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Effect of Heat Treatment on Smoothie Quality by Response Surface Methodology †

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Abstract: Smoothies are a popular and convenient way for to consume bioactive compounds from fruits and vegetables such as total phenolics, carotenoids and flavonoids, with the preservation treatment being an important action to guarantee the safety and extension of shelf-life. The main goal of this study was to evaluate the impact of heat treatment (HT) on smoothie prepared with "Fuji "apple (41%), pineapple (31%), cabbage (8%), pumpkin (10%) and banana (10%), by response surface methodology (RSM), where the temperature (70–100 °C) and treatment time (0.5–10.5 min), were the dependent variables. After optimization of HT conditions, a validation assay was performed to guarantee the minimal changes on color and reduction of 90% of polyphenoloxidase enzyme (PPO). Antioxidant activity (Ferric reducing antioxidant power assay (FRAP), DPPH, ABTS), total phenolics content (TPC), pH and solids soluble content were also analyzed. Predicted models of color parameters (L*, a*, ºh) and PPO enzymatic activity were found to be significant ($p < 0.05$) with regression coefficients (R^2) of 0.84, 0.86, 0.92 and 0.97, respectively. From the RSM-generated model, the HT conditions that ensure a minimal green loss of smoothie and inactivation of PPO enzyme was at 85 °C over 7 min. In the validation study, these conditions were tested and proved to be sufficient to achieve the main goals. In the heat-treated smoothie, increases in TPC (10%) and antioxidant capacity (ABTS: 50%, DPPH: 17%, FRAP: 13%) were attained. This study demonstrated that RSM was efficient to select the optimal conditions of HT and improve the important quality properties that influence the product quality and the potential consumer's health (TPC and antioxidant capacity).

Keywords: fruits; vegetable; heat treatment; total phenolic content; RSM; antioxidant capacity

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1. Introduction

Smoothies are a popular and convenient way of consuming fruit and vegetables and are semiprocessed, not refined and obtained by mechanical treatment (or, less often, by thermal treatment) of fruit followed by preservation [1]. Different ingredients such as fruit, vegetable, juice, ice, yogurt and milk can be parts of product formulation [2]. As smoothies contain a mixture of intracellular contents from different fruit components, they may exhibit very different biochemical behaviors to those of their individual components. Products color, texture and flavor are the key factors influencing consumer acceptability [3]. The activity of the oxidative enzyme polyphenoloxidase (PPO, EC 1.14.18.1)

can lead to the degradation of polyphenol contents and could decrease the nutritional status of the product as a significant portion of the anti-inflammatory and health promoting properties are related to polyphenolic compounds [4,5]. Furthermore, PPO activity and polyphenol content play a synergistic role in the development of enzymatic browning in fruit, which leads to a perceived loss of quality. Additionally, the process of blending could introduce oxygen into the smoothie mixture, leading to the degradation of nonenzymatically degraded components [6].

Preservation technologies are necessary to minimize quality changes and extend the shelf-life of foods. The conventional treatment usually applied is heat treatment, which promotes enzymatic and microbial inactivation resulting in organoleptic and nutritional quality losses of the product. Additionally, color and flavor are two quality attributes that are negatively affected during heat treatment [7].

The main goal of this study was to optimize, by response surface methodology, the conditions of heat treatment that guarantee the reduction in PPO enzymatic activity, leading to minimal color changes and a bioactive composition.

2. Materials and Methods

2.1. Raw Materials and Smoothie Preparation

The fruits and vegetables used in the present study were obtained from a company on the west coast of Portugal, Campotec S.A., and were: apple (cv. Fuji), pineapple, cabbage (cv. *galega*), pumpkin (cv. *menina*) and banana. After arriving in the laboratory, the products were selected and stored at a refrigerated temperature (4 ± 1 °C) until processing.

