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1 **QUALITY CHANGES AND SHELF-LIFE PREDICTION OF A FRESH FRUIT**
2 **AND VEGETABLES PURPLE SMOOTHIE**

3

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15

16 **Abstract**

17 The sensory, microbial and bioactive quality changes of untreated (CTRL) and mild
18 heat–treated (HT; 90 ºC/45 s) smoothies were studied and modelled throughout storage
19 (5, 15 and 25 ºC). The overall acceptability was better preserved in HT samples being
20 highly correlated (hierarchical clustering) with the flavour. The sensory quality data
21 estimated smoothie shelf–life (CTRL/HT) of 18/55 (at 5 ºC), 4.5/12 (at 15 ºC), 2.4/5.8
22 (at 25 ºC) days. The yeast and moulds growth rate was lower in HT compared to CTRL
23 while a lag phase for mesophiles/psychrophiles was observed in HT–5/15 ºC. HT and 5
24 ºC–storage stabilized the phenolics content. FRAP reported the best correlation
25 (R^2=0.94) with the studied bioactive compounds, followed by ABTS (R^2=0.81) while

DPPH was the total antioxidant capacity method with the lowest adjustment (R^2=0.49). Conclusively, modelling was used to estimate the shelf−life of a smoothie based on quality retention after a short time−high temperature heat treatment that better preserved microbial and nutritional quality during storage.

Keywords: modelling; anthocyanins; antioxidants; beverages; food safety; quality modelling.

1. INTRODUCTION

An adequate intake of fruit and vegetables is essential in the human diet since they are rich sources of essential nutrients and bioactive compounds which can reduce the risk of several chronic diseases (Boeing et al., 2012). Purple cabbage, beet, red grapes, broccoli and cucumber have high contents of such health−promoting compounds like phenolic compounds (polyphenols and phenolic acids), vitamin C and other antioxidant compounds (Shahidi, 2004; Souci et al., 2000). Anthocyanins are water−soluble vacuolar pigments (purple, dark blue and other colours) belonging to the polyphenol groups of flavonoids (Canuto et al., 2016). Anthocyanins together with phenolic acids and ascorbic acid are the main antioxidant compounds in fruit and vegetable smoothies (Lo Scalzo et al., 2004). Nevertheless, fruit and vegetables worldwide consumption is below the recommended daily intake (Hall et al., 2009). Accordingly, the food industry is developing new alternative presentations such as smoothies which may highly promote the fruit and vegetables consumption. Smoothies are non−alcoholic beverages prepared from fresh or frozen fruit and/or vegetables, which are blended and usually mixed with crushed ice to be immediately consumed. Smoothies may include other

50 components like yogurt, milk, ice−cream, lemon, water or tea. They have a milk
51 shake−like consistency that is thicker than slush drinks (Castillejo et al., 2016).
52 The smoothie preparation involves a breakdown of plant parenchyma, which leads to a
53 dispersed solution consisting in a liquid phase (including pectin and other soluble
54 solids) and a solid phase composed of insoluble solids (cell wall). Accordingly,
55 quality−degradative enzymes come easily in contact with their substrates and sugars are
56 more available for spoilage microorganisms, which highly limit the shelf−life of these
57 beverages (Rodríguez-Verástegui et al., 2015). In order to extend the shelf−life of these
58 beverages thermal treatments can be used (Houben et al., 2014) together with
59 subsequent low temperature storage, that would decrease the intensity of the
60 pasteurization (Castillejo et al., 2016; Rodríguez-Verástegui et al., 2015). However,
61 such thermal treatments can be detrimental to the smoothie quality, causing degradation
62 of thermolabile nutrients, and affecting sensorial properties such as texture, colour, taste
63 and aroma (Esteban et al., 2015). Accordingly, the thermal treatment should be as mild
64 as possible in order to preserve the nutritional and sensory quality of the smoothie while
65 achieving an appropriate microbial reduction and inactivation of quality−degradative
66 enzymes. In this way, thermal treatments at 80−95 ºC for less than 3 min (ensuring a
67 pasteurization treatment) together with subsequent low storage temperature have been
68 satisfactorily used to inactivate quality−degradative enzymes and to reach significant
69 microbial reductions while keeping acceptable sensory attributes (Castillejo et al., 2016;
70 Rodríguez-Verástegui et al., 2015; Sun-Waterhouse et al., 2014; Wang et al., 2014).
71 Optimum low storage temperature of 5 ºC in these products cannot be always ensured in
72 the retail surfaces. In addition, it is crucial to study the microbial, physicochemical,
73 sensory and nutritional/bioactive quality degradation of the smoothie throughout storage
74 at optimum low temperature (5 ºC), unfavourable room temperature (25 ºC) when no

