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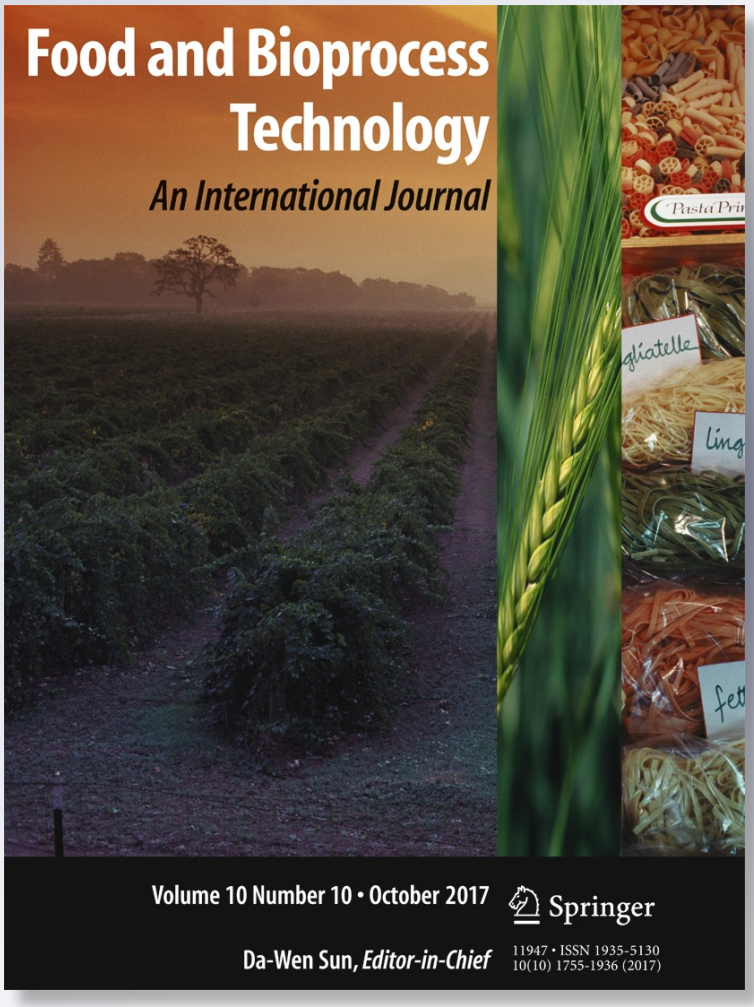

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ORIGINAL PAPER

Quality Changes and Shelf-Life Prediction of a Fresh Fruit and Vegetable Purple Smoothie

Gerardo A. González-Tejedor[1,2] · Ginés Benito Martínez-Hernández[2,3] · Alberto Garre[1,4] · Jose A. Egea[4] · Pablo S. Fernández[1,3] · Francisco Artés-Hernández[2,3]

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Abstract The sensory, microbial, and bioactive quality changes of untreated (CTRL) and mild heat-treated (HT; 90 °C/45 s) smoothies were studied and modelled throughout storage (5, 15 and 25 °C). The overall acceptability was better preserved in HT samples being highly correlated (hierarchical clustering) with the flavour. The sensory quality data estimated smoothie shelf-life (CTRL/HT) of 18/55 (at 5 °C), 4.5/12 (at 15 °C) and 2.4/5.8 (at 25 °C) days. The yeast and mould growth rate was lower in HT compared to CTRL while a lag phase for mesophiles/psychrophiles was observed in HT-5/15 °C. HT and 5 °C storage stabilised the phenolic content. Ferric reducing antioxidant power reported the best correlation (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.

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Keywords Modelling · Anthocyanins · Antioxidants · Beverages · Food safety · Quality modelling

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 vegetable consumption worldwide 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 vegetable 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 components like yogurt, milk, ice cream, lemon, water, or tea. They have a milk shake-like consistency that is thicker than slush drinks (Castillejo et al. 2016).

The smoothie preparation involves a breakdown of plant parenchyma, which leads to a dispersed solution consisting in a liquid phase (including pectin and other soluble solids) and a solid phase composed of insoluble solids (cell wall). Accordingly, quality-degradative

enzymes come easily in contact with their substrates and sugars are more available for spoilage microorganisms, which highly limit the shelf-life of these beverages (Rodríguez-Verástegui et al. 2015). In order to extend the shelf-life of these beverages, thermal treatments can be used (Houben et al. 2014) together with subsequent low-temperature storage that would decrease the intensity of the pasteurisation (Castillejo et al. 2016; Rodríguez-Verástegui et al. 2015). However, such thermal treatments can be detrimental to the smoothie quality, causing degradation of thermolabile nutrients, and affecting sensorial properties such as texture, colour, taste and aroma (Esteban et al. 2015). Accordingly, the thermal treatment should be as mild as possible in order to preserve the nutritional and sensory quality of the smoothie while achieving an appropriate microbial reduction and inactivation of quality-degradative enzymes. In this way, thermal treatments at 80–95 °C for less than 3 min (ensuring a pasteurisation treatment) together with subsequent low storage temperature have been satisfactorily used to inactivate quality-degradative enzymes and to reach significant microbial reductions while keeping acceptable sensory attributes (Castillejo et al. 2016; Rodríguez-Verástegui et al. 2015; Sun-Waterhouse et al. 2014; Wang et al. 2014). Optimum low storage temperature of 5 °C in these products cannot be always ensured in the retail surfaces. In addition, it is crucial to study the microbial, physicochemical, sensory and nutritional/bioactive quality degradation of the smoothie throughout storage at optimum low temperature (5 °C), unfavourable room temperature (25 °C) when no low storage temperature cabins are available and an intermediate one (15 °C) such as that of commercial retail cabins. Such quality changes at different storage temperatures should be modelled in order to establish the smoothie shelf-life to ensure a proper intake of nutritional and bioactive compounds while preserving its safety.

