

*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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Zhou, X., Yeomans, M., Thomas, A., Wilde, P., Linter, B. and Methven, L. (2021) 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. *Food Quality and Preference*, 90. 104116. ISSN 0950-3293 doi: <https://doi.org/10.1016/j.foodqual.2020.104116> Available at <https://centaur.reading.ac.uk/93942/>

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To link to this article DOI: <http://dx.doi.org/10.1016/j.foodqual.2020.104116>

Publisher: Elsevier

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

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18

19 **Abstract**

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

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

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

39 **Key words**

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

42

43 **Highlights**

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

49

50 **Introduction**

51 Dietary fat is the most energy-dense macronutrient in foods and contributes to food  
52 palatability. Fat is well-known to contribute to mouthfeel, whereas it is more recent that oral  
53 perception of free fatty acid has been recognised as a basic taste (Chale-Rush, Burgess, &  
54 Mattes, 2007a, 2007b; Stewart et al., 2010). Studies have suggested multiple candidate  
55 receptors on the tongue (CD36 and G protein coupled receptors (GPCRs)) which may be  
56 responsible for fat taste (Laugerette et al., 2005; Martin et al., 2011; Ozdener et al., 2014;  
57 Simons, Kummer, Luiken, & Boon, 2011). Although free fatty acids are only present in small  
58 amounts in foods, lingual lipase is reported to increase free fatty acid in the mouth by  
59 hydrolysing triglyceride (Kulkarni & Mattes, 2013; Pepino, Love-Gregory, Klein, & Abumrad,  
60 2012; Voigt et al., 2014).

61 Individuals have been reported to vary in fat taste sensitivity (Chale-Rush, Burgess, &  
62 Mattes, 2007a, 2007b; Martinez-Ruiz, Lopez-Diaz, Wall-Medrano, Jimenez-Castro, & Angulo,  
63 2014; Mattes, 2009a; Running & Mattes, 2014; Running, Mattes, & Tucker, 2013; Stewart et  
64 al., 2010; Stewart, Newman, & Keast, 2011; Tucker, Nuessle, Garneau, Smutzer, & Mattes,  
65 2015; Zhou, Shen, Parker, Kennedy, & Methven, 2016). This could be due to various factors,  
66 such as lipase activity (Kulkarni & Mattes, 2013; Pepino et al., 2012), genetic differences in  
67 fat taste receptors (Keller et al., 2012; Melis, Sollai, Muroi, Crnjar, & Barbarossa, 2015) and  
68 the quantity of fat taste receptors. Taste receptors are located within taste buds in papillae  
69 and, hence, research has suggested that variation in fungiform papillae density (FPD) can  
70 influence oral taste sensation (Bakke & Vickers, 2008; Dinnella et al., 2018; Masi, Dinnella,  
71 Monteleone, & Prescott, 2015; Melis et al., 2013; Miller & Bartoshuk, 1991; Miller & Reedy,  
72 1990). The influence of fungiform papillae in response to bitter taste perception of 6-n-  
73 propylthiouracil (PROP) is most well studied (Bajec & Pickering, 2008; Bakke & Vickers, 2008;  
74 Bartoshuk, Duffy, & Miller, 1994; Calo et al., 2011; Dinnella et al., 2018; Garneau et al., 2014;  
75 Melis et al., 2013; Shen, Kennedy, & Methven, 2016; Tepper & Nurse, 1997). As CD36 and  
76 GPCR120 are both found in human fungiform papillae (Ozdener et al., 2014), this raises the  
77 question whether FPD could also have an influence on fat taste sensitivity. Although one  
78 previous study has reported a relationship between FPD and fat perception, this mainly

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

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

102 The Graphic Mouth Behaviour Tool was developed by Jeltema, Beckley, and Vahalik (2014,  
103 2015) to characterize participants into four groups based on their preferred way of  
104 manipulating food in the mouth; Crunchers, Chewers, Smooshers and Suckers. Crunchers  
105 prefer to crunch and swallow food rapidly, whereas Chewers prefer to chew food for longer  
106 periods of time before swallowing and they prefer chewy foods. Smooshers tended to  
107 smooch the food in the mouth and Suckers prefer hard food which can be sucked for a long  
108 time. Such differences in mouth behaviour might change the structure of the food and  
109 hence result in different oral sensory perception, hence contributing to individual  
110 differences in mouthfeel perception.