75 low storage temperature cabins are available and an intermediate one (15 ºC) such as
76 that of commercial retail cabins. Such quality changes at different storage temperatures
77 should be modelled in order to establish the smoothie shelf−life to ensure a proper
78 intake of nutritional and bioactive compounds while preserving its safety.
79 The objective of this work was to study the effect of a mild heat treatment (ensuring
80 pasteurization) in a purple smoothie (pH <4.2) made of fresh horticultural products,
81 compared to fresh−blended untreated samples. Such quality changes were studied using
82 mathematical models allowing to estimate the potential shelf−life of such products at
83 different temperatures of 5 ºC (ideal), 15 ºC (maximum recommended) and 25 ºC
84 (misused) simulating shipping, distribution and retail sale periods. Accordingly, the
85 shelf-life prediction of this fruit and vegetables beverage will be of a high interest for
86 the related food industries, and also for consumers to improve the produce logistics all
87 over the chain and ensure lower costs and a better final quality of the product. To the
88 best of our knowledge, no previous studies have used mathematical tools to predict
89 quality (sensory, microbial and nutritional/bioactive) changes and shelf−life of
90 fresh−blended fruit/vegetables beverages treated with high temperature−short time
91 treatments and stored at different temperatures.
92

93 **2. MATERIAL AND METHODS**

94 **2.1. Plant material and smoothie preparation**

95 Fresh fruit and vegetables (purple seedless grapes, cucumber, beet and broccoli) were
96 obtained from a local market (Cartagena, Spain) and stored at 5 ºC and 90–95 % relative
97 humidity (RH) until the next day, when they were processed. The smoothie processing
98 was accomplished in a disinfected cold room at 10 ºC. First, plant material was washed
99 in chlorinated cold water (150 mg L^{-1}; 5 ºC; pH 6.5; 2 min) at a ratio of 300 g of plant

100 material to 5 L chlorinated water, rinsed with tap water (1 min; 5 ºC) and drained in a
101 perforated basket. Subsequently, cucumber and beet were peeled and all vegetables
102 were then cut and blended in a Thermomix food processor (TM 21, Vorwerk, Spain).
103 The blending program used was 1 min at level 4 followed by 1 min at maximum level
104 (10).
105 The smoothie formulation was 12 % beet, 45 % purple grapes, 35 % cucumber and 8 %
106 broccoli. The final formulation was selected according to a sensory evaluation of five
107 types of purple smoothies (selected based on common purple smoothie recipes on
108 books, internet, etc.) done with 30 participants (17 women/13 men, aged 20–48 years)
109 randomly chosen in the Universidad Politécnica de Cartagena. People were first asked
110 about their eating habits confirming that all of them consumed regularly fruit and
111 vegetables, and particularly liked all the ingredients that contained all smoothie types.
112 The participants were asked to scored smoothies appearance, flavour, texture and
113 overall acceptability according to a 5−point hedonic scale of acceptability (5: excellent,
114 4: good, 3: fair, limit of usability, 2: poor; 1: extremely bad). All five smoothie types
115 were given at a time in transparent plastic glasses (30 mL each one) coded with three
116 random digit numbers served in an arbitrary order. Participants were asked to drink still
117 mineral water as palate cleanser.
118 The nutritional composition of the smoothie was also determined with the software
119 DIAL 1.0 (Ortega-Anta et al., 2008) and it is presented in as Supplementary material.
120 pH of samples was always below 4.2 throughout all storage conditions.
121

122 **2.2. Smoothie treatments and storage conditions**

123 Heat treatment (HT) of the smoothie was carried out in a thermoresistometer Mastia
124 (Conesa et al., 2009) immediately after blending. Briefly, the sterilized vessel of the

125 thermoresistometer was filled with 400 mL of the smoothie. Then, the
126 thermoresistometer was programmed to increase the initial smoothie temperature (5±2
127 ºC) with a heating rate of 30 ºC min^{-1} to 90 ºC, followed by a holding period of 45 s and
128 cooled down to a final temperature of 35 ºC (heating rate of 30 ºC min^{-1}). This ensured
129 a pasteurization treatment. After the thermal treatment, the smoothie was cooled down
130 to the respective storage temperatures submerging the vessel in an ice−water bath while
131 continuously agitation was programmed in the thermoresistometer. Untreated samples
132 were used as control (CTRL). Samples were taken from the thermoresistometer through
133 a sampling port under aseptic conditions into 50−mL Falcon tubes. Samples were then
134 stored in darkness at 5, 15 and 25 ºC up to 28 days depending of storage temperature.
135 Five replicates per treatment, storage temperature and sampling day were prepared.
136 Samples for nutritional/bioactive compounds were taken on each sampling day and
137 stored at -80 ºC until further analysis.
138

