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Oleogustus: The Unique Taste of Fat

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1 Oleogustus: The unique taste of fat

2

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10

11 Abstract: Considerable mechanistic data indicate there may be a sixth basic taste: fat.
12 However, evidence demonstrating that the sensation of non-esterified fatty acids (the
13 proposed stimuli for “fat taste”) differs qualitatively from other tastes is lacking. Using
14 perceptual mapping, we demonstrate that medium and long-chain non-esterified fatty
15 acids have a taste sensation that is distinct from other basic tastes (sweet, sour, salty, and
16 bitter). While some overlap was observed between these NEFA and umami taste, this
17 overlap is likely due to unfamiliarity with umami sensations rather than true similarity.
18 Shorter chain fatty acids stimulate a sensation similar to sour, but as chain length
19 increases this sensation changes. Fat taste oral signaling, and the different signals caused
20 by different alkyl chain lengths, may hold implications for food product development,
21 clinical practice, and public health policy.

22

23 Keywords: oleogustus, fat taste, non-esterified fatty acid taste, fatty acid structure, basic
24 tastes

25

26

27 **Introduction:**

28 Despite more than two millennia of reflection, consensus is lacking on what
29 constitutes a “basic taste quality,” and whether taste is limited to a discrete set of taste
30 “primaries.” We and others have proposed criteria for “primary tastes,” including that
31 the sensation: 1) has ecological consequence, 2) is elicited by a distinctive class of
32 chemicals, 3) stems from activation of specialized receptors, 4) is detected through
33 gustatory nerves and is processed in taste centers, 5) has a quality non-overlapping with
34 other primary qualities, and 6) evokes a behavioral and/or physiological response (Mattes
35 2011; Kurihara and Kashiwayanagi 1998). Considerable evidence indicates oral
36 responses to non-esterified fatty acids (NEFA) meet criteria 1-4 and 6 (Gilbertson and
37 Khan 2014; Tucker *et al.* 2014; Running and Mattes 2015). However, documentation
38 that oral NEFA exposure elicits a perceptible and unique taste sensation, in addition to
39 their olfactory and somatosensory sensations, is weak overall and absent in humans.
40 Studies in rodent models indicate that taste aversions to nutritive oil and long chain fatty
41 acids do not generalize to other taste sensations or to textural qualities (Pittman 2010),
42 suggesting the sensation is unique in this species. In the two experiments that follow, a
43 perceptual sorting task was used to show that humans experience taste from short,
44 medium, and long chain fatty acids and that these sensations are different from other
45 recognized taste qualities, and from each other. The data were analyzed 3 ways for
46 consistency: 1) hierarchical clustering showed the predominant groups at various levels
47 of sorting; 2) multidimensional scaling (MDS) with bootstrapping generated perceptual
48 maps and 95% contours for each sample, and 3) Bhattacharyya coefficients were used to
49 determine the degree of overlap between pairs of samples (perfect overlap = 100%, no

50 overlap = 0%). These findings directly address the weakest link in the proposition that
51 fat is a basic taste quality and we suggest a new word to describe this taste: oleogustus.
52 Fat taste signaling may hold implications for food product development (e.g.,
53 composition of fat replacers), clinical practice (e.g., management of appetite, digestion,
54 taste disorders), and public health policy (e.g., dietary recommendations to moderate
55 postprandial lipemia).

56

57 Materials and methods

58 *Experiment 1*

59 The first experiment was designed to test whether short, medium, and long chain
60 NEFA were unique in sensation from each other as well as distinguishable from blanks
61 and sweet, sour, salty, bitter, and umami tastes. This experiment used 15 samples, as
62 described in Table 1. Concentrations were selected by conducting pilot tests to identify
63 samples of similar taste intensity to 0.54M glucose.

64

65 *Experiment 2*

66 Data from experiment 1 showed large perceptual overlap among bitter compounds
67 and medium to long chain NEFA, so this relationship was further explored to determine
68 if this similarity was attributable to hedonic (unpleasant) similarity or actual qualitative
69 similarity. This experiment used several bitter stimuli as described in Table 1, and
70 included two concentrations of urea and quinine to determine whether sorting patterns
71 were based on intensity rather than quality of sensation (despite explicit instructions to
72 sort on “quality or type” of sensation rather than intensity). Further, different types of

73 bitter compounds have different transduction mechanisms, so a variety of bitter chemicals
74 were included to ensure any perceptual similarities were not limited to specific classes of
75 bitter stimuli (Delwiche *et al.* 2001; Keast and Breslin 2002; Meyerhof *et al.* 2010).
76 Additionally, two blank solutions were included as internal controls and to identify PROP
77 tasters and non-tasters, which is a genetic trait that causes some individuals to taste this
78 compound as bitter while others experience little or no sensation (Bufe *et al.* 2005).
79 Participants were classified as PROP non-tasters if they grouped the PROP solution with
80 either blank solution in the first round of sorting (described below).

81

82 *Samples*

83 Oleic acid (C18:1, Spectrum Chemicals), linoleic acid (C18:2, Sigma Aldrich), 9-
84 decenoic acid (C10:1, Sigma Aldrich), *trans*-3-hexenoic acid (C6:1, SAFC Sigma
85 Aldrich), acetic acid (C2, Sigma Aldrich), citric acid monohydrate (Mallinckrodt
86 Chemicals), sodium chloride (Spectrum Chemicals), L-glutamic acid monosodium salt
87 monohydrate (MSG, Aldrich Chemistry), quinine sulfate dihydrate (Spectrum
88 Chemicals), urea (Mallinckrodt Chemicals), caffeine (Sigma Aldrich), 6-*n*-
89 propylthiouracil (PROP, Sigma Aldrich), sucrose octaacetate (SOA, Sigma Aldrich),
90 ethylenediaminetetraacetate (EDTA, Spectrum Chemicals), tert-butylhydroquinone
91 (TBHQ, Spectrum Chemicals), glucose and fructose (www.nuts.com) were all food grade
92 and purchased from commercial vendors. Disodium 5' inosinate (IMP) was a gift from
93 Ajinomoto Food Ingredients. Sodium caseinate was purchased from American Casein
94 Company (Burlington, NJ). Xanthan gum was purchased from local grocers (Bob's Red
95 Mill brand), and the same batch was used for all study procedures. Table 1 lists

96 concentrations used. Concentrations were selected based on pilot work indicating the
97 sensations were comparable in taste intensity. The blank was prepared by adding the
98 appropriate amounts of sodium caseinate, EDTA, TBHQ, and xanthan gum to distilled
99 water, mixing, and allowing the solution to sit overnight to fully hydrate the xanthan
100 gum. This blank was used as the base solution for all other mixtures in experiment 1
101 except for acetic acid, citric acid, and hexenoic acid, as the pH of these solutions would
102 have caused the sodium caseinate to precipitate out of solution. These solutions
103 contained xanthan gum, EDTA, and TBHQ in addition to the acids. In experiment 2, the
104 sodium caseinate, xanthan gum, EDTA, and TBHQ solution was again used as the base
105 solution for all samples, but 1% ethanol was added as it aided in the dissolution of several
106 of the less polar bitter compounds (PROP, SOA, quinine). These three bitter solutions
107 were first prepared as stock solutions in ethanol, and then diluted into the blank solution
108 of sodium caseinate, xanthan gum, and antioxidants.

