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Additional Information

1 **Influence of storage on the volatile profile, mechanical, optical properties and**
2 **antioxidant activity of strawberry spreads made with isomaltulose**

3
4 *Influence of storage on quality parameters of isomaltulose strawberry spreads*

5
6 Irene Peinado*^a, Estela Rosa^b, Ana Heredia^b, Isabel Escriche^b & Ana Andrés^b

7
8 ^a Faculty of Science and Technology, Free University of Bolzano, Piazza Università 5,
9 39100, BZ, Italy.

10 ^b Institute of Food Engineering for Development, Universitat Politècnica de València,
11 P.O. Box 46022 Valencia, Spain.

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13
14
15 *Corresponding author: Irene Peinado, T: +39 0471 017825; F: +39 0471 017009

16 e-mail address: Irene.PeinadoPardo@unibz.it / irpeipar@gmail.com

19 **Abstract**

20 This work represents the final step of a series of studies on the formulation of
21 strawberry products with partial replacement of sucrose by healthier sugars such as
22 fructose and isomaltulose. Previously, quality parameters of the formulated products
23 such as colour, texture, rheology, aromatic profile and sensory evaluation were
24 assessed. As a final step, in the present work, the volatile profile evolution of a
25 strawberry spread-product during 90 days of storage at room temperature (20°C), and its
26 relation with some physicochemical properties (a_w , pH, texture and colour) and
27 antioxidant activity as well as anthocyanin content were studied.

28 Most of the volatile compounds modified their concentration during storage; some of
29 them totally disappeared but 13 new compounds were formed: (methyl-2-methyl
30 butyrate, E-2-butenal, 2-butenal-2-methyl, 2-buten-1-ol, 2-penten-1-ol, 2-ethyl-1-hexanol,
31 6-methyl-5-hepten-2-one, acetic acid, propanoic-2-methyl acid, butyric acid and
32 butyric-2-methyl acid). Storage led to a dark pink colour and an increase in consistency
33 and adhesiveness while the antioxidant activity considerably increased (from 18 ± 2 to
34 $94 \pm 2\%$ DPPH inhibition).

35 Levels of citric acid and pectin influenced colour, texture and antioxidant activity as
36 well as retention and formation of aromatic compounds, especially in fructose-
37 isomaltulose products. Correlations via a PLS were found between the aromatic profile
38 of the products after storage and some of their quality parameters such as texture, colour
39 and antioxidant content. Future research might involve correlation and identification of
40 specific volatiles with different quality parameters.

41

42 **Keywords:** strawberry, fruit processing, isomaltulose, volatile profile, storage.

43

44 **Chemical compounds studied in this article**

45 Isomaltulose (PubChem CID: 439559); Sucrose (PubChem CID: 5988); Fructose
46 (PubChem CID: 5984); Citric acid (PubChem CID: 311); Potassium sorbate (PubChem
47 CID: 23676745); 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) (PubChem CID:
48 2735032); isopropyl acetate (PubChem CID: 7915); Propan-2-ol (PubChem CID 3776);
49 2-buten-1-ol (Pubchem CID 20024).

50

51

52 **1. Introduction**

53 Strawberry spread-product refers to a spread sweet food with improved functionality
54 due to the replacement of sucrose by healthier sugars, such as isomaltulose, and to the
55 incorporation of a higher content of fruit compared to jams for instance. The usual
56 cooking step of jam processing to reach a final soluble solid concentration is avoided in
57 fruit spread-product manufacturing. In our previous study, the influence of processing
58 parameters such as, fruit, sugars (isomaltulose-sucrose or isomaltulose-fructose), pectin
59 and citric acid, on the volatile profile of strawberry spread-product formulated with
60 isomaltulose and sucrose or fructose, was analysed (Peinado et al., 2013). A major
61 effect of the formulation parameters (mostly pectin and citric acid levels), and their
62 interactions, on the volatile fraction changes and aroma retention in the food gel was
63 found. Two different phenomena could occur as a consequence of processing: (a) the
64 modification (reduction or increase) of the compounds responsible of the aroma of fresh
65 strawberry, and (b) the generation of new aromatic compounds. Some steps involved in
66 processing of spreadable products, e.g. cutting or blending, sugar addition, etc., can lead
67 to an increase in the activity of enzymes responsible for the development of new
68 aromas. This fact has been also reported for many fruits under different condition such
69 as osmotic stress, UV light, pH variations or under contact with metal ions (Escriche et
70 al., 2000; Olías et al., 1993; Zabetakis & Holden, 1997).

71 In our previous study, 13 new compounds (6 aldehydes, 1 alcohol, 4 furans, 1 terpene
72 and 1 nitrile) were found in strawberry spread-product after twenty-four hours of
73 processing, compared to raw material. It has been previously reported that the
74 generation of new aldehydes might be related to the bio-oxidation of lipids as a
75 consequence of wall disruption (Yilmaz, 2001). Eucalyptol, the only generated alcohol,
76 appears during the heat treatment of fruit juices as a consequence of the hydrolysis of

77 their corresponding glycoside precursors (Barron & Etiévant, 1990). The activation of
78 Maillard reactions, or the catabolism of carotenoids and non-saturated fatty acids lead to
79 furans generation (van Boekel, 2006). In the studied spreads, the type of sugar
80 influenced the concentration of furfural (known as the most representative furan related
81 to cooked flavours) (van Boekel, 2006; Varlet, Prost, & Serot, 2007). From the 29
82 original volatile compounds detected in fresh strawberry, 5 of them completely
83 disappeared and 24 compounds (mostly esters and alcohols) varied their concentration
84 after processing. The retention of typical fresh strawberry volatile compounds was very
85 influenced by the blend of sugars, as well as percentage of pectin and citric acid; the
86 higher the percentage of both, pectin and citric acid, the higher the retention of volatile
87 profile of fresh strawberries, especially in those spreads formulated with isomaltulose-
88 sucrose. The strengthen of gelling structure increases with increasing amounts of pectin,
89 and citric acid, therefore, the gel has a closer structure where the exposure to external
90 factors and the possible enzymatic reactions, which induce changes in aroma profile, are
91 minimised. The role of the food matrix in controlling flavour retention, and even their
92 release, has been previously pointed out (Boland et al., 2006; Druaux & Voilley, 1997).
93 It is well known that volatile profile can continue evolving with storage time.
94 Nevertheless, a few number of studies have investigated the effect of storage conditions
95 on aroma changes of fruity products such as juices, jams, purées, etc. (Kopjar et al.,
96 2008; Torres, Chiralt, & Escriche, 2012). The majority of works have studied the effect
97 of postharvest conditions such as temperature, relative humidity, controlled-atmosphere,
98 etc. (Ayala-Zavala et al., 2004; Berna et al., 2007; Mo & Sung, 2007; Nielsen &
99 Leufven, 2008; Pelayo et al., 2003; Vandendriessche et al., 2013), or the influence of
100 osmotic treatment (Blanda et al., 2009; Rizzolo et al., 2007) on the modification of
101 strawberry aroma. Because foods are generally consumed after a certain time of storage,

102 the evaluation of the changes taking place during this period would be also suitable.
103 Since it is also expected that physicochemical properties such as mechanical and optical
104 ones change, there is a need to better understand the link between flavour profile and
105 texture or colour parameters evolution along storage, so colour and texture
106 determinations might help to control aroma changes. In this sense, this study analysed
107 the changes in the volatile profile occurring after 90 days of storage of 52 different
108 strawberry spread-products formulated with healthier sugars to replace sucrose partially
109 or totally. Additionally, the correlation between the aromatic compounds, some
110 physicochemical properties (pH, water activity, texture and colour) and antioxidant
111 activity as well as anthocyanin content were studied by means of a partial least squares
112 regression (PLS regression).

