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**The Effect of Consumption Volume on Profile and Liking of Oral Nutritional
Supplements of Varied Sweetness: Sequential Profiling and Boredom Tests**

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Running Title: Sequential Profile and Liking of Oral Nutritional Supplements

Abstract

Oral nutrition supplements (ONS) are routinely prescribed to those with, or at risk of, malnutrition. Previous research identified poor compliance due to taste and sweetness. This paper investigates taste and hedonic liking of ONS, of varying sweetness and metallic levels, over consumption volume; an important consideration as patients are prescribed large volumes of ONS daily. A sequential descriptive profile was developed to determine the perception of sensory attributes over repeat consumption of ONS. Changes in liking of ONS following repeat consumption were characterised by a boredom test. Certain flavour (metallic taste, soya milk flavour) and mouthfeel (mouthdrying, mouthcoating) attributes built up over increased consumption volume ($p \leq 0.002$). Hedonic liking data from two cohorts, healthy older volunteers ($n=32$, median age 73) and patients ($n=28$, median age 85), suggested such build-up was disliked. Efforts made to improve the palatability of ONS

27 must take account of the build up of taste and mouthfeel characteristics over increased
28 consumption volume.

29

30 **Keywords:** oral nutrition supplements, sensory attributes, sequential profile, boredom test

31

32 **1. Introduction**

33 Malnutrition is a recognised problem in the elderly population, especially in hospitalised
34 subjects; 60% of older people are at risk of malnutrition, or their situation worsening, in
35 hospital (Age Concern, 2006). Identification and treatment of malnutrition in this high-risk
36 group is extremely important to reduce the risk of disease, prevent worsening of any
37 existing conditions and to maintain an optimum quality of life (O'Flynn, Peake, Hickson,
38 Foster and Frost, 2005). Oral nutrition supplements (ONS) are supplementary oral dietary
39 "food" routinely prescribed in-between meals to help improve the nutritional status of those
40 with, or at risk of, malnutrition (Lochs et al, 2006). A review of sixty-two intervention trials
41 (10,187 participants) by Milner, Potter, Vivanti and Avenell (2002) found ONS
42 supplementation to produce consistent weight gain (in 42 trials), and decreased relative
43 risk for mortality in trials where participants were undernourished (n=2461).

44 It has been suggested that the success of ONS may depend on consumption of sufficient
45 quantities over an extended time period (Rahemtulla et al., 2005). A study investigating the
46 effect of quantity of ONS consumed on weight loss and lean tissue in cancer patients,
47 identified a failure to achieve the desired supplement intake prevented patients from
48 obtaining important clinical benefits (Fearon et al., 2003). Gosney (2003) indicated that
49 compliance of ONS can be low, thus limiting the success that can be achieved from
50 prescribing ONS. A 24-hour study of 96 elder care ward patients found that two-thirds of
51 the patients given ONS drank less than 50% of the carton resulting in 63% of ONS being
52 wasted. Poor compliance with ONS has been demonstrated previously; Nolan (1999)

53 reported average wastage of two different ONS to be 41 % and 44% and Stableforth
54 (1986) showed that elderly patients with femoral neck fractures only tolerated limited
55 amounts of ONS which meant that large calorie deficits remained. Bolton et al (1992)
56 compared the long term palatability of three commercial ONS products with cancer
57 patients and found that 54% of patients discontinued the trial for flavour reasons. In the
58 2003 study, Gosney (2003) found the greatest wastage of ONS was found in patients who
59 disliked the taste (72%). Of the 67% of patients who completed questionnaires, 56% said
60 they did not like the products and specific dislikes were taste (25%), texture (19%) and
61 sweetness (38%). Other factors that were thought to decrease compliance with ONS
62 include a lack of thirst, chemosensory changes associated with ageing, the unfamiliarity of
63 cartons to elderly people, in comparison to the frequently available cups of tea, and
64 frequent spillage from cartons as a result of decreased dexterity (Gosney, 2003). Taste
65 fatigue, which tends to occur when ONS are consumed regularly over prolonged periods,
66 is thought to contribute to poor compliance (Rahemtulla et al., 2005).

