Individual differences in oral tactile sensitivity and gustatory fatty acid sensitivity and their relationship with fungiform papillae density, mouth behaviour and texture perception of a food model varying in fat

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Abstract

Fat provides multimodal stimulation, particularly through mouthfeel and as a taste stimulant via free fatty acids. Individuals vary in perception of both mouthfeel and taste sensations from fat. Papillae number on the tongue can influence oral tactile and taste sensitivity. In addition, mouth behaviour (how foods are manipulated in the mouth during eating before swallowing) varies between individuals, and may influence mouthfeel perception. Limited research has explored the relationships between these factors.

Fatty acid (FA) taste sensitivity was measured at two levels of oleic acid. Oral tactile sensitivity was measured using von Frey filaments. Fungiform papillae density (FPD) was measured on the tongue anterior. Mouth behaviour (MB) was measured by Graphic Jeltema/Beckley Mouth Behaviour (JBMB) classification tool. Mouthfeel perception (hardness, crunchiness, and greasiness) in a biscuit model was measured to examine the influence of FPD, tactile sensitivity and MB on mouthfeel perception.

Higher FPD was significantly related to higher taste sensitivity to fatty acid and to higher oral tactile sensitivity. FPD and oral tactile sensitivity both significantly influenced mouthfeel perception of biscuits. The results demonstrate the need to characterise individual differences in oral sensory perception by more than one method, and suggest oral tactile sensitivity can be used as a marker of FPD. Further studies are required to understand the impact of MB on sensory perception. The BMI of participants in this study was negatively related to oral tactile sensitivity and the perception of greasiness.

Key words

Fatty acid sensitivity, fungiform papillae, tactile sensitivity, mouth behaviour, mouthfeel perception,

Highlights

- Individuals differ in papillae density, oral tactile and fat taste sensitivity
- Fungiform papillae density positively correlates with oral tactile sensitivity
- Higher fungiform papillae density related to higher fat taste sensitivity
- Fungiform papillae density and tactile sensitivity influence mouthfeel perception
- BMI related to oral tactile sensitivity and perception of greasy

Introduction

Dietary fat is the most energy-dense macronutrient in foods and contributes to food palatability. Fat is well-known to contribute to mouthfeel, whereas it is more recent that oral perception of free fatty acid has been recognised as a basic taste (Chale-Rush, Burgess, & Mattes, 2007a, 2007b; Stewart et al., 2010). Studies have suggested multiple candidate receptors on the tongue (CD36 and G protein coupled receptors (GPCRs)) which may be responsible for fat taste (Laugerette et al., 2005; Martin et al., 2011; Ozdener et al., 2014; Simons, Kummer, Luiken, & Boon, 2011). Although free fatty acids are only present in small amounts in foods, lingual lipase is reported to increase free fatty acid in the mouth by hydrolysing triglyceride (Kulkarni & Mattes, 2013; Pepino, Love-Gregory, Klein, & Abumrad, 2012; Voigt et al., 2014).

Individuals have been reported to vary in fat taste sensitivity (Chale-Rush, Burgess, & Mattes, 2007a, 2007b; Martinez-Ruiz, Lopez-Diaz, Wall-Medrano, Jimenez-Castro, & Angulo, 2014; Mattes, 2009a; Running & Mattes, 2014; Running, Mattes, & Tucker, 2013; Stewart et al., 2010; Stewart, Newman, & Keast, 2011; Tucker, Nuessle, Garneau, Smutzer, & Mattes, 2015; Zhou, Shen, Parker, Kennedy, & Methven, 2016). This could be due to various factors, such as lipase activity (Kulkarni & Mattes, 2013; Pepino et al., 2012), genetic differences in fat taste receptors (Keller et al., 2012; Melis, Sollai, Muroni, Crnjar, & Barbarossa, 2015) and the quantity of fat taste receptors. Taste receptors are located within taste buds in papillae and, hence, research has suggested that variation in fungiform papillae density (FPD) can influence oral taste sensation (Bakke & Vickers, 2008; Dinnella et al., 2018; Masi, Dinnella, Monteleone, & Prescott, 2015; Melis et al., 2013; Miller & Bartoshuk, 1991; Miller & Reedy, 1990). The influence of fungiform papillae in response to bitter taste perception of 6-n-propylthiouracil (PROP) is most well studied (Bajec & Pickering, 2008; Bakke & Vickers, 2008; Bartoshuk, Duffy, & Miller, 1994; Calo et al., 2011; Dinnella et al., 2018; Garneau et al., 2014; Melis et al., 2013; Shen, Kennedy, & Methven, 2016; Tepper & Nurse, 1997). As CD36 and GPCR120 are both found in human fungiform papillae (Ozdener et al., 2014), this raises the question whether FPD could also have an influence on fat taste sensitivity. Although one previous study has reported a relationship between FPD and fat perception, this mainly

focused on oiliness and fat content (Tepper & Nurse, 1997), therefore, it remain worthwhile to further explore the relationship between FPD and fatty acid taste sensitivity.

Fungiform papillae are surrounded by trigeminal neurons responsible for innervating somatosensory (tactile) perception (Whitehead, Beeman, & Kinsella, 1985), hence influencing on the mouthfeel perception of food (Hayes & Duffy, 2007; Nachtsheim & Schlich, 2013; Tepper & Nurse, 1997). Yackinous and Guinard (2001) applied von Frey filaments to measure oral tactile sensitivity, where elastic fibres are pressed vertically onto the tongue surface and the specific diameter of each filament is used to vary the applied force. Their results indicated that the tongue area containing more fungiform papillae was more sensitive in detecting the touch of filaments. Bangcuyo and Simons (2017) applied various sizes of different letters to measure lingual tactile sensitivity of participants and discovered tactile sensitivity was significantly associated with FPD. It has been previously reported that oral tactile sensitivity is related to PROP taste sensitivity, specifically that participants who were classified as "supertasters" to PROP showed greater tactile sensitivity (Yackinous & Guinard, 2001). This is perhaps indicative that a higher FPD may lead to both a greater number of both taste receptors and trigeminal neurons, rather than a more fundamental relationship between the genetic difference in bitter taste receptors (*TAS2R38*) and extent of trigeminal neurons. Tactile sensitivity measured by von Frey filament is predicted to influence oral mouthfeel perception, yet limited studies have investigated the influence of oral tactile sensitivity on mouthfeel perception of foods. One such recent study found that individuals with greater oral acuity (as measured by von Frey filaments) were able to discriminate chocolate of different particle sizes where individuals with lower oral sensitivity could not (Breen, Etter, Ziegler, & Hayes, 2019).

The Graphic Mouth Behaviour Tool was developed by Jeltema, Beckley, and Vahalik (2014, 2015) to characterize participants into four groups based on their preferred way of manipulating food in the mouth; Crunchers, Chewers, Smooshers and Suckers. Crunchers prefer to crunch and swallow food rapidly, whereas Chewers prefer to chew food for longer periods of time before swallowing and they prefer chewy foods. Smooshers tended to smoosh the food in the mouth and Suckers prefer hard food which can be sucked for a long time. Such differences in mouth behaviour might change the structure of the food and hence result in different oral sensory perception, hence contributing to individual differences in mouthfeel perception.

Therefore, the objectives of this study were to:

- Explore the relationship between fatty acid sensitivity and fungiform papillae density
- Elucidate the relationship between fungiform papillae density and oral tactile sensitivity
- Explore the influence of fungiform papillae number, tactile sensitivity, and mouth behaviour on oral mouthfeel perception of food

Through these objectives we aim to establish simple methods to characterize oral sensory differences of consumers, in addition to understanding how such factors could influence individual differences in oral sensory perception of foods.