109 For experiment 1, emulsions of 0.18 M oleic acid, 0.18 M linoleic acid (10 times
110 the final concentration), and 0.0059 M decenoic acid were prepared in 1L batches by
111 adding the appropriate amount of NEFA to the blank solution (sodium caseinate, EDTA,
112 and TBHQ) and mixing with an Ultra Turrax T18 homogenizer at 14,000 RPM for 10
113 minutes equipped with the S18N-19G dispersing element. Next, these mixtures were
114 fully homogenized in 3.75L batches using a two stage homogenizer (APV 15 15MR-
115 8TBA) with the cylinder pressure set to 3500psi. The homogenizer was set to loop the
116 solution back through the system for a total of 5 minutes before collecting the final
117 homogenate. This stabilized the emulsions against creaming over time and allowed for
118 larger batch productions. The 10x concentrated linoleic acid emulsion was then diluted

119 into the blank for final testing. Viscosities of fatty acid emulsions and blank were
120 checked with a DHR-3 hybrid rheometer equipped with a 40 mm 2° cone and plate
121 geometry, from 1-300s⁻¹ at 37°C, controlled by a Peltier plate, with 10 points per decade.
122 Data confirmed the fatty acid emulsions matched the viscosity of the blank (Figure 1).
123 Emulsion stability was checked using a Mastersizer 2000 equipped with a Hydro 200MU
124 dispersion unit. Mean droplet diameters (both surface and volume weighted) were less
125 than 0.5µm (Figure 2), despite the small peak in the 1-2.5 µm range for samples made for
126 experiment 2 using only the rotor stator mixer (Ultra Turrax T18). Hexenoic acid was
127 soluble at the concentration used but to ensure full dissolution it was mixed into sodium
128 caseinate-free blank using the Ultra Turrax T18 homogenizer at 14,000 RPM for 10
129 minutes equipped with the S18N-19G dispersing element in 1L batches. Other solutions
130 were prepared by adding the compounds to the blank (sodium caseinate-free for acetic
131 and citric acids), stirring, and allowing the solutions to sit overnight in the refrigerator to
132 fully dissolve. All solutions were brought to room temperature for the experiment.

133 For experiment 2, emulsions were prepared by small batch homogenization as in
134 previous studies (Running and Mattes 2014a; Running and Mattes 2015). Briefly, 100
135 mL of 0.18 M oleic, 0.18 M linoleic (ten times the final concentration), and 0.0059 M
136 decenoic acid were homogenized with an Ultra Turrax T18 homogenizer at 14,000 RPM
137 for 10 minutes equipped with the S18N-19G dispersing element. The linoleic acid
138 emulsion was then diluted to 0.018 M using the blank solution. These emulsions were
139 checked for particle size and viscosity as detailed above. Quinine, PROP, and SOA
140 samples were first made at 100x final concentration in ethanol, as these are poorly
141 soluble in water, then diluted into the blank solution. Caffeine was dissolved into hot

142 water at 2x final concentration then diluted into the blank. NEFA emulsions, caffeine,
143 urea, and blank solutions all had 1% ethanol added to match the level of ethanol needed
144 to dissolve the quinine, PROP, and SOA samples. Final solutions all contained 1%
145 sodium caseinate, 0.05% xanthan gum, 1% ethanol, and 0.01% each EDTA and TBHQ in
146 addition to the tastants listed in Table 1.

147

148 *Participants*

149 All protocols were approved by Purdue University's Human Subjects Institutional
150 Review Board. Subjects were recruited through public announcements and through
151 participant pools of the Laboratory for Sensory and Ingestive Studies and the Purdue
152 Sensory Evaluation Laboratory. Eligibility criteria included: between the ages of 18 and
153 60, normal taste function, healthy (by self-report), and not allergic to dairy (because of
154 the source of sodium caseinate). For experiment 1, panelists could not be allergic to nut
155 products because the glucose and fructose were purchased from a supplier who also
156 processes nuts. Panelists were screened for their ability to discriminate 0.018 M linoleic
157 acid emulsion from the blank using two sequential, tetrad tests. This required the
158 panelists to sort 4 samples (two each, linoleic acid emulsion and blank) into 2 groups
159 based on similarity. The odds of correctly sorting two tetrad tests sequentially is 1/9. For
160 the second experiment, we further restricted this criterion by requiring the panelists to
161 identify the group that contained a "flavor" (i.e., linoleic acid). The odds of correctly
162 sorting two sequential directed tetrad tests is 1/36.

163 Panelists wore nose clips during the tests and all samples were served in opaque
164 containers with lids. Nose clips have been previously demonstrated to adequately

165 prevent human ability to discriminate long chain fatty acids from blank solutions (Bolton
166 and Halpern 2010). Participants were provided with water for rinsing their mouths as
167 well as a cup to spit the samples into after tasting. The spit cup also had a lid, with a
168 small hole that panelists were instructed to spit through. Panelists had to successfully
169 complete both tetrads in order to qualify for the study. If panelists did not successfully
170 complete the tetrads, they were excused from further testing. Panelists who qualified for
171 the full studies provided written informed consent as well as data on their ethnic
172 background, age, and their habitual fat intake using a validated food frequency
173 questionnaire (Block *et al.* 2000); participants were classified as having a “high fat diet”
174 if they scored a 23 or higher on this questionnaire (value set by questionnaire and
175 corresponds to 35.9% fat diet for females and 33.6% fat diet for males). Heights and
176 weights were measured. Demographic data on the participants from both studies is given
177 in Table 2. Panelists who qualified in experiment 1 were invited back for experiment 2,
178 so some overlap is present among these groups. Panelists who participated in the full
179 study received financial compensation.