67 A recent study reported age-related differences in preferred sweetness level, which were
68 in-line with increased detection and recognition thresholds for sweetness; an overall dislike
69 of ONS and dislike of the sweetness level of ONS vanilla products (Law, Gosney and
70 Kennedy, 2006; Law 2006). Literature on age related taste threshold changes, and
71 potential affects on food preference are somewhat contradictory. A number of studies have
72 shown sweet taste threshold to increase with age (Zandstra and de Graaf, 1998; Mojet,
73 Heidema and Christ-Hazelhof, 2003; Fukunaga, Uematsu and Sugimoto, 2005), whilst
74 other studies have found no significant age-related decline in sweet perception (Kaneda et
75 al, 2000; Koskinen, Kälviäinen and Tuorila 2003). Mojet, Christ-Hazelhof and Heidema
76 (2005) found no correlation between threshold sensitivity and optimal liking concentration
77 for any basic taste stimuli; however Zandstra and de Graaf (1998) did find a trend for high

78 optimal concentrations of sucrose and orange flavour in drinks for elderly subjects
79 compared to younger adults.

80 Development of ONS with lower sweetness, by replacing sucrose with an alternative
81 saccharide, palatinoseTM (α -D-glucopyranosyl-1,6-fructose), led to segmentation in
82 preference between consumers who liked the less sweet variants, and those who liked the
83 sweeter control (Methven et al, 2008). The study noted that further work was needed to
84 investigate if there was a difference in liking between ONS of different sweetness levels on
85 consumption of greater quantities, in line with the typical pack size (200 ml). However,
86 there appears to be no study in the literature which examines the specific sensory
87 attributes of ONS or their affect on liking over increasing consumption volume; this latter
88 point is likely to be extremely important in identifying potential reasons for the rejection of
89 ONS, which may arise when greater quantities of ONS are consumed.

90 In order to measure change in sensory perception over consumption time, time intensity
91 profiling (TI) is typically used (Duizer, Bloom and Findlay, 1997), however TI can only
92 characterise a maximum of two attributes per sample. A temporal dominance method
93 (Labbe, Schlich, Pineau, Gilbert and Martin, 2009) has been developed recently, although
94 one potential drawback of this method for products such as ONS could be that attributes of
95 secondary rather than primary dominance might be important determinants of product
96 liking. A previous study used progressive profiling (Jack, Piggott and Paterson, 1994) to
97 profile the textural attributes of hard cheese during mastication. In the present study this
98 idea has been progressed, with the help of Compusense, to a sequential profiling method
99 where up to five attributes are scored over consecutive tastings, at regimented time
100 intervals.

101 The present study aimed to investigate the effect of consumption volume on the sensory
102 profile and liking of ONS. In addition the study aimed to investigate if modifications of
103 sweetness and metallic levels could improve the hedonic liking of ONS.

104 **2. Materials and methods**

105 The commercial ONS (CONS) used was Ensure Vanilla Plus (Abbott Nutrition,
106 Maidenhead, UK), and Lactisole (sodium 2-(4-methoxyphenoxy)-propanoate) was used as
107 a sweetness suppressor (Domino Sugar, American Sugar Refining, USA). Standard
108 ingredients used in the manufacture of ONS were as follows : glucose syrup (Cerestar
109 01921, Cargill, Manchester, UK), sucrose (Tate and Lyle, London, UK), high oleic
110 sunflower oil, canola oil, and rape seed oil (Cargill, Liverpool, UK), sodium caseinate
111 (Bacarel, Stone, UK), milk protein concentrate (MPC85, Bacarel, Stone, UK), soy protein
112 isolate (ProFit SI90, Food Ingredient Technologies, Bedfordshire, UK); soy lecithin
113 (Emulpur IP, Cargill, Hamburg, Germany); commercial blends of emulsifier, vanilla flavour,
114 vitamins and minerals were supplied by Abbott (Abbott Nutrition, Columbus, USA).
115 Mineral water (Harrogate Spa, UK) and medium sliced white bread (Hovis, Windsor, UK)
116 were used as palate cleansers in sensory testing. Sucrose (Tate and Lyle, London, UK)
117 and iron sulphate heptahydrate (Fluka, Sigma Aldrich, Germany) were used for taste
118 threshold tests.

