is suggested (Norton *et al*., 2021a, **Chapter 2**; 2021b, **Chapter 5**). Methven *et al*. (2016) highlighted

the simplicity and suitability of 2-AFC tests for older adults, which can also be used to determine thresholds such as just-noticeable difference (JND). JND refers to the intensity required to elicit a perceptual change (Lawless & Heymann, 2010). In addition, JND tests have previously been utilised to establish differences in texture sensitivity between age groups (Kremer *et al*., 2007a; Withers *et al*., 2013a).

Detection thresholds aim to determine the minimum intensity of a stimulus required to cause a perceptual response and can be either product or individual focused (Lawless & Heymann, 2010). However, to date there have been limited whey protein beverage (WPB) threshold related studies and no defined whey protein derived mouthdrying thresholds have been published. Previous studies have typically used one of the following: (a) no set ratio progression between protein levels; (b) scales (0-5-, 0-7- and 0-15-point scales) rather than alternative forced choice tests (2-AFC or 3-AFC); or (c) focused on taste and orthonasal, rather than mouthfeel due to possible confounding factors associated with model WPBs (Sano *et al*., 2005; Kelly *et al*., 2010; Childs & Drake, 2010; Ye *et al*., 2012). Since WPBs are associated with mouthdrying at a range of different protein concentrations (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012) defining a threshold could have useful product implications.

Whey protein derived mouthdrying studies have often investigated the causes rather than the extent of individual differences in sensitivity to such mouthdrying. This study hypothesises that: (a) a mouthdrying detection threshold (MDT) for whey protein derived mouthdrying can be established; (b) there will be individual differences in mouthdrying thresholds; (c) sensitivity to mouthfeel differences will increase with age, regardless of the food model; (d) the intensity of mouthdrying will increase with protein concentration in WPBs; and (e) consumers of varying age will perceive that adding a cream topping to a whey protein fortified scone will suppress mouthdrying. In order to test these

hypotheses this paper uses: (1) whey beverages to evaluate mouthdrying thresholds via sensory panels and/or younger and older adults; and (2) whey protein fortified scones (with and without cream topping) to assess liking and perception by younger and older adults.

7.4. Materials and methods

7.4.1. Study outline

This study consisted of two stages, as summarised in Figure 7.1. Stage one utilised the trained sensory panel at the Sensory Science Centre (University of Reading) (*n* = 10; 9 female and 1 male) to determine a mouthdrying detection threshold (MDT) for whey protein. Stage two involved 116 healthy volunteers (Table 7.1) varying in age: (a) 58 younger adults (18-30 years, 25.4 ± 3.2 years); and (b) 58 older adults (over 65 years, 69.5 ± 3.9 years) to investigate the influence of age on perception. Based on the primary outcome (2-AFC mouthdrying sensitivity) power calculations (alpha = 0.05, power = 0.9 and delta = 0.80) were carried out using the results from previous work (Withers *et al.*, 2013a) concluding a sample size of 49 (Ennis & Jesionka, 2011) within each age group. All volunteers were recruited from the surrounding Reading area (UK) and the study was a single blinded randomised crossover trial involving a one-day study at home. The study was performed as an at home study due to ongoing COVID-19 restrictions, conforming with social distancing and COVID-19 guidelines, as well as applicable risk assessments. All volunteers had the study fully explained, provided written consent and were informed that data would be anonymous and remain confidential, as well as there being a right to withdraw. In addition, all volunteers were screened in accordance with the inclusion criteria (meeting age requirements, healthy, no COVID-19 symptoms or not having had COVID-19 within the past month, minimal medication, non-smokers and not having had

diabetes, food intolerances and allergies, cancer, oral surgery or a stroke). The University of Reading Research Ethics Committee (UREC) provided a favourable opinion for conduct (UREC 20/35) and the study was recorded as NCT04869722 on the clinical trials database (www.clinicaltrials.gov).

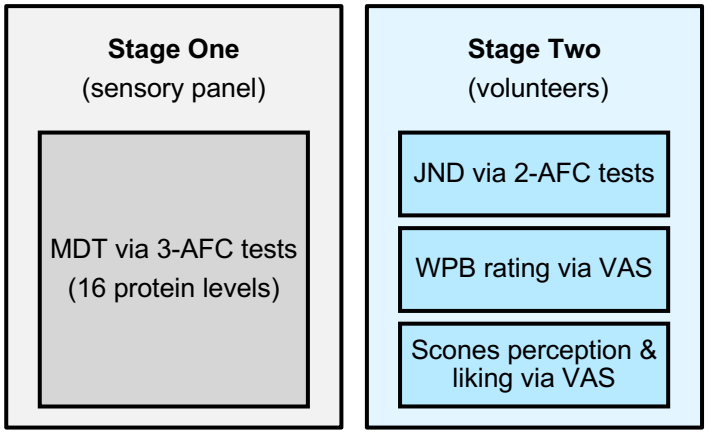


Figure 7.1. Study outline (MDT: mouthdrying detection threshold; 3-AFC: three-alternative forced choice; JND: just-noticeable difference; 2-AFC: two-alternative forced choice; WPB: whey protein beverage; VAS: visual analogue scale).

Table 7.1. Overview of volunteer's sex and medication (*n* and % represent number and percentage in each contributing group).

	Sex				**Medication**			
	Male		Female		Yes		No	
	n	%	*n*	%	*n*	%	*n*	%
Total (*n* = 116)	51	44	65	56	19	16	97	84
Younger Adults (*n* = 58)	22	38	36	62	2	3	56	97
Older Adults (*n* = 58)	29	50	29	50	17	29	41	71

7.4.2. Materials

All study materials are described in Table 7.2.

Table 7.2. Overview of study materials.

Product Description	Key Feature	Supplier
Volactose® Taw Whey Permeate (WPe)	89% lactose	Volac (Royston, UK)
Volactive® UltraWhey Sugar Free WPC (SF-WPC)	86% protein	Volac (Royston, UK)
Volactive® UltraWhey 80 Instant (WPC)	81% protein	Volac (Royston, UK)
Volactose® Edible Lactose (Lactose)	99% lactose	Volac (Royston, UK)
Nestle Resource Thicken Up Clear (Hydrocolloid)	n/a	NutriDrinks (London, UK)
Baking ingredients	n/a	Sainsburys (Reading, UK)
Rodda's Clotted Cream (Cream topping)	64% fat	Sainsburys (Reading, UK)

WPe: whey permeate; SF-WPC: sugar-free whey protein concentrate; WPC: whey protein concentrate; n/a: not applicable.

7.4.3. Study models preparation

7.4.3.1. Mouthdrying detection threshold (MDT) models

The control beverage was a whey permeate beverage (WPeB; 4.0% w/v, WPe powder in deionised water) considered a suitable non-protein whey control and a beverage well utilised in our previous work (Norton *et al*., 2020a, **Chapter 3**; 2021b, **Chapter 5**). The protein beverage consisted of 16 different protein levels (WPB, 0.14% to 10.0% w/v, SF-WPC powder in deionised water) based on ×1.33 progression, with the aim of representing a full spectrum of protein levels (up to 10.0% w/v) to establish a MDT for whey protein. Lactose was added to all protein levels to match the level found in the control beverage (in all beverages the lactose level was considered below the average lactose taste recognition threshold (4.19% w/v) (Belitz *et al*., 2004)).

7.4.3.2. Mouthdrying just-noticeable difference (JND) models

The formulations for JND thresholds were designed following the results of the MDT as mouthdrying was detectable at low protein levels (Section 7.5.1). Accordingly, six beverages were developed where the control beverage (WPB, 0.33% w/v, SF-WPC powder in deionised water) was considered a detectable mouthdrying sample based on the MDT results. Five additional protein levels (WPB, 0.41% to 1.00% w/v, SF-WPC powder in deionised water) were utilised using a ×1.25 progression (MDT results and initial testing within our laboratory concluded that a narrower progression than 1.33 was needed) to determine the level of increase in protein concentration required to cause a detectable difference in mouthdrying. All beverages were matched on lactose content as with the MDT model.

7.4.3.3. Whey protein beverages (WPB) rating models

Four different protein levels were selected (1.81%, 3.20%, 5.56% and 10.0% w/v; SF-WPC powder in deionised water) from the original 16 MDT levels. This was to cover a

range of protein levels from below and up to a typical WPB and to determine whether younger and older adults found increasing protein levels resulted in increased mouthdrying.

All model beverages are outlined in Table 7.3 and were stirred (Stuart^{TM} SM5 Bibby Fascia, UK) for 90-min at room temperature (19.2 ± 1.5 °C), as described in our previous work (Norton *et al*., 2020a, **Chapter 3**; 2021b, **Chapter 5**; 2021c, **Chapter 6**).

Table 7.3. Summary of mouthdrying detection threshold (MDT), just-noticeable difference (JND) and whey protein beverages (WPB) rating models.

Subset	Beverage¤	**Formulations** (per 100 mL)					**Composition** (per 100 mL)			
		Water (mL)	WPe (g)	SF-WPC (g)	Lactose (g)	Hydrocolloid (g)	Energy (kcal)	Fat (g)	Carbohydrate (g)	Protein (g)
MDT control	WPeB	96.0	4.0	-	-	0.150	14.7	0.008	3.65	0.10
MDT: WPBs varying in protein levels	0.14%	96.0	-	0.138	3.56	0.146	0.58	0.02	3.65	0.12
	0.18%	96.0	-	0.184	3.56	0.145	0.77	0.02	3.65	0.16
	0.25%	96.0	-	0.245	3.56	0.145	1.02	0.03	3.65	0.21
	0.33%	96.0	-	0.326	3.56	0.144	1.36	0.04	3.65	0.28
	0.43%	96.0	-	0.434	3.56	0.143	1.81	0.05	3.65	0.37
	0.58%	96.0	-	0.577	3.56	0.142	2.40	0.06	3.65	0.50
	0.77%	96.0	-	0.767	3.56	0.140	3.19	0.08	3.65	0.66
	1.02%	95.0	-	1.021	3.56	0.138	4.25	0.10	3.65	0.88
	1.36%	95.0	-	1.358	3.56	0.135	5.65	0.13	3.65	1.17
	1.81%[1]	95.0.	-	1.807	3.56	0.131	7.51	0.17	3.65	1.56
	2.40%	94.0	-	2.403	3.56	0.124	10.0	0.23	3.65	2.07
	3.20%[2]	93.0	-	3.196	3.56	0.117	13.3	0.30	3.65	2.75
	4.25%	92.0	-	4.251	3.56	0.107	17.7	0.40	3.65	3.66
	5.56%[3]	91.0	-	5.563	3.56	0.093	23.5	0.53	3.65	4.87
	7.52%	89.0	-	7.519	3.56	0.074	31.3	0.71	3.65	6.47
	10.0%[4]	86.0	-	10.00	3.56	0.042	41.6	0.95	3.64	8.60
JND control	0.33%	96.0	-	0.326	3.56	0.144	1.36	0.04	3.65	0.28
JND: WPBs varying in protein levels	0.42%	96.0	-	0.408	3.56	0.143	1.70	0.05	3.65	0.35
	0.51%	96.0	-	0.509	3.56	0.142	2.12	0.06	3.65	0.44
	0.64%	96.0	-	0.637	3.56	0.141	2.65	0.08	3.65	0.55
	0.80%	96.0	-	0.796	3.56	0.139	3.31	0.10	3.65	0.68
	1.00%	95.0	-	0.995	3.56	0.138	4.14	0.10	3.65	0.85

¤Beverage levels expressed as % w/v. Subscript numbers [(1-4)] denote models utilised in whey protein beverage (WPB) rating. Acronyms: whey permeate beverage (WPeB); whey permeate powder (WPe); sugar-free whey protein concentrate (SF-WPC). Data based on ingredients technical sheets. Dash (-) notes not applicable. Hydrocolloid (thicken up clear; maltodextrin and xanthan gum-based thickener) which was added to minimise viscosity differences between beverages (resulting viscosity measurements are presented in Figures S.7.1 and S.7.2). The grey shading demonstrates the control beverage for MDT and JND respectively.

7.4.3.4. Scone models

Whey protein fortified scones (30.0 g; 4.5 g protein per scone) with cream topping (8.0 g clotted cream providing 5.0 g fat and total fat level 9.0 g per scone) and without cream topping (total fat level 3.9 g per scone), were used as described in our previous work (Norton *et al*., 2021c, **Chapter 6**). In brief, the dry ingredients were added and mixed (Kenwood Titanium Major KMM020, Hampshire, UK) followed by wet ingredients (low speed, 2 to 10-min). Scones were formed (diameter: 4.5 cm cutter and 1.0 cm thickness), brushed with mixture (eggs and milk), baked (12-min at 200 °C in a pre-heated oven (Altas Salva, London, UK)), individually packaged (polypropylene pouches), frozen at -18 °C until consumption and underwent microbiological clearance testing (SGS analytics, Northumberland, UK).

7.4.4. Stage one: mouthdrying detection threshold (MDT)

The trained sensory panel used a series of three-alternative forced choice (3-AFC) tests to determine a MDT for whey protein; testing complied with ISO 13301:2018 (ISO, 2018). COVID-19 restrictions (February to March 2021) resulted in all sessions being carried out at panellists' homes; however, they conformed to COVID-19 guidelines and appropriate risk assessments. All sessions were completed remotely via Microsoft Teams (Version 1.3.00.28778, Washington, USA) individually on iPads (Apple, London, UK) with Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Ontario, Canada) in a quiet and aroma free location. The panellists were provided with samples (10 mL) (coded with a random three-digit number) in paper cups (113 mL) with sip lids (to mask any potential differences between samples) and tasted in a fixed ascending order, with each level allocated in a random sequential balanced order. Panellists completed a series of training sessions (3 × 30-min) to become familiar with the term mouthdrying (defined as the drying sensation in the mouth during or after consumption of

a product (and persists/builds for up to 30-s post swallow)) and were presented with three samples (two WPeBs and one WPB). Panellists were asked which sample was more mouthdrying and this procedure was repeated in triplicate for all 16 levels in different sessions. Panellists had an enforced 1-min break between pairs and used water (~ 40 °C, warm, filtered) for palate cleansing.

7.4.5. Stage two: at home tasting study

All tasting was carried out at volunteers' homes due to COVID-19 restrictions (April and May 2021) in a quiet and aroma free location. Tasting was completed on the same day (within 2-h) as they received the samples (all adhering to COVID-19 guidelines and risk assessments) and volunteers refrained from food or drink for 30-min prior to the test; volunteers recorded all results in paper booklets. For all tasks, volunteers were provided with detailed consumption instructions. All beverages were presented in paper cups with sip lids as outlined in Section 7.4.4. Volunteers were asked to consume: (a) all of the provided WPB; and (b) break each scone in half and consume two bites from the middle. In addition, all volunteers were provided with definitions for all perception attributes as summarised in Figure S.7.3.

7.4.5.1. Mouthdrying just-noticeable difference (JND)

Volunteers were provided with a series of five 2-AFC tests (with 1-min break in-between) to determine which sample was more mouthdrying within each pair (conforming with ISO 5495:2005) as summarised in Figure 7.2. All tasting was evaluated in a fixed ascending order with each pair allocated in a random sequential balanced order. The rationale for using 2-AFC tests (two samples: one control and one WPB) relates to 3-AFC (three samples: two controls and one WPB) can lead to fatigue (due to number of samples) and/or confusion (especially within a home setting). Accordingly, the 2-AFC test was used with volunteers since they were untrained and to increase suitability for the older adults.

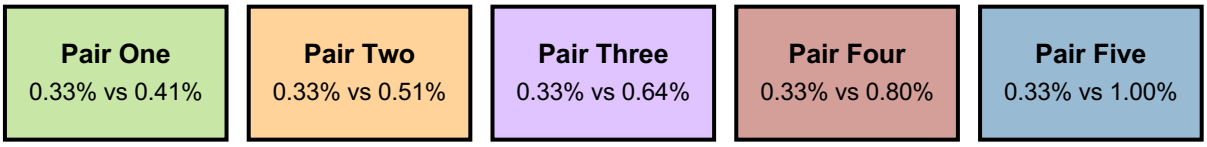


Figure 7.2. Overview of mouthdrying just-noticeable difference (JND) testing pairs (0.33% w/v protein denotes the control beverage and 0.41% to 1.00% w/v represents increasing protein levels within the WPB).

7.4.5.2. Whey protein beverages (WPB) rating

Volunteers were provided with four WPBs, differing in protein levels (1.81%, 3.20%, 5.56% and 10.0% w/v), in a random sequential balanced order (with 45-s break between samples). Volunteers rated all WPBs on visual analogue scales (VAS; 10 cm lines on paper, scale 0-100) for the following attributes: liking (dislike extremely to like extremely), easiness to consume (drink and swallow; very difficult to very easy), mouthdrying (not mouthdrying to very mouthdrying), appropriateness of flavour level (Just-About-Right, JAR) (five category labels; much too weak to much too strong) and added any comments relating to each sample. All volunteers completed a familiarisation exercise on how to use the VAS by non-food related questions (Norton *et al*., 2020b, **Chapter 4**) (Figure S.7.4).

7.4.5.3. Scones perception and liking

Volunteers were provided with two scones (with and without cream topping) in a random sequential balanced order (with 45-s break between samples). Volunteers rated scones on VAS for the following attributes: appearance liking (dislike extremely to like extremely), liking (dislike extremely to like extremely), easiness to consume (eat and swallow; very difficult to very easy), sweetness (not sweet to very sweet), moistness (not moist to very moist), mouthdrying (not mouthdrying to very mouthdrying), chewiness (not chewy to very chewy), rate of clearance (slow to fast), appropriateness of flavour level (Just-About-Right, JAR) (five category labels; much too weak to much too strong), added any comments relating to each sample and noted how often they consumed protein fortified

products. To finish, volunteers completed a single 2-AFC test to determine which sample was more mouthdrying.

7.4.6. Statistical analysis

MDT analysis was completed in R-package sensR (Christensen & Brockhoff, 2018) using binomial and beta-binomial models obtaining for all 16 individual protein levels: (a) proportion of correct responses (Pc; correct responses/number of total response); (b) proportion of discriminators ($Pd = \frac{P_c - P_g}{1 - P_g}$) (Jesionka *et al.*, 2014); and (c) significance of sample (*p* value). The Thurstonian model was also used to transform the number of correct responses into an estimate (d' value) of the underlying sensory difference. To capture any potential panellist variability (gamma - overdispersion) in the data (due to replication), the beta-binomial model was applied if there was a significant overdispersion, whilst if there was a non-significant result, the binomial model was utilised (Ennis & Bi, 1998; Liggett & Delwiche, 2005). Accordingly, all data was checked for overdispersion and for all WPBs the binomial model was sufficient (apart from two protein levels: 1.80% and 3.20% w/v, where the overdispersion was significant and the beta-binomial model was used). However, it should be noted that the d' value from both models were very similar, supporting no strong overdispersion in our data. Linear regression was fitted to determine a detection threshold (i.e. the overall 50% discriminator level) where the proportion of discriminators was plotted against the protein level natural logarithm (ln(protein%)) (ISO, 2018) in XLSTAT (version 2020.1.3, Addinsoft, New York, USA). Additionally, analysis was carried out using the Best Estimate Threshold (BET) approach (as described below) to determine both individual panellist and group sensitivity.

The BET method utilised the individual thresholds from MDT or JND by calculating the geometric mean of (a) the concentration at which the individual correctly identified the

WPB as more mouthdrying (with all subsequent levels deemed as mouthdrying); and (b) the highest concentration where the WPB was incorrectly identified as more mouthdrying (Lawless 2010; Lawless & Heymann, 2010). If an individual incorrectly identified the highest provided WPB level as mouthdrying; therefore, it was assumed that their individual threshold was equal to or greater than the next protein concentration presented based on the relevant subset progression (Lawless 2010; Lawless & Heymann, 2010). For example, equal to or greater than (a) MDT: 13.3% (×1.33) and (b) JND: 1.11% (×1.25) (w/v) protein and progression respectively. The group thresholds were calculated from the individual geometric means (MDT: panellists and JND: within an age group) (Lawless 2010; Lawless & Heymann, 2010).

JND data (using the BET approach to false positives (Lawless; 2010; Lawless & Heymann, 2010)) was also used to determine the: (a) proportion of correct responses; (b) proportion of discriminators (Jesionka *et al*., 2014); and (c) d' value using Thurstonian modelling in XLSTAT. Subsequent age group analysis was conducted in XLSTAT using a Mann-Whitney test due to non-normally distributed data (as defined by normality of residuals $p > 0.05$).

WPB and scones ratings (VAS; 0-100) were analysed in SAS® software (version 9.4, Cary, NC, USA) by linear mixed models (suitable for unbalanced data (Torrico *et al*., 2018)) as follows: (a) explanatory variables: age, sample, sex, medication and volunteer code (random effect); (b) dependent variables: liking, perception and JAR scores; (c) post hoc analysis (if the model demonstrated a significant value) applied Bonferroni; and (d) data denotes least square means (LSM) estimates. JAR data (0-100) was converted into category data (three levels: (1) too little (less than 45); (2) JAR (within 10% of midpoint (45-55)); and (3) too much (more than 55)) to relate perception of optimum flavour intensity to liking data. The resulting penalty analysis was then completed in XLSTAT, as

noted in our previous work (Norton *et al.*, 2021b, **Chapter 5**). Scone mouthdrying 2-AFC results were analysed by Binomial expansion and Thurstonian modelling (*p* values, power and d' value) in V-power (Ennis & Jesionka, 2011). A chi-square test on contingency tables was used to determine associations between age and categorical data (medication and protein consumption) in XLSTAT. For all analyses $p < 0.05$ was used to reflect sample significance.