Smoothie formulation was constituted by a mixture of apple (41%), pineapple (31%), cabbage (8%), pumpkin (10%) and banana (10%). Firstly, the products were washed in a conventional decontamination treatment with chlorinated water (HIPO, 150 ppm, 2 min at 5 °C) followed by washing tap water. Then, water excess was removed by absorbent paper and the fruits were peeled and sliced to appropriate dimensions for the next step. Apple slices were preheated by vapor (1.5 min) and cabbage and pumpkin were preheated by water immersion—100 °C/5 min and 90 °C during 6 min, respectively. After preheat treatment, the products were cooled in water/ice bath over 5 min and blended in a homogenizer (Robot Vorwerk, 9180 rpm) for 45 s. A mixture of 100 g of smoothie was transferred to laminated polyamide polyethylene bags (Eco-vac 40), vacuum sealed and heated in a water bath according to the description in Tables 1 and S1 (in Supplemental Materials). After heat treatment, the bags were removed from the bath and kept at a low temperature (3 °C) in a blast chiller temperature (SIMIL, Italy).

Table 1. Coded and decoded independent variables (temperature and time of treatment).

Coded Independent Variables		Decoded Independent Variables	
X1	**X2**	**Treatment (°C)**	**Time (min)**
−1.41421	−1.41421	70	0.5
−1	−1	75	2
0	0	85	5.5
1	1	95	9
1.41421	1.41421	100	10.5

2.2. Experimental Analysis and Validation of Optimized Condition of Heat Treatment

For optimization of heat treatment conditions (time and temperature), a central composite rotatable design (CCRD) was used as described in [8]. The range of interest of each independent variable was 0.5–10.5 min for treatment time (t) and 70–100 °C for treatment temperature (T). Additionally, color and PPO enzymatic activity were the dependent variables taken into account for quality optimization of the smoothie. In the validation study

of heat treatment, two smoothie samples were considered: heat-treated under the optimized conditions and untreated smoothie. As previously carried out, after treatment, smoothie samples were placed in a chiller temperature to quickly reduce temperature.

2.3. Physical-Chemical Analysis

2.3.1. Color, pH and Solid Soluble Contents

Color analysis was evaluated using a tristimulus colorimeter (Minolta chroma Meter, CR-300, Osaka, Japan), measuring the CIEL*a*b* parameters as described in [8]. From the CIELab coordinates, hue (ºh) and total color difference ($\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{0.5}$ were calculated as described in [9,10], respectively. Sixteen measurements were determined per treatment condition. Soluble solid content (SSC) and pH were determined in refractometer (DR-A1, ATAGO Co Ltd., Tokyo, Japan) and pH meter (SP70P, SympHony, Radnor, PA, USA), respectively. Two independent measures were taken per sample replicate.

2.3.2. Polyphenoloxidase (PPO) Enzymatic Activity

Enzyme extraction: Smoothie sample was homogenized with 0.1 M sodium phosphate buffer at pH 6.5 (1:3; *w*/*v*), 5% PVPP (*w*/*w*) and 5 µL Triton X-100, using a homogenizer (Grindomix GM200, Retsch GmbH&Co.KG, Germany) for 1 min. Homogenates were centrifuged at 8000 rpm over 20 min (4K15 Sigma Laboratory Centrifuges, rotor 11,150) and the supernatant was collected, filtered and used as crude extract. PPO enzymatic activity was assayed as described by [11] with some modifications. The increase rate of absorbance at 420 nm for 1 min was recorded using an ATI Unicam UV/Vis 4 spectrophotometer. The assay cuvette (3 mL) contained the substrate solution (110 mM of catechol prepared in 0.1 M sodium phosphate buffer at pH 6.5) and a given quantity of crude enzyme extract. The linear part of the curve absorbance/time was used to estimate the enzyme activity. One unit is defined as the change in 0.001 unit of absorbance per gram of smoothie. Two independent measures were taken per sample replicate.