The objective of this work was to study the effect of a mild heat treatment (ensuring pasteurisation) in a purple smoothie (pH <4.2) made of fresh horticultural products, compared to fresh-blended untreated samples. Such quality changes were studied using mathematical models allowing to estimate the potential shelf-life of such products at different temperatures of 5 °C (ideal), 15 °C (maximum recommended) and 25 °C (misused) simulating shipping, distribution and retail sale periods. Accordingly, the shelf-life prediction of this fruit and vegetable beverage will be of a high interest for the related food industries to improve the produce logistics all over the chain and ensure lower costs and a better final quality of the product. To the best of our knowledge, no previous studies have used mathematical tools to predict quality (sensory, microbial and nutritional/bioactive) changes and shelf-life of fresh-blended fruit/vegetable beverages treated with high-temperature, short-time treatments and stored at different temperatures.

Material and Methods

Plant Material and Smoothie Preparation

Fresh fruit and vegetables (purple seedless grapes, cucumber, beet and broccoli) were obtained from a local market (Cartagena, Spain) and stored at 5 °C and 90–95% relative humidity (RH) until the next day, when they were processed. The smoothie processing was accomplished in a disinfected cold room at 10 °C. First, plant material was washed in chlorinated cold water (150 mg L^{-1}; 5 °C; pH 6.5; 2 min) at a ratio of 300 g of plant material to 5 L chlorinated water, rinsed with tap water (1 min; 5 °C) and drained in a perforated basket. Subsequently, cucumber and beet were peeled and all vegetables were then cut and blended in a Thermomix food processor (TM 21, Vorwerk, Spain). The blending program used was 1 min at level 4 followed by 1 min at maximum level 10.

The smoothie formulation was 12% beet, 45% purple grapes, 35% cucumber and 8% broccoli. The final formulation was selected according to a sensory evaluation of five types of purple smoothies (selected based on common purple smoothie recipes on books, internet, etc.) done with 30 participants (17 women/13 men, aged 20–48 years) randomly chosen in the Universidad Politécnica de Cartagena. People were first asked about their eating habits confirming that all of them consumed regularly fruit and vegetables, and particularly liked all the ingredients that contained all smoothie types. The participants were asked to score smoothies' appearance, flavour, texture and overall acceptability according to a 5-point hedonic scale of acceptability (5, excellent; 4, good; 3, fair, limit of usability; 2, poor; 1, extremely bad). All five smoothie types were given at a time in transparent plastic glasses (30 mL each one) coded with three random digit numbers served in an arbitrary order. Participants were asked to drink still mineral water as palate cleanser. pH of samples was always below 4.2 throughout all storage conditions.

Smoothie Treatments and Storage Conditions

Heat treatment (HT) of the smoothie was carried out in a thermoresistometer Mastia (Conesa et al. 2009) immediately after blending. Briefly, the sterilised vessel of the thermoresistometer was filled with 400 mL of the smoothie. Then, the thermoresistometer was programmed to increase the initial smoothie temperature (5 ± 2 °C) with a heating rate of 30 °C min^{-1} to 90 °C, followed by a holding period of 45 s and cooled down to a final temperature of 35 °C (heating rate of 30 °C min^{-1}). This ensured a pasteurisation treatment. After

the thermal treatment, the smoothie was cooled down to the respective storage temperatures submerging the vessel in an ice-water bath while continuous agitation was programmed in the thermoresistometer. Untreated samples were used as control (CTRL). Samples were taken from the thermoresistometer through a sampling port under aseptic conditions into 50-mL Falcon tubes. Samples were then stored in darkness at 5, 15 and 25 °C up to 28 days depending of storage temperature. Five replicates per treatment, storage temperature and sampling day were prepared. Samples for nutritional/bioactive compounds were taken on each sampling day and stored at −80 °C until further analysis.

Sensory Evaluation

Sensory analyses were performed according to international standards (ASTM 1986). Tests were conducted in a standard room (ISO 2007) equipped with ten individual taste boxes using the white light. Samples (about 30 mL) were served at room temperature in transparent plastic glasses coded with three random digit numbers. Still mineral water was used as palate cleanser. The sensory panel consisted of 12 assessors (six women/six men, aged 22–68 years) which have specific sensory discriminative ability (colour, flavour, visual appearance and texture) on fruit and vegetable smoothies. A 5-point scale of damage incidence and severity was scored for off-colours, off-odours, lumpiness, turbidity and precipitation/phase separation (5, none; 4, slight; 3, moderate, limit of usability; 2, severe; 1, extreme). Visual appearance, aroma, flavour, texture and overall quality were assessed at the same time using a 5-point hedonic scale of acceptability (5, excellent; 4, good; 3, fair, limit of usability; 2, poor; 1, extremely bad).

The sensory data was rationalised to study proximal sensory parameters. Hierarchical clustering (Hartigan 1975) was applied in order to group similar parameters among a group of data. The degree of similitude between the different scores was quantified using the Euclidean distance.