139 **2.3. Sensory evaluation**

140 Sensory analyses were performed according to international standards (ASTM, 1986).
141 Tests were conducted in a standard room (ISO, 2007) equipped with ten individual taste
142 boxes using the white light. Samples (about 30 mL) were served at room temperature in
143 transparent plastic glasses coded with three random digit numbers. Still mineral water
144 was used as palate cleanser. The sensory panel consisted of twelve assessors (six
145 women/six men, aged 22–68 years) which have specific sensory discriminative ability
146 (colour, flavour, visual appearance and texture) on fruit and vegetables smoothies. A
147 5−point scale of damage incidence and severity was scored for off−colours, off−odours,
148 lumpiness, turbidity and precipitation/phase separation (5: none; 4: slight; 3: moderate,
149 limit of usability; 2: severe; 1: extreme). Visual appearance, aroma, flavour, texture and

150 overall quality were assessed at the same time using a 5−point hedonic scale of
151 acceptability (5: excellent, 4: good, 3: fair, limit of usability, 2: poor; 1: extremely bad).
152 The sensory data was rationalized to study proximal sensory parameters. Hierarchical
153 clustering (Hartigan, 1975) was applied in order to group similar parameters among a
154 group of data. The degree of similitude between the different scores was quantified
155 using the Euclidean distance.

156

157 **2.4. Microbial analysis**

158 To determine the mesophilic, psychrophilic and yeast and moulds (Y+M) growth,
159 standard enumeration methods were used according to Castillejo et al. (2016). Briefly,
160 10−fold dilution series were prepared in 9 mL of sterile peptone saline solution.
161 Mesophiles and psychrophiles were pour plated while Y+M were spread plated. The
162 following media/incubation conditions were used: plate count modified agar for
163 mesophiles and psychrophiles incubated at 37 ºC for 48 h and at 5 ºC for 7 days,
164 respectively; and rose bengal agar for Y+M incubated for 3–5 days at 25 ºC. All
165 microbial counts were reported as log colony forming units per gram of product (log
166 CFU mL^{-1}). The presence of *Salmonella* spp., *Listeria monocytogenes* and generic
167 *Escherichia coli* was monitored according to the European legislation (EC, 2007)
168 ensuring the food safety of the product. Each of the five replicates was analysed in
169 duplicate.

170

171 **2.5. Vitamin C**

172 The ascorbic (AA) and dehydroascorbic (DHA) acids were measured according to
173 Castillejo et al. (2017). Briefly, 5 g ground frozen (−80 ºC) smoothie was homogenized
174 (Ultraturrax T25 basic, IKA, Berlin, Germany) for 10 s with 10 mL of cold (4 ºC) buffer

175 (0.1 M citric acid, 0.05 % EDTA, 4 mM sodium fluoride and 5 % MeOH) under
176 water−ice bath. Then, the homogenate was immediately filtered (four−layer
177 cheesecloth) and the pH adjusted (6 N NaOH) to 2.35–2.4. Subsequently, 750 mL of
178 filtered (0.45 μm polyether sulphone filter; PTFE) purified extract (Sep−Pak cartridges
179 C18, Waters, Dublin, Ireland) was derivatised with 250 mL of 7.7 M
180 1,2−phenylenediamine for 37 min in darkness at room temperature and analysed by
181 HPLC. Accordingly, 20 mL of derivatised sample was injected onto a Gemini NX
182 (250mm×4.6 mm, 5 mm) C18 column (Phenomenex, Torrance CA, USA), using an
183 HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a
184 G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column
185 heater and G1315B photodiode array detector. AA and DHA were quantified using
186 commercial standards. Calibration curves were made with at least six data points for
187 each standard. Total vitamin C was calculated as the sum of AA and DHA and
188 expressed as mg kg^{-1} fresh weight (fw). Each of the five replicates was analysed in
189 duplicate.
190

191 **2.6. Total phenolic content and total antioxidant capacity**

192 Total phenolic content (TPC) and total antioxidant capacity (TAC) analysis was
193 conducted based on Rodríguez-Verástegui et al. (2015) with slight modifications.
194 Briefly, frozen samples of 1 g were placed in glass bottles, and 3 mL of MeOH was
195 added. The extraction was carried out in an orbital shaker (Stuart, Staffordshire, UK) for
196 1 h at 200×g in darkness inside a polystyrene box with an ice bed. The extracts were
197 then transferred in Eppendorf tubes and centrifuged at 15,000×g for 10 min at 4 ºC. The
198 supernatant was used as TPC and TAC extracts.

199 The TPC was determined based on Singleton and Rossi (1965) but with modifications
200 proposed by Martínez-Hernández et al. (2011). Briefly, 19 µL of extract was placed in a
201 96−well plate, and 29 µL of 1 N Folin–Ciocalteu reagent was added. The mix was
202 incubated for 3 min in darkness at room temperature. Then, 192 µL of a solution
203 containing Na_2CO_3 (0.4 %) and NaOH (2 %) was added. After 1 h of incubation at
204 room temperature in darkness, the absorbance was measured at 750 nm. The TPC was
205 expressed as mg gallic acid equivalents (GAE) kg^{-1} fw.