2.3.3. Antioxidant Capacity and Total Phenolic Content

Extraction: Smoothie extract was prepared in a ratio of 1:10 (m:v) of sample and methanol, following the homogenization in a Ultra-Turrax homogenizer (IKA LABORTECHNIK T25 basic, Janke & Kunkel GmbH&Co., Breisgau, Germany) at 8000 rpm for 2 min and incubated at 4 °C overnight. After, the extracts were centrifuged (HERMLE Z383K LABORTECHNIK, Germany) at 8000 rpm for 20 min (4 °C), and the supernatants were stored at 4 °C until analysis. DPPH scavenging activity assay was evaluated according to modified methodology of [12], as described in [13]. 2,20-Azino-bis (3-ethylbenzothiazoline6-sulphonic acid (ABTS) was determined following the modified methodology of [14,15], as shown in [13]. Ferric reducing antioxidant power assay (FRAP) was analyzed as [16], with some changes as observed in [13]. For expression of antioxidant capacity of smoothie samples, Trolox was used as standard for calibration curve and data were expressed as Trolox equivalent antioxidant capacity (TEAC; µmol Trolox per 100 g). Total phenolic content was determined according to modified methodology [17], as described in [13]. The obtained data were the average of three replicates and were expressed as mg GAE per 100 g. Two independent measures were taken per sample replicate.

2.4. Statistical Analysis

2.4.1. Model Fitting and Statistical Analysis

The obtained results were fitted to a second-order polynomial equation (Equation (1)) for each dependent variable (color and PPO enzymatic activity) as a function of independent variables X_j (T, t) by a stepwise multiple regression analysis, as detailed in [8].

$$Y = b_0 + \sum_{j=1}^{3} b_j X_j + \sum_{i<j}^{3} b_{ij} X_i X_j + \sum_{j=1}^{3} b_{jj} X_j^2 \quad (1)$$

Y—Predicted response;
Xj—independent variable;
b_0—intercept coefficient;
b_j—linear terms;
b_{jj}—squared terms;
b_{ij}—interaction terms.

where Y is the predicted response, X_j is the independent variable, b_0 is the intercept coefficient, b_j represents the linear terms, b_{jj} represents the squared terms and b_{ij} represents the interaction terms.

2.4.2. Quality Evaluation of Untreated and Heat-Treated Smoothies

The data obtained in validation study of heat treatment, as mean and standard deviation (SD), were subjected to analysis of variance at $p < 0.05$, with mean separation by Tukey's Honestly Significant Difference (HSD) test in order to analyze the effect of heat treatment on smoothie quality.

3. Results and Discussion

3.1. Model Fitting

The mathematical models for all attributes studied were developed by response surface methodology (RSM) and their adequacy was tested by the (analysis of variance ANOVA) technique. P-values were used as a tool to assess the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. For any of the terms in the model, a large regression coefficient and a small *P*-value would indicate a more significant effect on the respective response variables. The ANOVA analyses of L*, a* and hue color parameters and PPO enzymatic activity are shown in Table S2. The models equations (Equations (2)–(4)) resulted from the RSM and the corresponding correlation coefficient (R^2 and R^2_{adj}) are summarized at Table 2. Both values of R^2 and R^2_{adj} indicated the variation in color changes and inactivation of PPO activity explained by the models. The obtained results showed that the second-order polynomial model adequately represented the experimental data with values of R^2 and R^2_{aj} of 0.84, 0.86, 0.92 and 0.97 and 0.77, 0.79, 0.87 and 0.96 for L*, a*, hue and PPO, respectively.

Table 2. Model equations of L*, a* and hue color parameter and polyphenoloxidase enzyme (PPO) enzymatic activity with respective regression coefficient.

Eq.	Parameter	Model Equations	R^2	R^2_{adj}
(1)	**PPO**	**PPO** = 414.70 − 8.42 x **T** + 0.05 x **T²** − 1.22 x **t** + 0.55 x **t²** − 0.09 x **T** x **t**	0.97	0.96
(2)	**L***	**L*** = −12.52 + 1.57 x **T** − 0.010 x **T²** − 2.58 x **t** + 0.029 x **T** x **t**	0.84	0.77
(3)	**a***	**a*** = −28.56 + 0.18 x **T** + 1.89 x t − 0.021 x **t²** − 0.013 x **T** x **t**	0.86	0.79
(4)	**hue**	^{o}h = 166.43 − 0.99 x **T** + 0.004 x **T²** − 2.71 x **t** + 0.091 x **t²** + 0.01 x **T** x **t**	0.92	0.87

Eq.—equation; T—temperature (°C); t—time (min).

