1	The Effect of Consumption Volume on Profile and Liking of Oral Nutritional								
2	Supplements of Varied Sweetness: Sequential Profiling and Boredom Tests								
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13	Running Title: Sequential Profile and Liking of Oral Nutritional Supplements								
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15	Abstract								
16	Oral nutrition supplements (ONS) are routinely prescribed to those with, or at risk of,								
17	malnutrition. Previous research identified poor compliance due to taste and sweetness.								
18	This paper investigates taste and hedonic liking of ONS, of varying sweetness and metallic								
19	levels, over consumption volume; an important consideration as patients are prescribed								
20	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 ($p \le 0.002$). 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 must take account of the build up of taste and mouthfeel characteristics over increasedconsumption volume.

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

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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 hospital (Age Concern, 2006). Identification and treatment of malnutrition in this high-risk 35 36 group is extremely important to reduce the risk of disease, prevent worsening of any 37 existing conditions and to maintain an optimum guality of life (O'Flynn, Peake, Hickson, Foster and Frost, 2005). Oral nutrition supplements (ONS) are supplementary oral dietary 38 39 "food" routinely prescribed in-between meals to help improve the nutritional status of those with, or at risk of, malnutrition (Lochs et al, 2006). A review of sixty-two intervention trials 40 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 quantities over an extended time period (Rahemtulla et al., 2005). A study investigating the 45 effect of quantity of ONS consumed on weight loss and lean tissue in cancer patients, 46 identified a failure to achieve the desired supplement intake prevented patients from 47 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 the patients given ONS drank less than 50% of the carton resulting in 63% of ONS being 51 52 wasted. Poor compliance with ONS has been demonstrated previously; Nolan (1999)

reported average wastage of two different ONS to be 41 % and 44% and Stableforth 53 (1986) showed that elderly patients with femoral neck fractures only tolerated limited 54 amounts of ONS which meant that large calorie deficits remained. Bolton et al (1992) 55 56 compared the long term palatability of three commercial ONS products with cancer patients and found that 54% of patients discontinued the trial for flavour reasons. In the 57 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 sweetness (38%). Other factors that were thought to decrease compliance with ONS 61 62 include a lack of thirst, chemosensory changes associated with ageing, the unfamiliarity of cartons to elderly people, in comparison to the frequently available cups of tea, and 63 frequent spillage from cartons as a result of decreased dexterity (Gosney, 2003). Taste 64 fatigue, which tends to occur when ONS are consumed regularly over prolonged periods, 65 is thought to contribute to poor compliance (Rahemtulla et al., 2005). 66

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

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

80 Development of ONS with lower sweetness, by replacing sucrose with an alternative saccharide, palatinoseTM (α -D-glucopyranosyl-1,6-fructose), led to segmentation in 81 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 consumption of greater quantities, in line with the typical pack size (200 ml). However, 85 there appears to be no study in the literature which examines the specific sensory 86 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 secondary rather than primary dominance might be important determinants of product 95 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**

The commercial ONS (CONS) used was Ensure Vanilla Plus (Abbott Nutrition, 105 Maidenhead, UK), and Lactisole (sodium 2-(4-methoxyphenoxy)-propanoate) was used as 106 a sweetness suppressor (Domino Sugar, American Sugar Refining, USA). Standard 107 ingredients used in the manufacture of ONS were as follows : glucose syrup (Cerestar 108 01921, Cargill, Manchester, UK), sucrose (Tate and Lyle, London, UK), high oleic 109 110 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, vitamins and minerals were supplied by Abbott (Abbott Nutrition, Columbus, USA). 114 Mineral water (Harrogate Spa, UK) and medium sliced white bread (Hovis, Windsor, UK) 115 116 were used as palate cleansers in sensory testing. Sucrose (Tate and Lyle, London, UK) and iron sulphate heptahydrate (Fluka, Sigma Aldrich, Germany) were used for taste 117 118 threshold tests.

119 **2.1 Manufacture of ONS modifications**

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

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

132 **2.2. Sensory methods**

All sensory evaluation (sensory panel, healthy older volunteer and patient groups) was carried out at room temperature (25 °C +/- 2°C), product temperature was allowed to equilibrate to room temperature; actual product temperature at serving was 20 °C (+/- 3 °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 profiling techniques, all had over 1 years experience and had been given a minimum of 4 141 142 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 71-90) in hospital with a variety of medical conditions. Permission for the studies with the first 145 two panels was granted by the University of Reading Research Ethics committee and the 146 147 study with patients was approved by the Berkshire National Research Ethics committee (NRES 08H0505176). All participants gave written informed consent prior to taking part in 148 149 the study.