7.5. Results

7.5.1. Mouthdrying detection threshold (MDT)

Significant mouthdrying was detected at all protein levels tested compared with the whey permeate control (WPeB) and the d' value generally increased with increasing protein content as outlined in Table 7.4. The detection threshold for whey protein (defined as 50% discriminators level) was estimated at 0.41% w/v protein using the fitted regression model utilising all protein levels (Figure S.7.5). However, the lowest individual protein level detection threshold was 0.33% w/v (Table 7.4). The alternative BET approach resulted in a higher calculated mean detection threshold (1.37% w/v protein) and demonstrated the panellists individual range (0.12% to 5.92% w/v protein).

Table 7.4. Overview of mouthdrying detection threshold as identified by trained panel (*n* = 10).

Protein Level[¤]	Correct[1] (n)	Pc[2]	Pd[3]	Significance of sample (*p* value)[4]	d' value[5]
0.14%	17	0.57	0.35	0.007	0.77
0.18%	16	0.53	0.30	0.02	0.67
0.25%	16	0.53	0.30	0.02	0.67
0.33%	20	0.67	0.50	<0.0001	1.12
0.43%	21	0.70	0.55	<0.0001	1.24
0.58%	24	0.80	0.70	<0.0001	1.65
0.77%	22	0.73	0.60	<0.0001	1.37
1.02%	24	0.80	0.70	<0.0001	1.65
1.36%	26	0.87	0.80	<0.0001	2.01
1.81%#	20	0.68	0.52	0.04	1.16
2.40%	25	0.83	0.75	<0.0001	1.82
3.20%#	24	0.80	0.70	0.009	1.66
4.25%	26	0.87	0.80	<0.0001	2.01
5.56%	26	0.87	0.80	<0.0001	2.01
7.52%	29	0.97	0.95	<0.0001	2.96
10.0%	26	0.87	0.80	<0.0001	2.01

[¤]Protein levels expressed as % w/v; [1] refers to number of correct responses out of 30 (all data was collected in triplicate); [2] demonstrates the proportion of correct responses; [3] denotes the proportion of discriminators; [4] reflects the *p* value as defined by Binomial or beta-binomial model; [5] expresses the d' value as defined by Thurstonian modelling and # within the column highlights where the overdispersion was significant and data are reported as adjusted values from Beta-Binomial model.

7.5.2. Mouthdrying just-noticeable difference (JND)

The JND testing concluded a greater difference between WPBs resulted in more volunteers detecting differences in mouthdrying (Figure 7.3). For example, at 1.00% w/v protein (including all lower subsequent protein levels) the: (a) proportion of correct responses was 0.64; (b) proportion of discriminators reached only 0.26; therefore, a JND threshold (i.e. 50% discrimination) could not be established; and (c) maximum d' value was 0.50 and at lower protein levels a d' value was not possible to calculate as the guessing probability was higher than the number of correct responses. However, an age-related difference was present (calculated via the BET approach) where older adults had a significantly lower (p = 0.02) average JND threshold compared with younger adults (geometric mean: 0.75 ± 0.04% versus 0.90 ± 0.03% w/v protein respectively).

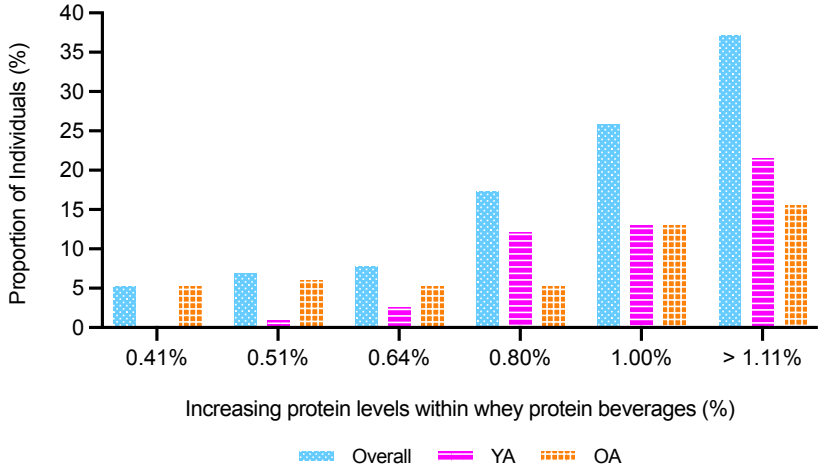


Figure 7.3. Just-noticeable difference (JND) mouthdrying thresholds frequency distribution (*n* = 116; younger adult (YA): *n* = 58; older adult (OA): *n* = 58) for each corresponding protein level (% w/v). Control was 0.33% w/v protein with increasing protein levels 0.41% to 1.00% w/v and > 1.11% w/v denotes individuals are above JND threshold.

7.5.3. Whey protein beverages (WPB) rating

Increasing protein from 1.81% to 10.0% (w/v) resulted in significantly increased mouthdrying, as well as significantly reduced liking and easiness to consume (Figure 7.4). Age had no significant effect on either liking or mouthdrying; however, older adults rated WPBs as significantly easier to consume compared with younger adults (Table 7.5). Flavour intensity became significantly closer to optimum (Just-About-Right; 50 on 0-100 scale) with increasing protein levels; age had no significant influence on JAR flavour ratings (Table 7.6). The impact of flavour intensity on subsequent liking was revealed by penalty analysis. For example, lower protein levels resulted in more individuals perceiving the WPBs as ‘too low’ in flavour, impacting liking, compared with ‘too much’ flavour. However, at higher protein levels both ‘too little’ and ‘too much’ flavour resulted in reduction in WPB liking. Older adults found the 10.0% (w/v) WPB having both ‘too little’ and ‘too much’ flavour which led to a reduction in liking whereas the younger adults only reported ‘too much’ flavour having an effect (Table 7.6). Other factors (such as sex and medication) had no significant effect on WPB ratings (Figure S.7.6). Comments were

provided relating to the WPBs with 245 comments recorded (32% positive and 68% negative) as described in Figure 7.5.

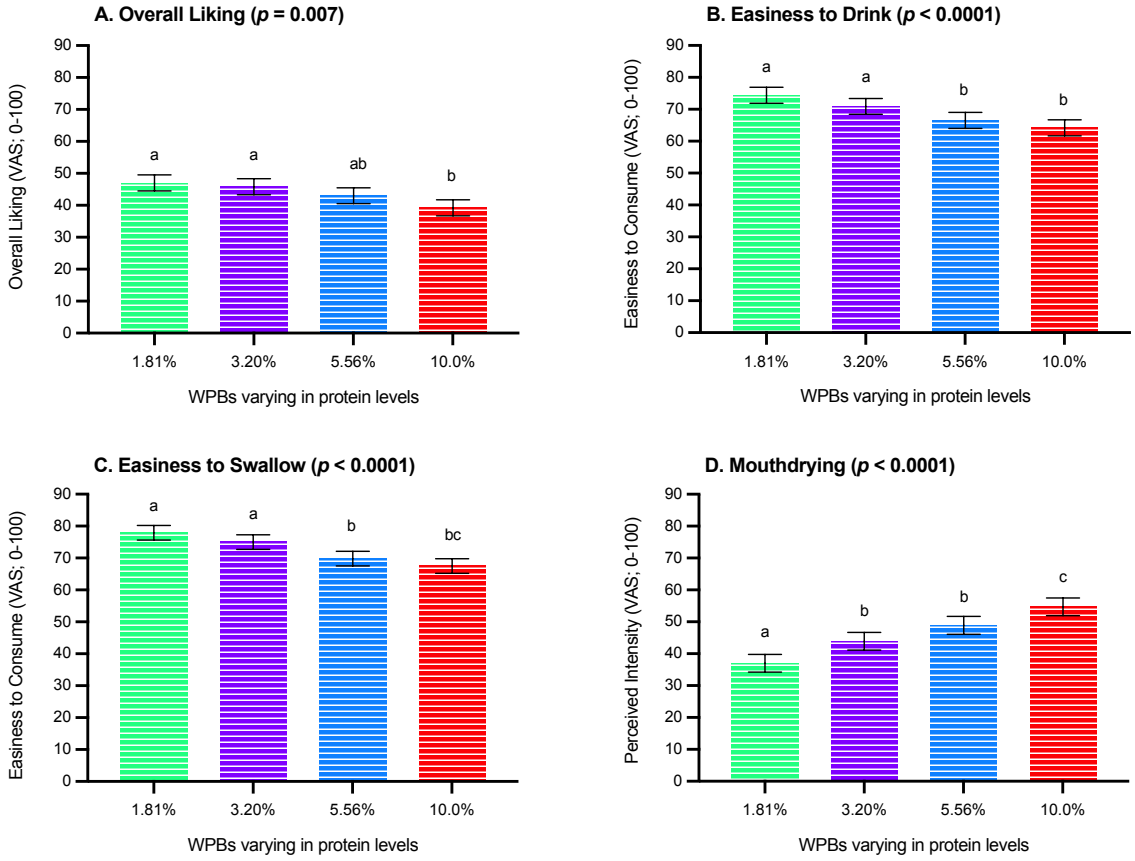


Figure 7.4. Mean whey protein beverages (WPB) ratings (± standard error) (*n* = 116; VAS: visual analogue scale 0-100) differing in protein levels (% w/v). Differing letters highlights sample significance from multiple comparisons.

There was a significant association ($p < 0.0001$) between medication and age, highlighting more older adults take medication than younger adults (Table 7.1). However, medication use had no significant effect on WPB ratings or perception and liking of scones (Section 7.5.4).

Table 7.5. Influence of age (YA: younger adult *n* = 58 and OA: older adult *n* = 58) on rating (± standard error) of differing protein levels (% w/v) in whey protein beverages (WPB).

	1.81%		**3.20%**		**5.56%**		**10.0%**	
	Younger Adults (*n* = 58)	Older Adults (*n* = 58)	Younger Adults (*n* = 58)	Older Adults (*n* = 58)	Younger Adults (*n* = 58)	Older Adults (*n* = 58)	Younger Adults (*n* = 58)	Older Adults (*n* = 58)
Overall Liking	47.9 ± 3.5	46.2 ± 3.1	43.8 ± 3.5	47.9 ± 3.1	45.9 ± 3.5	40.2 ± 3.1	37.8 ± 3.5	40.5 ± 3.1
Easiness to Drink	68.8 ± 3.5^{aA}	80.0 ± 2.9^{bA}	62.4 ± 3.5^{aAB}	79.3 ± 2.9^{bA}	61.5 ± 3.5^{aAB}	71.4 ± 2.9^{bAB}	57.2 ± 3.5^{aAB}	71.2 ± 2.9^{bAB}
Easiness to Swallow	74.3 ± 3.2^{A}	81.7 ± 2.7^{A}	68.6 ± 3.2^{aA}	81.4 ± 2.7^{bA}	66.4 ± 3.2^{AB}	73.1 ± 2.7^{B}	61.8 ± 3.2^{aB}	73.2 ± 2.7^{bB}
Mouthdrying	35.7 ± 4.0	38.3 ± 3.4	40.7 ± 4.0	47.1 ± 3.4	47.7 ± 4.0	50.1 ± 3.4	54.9 ± 4.0	54.7 ± 3.4

Significant differences between samples and age are noted by differing small letters (YA vs OA within sample) and capital letters (within age group across WPBs) respectively; no letter reflects no significance.

Table 7.6. Just-About-Right (JAR) flavour mean ratings (± standard error) and effect on liking (penalty analysis) by overall and age for whey protein beverages (WPB; % w/v) and scones.

	Overall (*n* = 116)		**Age**		**Penalty Analysis**							
		Significance of sample (*p* value)	Younger Adults (*n* = 58)	Older Adults (*n* = 58)	Too Little (YA)		Too Much (YA)		Too Little (OA)		Too Much (OA)	
					Mean Drop	Frequency (%)	Mean Drop	Frequency (%)	Mean Drop	Frequency (%)	Mean Drop	Frequency (%)
WPBs												
1.81%	39.6 ± 2.3^{a}	**<0.0001**	37.6 ± 3.3	41.5 ± 2.7	17.0#	59%	30.3†	14%	11.7#	53%	9.8†	17%
3.20%	42.3 ± 2.3^{a}		41.4 ± 3.3	43.1 ± 2.7	20.0#	55%	24.3#	21%	9.9†	38%	11.0†	12%
5.56%	43.2 ± 2.3^{ab}		41.2 ± 3.3	45.2 ± 2.7	18.3#	52%	9.3	21%	2.9	43%	13.1	26%
10.0%	52.3 ± 2.3^{c}		51.9 ± 3.3	52.7 ± 2.7	3.7	36%	18.1#	40%	18.0#	33%	36.3#	35%
Scones												
Protein Scone	42.8 ± 1.6	**0.0009**	43.4 ± 2.3	42.2 ± 1.8	15.7#	41%	10.7†	9%	16.3#	45%	26.0†	8%
Protein Scone + cream topping	46.6 ± 1.6		46.5 ± 2.3	46.8 ± 1.8	26.5#	31%	-2.9†	10%	19.6#	28%	18.8†	14%

Differing letters within WPBs overall column denotes within sample significance; no letter reflects no significance. # indicates significance difference from penalty analysis within each sample and age group; † denotes lower than group threshold (20%); frequency (%) represents percentage within too little or too much group.

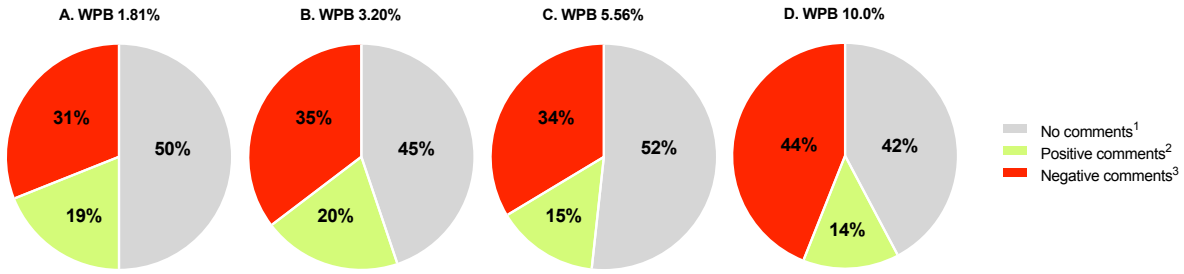


Figure 7.5. Percentage overview of volunteer comments relating to whey protein beverages (WPB) differing in protein levels (% w/v). [1] Refers to volunteers that did not provide any comments; [2] volunteers who provided positive comments (such as OK, great, preferred, tasty, nice, smooth, creamy, easy to consume and pleasant); [3] volunteers who provided negative comments (namely gritty, dislike, bland, horrible, unpleasant, mouthdrying, powdery, aftertaste, sickly, tacky, weak and watery).

7.5.4. Scones perception and liking

Scones fortified with whey protein and added cream topping significantly increased liking, easiness to consume, sweetness, moistness and rate of clearance, as well as significantly reduced mouthdrying and chewiness compared with the scone without cream topping (Figure 7.6). Older adults perceived scones as significantly chewier compared with younger adults; however, age had no significant effect on the remaining attributes (Figure 7.6). It should be noted there was a significant interaction between sample and age (p = 0.04) for sweetness; older adults perceived scones with cream topping less sweet (p = 0.01) than younger adults. The use of cream topping resulted in a scone closer to optimum flavour (JAR) than a scone without cream topping (Table 7.6). The penalty analysis highlighted that 'too little' flavour significantly related to lower liking for both scones (with and without cream topping); this trend was supported by both age groups (Table 7.6). Sex significantly altered sweetness perception, where males perceived scones to be significantly sweeter (p = 0.005) than females. However, all remaining additional factors (such as sex and medication) had no significant influence on scone perception and liking (Figure S.7.7).

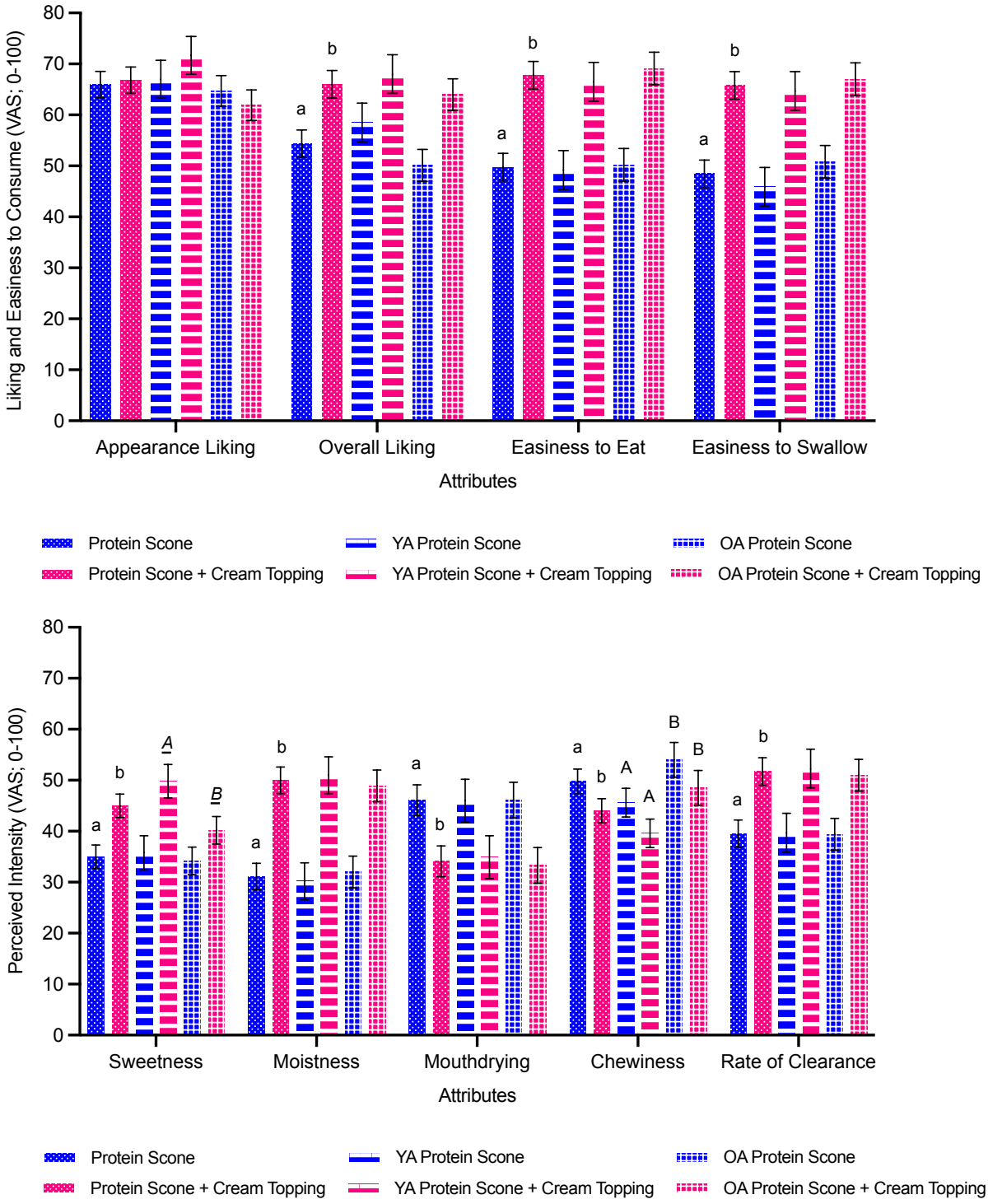


Figure 7.6. Volunteers' (*n* = 116) ratings of scones with and without cream topping by overall and age (YA: younger adults (*n* = 58); OA: older adults (*n* = 58)) (visual analogue scales; VAS 0-100). Data denotes means ± standard error. Significant differences between samples and age are noted by differing small letters and capital letters respectively. Differing capital letters in italics (sweetness) indicate a significant pairwise comparison between age groups for protein scone + cream topping (via a significant sample by age interaction (p = 0.04); however, age overall did not reach significance (p = 0.09)).

Volunteers provided 106 comments, where scones with cream topping had a greater number of positive comments (69%) compared with scones without cream topping (45%) as summarised in Figure 7.7. The mouthdrying discrimination test (2-AFC) supported the rating results, demonstrating that adding a cream topping to scones significantly reduced mouthdrying (p < 0.0001; d' value: 0.74; power: 1.00) compared with scones without cream topping. The proportion of individuals who identified the scone with cream as the less mouthdrying sample was 70%.

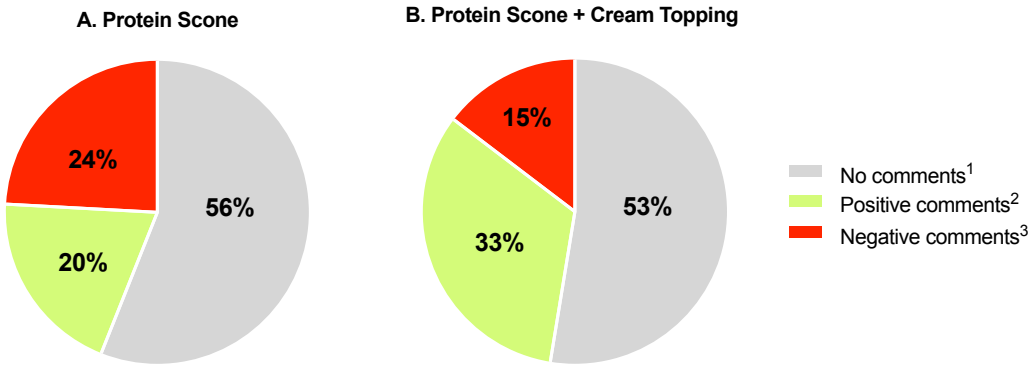


Figure 7.7. Percentage overview of volunteer comments relating to whey protein fortified scones with and without cream topping. [1] Refers to volunteers that did not provide any comments; [2] volunteers who provided positive comments (such as nice taste, delicious, easy to consume, enjoyed, good flavour, OK, sweetness, nice, soft, light, tasty, pleasant, palatable, better with cream); [3] volunteers who provided negative comments (namely sweetness, dry, tasteless, bitter, weak, grainy, dense, chewy, heavy, claggy, unpleasant, horrid, disappointing, rather messy with cream).