Microbial Analysis

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

Vitamin C

The ascorbic (AA) and dehydroascorbic (DHA) acids were measured according to Castillejo et al. (2017). Briefly, 5 g ground frozen (−80 °C) smoothie was homogenised (Ultraturrax T25 basic, IKA, Berlin, Germany) for 10 s with 10 mL of cold (4 °C) buffer (0.1 M citric acid, 0.05% EDTA, 4 mM sodium fluoride and 5% MeOH) under water-ice bath. Then, the homogenate was immediately filtered (four-layer cheesecloth) and the pH adjusted (6 N NaOH) to 2.35–2.4. Subsequently, 750 mL of filtered (0.45 μm polyether sulphone filter (PTFE)) purified extract (Sep-Pak cartridges C18, Waters, Dublin, Ireland) was derivatised with 250 mL of 7.7 M 1,2-phenylenediamine for 37 min in darkness at room temperature and analysed by HPLC. Accordingly, 20 mL of derivatised sample was injected onto a Gemini NX (250 mm × 4.6 mm, 5 mm) C18 column (Phenomenex, Torrance CA, USA), using an HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. AA and DHA were quantified using commercial standards. Calibration curves were made with at least six data points for each standard. Total vitamin C was calculated as the sum of AA and DHA and expressed as mg kg^{-1} fresh weight (fw). Each of the five replicates was analysed in duplicate.

Total Phenolic Content and Total Antioxidant Capacity

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

The TPC was determined based on Singleton and Rossi (1965) but with modifications proposed by Martínez-Hernández et al. (2011). Briefly, 19 μL of extract was placed in a 96-well plate, and 29 μL of 1 N Folin–Ciocalteu reagent was added. The mix was incubated for 3 min in darkness at room temperature. Then, 192 μL of a solution containing Na_2CO_3 (0.4%) and NaOH (2%) was added. After 1 h of incubation at room temperature in darkness, the absorbance

was measured at 750 nm. The TPC was expressed as mg gallic acid equivalents (GAE) kg^{-1} fw.

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

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

$$y = \beta_0 + \beta_1 vitC + \beta_2 phenol + \beta_3 anthocyanins + \sum inter \quad (1)$$

where y is the TAC method used, *vitC*, *phenol* and *anthocyanins* stand for the contents of vitamin C, phenols and anthocyanins, respectively, whereas $\sum inter$ represents a sum of first-order interaction terms between the concentrations of antioxidant compounds. β_i are undetermined coefficients to be estimated from the experimental data. Having three independent variables (i.e., *vitC*, *phenol* and *anthocyanins*), the first-order interaction terms among them (i.e., four more variables) and considering the independent term β_0, there are eight possible fitting parameter per model. A model selection procedure was carried out in order to avoid the overfitting of the models. This procedure aims to select only those independent variables which have a significant influence over the dependent variable, y, based on a performance index. A complete enumeration of models (128 possible models for each TAC method) was performed using the R programming language (R_Core_Team 2014), and the best among them was selected according to the Akaike information criterion (AIC) (Hirotogu 1998). The normality and independence of the residuals was tested using, respectively, the Shapiro-Willis and Durbin-Watson tests at the 95% confidence level. Their homoscedasticity was tested using a residual plot.

Anthocyanins

Anthocyanin extraction and determination were conducted as previously described (Barnes et al. 2009) but with modifications. Frozen smoothie sample (2.5 g) was homogenised with 5 mL MeOH and incubated under ultrasonic bath (Cole-Parmer, model 8890, IL, USA) for 10 min at 20 °C. Subsequently, the homogenate was centrifuged at 15,000×*g* for 15 min at 4 °C and the supernatant was collected in an amber bottle. The pellet previously obtained was resuspended with another aliquot of 5.0 mL MeOH, followed by ultrasounds and centrifugation as described. The latter procedure was repeated four times, and the supernatants were combined and make up to 25 mL with MeOH. The combined supernatants were then concentrated to dryness with a rotavapor (Hei-VAP Value, Schwabach, Germany) at 40 °C. The sample was resuspended with 2.5 mL of MeOH and filtered through a 0.22-μm PTFE filter.

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

Mathematical Modelling

Kinetics of the Sensory Quality Features of the Smoothie

The score assigned to the overall acceptance is a discrete variable. Therefore, the evolution of the overall acceptance of the smoothie was described using Poisson regression (McCullagh and Nelder 1989). This type of model can be written as shown in Eqs. 2 and 3, where y is the dependent variable (the quality attribute modelled), Poisson(μ) represents the Poisson distribution

of mean μ, x_i is the explanatory variable and β_i is the coefficient to estimate from the experimental data.

$$y = Poisson(\mu) \tag{2}$$

$$\log\mu = \beta_0 + \sum_i \beta_i x_i \tag{3}$$

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

Microbial Growth

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

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

$$\frac{dQ}{dt} = \mu_{max} \cdot Q(t) \tag{5}$$

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

$$\lambda \cdot \mu_{max} = ln\left(1 + \frac{1}{Q(0)}\right) \tag{6}$$

The model has been fitted to the experimental data using the Excel add-in DMfit. The goodness of the fit was evaluated using the RMSE and by visual inspection of the fitted curve.

