

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 °C/45 s) smoothies were studied and modelled throughout storage  
19 (5, 15 and 25 °C). 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 °C), 4.5/12 (at 15 °C), 2.4/5.8**  
22 **(at 25 °C) 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 °C. HT and 5  
24 °C–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

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

30

31 **Keywords:** **modelling;** anthocyanins; antioxidants; beverages; food safety; quality  
32 modelling.

33

## 34 **1. INTRODUCTION**

35 An adequate intake of fruit and vegetables is essential in the human diet since they are  
36 rich sources of essential nutrients and bioactive compounds which can reduce the risk of  
37 several chronic diseases (Boeing et al., 2012). Purple cabbage, beet, red grapes, broccoli  
38 and cucumber have high contents of such health-promoting compounds like phenolic  
39 compounds (polyphenols and phenolic acids), vitamin C and other antioxidant  
40 compounds (Shahidi, 2004; Souci et al., 2000). Anthocyanins are water-soluble  
41 vacuolar pigments (purple, dark blue and other colours) belonging to the polyphenol  
42 groups of flavonoids (Canuto et al., 2016). Anthocyanins together with phenolic acids  
43 and ascorbic acid are the main antioxidant compounds in fruit and vegetable smoothies  
44 (Lo Scalzo et al., 2004). Nevertheless, **fruit** and vegetables worldwide consumption is  
45 below the recommended daily intake (Hall et al., 2009). Accordingly, the food industry  
46 is developing new alternative presentations such as smoothies which may **highly**  
47 promote the fruit and vegetables consumption. Smoothies are non-alcoholic beverages  
48 prepared from fresh or frozen fruit and/or vegetables, which are blended and usually  
49 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 °C 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 °C 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 °C), unfavourable room temperature (25 °C) when no

75 low storage temperature cabins are available and an intermediate one (15 °C) 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 °C (ideal), 15 °C (maximum recommended) and 25 °C  
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 °C 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 °C. First, plant material was washed  
99 in chlorinated cold water (150 mg L<sup>-1</sup>; 5 °C; 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 °C) 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 score 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 ( $5\pm 2$   
127 °C) with a heating rate of  $30\text{ °C min}^{-1}$  to  $90\text{ °C}$ , followed by a holding period of 45 s and  
128 cooled down to a final temperature of  $35\text{ °C}$  (heating rate of  $30\text{ °C min}^{-1}$ ). 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  $25\text{ °C}$  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  $-80\text{ °C}$  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, psychophilic 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 psychophiles 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 psychophiles incubated at 37 °C for 48 h and at 5 °C for 7 days,  
164 respectively; and rose bengal agar for Y+M incubated for 3–5 days at 25 °C. All  
165 microbial counts were reported as log colony forming units per gram of product (log  
166 CFU mL<sup>-1</sup>). **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 °C) smoothie was homogenized  
174 (Ultraturrax T25 basic, IKA, Berlin, Germany) for 10 s with 10 mL of cold (4 °C) 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<sup>-1</sup> 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 °C. 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<sub>2</sub>CO<sub>3</sub> (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<sup>-1</sup> 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<sup>-1</sup>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).

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

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  $\beta_i$  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  $\beta_0$ ,

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) (Hirotoogu, 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 °C. Subsequently, the homogenate was  
240 centrifuged at 15,000×g for 15 min at 4 °C 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 °C. The sample was  
246 re–suspended with 2.5 mL of MeOH and filtered through a 0.22  $\mu$ m PTFE filter.  
247 Anthocyanins quantification was conducted by injection of 20  $\mu$ L 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 SPD-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 °C. 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<sup>-1</sup>. 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<sup>-1</sup> 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  $\mu$ ,  $x_i$  are the explanatory  
273 variables and  $\beta_i$  are coefficients to estimate from the experimental data.

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

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, psychophilic 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  $\mu_{max}$ , 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 ( $\lambda$ ) 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  $\beta_0$ . Values of  $\beta_1$  of  $-0.019\pm 0.010$ ,  $-0.093\pm 0.040$  and -  
321  $0.137\pm 0.071$  have been estimated for the CTRL samples at 5, 15 and 25 °C,