111 Therefore, the objectives of this study were to:

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

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

120

121 **Methods and Materials**

122 **Participants**

123 Participants were recruited from the local community (Reading, UK). The inclusion criteria  
124 were self-reported healthy, aged 18-70 years and weight stable in the last three months.  
125 Exclusion criteria included: smoking, drug abuse, food allergies (e.g. gluten, dairy) and  
126 intolerances (e.g. lactose), diagnosed with cardiovascular disease, diabetes, gastrointestinal,  
127 endocrine or renal disease, planning or currently on a weight reducing programme, pregnant  
128 or planned pregnancy or lactating. The study was given a favourable opinion for conduct by  
129 School of Chemistry, Food and Pharmacy research ethics committee (study number 14/17)  
130 (participants n=65) and later by the University of Reading Research Ethics Committee (study  
131 number 18/05) (participants n=29). During the testing of the initial 65 participants it became  
132 apparent that a finer von Frey filament would provide useful additional information. Hence  
133 9 of these participants were also tested with a finer (0.008g) filament. Of these initial 65, a  
134 further 9 participants (who had not been tested with the finer filament) returned for  
135 subsequent trials alongside a second group of 29 new participants. These 9 participants  
136 were retested for their fatty acid sensitivity to the low level of fatty acid and their tactile  
137 sensitivity to the thicker 0.02g filament; neither results changed. The second cohort were  
138 tested for their sensitivity to the higher level of fatty acid and a finer filament (0.008g).  
139 Therefore, in summary, there were 94 participants for each characterisation test except for  
140 the sensitivity tests to the higher level of fatty acid (n = 38) and to the finer filament (n = 47).  
141 Each participant was only tested once for each test. The details of participant numbers in  
142 each test are shown in Supplementary data 1.

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

147

148 **Fatty acid sensitivity**

149 *Sample preparation for fatty acid sensitivity*

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

161 **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%

162

163 *Procedure for fatty acid gustatory sensitivity*

164 To test gustatory fat sensitivity triplicate alternative forced choice (3-AFC) discrimination  
165 tests were carried out for the low oleic acid level (0.015% w/v). This concentration was  
166 selected based on the study of Zhou et al. (2016), of which the results indicated that 49% of  
167 participants (n=43/87) could detect this level. Participants (n=94) were served three samples  
168 (two controls and one oleic acid sample) each time and they were asked to taste the  
169 samples and identify the “odd” sample out. If the participant correctly identified the sample  
170 containing oleic acid from the control in each of the three 3-AFC tests they were defined as  
171 “passed” to 0.015% w/v oleic acid; the probability of incorrectly identifying an individual  
172 participant as a taster from three correct 3-AFC tests being 0.037 (3.7%). Participants who  
173 incorrectly identified the sample in one or more 3-AFC tests were defined as “failed” to  
174 0.015% oleic acid. During tasting participants wore nose clips to eliminate any olfactory  
175 effect. The test was conducted under red light to mask any visual variation between  
176 samples.

177 The same procedure was repeated for the high oleic acid level (0.105%) for 38 participants in  
178 order to compare the current results with the findings of Stewart et al. (2011). Participants  
179 were classified as “hypersensitive” if they “passed” the low concentration of oleic acid  
180 (0.015% w/v oleic acid) and as “hyposensitive” if they “failed” at the high concentration  
181 (0.105% w/v oleic acid).