206 TAC was determined using the same instruments and methodology described by
207 Rodríguez-Verástegui et al. (2015) using three different methods: free radical
208 scavenging capacity with 2,2−diphenyl−1−picrylhydrazil (DPPH) (Brand-Williams et
209 al., 1995), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999) and
210 2,20−azino−bis (3−ethylbenzothiazoline−6−sulphonicacid) (ABTS) (Cano et al., 1998).
211 TAC data were expressed as mg of Trolox equivalents kg^{-1}fw. Each of the five
212 replicates was analysed in duplicate.

213 Data from the three TAC methods were compared with the bioactive compounds
214 vitamin C, TPC and total anthocyanins to determine which TAC method better reflected
215 the content in antioxidant compounds. A linear regression model was used to study such
216 relationship (Equation 1).

217 $$y = \beta_0 + \beta_1 vitC + \beta_2 phenol + \beta_3 anthocyanins + \sum inter \quad \text{(Eq. 1)}$$

218 where *y* is the TAC method used, *vitC*, *phenol* and *anthocyanins* stand for the contents
219 of vitamin C, phenols and anthocyanins, respectively, whereas $\sum inter$ represents a sum
220 of first order interactions terms between the concentrations of antioxidant compounds.
221 β_i are undetermined coefficients to be estimated from the experimental data. Having 3
222 independent variables (i.e., *vitC, phenol* and *anthocyanins*), the first order interaction
223 terms among them (i.e., 4 more variables) and considering the independent term β_0,

224 there are 8 possible fitting parameter per model. A model selection procedure was
225 carried out in order to avoid the overfitting of the models. This procedure aims to select
226 only those independent variables which have a significant influence over the dependent
227 variable, *y*, based on a performance index. A complete enumeration of models (128
228 possible models for each TAC method) was performed using the R programming
229 language (R_Core_Team, 2014) and the best among them was selected according to the
230 Akaike Information Criterion (AIC) (Hirotogu, 1998). The normality and independence
231 of the residuals was tested using, respectively, the Shapiro−Willis and Durbin−Watson
232 tests at the 95 % confidence level. Their homoscedasticity was tested using a residuals
233 plot.

234

235 **2.7. Anthocyanins**

236 Anthocyanins extraction and determination were conducted as previously described
237 (Barnes et al., 2009) but with modifications. Frozen smoothie sample (2.5 g) was
238 homogenized with 5 mL MeOH and incubated under ultrasonic bath (Cole−Parmer,
239 model 8890, Illinois, U.S.A.) for 10 min at 20 ºC. Subsequently, the homogenate was
240 centrifuged at 15,000×g for 15 min at 4 ºC and the supernatant was collected in an
241 amber bottle. The pellet previously obtained was re−suspended with another aliquot of
242 5.0 mL MeOH, followed by ultrasounds and centrifugation as described. The latter
243 procedure was repeated four times and the supernatants were combined and make up to
244 25 mL with MeOH. The combined supernatants were then concentrated to dryness with
245 a rotavapor (Hei−VAP Value, Schwabach, Germany) at 40 ºC. The sample was
246 re−suspended with 2.5 mL of MeOH and filtered through a 0.22 μm PTFE filter.

247 Anthocyanins quantification was conducted by injection of 20 μL of filtered
248 anthocyanin extract in a Ultra High−Performance liquid chromatography (UPLC)

249 instrument (Shimadzu, Kyoto, Japan) equipped with a DGU−20A degasser, LC−30AD
250 quaternary pump, SIL−30AC autosampler, CTO−10AS column heater and SPDM−20A
251 photodiode array detector. The UPLC system was controlled by the software
252 LabSolutions (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out onto
253 a Kinetex C18 column (100 mm×4.6 mm, 2.6 μm particle size; Phenomenex,
254 Macclesfield, UK) with a KrudKatcher Ultra HPLC guard column (Phenomenex,
255 Macclesfield, UK). The column temperature was maintained at 40 ºC. The mobile
256 phases were water–formic acid (95:5, v/v) (A) and MeOH (B) with a flow rate of 1 mL
257 min^{-1}. The linear mobile phase gradient started with 2 % B, followed by 32 % B at 30
258 min, 40 % B at 40 min and 98 % B at 45 min, then isocratic for 5 min. For column
259 equilibration phase B was reduced to 2 % in 4 min and maintained at this concentration
260 for 10 min. Chromatograms were recorded at 520 nm. Anthocyanins were identified by
261 comparison of their retention times and absorption spectra with pure standards (Sigma
262 Aldrich, San Luis, USA). The calibration curves were made with at least six data points
263 for each standard. The results were expressed as g anthocyanin kg^{-1} fw. Each of the
264 three replicates were analysed in duplicate.
265

266 **2.8. Mathematical modelling**

267 *2.8.1 Kinetics of the sensory quality features of the smoothie*

268 The score assigned to the overall acceptance is a discrete variable. Therefore, the
269 evolution of the overall acceptance of the smoothie was described using Poisson
270 regression (McCullagh and Nelder, 1989). This type of model can be written as shown
271 in equations 2−3, where y is the dependent variable (the quality attribute modelled),
272 $Poisson(\mu)$ represents the Poisson distribution of mean μ, x_i are the explanatory
273 variablesand β_i are coefficients to estimate from the experimental data.

