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Optimization of Osmotic Dehydration of Chestnut (*Castanea sativa* Mill.) Slices Using Response Surface Methodology

Teresa Delgado[a, b], Bruna Paim[c], José Alberto Pereira[a], Susana Casal[b], and Elsa Ramalhosa[a*]

[a] CIMO - Mountain Research Center, School of Agriculture, Polytechnic Institute of Bragança, Campus de Stª Apolónia, 5300-253 Bragança, Portugal
[b] LAQV@REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia da Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal
[c] Instituto Federal de Educação, Ciência e Tecnologia Farroupilha Alegrete, Campus Alegrete, Alegrete, Brasil
*Corresponding author
elsa@ipb.pt
Tel: +351-273303308
Fax: +351-273325405

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Abstract

Osmotic dehydration of chestnut slices in sucrose was optimized for the first time by Response Surface Methodology (RSM). Experiments were planned according to a three-factor central composite design (α=1.68), studying the influence of sucrose concentration, temperature and time, on the following parameters: volume ratio, water activity, color variation, weight reduction, solids gain, water loss and normalized moisture content, as well as total moisture, ash and fat contents.
The experimental data was adequately fitted into second-order polynomial models with coefficients of determination (R^2) from 0.716 to 0.976, adjusted-R^2 values from 0.460 to 0.954, and non-significant lacks of fit. The optimal osmotic dehydration process conditions for maximum water loss and minimum solids gain and color variation were determined by the "Response Optimizer" option: 83% sucrose concentration, 20 °C and 9.2 hours. Thus, the best operational conditions corresponded to high sugar concentration and low temperature, improving energy saving and decreasing the process costs.

Keywords: Chestnut; *Castanea sativa* Miller; Osmotic Dehydration; Response Surface Methodology; Physicochemical properties

1 Introduction

Chestnut production is of great economic importance for some countries. In 2012, the main world producer was China, representing about 82.5% of the total production, followed by Europe, with 6.4% (Food and Agriculture Organization of the United Nations, 2014). Portugal accounts for about 15% of the European production (Food and Agriculture Organization of the United Nations, 2014), with the North area, mainly Trás-os-Montes region, contributing to 80.5% of the national production and representing 87.4% of chestnut national production area (30586 ha) (Instituto Nacional de Estatística, 2014).
Being a seasonal product, some problems may arise during chestnuts' storage, compromising its availability and quality throughout the year. One way to mitigate this problem is to use different post-harvest technologies such as low temperature storage or convection drying. However, a promising technology to preserve perishable

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Nomenclature

RSM	Response Surface Methodology
OD	Osmotic dehydration
WR	Weight Reduction (g g^{-1} fresh chestnut)
SG	Solids Gain (g g^{-1} fresh chestnut)
WL	Water Loss (g g^{-1} fresh chestnut)
NMC	Normalized Moisture Content
a_w	Water activity
M_0	Sample mass before osmotic dehydration (g)
M	Sample mass after osmotic dehydration (g)
m_0	Initial mass of the solids in chestnut sample (g)
m	Mass of the solids in chestnut sample after osmotic dehydration (g)
W, L, t	Axial dimensions (Width, Length and thickness, respectively (mm))
L^*, a^*, b^*	Color parameters (CIELab)
X	Moisture ratio
CCD	Central Composite Design

items and make them available to distant regions of a production area throughout the year is osmotic dehydration (OD) which is a simple and a low cost method (Rastogi, Raghavarao, Niranjan, & Knorr, 2002; Shi & Le Maguer, 2002). Furthermore, with this OD technology an interesting chestnut snack may be produced. As chestnut is naturally gluten-free, snacks of this nut may be a good option for celiac patients.

Osmotic dehydration occurs by immersion of the food in osmotic solutions. During this process, the cellular structure of the food allows water loss, while a gain of solute occurs simultaneously (Rastogi, Raghavarao, & Niranjan, 1997). Both mass flows are affected by diverse factors, including the nature of the food and its geometry, the composition and concentration of the osmotic solution, and several methodological parameters such as temperature, contact time and agitation (Kaymak-Ertekin & Sultanoglu, 2000; Singh, Kumar, & Gupta, 2007; Tonon, Baroni, & Hubinger, 2007).