113 **2. Material and Methods**

114 *2.1. Raw material*

115 For this study, fifteen different batches of raw strawberry (*Fragaria vesca*, Camarosa)
116 were acquired from a local supermarket from February to June 2010. A selection based
117 on colour, shape and ripeness were performed for each batch in order to reduce the
118 variability of the raw material. Each different batch was enough to produce 3 or 4
119 different spreadable products, depending on the batch. Then they were cut and washed
120 in chlorinated water to eliminate possible field residues.

121 *2.2. Experimental methodology*

122 Strawberry spread-products of 50 °Brix were formulated according to a statistical
123 central composite design 2^4 + start (Peinado et al., 2013, 2015). For the experimental
124 design, four independent variables were taken into account: percentages of isomaltulose,
125 pectin and citric acid, as well as time of heat treatment (table I). Concretely, two groups
126 of spread-product were obtained following two replicates of the above statistical design:

127 one group containing 26 spread-products formulated with isomaltulose-sucrose, and
128 another one of 26 spread-products formulated with isomaltulose-fructose. The
129 ingredients in the spreadable strawberry products were: strawberries, sugars (sucrose or
130 fructose, and isomaltulose), pectin (as gelling agent), citric acid, and potassium sorbate
131 (as microbiological preserver); the formulation of the products has already been
132 explained in detail and was the implementation of dry osmotic dehydration studied
133 previously (Rosa et al., 2008, Peinado et al., 2013 & 2015). Basically, ingredients were
134 directly mixed in the correct proportions to reach the established concentration of 50
135 °Brix, avoiding therefore the typical equilibrium stage which takes place during osmotic
136 dehydration. The quantities of raw strawberry and sugar were calculated according to
137 the correspondent mass balance and they were dependent on the soluble solids content
138 in the raw strawberries. Once the ingredients were mixed, the product was heated until it
139 reached 85°C. This temperature was necessary so the gel structure would not break up
140 during storage as well as to make “hot canning” effective. Then, the product was
141 placed in glass jars and some of the samples, depending on the statistical design, were
142 heated for 5, 10, 15 or 20 min in a bath of boiling water. Finally, they were stored at
143 20°C and darkness for 24 hours and 90 days and after that, the correspondent analyses
144 were performed.

145 2.3. *Analytical determinations*

146 All analytical determinations were performed in triplicate after 24h and 90 days of
147 storage.

148 2.3.1. Volatile profile characterization

149 Following the same methodology as Peinado et al. (2013), volatile compounds were
150 extracted by purge and trap thermal desorption using 2-pentanol as an internal standard.
151 GC–MS analyses were performed using a Finnigan TRACETM MS (TermoQuest,

152 Austin, USA) equipped with a BP-20 capillary column (SGE, Australia) (60 m x 0.32
153 mm i.d. x 1.0 mm film thickness). Helium at 1 ml·min⁻¹ was used as a carrier gas. The
154 ramp temperature was from 40°C (2 min hold time) to 190°C at 4°C ·min⁻¹ and finally to
155 230°C at 10°C min⁻¹.

156 Relative retention indices were determined by injection of a standard solution of normal
157 alkanes (C₈–C₂₀; by Fluka Buchs, Schwiez, Switzerland). The volatile compounds were
158 tentatively identified and semi-quantitative analyses were carried out (Peinado et al.,
159 2013; Soria et al., 2008). The data (µg/100 g) were expressed using the amount of
160 internal standard and the relative area of each compound with respect to that of the
161 internal standard, assuming a response factor equal to one.

162 2.3.2. Physicochemical determinations

163 All the physicochemical parameters were analysed following the methods explained
164 with more detail by Peinado et al. (2015). Basically, moisture content was determined
165 gravimetrically by drying to constant weight in a vacuum oven at 60°C (AOAC method
166 20.103, 2000). Soluble solids content (Brix) was measured, in previously homogenized
167 samples, with a refractometer at 20°C (ATAGO 3 T). Water activity (a_w) was
168 determined with a dew point hygrometer (FA-st lab, GBX). pH was determined with a
169 pH-meter (SevenEasy, Mettler Toledo). Texture properties of the spread-products were
170 performed by a back-extrusion test using a texturometer TA/XT/PLUS Texture
171 Analyser (García-Martínez et al., 2002; Sesmero et al., 2007). This assay provides a
172 typical curve with two characteristic areas. The positive area of the curve is taken as an
173 indication of consistency (N·s), while the negative area gives an indication of the
174 adhesiveness (N·s) but also of the consistency/viscosity of the sample. The optical
175 properties were conducted in a Minolta spectrophotometer (model CM-3600d), using a
176 black background, at room temperature and visible absorption spectra were recorded

177 between 380 and 770 nm by reflectance to obtain tristimulus values of CIEL*a*b*,
178 considering standard light source D₆₅ and standard observer 10° as references.

179 2.3.3. Antioxidant activity

180 Antioxidant activity was estimated by means of the DPPH method (Castelló et al.,
181 2011). As the aim of the present work was to correlate the evolution of the aromatic
182 profile with some quality parameters, antioxidant activity results were taking into
183 account, however, the detailed methodology as well as individual results for all
184 treatments can be found at (Peinado et al., 2015).

185 2.3.4. Total anthocyanin content

186 Finally, total anthocyanin content was measured at 520 nm using the
187 spectrophotometric method described by Alarcão-E-Silva et al. (2001) and results were
188 expressed as mg of pelargonidin-(Pgd)-3-glucoside in 100 g of spreadable product, on
189 the basis of the molar extinction coefficient ($E_{\text{molar}}=36,000 \text{ M}^{-1} \text{ cm}^{-1}$) (Vicente, et
190 al., 2002) and taking the molecular weight (433 g/mol) reported by Skrede et al. (1992)
191 for pelargonidin-(Pgd)- 3-glucoside. In the same way that for antioxidant activity, the
192 total anthocyanin content was correlated with the aromatic profile, but, the detailed
193 methodology as well as individual results for all treatments can be found at (Peinado et
194 al., 2015).