Volunteers' protein fortified products consumption habits were categorised into two groups: "yes, I consume protein fortified foods and/or beverages (less than once per month to once a day)" and "no, I do not eat/drink protein foods and/or beverages". There was a significant association (p < 0.0001) between protein fortified product consumption and age, where older adults infrequently consume protein fortified products compared with younger adults (Figure 7.8).

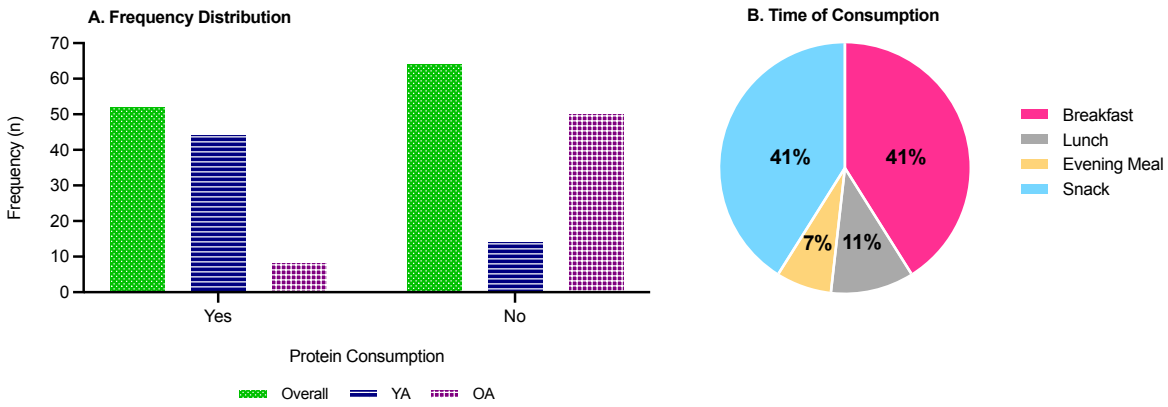


Figure 7.8. Overview of volunteers protein fortified consumption habits **(A)** frequency distribution (*n* = 116; younger adult (YA): *n* = 58; older adult (OA): *n* = 58) and **(B)** volunteers that consume protein fortified products (n = 52/116) time of consumption.

7.6. Discussion

7.6.1. Mouthdrying detection threshold (MDT)

The MDT demonstrated mouthdrying was detectable in all WPBs compared with the control (WPeB). The estimated whey protein detection threshold was 0.41% w/v protein, such levels are considerably lower than most commercial WPBs. The resulting threshold was analysed using binomial and beta-binomial models (suitable for a trained panel often having small sample size with replicated results) for all 16 individual protein levels and subsequently fitted into a linear regression to obtain a 50% discriminator level. However, the BET method resulted in a higher estimated threshold most likely due to this method being considered less accurate, which can lead to individual thresholds potentially being over-estimated (especially if individuals fail to correctly identify the highest protein level) (ISO, 2018). Therefore, regardless of the statistical approach, mouthdrying was detectable at low protein levels by a trained panel. In addition, confounding factors were minimised as the control (WPeB) was matched with all protein levels in terms of sweetness and viscosity. There were relatively small fat differences in samples (0.008% to 0.95% w/v); however, such small differences in fat are unlikely to contribute to

mouthdrying (Norton *et al*., 2021c, **Chapter 6**). Furthermore, all samples were presented in sealed cups with sip lids to mask any potential differences. Previous work in this area has used a range of low pH WPB models (β-lactoglobulin, lactoferrin, whey protein isolate (WPI), process whey protein (PWP) and acidic process whey protein (aPWP)) (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012). Such studies have utilised rating scales (0-5-, 0-7- and 0-15-point scales) and used no ratio set progression between protein levels; however, they have also demonstrated that mouthdrying can be detected at low protein levels (less than 3.0% protein) (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012). These studies focused on low pH WPB (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012), whereas our study used a neutral pH WPB. This could suggest that mouthdrying is detectable at low protein levels regardless of the potential differences in mechanisms between astringency (low pH WPB) and mouthdrying (neutral pH WPB) (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012; Norton *et al*., 2021b, **Chapter 5**). Mouthdrying can be detectable at low levels using: lactoferrin (0.05%) (Ye *et al*., 2012), aPWP (0.07%), PWP (0.10%) (Sano *et al*., 2005), WPI (0.15%) and β-lactoglobulin (0.25-3.0%) (Kelly *et al*., 2010; Ye *et al*., 2012) (all in low pH WPBs; % w/v or wt/wt). These levels are comparable to the 0.41% (w/v) demonstrated in our study using a neutral pH WPB (SF-WPC). The accuracy and/or differences in detectable protein levels could depend on the: (1) specific sensory test used (rating scales versus discrimination testing); (2) increments in protein level; and/or (3) protein type. It is also likely that once mouthdrying is detected individuals will subsequently find it more difficult to detect the differences between levels. This supports Kelly *et al*. (2010) that noted mouthdrying plateaus at higher levels (4.0-13.0% wt/wt protein). All these findings have important product implications since on-the-market WPBs are typically between 6.0-10.0% w/v protein, which is considerably higher than the ‘lowest’ detectable mouthdrying WPB.

7.6.2. Mouthdrying just-noticeable difference (JND)

The JND testing demonstrated individuals differ in mouthdrying thresholds; however, most individuals could tolerate a 0.67% w/v increase in protein level without registering an increase in mouthdrying. Moreover, older adults were more sensitive to WPB mouthdrying compared with younger adults. This supports previous mouthdrying research in dairy beverages which also used discrimination testing; therefore, highlighting the enhanced discriminating abilities of older adults compared with younger adults (Withers *et al.*, 2013a). It is suggested that older adults are more sensitive to mouthdrying due to potential age-related associated effects, such as increased protein retention (Norton *et al.*, 2020a, **Chapter 3**), reduced saliva flow (Vandenberghe-Descamps *et al*., 2016) and/or a dry mouth (Thomson, 2016).

This study was limited by the number of samples that could be provided within the JND subset; accordingly, at the 50% discriminators level the JND threshold was unable to be established. Therefore, subsequent testing with less tight protein progression would be recommended to determine a more accurate threshold than estimated by the BET method for those considered above threshold. However, as alluded to in a review on sensory methods for older adults, providing a balance between the number of samples versus sample fatigue is a key issue within older adults (Methven *et al*., 2016). In addition, the tight progression (i.e. ×1.25) between samples could have led to samples being considered too similar; therefore, resulting in less than 50% of individuals detecting a difference at each level. As noted within the MDT subset, once mouthdrying is detected, it is less easy to detect any increase in mouthdrying or difference between samples. This could be the reason why individuals found it challenging to select correctly the more mouthdrying WPB within all five pairs, despite the increasing protein content. Therefore, future work could focus on determining an exact JND threshold for whey protein derived

mouthdrying and to achieve this both optimising protein level progression and the number of samples is needed. It should also be noted that our study was unable to collect saliva samples (due to the ongoing COVID-19 pandemic) and differences in saliva flow have recently been correlated with mouthdrying build up in ONS (Lester *et al*., 2021). Therefore, such differences in mouthdrying sensitivity may relate to saliva flow groups; however, this needs further proof in older adult populations and using balanced saliva flow groupings. The individual differences in mouthdrying sensitivity could impact product compliance and understanding them could assist in providing product suitability for the ageing population. Our study also supports the use of 2-AFC tests as providing useful mouthdrying results in both a home setting (as per this current study) and a sensory laboratory (Withers *et al*., 2013a; Norton *et al*., 2021b, **Chapter 5**).

7.6.3. Whey protein beverages (WPB) rating

Increased protein levels in WPBs correlated with negative effects such as reduced liking and easiness to consume as well as increased mouthdrying. However, flavour intensity was closer to JAR with increased protein levels demonstrating WPBs, especially those with lower protein content, were perceived to lack flavour. This would be expected since the WPBs used in our study had no added flavour and accordingly adding flavour would be suggested in order to mask the associated undesirable whey related flavours which were more prevalent at the higher protein levels. This could also imply that texture related attributes (mouthdrying) had a greater effect than flavour related attributes on liking. Previous work, investigating differing protein levels in WPBs, has typically focused on low pH WPBs (as alluded to in Section 7.6.1). This demonstrated that increasing protein levels (0.01-5.0% w/v or wt/wt) in different WPBs models resulted in higher mouthdrying (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012) which subsequently plateaued at

higher levels (4.0-13.0% wt/wt) (Kelly *et al.*, 2010). These findings generally support our work in neutral WPBs which show that increasing protein levels increases mouthdrying.

Age-related effects were present between age groups, where older adults perceived all WPBs as easier to drink and swallow compared with younger adults. This is a relatively positive result, as it supports their suitability for an ageing population, despite the associated negative sensory attributes. This may be because the WPBs are a thin beverage (viscosity: 4.20-4.96 mPa·s) rather than a thicker beverage (above 50 mPa·s) and therefore easier to consume. In addition, this could also be explained by older adults having, in varying extents, altered taste and tactile sensations (Smith *et al*., 2006; Methven *et al*., 2012) and perceiving WPBs in a real life setting as easier to consume than their younger counterparts. No additional age-related significant differences were present; however, such differences could have been suppressed due to the following: (a) all sensory evaluation was conducted using single sips (10 mL) to maintain adherence in a home setting; therefore, negative attributes (such as mouthdrying) could not build up over consumption (mouthdrying is suggested to build with repeated consumption); and (b) all testing was carried out using VAS (0-100) which may lack test sensitivity compared with discrimination testing. It is noteworthy that in our current study we recruited healthy community based older adults (aged 65 years or over); however, the group age average was 69.5 years which is towards the lower end of this age group. Future work using different older adult populations (such as 65-74 years and over 75 years) is recommended, as recently done by Regan *et al*. (2021), as the effects are likely to intensify with increased age. JND testing (Section 7.6.2) via 2-AFC tests demonstrated that older adults are more sensitive to mouthdrying; however, when WPBs were presented monadically using VAS (0-100) these significant differences were not present. This suggests that in the future using short simple sensitive discrimination tests (such as

a 2-AFC) rather than rating scales (0-100) would be recommended when investigating age-related mouthdrying.

7.6.4. Scones perception and liking

Consumers of differing ages found adding cream topping to whey protein fortified scones to have a positive effect, both increasing liking and reducing mouthdrying. This supported our previous work involving a trained sensory panel and concluded that increasing fat (via cream topping), hence increasing lubrication, is an effective strategy to suppress perceived mouthdrying in a whey protein solid food model. Moreover, future work should focus on methods to increase lubrication (without the need to add cream) and subsequent effects on food bolus within such products. However, within the context of older adults, energy dense toppings (such as milk, cream and butter), which can be easily added to products, are often used to moisten food bolus (Cichero, 2016) and is a well utilised strategy within clinical settings to promote food intake (BAPEN, 2016). It should be noted that the cream topping was well received by the volunteers, as supported by their liking scores. Similarly in cream cheese (enriched with whey protein), added butter improved flavour and increased liking (Song *et al*., 2018). Furthermore, using 'familiar' foods has previously been considered a viable means of enhancing protein intake within an ageing population (Morilla-Herrera *et al*., 2016; Beelen *et al*., 2017a; 2017b; Mills *et al*., 2018). Clotted cream fits this remit well and makes a whey protein solid food matrix more palatable.

Age-related differences between age groups were noted where older adults perceived scones as chewier than younger adults. This suggests that within whey protein fortified foods texture sensitivity can increase with age. Currently, the extent of such effects in whey protein fortified foods are relatively unknown since age-related differences were unable to reach significance in whey protein fortified cakes and biscuits (Norton *et al*.,

2020b, **Chapter 4**). However, in other food models, such as nuts, older adults noted hardness as a more dominant sensation (Hutchings *et al*., 2014) and had increased brittleness preference (Miyagi & Ogaki, 2014) compared with younger adults. Vandenberghe-Descamps *et al*. (2018) developed an oral comfort questionnaire for the ageing population during food consumption. Products such as ground beef and protein enriched milk roll were perceived as 'less comfortable' and were associated with negative terms (i.e. hard/firm, dry, doughy and difficult to chew, swallow and humidify) (Vandenberghe-Descamps *et al*., 2018). Bolus properties also alter with age. For example, older adults have a more degraded bolus and perceived dryness as a more dominant attribute (during the latter stages of consumption only) as result of increased consumption time post sausage consumption than younger adults (Aguayo-Mendoza *et al*., 2020). It is likely that the reduced saliva flow and/or dental status in older adults leads to poor oral clearance (Turner & Ship, 2007; Razak *et al*., 2014; Vandenberghe-Descamps *et al*., 2016) or alternatively increased protein retention within the oral cavity (Norton *et al*., 2020a, **Chapter 3**) resulting in foods being perceived as chewier or harder. Interestingly, no other significant age-related effects were present in our study. This highlights the challenges of sensory testing with older adults when researching age-related differences. In addition, texture sensitivity with age may be attribute, product and segment (age or population) based (Song *et al*., 2016; Norton *et al*., 2021a, **Chapter 2**).

7.7. Conclusion

Mouthdrying was detectable regardless of the protein level and a MDT was estimated at 0.41% w/v protein. JND testing noted many naïve consumers could tolerate a 0.67% w/v increase in protein content without detecting an increase in mouthdrying; corresponding, this led to the JND threshold being unable to reach 50% discriminators. However, older adults were more sensitive to mouthdrying than younger adults. Such findings are

important since previous research has not typically focused on individual differences and could be key to ensure that whey protein products meet the needs of the consumer. Similarly, at higher protein levels (more relevant to commercial products) increasing protein content within WPBs increased mouthdrying and reduced liking. Accordingly, this work demonstrated that mouthdrying was clearly present in WPBs whatever the protein level. Therefore, future work should focus on proposed causes and methods to suppress mouthdrying, whilst taking account of individual differences, to maximise the benefits and encourage protein intake, especially in an ageing population. Scones with cream topping successfully improved palatability of whey protein fortified models, suppressed mouthdrying and increased liking in consumers of both age groups. This resulted from enhanced lubrication from fat; however, future work should focus on improved methods to increase lubrication within whey protein fortified foods. In addition, since older adults found the whey protein fortified scones chewier this also emphasises the importance of protein products being formulated to meet the needs of older consumers to enhance protein intake.

S.7. Supplementary

S.7.1. Whey protein liquid models viscosity

The resulting apparent viscosities from all beverages were measured using rheometer (Modular Compact Rheometer (MCR) 102, Anton Paar Ltd., UK) as described in our previous work (Norton *et al*., 2021b, **Chapter 5**; 2021c, **Chapter 6**). All beverages were considered matched at a shear rate 50 s^{-1} (4.20 to 4.98 mPa·s) as outlined in Figure S.7.1. In addition, the effect of increasing hydrocolloid concentration on subsequent viscosity was measured as summarised in Figure S.7.2.

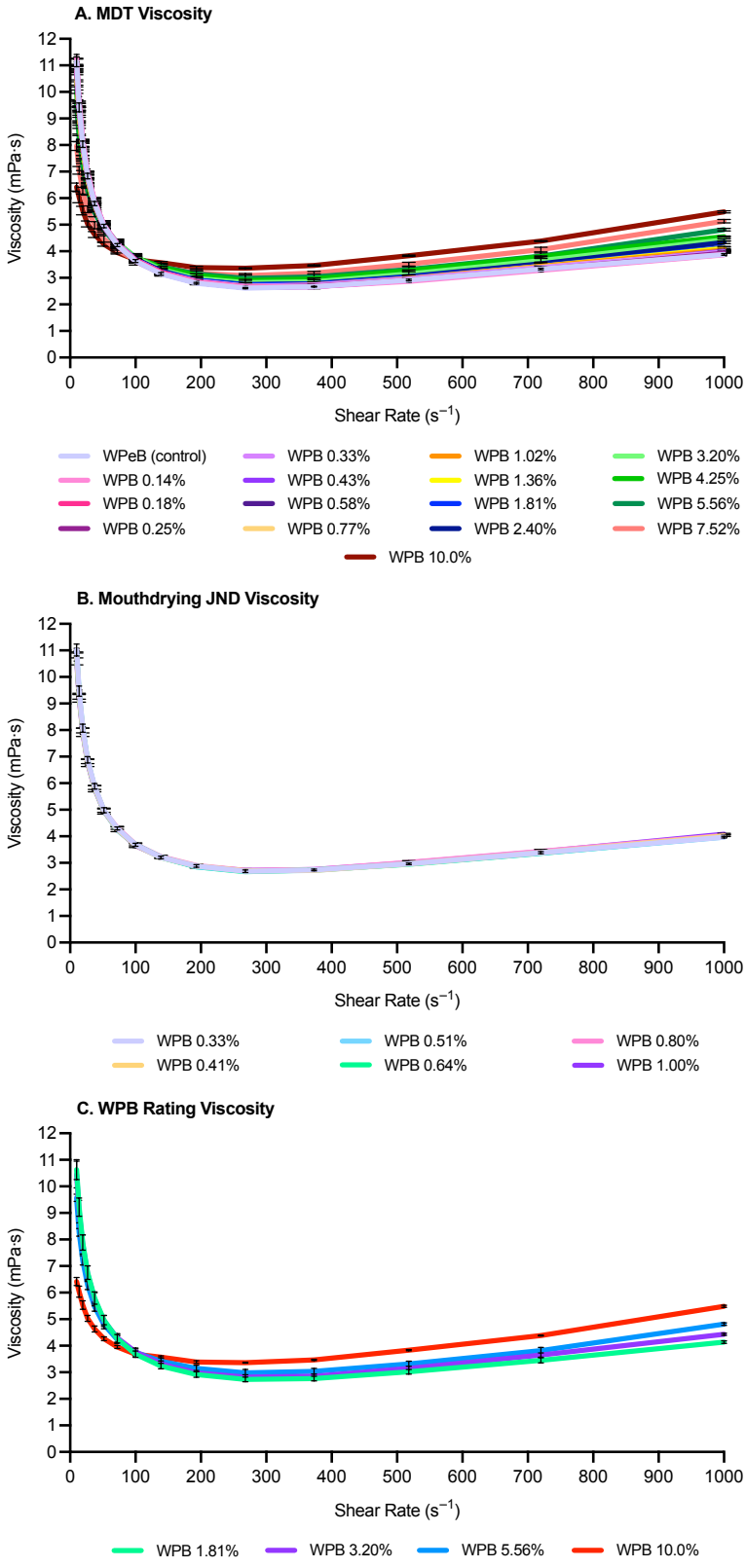


Figure S.7.1. Overview of apparent viscosities of all whey liquid models by (**A**) mouthdrying detection threshold (MDT) (WPeB: whey permeate beverage (control)); (**B**) mouthdrying just-noticeable difference (JND); and (**C**) whey protein beverage (WPB) rating. Data signifies means of six replicates ± standard error.

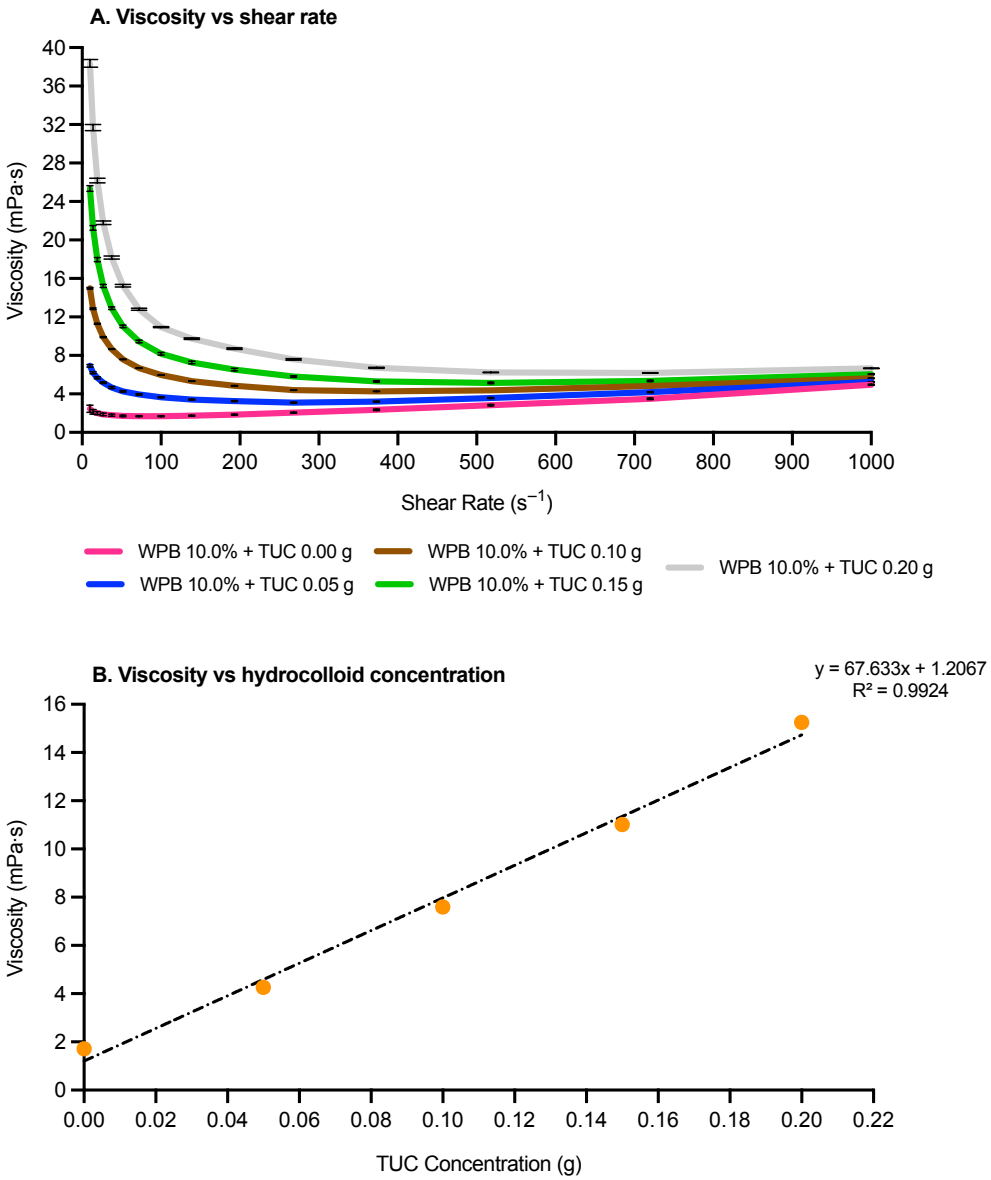


Figure S.7.2. Influence of increasing hydrocolloid (TUC: thicken up clear) on apparent viscosity of whey protein beverages (WPB) by (**A**) viscosity versus shear rate and (**B**) viscosity versus hydrocolloid concentration with dashed line (**— · -**) denoting linear trend line with corresponding equation. Data represents means of six replicates ± standard error.