182

183 *Fungiform papillae density*

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

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

202

### 203 ***Tactile sensitivity measurement***

204 Two von Frey filaments (Aesthesio, Danmic Global, LLC, US), 0.02g force (size 1.65) and  
205 0.008g force (size 2.35), were used to determine tactile sensitivity on the tongue. All  
206 participants were tested using the 0.02 g filament, whereas 47 were additionally tested  
207 using the 0.008g filament. The participants were blindfolded and asked to protrude their  
208 tongue over their bottom lip whilst allowing it to relax. The front area of their tongue was  
209 then touched with each filament. Each filament was used ten times, five times with the true  
210 touch (touch) and five times with the false touch (no touch), in a randomly allocated  
211 balanced order, either side of the tongue midline. The filament was held perpendicular to  
212 the surface of tongue. The tip of the filament was touched on the tongue surface until the  
213 fibre slightly bowed, and then the filament was removed. The participant was asked if they  
214 could detect the stimulus on their tongue (forced-choice) and additionally asked to indicate  
215 how sure they were about their answer. Hence, there were four possible answers; “yes,  
216 sure”, “no, sure”, “no, not sure” and “yes, not sure”. The answers were recorded to calculate  
217 the R index (see **equation 1**) which was the measure of oral tactile sensitivity. If an R-index  
218 of 1 was obtained it inferred that the participant could easily detect the filament. However,  
219 if the R index was 0.5 or less, it indicated that the participants could not detect that filament.

220

221 **Equation 1** formula of calculating the R index by using the results obtained from volunteer’s  
222 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	

223

224

225

226

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

227

228

### 229 ***Mouth behaviour measurement***

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

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



239

## 240 **Biscuit ratings**

### 241 *Biscuit preparation*

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

246 **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%

247

248 Plain flour (Co-operative, UK), egg (Free range, Co-operative, UK), baking powder (Dr.Oetker  
249 Baking Powder, Dr.Oetker Ltd., Leeds, UK), salt (Table salt, Co-operative, UK), unsalted  
250 butter (Co-operative, UK) and cheese (medium grated cheddar cheese, Co-operative, UK)  
251 were weighed and mixed for 90 s at speed 2 in a dough mixer (Kenwood Major Titanium  
252 KMM020, Kenwood Ltd., Havant, UK). Cold butter was added to the mixer at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ,  
253 warm butter was added at  $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , melted butter was melted using a water bath ( $50^{\circ}\text{C} \pm$   
254  $2^{\circ}\text{C}$ ) prior to mixing. The mixed dough was sheeted (Rondo sheeter STM-503, Rondo Ltd,  
255 Surrey, UK) to a uniform thickness of 3.25mm, cut into circles (4.25cm diameter) and placed  
256 on a baking tray. Biscuits were baked  $180^{\circ}\text{C}$  for 15 min in a pre-heated oven (Salva KWIK-CO  
257 convection oven, ATLAS equipment (London) Ltd, London, UK). After baking, biscuits were  
258 cooled to ambient ( $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and stored in sealed polyethylene bags for later use.

### 259 *Biscuit mouthfeel perception and texture measurements*

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

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

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

## 282 ***Statistical analysis***

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

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

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

301 The relationship between fatty acid sensitivity and FPD was tested by ANCOVA with fatty  
302 acid sensitivity (categorical data) fitted as the explanatory variable and BMI as the covariate.  
303 We note that the direction of the relationship expected is that FPD would influence fatty  
304 acid sensitivity (FA) rather than vica versa, therefore logistic regression was initially used  
305 with numerical data (FPD) as the independent variable and categorical data (fatty acid  
306 sensitivity) as the dependent variable (FA = FPD). The logistic regression concluded a  
307 significant relationship between fatty acid sensitivity and FPD ( $p = 0.003$ ; predictive AUC =  
308 0.76; data not shown). As the significance of the relationship was the same where the  
309 categorical data (fatty acid sensitivity) is fitted as the independent variable, and this allows  
310 for BMI to be fitted as the covariant, the final model reported is from ANCOVA (FPD = FA +  
311 BMI). The relationship between oral tactile sensitivity to the finer filament (0.008g) (F0.008)  
312 and FPD was examined by linear regression, fitting both FPD and BMI as explanatory  
313 variables (F0.008 = FPD + BMI). The relationship between tactile sensitivity to the 0.02g  
314 filament (F0.02) and FPD was examined by Spearman partial correlation, accounting for BMI  
315 within the analysis (F0.02 = FPD + BMI).