195

196 2.4. Statistical analysis

197 As previously mentioned, the number of samples was set by means of a central
198 composite design taking into account four independent variables: *X1* (% of
199 isomaltulose), *X2* (% of pectin), *X3* (% of citric acid) and *X4* (time of thermal
200 treatment), at five levels (table I).

201 Principal Component Analysis, (PCA) and Partial Least Squares regression (PLS
202 regression) (Unscrambler version 10.X; CAMO Process AS, Oslo, Norway) were
203 applied to describe the relation between the volatile compounds with the
204 physicochemical and antioxidant parameters as well as with the process variables (wt.%
205 of isomaltulose, wt.% of pectin, wt.% of citric acid and time of heat treatment.
206 PCA was used an orthogonal transformation to convert the obtained data of possibly
207 correlated variables (volatile compounds and different spread products) into a set of
208 values of linearly uncorrelated variables (called principal components). This
209 transformation is defined in such a way that the first principal component has the largest
210 possible variance (that is, accounts for as much of the variability in the data as possible),
211 and each succeeding component in turn has the highest variance possible under the
212 constraint that it is orthogonal to the preceding components. PLS was used used to find
213 the correlations between two matrices of data (X: volatiles and Y: physicochemical
214 parameters as well as antioxidant values) (Martens & Næs, 1989).

215

216 **3. Results and discussion**

217 *3.1. Volatile profile of spreadable strawberry products after storage.*

218 Table II shows the relative retention index (RI), the odour threshold values as well as
219 the odour descriptors for the volatile compounds identified in the raw strawberries as
220 well as the volatile compound generated 24 hours after processing. The volatile profile
221 of the samples changed as a consequence of storage time. In general terms, the
222 concentration of the majority of the volatile compounds was modified compared to their
223 concentration in the products after processing. Table III illustrates the relative retention
224 index (RI) calculated for each volatile compound identified as well as the maximum and
225 minimum net variations for the volatile compounds respect to the products after 24

226 hours of processing. Results are expressed as the ratio $(C_{90}-C_0)/C_0$, C_{90} being the
227 concentration of the volatile compound in the spreadable products stored during 90
228 days, and C_0 the initial concentration of this compound in its correspondent spreadable
229 product 24 hours after processing (μg of volatile compound /100 g of spreadable
230 product). The general trend for most of the volatile compounds was a decreased in their
231 concentration (table III illustrates just the lowest and highest value of the 26 products
232 formulated with fructose-isomaltulose and 26 products formulated with sucrose-
233 isomaltulose, therefore some increase can be observed as the maximum value), whereas
234 some compounds present after processing such as octanal, nonanal, decanal, mesifurane,
235 butyl butyrate, hexyl butyrate and octyl acetate, were not detected after 90 days of
236 storage. Nevertheless, 11 new compounds were identified for the fructose-isomaltulose
237 products and 13 for the sucrose-isomaltulose ones, within the volatile fraction of the
238 strawberry spreadable products after storage. These compounds may be classified in:
239 three esters (for the sucrose-isomaltulose samples) and one ester (fructose-isomaltulose
240 samples), two aldehydes, three alcohols, one ketone and four acids, as it can be seen in
241 table III which also illustrates the concentration of these new compounds expressed as
242 μg of volatile compound /100 g of spread (net variation cannot be calculated as the
243 initial concentration in the processed products after 24 hours would be equal to 0). It is
244 important to point out that some compounds as isopropyl acetate and some of the
245 alcohols (propan-2-ol, 3-methyl-butanol, 1-pentanol and 1-hexanol) were already
246 detected in some of the samples after processing, however, they were not found in all 52
247 samples, meaning that there was also a generation of them after storage in some of the
248 samples.

249 Esters decreased their concentration during storage, but three new esters were found,
250 isopropyl acetate, ethenyl acetate and methyl-2-methyl butyrate. Some authors have also

251 found a decrease in esters after storage of fruit jams at temperatures above refrigeration.
252 Esters, might in fact, potentially serve as precursor compounds for synthesis of
253 secondary volatile aroma compounds such as terpens, alcohols or aldehydes (Ayala-
254 Zavala et al., 2004; Lamikanra & Richard, 2002; Touati et al., 2014). However, Aguiló-
255 Aguayo et al. (2009), observed an increase in butyl acetate concentration in strawberry
256 juices after 35 days of storage at 4°C and in the same way, Ayala-Zavala et al. (2004),
257 reported higher concentration of most of the esters of strawberry stored at 0, 5 and 10°C.
258 These authors established a relation between storage temperature and volatile profile
259 development; the higher the storage temperature, the higher the increase in the
260 concentration of the esters. It has to be mentioned, that in the present study, products
261 were stored at 20°C, which is above the refrigeration temperatures (0-10°C) that seem to
262 have a positive effect on the stability and even increase of typical strawberry esters. The
263 results obtained in our study suggest that higher temperatures might contribute to a
264 decrease or loss of esters in strawberry spreads, but also on the availability of other
265 compounds such as acids and alcohols required for their formation as well as the
266 presence of some enzymes responsible of their catalysis (Azodanlou et al., 2003; Bood
267 & Zabetakis, 2002; Lamikanra & Richard, 2002; Touati et al., 2014).
268 Most of the alcohols increased their concentration after storage with the exception of
269 propan-2-ol that decreased, and linalool that disappeared. Alcohols have been identified
270 as constituents of the characteristic strawberry aroma (Pino et al., 2001), and some
271 authors detected an increase in this compounds after the storage of strawberries at
272 refrigeration temperatures; this increase might produce an undesirable aroma in the
273 product due to their strong taste and odour (Aguiló-Aguayo et al., 2009; Ayala-Zavala
274 et al., 2004). Furthermore, in the same way as in the present study, Von Sydow &
275 Karlsson (1971), observed a decrease in linalool concentration during storage of

276 samples after thermal treatment, which could be related to the conversion of linalool in
277 other terpens, by different mechanisms as hydration, restructuration or cyclation of the
278 double bounds.

279 Besides that the concentration of most of the aldehydes decreased and 1-penten-3-one
280 disappeared, two new aldehydes and one ketone appeared after storage. These
281 compounds are easily reduced or oxidized, leading to the formation of some other
282 derivate compounds. Specifically, hexanal can be produced by an oxidation catalysed
283 by LOX-lyase; therefore, the inactivation of this enzyme, that might occurred due to the
284 heat treatment, would lead to a decrease of this compound during storage (Hamilton-
285 Kemp et al., 1996).

286 For the furans presents in the strawberry products, mesifurane was not detected after 90
287 days of storage, while in general furfural, 2-acetilfurane and 5-methyl furfural
288 experimented an increase in their concentrations. This fact is coincident with that
289 observed by other authors with the similar type of compounds. For instance Touati et al.
290 (2014) reported a significant increase of HMF content for fruit nectars after 8 months
291 storage, at 25, 35 and 45 °C and Orruño et al. (2001), observed an increase of DMHF
292 (2,5-di-(hydroxyl-methyl)-furan) in strawberry juices thermally treated. The increase of
293 those compounds might be related to the increase of some enzymatic activity
294 responsible for the formation of compounds as β -glycosidase or also to sugar
295 degradation in acidic medium (Orruño et al., 2001; Touati et al., 2014).