180

181 *Free sorting task*

182 In the first experiment, demographic data and tetrad tests were administered with
183 CompuSense 5 software. In the second experiment, Qualtrics was used. After
184 completing the screening tests and the demographic questions, panelists received the
185 sorting samples in opaque, 4 oz cups with lids labeled with randomized three digit codes.
186 Samples were presented all at once on a tray in a randomized arrangement. Two sodium
187 chloride samples were used in experiment 1; this allowed an internal control for whether

188 panelists understood the task. For experiment 2, two blanks were included to verify the
189 success of the task. In experiment 1, panelists who did not sort the two identical sodium
190 chloride solutions together in the first round of sorting were removed from the data
191 analysis. Fifty-three of 78 participants passed screening, 4 panelists failed to sort the two
192 sodium chlorides together, and 1 panelist did not taste all the samples for sorting, leaving
193 48 panelists in the final analysis. In experiment 2, all qualified panelists (54) were
194 included in the final results, and the output data were analyzed to confirm substantive
195 overlap among the two identical blank samples.

196 The free-sorting task was modified from other published methods (Courcoux *et*
197 *al.* 2012). After panelists had donned the nose clips, they were instructed to taste each
198 sample, expectorate it into the waste cup, and rinse with water. Then, they sorted the
199 samples into groups they believed were similar in “quality or type” of sensation (caution
200 was used to avoid the use of the word “taste”). Groups could contain as many or as few
201 samples as desired, and participants could make as many or as few groups as they
202 desired. Panelists wrote a description for each group. After finishing this initial sorting
203 task, if panelists had more than two total groups, they were instructed to select the two
204 groups they believed were most similar to each other and combine them. If panelists still
205 had more than two total groups (the new, large group counting as a single group), they
206 were instructed to combine the two most similar groups again, either by adding a third
207 group to their new, large group or by creating another combined group of two previously
208 separate groups. This continued until panelists only had two groups remaining.

209

210 *Statistics*

211 Data were organized into dissimilarity matrices for each participant's groupings.
212 For the first study, the two identical sodium chloride samples were collapsed into one, as
213 participants were required to sort these two samples together in order for their data to be
214 included; these samples are referred to as "sodium chloride" or "salts" for the purpose of
215 analysis. Additionally, inspection of the data revealed that all participants also put both
216 glucose and fructose samples together in the first round of sorting. To reduce error in the
217 analysis, these samples were also collapsed into one for the analysis, and are referred to
218 as "sugars." The total number of rounds of combining groups was noted for each
219 panelist, and the dissimilarity matrices were normalized by dividing all group numbers by
220 each panelist's total number of groups. Thus, all data were on a scale of 0-1. SAS 9.4
221 was used for bootstrapping, multidimensional scaling, and procrustean transformations.
222 Random bootstrapping with replacement was conducted using panelist as a sampling unit.
223 Multidimensional scaling was conducted on each bootstrapped replicate with settings of
224 ordinal level data and 2 dimensions. Output from multidimensional scaling was put
225 through procrustean transformation to optimally align the sample coordinates. This
226 generated a dataset with 500 pairs of (X,Y) coordinates for each sample type. The 2D
227 binning procedure in OriginPro 2015 b9.2.214 was used to calculate the number of points
228 from each sample located in a 30x30 grid superimposed over the data map. For
229 experiment 1, the data map stretched from X: (-3,2) and Y: (-2,2). For experiment 2, the
230 data map stretched from X: (-2,2) to Y: (-2,2). The axes for both experiments are
231 completely arbitrary and are determined from the first multidimensional scaling output,
232 which was used as the basis for the procrustean transformations. The bin counts from
233 OriginPro were then entered into Excel spreadsheets, where the total number of each

234 sample in each bin (900 bins total) were counted. Probability of a sample having a point
235 in each bin was calculated as the bin count/500, since there were 500 points for each
236 sample. Bhattacharyya coefficients were calculated as: $\sum \sqrt{[\text{Prob}_{\text{bin}i}(\text{SampleA}) * \text{Prob}_{\text{bin}i}$
237 $(\text{SampleB})]}$ for $i= 1$ to 900 (sum of the probabilities for all of the bins). A Bhattacharyya
238 coefficient of 100% indicates perfect overlap and 0% indicates no overlap. In OriginPro,
239 2D Kernel densities were calculated using the Bivariate Kernel Density Estimator with 50
240 points in X/Y. The output matrices for each sample were then mapped using 3D surface
241 contour maps, showing horizontal lines at each 10th percentile. Additionally, 2D maps
242 were generated of the 95th percentile density contour for each sample. Hierarchical
243 cluster analysis using Ward's method was conducted in OriginPro using the participants'
244 dissimilarity matrices.

245

246 Results

247 Textural cues of fatty acids were adequately masked, as there were no measurable
248 differences in viscosity and particle sizes were, on average, below 1 μm (Figures 1 and 2)
249 (Running and Mattes 2014a; Running and Mattes 2015).

250 The first experiment's results show clear separation of sweet, salty, sour and bitter
251 stimuli, as predicted, in all three methods of analysis (Figure 3, Table 3). Consistently in
252 all three analyses, the short chain NEFA overlapped and was grouped with the sour
253 stimuli, which was expected as acetic acid is also a short chain fatty acid. Also in all
254 three analyses, some overlap occurred among umami compounds and the medium to long
255 chain NEFA, especially for IMP. MSG, which is the prototypical stimulus for umami, is
256 clearly distinct from the long chain NEFA in the perceptual contour maps (Figure 4),

257 Bhattacharyya's coefficients reveal minimal overlap with oleic acid (2.3%) or linoleic
258 acid (4.3%), and MSG is in a separate cluster in the hierarchical data (Table 3, Figure 3).

259 In the second experiment, all three analytical approaches revealed distinctions
260 among the NEFA, bitter, and blank compounds (Figure 5, Table 3), with clear separation
261 between the medium and the long chain NEFA. Hierarchical clustering (Figure 5b)
262 demonstrates that the three main sorting groups from this experiment are blank samples,
263 bitter samples, and NEFA samples. This pattern can also be seen in the perceptual
264 contour map (Figure 5a) where the bulk of the NEFA density is clustered in the upper
265 right hand portion of the map (axes are arbitrary). Additionally in the perceptual map,
266 there is no overlap between decenoic acid (medium chain) and any other sample.