Results and Discussion

Sensory Analysis

A dendrogram depicting the results of the hierarchical clustering performed on the quality data is shown in Fig. 1. As observed, there are two main groups in the sensory analysis with a large distance between them: visual appearance and flavour. The overall acceptability of the product is highly correlated with the flavour. Hence, flavour is the most relevant acceptability feature that the consumer assigns to the product, leading to the remaining sensory attributes in a second scenario. For that reason, horticultural produce selection in its optimum ripening stage for the smoothie preparation becomes a key factor for consumer acceptance. Similar dendrograms were constructed with the sensory data obtained for HT samples and fresh-blended unheated ones (CTRL), obtaining similar results (not shown).

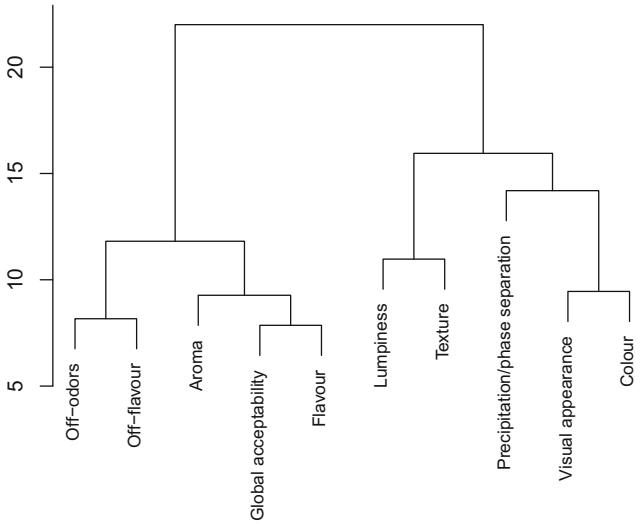

Fig. 1 Dendrogram of the hierarchical clustering of sensory attributes of smoothies

The thermal treatment did not generally affect the sensory attributes of the smoothie, except flavour, which was increased ($p < 0.05$) from a score of 3.9 to 4.6 (Fig. 2). The enhancement of the smoothie flavour after the thermal treatment may be explained by the thermal breakdown of plant cells leading to a leakage of compounds responsible for flavour.

The fitting of the generalised linear model to the overall acceptability data is summarised in Table 1. The fitted models predict similar scores at day 0, as shown in the values estimated for β_0. Values of β_1 of −0.019 ± 0.010, −0.093 ± 0.040 and −0.137 ± 0.071 have been estimated for the CTRL samples at 5, 15 and 25 °C, respectively, whereas for the HT samples, the models estimate values between a 48 and 63% lower. Therefore, the overall acceptability of the CTRL samples decreases more rapidly throughout storage than the equivalent HT samples. Accordingly, the shelf-life of HT samples, setting a score of 3 for the overall acceptability as a threshold value, can be predicted as 18 days for CTRL samples and 55 days for HT samples at 5 °C (Fig. 3). Similarly, a shelf-life of 4.5 and 2.4 days at 15

Fig. 2 Sensory scores of untreated (CTRL) and heat-treated (HT) smoothies during storage at 5, 15 and 25 °C ($n = 5$)

and 25 °C is estimated for CTRL samples, whereas a shelf-life of 12 and 5.8 days are estimated for HT samples at 15 and 25 °C. Hence, it can be concluded that the heat treatment applied effectively increased the

Table 1 Model parameters of the Poisson regression model fitted to the values of sensory quality of purple smoothies during storage

	CTRL		HT	
Temperature (°C)	β_0	β_1 (1/day)	β_0	β_1 (1/day)
5	1.448 ± 0.128	−0.019 ± 0.010	1.503 ± 0.111	−0.007 ± 0.006
15	1.522 ± 0.139	−0.093 ± 0.040	1.525 ± 0.135	−0.035 ± 0.035
25	1.425 ± 0.161	−0.137 ± 0.071	1.512 ± 0.152	−0.071 ± 0.063

sensory shelf-life of the smoothie at every storage temperature studied.

Microbial Analysis

The initial mesophilic, psychrophilic and Y+M counts of the fresh-blended smoothie 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 log units after the thermal treatment (Fig. 4) according to a Welch two-sample t test. The Baranyi model was able to describe the temporal evolution of the microbial data for every experiment. The values of the model parameters estimated, as well as their standard deviations, are shown in Table 2. For several experiments (CTRL-5 for mesophiles, HT-5 and HT-25 for psychrophiles, and CTRL-15 and HT-15 for Y+M), the fitting algorithm failed at estimating the values of λ and μ_{max} due to an insufficient number of measurements made during the exponential growth phase. The mesophilic growth rates of HT samples stored at 15 and 25 °C were 0.90 ± 0.06 and 2.29 ± 0.10, while CTRL samples reported 0.83 ± 0.11 and 1.95 ± 0.14, respectively. However, there were no significant differences among the growth rates of CTRL and HT samples at 15 and 25 °C. Nevertheless, when the storage temperature was reduced to 5 °C, the mesophilic growth rate was reduced to 0.20 as obtained for HT-5 samples. HT samples stored at 5 and 15 °C showed lag values of 2.18 ± 3.08 and 1.62 ± 0.51 days, respectively, while no lag was found for HT samples at 25 °C. As observed, the lag increased as the storage temperature decreased in HT samples. No lag was found for CTRL samples.