296 A PCA was conducted to evaluate the global effect of the storage on the volatile
297 fraction of the spreadable products, from a descriptive point of view. Figure 1 illustrates
298 the loadings for the different aromatic compounds as well as the sample scores of the
299 different strawberry samples after 24 hours of processing (S₀1–S₀26, and F₀1–F₀26),
300 and the samples after 90 days of storage (S₉₀-1–S₉₀-26, and F₉₀-1–F₉₀-26). The first two

301 principal components of the analyses explain 50% of the total variance of the
302 concentrations of the different volatile compounds among the different samples (PC1:
303 41 % & PC2: 9%). In the score plot, proximity between samples indicates similar
304 behaviour in terms of the volatile profile. PC1 (41%) clearly differentiates between
305 products after processing, located at the right side of the plot, and products after storage,
306 located at the left side. PC2 (9%), differentiates between those treatments that had
307 higher percentages of pectin and citric acid (treatments F-19, S-19, F20, S-20, F22, S-
308 22), being the differences clearly more noticeable after storage when the gel has become
309 stronger.

310 *3.2. Influence of formulation on the physicochemical parameters of spreadable*
311 *strawberry products.*

312 A PLS was conducted to analyse the relation between the aromatic profile of the
313 spreadable products and their physicochemical and antioxidant values. Figure 2
314 illustrates the sample loadings for the different aromatic compounds as well as
315 physicochemical and antioxidant parameters analysed within the strawberry spreadable
316 products after storage. In the plot, proximity between aromas demonstrates some
317 similarity in their concentration levels, while the parameters allocated inside the ring are
318 those that can be correlated with the volatile compounds. Figure 2 summarizes the
319 significant correlation coefficients and the r^2 obtained for the analysed parameters.
320 Antioxidant activity and anthocyanin content as well as texture values were highly
321 correlated with the aromatic profile, while concerning colour, only chrome (*C) seemed
322 to be correlated.

323 3.2.1. Stability of stored products in terms of a_w and pH

324 Table IV gathers the punctual data for the pH values and water activity (a_w) for every
325 single spreadable product 24 hours after processing as well as after 90 days of storage.

326 Their pH values ranged between 3.410, for the spreads formulated with the lowest
327 percentage of citric acid, and 2.703 for those with the highest percentage of citric acid,
328 after 90 days of storage, (regardless the sugar used in their formulations sucrose-
329 isomaltulose or fructose-isomaltulose). These values were in most of the samples
330 slightly lower when compared to their correspondent values for the products after 24
331 hours of processing; however, the differences were not statistically significant ($P_{\text{value}} >$
332 0.005). It is important to mention that, contrary to what might have been expected, no
333 correlation was found between pH or a_w and the volatile profile of the spreadable
334 products after storage; nevertheless, the differences in pH and a_w between the different
335 products should be taken into account because they could have a direct influence on the
336 stability and acceptance of the product even when they are not reflected on the volatile
337 profile.

338 3.2.2. Optical and mechanical properties

339 As reported in a previous study (Peinado et al., 2015), processing induced, in general, a
340 reduction of L^* , a^* and b^* parameters in all the spreadable products 24 hours after
341 processing compared to raw strawberries (lower Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$), and hue
342 ($h^* = \arctg(b^*/a^*)$) were observed as well). As an example, Figure 3 illustrates the
343 colorimetric maps (L^* - a^* and b^* - a^*) for the spreadable products formulated with the
344 higher and lower levels of the formulation variables (Tables I and IV) after 24 hours of
345 processing as well as after 90 days of storage. The higher the percentages of citric acid
346 and pectin, the lower the values of these coordinates. However, the influence of pectin
347 and citric acid disappeared after storage only for the spreadable formulated with the
348 blend fructose-isomaltulose, leading to products with darker colour, and a major
349 homogeneity regardless the process variables, so the type of sugar influenced the
350 reactions involved in colour changes such as anthocyanin degradation.

351 Chrome showed a negative correlation with the volatile compounds characteristics of
352 the spreads after processing (and so it did the anthocyanin content). Storage led to a
353 change from bright red colour to dark pink. These colour changes may be mainly
354 attributed to anthocyanin degradation, which are known to be very sensitive to different
355 factors such as light, oxygen, temperature, pH, sugar content, ascorbic acid or metals
356 (Golaszewski, et al., 1998; Garcia-Viguera et al., 1999; Renard et al., 2006). However,
357 there are other compounds that also suffer browning reactions, for instance, sugar and
358 ascorbic acid degradation during storage have been related to colour loss in strawberry
359 products as well as formation of aromatic compounds such as acetic acid and some
360 furans.

361 Regarding mechanical properties, an increase in consistency and adhesiveness after 90
362 days of storage was observed for all the spreadable products. Figure 4 illustrates the
363 consistency values obtained for the spreadable products formulated with the higher and
364 lower level of the formulation variables (Tables I and IV) after 24 hours of processing
365 as well as after 90 days of storage. This increase was higher as citric acid and pectin
366 levels augmented, while sugar became also significant at high levels of pectin. The PLS
367 plot, illustrates a positive correlation for the texture parameters with the volatile
368 compounds characteristics of storage such as acids and aldehydes (butanoic acid,
369 propanoic acid, benzaldehyde...), which are developed due to changes in the food
370 matrix as the gel becomes more rigid along the storage time. Nevertheless, the intensity
371 of aroma release in the samples stored will also depend on the amounts of pectin and
372 citric acid, determining the final consistency and rigidity of the gel, (Boland et al., 2006;
373 Golaszewski et al., 1998; Piccone et al., 2011).

374 3.2.3. Antioxidant activity and total anthocyanin content.

375 As it can be found in Peinado et al., (2015), the antioxidant activity (% DPPH
376 inhibition) of the different spreadable products showed a considerable increase after 90
377 days of storage for most products compared to their antioxidant values immediately
378 after processing, from 18 to 94 % DPPH (100 μ M trolox = 50 % DPPH, reference
379 value). Once again, citric acid and pectin levels were the variables that most influenced
380 the antioxidant activity. A high antioxidant capacity was associated with a high level of
381 pectin and citric acid in the samples after 24 hours of processing, while the antioxidant
382 activity became similar among all samples after storage, regardless the sugar used or the
383 amounts of pectin or citric acid.