Methods and Materials

Participants

Participants were recruited from the local community (Reading, UK). The inclusion criteria were self-reported healthy, aged 18-70 years and weight stable in the last three months. Exclusion criteria included: smoking, drug abuse, food allergies (e.g. gluten, dairy) and intolerances (e.g. lactose), diagnosed with cardiovascular disease, diabetes, gastrointestinal, endocrine or renal disease, planning or currently on a weight reducing programme, pregnant or planned pregnancy or lactating. The study was given a favourable opinion for conduct by School of Chemistry, Food and Pharmacy research ethics committee (study number 14/17) (participants n=65) and later by the University of Reading Research Ethics Committee (study number 18/05) (participants n=29). During the testing of the initial 65 participants it became apparent that a finer von Frey filament would provide useful additional information. Hence 9 of these participants were also tested with a finer (0.008g) filament. Of these initial 65, a further 9 participants (who had not been tested with the finer filament) returned for subsequent trials alongside a second group of 29 new participants. These 9 participants were retested for their fatty acid sensitivity to the low level of fatty acid and their tactile sensitivity to the thicker 0.02g filament; neither results changed. The second cohort were tested for their sensitivity to the higher level of fatty acid and a finer filament (0.008g). Therefore, in summary, there were 94 participants for each characterisation test except for the sensitivity tests to the higher level of fatty acid (n = 38) and to the finer filament (n = 47). Each participant was only tested once for each test. The details of participant numbers in each test are shown in Supplementary data 1.

Before participants being asking to taste any samples, demographic questions (age, gender, height, and weight) were collected. Height was measured by a wall mounted stadiometer and weight was measured on a glass electronic balance (Salter, UK). BMI was calculated by the Quetelet Index (kg/m^2).

Fatty acid sensitivity

Sample preparation for fatty acid sensitivity

Food-grade oleic acid (Sigma, UK) was used at two levels based on the previous research (Stewart et al., 2010; Zhou et al., 2016). The samples comprised oleic acid, milk (Long life skimmed milk, Co-operative, UK), water, liquid paraffin (Care, Thornton & Ross, Huddersfield, UK) and thickener (xanthan gum based, Nestlé Nutrition Resource, ThickenUp Clear, Liverpool, UK). The control samples consisted of the same ingredients but without addition of oleic acid. EDTA was included in the emulsion to prevent oxidation of free oleic acid. After mixing all ingredients, samples (100ml) were homogenised at 5000rpm for 3 min using a high-shear mixer (Silverson Laboratory L4RT Mixer, Silverson machines, Chesham, UK). Each sample was prepared on the day of consumption, 1 hour prior to testing and served at ambient temperature (23 ± 2°C) to each participant. Sample compositions are given in **Table 1**.

Table 1 Composition of samples used to test free fatty acid gustatory sensitivity

	Water (ml)	Milk (ml)	Thickener (g)	Liquid paraffin (g)	EDTA (g)	Oleic acid (g)	Oleic acid level (%w/w)

Control	80	20	1	3	0.01	n/a	n/a
Low level oleic acid	80	20	1	3	0.01	0.016	0.015%
High level oleic acid	80	20	1	3	0.01	0.11	0.105%

Procedure for fatty acid gustatory sensitivity

To test gustatory fat sensitivity triplicate alternative forced choice (3-AFC) discrimination tests were carried out for the low oleic acid level (0.015% w/v). This concentration was selected based on the study of Zhou et al. (2016), of which the results indicated that 49% of participants (n=43/87) could detect this level. Participants (n=94) were served three samples (two controls and one oleic acid sample) each time and they were asked to taste the samples and identify the "odd" sample out. If the participant correctly identified the sample containing oleic acid from the control in each of the three 3-AFC tests they were defined as "passed" to 0.015% w/v oleic acid; the probability of incorrectly identifying an individual participant as a taster from three correct 3-AFC tests being 0.037 (3.7%). Participants who incorrectly identified the sample in one or more 3-AFC tests were defined as "failed" to 0.015% oleic acid. During tasting participants wore nose clips to eliminate any olfactory effect. The test was conducted under red light to mask any visual variation between samples.

The same procedure was repeated for the high oleic acid level (0.105%) for 38 participants in order to compare the current results with the findings of Stewart et al. (2011). Participants were classified as "hypersensitive" if they "passed" the low concentration of oleic acid (0.015% w/v oleic acid) and as "hyposensitive" if they "failed" at the high concentration (0.105% w/v oleic acid).

Fungiform papillae density

In order to count fungiform papillae (FP) participants was asked to hold their tongue out to below their bottom lip and relax. Their tongue was dyed using blue food colouring (Dr.Oetker Blue Food Colouring Gel, Dr.Oetker Ltd, Leeds, UK), this procedure stains the tongue surface blue, however the FP remain unstained. Participants were asked to hold a ruler parallel to their tongue in order to provide a 1cm reference. A photo was taken using a digital SLR camera (Canon, E05 700D) with an EF-S 19-55mm lens. At least three photos were taken for each tongue, and the clearest photo was selected for FP counting. According to the study conducted by Eldeghaidy et al (2018), the mean number of FP detected using their automated method was highest in the first cm of the anterior 2 cm of the tongue. Therefore, two parallel $1cm^2$ squares were selected for FPD counting at the position 0.5 cm from the tongue tip. The two $1cm^2$ squares were next to each other To facilitate counting, these squares were drawn (by PowerPoint), using the ruler held next to each participant's tongue in the original image as a guide. Counting of fungiform papillae was conducted by three assessors for the majority of images (85%) and by two assessors for 15%; in all cases one assessor was the same for all images. All of the assessors conducted the counting blinded from the results of other assessors and also from participant's phenotype measurements.

In order to reduce bias each assessor counted independently and any discrepancies were resolved by discussion.

Tactile sensitivity measurement

Two von Frey filaments (Aesthesio, Danmic Global, LLC, US), 0.02g force (size 1.65) and 0.008g force (size 2.35), were used to determine tactile sensitivity on the tongue. All participants were tested using the 0.02 g filament, whereas 47 were additionally tested using the 0.008g filament. The participants were blindfolded and asked to protrude their tongue over their bottom lip whilst allowing it to relax. The front area of their tongue was then touched with each filament. Each filament was used ten times, five times with the true touch (touch) and five times with the false touch (no touch), in a randomly allocated balanced order, either side of the tongue midline. The filament was held perpendicular to the surface of tongue. The tip of the filament was touched on the tongue surface until the fibre slightly bowed, and then the filament was removed. The participant was asked if they could detect the stimulus on their tongue (forced-choice) and additionally asked to indicate how sure they were about their answer. Hence, there were four possible answers; "yes, sure", "no, sure", "no, not sure" and "yes, not sure". The answers were recorded to calculate the R index (see **equation 1**) which was the measure of oral tactile sensitivity. If an R-index of 1 was obtained it inferred that the participant could easily detect the filament. However, if the R index was 0.5 or less, it indicated that the participants could not detect that filament.

Equation 1 formula of calculating the R index by using the results obtained from volunteer's responses. Y-sure; Y-unsure; N-sure; N-unsure.

	Y-sure	Y-unsure	N-unsure	N-sure	Total	R-index
True touch	a	b	c	d	5	
False touch	e	f	g	h	5	

$$\text{R index} = \left[a\times(f+g+h)+b\times(g+h)+c\times h+\frac{1}{2}\times(a\times e+b\times f+c\times g+d\times h)\right]/(5\times 5)$$

Mouth behaviour measurement

Mouth behaviour was measured using the Graphic Jeltema/Beckley Mouth Behavior (JBMB) Classification Tool (Jeltema et al., 2014, 2015; Jeltema, Beckley, & Vahalik, 2016).