The psychrophilic growth rate of CTRL samples increased as the storage temperature did, reporting values of 0.55 ± 0.10, 1.25 ± 0.05 and 2.10 ± 0.05 for 5, 15 and 25 °C, respectively. CTRL-15 and HT-15 samples showed psychrophilic growth rates of 1.25 ± 0.05 and 1.80 ± 0.17, respectively, without significant ($p < 0.05$) differences among them. The Y+M growth rate of HT samples stored at 5 °C was lower than the one in CTRL samples with 0.20 ± 0.02 and 0.63 ± 0.24, respectively. As the storage temperature increased, the growth rates also increased with 1.72 ± 0.33 and 0.79 ± 0.27 for CTRL-25 and HT-25 samples, respectively.

Conclusively, the HT reduced the initial mesophilic, psychrophilic and Y+M loads of the smoothie. Furthermore, HT did not cause any significant variation in the microbial growth rates, although Y+M growth rate of HT samples was lower than CTRL samples. Nevertheless, it can be qualitatively stated than HT introduces a lag in mesophilic and psychrophilic data which was not observed in CTRL samples, increasing the time required for the microorganisms to reach hazardous levels.

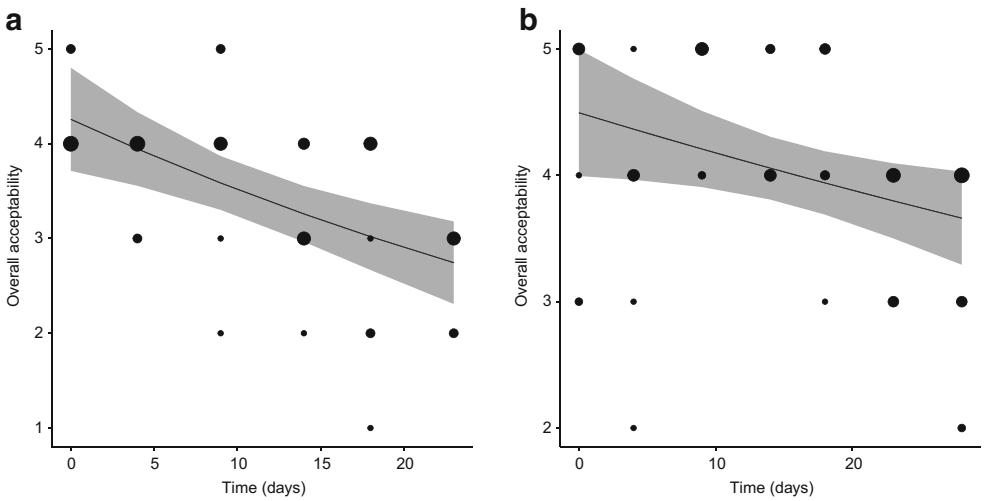

Fig. 3 Overall acceptability of untreated (**a**) and heat-treated (**b**) smoothies during storage at 5 °C. The overall acceptability was considered a discrete variable. The size of the symbols is proportional to the number of occurrences of a given value

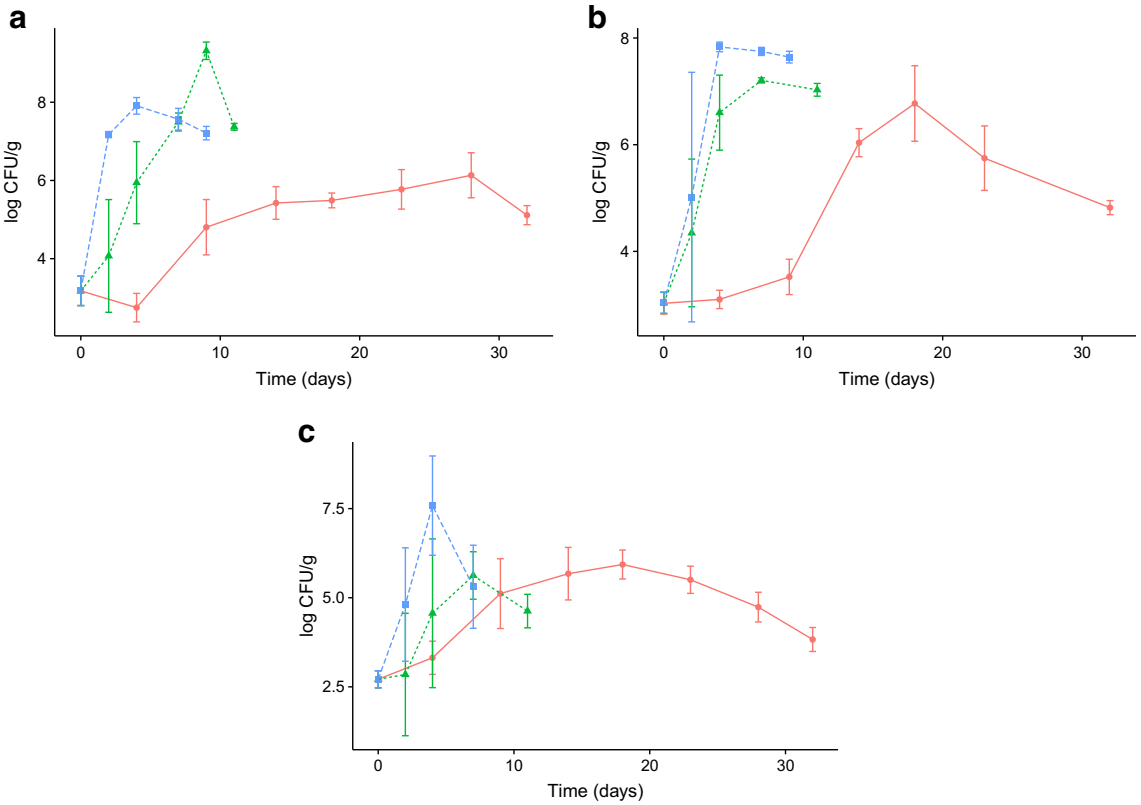

Fig. 4 Microbial growth of mesophiles (**a**), psychrophiles (**b**) and yeasts and moulds (**c**) in the heat-treated smoothie during storage at different temperatures: 5 °C (*red solid line*, *circles*), 15 °C (*green short dashed line*, *triangles*) and 25 °C (*blue long dashed line*, *squares*) ($n = 5 \pm$ SD)