384 On the contrary, there was a decrease in the anthocyanin content during storage, from
385 10.9 to 3.9, and from 10.0 to 1.7 mg pelargonidin-3-glucoside /100 g for sucrose-
386 isomaltulose and fructose-isomaltulose respectively, (individual values after 24 hours
387 and 90 days can be found at Peinado et al., 2015). Despite of this decrease, other
388 compounds with high antioxidant activity such for instance those derived from
389 degradation of phenolic, gallic or ellagic acid might result in an increase of antioxidant
390 activity during storage (Aaby et al., 2007; Lamikanra & Richard, 2002). Along the
391 storage time, the tri-dimensional network of the gel acquires more strength, becoming a
392 key-factor for the retention and protection of the antioxidant components against
393 oxidative agents (light, oxygen, etc.). In fact, the PLS correlated antioxidant activity
394 with some of the new compounds identified after storage such as propanoic and
395 butanoic acids.

396

397 **4. Conclusions.**

398 Storage influenced the colour, texture and antioxidant activity as well as the volatile
399 profile of the spreadable products. The percentages of pectin and citric acid were a key
400 factor on the retention of the aroma, especially in the fructose-isomaltulose products,
401 contrary to that happening after processing (Peinado et al., 2013). Most volatile
402 compounds in the spreads after storage decreased their concentration, whereas some
403 compounds disappeared, and some new compounds were identified after storage.

404 The PLS established good correlations between the aromatic profile of the products
405 after storage and some of their physicochemical parameters such as texture, colour and
406 antioxidant content. Mechanical properties and antioxidant activity were positively
407 correlated with some of the volatiles compounds after storage while anthocyanin
408 content and chrome were negatively correlated. It can be concluded then, that a
409 healthier spreadable strawberry product could be formulated replacing sucrose by
410 fructose-isomaltulose with high percentages of citric acid and pectin. Further work in
411 terms of which specific volatiles could be correlated with those parameters might be
412 required as a future field of research.

413

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418

419 **'Conflict of Interest Statement**

420 We wish to confirm that there are no known conflicts of interest associated with this
421 publication and there has been no significant financial support for this work that could
422 have influenced its outcome.

423

424 We confirm that the manuscript has been read and approved by all named authors and
425 that there are no other people who satisfied the criteria for authorship but are not listed.

426 We further confirm that the order of authors listed in the manuscript has been approved
427 by all of us.

428

429 We confirm that we have given due consideration to the protection of intellectual
430 property associated with this work and that there are no impediments to publication,
431 including the timing of publication, with respect to intellectual property. In so doing we
432 confirm that we have followed the regulations of our institutions concerning intellectual
433 property.

434

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437 responsible for communicating with the other authors about progress, submissions of
438 revisions and final approval of proofs. We confirm that we have provided a current,
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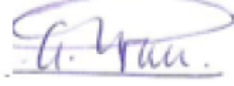
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447 Irene Peinado

Estela Rosa

Ana B Heredia

Ana Andrés

Isabel Escriche

448

449 Date: 29/06/2015

450

451

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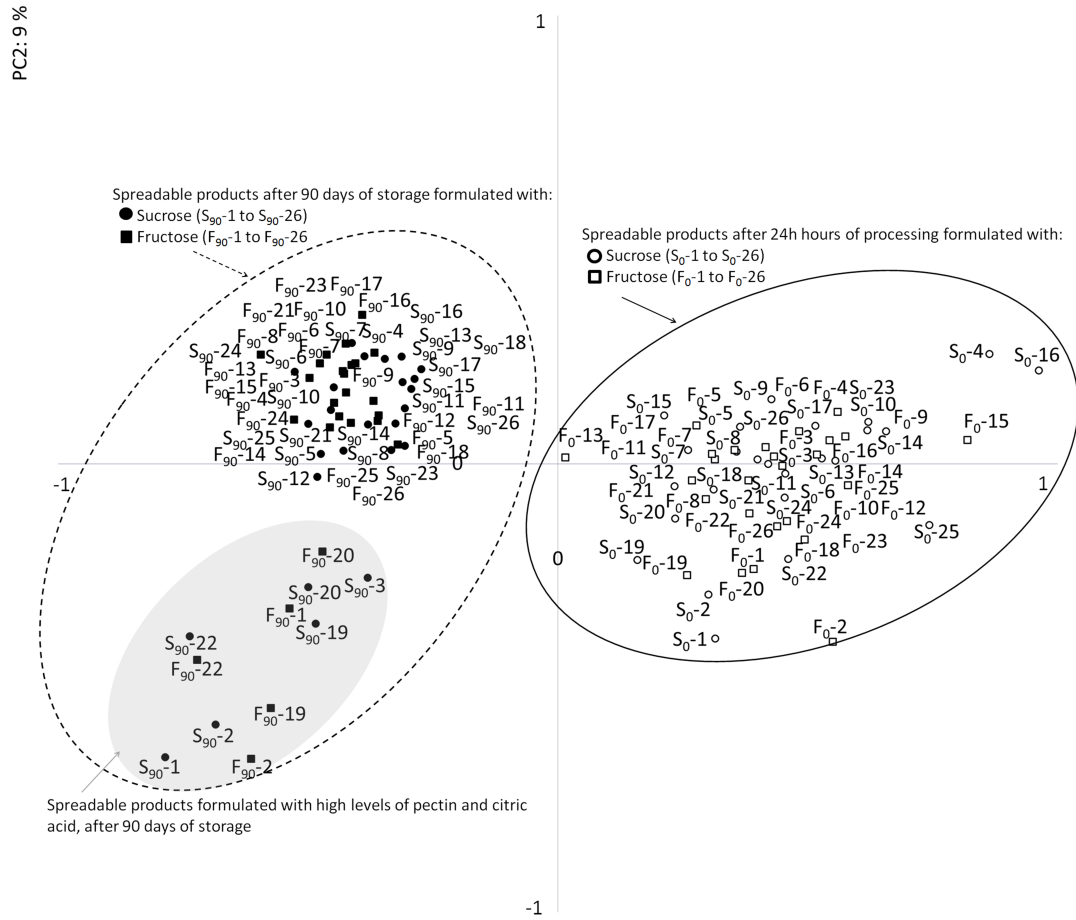


Figure 1

PC1: 41 %

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608 **Fig 1.** Biplot for the different volatile compounds and their relation with the different
 609 strawberry spreadable samples after 24 hours of processing (S₀1–S₀26, and F₀1–F₀26),
 610 and the samples after 90 days of storage (S₉₀-1–S₉₀-26, and F₉₀-1–F₉₀-26) (PC1: 41%
 611 and PC2: 9%) obtained by means of the PCA.