Quantification with the trained panel took place in isolated booths, under artificial daylight unless specified otherwise. Healthy older volunteer panels took place in a central location, using isolated tables; lighting was standard fluorescent lighting. Patients were studied 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 a sequential profile. This is a descriptive profiling method developed to determine the 156 perception of sensory attributes upon repeat consumption of ONS over time. Panellists 157 158 tasted eight consecutive aliquots (5 ml) of each ONS sample and were instructed to score the selected five attributes following each of the eight tastings. For each tasting, panellists 159 160 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.

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

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

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

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

187 **2.2.2.1 Sequential profiling method validation**

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

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 44.8mg/L, six sucrose solutions were prepared from 0.34 to 10.88g/L and ranges were 200 201 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 for the trained panel and sample cups with sip lids were used for the older volunteer panel. 204 Volunteers were instructed to choose the odd-one-out and comment on the taste which 205 206 they perceived in the most different sample. Individual detection thresholds were

207 calculated as the geometric mean of the detection threshold and the concentration208 preceding this.

209 **2.2.4 Hedonic tests**

210 Hedonic liking data was collected from 32 healthy older adults and 28 patients, using a modified boredom test (Köster and Mojet, 2007). This was used to characterise any 211 212 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 tasting the first four aliguots of the series of eight. Subjects subsequently tasted a further 218 219 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 aliquots were replaced by a central cup containing the full 40 ml of sample as it was 223 224 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**

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

- **3. Results and discussion**
- **3.1. Sensory data**
- 235 **3.1.1. Sequential Profile**

236 **3.1.1.1 Standard ONS and sweetness suppressed ONS**

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

Figure 1 illustrates how perception of the five selected sensory attributes varied with 244 repeat consumption of standard vanilla Ensure ONS (CONS). Mouthdrying, metallic, 245 mouthcoating and soya milk flavour built up significantly over time (p<0.0001, p=0.002, 246 247 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. Figure 2 compares the standard sweet (CONS) and the sweetness suppressed (SSONS) 249 250 variants for three attributes. The SSONS was perceived as significantly less sweet (p<0.0001; initial mean scores 24 and 48 respectively). It was also significantly more 251 mouthdrying (p<0.0001), although the difference was less substantial as tastings 252 progressed, (mean scores at second sip of 42 and 36 respectively). It is likely that the 253 254 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 its unsweetened counterpart (Courrelongue, Schlich and Noble, 1999). There was no 256 significant difference in the metallic perception of the two products, the soya milk flavour or 257 258 mouthcoating (data not shown).

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

It was hypothesised that the minerals added to ONS during manufacture may contribute to 260 261 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 zinc, are also known to impart astringent properties to solutions (Yang and Lawless, 2005). 263 To test the hypothesis, a control ONS formulation (PPSONS) that contained the full 264 265 mineral supplement and a formulation that had no mineral supplementation (PPNONS) 266 were manufactured. Figure 3 demonstrates the mouthdrying and metallic profiles of these two ONS products. As with commercial ONS; metallic and mouthdrying built up 267 268 significantly over consumption time (p=0.001 and p<0.0001 respectively) for both products. On first consumption (5 ml) the mineral free product had a lower mean for metallic taste 269 270 (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 significantly less metallic (p<0.0001), although the difference in overall means across time 272 273 was very small (25.2 and 26.7 respectively). It is therefore noted that although the minerals added to the ONS formulation do contribute to the metallic taste, expected as the 274 supplementation contains iron sulfate, this cannot be the only source of metallic taste in 275 276 the products. The mineral supplementation is not thought to be the major source of mouthdrying as the two products did not differ significantly in mouthdrying. It is 277 278 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 on β -casein can yield y-caseins which are associated with 282 perceived dryness of milks (Harwalkar, Cholette, McKellar and Emmons, 1993).

3.1.1.3 Validation of the sequential profile

Given that panellists were asked to score the same attributes over time during sequential 284 profiling, their expectation might be that certain attributes were expected to build up over 285 time. However, in the first and subsequent sequential profile sessions four attributes 286 287 (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 reference standard, it is expected that absolute values for the samples varied between the 295 296 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 the trends for both methods was the same. There was a significant difference between the 302 two methods (p<0.0001), but still a significant overall increase in metallic taste with time 303 304 (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 increased consumption overall (p=0.026, p<0.0001). There was no significant difference 308 309 between the methods for mouthcoating (data not shown) and an overall increase in

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

Panellists received two ONS samples in one sequential profiling session; they therefore consumed 80ml per session. This amount is in line with typical volumes of ONS consumed in hospitals, as previously reported; Gosney (2003) identified that only 37% of ONS were consumed, which is approximately 80ml, assuming a typical pack size of 220ml. The data from the commercial sequential profiles is therefore likely to represent the sensory 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**