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

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

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

336

## 337 **Results**

### 338 ***Characterization of participants***

339 Ninety-four participants participated in the study. There were 64 females (68%) and 30  
340 males (32%). Fifty-eight (62%) were Caucasian, twenty-nine (31%) were Asian and seven  
341 (7%) were African (**Table 3**). The BMI ranged from 15.6 kg/m<sup>2</sup> to 38.8 kg/m<sup>2</sup>.

342 All participants were tested for fatty acid sensitivity at the lower oleic acid level (0.015%  
343 w/v); 18 participants (19%) could successfully identify the sample and were hence deemed  
344 to have "passed" 0.015% w/v oleic acid, whereas 76 participants (81%) failed this  
345 concentration. Subsequently, 38 participants were tested at the higher level of free oleic  
346 acid (0.105% w/v), in which 13 of them (34%) "passed" at 0.105% w/v oleic acid and 25 (66%)  
347 "failed" (**Table 3**). Of these 13 volunteers sensitive to 0.105% w/v oleic acid, 6 (16%) had the  
348 ability to "pass" 0.015% w/v oleic acid implying their thresholds to oleic acid were lower than  
349 0.015% w/v; whilst 7 (18%) could not "pass" the 0.015% w/v oleic acid implying their  
350 thresholds were between 0.015% w/v and 0.105% w/v oleic acid.

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

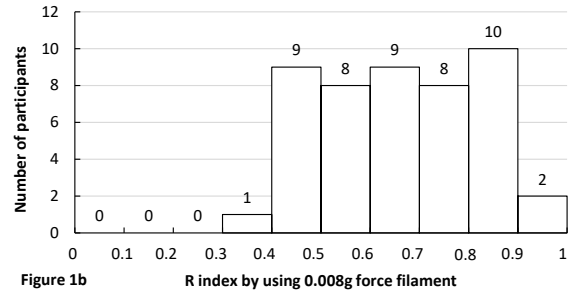
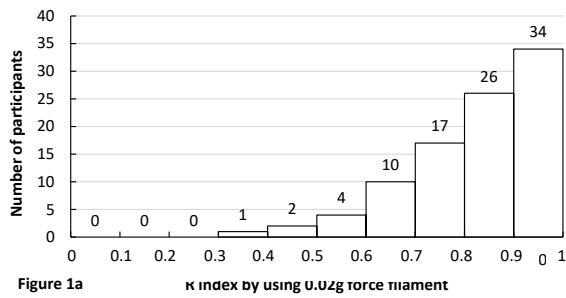
**Table 3** Demographic and characterization measurements of participants

	Characterization	Number	Proportion	BMI range (kg/m <sup>2</sup> )	BMI mean (kg/m <sup>2</sup> )	
<b>Gender</b>	Female	64	68%	15.6-38.8	22.7	
	Male	30	32%	16.3-30.0	24.1	
<b>Ethnicity</b>	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	
<b>Fatty acid sensitivity</b>	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
<b>Mouth behaviours</b>	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	

358

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



375

376

(Figure 1 goes here)

377

**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).

378

379

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

380

381

382

383

**Relationship between phenotypic measurements**

384

**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 ( $p = 0.37$ ).

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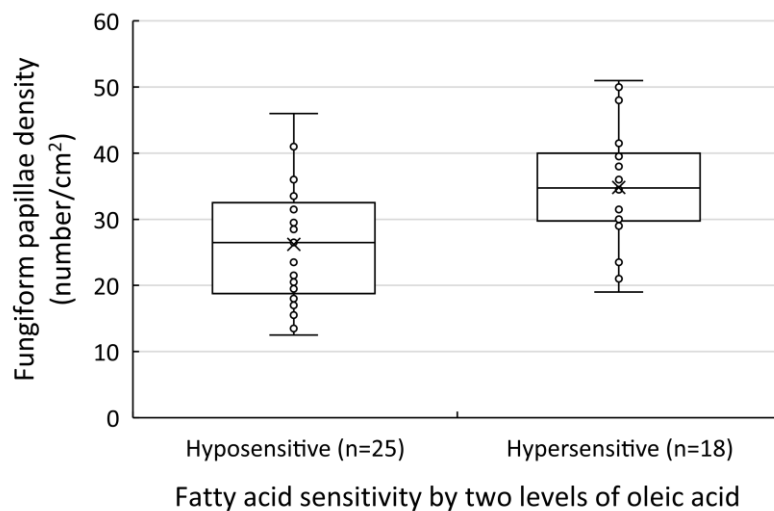
389