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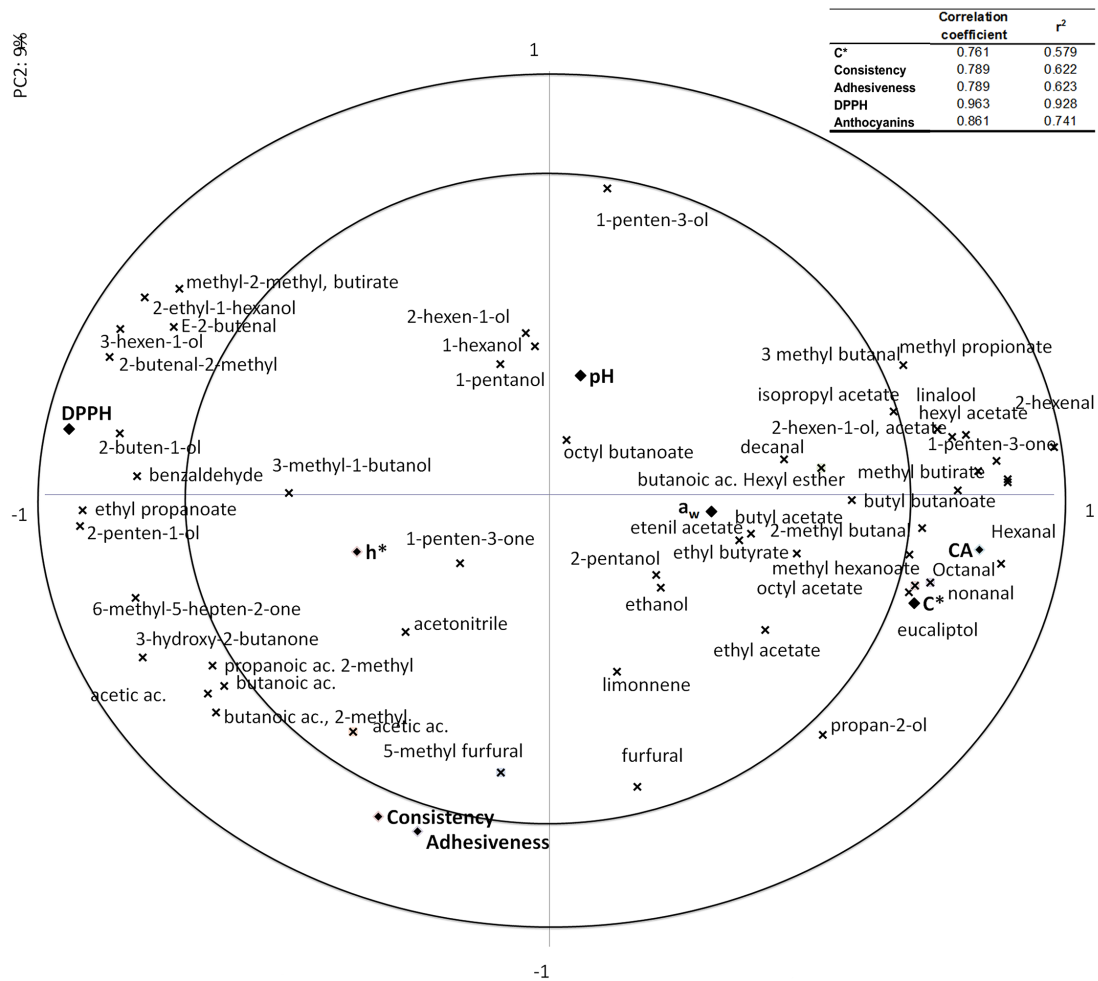