S.7.2. Additional study data

Sweetness: refers to the sweet taste of the product stimulated by sucrose

Moistness: refers to degree of slightly damp dough

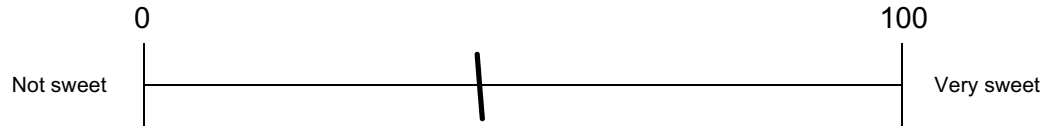
Mouthdrying: refers to the drying sensation in the mouth during or after consumption of a product (and persists/builds for up to 30-s post swallow)

Rate of clearance: refers to the rate of clearing the sample from the mouth

Chewiness: refers to ease of ability to chew

Figure S.7.3. Overview of attribute perception definitions provided to volunteers.

Before we move onto rating using line scales, we are going to do a familiarisation exercise on how to use the line scale. The scale (please see below) represents **0 to 100** increasing from left to right, for example not to very.



Please answer the following questions **BY MARKING** your response (with a dash) on each line scale:

TV Soaps: How much do you enjoy **TV SOAPS?**

Not ——— Very

Board Games: How much do you enjoy **PLAYING BOARD GAMES?**

Not ——— Very

Watching Sport: How much do you enjoy **WATCHING SPORT?**

Not ——— Very

Shopping: How much do you enjoy **SHOPPING?**

Not ——— Very

Figure S.7.4. Summary of volunteers' familiarisation exercise on how to use the line scale.

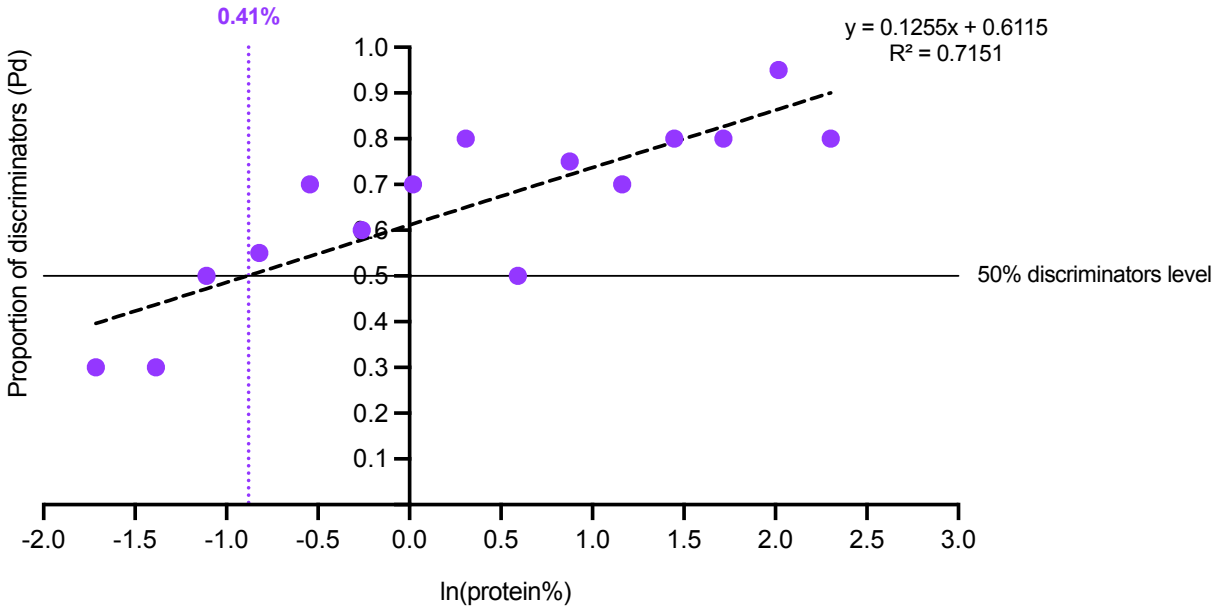


Figure S.7.5. Mouthdrying detection threshold linear regression equation plotting proportion of discriminators (Pd) against protein level natural logarithm (ln(protein%)). Data obtained from trained panel (*n* = 10) utilising binomial and beta-binomial models. Black dash line (– –) denotes linear trend line with corresponding equation, black solid line (——) represents 50% discriminator level and purple dotted line (·····) signifies whey protein detection threshold level (i.e. 0.41% w/v).

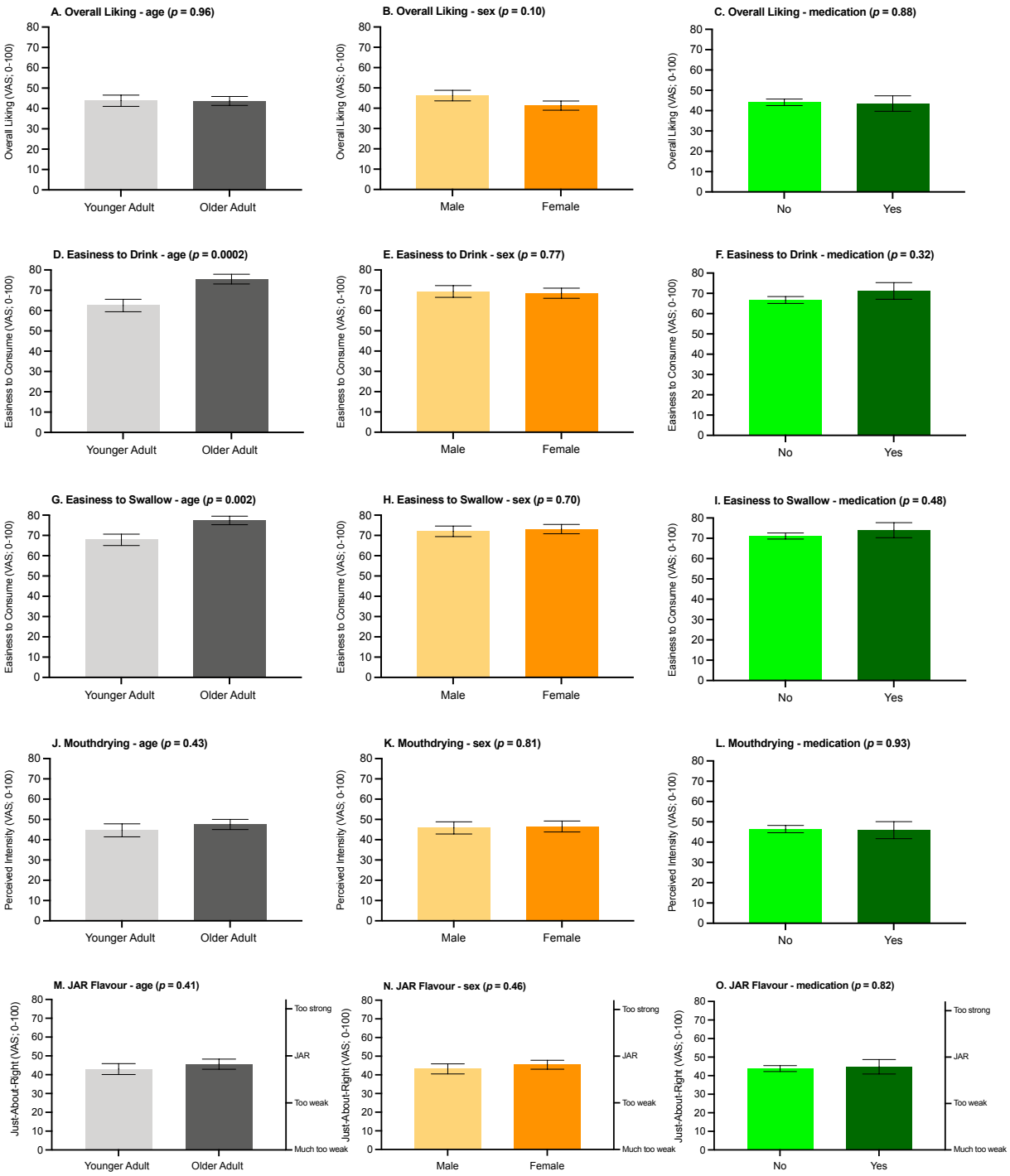


Figure S7.6. Additional factors (**A-C:** overall liking; **D-F:** easiness to drink; **G-I:** easiness to swallow; **J-L:** mouthdrying and **M-O:** Just-About-Right (JAR) Flavour) influencing mean whey protein beverage ratings (± standard error) (*n* = 116; VAS: visual analogue scale 0-100) differing in protein levels with relevant *p* value above each category.

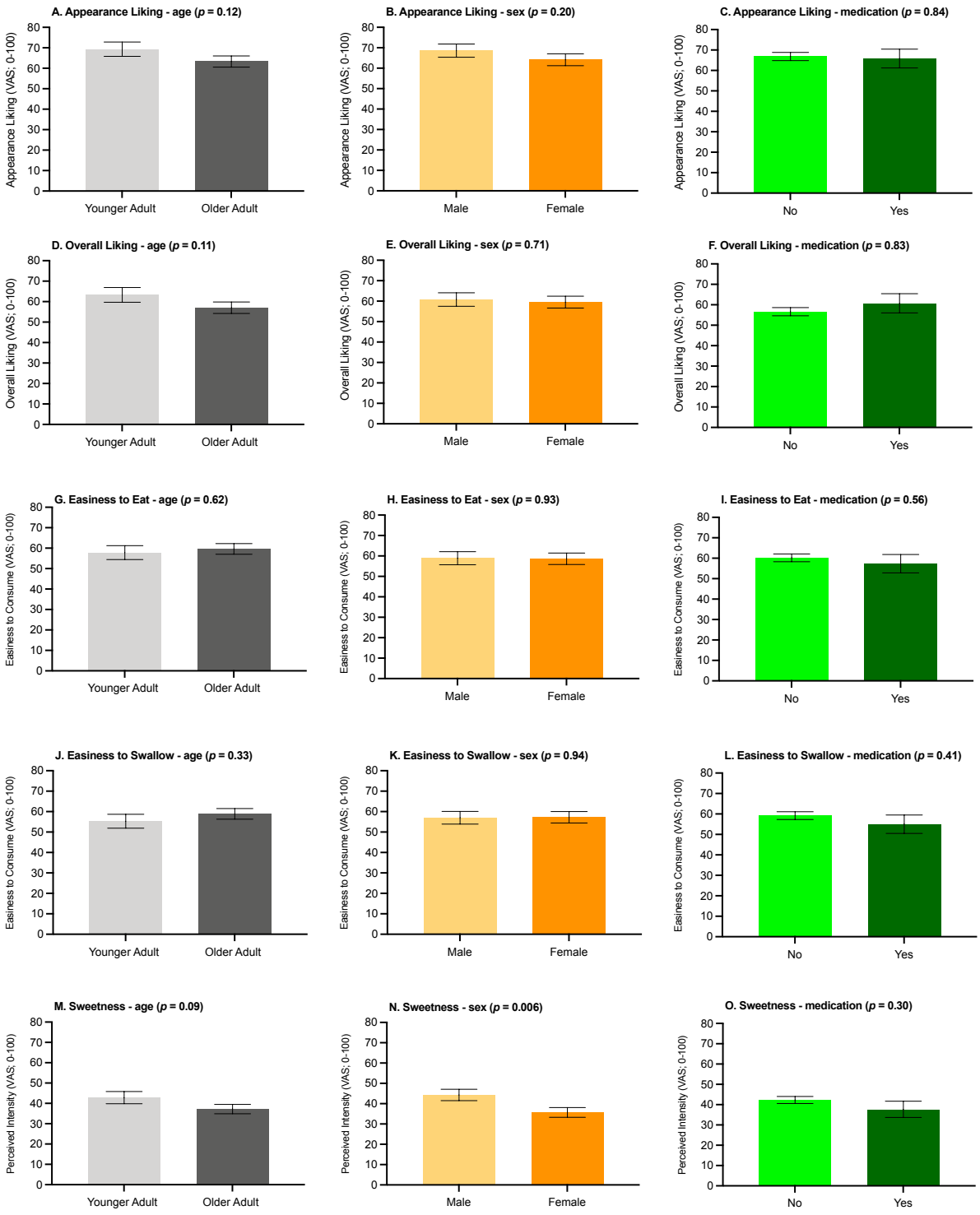


Figure S.7.7. Additional factors (**A-C:** appearance liking; **D-F:** overall liking; **G-I:** easiness to eat; **J-L:** easiness to swallow; **M-O:** sweetness) influencing mean scone ratings (± standard error) (*n* = 116; VAS: visual analogue scale 0-100) with relevant *p* value above each category.

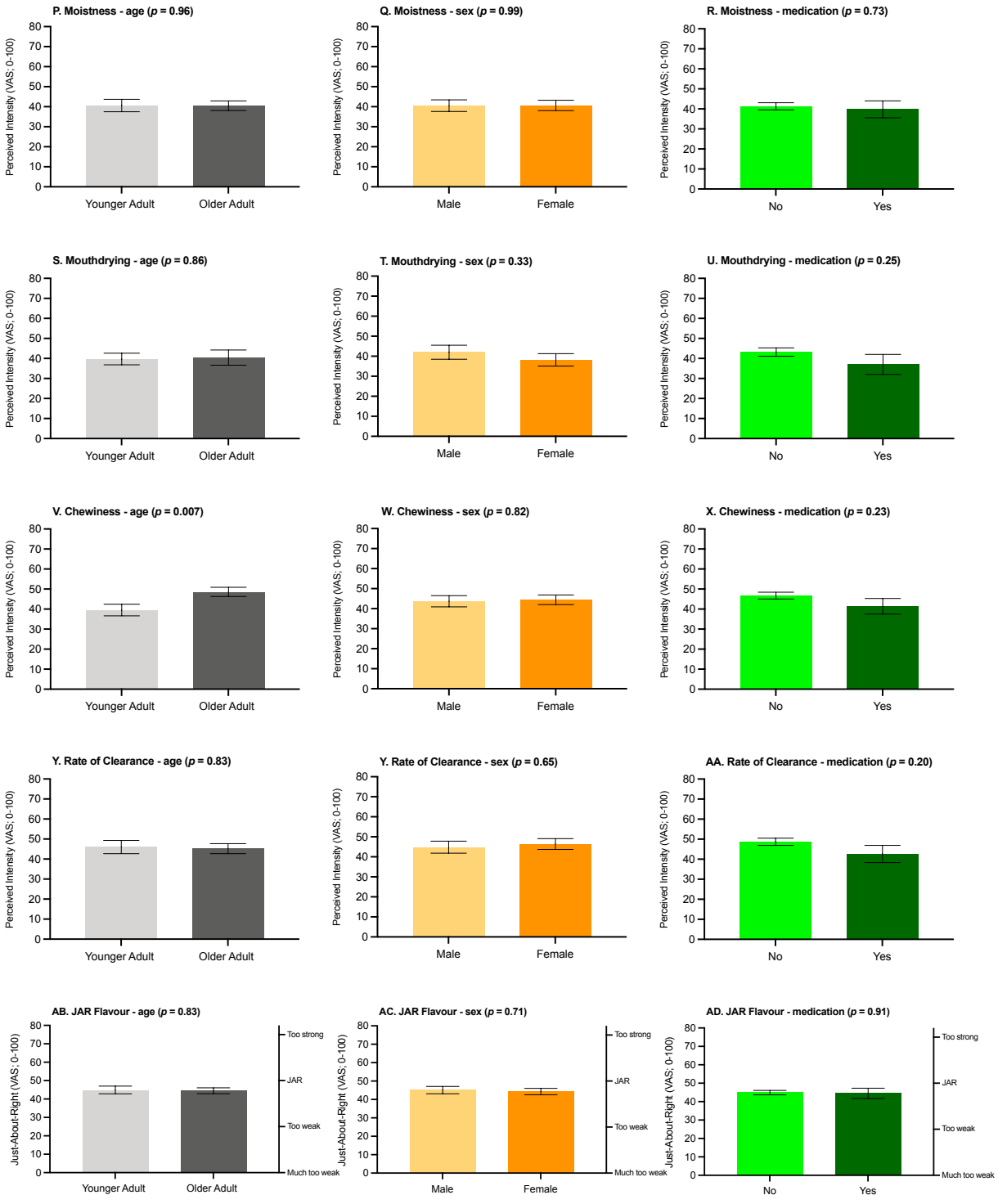


Figure S.7.7. continued…additional factors (**P-R:** moistness; **S-U:** mouthdrying; **V-X:** chewiness; **Y-AA:** rate of clearance and **AB-AD:** Just-About-Right (JAR) Flavour) influencing mean scone ratings (± standard error) (*n* = 116; VAS: visual analogue scale 0-100) with relevant *p* value above each category.

Chapter 8

General discussion

This final chapter summarises the thesis key findings, implications, limitations, suggested future work and conclusions. Broadly this thesis aimed to investigate the extent of perceived mouthfeel effects (predominately mouthdrying) derived from whey protein fortified products and the influence of individual differences. Improving knowledge in this area can enable optimisation of these products, subsequently promoting increased consumption and reduced waste. Key thesis findings are summarised in Figure 8.1.

8.1. Whey protein causes mouthdrying regardless of food model

Perceived mouthdrying is relatively well documented in whey protein beverages (WPB); however, less is known in whey protein solid models. In addition, research has usually focused on sensory panels rather than the naïve consumers. This is relevant since there is a growing whey protein market typically including the sport, health, lifestyle and/or older populations (Phillips *et al*., 2016). All tested WPBs were perceived as mouthdrying by consumers: (a) regardless of heat treatment (**Chapter 3**); (b) compared with a non-protein whey control (**Chapter 5**); and (c) intensifying with protein content (**Chapter 7**). The sensory panel also perceived WPBs as more mouthdrying than whey permeate beverages (WPeB) (**Chapter 5)** and detected mouthdrying at low protein levels regardless of the threshold method (**Chapter 7**)

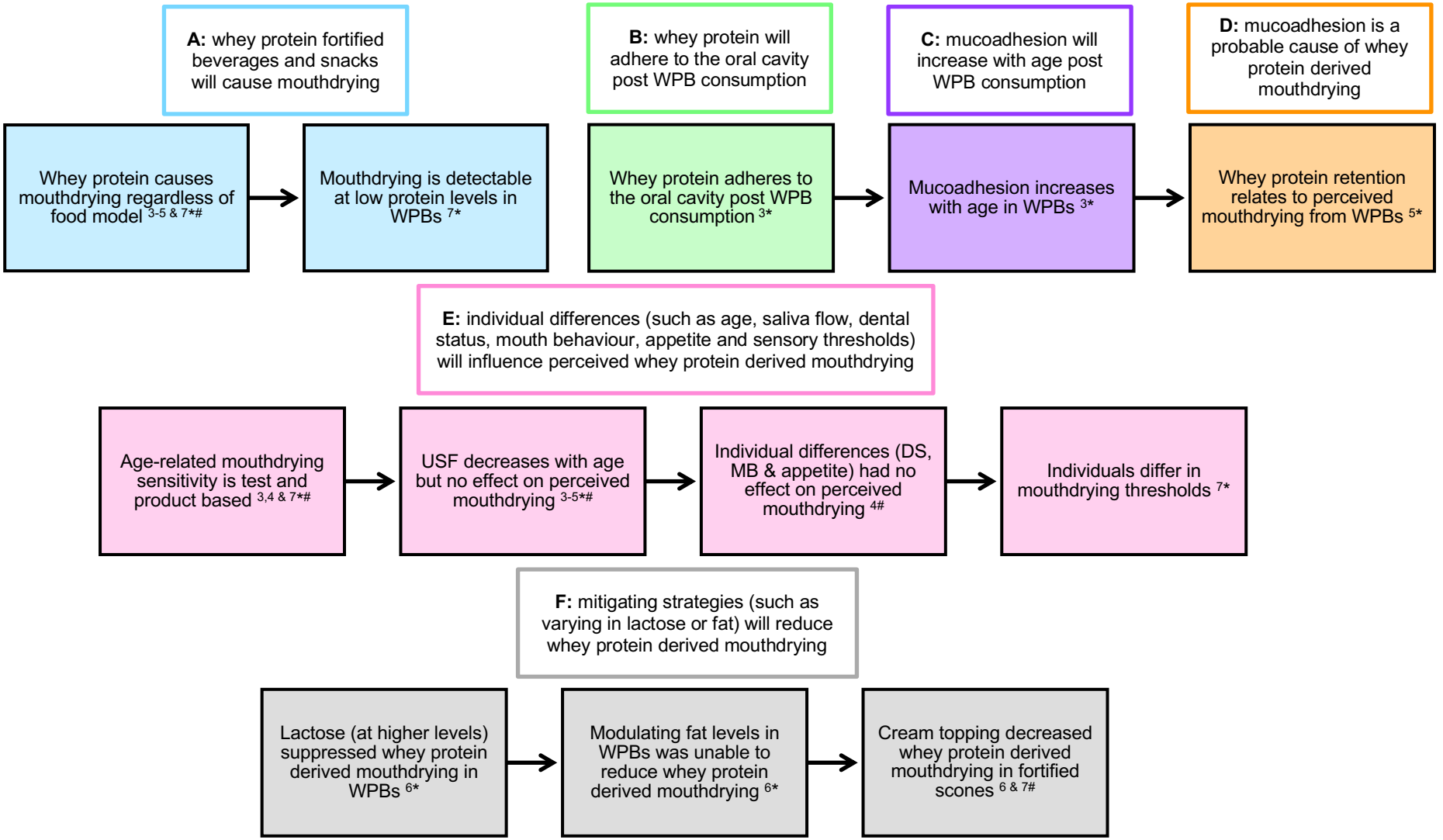


Figure 8.1. Overview of thesis hypotheses (non-shaded boxes; letters [A-F]) with key results (shaded coloured boxes; numbers [3-7] represent thesis chapter tested in; * refers to liquid model result; # indicates solid model result). Acronyms: whey protein beverages (WPB); unstimulated saliva flow (USF); dental status (DS); mouth behaviour (MB).