119 **2.1 Manufacture of ONS modifications**

120 Preparation of suppressed sweetness ONS (SSONS) was carried out by adding the
121 sweetness suppressor lactisole to the commercial vanilla ONS products (0.003mg
122 lactisole/100ml Ensure Plus Vanilla). In addition, ONS samples were manufactured on a
123 pilot scale ultra heat treatment (UHT) plant. The standard formulation (PPSONS)
124 consisted, per 100g, of glucose syrup (17g), sodium caseinate (3.5 g), sucrose (2 g), oil
125 blend (4.4g), milk protein concentrate (1.8 g), soy protein isolate (1.3 g) and a commercial
126 blend of emulsifier, flavour, vitamins and minerals. Ingredients were blended at 60 °C prior
127 to ultra heat treatment by indirect steam injection at 140°C for 27 seconds. Two
128 formulations were manufactured, the standard formulation (PPSONS) and a formulation
129 without mineral mix (PPNONS). The total solids content of all products measured by

130 refractometer, was 32%. The pH ranged from 6.6 to 6.8 and density ranged from 1.05 and
131 1.09 g/ml. All samples were stored at 4°C prior to tasting.

132 **2.2. Sensory methods**

133 All sensory evaluation (sensory panel, healthy older volunteer and patient groups) was
134 carried out at room temperature (25 °C +/- 2°C), product temperature was allowed to
135 equilibrate to room temperature; actual product temperature at serving was 20 °C (+/- 3
136 °C).

137 **2.2.1 Sensory, volunteer and patient groups**

138 This study employed three different groups to assess the products; a trained sensory
139 panel, a healthy older volunteer panel and a patient group. The trained sensory panel
140 comprised 12 adults (11 females, 1 male; median age 42 years, range 33-59), expert in
141 profiling techniques, all had over 1 years experience and had been given a minimum of 4
142 hours training on profiling of ONS. The healthy older volunteer panel comprised 32
143 healthy, older, free-living volunteers (20 females, 12 males; median age 73 years, range
144 66-88). The patients were 28 older adults (11 female, 17 male; median age 85, range 71-
145 90) in hospital with a variety of medical conditions. Permission for the studies with the first
146 two panels was granted by the University of Reading Research Ethics committee and the
147 study with patients was approved by the Berkshire National Research Ethics committee
148 (NRES 08H0505176). All participants gave written informed consent prior to taking part in
149 the study.

150 Quantification with the trained panel took place in isolated booths, under artificial daylight
151 unless specified otherwise. Healthy older volunteer panels took place in a central location,
152 using isolated tables; lighting was standard fluorescent lighting. Patients were studied
153 individually at their bedside, under standard hospital lighting conditions..

154 **2.2.2. Sequential profiling**

155 The trained sensory panel characterised five specific sensory attributes of various ONS in
156 a sequential profile. This is a descriptive profiling method developed to determine the
157 perception of sensory attributes upon repeat consumption of ONS over time. Panellists
158 tasted eight consecutive aliquots (5 ml) of each ONS sample and were instructed to score
159 the selected five attributes following each of the eight tastings. For each tasting, panellists
160 were also instructed to score the same five attributes as after-effects, following 30 s and
161 60 s time delays. A two minute time delay was enforced between samples. Panellists
162 scored each attribute on unstructured line scales with the appropriate anchors.
163 Compusense five was used to design and run the profile and capture data.

164 The five attributes scored were sweet, metallic, soya milk flavour, mouthcoating and
165 mouthdrying. In a previous full quantitative descriptive analysis (QDA) profile of four
166 commercial products (Ensure Vanilla Plus, Abbott Nutrition UK; Fortisip Vanilla, Nutricia
167 Clinical Care UK; Resource Shake Vanilla and Clinutren Vanilla, Nestle Nutrition France)
168 sweet taste was found to be significantly different between samples ($p=0.03$), soya milk
169 flavour was only found to be significant as an aftertaste ($p=0.03$) (data not shown). QDA
170 did not reveal significant differences in metallic taste, mouthdrying or mouthcoating; and
171 yet these characteristics were thought to be distinct in ONS. The trained panel commented
172 on this and noted that these attributes appeared to last in the mouth beyond the profiling
173 session. It was, therefore, decided to study metallic, mouthdrying and mouthcoating,
174 alongside sweet taste and soya milk flavour, using the sequential profile.

175 Sequential profile data was collected for the following ONS: standard commercial vanilla
176 ONS (CONS) (Ensure Plus), sweetness suppressed vanilla ONS (SSONS; Lactisole in
177 Ensure; 0.003g/100ml), pilot ONS control (PPSONS, with vitamins and minerals) and pilot
178 ONS with no mineral addition (PPNONS).