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 °C (Figure  
327 3). Similarly, a shelf-life of 4.5 and 2.4 days at 15 and 25 °C 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 °C. 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, psychophilic and Y+M counts of the fresh blended smoothie  
334 was 3.5, 3.2 and 2.9 log CFU g<sup>-1</sup>, 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 psychophiles, and CTRL-15 and HT-15 for Y+M)  
340 the fitting algorithm failed at estimating the values of  $\lambda$  and  $\mu_{max}$  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 °C were  $0.90\pm 0.06$  and  $2.29\pm 0.10$ , while  
343 CTRL samples reported  $0.83\pm 0.11$  and  $1.95\pm 0.14$ , respectively. However, there were  
344 not significant differences among the growth rates of CTRL and HT samples at 15 and  
345 25 °C. Nevertheless, when the storage temperature was reduced to 5 °C the mesophilic  
346 growth rate was reduced to 0.20 as obtained for HT-5 samples. HT samples stored at 5

347 and 15 °C showed lag values of  $2.18\pm 3.08$  and  $1.62\pm 0.51$  days, respectively, while no  
348 lag was found for HT samples at 25 °C. 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  $0.55\pm 0.10$ ,  $1.25\pm 0.05$  and  $2.10\pm 0.05$  for 5, 15 and 25 °C,  
352 respectively. CTRL-15 and HT-15 samples showed psychrophilic growth rates of  
353  $1.25\pm 0.05$  and  $1.80\pm 0.17$ , respectively, without significant ( $p < 0.05$ ) differences among  
354 them. The Y+M growth rate of HT samples stored at 5 °C was lower than the one in  
355 CTRL samples with  $0.20\pm 0.02$  and  $0.63\pm 0.24$ , respectively. As the storage temperature  
356 increased, the growth rates also increased with  $1.72\pm 0.33$  and  $0.79\pm 0.27$  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 that 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 ( $< 0.11 \text{ mg kg}^{-1}$ ) 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 ( $354.1 \text{ mg kg}^{-1}$ ) 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  $100 \text{ mg kg}^{-1}$  by the end of the experiment. Nevertheless,  
382 the decrease rate depended on the storage temperatures, with the samples stored at  $25 \text{ }^\circ\text{C}$   
383 requiring 4 days to reach  $100 \text{ mg kg}^{-1}$ , whereas samples stored at 15 and  $25 \text{ }^\circ\text{C}$  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  $70.7\text{--}108.6 \text{ mg kg}^{-1}\text{fw}$  after 14, 11 and 9 days at 5, 15 and  $25 \text{ }^\circ\text{C}$ , 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  $267.6 \text{ mg GAE kg}^{-1}\text{fw}$  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 °C 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 °C showed a TPC decrease up to 55 % on day 9 followed by an increase  
402 reaching final TPC of 174.7 mg GAE kg<sup>-1</sup> 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 °C 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 °C. 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 °C 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 °C showed a similar behaviour to those CTRL  
420 samples at 5 °C with a TPC reduction of approximately 60 % after 9 days. Particularly,  
421 the HT smoothie stored at 15 °C 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 °C due to the higher storage temperature. On the other  
425 side, the high phenolics degradation occurred at 25 °C probably masked the TPC  
426 enhancement observed at 15 °C.

427

### 428 **3.5. Anthocyanins content**

429 The major anthocyanins detected, from higher to lower amounts were (mg kg<sup>-1</sup>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 °C 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<sup>-1</sup>  
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 ( $0.36 \pm 0.10$ ) and the TPC ( $1.41 \pm 0.13$ ). Furthermore the total  
464 anthocyanins content had a synergistic effect with the phenolic content ( $0.0039 \pm 0.0010$ )  
465 and an antagonistic effect with respect to the vitamin C content ( $-0.0080 \pm 0.0014$ ).  
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 °C. 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 °C), 8**  
486 **(at 15 °C) and 3 days (at 25 °C) 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.