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**).

390

391

392



393

394

(Figure 2 goes here)

395 **Figure 2** Distribution of fungiform papillae density in “hypersensitive” (n=18) and “hyposensitive”  
396 (n=25) participants.

397

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

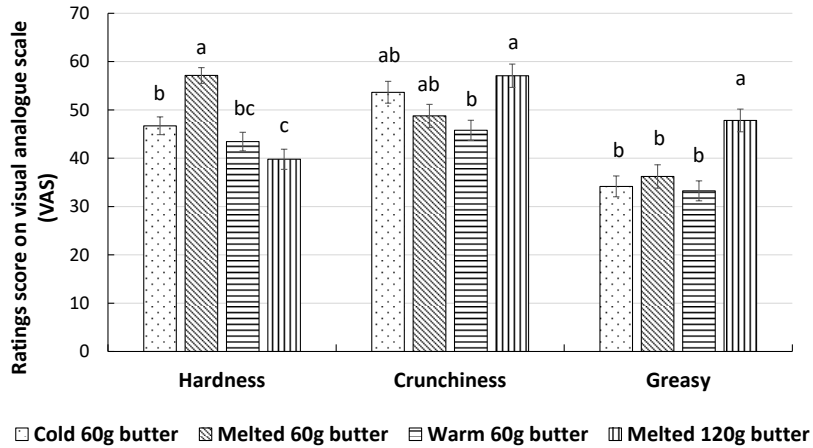
403 **Sensory phenotypes and demographic measurements:** There were no significant  
404 correlations between fatty acid sensitivity (at low or high level by using “pass/fail” to classify  
405 participants at one level of oleic acid) and any other individual characterisation parameter  
406 measured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

412 There was no relationship between BMI and fatty acid sensitivity using “pass/fail” to classify  
413 participants at one level of oleic acid (at 0.015% w/v:  $p=0.59$ ; at 0.105% w/v:  $p=0.24$ ), nor  
414 when using the combined “hyper/hypo” sensitive categorisation ( $p=0.71$ ). No correlation  
415 was found between FPD and BMI ( $p=0.43$ ), nor between BMI and tactile sensitivity  
416 measured using the finer 0.008g filament ( $p=0.38$ ). However, there was a negative  
417 correlation between BMI and tactile sensitivity measured using the 0.02g filament ( $\rho=-$   
418  $0.29$ ,  $p=0.006$ ). This suggests that a higher BMI is related to a lower oral tactile sensitivity,  
419 although it should be noted that a higher proportion of participants could detect this thicker  
420 filament (distribution substantially skewed, Figure 1a), perhaps limiting the application of  
421 this finding. There was no relationship between BMI and mouth behaviour ( $p=0.80$ ).

422

### 423 ***Influence of biscuit type on biscuit ratings***

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



434

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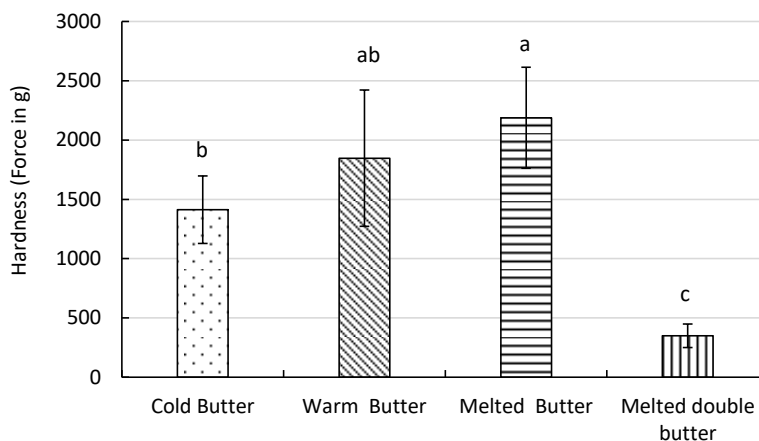
(Figure 3 goes here)