Previously, results suggested that whey protein heat treatment correlated with mouthdrying using sensory panels via rating scales (0-4) (Josephson *et al*., 1967), quantitative descriptive analysis (QDA) and sequential profiling (Bull *et al*., 2017). Hence, the initial experiments (**Chapter 3**) focused on WPB heat treatment using consumers (*n* = 84) and this demonstrated that such heating was associated with intensified mouthdrying sensations. These findings are in agreement with previous research; therefore, minimising subsequent heating within WPBs is recommended to reduce perceived mouthdrying.

Mouthdrying related studies have typically not used a non-protein whey control, hence, this thesis developed a WPeB (non-protein whey control) to fit this purpose. WPBs were consistently perceived as more mouthdrying than WPeBs regardless of sensory method or individual group used (**Chapters 5 and 7**). Accordingly, this demonstrated that the protein within WPBs is considered to drive mouthdrying rather than other whey constituents. Whey proteins are typically fortified into beverages up to a 10.0% protein level; therefore, it is relevant to investigate the psychophysical relationship between protein content and mouthdrying sensation (**Chapter 7**). Mouthdrying was detectable at low protein levels (less than 2.0%) in WPBs and such levels are well below the typical levels (6.0-10.0%) found in on-the-market products. In addition, consumers (*n* = 116) were able to perceive mouthdrying at differing protein levels and mouthdrying intensity increased with increasing protein levels. Such findings using neutral pH WPBs (in this thesis) are in line with previous work in low pH WPBs (Sano *et al*., 2005; Kelly *et al*., 2010; Ye *et al*., 2012). Therefore, suggesting that regardless of the amount of whey protein added, WPBs were still considered mouthdrying.

Whey protein fortified snacks can provide a familiar food to assist in enhancing protein intake within an ageing population; however, they need to have an acceptable mouthfeel.

Previous studies suggest that whey protein fortified into solid models can modulate texture (i.e. less smooth/soft, elastic, harder and drier texture) (Wendin *et al*., 2017; Hoglund *et al*., 2017; Song *et al*., 2018). However, the extent of perceivable mouthdrying within whey protein fortified solid models was relatively unclear. Accordingly, four different snack models were developed: cakes, biscuits, cupcakes and scones, with and without whey protein fortification (**Chapters 4 and 6**).

All tested whey protein fortified snacks were perceived as mouthdrying by the sensory panels and/or consumers; therefore, supporting results in WPBs. Having fortified four different snacks with whey protein, key themes were evident relating to: (a) blunted flavour (vanilla and lemon) via sensory panel; (b) modulated mouthfeel (increased chewiness, firmness of bite, hardness and mouthdrying and reduced moistness) by sensory panel and/or consumers; and (c) altered physical properties (increased hardness/fracturability and chewiness) using texture analysis. It should be acknowledged that whey protein physical changes might occur from heating (i.e. baking) (though this was not the focus in this thesis) which may modulate texture. Examples include: (a) protein aggregation could impact cell wall strength (Wilderjans *et al*., 2010); (b) water holding capability (Kew *et al*., 2020) could impact moistness; (c) protein denaturation temperature could result in air cells being more likely to overexpand (Arunepanlop *et al*., 1996); and (d) foaming properties could be altered by heating (Dissanayake & Vasiljevic, 2009). Most snack models (cupcakes and scones) used in this thesis had post-baking moisture content matched within 1.0%, although there were some small differences in water activity (**Chapter 4 and 6**). Such findings would suggest mouthdrying is possibly unrelated to differences in final moisture content. Potentially this could imply bolus hydration may have a greater impact on texture perception (Jourdren *et al*., 2016) or protein alters how moisture is bound in foods thereby resulting in a different perception.

In addition, despite changing the whey protein source (whey protein isolate (WPI) versus whey protein concentrate (WPC)) (**Chapter 4**) or modulating processing (standard versus heat-stable WPC) (**Chapter 6**), snacks still resulted in mouthdrying. Accordingly, this suggests such mouthdrying is perceived to the same extent regardless of different whey protein processing strategies.

In all experiments individuals were provided with a mouthdrying description rather than a physical reference for the following reasons: (a) typically the suggested physical references for mouthdrying relate to polyphenolic compounds (i.e. tannic acid and alum (Sano *et al*., 2005; Lee & Vickers, 2008; Beecher *et al*., 2008; Kelly *et al*., 2010; Vardhanabhuti *et al*., 2010)) which subsequently cause astringency (i.e. polyphenols bind to salivary proteins) considered a different mechanism and more relevant to low pH WPB than whey protein derived mouthdrying at neutral pH (i.e. protein adhering to the oral cavity); (b) other proposed physical references utilise dairy proteins (i.e. heat-treated rennet whey and milk with added whey protein (Withers *et al*., 2014; Lester *et al*., 2021)) and are considered a less pure standard, being potentially in lower intensity than our tested WPB; and (c) the trained panel could come to a consensus on a description based on the tested WPB, which was subsequently used as a description for consumers. It should be noted terms mouthdrying and astringency are used interchangeably in some cases across this thesis.

In summary, these experiments demonstrated that the protein within whey powders is the cause of the perceived mouthdrying in fortified products, regardless of matrix or method. This highlights that mouthdrying is a widespread problem, since it can be identified by sensory panels and/or consumers at a range of protein levels. Accordingly, the thesis focus shifted to two key areas to address this, namely mouthdrying mechanisms and mitigating strategies.

8.2. Mouthdrying mechanisms in whey protein beverage (WPB) models

8.2.1. Whey protein adheres to the oral activity post WPB consumption

Proteins are suggested to have adhesive and binding properties (Ye *et al*., 2012; Withers *et al*., 2013b; Hsein *et al*., 2015; Bull *et al*., 2020). However, cited studies can be associated with key methodology limitations (i.e. utilising animal models, small subject size in human studies or the absence of a non-protein whey control). Such properties could relate to whey protein derived mouthdrying in neutral pH WPBs (Withers *et al*., 2013b; Bull *et al*., 2020) but this needs proving. Accordingly, **Chapter 3** initially focused on method development utilising consumers and demonstrated that oral retention was a valid method to establish whether whey protein adheres to the oral cavity compared with a non-protein whey control. Despite a suitable 'physical measure' being developed, this study was unable to determine the link between protein retention and mouthdrying; thereby providing the basis for **Chapter 5**.

WPB heat treatment increased perceived mouthdrying (Section 8.1); however, this did not translate into a substantial difference in oral retention. This result was unexpected as WPB heat treatment was hypothesised to intensify mechanisms as a result of protein denaturation and aggregation (Wijayanti *et al*., 2014). Previously, one study using a small subject size (n = 5) demonstrated that heated WPBs increased protein weights in saliva samples post consumption compared with unheated WPBs (Bull *et al*., 2020). However, the findings in **Chapter 3** are considered valid and robust for the following reasons: (a) the pilot study focused on method development (i.e. addressing calculations and baseline value concerns); and (b) data was collected in duplicate from 84 consumers. Therefore, future experiments focused solely on the unheated WPB which was still perceived as mouthdrying and associated with mucoadhesion properties.

8.2.2. Mucoadhesion is a cause of mouthdrying in WPBs

Whey protein (a) causes mouthdrying (**Chapter 5**) and (b) adheres to the oral cavity (**Chapter 3**) compared with a non-protein whey control. However, the link between the two phenomena had not previously been investigated within the same experiment. **Chapter 5** established this, where WPB consumption increased both perceived mouthdrying and protein concentration in saliva samples (oral retention), compared with WPeB, in 40 consumers. It is likely that consuming WPBs results in whey protein interacting and adhering to the oral mucosa (Smart, 2005; Khutoryanskiy, 2011) leading to reduced lubrication and increased friction (Vardhanabhuti *et al*., 2011) subsequently perceived as mouthdrying (Figure 2.3 in **Chapter 2**). However, as suggested in **Chapter 2**, various factors could influence whey protein oral retention strength including age (Section 8.2.3) and saliva flow. **Chapter 3** demonstrated that saliva flow may relate to protein oral retention; however, results were only a trend (p = 0.06). Perhaps resulting from the relatively small number of volunteers (n = 84) and further exacerbated by grouping individuals into three (n = 27-29).

8.2.3. Mucoadhesion increases with age

The role of age on mucoadhesion post WPB consumption has not previously been investigated; however, it is highly relevant since: (a) whey protein has proven nutritional benefits (i.e. greater muscle synthesis and protein gain) in older adults (Dangin *et al*., 2003; Pennings *et al*., 2011); (b) older adults are more sensitive to mouthdrying in dairy beverages (Withers *et al*., 2013a); and (c) salivary flow rates can decline with age (Vandenberghe-Descamps *et al*., 2016). The latter two points could intensify mucoadhesion. The oral retention method was successfully utilised in an ageing population concluding older adults had increased mucoadhesion compared with younger adults (**Chapter 3)**. It is also worth noting that WPBs resulted in increased mucoadhesion

compared with WPeB in two studies using younger adults (**Chapters 3 and 5**); therefore, showing clear effects and it was subsequently considered unnecessary to repeat in older adults.

In summary, oral retention can be considered a suitable method to measure mucoadhesion in WPBs. Moreover, this advances knowledge in the area by providing a suggested mouthdrying mechanism (i.e. whey protein adhering to the oral cavity) hence indicating product redevelopment in WPBs should focus on reducing such adhesion and interactions to promote product consumption. Additionally, this warrants consideration since this mechanism intensifies with age.

8.3. Influence of individual differences on perceived mouthfeel

Typically, mouthdrying related research has focused on the product rather than individual differences; however, it hypothesised that such differences could intensity mouthfeel sensations.

Currently, studies suggest texture perception could be either suppressed, preserved or enhanced with age depending on the attribute and product. However, specifically in whey protein related models, one study demonstrated mouthdrying sensitivity can increase with age (Withers *et al*., 2013a). Accordingly, experiments were designed to build on this finding to enable whey protein fortified products to be designed to suit better the needs of older adults. However, results in **Chapters 3 and 4** were inconclusive in determining mouthdrying age-related effects. This potentially resulted from (a) the differences between age groups being negligible; (b) challenges with sensory methods relating to test sensitivity for older adults; or (c) lack of build-up methods (as mouthdrying proven to increase with repeated consumption (Methven *et al*., 2010)).

Subsequent age-related experiments were on hold due to the ongoing COVID-19 pandemic till the final experimental chapter (**Chapter 7**). Lack of test sensitivity had been highlighted as a concern and a potential rationale for minimal age-related differences in **Chapters 3 and 4**. Accordingly, method suitability is a key consideration in older adults and discrimination tests (such as 2-AFC) are recommended within an ageing population due to their simplicity (Methven *et al*., 2016). More specifically, **Chapter 7** used just-noticeable difference (JND) testing (intensity needed to elicit a perceptual change (Lawless & Heymann, 2010)) concluding older adults (*n* = 58) were more sensitive to WPB mouthdrying than younger adults (*n* = 58), supporting the Withers *et al*. (2013a) findings. Accordingly, the probable reasons older adults perceive WPBs as more mouthdrying than younger adults are: (a) saliva related changes such as reduced saliva flow (Vandenberghe-Descamps *et al*., 2016; **Chapters 3 and 4**), lower viscoelasticity (Pushpass *et al*., 2019b) and/or saliva being potentially less watery and more concentrated (Nagler & Hershkovich, 2005); (b) more familiarity with having a dry mouth (Thomson, 2016); (c) poor dental status (Razak *et al*., 2014; **Chapter 4**); and/or (d) increased protein adhesion (**Chapter 3**); all potentially contributing to poor oral clearance and resulting in mouthdrying being more likely to occur and/or linger. However, such mouthdrying age-related changes were not found when using rating scales (VAS; 0-100). This same cohort of older adults did, however, rate WPBs as easier to consume and scones as chewier compared with younger adults; accordingly, demonstrating that our older adults could reliably use rating scales. Therefore, it is probable that discrimination on rating scales may be insufficient where: (a) differences between products are small; or (b) rating an attribute where perception (increases) with repeated consumption. The differences between age groups could relate to the product type, for example: (a) a thin WPB would be expected to be relatively easy to consume; and (b) scones being

potentially less desirable than expected, so texture awareness may have increased (Szczesniak & Kahn, 1971).

Another potential reason for lack of age-related differences in this thesis could relate to our recruited older adults being: (1) relatively young within the older age bracket (73.6 ± 6.2 years (**Chapters 3 and 4**); 74.6 ± 5.7 years (**Chapter 4**); 69.5 ± 3.9 years (**Chapter 7**) and (2) considered as healthy since many recruited older adults in this thesis were not taking prescribed medication (78 out of 132). In addition, those on medication (*n* = 54) were taking an average of 2.04 ± 1.31 medications, which could be considered relatively low for an older population[53]. Previously, Withers *et al*. (2013a) in an age-related mouthdrying study, had recruited an older aged cohort (*n* = 28; average: 77 years; range 65-87 years). Other relevant factors could relate to the varying level of sensory study experience (none to lots) and impairments (physical, visual and hearing). Accordingly, all these differences could have impacted results, resulting in minimal age-related differences.

This thesis tested two whey models: (1) liquid model (whey beverages) and (2) solid model (snacks) to represent typical products used in clinical settings (Table 1.3). More broadly, sweet snacks (cakes, biscuits, cupcakes and scones) were utilised since familiar and popular products are considered a viable route to increase protein consumption within an ageing population (Morilla-Herrera *et al*., 2016; Mills *et al*., 2018). Older adults are also proven to have higher liking scores for cakes compared with younger adults (Michon *et al*., 2010); hence, supporting this type of snack for investigation. Additionally, beverages and/or snacks can easily be incorporated into the diets of older adults by providing a mid-morning or mid-afternoon drink or snack to enhance nutritional status.

[53] thesis medication use is summarised in **Appendix E**

Consuming a whey protein fortified: beverage (8.2 g protein per 100 mL) (**Chapter 3**), cake (5.3 g protein per 45.0 g slice) (**Chapter 4**), biscuit (2.7 g protein per 20.0 g biscuit) (**Chapter 4**), cupcake (4.1 g protein per 35.0 g cupcake) (**Chapter 4**) or scone (4.6 g protein per 30.0 g scone with clotted cream) (**Chapter 7**) will positively impact protein consumption. This thesis demonstrated all models can easily be fortified with whey protein and are suitable for an ageing population (**Chapters 3, 4 and 7**). However, in most cases, tested models would benefit from improved palatability and acceptability.

Saliva flow can decline with age (Vandenberghe-Descamps *et al*. 2016); therefore, this could be relevant for perception of whey protein fortified products in an ageing population. **Chapters 3 and 4** demonstrated older adults had significantly lower unstimulated salivary flow rates compared with younger adults; however, stimulated saliva flow remained unaltered by age. The latter result was in contrast to the Vandenberghe-Descamps *et al*. (2016) study which demonstrated both unstimulated and stimulated salivary flow rates decreased with age. The variation in results may relate to the shorter stimulated saliva flow collection time used in this thesis (2-min) compared 5-min used in Vandenberghe-Descamps *et al*. (2016). The rationale for using the shorter collection time was that it improved compliance and/or prevented volunteers swallowing saliva over collection time potentially resulting in false results. Indeed, unstimulated salivary flow may be more sensitive to metabolic and physiological changes; therefore, more likely to be impacted by age than stimulated salivary flow (Whelton, 2012).

It has been suggested that a reduced saliva flow could cause poor oral clearance (Turner & Ship, 2007), hence, food particles may linger and subsequently impact perception. This thesis demonstrated unstimulated saliva flow reduced with age (as noted above), yet unstimulated saliva flow was not significantly related to mouthdrying perception from WPBs, cakes, biscuits or cupcakes (**Chapters 3 and 4**). It should be noted some trends

were reported in these chapters; however, any suggested differences either lacked overall significance or the trends mentioned were actually very small. Moreover, it was recognised that differences in saliva flow rate might be much greater in an older population that was older or less healthy than recruited in our studies; therefore, subsequent focus was on stimulating differences in saliva flow. Accordingly, **Chapter 5** modulated saliva flow via two conditions: decreasing (using cotton wool rolls) and increasing (using parafilm), to evaluate their impact on perceived mouthdrying from WPBs. However, neither condition significantly altered mouthdrying. Ongoing COVID-19 restrictions resulted in this study being carried out with younger adults (*n* = 40; 18-30 years) and modulating saliva flow may be more relevant in an ageing population. Furthermore, the role of saliva flow in consuming thin beverages (less than 2.0 mPa·s at 50 s^{-1}) could potentially be fairly minimal compared with a thicker beverage and/or dry chewy and hard food.

The lack of differences in **Chapters 3-5** could relate to: (a) low numbers (*n* = 23-29) and high variability within each group (**Chapters 3 and 4**); (b) our statistical approach using tertiary analysis (three balanced groups) (**Chapters 3 and 4**) rather than set values to categorise flow rate (based on proposed low or high saliva flow values); (c) using only younger adults (**Chapter 5**) as older adults may take longer to re-stimulate saliva flow; and (d) lack of method (single consumption rather than build-up methods) or model (focus was more on WPBs than solid models) suitability to find differences (**Chapters 3-5**). Similarly, another study with older adults also struggled to demonstrate saliva flow related effects in perception of cereal and meat-based products (Vandenberghe-Descamps *et al*., 2017). More recently, Lester *et al*. (2021) demonstrated low saliva flow correlated with mouthdrying build-up in oral nutritional status (ONS). However, there are some noteworthy differences in methodology and population that could explain these results.

For example, Lester *et al*. (2021) used: (a) stimulated saliva flow with a measured range: 0.3-1.2 mL/min, such values were lower than expected (unstimulated saliva flow in this thesis had a greater measured range: 0.04-2.18 mL/min); (b) saliva flow was collected over 15-min (potentially poor method adherence resulted in low values as saliva was collected for only 5-min in this thesis with higher unstimulated values); (c) unbalanced saliva flow grouping (low n = 5; medium n = 16; high n = 9) (potentially this could have resulted in more favourable findings as there was less variability within each group); (d) younger adults (18-40 years; healthy) rather than an older population; (e) build-up methods (8 × 15 mL sips) (mouthdrying more likely to intensify with repeated consumption) rather than single sips as used in this thesis; and (f) ONS (typically a thicker beverage) rather a WPB (described as thin and watery used in this thesis).

Ageing is associated with poor oral health which may subsequently impact food consumption (Razak *et al*., 2014). **Dental status** in this thesis was quantified using a self-report questionnaire. As expected, dental status declined with age in **Chapter 4**; however, any relationship between tested sensory perception was relatively small. Vandenberghe-Descamps *et al*. (2017) also found few significant effects of dental status on the sensory perception of cereal and meat products. Moreover, the older adults in **Chapter 4** were: (a) in the great majority, not denture wearers (denture use can impact texture perception in ONS (Regan *et al*., 2021)) and (b) relatively young and healthy older adults (as highlighted above); all these findings may have minimised any dental status effects relating to sensory perception.

Mouth behaviour is a self-report food texture grouping tool and has been suggested to have implications for older adults (Jeltema *et al*., 2015; 2016). Mouth behaviour was considered more relevant for solids (cakes, biscuits and cupcakes) than WPBs; therefore, it was evaluated in **Chapter 4**. However, mouth behaviour groupings were not

significantly influenced by age and only impacted the appearance liking of cakes and biscuits in the pilot study. Accordingly, it was concluded, despite differences in mouth behaviour, that this was unable to translate into perception differences in this thesis.

Appetite *per se* declines with age; however, some broader aspects of subjective appetite ratings (i.e. thirst) may relate to sensory perception. This warranted investigation in **Chapter 4** and cupcakes fortified with whey protein significantly increased perceived thirst compared with the control. This result correlates with whey protein cupcakes also being more mouthdrying than the control version. This could suggest there is potential link between perceived mouthdrying and thirst. Similar results have been established in ONS consumption (multiple sips) which resulted in both increased thirst and drying sensations (Thomas *et al*., 2018).

Sensory thresholds provide a measure of individual sensitivity and can also be modulated by age (Methven *et al*., 2012). Surprisingly, studies have typically not investigated individual mouthdrying sensitivity. Results in **Chapter 7** revealed individuals vary in mouthdrying sensitivity to increasing protein levels (0.41-1.00%); however, developing a new method can often result in challenges. Initial testing in the laboratory (by researchers within our group) noted the ×1.33 progression (as used in the mouthdrying detection threshold (MDT)) was potentially too easy to discriminate between protein levels. Therefore, a narrower progression (×1.25) was utilised for consumers aiming to establish a mouthdrying JND threshold. Conversely, this resulted in tested WPBs being considered too similar, likely to be caused by a relatively small maximum protein difference between levels (i.e. overall difference: 0.67% and range: 0.33% (control) to 1.00% (highest level) w/v protein), resulting in 50% discrimination not being reached, despite using 116 consumers.