274 $$y = Poisson(\mu) \quad \text{(Eq.2)}$$

275 $$\log \mu = \beta_0 + \sum_i \beta_i x_i \quad \text{(Eq.3)}$$

276 The model was fitted independently for each experimental conditions (storage
277 conservation and CTRL/HT samples) using the functions implemented in the *stats*
278 package of the R programming language (R_Core_Team, 2014). Therefore, in our case,
279 the only explanatory variable considered is the storage time.

280

281 *2.8.2. Microbial growth*

282 The growth kinetics of the microorganisms studied (mesophilic, psychrophilic and
283 Y+M) have been described using the Baranyi model (Baranyi and Roberts, 1994). The
284 system of differential equations describing this model is shown in Eq. 4−5, where *N*
285 stands for the microbial count at time *t*. The exponential phase is described by
286 parameter μ_{max}, which defines the maximum growth rate. The lag phase is introduced
287 through an hypothetical substance, *Q(t)*, which must reach a certain level before the
288 microbial population can grow exponentially. The maximum number of microorganism
289 is limited by N_{max}. Finally, model parameter *m* defines the sharpness of the transition
290 between the exponential and the stationary growth phases.

291

292 $$\frac{dN}{dt} = \frac{Q(t)}{1+Q(t)} \cdot \mu_{max} \cdot \left(1 - \frac{N(t)}{N_{max}}\right)^m \cdot N(t) \quad \text{(Eq. 4)}$$

293 $$\frac{dQ}{dt} = \mu_{max} \cdot Q(t) \quad \text{(Eq. 5)}$$

294 The duration of the lag phase (λ) can be calculated from the values of the model
295 parameters as shown in Eq. 6.

296 $$\lambda \cdot \mu_{max} = \ln\left(1 + \frac{1}{Q(0)}\right) \quad \text{(Eq. 6)}$$

297 The model has been fitted to the experimental data using the excel add−in DMfit. The
298 goodness of the fit was evaluated using the RMSE and by visual inspection of the fitted
299 curve.
300

301 3. RESULTS AND DISCUSSION

302 3.1. Sensory analysis

303 A dendrogram depicting the results of the hierarchical clustering performed on the
304 quality data is shown in Figure 1. As observed, there are two main groups in the sensory
305 analysis with a large distance between them: visual appearance and flavour. The overall
306 acceptability of the product is highly correlated with the flavour. Hence, flavour is the
307 most relevant acceptability feature that the consumer assigns to the product, leading to
308 the remaining sensory attributes in a second scenario. For that reason, horticultural
309 produce selection in its optimum ripening stage for the smoothie preparation becomes a
310 key factor for consumer acceptance. Similar dendrograms were constructed with the
311 sensory data obtained for HT samples and fresh blended unheated ones (CTRL),
312 obtaining similar results (not shown).
313 The thermal treatment did not generally affect the sensory attributes of the smoothie,
314 except flavour, which was increased (p<0.05) from a score of 3.9 to 4.6 (Figure 2). The
315 enhancement of the smoothie flavour after the thermal treatment may be explained by
316 the thermal breakdown of plant cells leading to a leakage of compounds responsible of
317 flavour.
318 The fitting of the generalized linear model to the overall acceptability data is
319 summarized in Table 1. The fitted models predict similar scores at day 0, as shown in
320 the values estimated for β_0. Values of β_1 of -0.019±0.010, -0.093±0.040 and -
321 0.137±0.071 have been estimated for the CTRL samples at 5, 15 and 25 ºC,