179 The commercial products (with and without lactose) were tasted in one week, in replicate,
180 samples presented in a balanced order. The pilot plant products were presented in

181 replicate in a separate week, in balanced order. Samples were coded with 3-digit numbers;
182 however, all samples which were the same received the same code (panellist not blinded
183 to sequential protocol). Still mineral water and bread were provided as palate cleansers in-
184 between product samples (not between the eight consecutive aliquots of the same
185 sample). Panellists were instructed to drink all the sample volume presented and were not
186 permitted to drink water during sequential profiling.

187 **2.2.2.1 Sequential profiling method validation**

188 In order to validate the sequential profiling method a further evaluation of CONS was
189 carried out, where panellists were given eight consecutive aliquots (5 ml) of the same
190 sample, however, they were blinded to the test procedure, each aliquot had a unique three
191 digit code and these were presented in a balanced order. Time of tastings and scoring
192 after-effects were controlled in the same manner as the sequential profile.

193 **2.2.3. Taste detection threshold tests**

194 For all groups, taste thresholds were determined by forced-choice ascending
195 concentration method (ASTM, 1997). Each assessor received a series of 3-alternative
196 forced choice (3-AFC) sets, each set comprised a taste solution (prepared in mineral
197 water) and two water samples at room temperature (balanced presentation order). Sets
198 were presented once, in order of increasing concentration, increased by a geometric
199 progression of two. Five iron sulfate (metallic) solutions were prepared from 2.8 to
200 44.8mg/L, six sucrose solutions were prepared from 0.34 to 10.88g/L and ranges were
201 within those recommended by ISO 3972 (ISO, 1991). Patients received only sweet
202 solutions and only in five different concentrations, from 0.68 to 10.88 g/L. Samples were
203 coded with 3-digit random numbers. For metallic solutions, red light conditions were used
204 for the trained panel and sample cups with sip lids were used for the older volunteer panel.
205 Volunteers were instructed to choose the odd-one-out and comment on the taste which
206 they perceived in the most different sample. Individual detection thresholds were

207 calculated as the geometric mean of the detection threshold and the concentration
208 preceding this.

209 **2.2.4 Hedonic tests**

210 Hedonic liking data was collected from 32 healthy older adults and 28 patients, using a
211 modified boredom test (Köster and Mojet, 2007). This was used to characterise any
212 changes in liking of ONS following repeat consumption and to compare the liking of pairs
213 of samples. All subjects began by tasting 5ml of each of two samples (random 3 digit
214 coded, balanced presentation order) and scored liking for each on a 9-point hedonic scale
215 (initial liking), scaled from dislike extremely to like extremely. They then tasted a series of
216 eight consecutive 5ml aliquots of one sample (balanced presentation across volunteers,
217 samples coded by symbol) and were permitted to drink mineral water, if desired after
218 tasting the first four aliquots of the series of eight. Subjects subsequently tasted a further
219 5ml of each of the two samples (random 3 digit coded, balanced presentation order), re-
220 scored their liking for each on the 9-point hedonic scale (final liking) and were asked to
221 state the sweetest sample of the final two samples. Subjects consumed 60ml of ONS in
222 total. The boredom trial was modified for the patient group in that the central eight 5 ml
223 aliquots were replaced by a central cup containing the full 40 ml of sample as it was
224 impractical to present 12 small cups on one tray at a patient's bedside. Patients were also
225 their sugar usage in tea and/or coffee.

226 **2.3. Statistical analysis**

227 SENPAQ (version 3.2) was used to carry out analysis of variance (ANOVA) and principal
228 component analysis (PCA) of sensory panel profiling data. In order to determine the
229 effects of time from the sequential profiling, three-way ANOVA was carried out in XLSTAT
230 (version 2009.1.02), using sample (n=2), assessors (n=12) and time (n=8) as explanatory
231 variables. Non-parametric testing on the liking data and ANOVA on taste threshold data
232 were also carried out in XLSTAT (version 2009.1.02).

233 3. Results and discussion

234 3.1. Sensory data

235 3.1.1. Sequential Profile

236 3.1.1.1 Standard ONS and sweetness suppressed ONS

237 Sequential profile data was collected for commercial ONS (CONS) to characterise if
238 changes in perception of sensory attributes occurred over repeat consumption of a typical
239 commercial ONS. Sequential profiling was also carried out on the sweetness suppressed
240 variant (SSONS) to determine the effect of sweetness suppression on the perception of
241 sensory attributes over repeat consumption; the interest in sweetness suppression was
242 triggered by previous research which identified a disliking for the sweet taste of ONS
243 (Gosney, 2003; Methven et al, 2008).