In summary, this contributes to the literature as age-related effects were demonstrated in a relatively young and healthy older adult cohort. Such results suggested whey protein fortified products need to be reformulated to meet the needs of older adults. However, other individual differences tested in this thesis had limited effects on subsequent perception of whey protein fortified products.

8.4. Can mitigating strategies reduce whey protein derived mouthdrying?

As alluded to in Section 8.1, mouthdrying is a widespread problem in whey protein models, being perceivable by both sensory panels and/or consumers. Therefore, methods to reduce whey protein derived mouthdrying are fundamental, since whey proteins are associated with numerous functional and nutritional benefits. Surprisingly, despite mouthdrying being an established phenomenon (to varying extents depending on the specific whey protein model) there are relatively few successful published strategies in this area. Previously, the tested strategies have typically focused on viscosity, sweetness and fat in whey protein liquid models (Withers *et al*., 2014), whereas in solid food models they have utilised fat or toppings to modulate chewing or sensory perception (Engelen *et al*., 2005; Song *et al*., 2018; van Eck *et al*., 2019). In addition, as demonstrated in Table 1.5 (**Chapter 1**), the key difference between the thesis tested WPB and ONS relate to the additional ingredients, in terms of added flavour, carbohydrate and fat levels.

Accordingly, **Chapters 5-7** focused on putting thesis tested models back into context. Initial experiments (**Chapter 5**) focused on sample palatability by adding sucrose and flavour (vanilla), subsequently reduced mouthdrying for both the sensory panel and consumers. Whey protein still adhered to the oral cavity in sweetened WPBs (**Chapter 5**); therefore, a physical change is unlikely to be driving the change in mouthdrying in this specific case. Additional experiments (**Chapter 6**) were centred around two suggested

mechanisms, namely cross-modal suppression (via sweetness) and lubrication (by fat), to determine whether they could suppress whey protein derived mouthdrying. **Chapter 6** utilised four different mouthdrying mitigating strategies involving a sensory panel with mixed results:

(1) lactose significantly reduced mouthdrying to a small extent (at higher lactose levels) in WPBs supporting cross-modal suppression.

(2) fat had no significant reduction on mouthdrying, demonstrating lubrication potentially is less relevant in WPBs. Both points (1) and (2) are considered a limitation of previous research within this area. Therefore, despite adding a range of lactose or fat levels in WPBs, these were at levels still below typical ONS levels suggesting either: (a) lactose and/or fat may only be effective if added in substantial quantities (like in a ONS) and (b) lactose and/or fat may have a relatively small role on mouthdrying and/or a greater effect on improving palatability.

(3) despite cupcakes being fortified with different WPC powders (standard and heat-stable) mouthdrying was perceived to the same extent. This could suggest whey protein has a greater effect on mouthdrying than processing differences in this case.

(4) adding cream topping to whey protein fortified scones (i.e. adding fat to increase lubrication) significantly suppressed mouthdrying. Moreover, such findings were also demonstrated in **Chapter 7** using consumers (*n* = 116). Our findings are in line with previous studies utilising toppings or incorporating fat in food models to modulate subsequently the number of chews, flavour, dryness, firmness and/or liking (Engelen *et al*., 2005; Song *et al*., 2018; van Eck *et al*., 2019).

In summary, such findings highlighted the challenges in reducing whey protein derived mouthdrying. However, modulating lubrication (demonstrated via adding a cream

topping) in a whey protein solid model was a promising strategy to reduce mouthdrying and increase liking.

8.5. Limitations and suggested future work

This thesis demonstrated some useful findings; however, there remain further areas to be explored which, in some cases, were considered to be outside the scope of this thesis, or not possible due to time constraints. In addition, the COVID-19 pandemic proved to be the most unexpected limitation, subsequently impacting two key research themes within the thesis, namely age and saliva flow. Consequently, planned experiments were either revised, or in some cases were no longer considered possible. Accordingly, future work should be focused on the following four areas: (1) optimising assessment methods; (2) mitigation strategies; (3) mechanisms; and (4) individual differences, to address key mouthdrying related limitations.

8.5.1. Mouthdrying methods

This thesis focused on four methods: rating scales, discrimination testing, sensory profiling and thresholds using a single portion approach. However, it should be acknowledged that using multiple sips methods (such as sequential profiling) could allow mouthdrying build-up which would most likely intensify findings, especially in liquid models. In addition, it is yet to be established in solids models whether perceived mouthdrying is present and/or builds post consumption or just occurs during consumption soley as result of the physical dry texture. Surprisingly sensory threshold methods have not commonly been utilised in mouthdrying studies. Accordingly, based on these findings in this thesis, additional methodology improvements relating to: (1) number of samples; (2) progression selection; (3) comparing threshold methods; and (4) broadening findings into different food models, would be suggested.

8.5.2. Mitigating strategies

Methods to reduce whey protein derived mouthdrying are key, regardless of the food model; however, establishing such methods can be challenging. For example, WPBs can be optimised for sweetness, fat, thickness and flavour to reduce mouthdrying. However, such optimisation is unlikely to eliminate mouthdrying since ONS are sweet, relatively thick and contain fats and flavour, yet are still perceived as mouthdrying (Methven *et al*., 2010; Thomas *et al*., 2016; 2018; Lester *et al*., 2021). Since adding fat in this thesis was unable to reduce successfully mouthdrying in WPBs; accordingly, other ingredients with lubricating properties (such as hydrogels or oleogels) (Krop *et al*., 2019b; Park & Maleky, 2020) could be explored. It is also suggested that carbonated liquids could impact positively saliva flow and swallowing (Dawes *et al*., 2000; Bozorgi *et al*., 2020). Therefore, carbonated WPBs could be explored with older adults and similarly, fruit could be incorporated into WPBs (since acidity can increase saliva flow (Dawes *et al*., 2000)) to determine whether these are suitable strategies to reduce perceived mouthdrying.

This thesis noted increasing lubrication (via cream topping) can suppress mouthdrying in whey protein fortified scones, but ideally the fat source needs to be incorporated within the product rather than added on top. It is unclear whether these findings can be translated into solid models which are higher in fat (i.e. cakes and biscuits) or are only applicable to low fat solid models. However, within clinical settings energy dense toppings can provide a simple method to improve food intake within older adults (BAPEN, 2016; Cichero, 2016). Next steps could include testing different lubricating ingredients such as alternative fats, oils or emulsions (e.g. hydrogels or oleogels); varying the droplet size and/or concentration has been shown to be relevant for in-mouth lubrication (de Wijk & Prinz, 2006). More broadly, oral films provide a promising drug delivery system (Sevinc Ozakar & Ozakar, 2021). However, the potential for such films within the food industry

has not been fully explored and they could have a role in sensory properties (Kumar *et al*., 2021) as the oral mucosa (i.e. surface properties) can impact lubrication (de Wijk & Prinz, 2006). Such factors need to be considered in future work to understand better the role of lubrication in reducing whey protein derived mouthdrying.

Lemon zest was utilised in this thesis to improve acceptability (biscuits) and reduce off-flavours (cupcakes) in whey protein fortified snacks. More broadly, foods (such as lemon juice) can naturally stimulate saliva flow (Batubara & Lindawati, 2019). Therefore, subsequently increasing saliva flow could lead to reduced protein adhesion, thereby potentially enabling products to be perceived as less mouthdrying. Accordingly, consideration of how such foods can be successfully incorporated into whey protein fortified products is suggested so as to enhance the eating experience. For example, adding lemon sauce or similar into the middle of whey protein fortified cakes or as a topping could be recommended. In addition, yoghurt may provide similar saliva stimulating abilities (Murugesh *et al*., 2015) and could be added to baking products.

8.5.3. Mouthdrying mechanisms

This thesis focused on mouthdrying mechanisms in WPBs and provided several useful and novel findings. However, some additional research building on these findings would be suggested. For example, demonstrating that both mouthdrying and mucoadhesion could be modulated together. Currently, a suitable WPB model needs to be developed to enable this, coupled with a more sensitive mouthdrying test (i.e. discrimination testing) rather than rating scales (like gLMS). Whilst **Chapter 3** aimed to address oral retention methodology limitations, this can still be expanded (especially relating to baseline saliva values) to minimise the number of samples presented. As alluded to in Figure 2.3 (**Chapter 2**), various factors could strengthen mucoadhesion and this should be further explored. It is clear that focus should now move to the solid model; however, this is not

without its challenges. For example, snacks: (a) can crumble resulting in food debris; (b) are not held in the mouth; and (c) contain additional ingredients (i.e. more than water and a powder found in a WPB). It is, however, proposed that mucoadhesion strength could be greater in solid models (Cook *et al*., 2017). Therefore, method development would be necessary to apply the validated oral retention in WPBs to allow for solid models. Accordingly, a semi-solid whey protein model could be considered as a starting point. In addition, there are other directions which could be suggested to progress mouthdrying mechanisms:

- In-mouth methods such as post consumption bolus analysis (such as content (saliva, protein and moisture), physical properties analysis and at different chewing times). Bolus analysis of solid foods could help to explain variations in rate of breakdown leading potentially to: (a) age-related and sensory perception differences; (b) the role of saliva; and (c) physical differences resulting from whey protein fortification.
- *In vivo* methods utilising whey models (whey permeate versus whey protein; powders and products) and/or artificial saliva using: (a) rheological and tribological analysis; (b) dissolution rates via turbidity, particle size (dynamic light scattering (DLS)) and/or zeta-potential; and/or (c) interactions analysis between mucosa and whey protein (such as Ellman assay). As evidenced in **Chapter 2** (Table 2.3), a fair amount of physiochemical analysis has already been done relating to WPBs; however, developing a non-protein whey control (WPe) (as done in this thesis) could potentially lead to comparative analysis with different whey protein powders commonly used in WPBs. These could also provide data on the extent of interactions (i.e. exposure of thiol groups) between protein type within whey protein powders and the oral cavity, which could be relevant to oral retention and/or

mouthdrying. Additionally, the role of hydrophobicity could also be relevant since potentially a more hydrophobic whey material could lead to poor dispersion between whey protein and saliva, causing increased protein retention which subsequently causes mouthdrying.

8.5.4. Individual differences

Surprisingly, individual differences had a smaller impact on whey protein fortified products than expected, potentially due to the small sample size within each category and the older adults recruited being too young and healthy. Despite this some useful and relevant findings were demonstrated; accordingly, these will help provide a basis for future work.

This thesis highlighted the challenges with determining **age** group differences in whey protein models. It could, therefore, be proposed that age-related mouthdrying research should focus on the following:

(1) Short simple sensitive discrimination testing would be suggested and more findings in other food models are recommended to validate the liquid model results.

(2) Use of dynamic and build-up related methods. Since potentially there could be age-related differences in the later consumption stages that may have a greater effect on subsequent perception than single timepoint approaches.

(3) Product consideration is also needed since age-related differences may be easier to identify in some products than others.

(4) This thesis used healthy older adults (aged 65 years or over) based in the community; therefore, future work should focus on using (a) clinical settings based older adults and/or (b) research by age group (i.e. 65-74 years and over 75 years) since they are the older cohort most likely to need such products and confirm whether the findings intensify. This population change would also be relevant for

other tested individual differences (saliva flow, mouth behaviour, dental status, appetite and sensory thresholds) which also are likely to be modulated.

(5) Going forward, a broader range of familiar whey protein fortified products (such as bread, cereals, pasta, sauces, mashed potatoes, soups, chocolate, custard, rice pudding, ice cream, fruit juices) would be suggested. Testing a more extensive range of foods could provide useful insights into the extent of mouthdrying effects and whether age-related differences are specific to certain or all whey protein fortified products. Based on this thesis, it could be suggested that solid models (such as cakes) could have greater mouthdrying effects than liquids; however, improving lubrication could be a proposed mechanism that would benefit from additional research (as highlighted in Section 8.5.2).

(6) More broadly, improved awareness and education, potentially in advance of reaching the 'older adult' stage, could encourage older adults to: (a) realise the benefits of protein consumption; (b) incorporate such foods into their diet; and (c) result in more commercial fortified products being available in supermarkets.

Saliva flow could not be fully investigated to the extent originally planned due to the ongoing COVID-19 pandemic. For example:

- After **Chapter 4** no additional studies could utilise saliva flow in older adults as this was deemed unsuitable and a high-risk activity.
- Following **Chapter 5** further national lockdowns resulted in younger adults (18-30 years) also being unable to come onto campus and this meant a planned study (ethical approval number SCFP 46/20) aiming to investigate saliva flow changes in liquid and solid models was not considered possible.

Overall, this thesis demonstrated minimal effects from saliva flow on sensory perception, potentially as result of methodology challenges. Accordingly, additional investigation to

confirm conclusively the role of saliva flow in sensory perception of whey protein fortified products is suggested. This thesis used unstimulated saliva flow (since it is more impacted by age) for sensory perception and stimulated saliva flow (more relevant to food consumption) for oral retention. More research is considered necessary in the following areas:

- Understanding which method (unstimulated saliva flow versus stimulated saliva flow; including length of collection time) is better to correlate with sensory perception since there is some discussion in the literature as to which is more suitable.
- How best to modulate saliva flow (increase or decrease) to reflect natural variation (including the speed of restimulation, which is potentially relevant in an ageing population and stimulating saliva pre and post food consumption may be related to mouthfeel perception).
- If the specific sensory test could intensify findings (such as more sensitive tests than were used in this thesis or more dynamic methods rather than single timepoint methods).
- Creating a standardised approach to enable easier comparisons between studies.

There are additional saliva analyses that might influence oral retention and/or sensory perception; however, such approaches were considered outside the scope of this thesis. Examples include: (a) rheological analysis (i.e. viscoelasticity (via Spinnbarkeit) could be impact mouthfeel perception and decline with age (Pushpass *et al*., 2019b) or viscosity (more viscous saliva could lead to poor oral clearance (Lester *et al*., 2021)); (b) protein composition analysis (sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (age-related differences in protein composition could relate to oral retention and/or mouthdrying); and (c) artificial saliva models as outlined in Section 8.5.3

(assessing differences in structure between whey models and correlating with sensory perception).

Dental status, mouth behaviour and **appetite** were all self-report measures via questionnaires or subjective ratings. Therefore, next steps should focus on broader: (a) oral parameters (such as teeth counting, functional unit counting, chewing capacity/efficiency, tongue pressure, oral cavity volume) and (b) food oral processing methods (such as post consumption bolus analysis (as outlined in Section 8.5.3) and eating behaviour and muscle activity i.e. bite size, consumption time, number of chews/swallows, eating rate via video recordings, electromyography and jaw tracking). Subsequently such findings could be used to explain individual differences in perception of whey protein fortified products. In addition, the effect of consuming whey protein fortification beverages and/or snacks between meals on appetite responses at subsequent meals could be investigated (i.e. gastric emptying and satiety hormones).

Despite best intentions, in some cases there were sex imbalances within studies (i.e. greater number of females than males and this was predominately in the younger age group) and this could have resulted in sex related effects. It should also be noted that whilst fluid intake was controlled in terms of timings in all studies, there was no control on the volume. Accordingly, future studies should aim to control fluid intake during testing to establish the relationship between perceived mouthdrying and thirst. More broadly, it was evident that volunteers in this thesis, regardless of the study, typically lacked familiarity with protein fortified products and this may have influenced sensory perception. Moreover, typical protein consumption was not recorded and could be relevant. To date there are limited studies that have investigated whether frequent protein consumption could modulate sensitivity to the perception of mouthdrying from protein fortified foods.

8.6. Conclusion

This thesis primarily focused on testing mouthfeel perception from whey protein fortified products, using consumers of varying age, via sensory related methods combined in some cases with two oral physical measures. This aimed to progress whey protein derived mouthdrying related research and addressed key research gaps cited within the area. For example, improving oral retention methodology, identifying a suitable 'physical measure' for mouthdrying, investigating individual differences in consumers within the target population and testing mouthdrying mitigation strategies. However, future work could be suggested based on this thesis in certain fundamental mouthdrying areas: (a) causes of whey protein derived mouthdrying (developing whey models that can modulate both oral retention and mouthdrying); (b) individual differences (broader saliva and food oral processing methods); and (c) mouthdrying mitigating methods (lubrication and foods that can naturally stimulate saliva). In addition, this thesis supports a call to industry to reduce negative sensory attributes (such as mouthdrying) associated with whey protein products, since such effects are perceived by both younger and older consumers. More specifically, concluding older adults had: (1) reduced saliva flow; (2) increased whey protein adhesion; (3) increased sensitivity to whey protein derived mouthdrying (via thresholds) and chewiness in fortified scones; and (4) poor dental status, compared with younger adults. Therefore, to cater for these age-related changes, it would be suggested that whey protein fortified: (a) beverages need to be less mouthdrying and adhesive as well as optimised for sweetness, fat and thickness levels, coupled with familiar or popular flavours and (b) snacks need to be softer, less chewy and dry, subsequently promoting consumption and enjoyment. The potential impact of this thesis is widespread for our ageing population as enabling increased protein consumption (via fortified beverages

and/or snacks) could help to reduce susceptibility to malnutrition and sarcopenia, thereby improving nutritional status in older adults.

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Appendix A

A.1. Ethics related information

For all consumer-based studies, favourable opinion for conduct was either required from the University Research Ethics Committee (UREC) or the School of Chemistry, Food and Pharmacy (SCFP) Research Ethics Committee (depending on the exact study nature) in the form of a letter (UREC) or email (SCFP Research Ethics Committee). VN drafted ethics applications with LM and SL providing guidance and feedback. In all studies volunteers were recruited via the following routes: (a) databases (Hugh Sinclair Unit of Nutrition, Psychology and Clinical Language Sciences and/or Sensory Science Centre); (b) departmental email circulation lists; (c) word of mouth; (d) posters placed on University campus; and/or (e) leaflets distributed in the local Reading area. Examples of corresponding emails and letters are outlined in Figures A.1 and A.2 with Table A.1 summarising all thesis ethics applications approval codes and dates.

Dear Lisa, Stella and Vic

I am pleased to inform you that Professor Richard Frazier has given a favourable opinion for conduct for your study "Investigation of the Perception of Protein Fortified Foods and Beverages" via the in-School exceptions route. This email constitutes your permission to proceed with the studies as described in your application. The following study number has been assigned to your study and you should quote this number in any correspondence you undertake about your studies.

STUDY Number – 28/19

If you feel that you need to make changes to the way your studies are run, please let us know at the earliest opportunity and we can advise you of whether a formal amendment to your proposal is required or not.

I wish you the best of luck with the projects and finish by reminding you of the need for safe custody of project data at all times (a service that Barbara Parr, copied in, can provide if you require it).

Kind regards
Julie

Professor Julie Lovegrove
Chair SCFP Research Ethics Committee

Figure A.1. Example of School of Chemistry, Food and Pharmacy (SCFP) Research Ethics Committee approval email.

University of Reading

Coordinator for Quality Assurance in Research
Dr Mike Proven, BSc(Hons), PhD

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Dr Lisa Methven
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21 December 2018

Dear Lisa,

UREC 18/46: Investigation of the Perception of Protein Fortified Foods and Beverages. *Favourable opinion*

Thank you for the response (your email, dated 18 December 2018, refers) addressing the issues raised by the UREC Sub-committee at its November 2018 meeting (*my Favourable Opinion with Conditions email of 29 November including attachments refers*), and the additional amendments *(including; amendment to consent form to include storage of saliva samples; clarification on the Participant Information Sheet that three study visits will be required; addition of Q10 on the screening questionnaire).* On the basis of these responses and amendments, I can confirm that the Chair is pleased to confirm a favourable ethical opinion.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here:

http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx.

Yours sincerely

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)

Figure A.2. Example of University Research Ethics Committee (UREC) approval letter.

Table A.1. Overview of thesis ethics applications.

Chapter	Type	Study Number	Approval Date
3	SCFP	28/19	11th October 2019
3 & 4	UREC	18/46	21st December 2018
4	UREC	19/67	27th January 2020
5	SCFP	32/20	13th July 2020
7	UREC	20/35	29th January 2021
-	SCFP	46/20	10th November 2020

Dash (-) denotes study was unable to be carried out due to ongoing COVID-19 restrictions. SCFP: School of Chemistry, Food and Pharmacy Research Ethics Committee and UREC: University Research Ethics Committee.

All sensory panel work (i.e. descriptive sensory profiling (DSP) or three alternative forced choice (3-AFC) test; **Chapters 4-7**) was neither subjected to a specific ethical review nor additional consent, as the trained sensory panel were tasting products made from standard commercial food ingredients. However, it should be noted that all panellists had consented to evaluate different food and beverage products as part of their employment contract.