322 respectively, whereas for the HT samples, the models estimate values between a 48 and
323 63 % lower. Therefore, the overall acceptability of the CTRL samples decreases more
324 rapidly throughout storage than the equivalent HT samples. Accordingly, the shelf−life
325 of HT samples, setting a score of 3 for the global acceptability as a threshold value, can
326 be predicted as 18 days for CTRL samples and 55 days for HT samples at 5 ºC (Figure
327 3). Similarly, a shelf−life of 4.5 and 2.4 days at 15 and 25 ºC is estimated for CTRL
328 samples, whereas a shelf−life of 12 and 5.8 days are estimated for HT samples at 15 and
329 25 ºC. Hence, it can be concluded that the heat treatment applied effectively increased
330 the sensory shelf−life of the smoothie at every storage temperature studied.
331
332 **3.2. Microbial analysis**
333 The initial mesophilic, psychrophilic and Y+M counts of the fresh blended smoothie
334 was 3.5, 3.2 and 2.9 log CFU g^{-1}, which were decreased (p<0.05) by 0.7, 0.3 and 0.4
335 log units after the thermal treatment (Figure 4) according to a Welch two sample T−test.
336 The Baranyi model was able to describe the temporal evolution of the microbial data for
337 every experiment. The values of the model parameters estimated, as well as their
338 standard deviations, are shown in Table 2. For several experiments (CTRL−5 for
339 mesophiles, HT−5 and HT−25 for psychrophiles, and CTRL−15 and HT−15 for Y+M)
340 the fitting algorithm failed at estimating the values of λ and μ_{max} due to an insufficient
341 number of measurements made during the exponential growth phase. The mesophilic
342 growth rates of HT samples stored at 15 and 25 ºC were 0.90±0.06 and 2.29±0.10, while
343 CTRL samples reported 0.83±0.11 and 1.95±0.14, respectively. However, there were
344 not significant differences among the growth rates of CTRL and HT samples at 15 and
345 25 ºC. Nevertheless, when the storage temperature was reduced to 5 ºC the mesophilic
346 growth rate was reduced to 0.20 as obtained for HT−5 samples. HT samples stored at 5

347 and 15 ºC showed lag values of 2.18±3.08 and 1.62±0.51 days, respectively, while no
348 lag was found for HT samples at 25 ºC. As observed, the lag increased as the storage
349 temperature decreased in HT samples. No lag was found for CTRL samples.
350 The psychrophilic growth rate of CTRL samples increased as the storage temperature
351 did, reporting values of 0.55±0.10, 1.25±0.05 and 2.10±0.05 for 5, 15 and 25 ºC,
352 respectively. CTRL−15 and HT−15 samples showed psychrophilic growth rates of
353 1.25±0.05 and 1.80±0.17, respectively, without significant ($p<0.05$) differences among
354 them. The Y+M growth rate of HT samples stored at 5 ºC was lower than the one in
355 CTRL samples with 0.20±0.02 and 0.63±0.24, respectively. As the storage temperature
356 increased, the growth rates also increased with 1.72±0.33 and 0.79±0.27 for CTRL−25
357 and HT−25 samples, respectively.
358 Conclusively, the HT reduced the initial mesophilic, psychrophilic and Y+M loads of
359 the smoothie. Furthermore, HT did not cause any significant variation in the microbial
360 growth rates, although Y+M growth rate of HT samples was lower than CTRL samples.
361 Nevertheless, it can be qualitatively stated than HT introduces a lag in mesophilic and
362 psychrophilic data which was not observed in CTRL samples, increasing the time
363 required for the microorganisms to reach hazardous levels.
364

365 **3.3. Total vitamin C**

366 Low AA levels (<0.11 mg kg^{-1}) were detected in the samples. The AA oxidation to
367 DHA is rapidly catalysed by the enzymes ascorbate oxidase and ascorbic acid
368 peroxidase. Accordingly, the AA absence may be explained since during the smoothie
369 blending, plant cells are disrupted easily allowing enzymes to access their substrates
370 located in different plant cell locations. However, DHA also exhibits antioxidant
371 properties in addition to antiscorbutic activity equivalent to that of AA being total

372 vitamin C considered as the sum of AA and DHA (Munyaka et al., 2010). The initial
373 total vitamin C content of samples (354.1 mg kg^{-1}) was not significantly ($p<0.05$)
374 affected on processing day after the thermal.
375 The effect of the storage time and the thermal treatment on the total vitamin C
376 degradation rate was assessed using an ANCOVA analysis. The results show that the
377 storage time significantly ($p<0.05$) affects the degradation rate, whereas no significant
378 differences ($p<0.05$) were observed between the inactivation rates observed for the
379 CTRL and HT samples. Figure 5 represents the DHA degradation observed in the
380 sample at the different storage temperatures tested. In every case, the DHA content
381 decreased to values lower than 100 mg kg^{-1} by the end of the experiment. Nevertheless,
382 the decrease rate depended on the storage temperatures, with the samples stored at 25 ºC
383 requiring 4 days to reach 100 mg kg^{-1}, whereas samples stored at 15 and 25 ºC required
384 7 and 14 days, respectively. A quantitative comparison through a kinetic model has not
385 been performed due to the dispersion of the data. DHA contents of samples ranged
386 among 70.7−108.6 mg kg^{-1}fw after 14, 11 and 9 days at 5, 15 and 25 ºC, respectively. A
387 portion of 250 g of the smoothie at the end of last storage periods still ensured the
388 40−60 % of the recommended vitamin C daily intake by the FAO/WHO (2004).
389

390 **3.4. Total phenolic and anthocyanins content**

391 The smoothie showed an initial TPC of 267.6 mg GAE kg^{-1}fw being considered as a
392 good source of phenolic compounds as other red and green vegetables smoothies
393 (Castillejo et al., 2017; Rodríguez-Verástegui et al., 2015). In general, phenolics
394 degradation may occur after thermal treatments and during storage due to chemical and
395 enzymatic oxidation, which can also lead to changes in bioavailability or biological
396 activity (Tomás-Barberán and Espín, 2001). However, the mild heat treatment applied