Participants were shown the JBMB tool which provides food images in each of 4 quadrants, alongside 4 headings ("I like foods that I can crunch", "I like foods that I can chew", "I like foods I can suck on for a long time" and "I like foods I can smoosh"). They were asked two questions, "which is most like you" and "which is not like you at all". After this, there were nine questions to validate each group characteristics (shown in Supplementary data 2 and 3). Participants were classified into four groups based on their answers to the question of "which is most like you".

Biscuit ratings

Biscuit preparation

Four savoury biscuits were formulated to provide small differences in mouthfeel based on differences in processing of the fat (butter) and fat quantity (**Table 2**). Three biscuits were made with the same butter level but varying the temperature of butter. One biscuit was made using a higher level of butter.

Table 2 Composition of biscuits used to rate mouthfeel of a food model

Sample	Butter (%w/w)	Flour (%w/w)	Cheese (%w/w)	Baking powder (%w/w)	Salt (%w/w)	Egg (%w/w)
Cold Butter	18.3%	42.8%	18.3%	1.8%	0.3%	18.3%
Warm Butter	18.3%	42.8%	18.3%	1.8%	0.3%	18.3%
Melted Butter	18.3%	42.8%	18.3%	1.8%	0.3%	18.3%
Melted Double Butter	31.0%	36.2%	15.5%	1.6%	0.3%	15.5%

Plain flour (Co-operative, UK), egg (Free range, Co-operative, UK), baking powder (Dr.Oetker Baking Powder, Dr.Oetker Ltd., Leeds, UK), salt (Table salt, Co-operative, UK), unsalted butter (Co-operative, UK) and cheese (medium grated cheddar cheese, Co-operative, UK) were weighed and mixed for 90 s at speed 2 in a dough mixer (Kenwood Major Titanium KMM020, Kenwood Ltd., Havant, UK). Cold butter was added to the mixer at 4°C ± 2°C, warm butter was added at 22°C ± 2°C, melted butter was melted using a water bath (50°C ± 2°C) prior to mixing. The mixed dough was sheeted (Rondo sheeter STM-503, Rondo Ltd, Surrey, UK) to a uniform thickness of 3.25mm, cut into circles (4.25cm diameter) and placed on a baking tray. Biscuits were baked 180°C for 15 min in a pre-heated oven (Salva KWIK-CO convection over, ATLAS equipment (London) Ltd, London, UK). After baking, biscuits were cooled to ambient (22°C ± 2°C) and stored in sealed polyethylene bags for later use.

Biscuit mouthfeel perception and texture measurements

Three attributes were used to rate the mouthfeel of biscuits: hardness of the initial bite, crunchiness after two bites and the greasiness of the mouthfeel. A definition for each attribute was given to the participants to aid their understanding. “Hardness” was defined as “the hardness at the first bite of biscuit”, “Crunchiness” as “the low frequency noise when biting the product” and “Greasy” was defined as “the greasy feeling or oily feeling after tasting the sample”. Participants were asked to taste each biscuit type (Table 2) once and rate these attributes on a structured line scale (“not at all”, “a little”, “some” and “very” anchors at 0, 33, 66 and 100 out of 100). The biscuits were served in a randomly allocated balanced order under red light in order to mask any visual differences. There was a 30 s time interval between samples and participants were instructed to clean their palate with water during the time delay.

The hardness of biscuits was measured by Texture Analyser (Stable Micro Systems, TAXT2) to relate the physical texture to the perception of hardness. Each biscuit was placed on two stationary supports of the rig base plate with a 3 cm gap. The base plate was secured to a

heavy-duty platform. The probe was a three-point bend rig (HDP/3PB), and the test mode was compression. The test speed was set at 3 mm/sec and the strain was set at 60%. The data were captured by Exponent (version 6.1.4.0, Stable Micro Systems Ltd, Surrey, UK). Each processing batch of each biscuit formulation (Table 2) was stored for a maximum of 5 days after baking. Hardness (force (g)) was measured from two separately prepared batches of biscuits, these duplicate measurements were taken on each of 5 consecutive storage days, in order to examine the texture stability. The hardness differences between storage days, batches, and biscuit types were examined.

Statistical analysis

The results of demographic questions, mouth behaviour questionnaire and biscuit ratings were collected by Compusense at-hand (Compusense, Canada). Data were analysed by XLSTAT (version 2018.5, Addinsoft), except for the Spearman partial correlation analysis which was conducted using SPSS Statistics (version 22, IBM).

Outlier analysis in all data sets was examined by Grubbs test. Chi-square analysis was conducted to examine associations between categorical data: gender, ethnicity, fatty acid sensitivity group and mouth behaviour.

The residuals of all continuous numerical data were tested for normality using the Shapiro-Wilk test, histograms, and Q-Q plots. Residuals of tactile sensitivity using 0.008g filament (R-index) and FPD were normally distributed. The residuals of BMI and biscuit perception data (hardness, crunchiness, and greasiness) were not normally distributed according to the Shapiro-Wilk test; however, the residual Q-Q plot approximated linearity and the distributions of residuals were bell shaped. In addition, the skewness values from the Pearson skewness test for all four of these factors were between -0.5 and 0.5, which indicates the data is symmetrical,hence data from these factors were considered to be sufficiently robust for parametric analysis. Residuals of tactile sensitivity using 0.02g filament (R-index) were not normally distributed and the data were substantially skewed (skewness value -1.17) toward R-index values of 1.0, hence these data were treated as non-parametric.

The relationship between fatty acid sensitivity and FPD was tested by ANCOVA with fatty acid sensitivity (categorical data) fitted as the explanatory variable and BMI as the covariate. We note that the direction of the relationship expected is that FPD would influence fatty acid sensitivity (FA) rather than vica versa, therefore logistic regression was initially used with numerical data (FPD) as the independent variable and categorical data (fatty acid sensitivity) as the dependent variable (FA = FPD). The logistic regression concluded a significant relationship between fatty acid sensitivity and FPD ($p = 0.003$; predictive AUC = 0.76; data not shown). As the significance of the relationship was the same where the categorical data (fatty acid sensitivity) is fitted as the independent variable, and this allows for BMI to be fitted as the covariant, the final model reported is from ANCOVA (FPD = FA + BMI). The relationship between oral tactile sensitivity to the finer filament (0.008g) (F0.008) and FPD was examined by linear regression, fitting both FPD and BMI as explanatory variables (F0.008 = FPD + BMI). The relationship between tactile sensitivity to the 0.02g filament (F0.02) and FPD was examined by Spearman partial correlation, accounting for BMI within the analysis (F0.02 = FPD + BMI).

To examine any relationships between BMI and sensory phenotypes with category data (fatty acid sensitivity, FA and mouth behaviours, MB) ANOVA was carried out (BMI = FA + MB). To examine any relationship between BMI and oral tactile sensitivity, linear regression was used for R-index data collected from the 0.008g filament (BMI = F0.008), and Spearman's correlation test for R-index data collected from the 0.02g filament (BMI = F0.02).

Differences in perception of biscuits between different biscuit types were analysed using ANCOVA with Tukey's HSD for pairwise comparisons where biscuit type was regarded as the fixed factor (categorical data) and BMI as a covariate (numeric data) (Hardness, Crunchy or Greasy = Biscuit Type + BMI) . To further test any relationship between biscuit perception ratings and sensory phenotypes separate ANCOVA where carried out, in all cases biscuit type was fitted as a fixed factor (categorical data), BMI as a covariate (numeric data); FPD and oral tactile sensitivity measurements (R-indices) were fitted, separately, as covariates (numeric data) (Hardness, Crunchy or Greasy = Biscuit Type + BMI+ either FPD; F0.02, or F0.008); mouth behaviour and fatty acid sensitivity were fitted, separately, as fixed factors (categorical data) (Hardness, Crunchy or Greasy = Biscuit Type + BMI+ either mouth behaviour or fatty acid sensitivity).

