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

Joaquina Pinheiro[1]*, Diana I. Santos[2], Elsa M. Gonçalves[3, 4], Marta Abreu[2,3] and Margarida Moldão-Martins[2]

[1] MARE—Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, 2520-641 Peniche, Portugal; joaquina.pinheiro@ipleiria.pt or joaquina.pinheiro@gmail.com (J.P.)
[2] LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia Universidade de Lisboa, 1349-017 Lisboa, Portugal; dianaisas@gmail.com or dianaisasantos@isa.ulisboa.pt (D.I.S.); marta.abreu@iniav.pt (M.A.); mmoldao@isa.utl.pt (M.M.-M.)
[3] UTI—Unidade de Tecnologia e Inovação, Instituto Nacional de Investigação Agrária e Veterinária, Avenida da República, Quinta do Marquês, 2780-157 Oeiras, Portugal; goncalves.melsamail.com or elsa.goncalves@iniav.pt (E.M.G.)
[4] GeoBiotec—Geobiociências, Geoengenharias e Geotecnologias, – Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa Campus de Caparica, 2829-516 Caparica, Portugal
* Correspondence: joaquina.pinheiro@ipleiria.pt or joaquina.pinheiro@gmail.com (J.P.)
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Abstract: Smoothies are a popular and convenient way for bioactive compounds consumption from fruits and vegetables such as, total phenolics, carotenoids and flavonoids, being the preservation treatment 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 colour and reduction of 90% of polyphenoloxidase enzyme (PPO). Antioxidant activity (FRAP, DPPH, ABTS), total phenolics content (TPC), pH and solids soluble content, were also analyzed. Predicted models of colour 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 ensures a minimal green loss of smoothie and inactivation of PPO enzyme was at 85 °C during 7 min. In the validation study, these conditions were tested and proved to be sufficient to achieve the main goals. In heat-treated smoothie an increase of TPC (10%) and antioxidant capacity (ABTS: 50%, DPPH: 17%, FRAP: 13%) was attained. This study demonstrated that RSM was efficient to select the optimal conditions of HT and improvement the important quality properties that influences the product quality and the potential consumer health (TPC and antioxidant capacity).

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

1. Introduction

Smoothies are a popular and convenient way of fruit and vegetables consuming and are defined as semi-processed, not refined, obtained by mechanical treatment (or, less often, by thermal treatment) of fruit followed by their preservation [1]. Different ingredients like fruit, vegetable, juice,

ice, yogurt, and milk can be part in 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 colour, texture and flavour are the key factors influencing the consumer acceptability [3]. The oxidative enzyme polyphenoloxidase (PPO, EC 1.14.18.1) activity leading to the degradation of polyphenols content and could decrease the nutritional status of the product as a significant portion of the anti-inflammatory and health promoting properties attributes 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 non-enzymatically 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 the enzymatic and microbial inactivation resulting in to organoleptic and nutritional quality losses of product. Also, colour and flavour 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 colour changes and 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.: apple (cv. Fuji), pineapple, cabbage (cv. *galega*), pumpkin (cv. *menina*) and banana. After arrived in the laboratory, the products were selected and stored at 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, sliced to appropriate dimensions to the next step. Apple slices were pre-heated by vapour (1.5 min), cabbage and pumpkin by water immersion, 100 °C/5 min and 90 °C during 6 min, respectively. After pre-heat treatment, the products were cooled in water/ice bath during 5 min and blended in a homogeniser (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 description in Table 1 and Table S1 (in supplemental material). After heat treatment, the bags were removed from bath and kept at low temperature (3 °C) in a blast chiller temperature (SIMIL, Italy).

Table 1. Coded and decoded of 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 were 0.5–10.5 min for treatment time (t) and 70–100 °C for treatment temperature (T). Also,

colour and PPO enzymatic activity were the dependent variable taken into account for quality optimization of smoothie. In the validation study of heat treatment, two smoothie samples was considered: heat-treated under the optimized conditions and untreated smoothie. As previously proceed, after treatment, smoothie samples was placed in a chiller temperature to quickly reduce temperature.