Diverse statistical and mathematical techniques have been applied to optimize and improve the development of these processes, combining and analyzing the role of different factors such as temperature, solute concentration and time, while minimizing analyses' error and the amount of necessary experiments. Response Surface Methodology (RSM) is one of these techniques, aiming to optimize response-variables of interest by studying the influence of a defined number of independent variables. Besides having the advantage of analyzing the effects of independent variables, this methodology generates a mathematical model that describes the chemical or biochemical processes under study (Anjum, Tasadduq, & AlSultan, 1997). In particular, RSM has been applied to osmotic dehydration studies of some fruits and vegetables, including apples (Azarpazhooh & Ramaswamy, 2012), bananas (Atares, Gallagher, & Oliveira, 2011), carrots (Changrue, Orsat, Raghavan, & Lyew, 2008), cherry tomatoes (Derossi, Severini, Del Mastro, & De Pilli, 2015), figs (Vasconcelos, Andrade, Maciel, Guerra, & Vasconcelos, 2012), green peppers (Ozdemir, Ozen, Dock, & Floros, 2008), kiwi (Cao, Zhang, Mujumdar, Du, & Sun, 2006), peaches (Yadav, Yadav, & Jatain, 2012), plums (Koocheki & Azarpazhooh, 2010) and strawberries (Changrue et al., 2008). Generally, three factors are studied, namely, temperature, time and concentration of the osmotic solution (Azarpazhooh & Ramaswamy, 2012; Cao et al., 2006; Changrue et al., 2008; Koocheki & Azarpazhooh, 2010; Vasconcelos et al., 2012; Yadav et al., 2012).

Regarding chestnut, most studies of OD have

been focused on whole fruits of Spanish chestnut varieties, and primarily on diverse osmotic agents and temperatures (Chenlo, Moreira, Fernández-Herrero, & Vázquez, 2006b, 2006a, 2007; Moreira, Chenlo, Chaguri, & Oliveira, 2007; Moreira, Chenlo, Chaguri, & Fernandes, 2008; Moreira, Chenlo, Chaguri, & Vazquez, 2011; Moreira, Chenlo, Chaguri, & Mayor, 2011). The osmotic agents studied included sodium chloride (17 to 26.5%), glucose and sucrose (40 to 60%) at different concentrations and submitted to several temperatures in the range of 25 and 65 °C.
Nevertheless, none of these studies performed the optimization of the OD process taking into account several factors and responses simultaneously. Thus, the aims of our work were: i) to evaluate by RSM the role of the three main parameters, temperature, time and concentration of the osmotic solution (sucrose), in affecting some physicochemical properties of chestnut slices; and ii) to optimize these parameters for the industrial production of an interesting chestnut based snack in the near future. Sucrose was the first osmotic agent to be tested by RSM because it is more common to use this compound in OD processes of fruits than sodium chloride that can induce high blood pressure (Appel et al., 2012). Furthermore, sucrose is also cheaper than glucose.

2 Materials and Methods

2.1 Plant material

Castanea sativa Miller (European chestnut) fruits, variety Longal, were acquired in Bragança (NE Portugal) in November 2013, and stored in cold chambers (4±1 °C) until the osmotic dehydration experiments were performed. Before doing these experiments, chestnuts were carefully unshelled and sliced (approximately 4-6 mm of thickness).

2.2 Osmotic Dehydration (OD)

The osmotic solutions were prepared with food-grade sucrose and potable water. The OD experiments were carried out in 1L beakers. For each condition, 70 g of fresh sliced chestnuts were added to 700 mL of sugar solution and mixed with a magnetic stirrer at 310 rpm in a temperature controlled water bath. At specific times, the dehydrated chestnut slices were removed from the solution, drained, and gently cleaned with absorbent paper to remove any sugar solution in excess. For each condition, the assays were performed in duplicate.
In order to adequately follow the OD kinetics, several parameters were analyzed, namely weight reduction (WR), solids gain (SG), water loss (WL) and normalized moisture content (NMC). These were determined according to the following equations (Eq. 1 to 4) (Lerici, Pinnavaia, Rosa, & Bartolucci, 1985):

$$WR = \frac{M_0 - M}{M_0} \tag{1}$$

$$SG = \frac{m - m_0}{M_0} \tag{2}$$

$$WL = WR + SG \tag{3}$$

$$NMC = \frac{1 - \frac{m}{M}}{1 - \frac{m_0}{M_0}} = \frac{X}{X_0} \tag{4}$$

where M_0 and M represents the total mass of sample before and after OD, respectively; m_0 and m are the mass of the solids before and after OD, respectively; and X_0 and X correspond to the moisture contents of the samples before and after the OD treatment, respectively.

2.3 Physicochemical characterization

Volume

The three axial dimensions (Width, W; Length, L; and thickness, h) of all chestnut slices were measured using a digital caliper, before and after the OD experiments. Volume was calculated by the following equation:

$$V = Area\ of\ the\ elipse \times h = \pi \times \frac{W}{2} \times \frac{L}{2} \times h \tag{5}$$

The volume was calculated by considering the dimensions before (V_0) and after (V) OD, enabling the calculation of the volume ratio ($\frac{V}{V_0}$).