Significance level (p value) was set at 0.05, two tailed. It is noted that where factors were significantly correlated (FPD, tactile sensitivity, mouth behaviour and fatty acid sensitivity) they could not be combined into a single ANCOVA. Where BMI fitted as a covariate in any ANCOVA it had a non-significant effect unless stated otherwise in the results section.

Results

Characterization of participants

Ninety-four participants participated in the study. There were 64 females (68%) and 30 males (32%). Fifty-eight (62%) were Caucasian, twenty-nine (31%) were Asian and seven (7%) were African (**Table 3**). The BMI ranged from 15.6 kg/m^2 to 38.8 kg/m^2.

All participants were tested for fatty acid sensitivity at the lower oleic acid level (0.015% w/v); 18 participants (19%) could successfully identify the sample and were hence deemed to have "passed" 0.015% w/v oleic acid, whereas 76 participants (81%) failed this concentration. Subsequently, 38 participants were tested at the higher level of free oleic acid (0.105% w/v), in which 13 of them (34%) "passed" at 0.105% w/v oleic acid and 25 (66%) "failed" (**Table 3**). Of these 13 volunteers sensitive to 0.105% w/v oleic acid, 6 (16%) had the ability to "pass" 0.015% w/v oleic acid implying their thresholds to oleic acid were lower than 0.015% w/v; whilst 7 (18%) could not "pass" the 0.015% w/v oleic acid implying their thresholds were between 0.015% w/v and 0.105% w/v oleic acid.

Combining results from all volunteers that carried out sensitivity tests at both levels of oleic acid; participants were classified as "hypersensitive" where they "passed" the lower level of oleic acid (0.015% w/v oleic acid), and as "hyposensitive" where they "failed" to distinguish the higher level of oleic acid (0.105% w/v) once, or more than once, in three triangle tests. In summary this combined approach resulted in 18 hypersensitive and 25 hyposensitive participants.

357 **Table 3** Demographic and characterization measurements of participants

Characterization			Number	Proportion	BMI range (kg/m^2)	BMI mean (kg/m^2)
Gender		Female	64	68%	15.6-38.8	22.7
		Male	30	32%	16.3-30.0	24.1
Ethnicity		Caucasian	58	62%	15.6-38.8	23.6
		Asian	29	31%	16.8-29.4	22.3
		African	7	7%	16.4-28.4	23.2
Fatty acid sensitivity	0.105% w/v oleic acid	"Passed" at 0.105% w/v oleic acid	13	34%	18.0-28.4	21.9
		"Failed" at 0.105% w/v oleic acid (HYPOSENSITIVE)	25	66%	18.5-29.4	23.1
	0.015% w/v oleic acid	"Passed" at 0.015% w/v oleic acid (HYPERSENSITIVE)	18	19%	15.6-38.0	22.6
		"Failed" at 0.015% w/v oleic acid	76	81%	16.4-38.8	23.3
Mouth behaviours		Chewers	33	35%	18.1-38.0	22.9
		Crunchers	49	52%	15.6-38.8	23.5
		Smooshers	11	12%	16.4-29.4	22.4
		Suckers	1	1%	n/a	22.2

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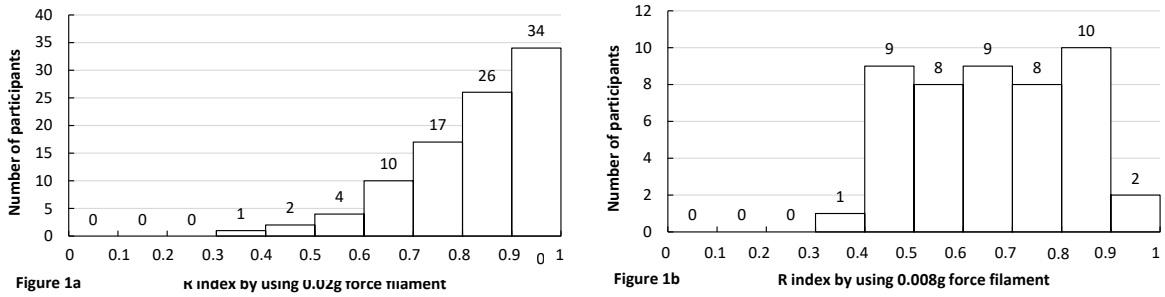
359 The fungiform papillae density on the left $1cm^2$ of the tongue varied from 10 to 85, with an
360 average of 32 (median 31); the right $1cm^2$ varied from eight to 119 with an average of 33
361 (median 30). The fungiform papillae number on the left $1cm^2$ was positively correlated to
362 the number on the right $1cm^2$ ($p<0.0001$, $r^2=0.85$), therefore the average FPD from the left
363 $1cm^2$ and right $1cm^2$ measurements was used in subsequent analysis.

364 Oral tactile sensitivity of all participants was measured by 0.02g force filament, and 47
365 participants were additionally measured by 0.008g force filament. Using the 0.02g force
366 filament the R index varied from 0.38 to 1, with an average of 0.87 (median 0.9). However as
367 shown in figure 1 the distribution was skewed to the right with 36% of participants (n=34)
368 having complete discrimination (R index = 1) and only 3% having an R index at, or below, 0.5.
369 The R index obtained from 0.008g force filament varied from 0.36 to 1, with an average of
370 0.69 (median 0.7). As mentioned in the method section, when the R index is 0.5 or less, it
371 indicates that the participants cannot detect the presence of that filament. This finer
372 filament was less easily detected and measured greater variation of R index values between
373 participants, with only 2% of participants having complete discrimination (R index = 1) and
374 21% having an R index at or below 0.5.



(Figure 1 goes here)

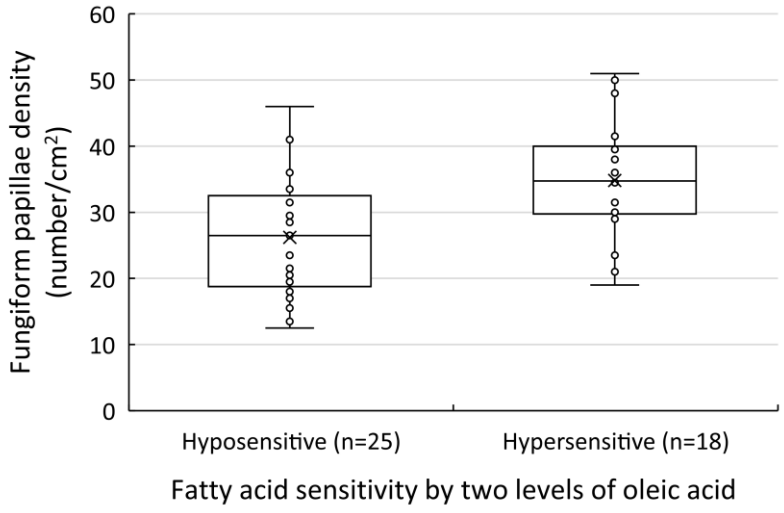
Figure 1 Distribution of tactile sensitivity (R index values) in 94 participants by using 0.02g force filament (1a, left) and in 47 participants by using 0.008g force filament (1b, right).

Regarding mouth behaviour, 33 participants were classified as "Chewers" (35%), 49 were "Crunchers" (52%), 11 participants were "Smooshers" (12%) and only one was classified as "Sucker" (1%).

Relationship between phenotypic measurements

Fatty acid sensitivity and FPD: At the low fatty acid concentration (0.015% w/v oleic acid) where 80% of participants "failed" to distinguish this level, there was no significant relationship between oral fatty acid sensitivity and FPD (p=0.19). Similarly, at the higher fatty acid concentration (0.105% w/v oleic acid), where 66% of participants "failed" to distinguish this level, there was no significant relationship with FPD (p = 0.37).