494

495

496

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505

506 **REFERENCES**

- 507 ASTM (1986). *Physical requirements guidelines for sensory evaluation laboratories* (Vol. 913,  
508 ASTM Special Technical Pub. 913). Philadelphia, USA: American Society for Testing  
509 Materials.
- 510 Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food.  
511 *International Journal of Food Microbiology*, 23(3-4), 277-294, doi:10.1016/0168-  
512 1605(94)90157-0.
- 513 Barnes, J. S., Nguyen, H. P., Shen, S., & Schug, K. A. (2009). General method for extraction of  
514 blueberry anthocyanins and identification using high performance liquid  
515 chromatography–electrospray ionization-ion trap-time of flight-mass spectrometry.  
516 *Journal of Chromatography A*, 1216(23), 4728-4735,  
517 doi:10.1016/j.chroma.2009.04.032.
- 518 Benzie, I. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of  
519 total antioxidant activity of biological fluids and modified version for simultaneous  
520 measurement of total antioxidant power and ascorbic acid concentration. *Methods in*  
521 *Enzymology*, 299, 15-27, doi:10.1016/S0076-6879(99)99005-5.
- 522 Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., et al. (2012). Critical review:  
523 Vegetables and fruit in the prevention of chronic diseases. *European Journal of*  
524 *Nutrition*, 51(6), 637-663, doi:10.1007/s00394-012-0380-y.
- 525 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to  
526 evaluate antioxidant activity. *LWT- Food Science and Technology*, 28(1), 25-30,  
527 doi:10.1016/S0023-6438(95)80008-5.
- 528 Cano, A., Hernández-Ruíz, J., García-Cánovas, F., Acosta, M., & Arnao, M. B. (1998). An end-  
529 point method for estimation of the total antioxidant activity in plant material.  
530 *Phytochemical Analysis*, 9(4), 196-202, doi:10.1002/(SICI)1099-  
531 1565(199807/08)9:4<196::AID-PCA395>3.0.CO;2-W.
- 532 Canuto, G. A. B., Oliveira, D. R., da Conceição, L. S. M., Farah, J. P. S., & Tavares, M. F. M.  
533 (2016). Development and validation of a liquid chromatography method for  
534 anthocyanins in strawberry (*Fragaria* spp.) and complementary studies on stability,  
535 kinetics and antioxidant power. *Food Chemistry*, 192, 566-574,  
536 doi:10.1016/j.foodchem.2015.06.095.
- 537 Castillejo, N., Martínez-Hernández, G. B., Gómez, P. A., Artés, F., & Artés-Hernández, F. (2016).  
538 Red fresh vegetables smoothies with extended shelf life as an innovative source of  
539 health-promoting compounds. *Journal of Food Science and Technology*, 53(3), 1-12,  
540 doi:10.1007/s13197-015-2143-2.
- 541 Castillejo, N., Martinez-Hernandez, G. B., Monaco, K., Gomez, P. A., Aguayo, E., Artes, F., et al.  
542 (2017). Preservation of bioactive compounds of a green vegetable smoothie using  
543 short time-high temperature mild thermal treatment. *Food Science and Technology*  
544 *International*, 23(1), 46-60, doi:10.1177/1082013216656240.
- 545 Conesa, R., Andreu, S., Fernandez, P. S., Esnoz, A., & Palop, A. (2009). Nonisothermal heat  
546 resistance determinations with the thermoresistometer Mastia. *Journal of Applied*  
547 *Microbiology*, 107(2), 506-513, doi:10.1111/j.1365-2672.2009.04236.x.
- 548 EC (2007). Commission Regulation (EC) No 1441/2007. *Official Journal of the European Union*,  
549 322, 12-29.
- 550 Esteban, M. D., Conesa, R., Huertas, J. P., & Palop, A. (2015). Effect of thymol in heating and  
551 recovery media on the isothermal and non-isothermal heat resistance of *Bacillus*  
552 spores. *Food Microbiology*, 48, 35-40, doi:10.1016/j.fm.2014.11.016.
- 553 FAO/WHO (2004). *Vitamin and mineral requirements in human nutrition* (2ed., Code of Federal  
554 Regulations). Bangkok: World Health Organization and Food and Agriculture  
555 Organization of the United Nations.

556 Hall, J. N., Moore, S., Harper, S. B., & Lynch, J. W. (2009). Global variability in fruit and  
557 vegetable consumption. *American Journal of Preventive Medicine*, 36(5), 402-409,  
558 doi:10.1016/j.amepre.2009.01.029.

559 Hartigan, J. A. (1975). *Clustering Algorithms*. New York: John Wiley & Sons.

560 Hirotogu, A. (1998). Information theory and an extension of the maximum likelihood principle.  
561 In E. Parzen, K. Tanabe, & G. Kitagawa (Eds.), *Selected papers of Hirotogu Akaike* (pp.  
562 199–213). New York: Springer.

563 Houben, K., Jamsazzadeh Kermani, Z., Van Buggenhout, S., Van Loey, A. M., & Hendrickx, M. E.  
564 (2014). Thermal and high-pressure stability of pectin-converting enzymes in broccoli  
565 and carrot purée: Towards the creation of specific endogenous enzyme populations  
566 through processing. *Food and Bioprocess Technology*, 7(6), 1713-1724,  
567 doi:10.1007/s11947-013-1166-9.

568 ISO (2007). Sensory analysis-General guidance for the design of test rooms. In ISO (Ed.), (Vol.  
569 8589:2007): ISO.