Table 2 Model parameters of the Baranyi model fitted to microbial counts of purple smoothies during storage

Microbial group	Treatment	λ (min)	μ_{max} (1/min)	logN_{max} (CFU mL^{-1})	RMSE
Mesophiles	CTRL 5 °C	[a]	[a]	5.53 ± 0.07	0.25
	HT 5 °C	2.18 ± 3.08	0.20 ± 0.04	6.20 ± 0.16	0.41
	CTRL 15 °C	[b]	0.83 ± 0.11	8.20 ± 0.18	0.68
	HT 15 °C	1.62 ± 0.51	0.90 ± 0.06	[c]	0.43
	CTRL 25 °C	[b]	1.95 ± 0.14	7.70 ± 0.09	0.34
	HT 25 °C	[b]	2.29 ± 0.10	7.42 ± 0.05	0.20
Psychrophiles	CTRL 5 °C	8.23 ± 0.95	0.55 ± 0.10	6.76 ± 0.14	0.44
	HT 5 °C	[a]	[a]	5.54 ± 0.10	0.47
	CTRL 15 °C	[b]	1.25 ± 0.05	7.14 ± 0.04	0.15
	HT 15 °C	2.28 ± 0.17	1.80 ± 0.17	7.17 ± 0.02	0.07
	CTRL 25 °C	[b]	2.10 ± 0.05	7.80 ± 0.03	0.13
	HT 25 °C	[a]	[a]	7.67 ± 0.04	0.11
Yeasts and moulds	CTRL 5 °C	3.13 ± 0.82	0.63 ± 0.24	6.10 ± 0.09	0.33
	HT 5 °C	[b]	0.20 ± 0.02	5.21 ± 0.10	0.37
	CTRL 15 °C	[a]	[a]	6.39 ± 0.11	0.24
	HT 15 °C	[a]	[a]	4.59 ± 0.23	0.56
	CTRL 25 °C	[b]	1.72 ± 0.33	7.64 ± 0.32	0.99
	HT 25 °C	[b]	0.79 ± 0.27	5.11 ± 0.33	0.89

[a] Unsuccessful fit

[b] Lag phase was not observed in this experiment

[c] Stationary phase was not reached in this experiment

Total Vitamin C

Low AA levels (<0.11 mg kg^{-1}) were detected in the samples. The AA oxidation to DHA is rapidly catalysed by the enzymes ascorbate oxidase and ascorbic acid peroxidase. Accordingly, the AA absence may be explained since during the smoothie blending, plant cells are disrupted easily allowing enzymes to access their substrates located in different plant cell locations. However, DHA also exhibits antioxidant properties in addition to antiscorbutic activity equivalent to that of AA being total vitamin C considered as the sum of AA and DHA (Munyaka et al. 2010). The initial total vitamin C content of samples (354.1 mg kg^{-1}) was not significantly ($p < 0.05$) affected on processing day after the thermal treatment.

The effect of the storage time and the thermal treatment on the total vitamin C degradation rate was assessed using an ANCOVA analysis. The results show that the storage time significantly ($p < 0.05$) affects the degradation rate, whereas no significant differences ($p < 0.05$) were observed between the inactivation rates observed for the CTRL and HT samples. Figure 5 represents the DHA degradation observed in the sample at the different storage temperatures tested. In every case, the DHA content decreased to values lower than 100 mg kg^{-1} by the end of the experiment. Nevertheless, the decrease rate depended on the storage temperatures, with the samples stored at 25 °C requiring 4 days to reach 100 mg kg^{-1}, whereas samples stored at 15 and 25 °C required 7 and 14 days, respectively. A quantitative comparison through a kinetic model has not been performed due to the dispersion of the data. DHA contents of samples ranged among 70.7 to 108.6 mg kg^{-1} fw after 14, 11 and 9 days at 5, 15 and 25 °C, respectively. A portion of 250 g of the smoothie at the end of last storage periods still ensured the 40–60% of the recommended vitamin C daily intake by the FAO/WHO (2004).

Total Phenolic and Anthocyanin Content

The smoothie showed an initial TPC of 267.6 mg GAE kg^{-1} fw being considered as a good source of phenolic compounds as other red and green vegetable smoothies (Castillejo et al. 2017; Rodríguez-Verástegui et al. 2015). In general, phenolic degradation may occur after thermal treatments and during storage due to chemical and enzymatic oxidation, which can also lead to changes in bioavailability or biological activity (Tomás-Barberán and Espín 2001). However, the mild heat treatment applied did not induce significant ($p < 0.05$) TPC changes similarly to what is reported in other vegetable beverages treated at 70–90 °C for 1–2 min (Odriozola-Serrano et al. 2008; Patras et al. 2009).