267 Linoleic acid and oleic acid have very similar contours (Figure 6), with limited low
268 density overlap with the bitter compounds. Considering Bhattacharyya's coefficients
269 (Table 3), again there is no overlap between decenoic acid and any other sample (all
270 below 5%), and overlap is greatest between oleic acid and linoleic acid (86.2%). There
271 were low levels of overlap between oleic acid and caffeine (14.8%), low concentration
272 quinine (6.5%), and both concentrations of urea (low: 8.1%, high: 15.3%) as well as
273 between linoleic acid and caffeine (16.6%), low concentration quinine (5.8%), SOA
274 (8.7%), and both concentrations of urea (low: 5.9%, high: 20.2%). However, overlap
275 among the bitter samples is much greater than the overlap between bitter compounds and
276 long chain NEFA, and t-tests conducted on the percent overlap among all bitter
277 compounds compared to percent overlap between bitter compounds and long chain
278 NEFA indicated greater overlap among the bitter compounds (34.4% mean overlap
279 among bitters, 8.4% mean overlap between bitter and long chain NEFA, $p=0.0003$;

280 excluding PROP from this analysis yields 43.2% and 9.5% overlap respectively,
281 $p=0.0002$; unequal variance assumed for both tests). Findings from PROP reflect the
282 presence of tasters and non-tasters in this analysis, as expected, and the multidimensional
283 scaling procedure averages over the groupings from these two populations. Analyzing
284 the data separately for tasters and non-tasters displayed only small changes in the
285 perceptual maps, except for the movement of the PROP solution, which overlaps with
286 blank for non-tasters and with bitter compounds for tasters (Figure 6). The only
287 noticeable shift for the NEFA was more overlap between the medium and both long chain
288 NEFA for the tasters compared to non-tasters, and more overlap among the long chain
289 NEFA and PROP for tasters. Similarly, comparing participants reporting consumption of
290 a high fat diet to those with a lower fat diet (N=29 and 25 respectively), a small shift was
291 observed with more overlap between the medium and both long chain NEFA as well as
292 between PROP and both long chain NEFA for participants on the low fat diet compared
293 to those on a high fat diet (Table 3).

294

295 Discussion

296 The data from these studies provide substantial new evidence not only that fat, in
297 the form of long-chain, non-esterified fatty acids, has a percept we believe is taste
298 ($64\pm 5\%$ of people in experiment 1 could identify the linoleic acid emulsion compared to
299 the blank with no prior training, and olfactory and somatosensory cues are inconsistent
300 with the findings), and there was no overlap in any of the three analyses between the
301 blanks and the fatty acids in experiment 2), but also that the oral sensations of fatty acids

302 are altered according to alkyl chain lengths. The findings for the unique qualities of
303 short, medium, and long chain NEFA are discussed below.

304 Our first study shows that short chain fatty acids have a sour note. This is
305 unsurprising as acetic acid itself is actually a short chain fatty acid (C2). At some point,
306 extending the alkyl chain of NEFA creates a perceptual shift from the sourness of short
307 chain NEFA to the quality experienced at a length of ten carbons, which was clearly
308 distinct as seen in experiment 1. Medium chain fatty acids such as decenoic acid may
309 have their own unique sensation from both short and long chain NEFA. From the
310 descriptions given during the second sorting experiment and from prior work, this
311 sensation could be irritating or pungent (Running and Mattes 2014). Considerable
312 overlap was observed among decenoic acid, IMP, and MSG in the first study, but this is
313 likely due to less experience by participants with pure umami sensations, rather than a
314 true perceptual overlap. Further, IMP and MSG in combination potentiate the umami
315 signal, so if participants did not thoroughly rinse between such samples, the intensity of
316 the flavor from these solutions could have varied (Kurihara and Kashiwayanagi 1998).
317 This could have led to greater discrimination of the MSG sample from the other samples,
318 but left a wider distribution for sorting of IMP, as observed in both analyses of the
319 multidimensional scaling data from experiment 1.

320 In our prior studies, self-reported qualitative descriptions indicated medium chain
321 fatty acids are more potent irritants than long chain fatty acids (Running and Mattes
322 2014). Considering that no other irritants were included in the sample set, the diffuse
323 sorting of decenoic acid in the first study and overlap with less familiar umami sensations
324 may reflect participants' confusion on how to sort sensations that did not have obvious

325 matches among the other samples. Additionally, many participants sorted the decenoic
326 acid with the blank solution in the first study, as demonstrated by the overlap between
327 these samples in all three methods of analysis. Potentially, there could be a bimodal
328 distribution of perception for medium chain fatty acids such as decenoic acid, where
329 some individuals perceive an unpleasant sensation and others perceive no sensation from
330 the stimulus.

331 Given the variety of samples presented in the first sorting study, participants may
332 have initially sorted out the familiar sensations of sweet, salty, and sour, and then
333 grouped the others together based on low palatability (descriptive terms reflect this).
334 Data from the second study show the medium chain NEFA was clearly unique from
335 bitter, long chain NEFA, and blank solutions. Analyzing the data separately for PROP
336 tasters and non-tasters, there is still evidence that non-tasters may experience less
337 sensation from this compound (Table 3). However, PROP tasters and consumers of a
338 low-fat diet appear to have grouped the medium chain NEFA with the long chain NEFA
339 more frequently than non-tasters and consumers of high fat diets, respectively. As noted
340 in Table 2, tasters and non-tasters were fairly evenly split among the high and low fat diet
341 categories, so these similarities in groups are not due to do confounding of these two
342 factors. The mechanism for such a similarity is unclear, as the medium chain NEFA have
343 very low affinity for proposed fatty acid taste receptors (Galindo *et al.* 2012; Hirasawa *et*
344 *al.* 2005; Hajri and Abumrad 2002; Briscoe *et al.* 2003), and medium chain fatty acid
345 receptors such as GPR 40 have not been identified in human taste cells (Galindo *et al.*
346 2012).