397 did not induce significant (*$p<0.05$*) TPC changes similarly to what is reported in other
398 vegetable beverages treated at 70−90 ºC for 1−2 min (Odriozola-Serrano et al., 2008;
399 Patras et al., 2009).
400 An accurate kinetic model was not developed due to the dispersion of the data. Samples
401 stored at 5 ºC showed a TPC decrease up to 55 % on day 9 followed by an increase
402 reaching final TPC of 174.7 mg GAE kg^{-1} fw after 18 days (Figure 6). The latter
403 behaviour may be explained by an initial phenolic degradation through
404 phenolic−degradative enzymes followed by a possible increment of the phenylalanine
405 ammonia lyase (PAL) activity, the key enzyme in the biosynthetic pathway of phenolic
406 compounds. Similarly, PAL activity and TPC enhancements were observed in untreated
407 red vegetables smoothies stored at 5 ºC probably owed to the wounding abiotic stress
408 occurred during the smoothie preparation (Rodríguez-Verástegui et al., 2015). Contrary,
409 HT samples did not show significant (*$p<0.05$*) changes after 18 days at 5 ºC. Therefore,
410 the heat treatment and the low storage temperature stabilized the TPC levels probably
411 due to the reduction of the activity of those enzymes responsible of phenolic
412 degradation as previously reported (Rodríguez-Verástegui et al., 2015). Nevertheless,
413 when CTRL and HT samples were stored at 25 ºC the TPC levels were highly reduced
414 by 70 and 90 % after 9 days, respectively. The high phenolics degradation may be
415 explained by a high activity at such high storage temperature of those
416 phenolic−degradative enzymes. Furthermore, the latter enzymatic activities were even
417 favoured in those HT samples due to a higher enzymatic substrates availability
418 enhanced by the plant cells disruption after the thermal treatment. CTRL samples stored
419 at the intermediate temperature of 15 ºC showed a similar behaviour to those CTRL
420 samples at 5 ºC with a TPC reduction of approximately 60 % after 9 days. Particularly,
421 the HT smoothie stored at 15 ºC showed a TPC enhancement of 71 % after 4 days

422 followed by a decrease reaching after 9 days similar levels to processing day. The latter
423 phenolic enhancement could be explained by an increase of the PAL activity earlier
424 than CTRL samples stored at 5 ºC due to the higher storage temperature. On the other
425 side, the high phenolics degradation occurred at 25 ºC probably masked the TPC
426 enhancement observed at 15 ºC.

427

428 **3.5. Anthocyanins content**

429 The major anthocyanins detected, from higher to lower amounts were (mg kg^{-1}fw):
430 pelargonidin 3−O−glucoside (Pg 3−GLU; 28.98), cyanidin 3−O−galactoside (Cy
431 3−GA; 4.83), cyanidin 3−O−glucoside (Cy 3−GLU; 3.46), and cyanidin
432 3,5−O−diglucoside (Cy 3,5−GLU; 0.17) (data not shown). Such anthocyanins contents
433 found in the purple smoothie are due to the high proportion of red grapes which have
434 high contents of these phenolic compounds as previously reported (Picariello et al.,
435 2014).

436 The anthocyanins changes during storage could not be modelled due to the dispersion of
437 the data. The Pg 3−GLU was reduced by 38 % on processing day after the thermal
438 treatment. The remaining anthocyanins were not significantly ($p<0.05$) changed after
439 the thermal treatment. Pg 3−GLU contents of CTRL samples were highly decreased by
440 76−94 % after 4 days of storage except samples stored at 15 ºC which were reduced by
441 40 %. The latter lower reduction may be a result of a phenolics enhancement due to
442 PAL activation, as observed for TPC at such temperature, which counterbalanced the
443 other high Pg 3−GLU decreases. The same trend was observed for Cy 3−GA while the
444 other anthocyanins did not show significant ($p<0.05$) changes during storage. However,
445 the latter Pg 3−GLU and Cy 3−GA decrements during storage were minimized up to
446 2.3−fold in those HT samples.

447

448 **3.6. Total antioxidant capacity**

449 CTRL smoothie showed an initial TAC of 517.0, 445.2 and 480.4 mg Trolox kg^{-1}
450 reported by FRAP, ABTS and DPPH method, respectively. The thermal treatment did
451 not affect significantly ($p<0.05$) the TAC of the samples at day 0.