An accurate kinetic model was not developed due to the dispersion of the data. Samples stored at 5 °C showed a TPC decrease up to 55% on day 9 followed by an increase reaching final TPC of 174.7 mg GAE kg^{-1} fw

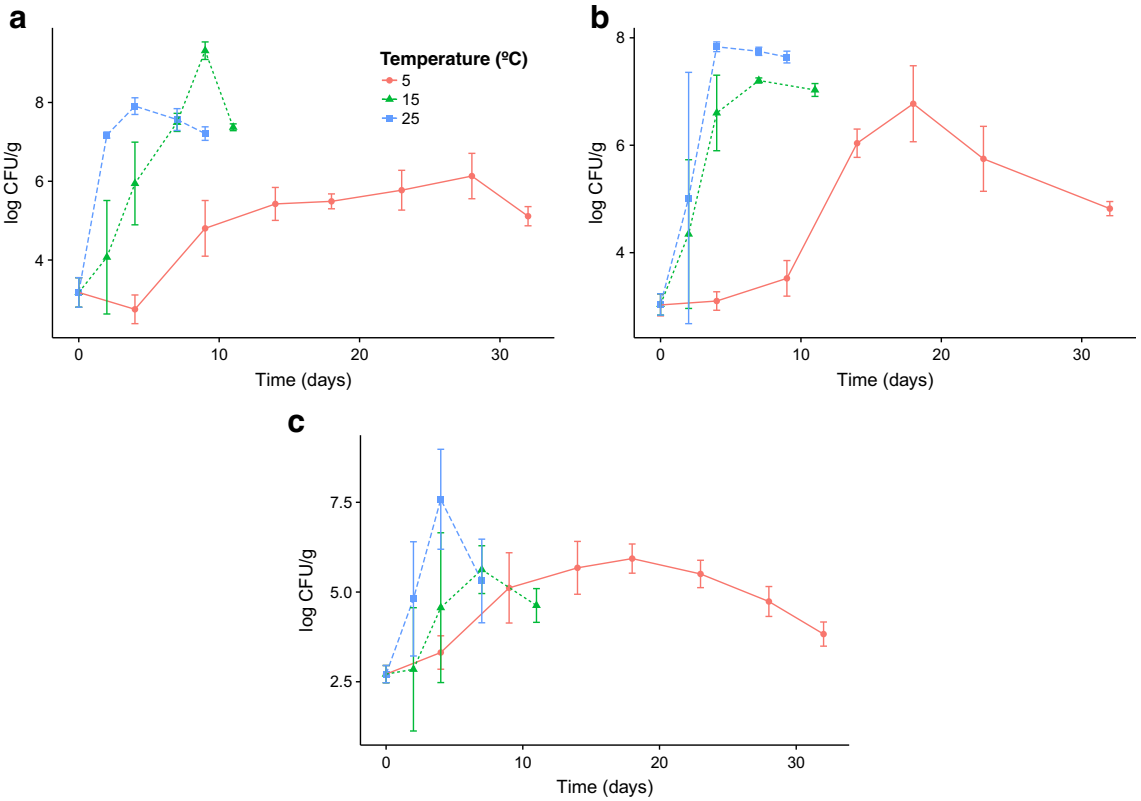

Fig. 5 Vitamin C (dehydroascorbic acid (DHA)) of untreated (CTRL) and heat-treated (HT) smoothies during storage at 5, 15 and 25 °C ($n = 5 \pm$ SD)

after 18 days (Fig. 6). The latter behaviour may be explained by an initial phenolic degradation through phenolic-degradative enzymes, followed by a possible increment of the phenylalanine ammonia lyase (PAL) activity, the key enzyme in the biosynthetic pathway of phenolic compounds. Similarly, PAL activity and TPC enhancements were observed in untreated red vegetable smoothies stored at 5 °C probably owed to the wounding abiotic stress occurred during the smoothie preparation (Rodríguez-Verástegui et al. 2015). Contrary, HT samples did not show significant ($p < 0.05$) changes after 18 days at 5 °C. Therefore, the heat treatment and the low storage temperature stabilised the TPC levels probably due to the reduction of the activity of those enzymes responsible for phenolic degradation as previously reported (Rodríguez-Verástegui et al. 2015). Nevertheless, when CTRL and HT samples were stored at 25 °C, the TPC levels were highly reduced by 70 and 90% after 9 days, respectively. The high phenolic degradation may be explained by a high activity at such high storage temperature of those phenolic-degradative enzymes. Furthermore, the latter enzymatic activities were even favoured in those HT samples due to a higher enzymatic substrate availability enhanced by the plant cell disruption after the thermal treatment. CTRL samples stored at the intermediate temperature of 15 °C showed a similar behaviour to those CTRL samples at 5 °C with a TPC reduction of approximately 60% after 9 days. Particularly, the HT smoothie stored at 15 °C showed a TPC enhancement of 71% after 4 days followed by a decrease reaching after 9 days similar levels to processing day. The latter phenolic enhancement could be explained by an increase of the PAL activity earlier than CTRL samples stored at 5 °C due to the higher storage temperature. On the other side, the high phenolic degradation occurred at 25 °C probably masked the TPC enhancement observed at 15 °C.