347 The present data suggest that long chain fatty acids stimulate their own unique
348 taste, which is unpalatable but very similar between oleic and linoleic acid when matched
349 for intensity. This observation is in agreement with the mechanistic literature on fatty
350 acid taste indicating the putative fat taste receptors interact predominantly with long
351 chain fatty acids (Galindo *et al.* 2012; Hirasawa *et al.* 2005; Hajri and Abumrad 2002;
352 Briscoe *et al.* 2003), though a diffusion mechanism could also still be possible (Tucker *et*
353 *al.* 2014). While these compounds also activate trigeminal neurons (Yu *et al.* 2012), the
354 distinction between these two NEFA and the medium chain NEFA, which should be a
355 more potent irritant (Stillman *et al.* 1975), would indicate another quality is dominant
356 with the long chain NEFA. While some overlap was observed among the long chain
357 NEFA and various bitter compounds, overlap was much greater within just the bitter
358 compounds or between the two long chain NEFA. In both studies, the overlap between
359 linoleic and oleic acid was consistently high in all analyses, and the only percentage of
360 overlap that was greater based on Bhattacharyya's coefficients was overlap of acetic and
361 citric acids in the first experiment (90.8%, data not shown). This indicates that the
362 sensations from oleic and linoleic acids are very comparable, and also gives additional
363 evidence that the sensation from the NEFA is unlikely to be predominantly textural in
364 nature. While the tests in this study did not show any textural difference among the
365 emulsions, there are many textural properties not fully evaluated by these methods (such
366 as tribology or salivary induced flocculation (Silletti *et al.* 2008; van Aken *et al.* 2011;
367 Vingerhoeds *et al.* 2005; Vingerhoeds *et al.* 2008; Vingerhoeds *et al.* 2009). However,
368 any textural sensation from the oleic acid emulsion should have been very different from
369 the linoleic acid emulsion, as the concentration of oleic acid was 10 fold higher (5%

370 compared to 0.5% w/w). As these two compounds mapped almost completely together in
371 all three assessments, texture would not explain the similarity in sensation. Qualitative
372 descriptions from panelists did not indicate the similarity would be explained by irritant
373 sensations. Further if irritancy were the dominant quality, greater overlap among the
374 medium chain NEFA and long chain NEFA would be expected, as previous work would
375 indicate the medium chain NEFA would be the most irritating of the stimuli.
376 Additionally, if the NEFA were irritating due to their nature as acids, the NEFA should
377 have been grouped with the sour compounds in the first experiment, which was clearly
378 not the case. Still, further work should be conducted to clarify whether the two long chain
379 NEFA may be perceived primarily as irritating by some participants and at what
380 concentrations the fatty acid taste becomes dominated by an irritant quality.

381

382 Conclusions

383 Overall, these experiments provide definitive evidence that long chain fatty acids
384 elicit a unique, perceptible sensation at concentrations relevant to our food supply
385 (Kulkarni and Mattes 2013; Chang and Chow 2008). The concentrations of fatty acids
386 tested are relatively high compared to those customarily encountered in the food supply,
387 but levels of non-esterified fatty acids can reach concentrations the in low percentiles
388 (5%=0.18M for oleic acid) in many fermented or rancid products, as well as in cooking
389 oils (Chang and Chow 2008). Medium and short chain fatty acids stimulate different
390 sensations from long chain fatty acids, with short chain species producing a sour
391 sensation and medium chain fatty acids characterized by potentially by irritancy, yet both
392 may have an uncertain fat quality. Further analyses should determine at what specific

393 chain length the perceptual differences among short, medium and long chain NEFA
394 occur. These data added to the totality of evidence on “fat taste” now provide a
395 comprehensive body of evidence supporting the existence of another basic or primary
396 taste quality for selected fatty acids (fat taste), whose oral activity should thus be
397 considered when examining the health consequences of fatty acid signaling.

398 Notably, the taste sensation elicited by long chain fatty acids is not wholly
399 consistent with the expectations of “fattiness.” Given the clear unpleasantness of the
400 sensation in isolation, and the incongruity with the term “fatty,” which has strong textural
401 context, we propose a new term to describe the taste of long chain NEFA. The term
402 “pinguis” was used to describe fattiness as early as the 16th century (Reed and Knaapila
403 2010; Fernel 1581), but this term refers more to a fatty or dense characteristic without
404 specificity to taste. Following the precedent set for umami which was derived from
405 Japanese to mean delicious taste (umai: delicious/savory, mi: taste), we propose the term
406 “oleogustus.” The latin term, “oleo” is a root for oily or fatty and “gustus” refers to taste.
407 The term oleogustus would provide a word easily recognized as pertaining to taste by
408 those in the field, but not easily confused with other sensations of fat perception.

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418

419 Figure 1: Viscosity of emulsions and blank.

420

421 Figure 2: Particle sizes of highest concentration emulsions.

422

423 Figure 3. A) Kernel density of experiment 1 samples in perceptual map representing 500

424 bootstrapped replicates of the multidimensional scaling data generated with panelists'

425 dissimilarity matrices. Horizontal lines are 10% increments of density; X and Y

426 dimensions are arbitrary. B) Dendrogram from hierarchical clustering of all participant

427 (N=48) dissimilarity matrices using Ward's method. C) Histogram of number of groups

428 created in first round of sorting.

429

430 Figure 4: 95% contours experiment 1

431 A: Sugars (white), sodium chloride (dark yellow), blank (grey), acetic acid (light blue),

432 citric acid (dark blue), hexenoic acid (green)

433 B: Blank (grey), decenoic acid (orange), oleic acid (yellow), linoleic acid (red)

434 C: Decenoic acid (orange), quinine (dark purple), urea (light purple)

435 D: Oleic acid (yellow), linoleic acid (red), quinine (dark purple), urea (light purple)

436 E: Decenoic acid (orange), inosine monophosphate (light pink), monosodium glutamate

437 (bright pink)

438 F: Oleic acid (yellow), linoleic (red), inosine monophosphate (light pink), monosodium

439 glutamate (bright pink)

440

441 Figure 5. A) Kernel density of experiment 2 samples in perceptual map representing 500
442 bootstrapped replicates of the multidimensional scaling data generated with panelists'
443 dissimilarity matrices. Horizontal lines are 10% increments of density; X and Y
444 dimensions are arbitrary. B) Dendrogram from hierarchical clustering of all participant
445 (N=48) dissimilarity matrices using Ward's method. C) Histogram of number of groups
446 created in first round of sorting.