244 Figure 1 illustrates how perception of the five selected sensory attributes varied with
245 repeat consumption of standard vanilla Ensure ONS (CONS). Mouthdrying, metallic,
246 mouthcoating and soya milk flavour built up significantly over time ($p<0.0001$, $p=0.002$,
247 $p<0.0001$ and $p<0.0001$ respectively). Unlike the aforementioned attributes, sweetness did
248 not build over repeat consumption, it peaked at sips and decreasing as after-effects.
249 Figure 2 compares the standard sweet (CONS) and the sweetness suppressed (SSONS)
250 variants for three attributes. The SSONS was perceived as significantly less sweet
251 ($p<0.0001$; initial mean scores 24 and 48 respectively). It was also significantly more
252 mouthdrying ($p<0.0001$), although the difference was less substantial as tastings
253 progressed, (mean scores at second sip of 42 and 36 respectively). It is likely that the
254 sweeter sample is perceived as less mouthdrying due to the sweet taste interfering with
255 the drying perception; a previous study found sweetened soymilk to be less astringent than
256 its unsweetened counterpart (Courrelongue, Schlich and Noble, 1999). There was no
257 significant difference in the metallic perception of the two products, the soya milk flavour or
258 mouthcoating (data not shown).

259 **3.1.1.2 ONS control and No-Mineral ONS formulations**

260 It was hypothesised that the minerals added to ONS during manufacture may contribute to
261 both astringent and metallic tastes. The mineral supplementation added to ONS contains
262 iron sulfate, known to impart metallic taste (Lim and Lawless, 2006). Minerals, particularly
263 zinc, are also known to impart astringent properties to solutions (Yang and Lawless, 2005).
264 To test the hypothesis, a control ONS formulation (PPSONS) that contained the full
265 mineral supplement and a formulation that had no mineral supplementation (PPNONS)
266 were manufactured. Figure 3 demonstrates the mouthdrying and metallic profiles of these
267 two ONS products. As with commercial ONS; metallic and mouthdrying built up
268 significantly over consumption time ($p=0.001$ and $p<0.0001$ respectively) for both products.
269 On first consumption (5 ml) the mineral free product had a lower mean for metallic taste
270 (21.5 compared to 24.2) although the difference was not significant. Over all of the
271 consumption period (eight 5 ml samples) the mineral free product (PPNONS) was
272 significantly less metallic ($p<0.0001$), although the difference in overall means across time
273 was very small (25.2 and 26.7 respectively). It is therefore noted that although the minerals
274 added to the ONS formulation do contribute to the metallic taste, expected as the
275 supplementation contains iron sulfate, this cannot be the only source of metallic taste in
276 the products. The mineral supplementation is not thought to be the major source of
277 mouthdrying as the two products did not differ significantly in mouthdrying. It is
278 hypothesised that another source of mouthdrying could be the milk proteins typically used
279 in ONS formulations. Previous studies have shown whey proteins to cause mouthdrying
280 through precipitation onto the tongue (Sano, Egashira, Kinekawa and Kitabatake, 2005);
281 alternatively proteolysis of β -casein can yield γ -caseins which are associated with
282 perceived dryness of milks (Harwalkar, Cholette, McKellar and Emmons, 1993).

283 **3.1.1.3 Validation of the sequential profile**

284 Given that panellists were asked to score the same attributes over time during sequential
285 profiling, their expectation might be that certain attributes were expected to build up over
286 time. However, in the first and subsequent sequential profile sessions four attributes
287 (mouthdrying, metallic, mouthcoating, and soya milk flavour) were found to build with time,
288 whereas sweetness did not. It was not thought likely that the panellists anticipated that
289 certain attributes would build over time and others would not. To further validate the
290 sequential profiling method, panellists were given eight consecutive aliquots of the same
291 sample, and blinded to the fact that the samples were identical. Figure 4 demonstrates that
292 the two methods did not give identical results. The panellists contributing to the data
293 acquired by both methods were the same, however, the batch codes of the samples were
294 different and the methods were run in different weeks. As the panellists were not using any
295 reference standard, it is expected that absolute values for the samples varied between the
296 methods; it is whether the trends differ that is important.