However, by combining the data from both fatty acid tests into the single "hyper-/hypo-sensitivity" classification, there was a significant relationship between sensitivity and FPD (p = 0.003). The fatty acid-hypersensitive participants had a higher mean FPD than the hyposensitive participants (**Figure 2**).



(Figure 2 goes here)

Figure 2 Distribution of fungiform papillae density in "hypersensitive" (n=18) and "hyposensitive" (n=25) participants.

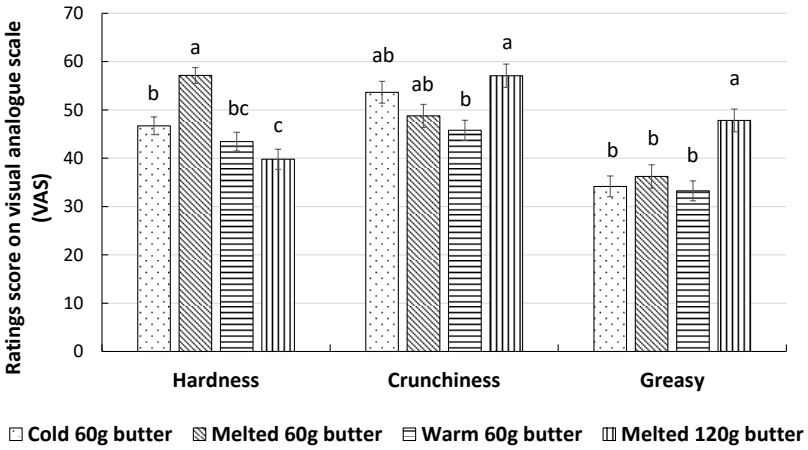
FPD and oral tactile sensitivity: Linear regression found a significant positive correlation between FPD and tactile sensitivity using the finer filament (R-index at 0.008g) ($r=0.41$, $p=0.008$). Although there R-indices were overall closer to 1 for the thicker (0.02g) filament (Figure 1a) there was a weak but significant correlation (Spearman rho=0.28, $p=0.008$) between sensitivity to this filament and FPD.

Sensory phenotypes and demographic measurements: There were no significant correlations between fatty acid sensitivity (at low or high level by using "pass/fail" to classify participants at one level of oleic acid) and any other individual characterisation parameter measured (with gender $p=0.89$ and $p=0.75$ respectively; with ethnicity $p=0.79$ and $p=0.56$ respectively; with mouth behaviour $p=0.29$ and $p=0.22$ respectively). Similarly, when using the combined "hyper-/hypo-sensitivity" classification, there were no significant correlations between fatty acid sensitivity and any other characterisation measured (with gender $p=0.86$; with ethnicity $p=0.66$; with mouth behaviour $p=0.18$). Mouth behaviour did not correlate with gender ($p=0.43$) nor ethnicity ($p=0.42$) in the population studied.

There was no relationship between BMI and fatty acid sensitivity using "pass/fail" to classify participants at one level of oleic acid (at 0.015% w/v: $p=0.59$; at 0.105% w/v: $p=0.24$), nor when using the combined "hyper/hypo" sensitive categorisation ($p=0.71$). No correlation was found between FPD and BMI ($p=0.43$), nor between BMI and tactile sensitivity measured using the finer 0.008g filament ($p=0.38$). However, there was a negative correlation between BMI and tactile sensitivity measured using the 0.02g filament (rho=-0.29, $p=0.006$). This suggests that a higher BMI is related to a lower oral tactile sensitivity, although it should be noted that a higher proportion of participants could detect this thicker filament (distribution substantially skewed, Figure 1a), perhaps limiting the application of this finding. There was no relationship between BMI and mouth behaviour ($p=0.80$).

Influence of biscuit type on biscuit ratings

Overall the participants found significant differences in hardness, crunchiness and greasiness between the four biscuit types ($p<0.0001$, $p=0.004$, $p<0.0001$ respectively: Figure 3). Biscuits with melted butter (18.3% fat level) perceived significantly harder than the other three biscuits ($p=\leq 0.001$). Biscuits produced with the higher level of melted butter (31% fat) were significantly crunchier than those produced with warm butter ($p=0.004$). Biscuits with the higher level of melted butter were significantly greasier than all other biscuits ($p=\leq 0.001$). There was no influence of BMI (fitted as covariate) on the perception of hardness or crunchiness ($p=0.11$, $p=0.70$ respectively). However, there was a negative relationship between BMI and greasy perception ($p=0.005$, value of BMI in the model -0.75), indicating that participants with a higher BMI tended to rate their perception of greasy as lower.



434

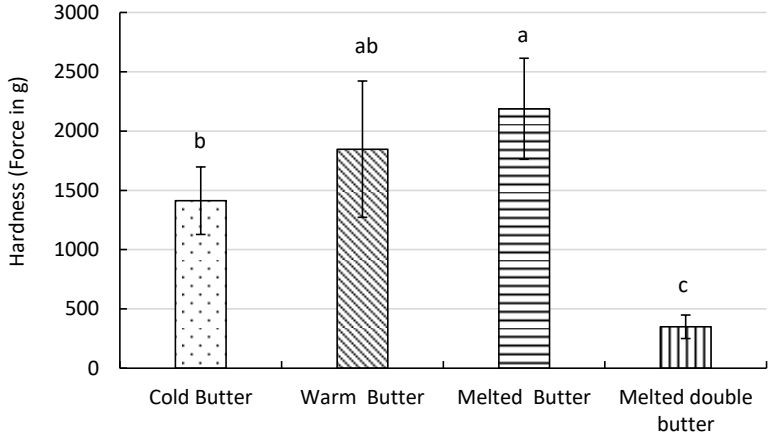
435 *(Figure 3 goes here)*

436 **Figure 3** Hardness, crunchiness, and greasy ratings of four types of biscuits. The results are expressed
437 as mean ± standard error. Bars not sharing a common letter indicate a significant difference between
438 biscuits within each attribute ($p<0.05$).

439

440 ***Texture analysis of biscuit hardness***

441 The texture analysis results showed that there was no significant difference between the
442 two biscuit batches ($p=0.82$), and storage day did not influence the hardness of biscuits
443 ($p=0.73$). There was a significant difference in hardness between biscuit types ($p<0.0001$,
444 Figure 4). The biscuits with melted double butter showed the least hardness, which was
445 significantly lower than other three types of biscuits ($p<0.0001$). The biscuits with melted
446 butter showed the highest hardness in average, which was significantly higher than the
447 biscuits with cold butter ($p=0.001$) and biscuits with melted double butter ($p<0.0001$).



448

449 (*Figure 4 goes here*)

450 **Figure 4** Hardness of four biscuit types by using three-point bend test in Texture Analyser. The bars
451 are expressed as mean ± standard deviation. Bars not sharing a common letter indicate a significant
452 difference between biscuits in each attribute ($p<0.05$).

The influence of phenotypic measurements on biscuit ratings

Gustatory fatty acid sensitivity (when used "pass" and "fail" to group participants at 0.015% and 0.105%w/v oleic acid) had no significant influence on perception of biscuit ratings (for hardness p=0.062, p=0.097 respectively; for crunchiness p=0.46, p=0.74 respectively; for greasy p=0.25, p=0.33 respectively). Similarly, using the combined "hyper-/hypo-sensitivity" classification, there was no relationship between fatty acid sensitivity and crunchiness or greasy perception (p=0.17, p=0.80 respectively); however the overall mean rating for biscuit hardness was significantly greater for hypersensitive compared to hyposensitive participants (mean rating 50.7 versus 44.6, p=0.031).