447

448 Figure 6: 95% contours experiment 2

449 A: Oleic acid (yellow), linoleic acid (red), decenoic acid (orange), and blanks (grey)

450 B: Oleic acid (yellow), linoleic acid (red), decenoic acid (orange), blanks (grey), and
451 caffeine (white)

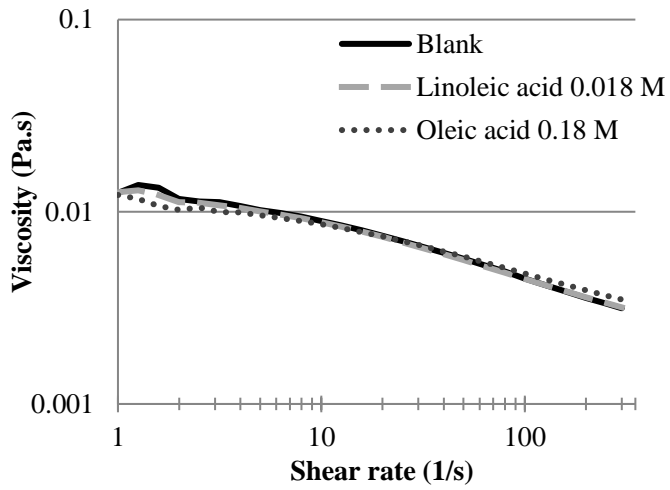
452 C: Oleic acid (yellow), linoleic acid (red), decenoic acid (orange), blanks (grey), quinine
453 low concentration (light purple), and quinine high concentration (dark purple)

454 D: Oleic acid (yellow), linoleic acid (red), decenoic acid (orange), blanks (grey), and
455 sucrose octaacetate (green)

456 E: Oleic acid (yellow), linoleic acid (red), decenoic acid (orange), blanks (grey), urea low
457 concentration (light blue), and urea high concentration (dark blue)

458 F: Tasters (N=28), oleic acid (yellow), linoleic acid (red), decenoic acid (orange), 6-n-
459 propylthiouracil (pink), blanks (grey); greater overlap overall was observed because of
460 smaller sample size

461 G: Non-tasters (N=26), oleic acid (yellow), linoleic acid (red), decenoic acid (orange), 6-
462 n-propylthiouracil (pink), blanks (grey); greater overlap overall was observed because of
463 smaller sample size

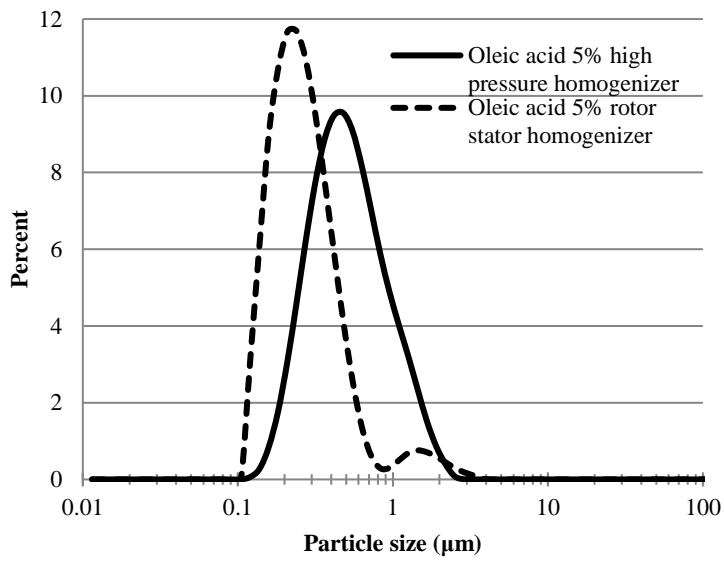


464

465 Figure 1

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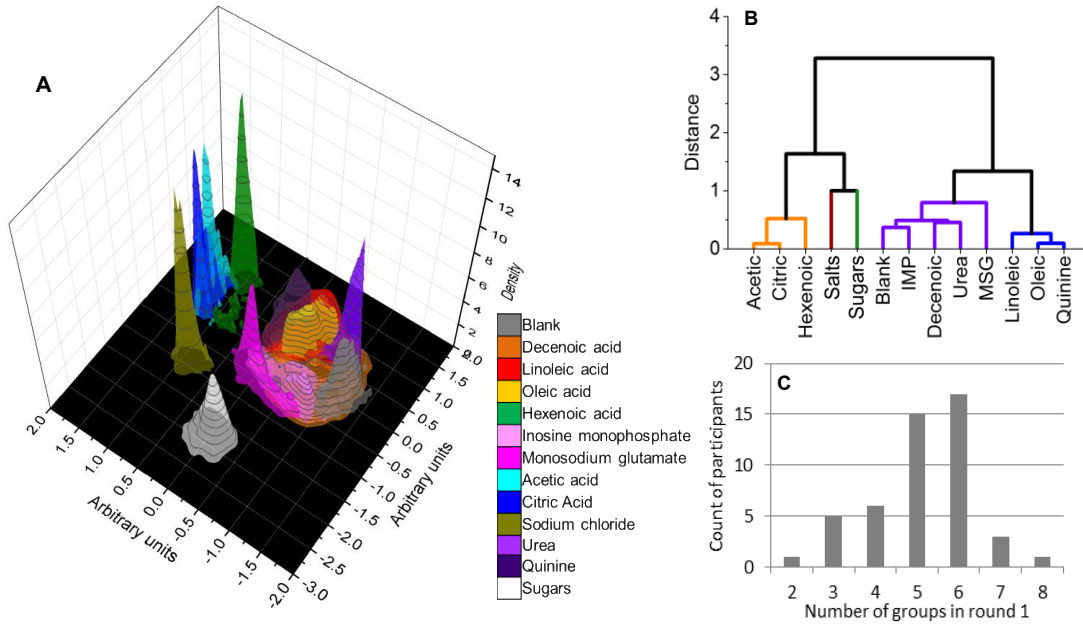
469 Figure 2

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475 Figure 3

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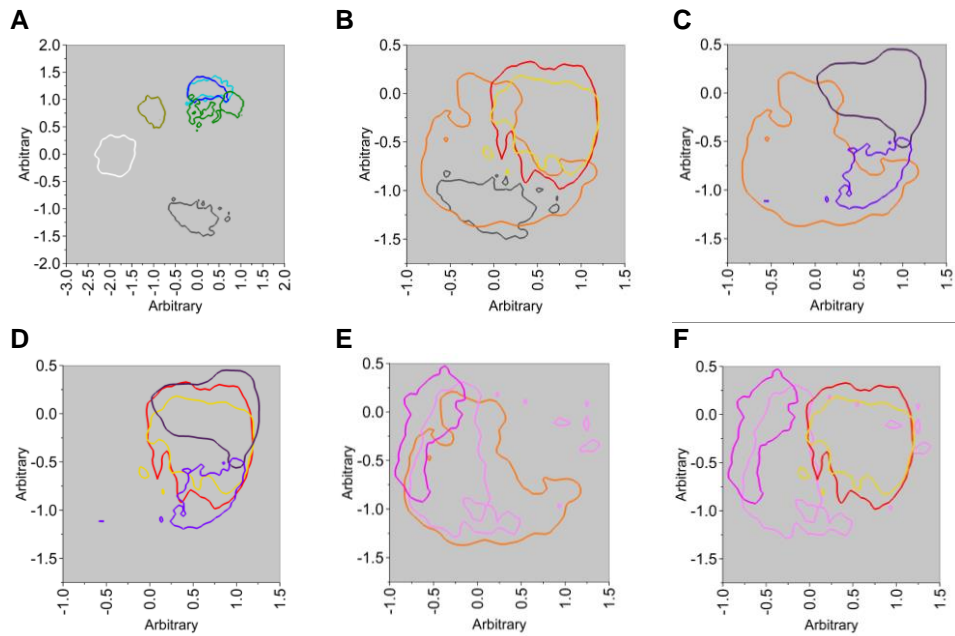