3.2. Response Surface Analysis

Figure 1A shows the effects of temperature (T) and time (t) of heat treatment on color (L* and a* color value) and PPO enzymatic activity (C) of smoothies, respectively. The highest values of luminosity were obtained after treatments at temperature range of 75–85 °C over a period of less than 6 min. A visual assessment of heat-treated smoothies confirmed the darkness as a consequence of heat treatment intensity. Usually, increased color degradation is associated with thermal processing enhancing the formation of degradation products affecting the color perception. The a* color parameter was significantly affected ($p < 0.05$) by increases in temperature and time treatment, leading to the highest a* value, which reflects the loss of the green color (Figure 1B). PPO enzymatic activity was significantly ($p < 0.05$) reduced by exposure of the smoothie to heat treatment (Figure 1C). The quadratic effect of temperature and time contributed to a significant effect ($p < 0.05$)

on this enzymatic activity. The temperature and the time between 75 and 90 °C and 5 and 10 min led to the reduction in PPO enzymatic activity, an important enzyme that contributes to enzymatic browning by oxidation of phenolic compounds [18].

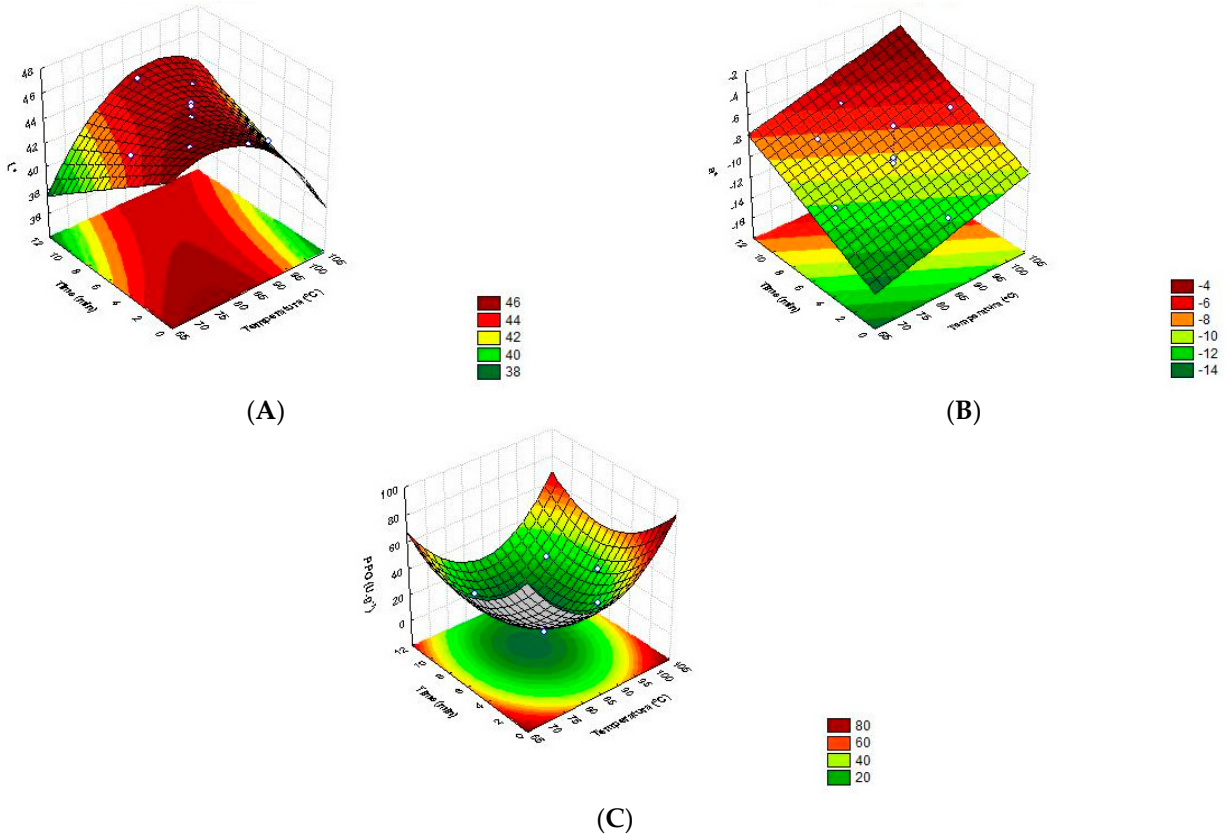

Figure 1. Response surface plots reflecting the effects of temperature (T, °C) and time treatment (t, min) on L* (**A**), a* (**B**) color parameters and PPO enzymatic activity (**C**) of smoothie.

3.3. Validation Study of Optimzed Heat Treatment

The optimum heat treatment conditions applied to the smoothie should lead to inactivation of 90% of PPO enzymatic activity and minimal changes in color and bioative composition of the product. Regarding RSM analysis, the selected optimum condition of heat treatment was 85 °C for 7 min. As observed in Table 3, the heat-treated smoothie denoted a reduction in PPO enzymatic activity (90%), an important achievement since this enzyme is responsible for browning of the product. Additionally, after heat treatment, a significantly ($p < 0.05$) enhanced bioactive component was achieved in all the realized methodologies (FRAP, DPPH and ABTS).