Figure 2

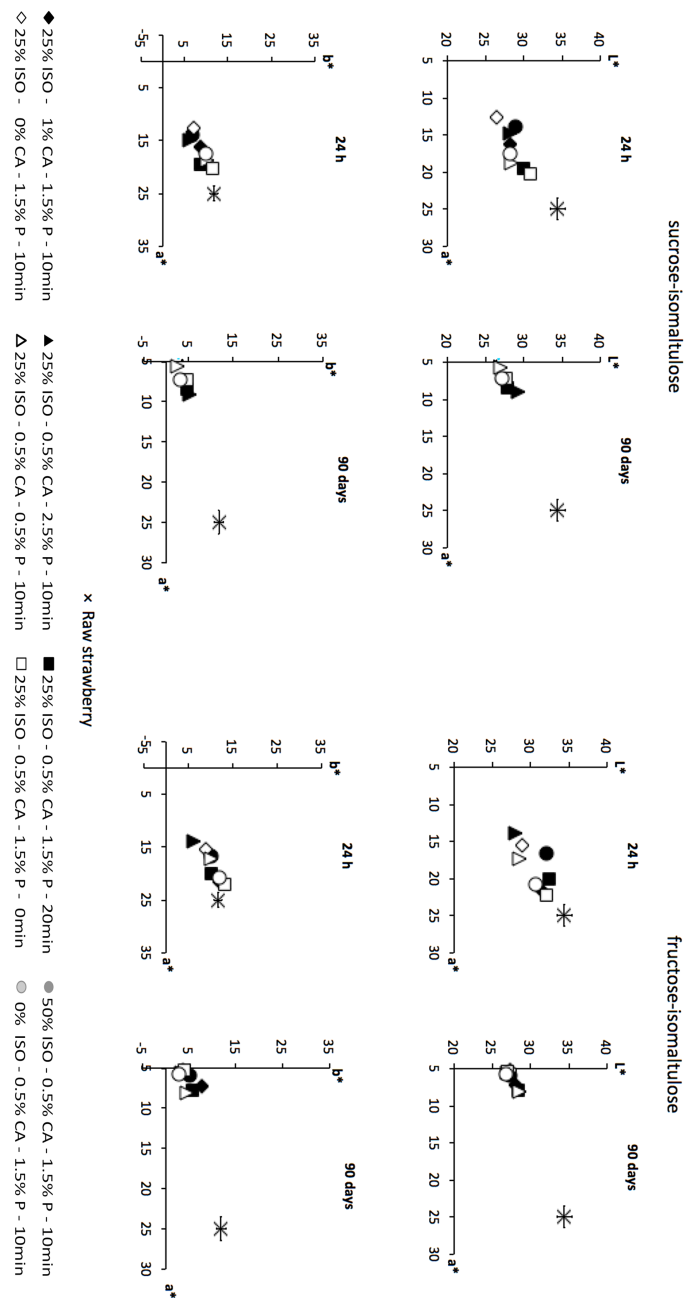
PC1: 41 %

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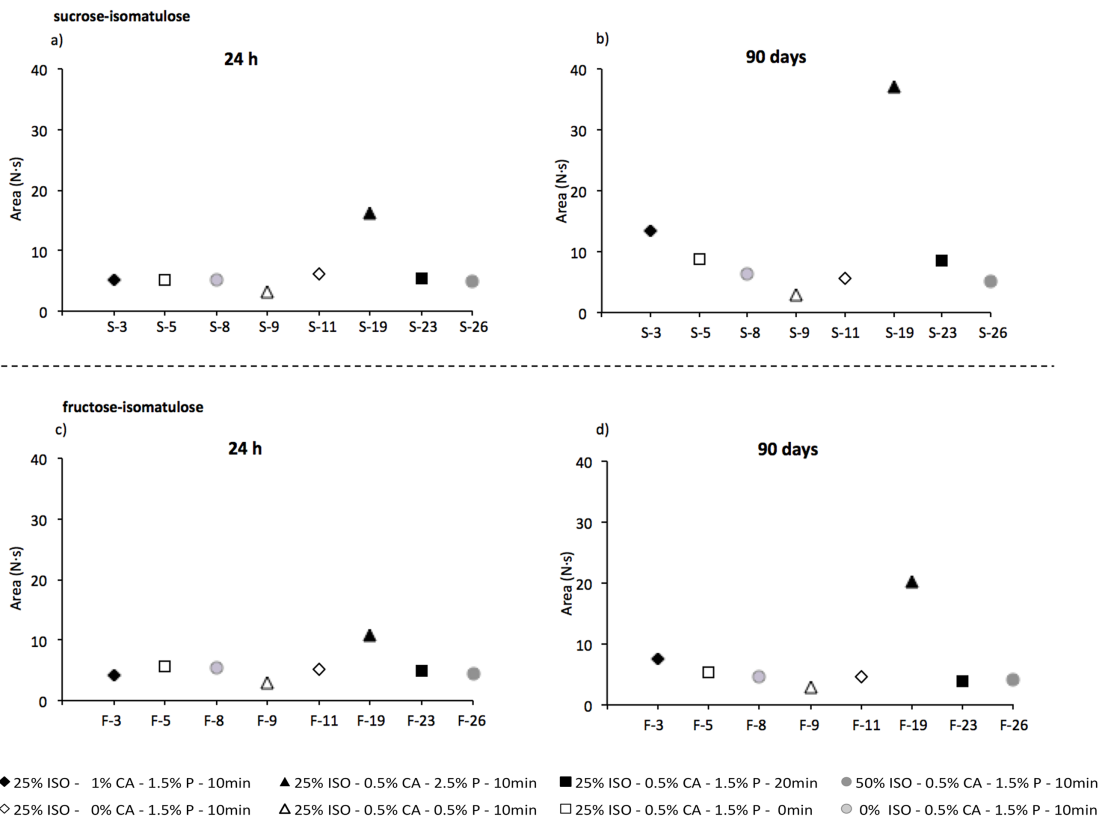
614 **Fig 2.** Biplot for the different volatile compounds and their relation with the
 615 physicochemical parameters (a_w , pH, colour [h*: hue; C*: chrome] and texture
 616 [Consistency & Adhesiveness]), antioxidant activity (DPPH) and anthocyanin
 617 concentration (CA). Correlation coefficients and the r^2 obtained by means of PLS.
 618 DPPH and total anthocyanin content results of all treatments can be found at Peinado
 619 et al., (2015).

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641 **Fig 3.** Colorimetric parameters L^* , a^* and b^* of the raw material and strawberry
 642 spreadable products. Illustrated values correspond with the higher and lower level of
 643 each formulation variable (Table 1: 0-50% isomaltulose; 0-1% citric acid; 0.5-2.5%
 644 pectin and 0-20 minutes of thermal treatment) after 24 hours of processing as well as
 645 after 90 days of storage.



646

647 **Fig 4.** Consistency values (Ns) of strawberry spreadable products. Illustrated values

648 correspond with the higher and lower level of each formulation variable (Table 1: 0-

649 50% isomaltulose; 0-1% citric acid; 0.5-2.5% pectin and 0-20 minutes of thermal

650 treatment) after 24 hours of processing as well as after 90 days of storage.

651

652 **Table I.** Independent variables and their level used for central
 653 composite design.

Independent variables	Symbol ^c	Coded variable levels				
		-2	-1	0	1	2
Isomaltulose (%)^a	X_1	0	12.5	25	37.5	50
Pectin (%)^b	X_2	0.5	1	1.5	2	2.5
Citric acid (%)^b	X_3	0	0.25	0.5	0.75	1
Heat treatment time (min)	X_4	0	5	10	15	20

654 **a:** % of isomaltulose in the total amount of sugar mix (sucrose-
 655 isomaltulose or fructose-isomaltulose).

656 **b:** in final product.

657 **c:** Symbol with which each independent variable is cited in the text.

658

659 **Table II.** Retention index, odour thresholds and odour descriptors of volatile
660 compounds identified in raw strawberries and generated after processing; (n=3).

	RI	Odour threshold	Identification	Odour description
<u>In Raw strawberries</u>				
<i>Esters</i>				
ethyl acetate	904	5-5,000 ^D	MS, RI Std	Caramel, Sweet, Solvent, Fruity, Acid, Buttery, Pungent, Orange ^C
isopropyl acetate	911	-	MS, RI Std	Ethereal, Fruity, Sweet, Banana, Chemical
methyl propionate	926	-	MS, RI Std	Ethereal, Rum, Fruity ^C
ethyl propionate	972	10 ^D	MS, RI Std	Fruity, Solvent, Acetone ^C
methyl butyrate	1005	60-76 ^D	MS, RI Std	Fruity, Sweet, Ester, Green ^C
ethyl butyrate	1054	1 ^D	MS, RI Std	Fruity, Banana, Strawberry, Sweet, Bubblegum, Pin Acetone, Caramel ^C
butyl acetate	1093	66 ^D	MS, RI Std	Bitter, Green, Sweaty, Strong, Sweet
methyl hexanoate	1205	70-80 ^D	MS, RI Std	Fruity, Thinner, Acetone ^C
butyl butyrate	1217	100 ^D	MS, RI Std	Fresh, Sweet, Fruity ^C
ethyl hexanoate	1226	1 ^D	MS, RI Std	Fruity, Strawberry, Anise, Wine gum, Sweet ^C
hexyl acetate	1248	2 ^D	MS, RI Std	Fruity, Spicy, Herbal, Sweet wine, Rubbery, Tobacco, Acid, Citrus, Green ^C
3-hexen-1-yl, acetate	1266	-		-
2-hexen-1-yl, acetate	1277	-		-
hexyl butyrate	1432	250 ^D		Fruity ^C
octyl acetate	1494	12 ^D		Waxy, Floral, Apple ^C
butyl octanoate	1634	1 ^D	MS, RI Std	Fruity, Green, Oily, Floral ^C
<i>Aldehydes</i>				
hexanal	1106	4.5-5 ^D	MS, RI Std	Fishy, grass ^{A,B,C}
2-hexenal	1224	2-17 ^D	MS, RI Std	Apple, Fruity, Strawberry, Cherry, Green, Almond, Herbal, Leafy ^C
<i>Alcohols</i>				
propan-2-ol	943	-	MS, RI Std	Ethereal, Alcohol ^C
ethanol	953	100,000 ^D	MS, RI	Ethanol, Pungent, Sweet ^C
1-butanol	1169	500 ^{A,D}	MS, RI Std	Fruity, Medicinal, Cheesy
1-penten-3-ol	1182	350-400 ^{A,D}	MS, RI Std	Burnt, meaty ^A , paint like chemical like ^B grassy-green ^C
3-methyl-1-butanol	1210	250-300 ^D	MS, RI, Std	Pungent, Balsamic, Alcohol, Fruity, Malty, Ripe o Cheesy, Bitter, Harsh ^C
1-pentanol	1236	4,000 ^D	MS, RI, Std	Fruity, Green, Sweet, Pungent
1-hexanol	1286	2,500 ^D	MS, RI, Std	Flowery, Toasty, Dry, Fruity, Herbal, Mild, Woody, Sweet, Green, Leafy ^C
3-hexen-1-yl	1302	70 ^D	MS, RI, Std	Fresh, Green, Grassy, Leafy ^C
2-hexen-1-yl	1425	-	MS, RI, Std	Walnut, Medicinal, Cooked butter, Green, Leafy
linalool	1564	6 ^D	MS, RI Std	Muscat, Sweet, Green, Floral, Lemon, Parsley, Lavender, Fruity ^C