Figure 4

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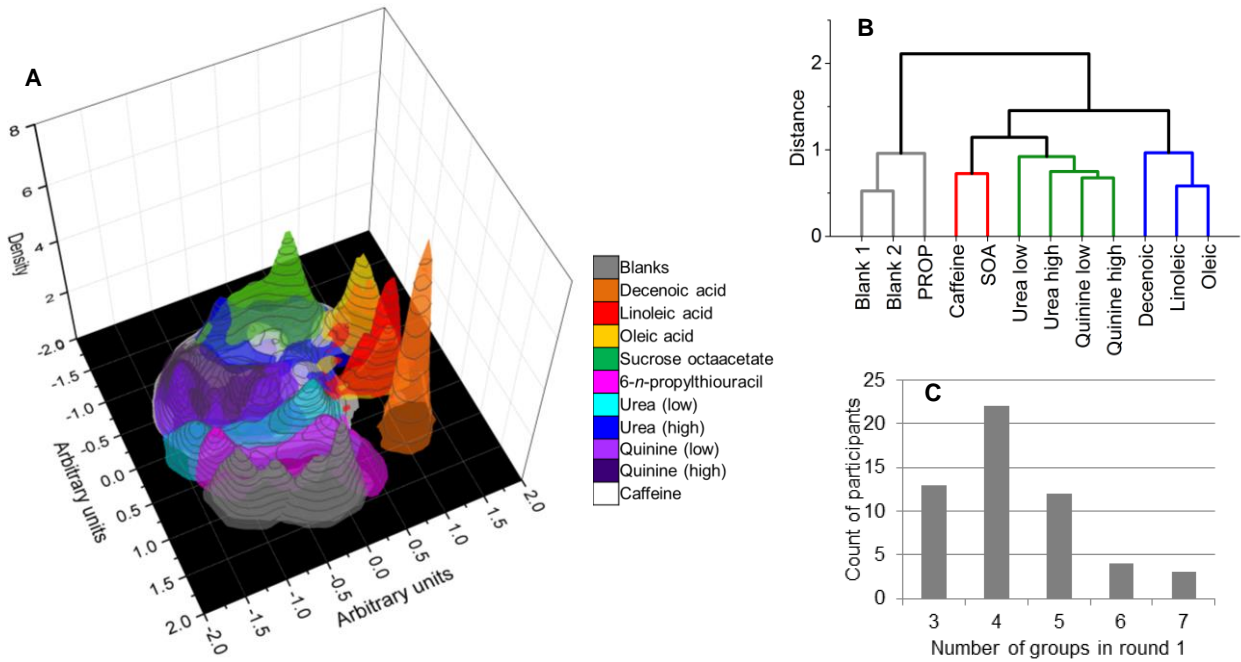


Figure 5

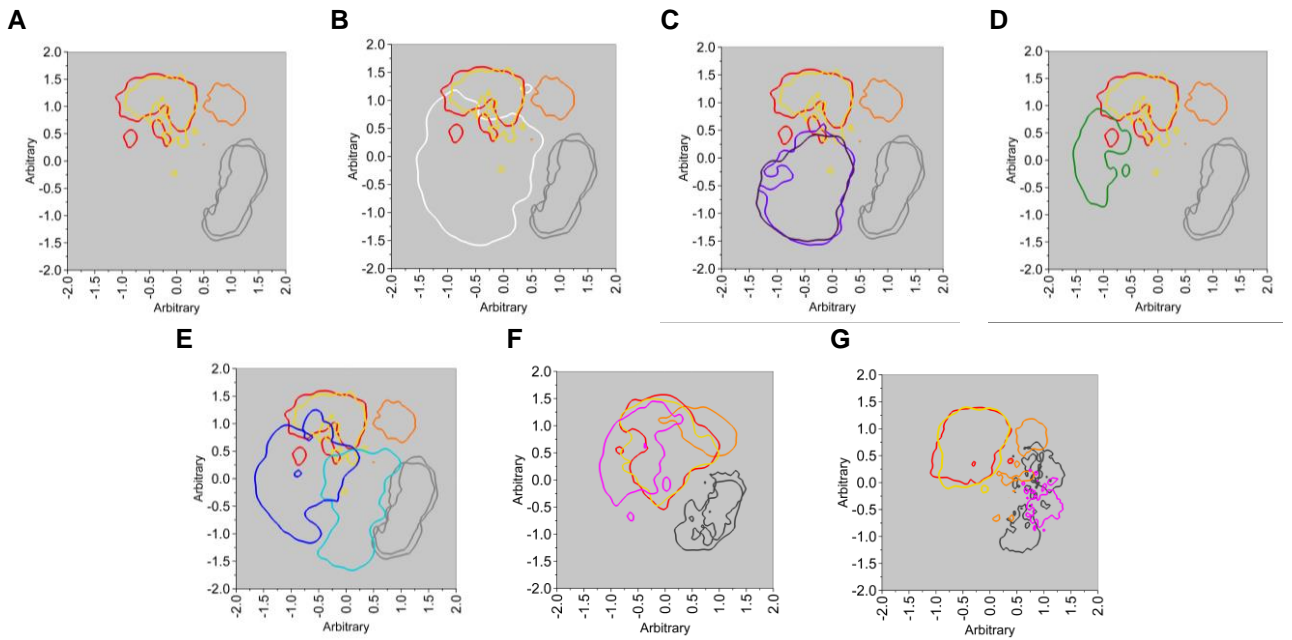


Figure 6

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Table 1: Concentrations of tastants and fatty acids