2.3. Physical-Chemical Analysis

2.3.1. Colour, pH and Solids Soluble Content

Colour 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 (^{o}h) and total colour difference ($\Delta E = ((\Delta L)^2+(\Delta a)^2+(\Delta b)^2)^{0.5}$ were calculated as described in [9] and [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 during 20 min (4K15 Sigma Laboratory Centrifuges, rotor 11,150) and the supernatant collected, filtered and used as crude extract. PPO enzymatic activity was assayed as described by [11] with some modifications. The increase rate in 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 homogeneization in a Ultra-Turrax homogenizer (IKA LABORTECHNIK T25 basic, Janke & Kunkel GmbH &Co., Germany) at 8000 rpm for 2 min and incubated at 4 °C overnight. After, ehe 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]. ABTS (2,20 -azino-bis(3-ethylbenzothiazoline6-sulphonic acid) was determined following the modified methodology of [14,15] as shown in [13]. Ferric reducing antioxidant power assay (FRAP) was analysed as [16], with some changes as observed in [13]. For expression of antioxidant capacity of smoothie samples, was used the Trolox as standard for calibration curve and data was expressed as Trolox equivalent antioxidant capacity (TEAC; µmol Trolox per 100 grams). Total phenolic content was determined according to modified methodology [17] as described in [13]. The obtained data resulted of average of three replicates and was expressed as mg GAE per 100 grams. 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 second –order polynomial equation (Equation 1) for each dependent variable (colour and PPO enzymatic activity) as a function of independent variables X_j (T, t) by a stepwise multiple regression analysis, as detailed in [8].

$$Y = b_0 \cdot \underbrace{\sum^{2}}_{j=1} {}_{j}X_j \quad \underbrace{\sum^{2}}_{i<j} {}_{ij}X_i X_j \quad \underbrace{\sum^{2}}_{j=1} {}_{jj}X_j^2 \qquad \text{(Equation 1)}$$

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 is the linear terms, bjj is the squared terms, and bij is the interaction terms.

2.4.2. Quality evaluation of untreated and heat-treated smoothie

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 have been developed by response surface methodology (RSM) and its adequacy was tested by analysis of variance technique (ANOVA). P-values were used as a tool to check 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 analysis of L*, a* and hue colour parameters, and PPO enzymatic activity are shown in Table S2. The models equations (Eq. 2–4) resulted from 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 colour 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, 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 colour parameter and PPO enzymatic activity with respective regression coefficient.

Eq.	Parameter	Model Equations	R^2	R^2_{adj}
(1)	**PPO**	**PPO** = 414.70-8.42***T**+0.05***T²**-1.22***t**+0.55***t²**-0.09***T***t**	0.97	0.96
(2)	**L***	**L*** = -12.52+1.57***T**-0.010***T²**-2.58***t**+0.029***T***t**	0.84	0,77
(3)	**a***	**a*** = -28.56+0.18***T**+1.89*t-0.021***t²**-0.013***T***t**	0.86	0.79
(4)	**hue**	^{o}h = 166.43-0.99***T**+0.004***T²**-2.71***t**+0.091***t²**+0.01***T***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 colour (L* and a* colour value) and PPO enzymatic activity (C) of smoothie, respectively. The highest values of luminosity were obtained after treatments at temperature range of 75–85 °C during period less than 6 min. Visual assessment of heat-treated smoothies confirm the darkness as consequence of heat treatment intensity. Usually, increased colour degradation is associated with thermal processing as enhancing the formation of degradation products affecting the colour perception. The a* colour

parameter was significantly affected ($p < 0.05$) with increase of temperature and time treatment, leading to highest a* value, which reflects the loss of green colour (Figure 1B). PPO enzymatic activity was significant ($p < 0.05$) reduced by exposure of 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 75–90 °C and 5–10 min led to the reduction of PPO enzymatic activity, an important enzyme that contributes to the enzymatic browning by oxidation of phenolic compounds [18].