Appendix B

B.1. Baking hazard analysis and critical control point (HACCP)

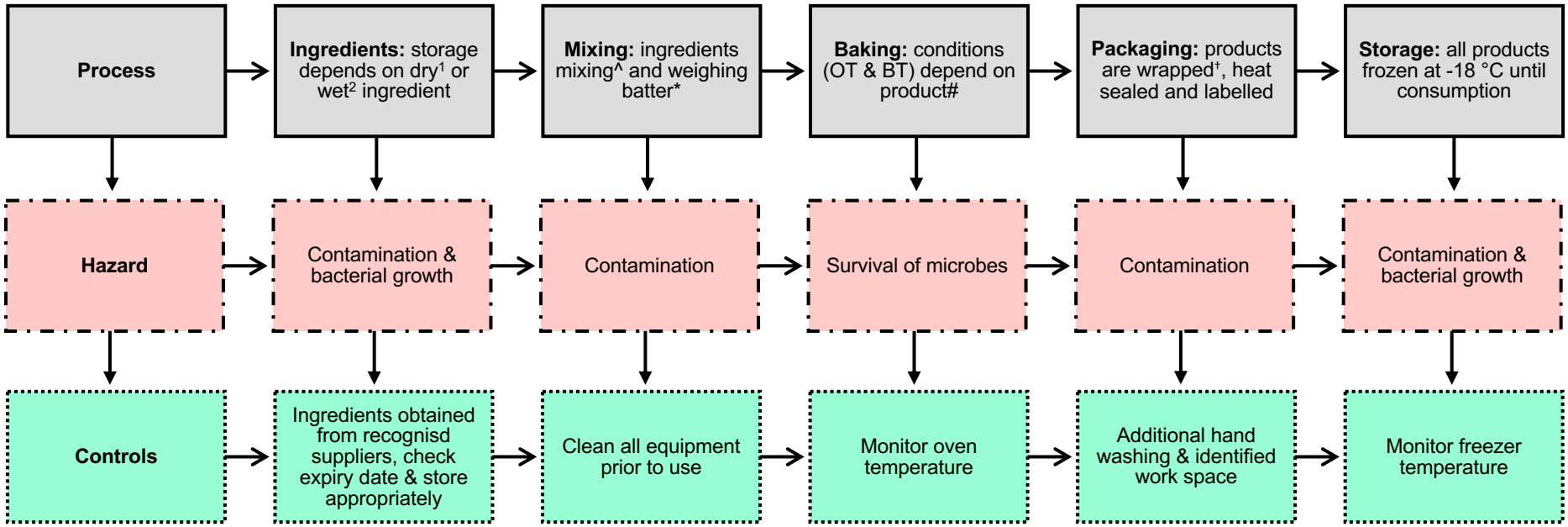


Figure B.1. Hazard analysis and critical control point (HACCP) product summary for production of cakes, biscuits, cupcakes and scones at the University of Reading pilot plant bakery. Acronyms: oven temperature (OT) and baking time (BT). Dry[1] stored in cool and dry conditions at ambient temperature; wet[2] (i.e. butter, egg, milk) stored at 4 °C until required for use; ^ cakes & biscuits: three-stage mixing, cupcakes: all-in-one and scones: two-stage mixing; * denotes batter into foil tins (cakes), paper cases (cupcakes) and onto sheeted baking trays (biscuits and scones). # cakes (OT: 170 °C; BT: 30-min), cupcakes (OT: 170 °C; BT: 20-min), biscuits (OT: 190 °C; BT: 9-min) and scones (OT: 200 °C; BT: 12-min). † all individually wrapped into cling film and heat sealed in a polypropylene stock bag (cakes, cupcakes and scones) or aluminium foil bags (biscuits only). In addition, general requirements were adhered to such as hygiene controls, food safety, ensuring all equipment was checked prior to use (yearly portable appliance testing (PAT)) and user to wear appropriate personal protective equipment (lab coat, hairnet, gloves and suitable footwear).

B.2. Baking microbiological clearance testing

Post baking, a 150 g sample from each batch was placed aside for microbiological clearance testing to ensure product safety. In brief, this involved sending samples to an accredited laboratory (SYNLAB or SGS analytics[54], Northumberland, UK) prior to consumption by the sensory panel and/or consumers. Approximately seven to ten days later a detailed report was provided by the lab (see example certificate of analysis in Figure B.2). All snacks (cakes, biscuits, cupcakes and scones) presented in this thesis passed ( ) as summarised in Table B.1.

Table B.1. Summary of microbiological clearance testing results.

Snack Description	Report Number	Lab Number	Result
Control cake	19-75184	1454573	Passed
Protein cake	19-75184	1454574	Passed
Control biscuit	19-75184	1454575	Passed
Protein biscuit	19-75184	1454576	Passed
Control biscuit	19-78849	1488358	Passed
Protein biscuit	19-78849	1488359	Passed
Control cake	19-11202	1509827	Passed
Protein cake	19-11202	1509828	Passed
Control biscuit	19-16245	1552749	Passed
Protein biscuit	19-16245	1552750	Passed
Control cake	19-16850	1557528	Passed
Protein cake	19-16850	1557529	Passed
Control cupcake	20-40830	1777959	Passed
Protein cupcake	20-40830	1777960	Passed
Control cupcake	20-73441	2109825	Passed
WPC cupcake	20-73441	2109826	Passed
HS-WPC cupcake	20-73441	2109827	Passed
Control scone	21-14810	2239954	Passed
Protein scone	21-14810	2239955	Passed
Protein scone	21-16265	2256005	Passed
Protein scone	21-19364	2288137	Passed

Acronyms: whey protein concentrate (WPC); heat-stable whey protein concentrate (HS-WPC).

[54] SYNLAB was subsequently sold to SGS analytics in 2021; however, all procedures, analysis and reports remained the same

SYNLAB

UKAS TESTING 4412

CERTIFICATE OF ANALYSIS

FAO:	Dr Sameer Khalil Ghawi	Order Number:	3256123
Company:	University of Reading	Date Received:	22/01/2019
Address:	Department of Food and Nutritional Sciences University of Reading Whiteknights	Analysis Started	22/01/2019
		Report Date:	27/01/2019

Reference: Report Number: 19-75184

Lab No	Client Reference	Sample Description	Specificati
1454573	Sample 1	Control Cake	
1454574	Sample 2	Protein Cake	
1454575	Sample 3	Control Biscuit	
1454576	Sample 4	Protein Biscuit	

Lab No.	Test Ref	Analysis	Result
1454573	MIC1004	Aerobic Colony Count 72h at 30ºC	< 100 cfu/g
1454573	MIC1005	Osmophilic Moulds	< 20 cfu/g
1454573	MIC1005	Osmophilic Yeasts	< 20 cfu/g
1454573	MIC1018	Enterobacteriaceae (presumptive)	< 10 cfu/g
1454573	MIC1021	Coagulase Pos Staphylococci	< 20 cfu/g
1454573	MIC1022	E. coli (presumptive)	< 10 cfu/g
1454573	MIC1023	Salmonella spp. (detection)	Not Detected in 25g
1454574	MIC1004	Aerobic Colony Count 72h at 30ºC	100 cfu/g
1454574	MIC1005	Osmophilic Moulds	< 20 cfu/g
1454574	MIC1005	Osmophilic Yeasts	< 20 cfu/g
1454574	MIC1018	Enterobacteriaceae (presumptive)	< 10 cfu/g
1454574	MIC1021	Coagulase Pos Staphylococci	< 20 cfu/g
1454574	MIC1022	E. coli (presumptive)	< 10 cfu/g
1454574	MIC1023	Salmonella spp. (detection)	Not Detected in 25g
1454575	MIC1004	Aerobic Colony Count 72h at 30ºC	< 100 cfu/g
1454575	MIC1005	Osmophilic Moulds	< 20 cfu/g

Tests marked with * do not form part of our current UKAS Scope of Accreditation.
The results reported relate only to the items tested.
Tests marked with ‡ are sub-contracted to a UKAS accredited Laboratory
Results marked with an "E" are estimated counts.

1454575	MIC1005	Osmophilic Yeasts	< 20 cfu/g
1454575	MIC1018	Enterobacteriaceae (presumptive)	< 10 cfu/g
1454575	MIC1021	Coagulase Pos Staphylococci	< 20 cfu/g
1454575	MIC1022	E. coli (presumptive)	< 10 cfu/g
1454575	MIC1023	Salmonella spp. (detection)	Not Detected in 25g
1454576	MIC1004	Aerobic Colony Count 72h at 30ºC	< 100 cfu/g
1454576	MIC1005	Osmophilic Moulds	< 20 cfu/g
1454576	MIC1005	Osmophilic Yeasts	< 20 cfu/g
1454576	MIC1018	Enterobacteriaceae (presumptive)	< 10 cfu/g
1454576	MIC1021	Coagulase Pos Staphylococci	< 20 cfu/g
1454576	MIC1022	E. coli (presumptive)	< 10 cfu/g
1454576	MIC1023	Salmonella spp. (detection)	Not Detected in 25g
			cfu = colony forming units

END

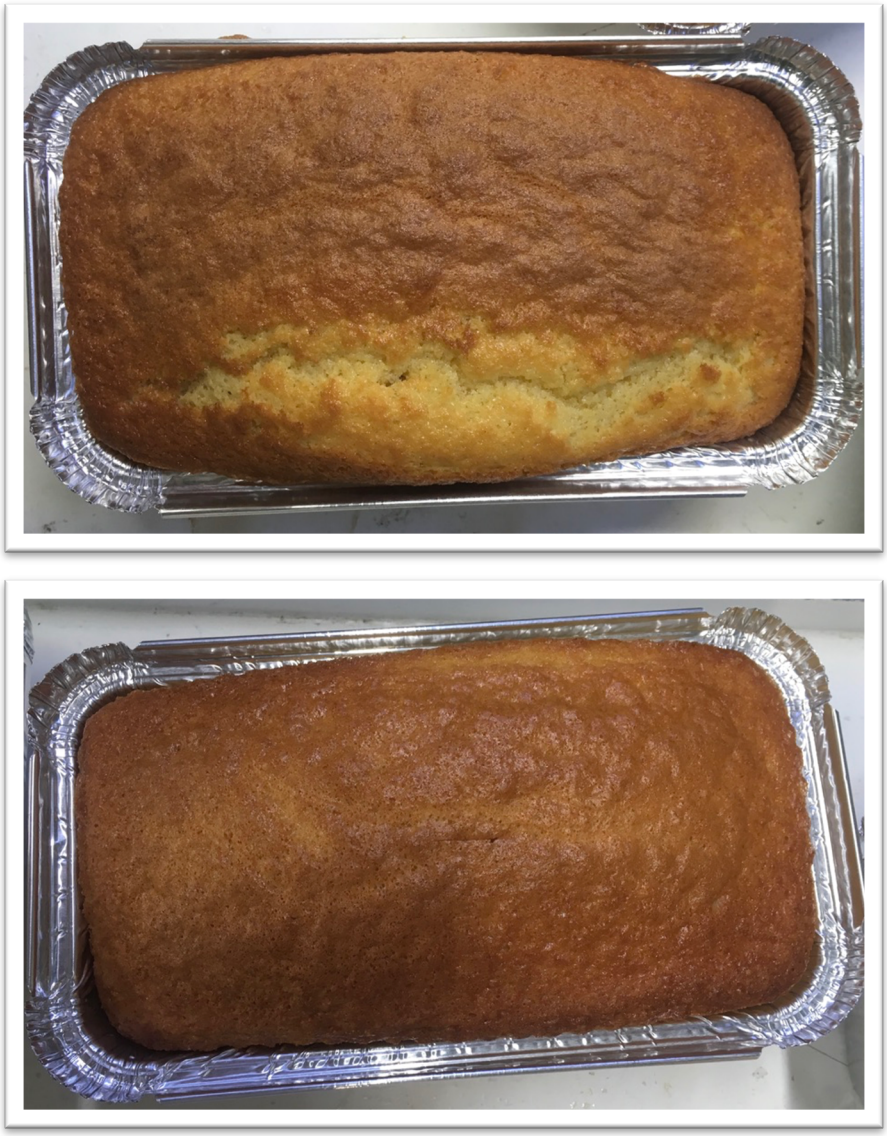
Signed for and on behalf of SYNLAB

Lee Ward, Micro Lab Team Leader

Figure B.2. Example of microbiological clearance report from SYNLAB.

B.3. Baking images

(**A**) Cake (top: control cake; bottom: protein cake)



(**B**) Biscuits (left: control biscuit; right: protein biscuit)

(**C**) Cupcakes (left: control cupcake; middle: protein cupcake; right: HS-protein cupcake)

(**D**) Scones (left: scone without cream topping; right: scone with cream topping)

Figure B.3. Image overview of baked solid models used in this thesis **(A)** cakes; **(B)** biscuits; **(C)** cupcakes and **(D)** scones.

Appendix C

C.1. Chapter 4 denture use

As alluded to in **Chapter 4**, dental status was monitored in both studies via a dental status questionnaire (as outlined in Section C.2.1). Denture use was evaluated since this could alter perception; however, as highlighted in Figure C.1, the majority of volunteers (53 out of 74) were not denture wearers. Accordingly, due to the small number of wearers within each study it was impossible to include 'dentures' within statistical analysis; though, the data was checked for potential outliers. In addition, as the two studies used different scales (generalised labelled magnitude scale (gLMS) versus visual analogue scale (VAS)), it was not possible to combine both studies data set for additional statistical analysis. Furthermore, it should be noted from the knowledge gained in the pilot study that it was decided to add more questions (Q3-7) relating to dentures as outlined in Table C.1. Consequently, based on these results (i.e. minimal effect of dental status in healthy volunteers), dental status was not subsequently investigated in the remaining chapters.

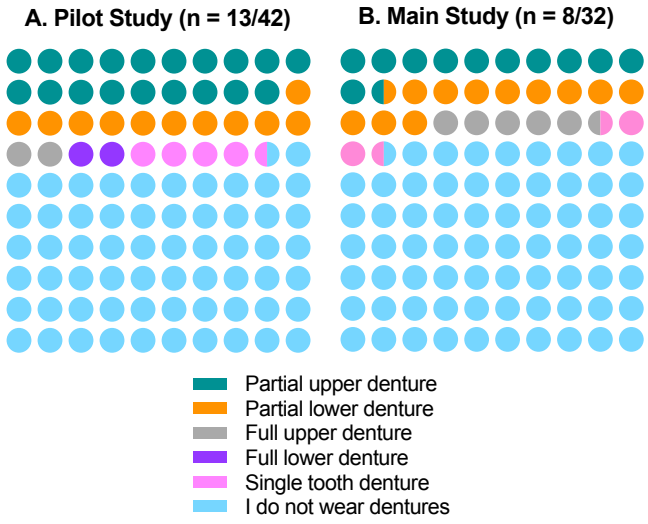


Figure C.1. Summary of older adults denture use in **Chapter 4**. Data expressed as % with each coloured circle representing 1.0%.

Table C.1. Summary of older adults denture use (*n* = 8) in the main study.

Code	Denture type[1]					Denture attachment[2]		Years worn[3]	Denture fit[4]			Alter perception whilst wearing dentures[5]			Perception with vs without dentures[6]		
	PUD	PLD	FUD	FLD	STD	PIP	MC		Yes	No	Other	Yes	No	Other	Yes	No	Not sure
v54		✓	✓			✓		20 years +		✓		✓			-	-	-
v63	✓					✓		6 years		✓			✓			✓	
v66	✓					✓		-			Fairly well			Chewing hard without	✓		
v68	✓					✓		20 years +	✓				✓				✓
v69					✓	✓		65 years +	✓				✓		✓		
v74		✓	✓			✓	✓	4 years	✓				✓		✓		
v77		✓					✓	-	✓				✓		✓		
v78	✓	✓				✓		10 years	✓				✓		✓		

Code denotes volunteer code and (-) denotes volunteer did not answer question. Acronyms: partial upper denture (PUD); partial lower denture (PLD); full upper denture (FUD); full lower denture (FLD); single tooth denture (STD); plastic plate (PIP) and metal clip (MC). Questions: [1]do you have any removeable dentures?; [2]what type of denture attachment do you have?; [3]how long have you worn dentures for? [4]do your dentures fit well?; [5]do you feel your perception of food is altered whilst wearing your dentures?; [6]do you feel you can perceive hot and cold foods the same as when you are not wearing your dentures?

C.2. Questionnaire examples

C.2.1. Dental status questionnaire

University of Reading

Volunteer Code _____

Dental Status Questionnaire

(Study Number: UREC 19/67)

Please answer the following questions relating to your dental status.

1. How many natural teeth do you have?

Please select the correct response

No natural teeth	☐
1-9 teeth	☐
10-19 teeth	☐
20 teeth or more	☐

2. Do you have any removeable dentures?

Please select the correct response

Partial upper denture	☐
Partial lower denture	☐
Full upper denture	☐
Full lower denture	☐
Single tooth denture	☐
I do not wear dentures (please go to question 8)	☐

3. What type of denture attachment do you have?

Please select the correct response

Plastic plate	☐
Metal clip	☐
Other (please comment)	☐

4. How long have you worn your dentures for?

Please comment in the space below

5. Do your dentures fit well?

Please select the correct response

Yes	☐
Not sure	☐
No	☐
Other (please comment)	☐

6. Do you feel your perception of food is altered whilst wearing your dentures?

Please select the correct response

Yes	☐
Not sure	☐
No	☐
Other (please comment)	☐

7. Do you feel you can perceive hot and cold foods the same as when you are not wearing your dentures?

Please select the correct response

Yes	☐
Not sure	☐
No	☐
Other (please comment)	☐

8. Do all your teeth have its opposite pair? (functional unit counting)

Please select the correct response

Yes	☐
No	☐

If no, how many teeth are missing?

0-4 teeth	☐
5-8 teeth	☐
9-12 teeth	☐
13-16 teeth	☐
I do not know	☐

9. How often do you clean your teeth?

Please select the correct response

Twice or more a day	☐
Once a day	☐
Two to six times a week	☐
Never	☐
Other, please comment	☐

10. Do you use any of the following to clean your teeth?

Toothpicks

Interdental brush

Dental floss

Manual toothbrush

Electronic toothbrush

Please tick below all that apply

Manual toothbrush	☐
Electronic toothbrush	☐
Toothpicks	☐
Dental floss	☐
Interdental brush	☐
Other, please comment	☐

11. Do you use toothpaste to clean your teeth?

Please select the correct response

Yes	☐
No	☐
Other, please comment	☐

12. How long has it been since you last saw a dentist?

Please select the correct response

Less than 6 months	☐
6-12 months	☐
More than 1 year but less than 2 years	☐
2 years or more but less than 5 years	☐
5 years or more	☐
Never received dental care	☐

13. Because of the state of your teeth or mouth, how often have you experienced any of the following during the past 12 months?

Please select the correct response	Very often	Fairly often	Sometimes	No	Don't know
Difficulty in biting foods	☐	☐	☐	☐	☐
Difficulty chewing foods	☐	☐	☐	☐	☐
Difficulty with speech/trouble pronouncing words	☐	☐	☐	☐	☐
Dry mouth	☐	☐	☐	☐	☐
Difficult doing usual activities	☐	☐	☐	☐	☐

C.2.2. Mouth behaviour questionnaire

University of Reading

Volunteer Code_____

Mouth Behaviour Questionnaire
(Study Number: UREC 19/67)

This questionnaire is completed online at
https://www.surveygizmo.com/s3/2795244/Mouth-Behavior

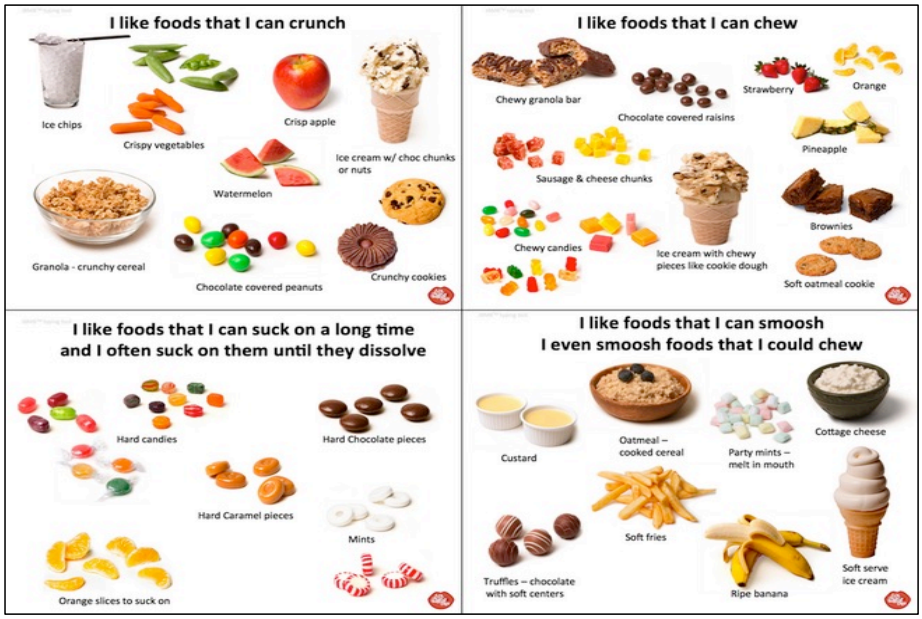
You will be shown four pictures (groupings of products)

Read the description at the top of each picture.

Think about the texture of foods in each group - which picture is most like you?

The description and food textures are those you most enjoy.

Using the diagram below please answer the following questions.



1. Which of the four groups BEST DESCRIBES YOU, MOST LIKE YOU.

You may ONLY select ONE response

Cruncher	☐
Chewer	☐
Sucker	☐
Smoosher	☐

2. Now looking again at the groupings of products, are there any that are NOT LIKE you at all? In other words, types of foods that you don't enjoy at all?

Please tick all that apply

Cruncher	☐
Chewer	☐
Sucker	☐
Smoosher	☐
There are none of these that are not like me	☐

3. How difficult was it for you to choose between the four sets of images for which images was most like you (i.e. the first question)?

Please select the correct response

Not at all difficult	☐
A little difficult	☐
Moderately difficult	☐
Very difficult	☐

4. Which story is most like you…when I eat food…

Please select ONE response only

Response One: ☐

The food can be either crunchy or tender, but I want to be able to get my teeth easily through it. Then as I am biting the food, it disappears quickly from my mouth. I love crunchy food, where I can hear the crunch. It stays crunchy until the last bite and is then gone - no particles or mush hanging around in my mouth.

Response Two: ☐

The food is either chewy from the start or becomes chewy as I eat it – it forms a mass that I can spend some time chewing before I swallow. The food should 'fight back' a little as I eat it.