297 The two profiles (where panelists blinded to the sequential nature of the profile, and where
298 they were not blinded) gave very similar trends for sweetness; there was a significant
299 difference between results from the two methods ($p=0.001$) and no significant change over
300 consumption time. For metallic taste, the panellists record a more substantial increase in
301 metallic taste over consumption time when not blinded to the sequential profiling, however
302 the trends for both methods was the same. There was a significant difference between the
303 two methods ($p<0.0001$), but still a significant overall increase in metallic taste with time
304 ($p<0.0001$), with the not-blinded sequential profile finding a mean increase of 19 (from 18
305 to 37) and the blinded sequential profile a mean increase of 9 (from 13 to 21). Similarly for
306 mouthdrying and soy milk flavour (data not shown), there were significant differences in
307 the results from the two methods ($p=0.01$, $p=0.05$), but a significant increase with
308 increased consumption overall ($p=0.026$, $p<0.0001$). There was no significant difference
309 between the methods for mouthcoating (data not shown) and an overall increase in

310 mouthcoating with increasing consumption ($p=0.001$). In conclusion, it was found that
311 panellists may exaggerate increase in perception were they aware that they had
312 performed a sequential profile; however, the significant changes found over time were the
313 same whether panelists were blinded to the sequential nature or not.

314 Panellists received two ONS samples in one sequential profiling session; they therefore
315 consumed 80ml per session. This amount is in line with typical volumes of ONS consumed
316 in hospitals, as previously reported; Gosney (2003) identified that only 37% of ONS were
317 consumed, which is approximately 80ml, assuming a typical pack size of 220ml. The data
318 from the commercial sequential profiles is therefore likely to represent the sensory
319 characteristics perceived by patients consuming similar volumes of these products.

320 **3.1.2 Taste threshold tests**

321 **3.1.2.1 Metallic taste thresholds**

322 Metallic detection threshold tests were conducted to identify whether older consumers
323 could potentially identify the metallic attribute in the ONS, and to determine any difference
324 in metallic threshold between younger and older adults (Figure 5). However, it was
325 surprising that only 60% of the trained sensory panellists (median age 42) correctly
326 identified any sample differences in the metallic threshold test; the group best estimated
327 metallic threshold (geometric mean) for these panellists was 16mg/L. Forty percent of the
328 sensory panellists could not detect metallic at the maximum concentration of 45mg/L. In
329 comparison, only 32% of the healthy older volunteers (median age 73) correctly identified
330 any sample differences in the metallic threshold test; the group best estimated metallic
331 threshold for these volunteers was 26mg/L. The higher metallic detection threshold and
332 higher proportion of non-detectors observed in the group of healthy older volunteers was
333 expected as several studies have found elevated thresholds for taste and a diminished
334 ability to discriminate between suprathreshold stimuli (Schiffman and Graham, 2000).
335 However, the present study also questions the validity of using the 3-AFC test as a

suitable test for metallic taste threshold determination. Metallic taste tends to be noticed as an aftertaste and, as shown in the sequential profiling results, it builds with time and is difficult to clear from the palate. Therefore, false identification is likely to arise from the 3-AFC tests as a result of build up from previous samples tasted. If the sensory panellists were truly unable to detect iron sulfate as metallic at 45 mg/L, it is unlikely that they would detect metallic taste in the ONS where the iron levels are typically around 20 mg/L, unless most of the metallic taste perceived is not attributed to the iron sulfate. In a previous study (n=18, mean age 24) the group best estimated threshold for iron sulfate was 27.5 mg/L (99 mmol/L), with a large standard deviation of 125mg/L (452mmol/L) (Lim and Lawless, 2006); this study also used the 3-AFC test method.

3.1.2.2 Sweet taste thresholds

The mean sweet detection thresholds for the sensory panel (median age 42), healthy older volunteers (median age 73) and patients (median age 85) were 2g/L, 3g/L and 5.5 g/L respectively. The median age of the older volunteers and patients combined was 78 years. The distribution of sweetness thresholds is given in Figure 6, which suggests an increase in sweet taste threshold with increasing age, as supported by previous literature (Zandstra and de Graaf, 1998; Mojet et al, 2003; Fukunaga et al 2005). Indeed, when the healthy older volunteers were divided into two age categories; 66 to 77 and 78 to 88 (below and above overall median age), the sweet taste thresholds were 2.6 and 4.1 g/L respectively, although this difference was not significant. The higher taste thresholds of the patients compared to the older volunteers cannot be explained by age alone. Combining the healthy older volunteer and patient data together and analysing for the effect of group (healthy or patient) and age (< or > 78) by ANOVA; the group had a significant effect on sweet taste threshold (p=0.005), whereas the age did not. It is, therefore, hypothesised that illness and medication have a greater effect on sweetness thresholds than age. Illness and medication are known to taste thresholds increases as well as a wide range of taste