When considering FPD as a covariate in the analysis of biscuit ratings, it was found to have a significant impact on hardness ratings (p=0.033), and on crunchiness (p=0.027), but not on greasy perception (p= 0.10). Higher FPD was related to higher ratings of biscuit hardness and crunchiness, however the scale of impact of these linear models was low (values of +0.16 and +0.21 respectively).

Oral tactile sensitivity, as evaluated using the 0.02g filament, had a significantly positive relationship with the rating of biscuit hardness (p=0.019), with a similar effect size on the model as FPD (value +15.5). There were no significant relationships between sensitivity measured using this thicker filament and ratings of biscuit crunchiness or greasiness (p=0.063, p=0.25 respectively). Regarding the influence of tactile sensitivity measured by the 0.008g force filament on biscuit ratings, there were no significant relationships with biscuit ratings (hardness: p=0.086; crunchiness: p=0.29; greasy: p=0.84)

In order to investigate the influence of mouth behaviour on biscuit ratings, as only one "Sucker" was found the data of this subject was excluded from data analysis. Mouth behaviour had no significant influence on biscuit ratings (hardness p=0.32, crunchiness p=0.33, greasy p=0.09, respectively).

In summary, it was the perception of biscuit hardness that was most significantly influenced by sensory sensitivity, and although FA sensitivity, FPD and oral tactile sensitivity were all found to have significant effect, these were all tested in separate statistical models due to the correlations between measures. Therefore, we cannot conclude that each sensory sensitivity measured is having a separate effect on the perception of the biscuits, merely that increased oral sensitivity did, overall, have a significant effect.

Discussion

As anticipated, participants tested in this study were found to vary in their fungiform papillae density, their gustatory sensitivity to free fatty acids, their oral tactile sensitivity to von Frey filaments, and in their preferred mouth behaviour. This study examined the relationships between these factors, and their impacts on mouthfeel perception of a food model. We found that fungiform papillae density was positively related to higher fat taste sensitivity, and positively correlated with oral tactile sensitivity. Both fungiform papillae density and tactile sensitivity influenced mouthfeel perception of the biscuit model,

although it unlikely that these were independent effects. Moreover, BMI influenced oral tactile sensitivity and perception of greasiness.

The relationship between fat taste sensitivity and fungiform papillae density

The influence of fungiform papillae density on taste perception has mostly been studied with bitter taste, particularly in relation to 6-n-propylthiouracil (PROP) (Bajec & Pickering, 2008; Bakke & Vickers, 2008; Bartoshuk et al., 1994; Calo et al., 2011; Dinnella et al., 2018; Garneau et al., 2014; Melis et al., 2013; Shen et al., 2016; Tepper & Nurse, 1997). Several studies reported that higher FPD resulted in greater bitterness perception from PROP (Bakke & Vickers, 2008; Bartoshuk et al., 1994; Calo et al., 2011; Melis et al., 2013; Shen et al., 2016; Tepper & Nurse, 1997). More fungiform papillae on the tongue is proposed to lead to more taste receptors and a stronger taste signal generation, although there are limited studies that have directly meassured this association.

Fat taste has been proposed as the sixth basic taste. Receptors such as CD36 and GPCRs on the tongue in both animals and humans have been proposed to be responsible for fat taste (Abdoul-Azize, Selvakumar, Sadou, Besnard, & Khan, 2014; Martin et al., 2011; Ozdener et al., 2014). Free fatty acid is proposed as the effective stimuli to activate the receptors on the tongue and hence generate the fat taste sensation (Chale-Rush et al., 2007a, 2007b; Mattes, 2009a, 2009b; Running, Craig, & Mattes, 2015; Running & Mattes, 2014; Running et al., 2013; Stewart et al., 2010; Zhou et al., 2016). CD36 and relevant G protein coupled receptors have both been found in fungiform papillae (Liu et al., 2018; Ozdener et al., 2014; Simons et al., 2011). Therefore, it was hypothesised that the participants who have more fungiform papillae may have more fat taste receptors and hence be more sensitive to fat taste.

In this study, two different concentrations of oleic acids were used. As noted in the methods, if a participant correctly identified the sample containing a specific level of oleic acid from the control in each of three 3-AFC tests they were defined as “passed” for that level of oleic acid. However, overall participants were classified as “hypersensitive” if they “passed” the low concentration of oleic acid (0.015% w/v oleic acid) and as "hyposensitive" if they “failed” at the high concentration (0.105% w/v oleic acid). Our results did not observe any relationship between FPD and fatty acid sensitivity by using “pass/fail” at one level of oleic acid. However, there was a relationship between FPD and fatty acid sensitivity by using the combined “hyper/hypo sensitivity” classification from the two different levels of oleic acid. Participants “hypersensitive” to oleic acid had higher FPD than those "hyposensitive", supporting the hypothesis that more fungiform papillae would result in more fat taste receptors and increased gustatory sensitivity to oleic acid.

However, the method used to classify participant’s fatty acid sensitivity is very important. When using one concentration of fatty acid as a “cut-off” point, the number of participants needs to be large. Two thirds of participants “failed” to distinguish the higher level of oleic acid used in this study (0.105% w/v), this proportion increasing to 81% at the lower oleic acid level (0.015% w/v). With such a high proportion of people failing a single cut-off test it is perhaps not surprising that there remains a broad range of FPD in the “fail” group. This may suggest that a higher level of oleic acid is needed for a single cut-off method, or that a greater participant sample size is needed. However, it does also infer that using more than one level of oleic acid leads to better discrimination between participants. The main

limitation of this approach is it is more time consuming and can increase participant fatigue. A large sample size in future studies is needed to confirm that using a two-concentration method leads to better discrimination between participants than a "cut-off" method using a single concentration of oleic acid.

In a previous study from our group (Zhou et al., 2016), a modified 3-AFC staircase method was used to measure the detection threshold of participants to free oleic acid. This modified method was developed by Allen, Withers, Hough, Gosney, and Methven (2014), which reduced the number of samples being tasted by participants to some extent, compared to the traditional 3-AFC staircase methods which has been used in other studies (Chale-Rush et al., 2007b; Mattes, 2009a; Running, 2015; Stewart et al., 2010). Both 3-AFC staircase methods provide an accurate outcome of the fat taste sensitivity, which can provide the distribution of different taste sensitivity in population, however, both are time-consuming and can cause participant fatigue. This is the reason why cut-off concentrations of oleic acid were used in this study.

Single "cut-off" concentrations have been used before in the studies of Stewart et al. (2010) and Stewart et al. (2011). Stewart et al. (2010) used a cut-off concentration of oleic acid of 1.4mM (0.04% w/v) concluding that 22% (n=12) of participants were hypersensitive whereas 78% (n=42) were hyposensitive. In the later study of Stewart et al. (2011), a higher concentration of oleic acid of 3.8mM (0.11% w/v) was used which resulted in 25% hypersensitive participants (n=13) and 75% (n=38) hyposensitive. By using similar concentration as Stewart as a cut-off (0.105% w/v), the proportion of "passed" participants in our study was higher than in the Stewart et al. (2011) paper, 34 % versus 25%. This is perhaps due to the different populations sampled in these studies; however, it may also be due to the relatively small number of participants in each study. This triplicate forced choice discrimination method with a cut-off concentration of oleic acid provides a quick approach to characterise the sensitivity of participants to fat taste, however, it loses accuracy compared to the detection threshold method. In addition, the cut-off concentration of 0.015% w/v was selected based on our previous study (Zhou et al., 2016) where the sample size was merely 51; the cut-off concentration of 0.105% w/v was selected based on Stewart et al. (2011) which similarly tested 51 participants. Therefore, future studies require a large sample size in order to conclude the distribution of fat taste thresholds in a population and subsequently to establish the most appropriate levels for a rapid discrimination method to characterize consumers' sensitivity.