If you select response two, please answer the following:

a) I like the food to stay chewy for a while and take some time to chew before I can swallow ☐

b) The food should be soft and not take a long time to chew ☐

Response Three: ☐

The food should be comfortable in my mouth - something that I want to hold in my mouth and suck on for a long time. If needed I will chew or crunch some, but I want to spend time sucking on the food.

Response Four: ☐

I like soft foods that spread throughout my mouth. If I don't need to chew or use my teeth to eat, so much the better. The food should be really easy to manipulate between my tongue and the roof of my mouth. Some people think I am a slow eater.

Appendix D

D.1. Summary of descriptive sensory profiling (DSP) panel performance

Panellists were drawn from an experienced trained commercial sensory panel (in accordance with ISO 8586:2012). Panellists received at least nine hours specific training on profiling whey protein fortified products during this thesis and were subject to performance monitoring (ISO 1132:2012). The main purpose of descriptive sensory profiling (DSP) was to confirm analytically the sensory differences between the products.

In all experiments panel performance was monitored as summarised in Table D.1, where a lower response is a preferable result. Therefore, there was good repeatability (5-13%) in all experiments across panellists; however, in some cases individual panellists did struggle to discriminate between samples. This mostly related to the samples being very similar in some attributes rather than poor panel performance. There were in some cases significant panellist interactions observed relating to the products, as outlined in Table D.2. The number of significant panellist interactions is likely to be explained by differences in the use of the scale (i.e. narrow versus broad); however, in most cases, the direction of scoring was consistent between the panellists and this is demonstrated in Figure D.1. Additionally, some variation is expected due to individual differences in levels of sensitivity and perception. Moreover, the statistical approach utilised (i.e. ANOVA in SenPAQ where the main effects (product and panellist) were tested against the product by panellist interaction (with product and panellists as fixed and random effects respectively)) takes into account the sample by assessor interaction; therefore, any sample significant *p* values remain valid despite the noted interactions. More broadly, it should be noted that experiments one to three were conducted prior to COVID-19 in accordance with standard sensory testing. However, subsequent experiments (four to nine) were completed under varying COVID-19 restrictions either in person with social

distancing and serving restrictions (experiment four) or at home via teams (experiments five to nine). Accordingly, this could have contributed to why panel performance was not continuing to improve across the thesis, despite training. Moreover, the panel utilised in this thesis was an experienced panel suitably trained and performance was validated between laboratory versus home setting by the commercial company.

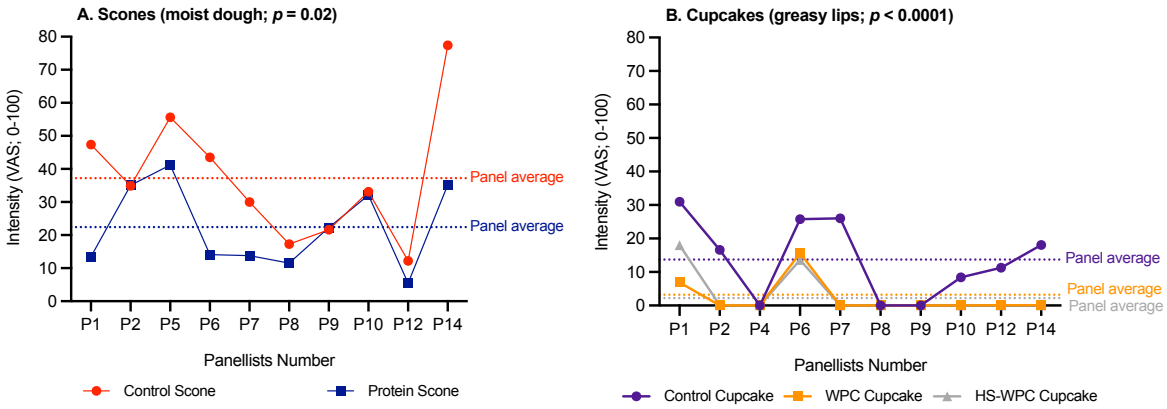


Figure D.1. Examples of sample by assessor interaction plots (**A**) showing different line scale use by the panellists and (**B**) demonstrating differences in perception between panellists. Data obtained from SenPAQ (via ANOVA) and expressed as means of two replicates from 10 panellists in both experiments.

Table D.1. Summary of descriptive sensory profiling (DSP) panellists' (*n* = 8-12) performance for all experiments.

Panellist	% non-discrimination of attributes[a]									% non-reproducibility of attributes[b]									% attributes causing interactions[c]								
number^	E1	E2	E3	E4	E5	E6	E7	E8	E9	E1	E2	E3	E4	E5	E6	E7	E8	E9	E1	E2	E3	E4	E5	E6	E7	E8	E9
P1	53	50	88	52	95	67	93	81	100	6	0	9	5	22	33	71	6	14	21	22	24	14	19	33	50	25	14
P2	47	44	70	-	73	100	86	66	100	3	0	0	-	14	22	14	3	0	18	22	18	-	38	6	21	25	0
P3	62	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-
P4	68	-	100	76	78	78	71	-	100	24	-	9	19	0	11	0	-	0	29	-	3	29	11	39	0	-	0
P5	76	92	97	-	-	61	86	94	86	3	0	0	-	-	0	0	0	0	9	11	0	-	-	11	7	9	0
P6	79	94	88	76	81	-	100	88	86	12	3	9	10	11	-	0	9	29	24	22	9	19	14	-	0	22	0
P7	35	100	-	62	78	78	93	75	-	0	22	-	48	11	6	7	3	-	56	56		57	30	17	21	19	-
P8	82	81	97	62	78	72	100	91	86	0	0	6	10	0	6	0	3	0	12	8	3	24	14	17	14	13	0
P9	76	83	88	57	84	89	93	84	100	3	6	0	5	5	0	0	13	29	18	11	21	33	14	17	36	22	0
P10	50	81	-	33	65	89	86	97	100	0	0	-	0	0	0	0	6	0	12	11		33	11	0	0	6	0
P11	79	-	88	-	-	-	-	-	-	41	-	3	-	-	-	-	-	-	32	-	21	-	-	-	-	-	-
P12	53	83	88	57	73	78	64	75	-	0	6	9	14	11	0	0	3	-	15	11	9	43	19	0	21	22	-
P13	-	92	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-
P14	-	81	-	67	78	89	100	94	-	-	47	-	5	5	17	0	13	-	-	19	-	43	16	22	0	19	-
Total	63	80	89	60	78	80	88	84	95	8	8	5	13	8	9	8	6	9	22	19	12	33	18	16	16	18	2

Data obtained from SenPAQ (via ANOVA) and expressed as means of two replicates as %. Panellists number^ refers to number of panellists (*n* = 8-12) and not all panellists were present during every experiment as noted by a dash (-); [a] denotes the number of attributes where the panellist was not able to significantly discriminate between samples; [b] highlights the number of attributes where the panellist was not reproducible in their scoring between replicates; [c] demonstrates the number of attributes where an assessor contributed significantly to the sample by assessor interaction; in all cases a lower response is a preferable result. Numbers (E1-9) represent differing experiments: E1 = cakes (control and protein; **chapter 4**); E2 = cupcakes (control and protein; **chapter 4**); E3 = biscuits (control and protein; **chapter 4**); E4 = whey beverages (whey permeate beverages (WPeB), whey permeate beverages sweetened (WPeBS); whey protein beverage (WPB) and whey protein beverage sweetened (WPBS); **chapter 5**); E5 = cupcakes (control and two × protein; **chapter 6**); E6 = whey protein beverages (WPB) with lactose (two controls and five lactose levels; **chapter 6**); E7 = whey protein beverages (WPB) with fat (control and three fat levels; **chapter 6**); E8 = scones (control and protein; **chapter 6**); E9 = scones (with and without cream topping; **chapter 6**).

Table D.2a. Summary of descriptive sensory profiling (DSP) sample by assessor interactions in whey protein solid models.

Modality	Attributes	E1: Cake	E2: Cupcakes	E3: Biscuits	E5: Cupcakes	E8: Scones	E9: Scones
		Sample*Assessor *p* value	Sample*Assessor *p* value	Sample*Assessor *p* value	Sample*Assessor *p* value	Sample*Assessor *p* value	Sample*Assessor *p* value
Appearance	Moist appearance[E1,2,5,8] | Smoothness of surface[E3]	**<0.0001**	0.81	0.35	0.07	0.14	-
	Dense appearance of sponge/dough [E1,2,5,8] | Colour (top)[E3]	0.13	0.40	0.42	**0.0009**	**0.001**	-
	Appearance of large holes in sponge/dough[E1,2,5,8]	**0.02**	0.21	-	0.24	0.33	-
	Yellow colour of crumb/dough (inside)[E1,2,5,8] | Colour (inside)[E3]	**0.003**	0.57	0.60	0.55	0.16	-
	Colour (bottom)[E3]	-	-	0.95	-	-	-
	Evenness of shape[E3]	-	-	0.69	-	-	-
	Thickness[E3]	-	-	0.95	-	-	-
	Crumb/aeration[E3]	-	-	0.25	-	-	-
Aroma	Overall aroma intensity[E1,2,5,8] | Baked[E3]	**0.02**	0.17	0.18	**0.03**	**0.03**	-
	Sweet[E1,2,3,5,8]	0.35	0.47	0.08	**0.006**	0.94	-
	Vanilla[E1] | Lemon[E2,5] | Lemony[E3] | Savoury/cheesey[E8]	0.09	**0.03**	0.053	**0.01**	0.43	-
	Buttery[E1,2,5,8] | Fatty[E3]	0.81	**0.03**	**0.002**	0.29	**0.02**	-
	Eggy[E1,2,5] | Oaty[E3] | Floury[E8]	0.53	0.69	**0.03**	**0.008**	0.18	-
	Rancid[E1] | Off-flavours[E2,5,8] | Sulfate off note[E3]	0.75	**0.02**	**<0.0001**	**0.002**	**0.003**	-
Flavour	Overall flavour intensity[E1,2,5,8]	**0.04**	**0.008**	-	**0.0008**	**0.01**	-
	Sweet[E1,2,3,5,8]	0.09	**0.02**	**0.05**	**0.04**	**0.01**	-
	Metallic[E1,2,3,5,8]	0.25	**0.005**	0.99	**0.02**	1.00	-
	Vanilla[E1] | Lemon[E2,5] | Lemony[E3] | Savoury/cheesey[E8]	**0.003**	**0.002**	0.10	**<0.0001**	**0.005**	-
	Buttery [E1,2,5,8] | Fatty[E3]	**<0.0001**	**0.0002**	0.29	0.06	**0.0008**	-
	Eggy[E1,2,5] | Oaty[E3] | Floury[E8]	**0.008**	0.89	0.13	0.10	**0.02**	-
	Liquorice[E2,5] | Bitter[E3]	0.35	**<0.0001**	0.64	**0.003**	-	-
	Rancid[E1] | Off-flavours[E2,5,8] | Sulfate off note[E3]	-	0.44	0.76	0.20	**0.002**	-
Mouthfeel	Firmness of Bite[E1,2,5,8,9] | Hardness[E3]	**0.05**	0.73	0.72	0.43	**0.02**	0.73
	Moist sponge/dough[E1,2,5,8,9] | Crumbly[E3]	**0.004**	0.69	0.31	0.70	**0.02**	0.26
	Chewy[E1,2,5,8,9] | Crunchy[E3]	**<0.0001**	**0.03**	0.28	0.07	**0.006**	0.73
	Mouthdrying[E3,5,8,9]	**-**	-	**0.03**	0.07	0.39	0.39
	Greasy lips[E1,2,5]	**0.004**	**0.0002**	-	**<0.0001**	-	-
	Crumbliness of sponge/dough [E1,2,5,8]	**<0.0001**	**0.04**	-	**0.0001**	0.26	0.72
	Crumb size[E1,2,5] | Grainy[E3]	**0.001**	0.16	-	0.34	-	-
	Pasty (cohesive)[E1,2,5,8,9] | Teeth packing[E3]	**0.006**	**0.03**	0.29	**0.0001**	0.32	0.45
	Rate of breakdown & clearance[E1,2,5,8,9] | Melt rate[E3]	**<0.0001**	0.07	0.67	0.49	0.32	0.30
	Cooling Sensation (numbing)[E1,2,5]	**0.002**	**<0.0001**	-	0.26	-	
Aftertaste	Mouthdrying[E1,2,3,5,8]	**0.02**	0.10	0.09	0.57	0.25	-
	Sweet[E1,2,3,5,8]	**0.03**	0.37	**0.05**	0.39	**0.005**	-
	Vanilla[E1] | Lemon[E2,5] | Lemony[E3] | Savoury/cheesey[E8]	**0.0002**	0.07	0.29	0.14	0.39	-
	Buttery[E1,2,5,8]	**0.001**	**<0.0001**	-	0.61	**0.003**	-
	Rancid[E1] | Off-flavours[E2,5,8] | Sulfate off note[E3]	**0.05**	0.21	0.64	0.25	0.07	-
	Salty[E1,2,5,8]	0.21	0.09	-	0.09	0.28	-
	Salivating[E1,2,5,8] | Teeth packing (residue)[E3]	0.13	0.88	**0.03**	0.31	0.32	-
	Metallic[E1,2,5,8]	**<0.0001**	**<0.0001**	-	**0.008**	0.08	-
	Liquorice[E2,5] | bitter[E3]	-	**<0.0001**	1.00	0.47	-	-

Table D.2b. Summary of descriptive sensory profiling (DSP) sample by assessor interactions in whey protein liquid models.

Modality	Appearance	E4: Whey Beverages	E6: WPBs with lactose	E7: WPBs with fat
		Sample*Assessor *p* value	Sample*Assessor *p* value	Sample*Assessor *p* value
Aroma	Cooked milk	**0.002**	0.19	-
	Powdered milk (wet)	**0.04**	0.14	-
	Whey isolate	0.09	0.14	-
	Vanilla[E4]	**0.0006**	-	-
Flavour	Sour	**0.0002**	**0.05**	0.28
	Metallic	0.18	0.77	0.76
	Salty	**0.03**	0.19	0.20
	Sweet	**0.03**	0.06	**0.0004**
	Cooked butter	**0.03**	**0.0006**	**<0.0001**
	Cooked milk	**<0.0001**	0.13	**0.002**
	Powdered milk (wet)	**0.0003**	**<0.0001**	0.67
	Whey isolate	**0.002**	0.74	**<0.0001**
	Vanilla[E4]	**<0.0001**	-	-
Mouthfeel	Body	**0.002**	0.26	**0.008**
	Chalky[E4] | Powdery[E6,7]	**0.001**	0.23	0.21
	Mouthdrying	0.10	0.33	0.98
Aftertaste	Aftertaste strength	0.17	0.77	0.95
	Mouthdrying	**0.005**	0.13	0.52
	Metallic	0.13	0.35	**0.01**
	Vanilla[E4]	**<0.0001**	-	-
	Sweet	**0.0006**	0.66	-

Data obtained from SenPAQ (via ANOVA) combining two replicates from 8-12 panellists where a significant *p* value indicates poor panel consensus. Numbers (E1-9) represent differing experiments: E1 = cakes (control and protein; **chapter 4**); E2 = cupcakes (control and protein; **chapter 4**); E3 = biscuits (control and protein; **chapter 4**); E4 = whey beverages (whey permeate beverages (WPeB), whey permeate beverages sweetened (WPeBS); whey protein beverage (WPB) and whey protein beverage sweetened (WPBS); **chapter 5**); E5 = cupcakes (control and two × protein; **chapter 6**); E6 = whey protein beverages (WPB) with lactose (two controls and five lactose levels; **chapter 6**); E7 = whey protein beverages (WPB) with fat (control and three fat levels; **chapter 6**); E8 = scones (control and protein; **chapter 6**); E9 = scones (with and without cream topping; **chapter 6**).

Going forward, it would be beneficial to carry out further panel training to reduce attribute interactions as well as to ensure consistent and reproducible scoring. This thesis utilised visual analogue scales (VAS; 0-100) (i.e. a scale without any structure); accordingly, structured scales using reference standards to indicate attribute intensity at set anchors could be suggested. However, it could be challenging for neutral pH WPBs to source a suitable standard (typically either considered a less pure standard or containing polyphenolic compounds). In addition, further information on individual perception and sensitivity would be useful. For example, salivary flow rates, oral tactile sensitivity,

mouthdrying sensitivity and mouth behaviour. This information could be used to explain individual differences in scoring and identify the panellists more likely to cause interactions between samples.

Appendix E

E.1. Thesis medication use

Medication use was recorded in all consumer studies (**Chapters 3-5 and 7**) (Table E.1). In summary, across the thesis from 332 volunteers only 58 volunteers (younger adults *n* = 4; older adults *n* = 54) were taking medication and of those 47% were taking only one medication, as summarised in Figure E.1. More specifically, within any individual study the maximum number of volunteers on medication was 26% (range: 0-26%). Additionally, all individual medications (*n* = 56) were checked for potential side effects likely to influence main study outcomes (perception and saliva flow) (Figure E.1).

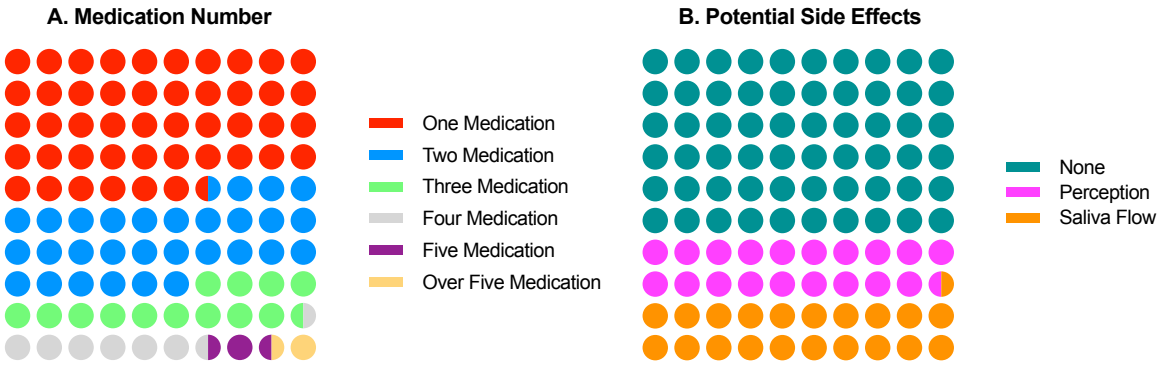


Figure E.1. Overall volunteers thesis medication use (*n* = 58) (**Chapters 3-5 & 7**) by (**A)** number of medications per volunteer and (**B**) individual medication (*n* = 56) potential side effects[55]. Data expressed as % with each coloured circle representing 1.0%.

[55] perception (**Chapters 3-5 & 7**) denotes taste related side effects (*n* = 22 medications; common *n* = 9; uncommon *n* = 5; rare/very rare *n* = 4; frequency not known *n* = 4) and saliva flow (**Chapters 3-5**) describes dry mouth or similar effects (*n* = 23 medications; common *n* = 13; uncommon *n* = 4; rare/very rare *n* = 1; frequency not known *n* = 5). Data was obtained from British National Formulary (2019) and frequency defined by: common (1 in 100 to 1 in 10); uncommon (1 in 1000 to 1 in 100); rare/very rare (1 in 10000 to 1 in 1000; less than 1 in 10000); frequency not known (either incidence rate not defined by literature or reported from post-marketing surveillance) (NICE, 2019). Moreover, data from volunteers on medication was checked for potential outliers.

Table E.1. Thesis summary of volunteers (*n* = 58) medication types.

Medicine Type*	Use Rationale#	Frequency^		Chapter
		n	%	
5-alpha reductase inhibitors	Benign prostate enlargement	1	0.9	7
Alpha-adrenoceptor blockers	Urological & benign prostate enlargement	3	2.7	4 & 7
Antidepressant	Anxiety, panic attacks & relaxation at night	4	3.5	3-5 & 7
Antihistamines	Perennial rhinitis	1	0.9	3 & 4
Antihypertensive	Lower blood pressure	20	17.7	3,4 & 7
Antimuscarinics	COPD (mild)	1	0.9	3 & 4
Antispasmodics	Overactive bladder	2	1.8	3 & 4
Beta blocking agents	Ocular hypertension & glaucoma	3	2.7	3,4 & 7
Bisphosphonates	Osteoporosis	1	0.9	7
Calcium-channel blockers	Atrial fibrillation	1	0.9	4
Cardiac glycosides	Heart problems	1	0.9	3 & 4
Corticosteroids	Asthma preventative & narrow & convoluted sinuses	5	4.4	3,4 & 7
Dopamine receptor agonists	Restless leg syndrome	1	0.9	4
Loop diuretics	Fluid retention	1	0.9	3 & 4
Macrolides	Asthma preventative	1	0.9	7
Non-steroidal anti-inflammatory drugs	Pain relief	3	2.7	3,4 & 7
Opioids	Osteoarthritis	2	1.8	3 & 4
Proton pump inhibitors	Lower stomach acid	12	10.6	3,4 & 7
Selective beta2-agonists (short-acting)	COPD	1	0.9	4
Statins	Lower cholesterol	16	14.2	3,4 & 7
Supplements	Bone health & well-being	10	8.8	3,4 & 7
Thyroid hormones	Underactive thyroid	11	9.7	3-5 & 7
Vitamin D and analogues	Psoriasis (very mild)	1	0.9	7
Vitamin K antagonists	Atrial fibrillation	2	1.8	3 & 4
Xa inhibitors	Blood thinner	1	0.9	3 & 4
Xanthine oxidase inhibitors	Gout	3	2.7	3 & 4
Other	Various	5	4.4	3,4 & 7

* Medicine type as obtained from British National Formulary (2019) and various includes: night cramps and polycystic ovary syndrome (PCOD); # relates to use rationale for specific medication stated by volunteer in screening questionnaire; ^ denotes frequency of medication used within thesis. Acronyms: chronic obstructive pulmonary disease (COPD).