Metallic detection threshold tests were conducted to identify whether older consumers 322 could potentially identify the metallic attribute in the ONS, and to determine any difference 323 324 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 identified any sample differences in the metallic threshold test; the group best estimated 326 327 metallic threshold (geometric mean) for these panellists was 16mg/L. Forty percent of the sensory panellists could not detect metallic at the maximum concentration of 45mg/L. In 328 comparison, only 32% of the healthy older volunteers (median age 73) correctly identified 329 any sample differences in the metallic threshold test; the group best estimated metallic 330 331 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 expected as several studies have found elevated thresholds for taste and a diminished 333 ability to discriminate between suprathreshold stimuli (Schiffman and Graham, 2000). 334 335 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 336 an aftertaste and, as shown in the sequential profiling results, it builds with time and is 337 338 difficult to clear from the palate. Therefore, false identification is likely to arise from the 3-339 AFC tests as a result of build up from previous samples tasted. If the sensory panellists 340 were truly unable to detect iron sulfate as metallic at 45 mg/L, it is unlikely that they would 341 detect metallic taste in the ONS where the iron levels are typically around 20 mg/L, unless 342 most of the metallic taste perceived is not attributed to the iron sulfate. In a previous study 343 (n=18, mean age 24) the group best estimated threshold for iron sulfate was 27.5 mg/L (99 344 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. 345

346 **3.1.2.2 Sweet taste thresholds**

347 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 348 349 respectively. The median age of the older volunteers and patients combined was 78 years. 350 The distribution of sweetness thresholds is given in Figure 6, which suggests an increase 351 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 352 353 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, 354 although this difference was not significant. The higher taste thresholds of the patients 355 compared to the older volunteers cannot be explained by age alone. Combining the 356 357 healthy older volunteer and patient data together and analysing for the effect of group 358 (healthy or patient) and age (< or > 78) by ANOVA; the group had a significant effect on 359 sweet taste threshold (p=0.005), whereas the age did not. It is, therefore, hypothesised 360 that illness and medication have a greater effect on sweetness thresholds than age. Illness 361 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.

368 **3.2. Hedonic data**

369 3.2.1. Boredom test

370 Mean liking scores for standard ONS (CONS) and the sweetness suppressed ONS 371 (SSONS), at start and end of the boredom test, are given in Table 3. With both older 372 cohorts, the mean initial liking of the standard vanilla ONS was significantly higher than the initial liking of the sweetness suppressed ONS (p≤0.05). However, there was a difference 373 374 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 375 376 boredom test from 6.3 to 5.0 (p≤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 377 378 and final liking pairs). The liking of the SSONS did not change over time for the volunteer 379 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 380 significantly over the boredom test, irrespective of the boredom sample (p≤0.05). One 381 point to note in carrying out the boredom trials with the patient group, as the central 382 383 boredom sample was contained in one cup as a 40 ml sample, rather than as eight 384 individual 5 ml samples, there was a tendency for patients not to consumer the full 40 ml 385 which is likely to have reduced any effect of change in liking over the boredom test.

386 The main conclusions from the boredom liking tests were that overall liking of the CONS 387 was greater than the SSONS. As liking was found to decrease with repeat consumption, it

is likely that consumption of a typical pack volume may reduce liking of the products even further. It is hypothesised that the attributes of mouthdrying and metallic which were found in the sequential profiling study to build substantially over consumption volume may, in 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 the standard ONS sample was sweeter than the SSONS (p<0.0001; 26 out of 32 399 volunteers; 25 out of 27 patients). Healthy older volunteers and patients who incorrectly 400 401 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**

Sequential profiling was used to characterise five attributes of vanilla dairy-based ONS 406 407 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 patients are often encouraged to drink up to 600ml daily. Liking of ONS, with both healthy 411 older and older patient groups, was found to diminish over repeat consumption (60ml), 412 413 suggesting that build up of taste and mouthfeel attributes over repeat consumption was

disliked. The combined use of sequential profiling and liking over repeat consumption (using a boredom test approach) is recommended as a methodology suitable for the 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 proteins, may contribute to these attributes. In support of this, calcium salts have been 420 421 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 research into the properties of ONS ingredients may help to elucidate potential causes of 424 425 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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511 Figure 1: Seguential 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:
- ^(a)Sip 1–8, consumption point of 5 ml aliquots; AE1, AE2, after-effects at 30 s and 60 s post consumption 518 519 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:**

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

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

Table 1. Liking Scores for Standard Commercial (CONS) and Sweetness Suppressed (SSONS) ONS at Initial and End Tasting, 540

following a Boredom Test, for a healthy older volunteer cohort (n=32) and a patient cohort (n=28) 541

542

		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)		
Cohort	Product	Initial	Final	Sig [₫]	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 543

during the boredom phase, ^c14 volunteers and 16 patients consumed SSONS during the boredom phase ^dSignificance of difference between initial and final liking, 544 545

as shown by ANOVA: p<0.001 (***), p<0.01 (**), P<0.05 (*), not significant (ns). . ^eSignificance of difference between CONS and SSONS.