It is reported that CD36 are not only located in fungiform papillae (Ozdener et al., 2014), but have also been found in circumvallate and foliate papillae (Simons et al., 2011). In addition, GPCR120 has been found in both fungiform papillae and circumvallate papillae (Galindo et al., 2012). Therefore, future work should consider counting all papillae types when relating papillae density to fat-taste sensitivity.

The current volunteers had diverse sensitivity to fatty acid which was in common with previous studies (Mattes, 2009; Stewart et al., 2010; Stewart & Keast, 2012; Stewart, Newman, & Keast, 2011; Tucker, Edlinger, Craig, & Mattes, 2014; Zhou et al., 2016). Such individual variation may be influenced by numerous factors, such as genetic variation in receptors and dietary fat intake. Some studies imply that dietary intake of fat may have a greater impact on altering fat taste sensitivity compared to other factors (such as genetic variation) (Costanzo et al., 2018; Heinze et al., 2018).

582

583 ***Tactile sensitivity positively correlates to fungiform papillae density***

584 Participants varied in FPD and oral tactile sensitivity, and these measures were positively
585 correlated; participants with higher FPD showed higher oral tactile sensitivity. As trigeminal
586 nerves surround fungiform papillae and are responsible for the mouthfeel perception
587 (Whitehead et al., 1985), FPD can be regarded as an indicator for oral tactile sensitivity.

588 Previous studies have examined the relationship between FPD and oral tactile sensitivity
589 (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone, 2003; Linne & Simons, 2017;
590 Nachtsheim & Schlich, 2013), or oral tactile sensations (e.g. roughness or astringency) (Bakke
591 & Vickers, 2008; Linne & Simons, 2017). However, findings are conflicting. Bangcuyo and
592 Simons (2017) measured the lingual tactile sensitivity using capitalized letters of different
593 sizes in forty-eight participants and concluded that oral tactile sensitivity was associated
594 with FPD ($p<0.001$, $r=0.51$). This was consistent with the study conducted by Essick et al.
595 (2003), in which they found that the variation of the tactile sensitivity using capitalized
596 letters with different sized could be influenced by the FPD in Asian participants (n=52).
597 However, Linne and Simons (2017) measured the tactile sensitivity using staircase method
598 with surface roughness from stainless steel coupons, but they did not observe any
599 relationship between FPD and tactile sensitivity. Similarly, the study of Nachtsheim and
600 Schlich (2013) did not find any relationship between FPD and intensity ratings of pressures
601 delivered by different sizes of von Frey filament in 116 volunteers. An earlier study of Bakke
602 and Vickers (2008) measured FPD in 37 participants and asked them to rate the roughness of
603 the breads which was used to reflect the tactile perception in the participants, but they did
604 not observe any relationship between the two.

605 The strength of correlation found between FPD and oral tactile sensitivity measure by
606 capitalised letters in the Bangcuyo and Simons (2017) study ($r=0.51$) was of a similar
607 magnitude to the relation found in the current study between FPD and sensitivity measured
608 by the 0.008g filament ($r=0.41$). As noted above there are various methods to measure the
609 tactile sensitivity. Von Frey filaments are used to deliver a specific force via punctate stimuli
610 (Nachtsheim & Schlich, 2013; Yackinous & Guinard, 2001) whereas the letter recognition
611 task used letters of various sizes (Bangcuyo & Simons, 2017; Essick et al., 2003). Another
612 approach used gratings that have different defined patterns onto the tongue (Linne &
613 Simons, 2017). The von Frey filament can only stimulate a very small area on the tongue,
614 which might not reflect the sensitivity of the whole tongue. Different methodologies of
615 measuring oral tactile sensitivity might result in different findings and future studies are
616 needed to standardize a quick and reliable approach for measuring the oral tactile acuity.

617 Fungiform papillae in this study were manually counted and yet previous authors have noted
618 issues with manual counting such as amorphous papillae on un-flattened tongues, small
619 papillae sizes being ignored during counting and improper staining of papillae (Garneau et al.
620 (2014)). All these issues can introduce bias in papillae counting. In this study the counting of
621 fungiform papillae was conducted independently by at least two researchers to reduce bias.
622 Several approaches on automated counting for fungiform papillae have been developed
623 (Eldeghaidy et al., 2018; Piochi et al., 2017), which can reduce inter-assessor bias and
624 increase counting accuracy. Therefore, future studies could use automated counting on
625 fungiform papillae to obtain, potentially, more accurate results coupled with saving time.
626 However, automatic counting using image analysis also has limitations, such as the

consistency of the photo brightness and whether the tongue needs to be dyed/un-dyed, which needs to be improved in the future.

The Influence of biscuit type on mouthfeel perception of biscuits

One of the study aims was to examine the relationship between individual differences in mouthfeel perception of biscuits and the sensory phenotype measurements. In particular, oral tactile sensitivity measured by von Frey filaments is predicted to influence oral mouthfeel perception of foods, and yet limited studies have investigated this influence, especially for solid foods which involve mastication. Therefore, the biscuit model was developed for this study.

In biscuit making, fat and starch are the ingredients considered to contribute predominantly to structure. Fat has a shortening role in biscuit making, which can lubricate, weaken, or shorten the structure of gluten. During mixing, water can interact with flour protein to form a gluten network which provides cohesive and extensible characteristics to the dough. However, gluten development is restricted in most types of biscuit. For example, fat can isolate the protein and starch granules from water, hence breaking the continuity of protein/starch structure (Ghotra, Dyal, & Narine, 2002). Therefore, the addition of fat has a strong impact on the final product. Biscuits produced from liquid oil have a harder texture than those produced using bakery fat (Jacob & Krishnarau, 2007). Mamat and Hill (2014) reported that different types of fat influence the textural properties of biscuits. They used palm oil (semi-solid), palm olein (liquid) and palm mid-fraction (solid) to produce developed dough ("rich tea" type) biscuits and concluded that the dough with palm mid-fraction (solid fat) resulted in the highest hardness (measured by texture profile analysis) and highest breaking force compared to other biscuits. Fat and water compete for the surface of flour particles, therefore, if the fat coats the flour before it is hydrated, the gluten network is interrupted and softer biscuits result (Mamat and Hill, 2014).

As cold butter stays in a solid state whereas melted butter is in a liquid state during biscuit processing, melted butter might be more effective in competing with water to prevent development of gluten, which may result in softer biscuits. This was indeed supported by the results of this study, from both the perception and physical properties data, although there was no difference in biscuit hardness between "cold" and "warm" butter. Doubling the proportion of butter (fat) used significantly reduced perceived and measured hardness, as well as increasing greasy perception.

The influence of fungiform papillae density, oral tactile sensitivity, and fatty acid sensitivity on mouthfeel perception of biscuits

Our study aimed to directly explore the influence of fungiform papillae density and oral tactile sensitivity on mouthfeel perception of the biscuit food model. Higher FPD was found to lead to significantly higher mean ratings of biscuit hardness and crunchiness. Similarly, greater oral tactile sensitivity (R-index using 0.02g filament) led to significantly higher ratings of hardness perception from biscuits. Although, we did not observe an influence of oral tactile sensitivity measured using the 0.008g force filament on mouthfeel perception, it is likely that this was due to the small sample size tested with the 0.008g filament (n=47;

showing a tendency for R-index measured by 0.008g filament to influence perception of biscuit hardness at p=0.086). Hypersensitivity to oleic acid was significantly related to higher hardness perception of biscuits, however this was considered an indirect relationship as increased fatty acid sensitivity was also positively related to higher FPD, which would influence both tactile and fatty acid perception. In order to determine whether gustatory fatty acid sensitivity influences fat-taste perception of a food model perhaps requires a semi-solid food model varying in fatty-acid level.