570 Lo Scalzo, R., Iannocari, T., Summa, C., Morelli, R., & Rapisarda, P. (2004). Effect of thermal  
571 treatments on antioxidant and antiradical activity of blood orange juice. *Food*  
572 *Chemistry*, 85(1), 41-47, doi:10.1016/j.foodchem.2003.05.005.

573 Martínez-Hernández, G. B., Gómez, P. A., Pradas, I., Artés, F., & Artés-Hernández, F. (2011).  
574 Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi®  
575 broccoli. *Postharvest Biology and Technology*, 62(3), 327-337,  
576 doi:10.1016/j.postharvbio.2011.06.015.

577 McCullagh, P., & Nelder, J. A. (1989). *Generalized Linear Models*. London: Chapman and Hall.

578 Munyaka, A. W., Makule, E. E., Oey, I., Van Loey, A., & Hendrickx, M. (2010). Thermal stability  
579 of L-ascorbic acid and ascorbic acid oxidase in broccoli (*Brassica oleracea* var. *italica*).  
580 *Journal of Food Science*, 75(4), C336-340, doi:10.1111/j.1750-3841.2010.01573.x.

581 Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Changes of health-related  
582 compounds throughout cold storage of tomato juice stabilized by thermal or high  
583 intensity pulsed electric field treatments. *Innovative Food Science and Emerging*  
584 *Technologies*, 9(3), 272-279, doi:10.1016/j.ifset.2007.07.009.

585 Ortega-Anta, R., López-Sobaler, A., Andrés-Carvajales, P., Requejo-Marcos, A., Aparicio-  
586 Vizuete, A., & Molinero-Casares, L. (2008). Programa para evaluación de dietas y  
587 gestión de datos de alimentación DIAL 1.0©. *ALCE Ingeniería (Madrid, Spain)*.

588 Patras, A., Brunton, N., Da Pieve, S., Butler, F., & Downey, G. (2009). Effect of thermal and high  
589 pressure processing on antioxidant activity and instrumental colour of tomato and  
590 carrot purées. *Innovative Food Science and Emerging Technologies*, 10(1), 16-22,  
591 doi:10.1016/j.ifset.2008.09.008.

592 Picariello, G., Ferranti, P., Garro, G., Manganiello, G., Chianese, L., Coppola, R., et al. (2014).  
593 Profiling of anthocyanins for the taxonomic assessment of ancient purebred *V. vinifera*  
594 red grape varieties. *Food Chemistry*, 146, 15-22, doi:10.1016/j.foodchem.2013.08.140.

595 R\_Core\_Team (2014). *R: A language and environment for statistical computing*. Vienna,  
596 Austria: R Foundation for Statistical Computing.

597 Rodríguez-Verástegui, L. L., Martínez-Hernández, G. B., Castillejo, N., Gómez, P. A., Artés, F., &  
598 Artés-Hernández, F. (2015). Bioactive compounds and enzymatic activity of red  
599 vegetable smoothies during storage. *Food and Bioprocess Technology*, 9(1), 137-146,  
600 doi:10.1007/s11947-015-1609-6.

601 Shahidi, F. (2004). *Phenolics in food and nutraceuticals*. Boca Raton FL, USA: CRC Press LLC.

602 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-  
603 phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3),  
604 144-158.

605 Souci, S. W., Fachmann, W., & Kraut, H. (2000). *Food Composition and nutrition tables*.  
606 Stuttgart, Germany: Medpharm Scientific Publishers Stuttgart.

607 Sun-Waterhouse, D., Bekkour, K., Wadhwa, S. S., & Waterhouse, G. I. N. (2014). Rheological  
608 and chemical characterization of smoothie beverages containing high concentrations  
609 of fibre and polyphenols from apple. *Food and Bioprocess Technology*, 7(2), 409-423,  
610 doi:10.1007/s11947-013-1091-y.

611 Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as  
612 determinants of quality in fruits and vegetables. *Journal of the Science of Food and*  
613 *Agriculture*, 81(9), 853-876, doi:10.1002/jsfa.885.

614 Wang, S., Lin, T., Man, G., Li, H., Zhao, L., Wu, J., et al. (2014). Effects of anti-browning  
615 combinations of ascorbic acid, citric acid, nitrogen and carbon dioxide on the quality of  
616 banana smoothies. *Food and Bioprocess Technology*, 7(1), 161-173,  
617 doi:10.1007/s11947-013-1107-7.

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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 °C. 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 °C (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: 5°C  
633 (red solid line, circles), 15°C (green short dashed line, triangles) and 25°C (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 °C (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 °C (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 °C. 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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