	Sample	Molarity
Experiment 1	<i>trans</i> -3-Hexenoic	0.0061 M
	9-Decenoic acid	0.0059 M
	Oleic acid	0.18 M
	Linoleic acid	0.018 M
	Acetic acid	0.0083 M
	Citric acid	0.0048 M
	Sodium chloride (in duplicate)	0.094 M
	Inosine monophosphate	0.0013 M
	Monosodium glutamate	0.0069 M
	Glucose	0.54 M
	Fructose	0.31 M
	Quinine	4.5E-05 M
	Urea	0.20 M
	Blank	--
Experiment 2	Oleic acid	0.18 M
	Linoleic acid	0.018 M
	9-Decenoic acid	0.0059 M
	Urea (low)	0.20 M
	Urea (high)	0.40 M
	Quinine (low)	3.3E-05 M
	Quinine (high)	4.9E-05 M
	Caffeine	0.0046 M
	PROP	8.2E-05 M
	Sucrose octaacetate	2.2E-05 M
Blank (in duplicate)	--	

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Table 2: Participant characteristics

	Experiment 1		Experiment 2		
			Non-taster	Taster	Total
Total	48		26	28	54
Low/ High fat	24/24		10/16	15/13	25/29
Male/Female	23/30		9/17	9/19	18/36
Mean age in years	28.4		27.6	29.6	28.6
(range)	(18-51)		(18-54)	(19-52)	(18-54)
BMI in kg/m ² (range)	26.3 (18.5-46.6)		27.6 (19.7-54.4)	27.8 (19.0-48.0)	27.7 (19.0-54.4)

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Table 3: Bhattacharyya’s coefficients (overlap between two non-parametric distributions)

Experiment 1													
	Acetic Acid	Blank	Citric Acid	9-Decenoic	<i>trans</i> -3-Hexenoic acid	IMP	Linoleic Acid	MSG	Oleic Acid	Quinine	Sodium Chloride	Sugars	Urea
9-Decenoic acid	0.0%	53.7%	0.0%	--	0.0%	72.6%	22.7%	25.3%	14.7%	8.2%	0.0%	0.0%	38.8%
<i>trans</i> -3-Hexenoic acid	23.3%	0.0%	23.5%	0.0%	--	0.2%	0.3%	0.7%	0.0%	0.4%	0.0%	0.0%	0.0%
Linoleic acid	0.0%	6.8%	0.0%	22.7%	0.3%	20.3%	--	4.3%	87.6%	70.5%	0.0%	0.0%	40.2%
Oleic acid	0.0%	3.6%	0.0%	14.7%	0.0%	13.8%	87.6%	2.3%	--	57.0%	0.0%	0.0%	25.8%

Experiment 2													
	9-Decenoic acid	Linoleic acid	Oleic acid	Blank 1	Blank 2	Caffeine	PROP	Quinine low	Quinine high	Sucrose octaacetate	Urea low	Urea high	
9-Decenoic acid	--	1.8%	3.0%	0.0%	0.6%	2.0%	4.1%	0.3%	0.2%	0.3%	4.3%	0.0%	
Linoleic acid	1.8%	--	86.2%	0.0%	0.0%	16.6%	1.5%	5.8%	4.4%	8.7%	5.9%	20.2%	
Oleic acid	3.0%	86.2%	--	0.0%	0.2%	14.8%	1.6%	6.5%	4.4%	3.4%	8.1%	15.3%	
Blank 1	0.0%	0.0%	0.0%	--	85.6%	0.5%	20.3%	0.0%	0.0%	0.0%	1.8%	0.0%	
Blank 2	0.6%	0.0%	0.2%	85.6%	--	0.2%	22.1%	0.0%	0.0%	0.0%	3.3%	0.0%	
Caffeine	2.0%	16.6%	14.8%	0.5%	0.2%	--	10.4%	66.8%	68.1%	49.4%	36.9%	66.7%	
PROP	4.1%	1.5%	1.6%	20.3%	22.1%	10.4%	--	9.8%	5.9%	0.0%	46.8%	2.2%	
Quinine low	0.3%	5.8%	6.5%	0.0%	0.0%	66.8%	9.8%	--	84.3%	13.0%	43.0%	35.4%	
Quinine high	0.2%	4.4%	4.4%	0.0%	0.0%	68.1%	5.9%	84.3%	--	18.3%	34.5%	40.7%	
Sucrose octaacetate	0.3%	8.7%	3.4%	0.0%	0.0%	49.4%	0.0%	13.0%	18.3%	--	3.2%	75.2%	
Urea low	4.3%	5.9%	8.1%	1.8%	3.3%	36.9%	46.8%	43.0%	34.5%	3.2%	--	12.5%	

Nontasters(N)/Tasters (T)													
	9-Decenoic acid		Linoleic acid		Oleic acid		Blank 1		Blank 2				
	N	T	N	T	N	T	N	T	N	T			
9-Decenoic acid	--	--	6.5%	26.1%	5.1%	21.0%	20.4%	0.2%	13.1%	0.3%			
Linoleic acid	6.5%	26.1%	--	--	87.3%	83.7%	0.0%	0.0%	0.5%	0.5%			
Oleic acid	5.1%	21.0%	87.3%	83.7%	--	--	0.0%	0.0%	0.3%	0.3%			
Blank 1	20.4%	0.2%	0.0%	0.0%	0.0%	0.0%	--	--	31.3%	68.5%			
Blank 2	13.1%	0.3%	0.5%	0.5%	0.3%	0.3%	31.3%	68.5%	--	--			
PROP	5.5%	5.6%	0.0%	41.1%	0.0%	45.2%	43.3%	0.0%	32.0%	0.0%			

High(H)/Low(L) fat diet consumers													
	9-Decenoic acid		Linoleic acid		Oleic acid		Blank 1		Blank 2				
	H	L	H	L	H	L	H	L	H	L			
9-Decenoic acid	--	--	2.9%	15.6%	5.5%	31.2%	3.6%	4.6%	3.5%	2.8%			
Linoleic acid	2.9%	15.6%	--	--	82.9%	65.0%	0.0%	2.3%	0.0%	0.7%			
Oleic acid	5.5%	31.2%	82.9%	65.0%	--	--	0.0%	4.6%	0.5%	1.9%			
Blank 1	3.6%	4.6%	0.0%	2.3%	0.0%	4.6%	--	--	84.9%	73.9%			
Blank 2	3.5%	2.8%	0.0%	0.7%	0.5%	1.9%	84.9%	73.9%	--	--			
PROP	8.2%	45.5%	0.0%	35.5%	0.5%	60.8%	42.2%	13.5%	50.2%	8.7%			

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