Ketones

1-penten-3-one	1042	1-1.3 ^D	MS, RI, Std	Pungent, fish-like, rotten, fruity, plastic, leather ^{A,B,C}
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Generated**Aldehydes**

2-methyl butanal	930	1 ^D	MS, RI Std	Green, Almond, Strong burnt, Malty, Cocoa ^C
3-methyl butanal	934	0.2-2 ^D	MS, RI Std	Cashew, apple ^A , almond-like, toasted, malty, green ^D
Octanal	1256	0.7 ^D	MS, RI Std	Lemon, stew-like, boiled meat-like, rancid, soapy, citrus, green, flower, fruit, orange ^{A,B,C}
Nonanal	1418	1 ^D	MS, RI Std	Gravy, green, fruity, gas, chlorine, floral, waxy, sweet, melon, soapy, fatty, citrus fruit ^{A,B,C}
Decanal	1524	0.1-2 ^D	MS, RI	Stewed, Burnt, Green, Waxy, Floral, Lemon, Fatty, Herbal, Soapy ^C
Benzaldehyde	1571	350-3,500 ^D	MS, RI	Bitter almond ^{A,B} , Burnt sugar, Woody ^C

Alcohols

Eucalyptol	1215	12 ^D	MS, RI, Std	Camphor, Minty, Sweet, Liquorices, Menthollic, Pine ^C
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Furans

Furfural	1503	3,000-23,000 ^{A,D}	MS, RI	Woody, Almond, Sweet, Fruity, Flowery ^C
2-Acetilfurane	1539	10,000-15,000 ^{A,D}	MS, RI	Tobacco, Smoky ^A , Balsamic, Cinnamon, Sweet ^C
5-Methyl furfural	1617	10,000 ^D	MS, RI, Std	Sweet, caramellic, bready, brown, coffee-like ^C
Mesifurane	1632	0.03 ^D	MS, RI,	Caramel, Cotton, Candy ^C

Terpens

Limonene	1208	10 ^D	MS, RI, Std	Green, Citrus, Ethereal, Fruit ^C
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661 ^A Giri et al., 2010; ^B Ganeko et al., 2008; ^C pherobase.org; ^D <http://www.leffingwell.com/odorthre.htm>

662 Odour tresholds in water (µg/L)

663

664 **Table III:** Changes of volatile compounds in the strawberry spreadable products after
 665 90 days of storage at 20°C. (n=3)

	Detected after 90 days of storage compared to spreadable products after 24 h of processing: $\Delta C=(C_{90}-C_0)/C_0$					Detected after 90 days of storage C_{90}			
	sucrose-isomaltulose (S)		fructose-isomaltulose (F)			sucrose-isomaltulose (S)		fructose-isomaltulose (F)	
	RI	Min	Max	Min	Max	Min	Max	Min	Max
Esters									
propyl acetate	911	G	G	-0.59	-0.01	1.45	5.67	1.78	7.37
ethyl acetate	1000	G	G	-0.73	41.99	7.54	23.15	8.26	13.78
ethyl-2-methyl butyrate	1028	G	G	G	G	0.00	1.74	0.26	1.50
Aldehydes									
methyl butanal	930	-0.80	0.30	-0.73	0.86	0.86	5.31	0.43	6.86
methyl butanal	934	ND	ND	-1.00	23.13	ND	ND	0.00	29.87
2-butenal	1072	G	G	G	G	0.55	3.02	0.06	4.56
butenal-2-methyl	1126	G	G	G	G	0.51	1.41	0.04	1.74
hexanal	1571	1.09	7.83	0.91	5.59	0.49	3.78	1.2	3.10
Alcohols									
propan-2-ol	943	-0.68	1.01	-0.69	-0.11	45.53	121.76	21.46	87.00
caliitol	1215	-1.00	1.62	-1.00	-0.56	0.00	2.12	0.00	0.65
methyl butanol	1210	-0.31	54.54	-0.28	25.15	0.92	3.33	0.92	3.19
pentanol	1236	-0.49	1.16	-0.49	0.97	0.78	1.72	0.59	1.58
hexanol	1286	-0.74	1.65	-0.69	1.22	1.96	14.15	2.26	14.61
buten-1-ol	1222	G	G	G	G	0.09	0.64	0.00	0.61
penten-1-ol	1272	G	G	G	G	0.71	1.76	0.84	2.43
ethyl-1-hexanol	1506	G	G	G	G	0.43	13.40	0.00	11.91
Ketones									
methyl-5-hepten-2-one	1283	G	G	G	G	0.09	0.93	0.12	0.54
Acids									
acetic ac.	1478	G	G	G	G	0.54	16.43	0.17	25.74
propanoic-2-methyl ac.	1591	G	G	G	G	0.00	3.15	0.18	2.18
acetic ac.	1653	G	G	G	G	0.00	2.73	0.03	1.69
acetic -2-methyl ac.	1696	G	G	G	G	0.00	4.15	0.23	2.61
Furans									
furfural	1503	-0.70	0.72	-0.61	0.06	4.94	130.01	11.42	157.96
acetylfurane	1617	-0.68	52.15	0.40	4.12	0.32	6.17	0.75	16.90
methyl furfural	1632	-0.61	2.44	-0.43	3.02	0.07	5.28	0.15	10.96
Terpens									
linalool	1208	-0.78	2.51	-0.88	1.90	0.16	0.97	0.11	0.85
Nitriles									
acetonitrile	1031	-0.81	30.33	-0.75	6.52	0.36	6.81	0.20	4.78

666 RI: Retention Index (Kovats Index) obtained for the volatile compounds.

667 $[(C_{90}-C_0)/C_0]$: Minimum and maximum concentration ratio for the volatile compounds that were
 668 present in the spreadable products after 24 hours of processing, and increased or decreased during
 669 storage.

670 G: volatile compounds generated after 90 days of storage at 20°C (concentration cannot be expressed
 671 as the ratio (ΔC)).

672 ND: not detected

673 C_0 : concentration of volatile compound in the strawberry spreadable product after 24 hours of
 674 processing expressed as $\mu\text{g} / 100 \text{ g}$ of spreadable product.

675 C_{90} : concentration of volatile compound in the strawberry spreadable product after 90 days of storage
 676 at 20°C expressed as $\mu\text{g} / 100 \text{ g}$ of spreadable product.

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