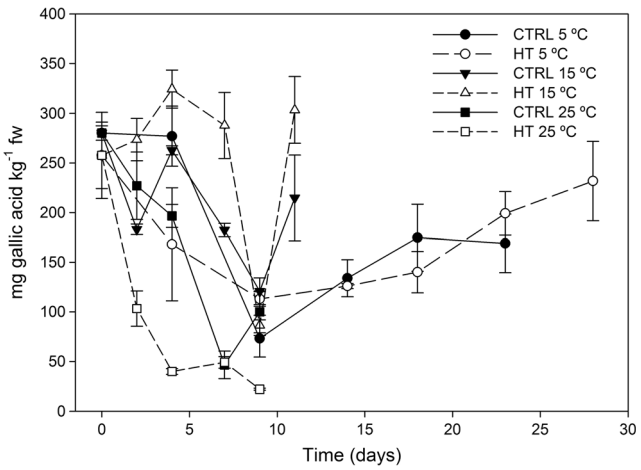

Fig. 6 Total phenolic content of untreated (CTRL) and heat-treated (HT) smoothies during storage at 5, 15 and 25 °C ($n = 5 \pm SD$)

Anthocyanin Content

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

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

Total Antioxidant Capacity

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

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

According to the fitted model, FRAP has an excellent linear relationship with the vitamin C concentration (0.36 ± 0.10) and the TPC (1.41 ± 0.13). Furthermore the total anthocyanins content had a synergistic effect with the phenolic content (0.0039 ± 0.0010) and an antagonistic effect with respect to

Table 3 Model parameters of the best linear models describing the antioxidant capacity of purple smoothie during storage as a function of the content in vitamin C (subscript 1), total phenolic compounds (subscript 2) and total anthocyanins (subscript 3)

	DPPH	ABTS	FRAP
β_0	563.25 ± 44.63	183.81 ± 17.94	119.63 ± 19.55
β_1	−0.46 ± 0.14	1	0.36 ± 0.10
β_2	0.46 ± 0.28	0.59 ± 0.14	1.41 ± 0.13
β_3	[a]	[a]	[a]
β_{12}	[a]	[a]	[a]
β_{13}	[a]	[a]	$(-7.97 \pm 1.44)10^{-3}$
β_{23}	[a]	$(5.13 \pm 1.09)10^{-3}$	$(3.86 \pm 1.04)10^{-3}$
β_{123}	$(-2.37 \pm 7.38)10^{-6}$	$(-2.37 \pm 0.52)10^{-5}$	[a]
R^2	0.45	0.81	0.94

Parameters with more than one subscript are interaction terms

[a] Model parameter not included in the model selected; 1, vitamin C; 2, total phenolic content; 3, total anthocyanins

the vitamin C content (−0.0080 ± 0.0014). Nevertheless, further data is required to test whether these conclusions can be extrapolated for experimental conditions different to the ones tested.

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

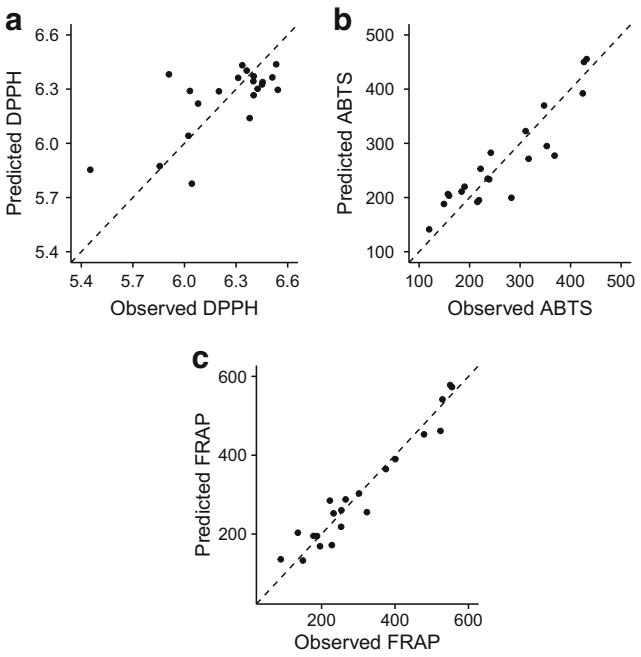

Fig. 7 Observed and predicted total antioxidant capacity data from untreated (CTRL) and heat-treated (HT) smoothies during storage at 5, 15 and 25 °C. The *dashed line* shows where points with a perfect fit would fall

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

Conclusions

The kinetic of the sensory, microbial and bioactive quality changes of a purple smoothie, made of fresh fruits and vegetables, heat-treated or not during processing, was studied throughout storage at 5, 15 and 25 °C. A hierarchical clustering of sensory quality attributes showed that the overall acceptability was highly correlated with the flavour. The shelf-life of the smoothies was approximately increased by 37 (at 5 °C), 8 (at 15 °C) and 3 days (at 25 °C) in heat-treated samples compared to untreated fresh-blended ones. Such mild heat treatment did not alter the initial vitamin C and phenolic content of samples on processing day, while such nutritional quality attributes were better preserved during storage at low temperature. The latter antioxidant compounds were highly correlated ($R^2 = 0.94$) with the FRAP total antioxidant capacity method. The purple smoothie still presented high health-promoting compound contents after the storage periods, particularly ensuring a 250-g portion of the smoothie, the 40–60% of the recommended vitamin C daily intake. The obtained results will be of high interest for the food industry to predict/estimate the shelf-life of this kind of fruit and vegetable beverages being the produce logistics improved all over the chain and ensuring lower costs and better final quality of the product. The modelling methodology used in this study represents an added value coming from its proper use (validation, goodness of fit, hypotheses checking), which makes the results reliable enough to describe the process relationships and to make right decisions by the *R+D* departments of the food-related industries.

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