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

453

#### 454 ***The influence of phenotypic measurements on biscuit ratings***

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

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

468 Oral tactile sensitivity, as evaluated using the 0.02g filament, had a significantly positive  
469 relationship with the rating of biscuit hardness ( $p=0.019$ ), with a similar effect size on the  
470 model as FPD (value +15.5). There were no significant relationships between sensitivity  
471 measured using this thicker filament and ratings of biscuit crunchiness or greasiness  
472 ( $p=0.063$ ,  $p=0.25$  respectively). Regarding the influence of tactile sensitivity measured by the  
473 0.008g force filament on biscuit ratings, there were no significant relationships with biscuit  
474 ratings (hardness:  $p=0.086$ ; crunchiness:  $p=0.29$ ; greasy:  $p=0.84$ )

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

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

#### 485 **Discussion**

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



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

495

#### 496 ***The relationship between fat taste sensitivity and fungiform papillae density***

497 The influence of fungiform papillae density on taste perception has mostly been studied  
498 with bitter taste, particularly in relation to 6-n-propylthiouracil (PROP) (Bajec & Pickering,  
499 2008; Bakke & Vickers, 2008; Bartoshuk et al., 1994; Calo et al., 2011; Dinnella et al., 2018;  
500 Garneau et al., 2014; Melis et al., 2013; Shen et al., 2016; Tepper & Nurse, 1997). Several  
501 studies reported that higher FPD resulted in greater bitterness perception from PROP (Bakke  
502 & Vickers, 2008; Bartoshuk et al., 1994; Calo et al., 2011; Melis et al., 2013; Shen et al., 2016;  
503 Tepper & Nurse, 1997). More fungiform papillae on the tongue is proposed to lead to more  
504 taste receptors and a stronger taste signal generation, although there are limited studies  
505 that have directly measured this association.

506 Fat taste has been proposed as the sixth basic taste. Receptors such as CD36 and GPCRs on  
507 the tongue in both animals and humans have been proposed to be responsible for fat taste  
508 (Abdoul-Azize, Selvakumar, Sadou, Besnard, & Khan, 2014; Martin et al., 2011; Ozdener et  
509 al., 2014). Free fatty acid is proposed as the effective stimuli to activate the receptors on the  
510 tongue and hence generate the fat taste sensation (Chale-Rush et al., 2007a, 2007b; Mattes,  
511 2009a, 2009b; Running, Craig, & Mattes, 2015; Running & Mattes, 2014; Running et al.,  
512 2013; Stewart et al., 2010; Zhou et al., 2016). CD36 and relevant G protein coupled receptors  
513 have both been found in fungiform papillae (Liu et al., 2018; Ozdener et al., 2014; Simons et  
514 al., 2011). Therefore, it was hypothesised that the participants who have more fungiform  
515 papillae may have more fat taste receptors and hence be more sensitive to fat taste.