Fungiform papillae are surrounded by trigeminal nerves which can be responsible for innervating somatosensory (tactile) perception (Whitehead et al., 1985), hence the number of fungiform papillae on the tongue has been reported to influence mouthfeel perception of products (Hayes & Duffy, 2007; Nachtsheim & Schlich, 2013; Tepper & Nurse, 1997). Both Tepper and Nurse (1997) and Nachtsheim and Schlich (2013) found participants with higher FPD gave higher ratings for fat content of milk-cream samples compared to those with low FPD; similarly Hayes and Duffy (2007) found participants with high FPD gave higher scores for perceived creaminess in a sugar and fat model food matrix. These studies, that reported a relationship between FPD and oral perception, tended to be in less solid food matrices (Hayes & Duffy, 2007; Nachtsheim & Schlich, 2013, Tepper & Nurse, 1997). The study by Bakke and Vickers (2008) used solid food matrix (breads), but did not observe a relationship between FPD and mouthfeel (roughness) perception of breads, although their sample size was small (n=37). In addition, the functionalities and morphology (such as shape and size) of FP might have an impact on mouthfeel perception (Piochi, Dinnella, Prescott, & Monteleone, 2018), however the counting of FP on the tongue cannot reflect such information.

One recent study has taken a similar approach in relating oral tactile sensitivity to the perception of particles in chocolate (Breen et al., 2019). This research group used fifteen von Frey filaments rather than only the smallest two (0.02 and 0.008 g). Using all filaments, the researchers were able to calculated detection thresholds for each subject. In agreement with our study they found almost all participants were able to detect the stimuli at 0.02g, and the lack of substantial difference in detection thresholds between participants meant that these thresholds could not be related to product perception. However, they also collected discrimination thresholds between the von Frey filaments, which were found to vary more substantially between individuals. Participants that were categorised as having greater discrimination sensitivity at the centre of the tongue were able to discriminate differences in particle size between two chocolates which those with lower oral discrimination sensitivity could not; however, this relationship did not hold true for acuity at the lateral edges of the tongue. As the authors of this study point out, detection and discrimination are different cognitive tasks and hence further work could be done using both the discrimination approach of the Breen study, as well as the R-index sensitivity approach of our study, to collect oral tactile sensitivity date from larger population groups and relate them to product perception. Attention should be paid to the fact that texture/mouthfeel perception from a food results from the combination of the tactile inputs both from the tongue and the soft palate (Engelen & van der Bilt, 2008). However, von Frey filament can only stimulate a very small area of the tongue which cannot reflect the tactile sensitivity in the whole mouth. Therefore, other tactile sensitivity measurements should be considered in future studies.

Investigation of mouth behaviour and mouthfeel perception of biscuits

Participants varied in mouth behaviour and most of the participants were classified as Crunchers or Chewers. Smooshers and Suckers were the minor groups, consistent with the findings of Jeltema et al. (2014, 2015). Jeltema et al. (2014, 2015) demonstrated that participants could be classified by their mouth behaviour when manipulating food in the mouth. In addition, the later study by Jeltema et al. (2016) showed that participants in different mouth behaviour groups had diverse preferences in food texture. Our study examined the influence of mouth behaviour on oral mouthfeel perception of biscuits, however no impact of mouth behaviour on biscuit perception ratings was found.

According to studies of Jeltema et al. (2014, 2015, 2016), Crunchers and Chewers prefer to use their teeth to break the foods down, whereas Smooshers and Suckers prefer to manipulate the foods between tongue and the roof of the mouth. Smooshers like foods that can be spread throughout the mouth and can be held in mouth for a long time. The cheese biscuits developed in the present study would have been bitten by vertical compression of the teeth and then softened by saliva. It was hypothesized that fat would be released from the biscuit where participants tried to spread the biscuit fractions throughout their mouth, which might have led to the tendency for Smooshers to perceive stronger greasy mouthfeel. However, this was not concluded from the study. This might be influenced by the small sample size of Smooshers in the present study (n=11). Future research should consider a larger sample size and a wider range of food models to gain a better understanding of the influence of mouth behaviour on mouthfeel perception of foods, and to determine whether the mouth behaviour questionnaire can be used as a quick and effective tool to understand and characterize mouthfeel perceptual differences of consumers.

Relationships between BMI and oral sensory perception

Although this study was not deigned to determine relationships between BMI and sensory perception as its primary objective, two significant relationships with BMI were found. Higher BMI correlated with lower oral tactile sensitivity and to a lower perception of greasiness in biscuits. These findings are limited by the relatively low number of participants in this study (n=93) to investigate BMI which is clearly influenced by numerous factors. In addition, the relationship with oral tactile sensitivity was only found with the 0.02g von Frey filament, despite the responses to this filament being highly skewed as most participants could detect the thicker filament. In future studies it would be useful to test the relationship between sensitivity to the finer von Frey filament (0.008g) with BMI in a larger study, as this filament led to greater discrimination between participant sensitivity but was limited by testing in only 47 participants. Despite these limitations, the conclusions drawn are somewhat in-line, indirectly, with previous studies.

Several studies examined the relationship between BMI and fatty acid sensitivity (Chevrot et al., 2014; Karmous et al., 2018; Keast, Azzopardi, Newman, & Haryono, 2014; Kindleysides et al., 2017; Newman, Torres, Bolhuis, & Keast, 2016; Stewart et al., 2010; Tucker et al., 2014; Tucker et al., 2015; Zhou et al., 2016). In these studies, Stewart et al. (2010) found that subjects hypersensitive to oleic acid had a lower BMI and proposed that oral fatty acid hypersensitivity was associated with lower energy and fat intakes and lower BMI. Similarly Kindleysides et al. (2017), in a study with female participants, found BMI to be higher in women who were hyposensitive to oleic acid taste. Despite low participant numbers in these previous studies (n=54, n=50 respectively) we were not able to replicate the relationship between sensitivity to oleic acid and BMI in the current study. However, the principle for the significant relationships between BMI and other sensory factors found in the current study

are the same. Reduced oral tactile sensitivity is expected to lead to reduced mouthfeel perception from fats, which could lead to higher fat intake and result in higher BMI, as concluded in the current study. Similarly, the reduced perception of greasiness from biscuits might lead to a higher intake of greasier high-fat foods, resulting in higher BMI as concluded from the results of the current study.

Conclusion

This study clearly demonstrated individual differences in fatty acid sensitivity, fungiform papillae density, oral tactile sensitivity, and mouth behaviour. Many of these individual differences, except mouth behaviour, led to differences in product perception within the biscuit model tested. FPD had a significant positive relationship with perceived hardness and crunchiness, and similarly oral tactile sensitivity had a significant positive relationship with perceived hardness. A systematic approach relating attributes within different matrices to individual differences in oral tactile sensitivity is called for.

The characterisation methods used in this paper provide quick approaches to determine differences in oral sensory characteristics of individuals. A relationship between fatty acid taste sensitivity and fungiform papillae density was found, however this was largely dependent on the approach used to categorise the participants fatty acid taste sensitivity. FPD significantly correlated with oral tactile sensitivity, implying that oral tactile sensitivity could be used as a quick method to characterise participants. This may prove useful, for example when aiming to interpret individual perception of products varying in fat content that will subsequently influence perception within both taste and mouthfeel modalities.

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Conflict of interest

Bruce Linter is employed by PepsiCo, Inc. The views expressed in this paper are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc. All other authors declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of University of Reading Research Ethics Committee and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all participants prior to study commencement.

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