Table 3. Physical-chemical characterization of untreated and heat-treated smoothies (average ± standard deviation).

Quality Parameter	Untreated	Heat-Treated
CIE Lab		
L*	42.14 ± 0.35 [a]	43.94 ± 0.60 [b]
a*	−16.14 ± 0.49 [a]	−7.73 ± 0.42 [b]
b*	29.95 ± 1.14 [a]	29.51 ± 0.59 [a]
hue	118.33 ± 0.30 [a]	104.67 ± 0.60 [b]
Antioxidant capacity (μmol Trolox.100g $^{-1}$)		
FRAP	5230.49 ± 177.10 [a]	5911.44 ± 216.81 [b]
DPPH	6321.29 ± 441.15 [a]	7443.79 ± 448.85 [b]
ABTS	1564.32 ± 183.00 [a]	2350.56 ± 82.07 [b]

Total phenolic content (mg GAE.100g $^{-1}$)	77.68 ± 2.05 ^{a}	85.34 ± 4.51 ^{b}
PPO activity (U.g $^{-1}$)	28.12 ± 2.66 ^{a}	2.46 ± 0.96 ^{b}
pH	3.57 ± 0.01 ^{a}	3.57 ± 0.01 ^{a}
Solids soluble content (°Brix)	10.51 ± 0.06 ^{a}	10.61 ± 0.06 ^{b}

Different subscript letters in the same line represent significant differences ($p < 0.05$, Tukey test).

4. Conclusions

Smoothies are a mixture of fruits and vegetables offering the consumer essential nutrients and bioactive compounds leading to health benefits. So, the maintenance of their quality is of interest for all stakeholders of the food chain. The optimum heat treatment condition at 85 °C for 7 min was attained by response surface methodology and validated to guarantee the reduction in PPO enzymatic activity (90%), minimal color alteration and augmented antioxidant capacity. Therefore, this study helps elevate the potential of fruit and vegetable consumption through food development with remarkable bioactive compounds, which can be positive for maintenance of smoothie quality during refrigerated storage.

Supplementary Materials: The following are available online at https://www.mdpi.com/2504-3900/70/1/6/s1, Table S1: Codex and decodex matrix of independent variables, Table S2: Analysis of variance of the second-order polynomial model for color parameters (L*, a* and hue) and PPO enzymatic activity of heat-treated smoothie.

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Conflicts of Interest: The authors declare no conflict of interest.

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