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

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

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

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

551 Single “cut-off” concentrations have been used before in the studies of Stewart et al. (2010)  
552 and Stewart et al. (2011). Stewart et al. (2010) used a cut-off concentration of oleic acid of  
553 1.4mM (0.04% w/v) concluding that 22% (n=12) of participants were hypersensitive whereas  
554 78% (n=42) were hyposensitive. In the later study of Stewart et al. (2011), a higher  
555 concentration of oleic acid of 3.8mM (0.11% w/v) was used which resulted in 25%  
556 hypersensitive participants (n=13) and 75% (n=38) hyposensitive. By using similar  
557 concentration as Stewart as a cut-off (0.105% w/v), the proportion of “passed” participants  
558 in our study was higher than in the Stewart et al. (2011) paper, 34 % versus 25%. This is  
559 perhaps due to the different populations sampled in these studies; however, it may also be  
560 due to the relatively small number of participants in each study. This triplicate forced choice  
561 discrimination method with a cut-off concentration of oleic acid provides a quick approach  
562 to characterise the sensitivity of participants to fat taste, however, it loses accuracy  
563 compared to the detection threshold method. In addition, the cut-off concentration of  
564 0.015% w/v was selected based on our previous study (Zhou et al., 2016) where the sample  
565 size was merely 51; the cut-off concentration of 0.105% w/v was selected based on Stewart  
566 et al. (2011) which similarly tested 51 participants. Therefore, future studies require a large  
567 sample size in order to conclude the distribution of fat taste thresholds in a population and  
568 subsequently to establish the most appropriate levels for a rapid discrimination method to  
569 characterize consumers’ sensitivity.

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

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

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

629

### 630 ***The Influence of biscuit type on mouthfeel perception of biscuits***

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

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

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

660

### 661 ***The influence of fungiform papillae density, oral tactile sensitivity, and fatty acid*** 662 ***sensitivity on mouthfeel perception of biscuits***

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

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

677 Fungiform papillae are surrounded by trigeminal nerves which can be responsible for  
678 innervating somatosensory (tactile) perception (Whitehead et al., 1985), hence the number  
679 of fungiform papillae on the tongue has been reported to influence mouthfeel perception of  
680 products (Hayes & Duffy, 2007; Nachtsheim & Schlich, 2013; Tepper & Nurse, 1997). Both  
681 Tepper and Nurse (1997) and Nachtsheim and Schlich (2013) found participants with higher  
682 FPD gave higher ratings for fat content of milk-cream samples compared to those with low  
683 FPD; similarly Hayes and Duffy (2007) found participants with high FPD gave higher scores  
684 for perceived creaminess in a sugar and fat model food matrix. These studies, that reported  
685 a relationship between FPD and oral perception, tended to be in less solid food matrices  
686 (Hayes & Duffy, 2007; Nachtsheim & Schlich, 2013, Tepper & Nurse, 1997). The study by  
687 Bakke and Vickers (2008) used solid food matrix (breads), but did not observe a relationship  
688 between FPD and mouthfeel (roughness) perception of breads, although their sample size  
689 was small ( $n=37$ ). In addition, the functionalities and morphology (such as shape and size) of  
690 FP might have an impact on mouthfeel perception (Piochi, Dinnella, Prescott, & Monteleone,  
691 2018), however the counting of FP on the tongue cannot reflect such information.

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

714

715 ***Investigation of mouth behaviour and mouthfeel perception of biscuits***

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

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

### 738 ***Relationships between BMI and oral sensory perception***

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

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

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

## 767 **Conclusion**

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

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

783

## 784 **Acknowledgement**

785 This study is funded by the UK Biotechnology and Biological Sciences Research Council  
786 (BBSRC) (BB/P023916/1, BB/P023932/1, BB/ P023924/1) in collaboration with Unilever,  
787 PepsiCo, Arla, Mars Wrigley, Mondelēz International, Pladis and Premier Foods. We would  
788 like to thank BBSRC and all industrial sponsors. The views expressed in this paper are those  
789 of the authors and do not necessarily reflect the position or policy of any of the collaborative  
790 companies. In addition, we would like to thank all participants in this work, and thank all  
791 project students for helping to recruit and test participants. We thank the Food Processing  
792 Centre and Sensory Science Centre of the University of Reading for providing facilities to  
793 conduct this work. We also would like to acknowledge the contribution of the staff of JBMB  
794 questionnaire.

795

## 796 **Conflict of interest**

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

800

801 All procedures followed were in accordance with the ethical standards of University of  
802 Reading Research Ethics Committee and with the Helsinki Declaration of 1975, as revised in  
803 2008. Informed consent was obtained from all participants prior to study commencement.

804

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