disturbances; this area has been previously reviewed by Schiffman (Schiffman and Zervakis, 2002). The patient cohort were prescribed an average of 4.5 medications (range 0-11) of which an average of 1.4 (range 0 to 3) were known have the capacity to cause taste disturbance (British National Formulary, 2009). The healthy older volunteers were prescribed an average of 2.1 medications (range 0-11) of which an average of 0.7 (range 0 to 3) had the capacity to cause taste disturbance.

3.2. Hedonic data

3.2.1. Boredom test

Mean liking scores for standard ONS (CONS) and the sweetness suppressed ONS (SSONS), at start and end of the boredom test, are given in Table 3. With both older cohorts, the mean initial liking of the standard vanilla ONS was significantly higher than the initial liking of the sweetness suppressed ONS ($p \leq 0.05$). However, there was a difference between the cohorts in their change in liking from start to end of the boredom test. The healthy volunteer mean liking of the standard ONS significantly decreased during the boredom test from 6.3 to 5.0 ($p \leq 0.001$). This was irrespective of whether they received 40 ml of CONS or SSONS during the boredom test (sample received in-between the initial and final liking pairs). The liking of the SSONS did not change over time for the volunteer cohort. In contrast, there was not a decrease in liking of the standard product during the boredom test for the patient cohort. However, their liking of the SSONS did decrease significantly over the boredom test, irrespective of the boredom sample ($p \leq 0.05$). One point to note in carrying out the boredom trials with the patient group, as the central boredom sample was contained in one cup as a 40 ml sample, rather than as eight individual 5 ml samples, there was a tendency for patients not to consumer the full 40 ml which is likely to have reduced any effect of change in liking over the boredom test. The main conclusions from the boredom liking tests were that overall liking of the CONS was greater than the SSONS. As liking was found to decrease with repeat consumption, it

388 is likely that consumption of a typical pack volume may reduce liking of the products even
389 further. It is hypothesised that the attributes of mouthdrying and metallic which were found
390 in the sequential profiling study to build substantially over consumption volume may, in
391 part, cause the reduction in liking.

392 **3.2.2 Consideration of sugar usage and sweetness thresholds on ONS liking**

393 There were 17 patients who regularly took sugar in their tea or coffee and 11 who did not.
394 There was no correlation between sugar usage and sweetness threshold. In addition,
395 there was no correlation between sugar usage or sweetness threshold and liking scores
396 for the standard ONS in comparison to the sweetness suppressed variant. The later point
397 supports the previous study by Mojet et al (2005) which found sweetness thresholds not to
398 correlate with preferred sweetness level. The volunteers and patients could determine that
399 the standard ONS sample was sweeter than the SSONS ($p < 0.0001$; 26 out of 32
400 volunteers; 25 out of 27 patients). Healthy older volunteers and patients who incorrectly
401 identified which sample was the sweetest, did not have the highest sweetness thresholds;
402 implying that the sweetness of the products was above each individuals sweetness
403 threshold. This also demonstrates that sugar consumption in hot beverages did not impact
404 upon ONS sweetness perception.

405 **4. Conclusions**

406 Sequential profiling was used to characterise five attributes of vanilla dairy-based ONS
407 over repeat consumption. This highlighted a significant build up of mouthdrying, metallic
408 and mouthcoating attributes over a total consumption volume of 40 ml, which would not
409 have been found though a standard profiling study. Such build may have major
410 implications on the long-term, repeat consumption of these products, especially since
411 patients are often encouraged to drink up to 600ml daily. Liking of ONS, with both healthy
412 older and older patient groups, was found to diminish over repeat consumption (60ml),
413 suggesting that build up of taste and mouthfeel attributes over repeat consumption was

414 disliked. The combined use of sequential profiling and liking over repeat consumption
415 (using a boredom test approach) is recommended as a methodology suitable for the
416 exploration of products such as ONS which are known to have aftertastes.