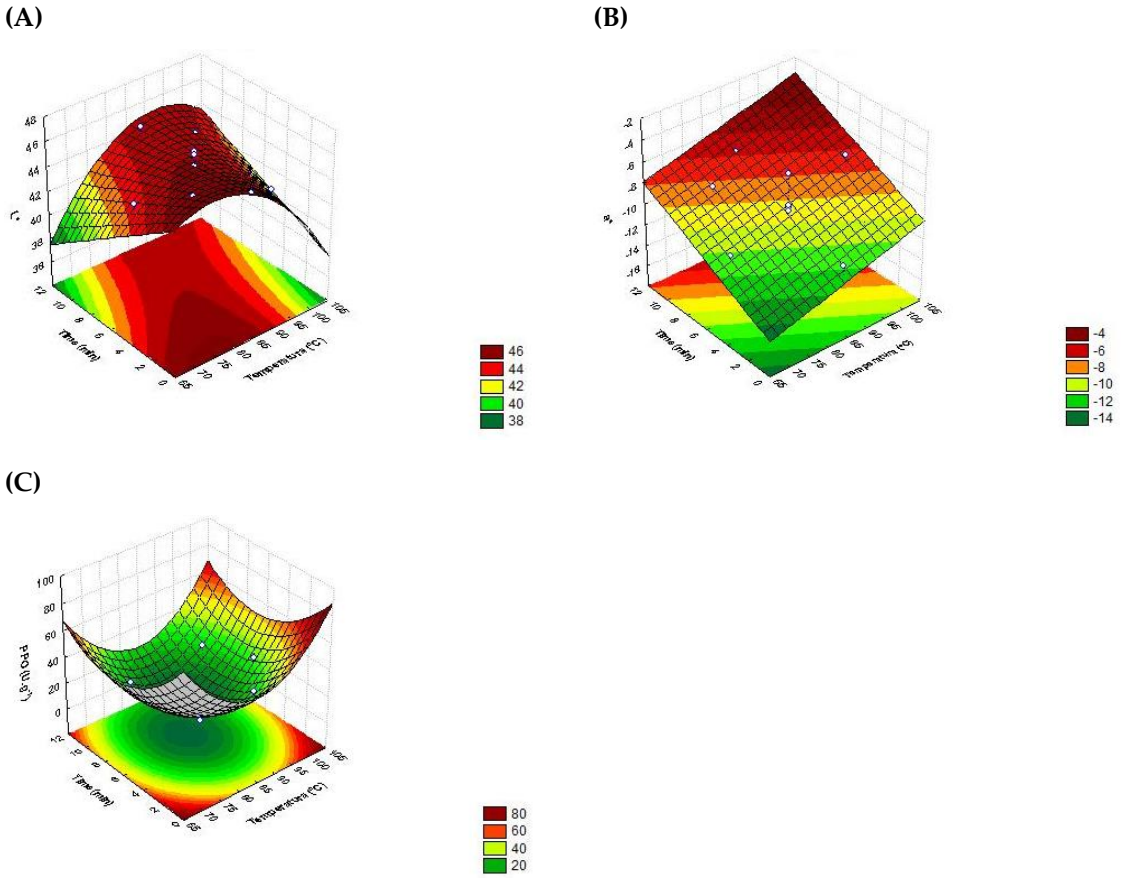

Figure 1. Response surface plots reflecting the effects of temperature (T, °C) and time treatment (t, min) on L* (A), a* (B) colour parameters and PPO enzymatic activity (C) of smoothie.

3.3. Validation Study of Optimzed Heat Treatment

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

Table 3. Physical-chemical characterization of untreated and heat-treated smoothie (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]
^{o}h	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 letters in the same line represent significant differences ($p < 0.05$, Tukey test).

4. Conclusions

Smoothie is a mixture of fruits and vegetables offering to consumer the 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 of PPO enzymatic activity (90%), minimal colour alteration and augmented of antioxidant capacity. Therefore, this study contributed to elevate the potential of fruits and vegetables consumption by food development with remarkable bioactive compounds, which can be positive for maintenance of smoothie quality during refrigerated storage.

Supplementary Materials: Table S1: Codex and decodex matrix of independent variables, Table S2 – Analysis of variance of the second order polynomial model for colour parameters (L*, a* and ^{o}h) and PPO enzymatic activity of heat-treated smoothie.

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