452 The model parameters included in the model which best describes the data according to
453 the AIC are summarized in Table 3. The best correlation with bioactive compounds was
454 achieved with FRAP with an excellent R^2=0.94, followed by ABTS with R^2=0.81, while
455 DPPH showed the poorest fitting with R^2=0.45. Therefore, according to the collected
456 data, FRAP is the method which best reflects the concentration of antioxidant
457 compounds in the smoothie. On the other hand, DPPH is the method whose values show
458 the lowest correlation with the antioxidant compounds. Figure 7 illustrates the model
459 fitting for each one of the selected models. It is in accordance with the conclusions
460 drawn from the obtained values of R^2: the model for DPPH shows the highest
461 dispersion, whereas the fit for FRAP is excellent.

462 According to the fitted model, FRAP has an excellent linear relationship with the
463 vitamin C concentration (0.36±0.10) and the TPC (1.41±0.13). Furthermore the total
464 anthocyanins content had a synergistic effect with the phenolic content (0.0039±0.0010)
465 and an antagonistic effect with respect to the vitamin C content (-0.0080±0.0014).
466 Nevertheless, further data is required to test whether these conclusions can be
467 extrapolated for experimental conditions different to the ones tested.

468 Similar conclusions can be drawn from the models constructed for the ABTS and DPPH
469 methods. However, due to the lower quality of the fitting for these models, they would
470 be strongly affected by the experimental error. Hence, they are not reported in this
471 work.

472 Since anthocyanins are phenolic compounds included in the flavonoids group, which
473 confer the characteristic purple colour to beet and purple grapes, it was also studied
474 which either TPC or total anthocyanin content, better contributed to TAC correlated
475 with the other great antioxidant present in the smoothie like vitamin C. Therefore, TPC
476 or total anthocyanin content terms were removed from the model to study their
477 contribution to TAC correlation. However, the omission of any of latter terms from the
478 model highly reduced the quality of the fitting (data not shown).
479

480 **4. CONCLUSIONS**

481 The kinetic of the sensory, microbial and bioactive quality changes of a purple
482 smoothie, made of fresh fruit and vegetables, heat−treated or not during processing, was
483 studied throughout storage at 5, 15 and 25 ºC. A hierarchical clustering of sensory
484 quality attributes showed that the overall acceptability was highly correlated with the
485 flavour. The shelf−life of the smoothies was approximately increased by 37 (at 5 ºC), 8
486 (at 15 ºC) and 3 days (at 25 ºC) in heat−treated samples compared to untreated fresh
487 blended ones. Such mild heat treatment did not alter the initial vitamin C and phenolics
488 content of samples on processing day, while such nutritional quality attributes were
489 better preserved during storage at low temperature. The latter antioxidant compounds
490 were highly correlated (R^2=0.94) with the FRAP total antioxidant capacity method. The
491 purple smoothie still presented high health−promoting compounds contents after the
492 storage periods, particularly ensuring a 250g−portion of the smoothie, the 40−60 % of
493 the recommended vitamin C daily intake.
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495
496

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505

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618

619

620 **FIGURE AND TABLE CAPTIONS**

621

622 **Figure 1.** Dendrogram of the hierarchical clustering of sensory attributes of smoothies.

623

624 **Figure 2.** Overall acceptability of untreated (A) and heat−treated (B) smoothies during

625 storage at 5 ºC. The global acceptability was considered a discrete variable. The size of

626 the symbols is proportional to the number of occurrences of a given value.

627

628 **Figure 3.** Sensory scores of untreated (CTRL) and heat−treated (HT) smoothies during

629 storage at 5, 15 and 25 ºC (n=5).

630

631 **Figure 4.** Microbial growth of mesophiles (A), psychrophiles (B) and yeasts and

632 moulds (C) in the heat−treated smoothie during storage at different temperatures: 5ºC

633 (red solid line, circles), 15ºC (green short dashed line, triangles) and 25ºC (blue long

634 dashed line, squares) (n=5±SD).

635

636 **Figure 5.** Vitamin C (dehydroascorbic acid; DHA) of untreated (CTRL) and

637 heat−treated (HT) smoothies during storage at 5, 15 and 25 ºC (n=5±SD).

638

639 **Figure 6.** Total phenolic content of untreated (CTRL) and heat−treated (HT) smoothies

640 during storage at 5, 15 and 25 ºC (n=5±SD).

641

642 **Figure 7.** Observed and predicted total antioxidant capacity data from untreated

643 (CTRL) and heat−treated (HT) smoothies during storage at 5, 15 and 25 ºC. The dashed

644 line shows where points with a perfect fit would fall.

645

646 **Table 1.** Model parameters of the Poisson regression model fitted to the values of

647 sensory quality of purple smoothies during storage.

648

649 **Table 2.** Model parameters of the Baranyi model fitted to microbial counts of purple

650 smoothies during storage.

651

652 **Table 3.** Model parameters of the best linear models describing the antioxidant capacity

653 of purple smoothie during storage as a function of the content in vitamin C (subscript

654 1), total phenolic compounds (subscript 2) and total anthocyanins (subscript 3).

655 Parameters with more than one subscript are interaction terms.

656

657 **Supplementary Table.** Nutritional content of the vegetables purple smoothie.

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