417 Removal of the minerals from an ONS formulation did not significantly reduce mouthdrying
418 and although the effect on metallic taste perception was significant, it was not substantial.
419 Components other than iron sulfate, intrinsic to ONS, such as the calcium and milk
420 proteins, may contribute to these attributes. In support of this, calcium salts have been
421 shown to exhibit both astringent and metallic taste properties (Lawless, Rapacki, Horne
422 and Hayes, 2003) and both whey protein precipitation and casein proteolysis products
423 have been associated with mouthdrying (Sano et al, 2005; Harwalkar et al, 1993). Further
424 research into the properties of ONS ingredients may help to elucidate potential causes of
425 the build up of attributes over repeat consumption. If the build up can be reduced this may
426 lead to improve palatability and consumption of ONS.

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510

511 **Figure 1: Sequential profile of commercial vanilla ONS**

512 **Footnote figure 1:**
 513 ^(a)Sip 1–8, consumption point of 5 ml aliquots; AE1, AE2, after-effects at 30 s and 60 s post consumption
 514 of aliquots 1–8.
 515

516 **Figure 2: Sequential profiles of two sweetness variants of vanilla ONS**

517 **Footnote figure 2:**
 518 ^(a)Sip 1–8, consumption point of 5 ml aliquots; AE1, AE2, after-effects at 30 s and 60 s post consumption
 519 of aliquots 1–8. CONS = Standard commercial ONS ; SSONS = Sweetness Suppressed commercial
 520 ONS
 521

522 **Figure 3: Sequential profiles of two mineral variants of vanilla ONS**

523 **Footnote figure 3:**
 524 ^(a)Sip 1–8, consumption point of 5 ml aliquots; AE1, AE2, after-effects at 30 s and 60 s post consumption
 525 of aliquots 1–8. PPSONS = control pilot plant ONS; PPNONS = No mineral pilot plant ONS
 526

527 **Figure 4: Validation of sequential profiling, used to quantify three attributes of**
528 **commercial vanilla ONS over eight consecutive (5 ml) consumptions**

529 **Footnote figure 4:**

530 ^(a)Sip 1–8, consumption point of 5 ml aliquots; AE1, AE2, after-effects at 30 s and 60 s post consumption
531 of aliquots 1–8. Blind= panellists not aware that consecutive samples were the same sample; Sequential
532 = panelists aware profile was sequential
533

534 **Figure 5: Frequency distribution of metallic taste detection thresholds for sensory**
535 **panellists and healthy older volunteers**

536

537 **Figure 6: Frequency distribution of sweet taste detection thresholds for sensory**
538 **panellists, healthy older volunteers and patients**

539

Table 1. Liking Scores for Standard Commercial (CONS) and Sweetness Suppressed (SSONS) ONS at Initial and End Tasting, following a Boredom Test, for a healthy older volunteer cohort (n=32) and a patient cohort (n=28)

Cohort	Product	Mean Liking ^a (Irrespective of sample used for 40ml boredom)			Mean Liking ^b (Participants consuming CONS for Boredom phase)			Mean Liking ^c (Participants consuming SSONS for Boredom phase)		
		Initial	Final	Sig ^d	Initial	Final	Sig ^d	Initial	Final	Sig ^d
Healthy	CONS	6.3±1.7	5.0±1.9	***	6.4±1.4	5.6±1.4	*	6.1±2.0	4.3±2.2	**
Older	SSONS	5.2±1.9	5.5±1.9	ns	5.2±1.7	5.0±1.6	ns	5.3±2.2	6.1±2.1	ns
Volunteers	Sig ^e	*	ns		*	ns		ns	ns(p=0.08)	
Patients	CONS	6.8±1.8	6.7±1.8	ns	6.9±1.3	6.3±1.2	ns (p=0.06)	6.8±2.2	7.0±2.1	ns
	SSONS	6.1±1.9	5.2±2.3	*	6.2±1.3	4.9±2.0	*	6.1±2.2	5.4±2.5	ns
	Sig ^e	*	***		ns	*		ns (p=0.06)	*	

^aPreference data represents mean scores ± standard deviation, from a 9-point hedonic scale for all volunteers; ^b18 volunteers and 12 patients consumed CONS during the boredom phase, ^c14 volunteers and 16 patients consumed SSONS during the boredom phase ^dSignificance of difference between initial and final liking, as shown by ANOVA: p<0.001 (***), p<0.01 (**), P<0.05 (*), not significant (ns). . ^eSignificance of difference between CONS and SSONS.