Color

Color analyses were carried out on chestnut slices before and after being subjected to OD. A Minolta CR-400 colorimeter was used, in CIE*Lab* color space, through the coordinates *L**, *a** and *b**, using the Spectra Magic Nx software (version CM-S100W 2.03.0006, Konica Minolta Company, Osaka, Japan), as already described in previous work (Delgado, Pereira, Baptista, Casal, & Ramalhosa, 2014).
In order to analyze the color changes due to the OD process, the total color difference (ΔE^*) was calculated according to:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (6)$$

All color determinations were made on 20 slices, before (the color of fresh chestnuts was considered as reference) and after the OD process, each time in duplicate.

2.4 Water activity (a_w), moisture, ash and crude fat contents

Water activity was determined by means of a LabSwift- a_w instrument (Novasina AG, Lachen, Switzerland). The instrument was calibrated with three water activity standards, namely 11% (a_w =0.112), 58% (a_w =0.587) and 84% (a_w =0.845).
Moisture, ash and crude fat contents were determined using standard procedures (Association of Official Analytical Chemists, 1995) in duplicate on samples of each osmotic dehydration assays (n=4). All reagents were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Crude fat was determined on 5 g of sample, using petroleum ether for 24 h in a Soxhlet apparatus (P Selecta, Abrera, Barcelona). Moisture was determined on 5 g of sample at 105 °C in an oven (Memmert UNB 500, Schwabach, Germany), until constant weight, while total ash was obtained by incineration at 550 °C (Lenton Thermal Designs Ltd, Hope Valley, United Kingdom). The ash and crude fat contents were expressed on g 100 g^{-1} dry matter.

2.5 Experimental design and statistical analysis

In order to determine the effect of selected operational parameters in the above mentioned chestnut properties, as well as to establish the best conditions to perform OD of chestnut slices, the Response Surface Methodology (RSM) was used through Minitab® software (USA). A one block with an α-value equal to 1.68 and a central composite design (CCD) was constructed to investigate the influence of the following three independent factors: sucrose concentration, temperature and time. The response variables were a_w, $\frac{V}{V_0}$, ΔE^*, *WR*, *SG*, *WL*, *NMC*, moisture, ash and crude fat contents. Each variable was coded at five levels: -1.68, -1, 0, +1 and +1.68. The correspondence between coded and uncoded variables is indicated in Table 1. Each point of the CCD was carried out in duplicate.
The relationship found between the dependent variables (a_w, $\frac{V}{V_0}$, ΔE^*, *WR*, *SG*, *WL*, *NMC*, moisture, total ash and crude fat contents) and the operational variables was established by the following second order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i>j}^{k} \beta_{ij} X_i X_j \quad (7)$$

where Y is the predicted dependent variable; β_0 is a constant that fixes the response at the central point of the experiment (constant); β_i are the regression coefficients for the linear effect terms; β_{ij} are the quadratic effect terms; β_{ii} are the interaction effect terms of variables i and j; X_i and X_j are independent variables (X_1 – sucrose concentration; X_2 – temperature; X_3 – time); and k the total number of independent factors (k=3).
Twenty experiments, with six replications in the central point (Experiments 1, 5, 14, 15, 19 and 20), were performed (Table 2). In order to limit the influence of systematic errors, the sequence of the experiments was randomized. The experiments performed in the central point allowed an estimate of experimental error, whereas the other experiments allowed the calculation of the regression coefficients of the model. The adequacy of the models was assessed through the coefficient of determination (R^2), the adjusted-R^2 (adj-R^2) and the analysis of variance (ANOVA).

Table 1: Independent variables and their coded and uncoded values for optimization

Coded value	Sucrose concentration (%, w/v) X_1	Temperature (°C) X_2	Time (h) X_3
-1.68	53	20	0.8
-1	60	30	2.5
0	70	45	5.0
1	80	60	7.5
1.68	87	70	9.2

Furthermore, the lack of fit of the models was used to check the quality of second-order polynomial models. If the p-value of the lack of fit is less than 0.05, evidence exists that the model does not accurately fit the data.
In order to obtain useful information about the behavior of the system within the experimental design, response surface plots were generated for different interactions of any two independent variables, while holding the value of a third variable constant. Furthermore, at the end an optimization of the osmotic dehydration process was performed by using the "Response Optimizer" option of Minitab® software, in order to define the levels of the independent variables that would give maximum water loss and the lowest solids gain and ΔE^* (these response-variables are very important to achieve a product that will be well accepted by consumers). The optimization procedure picks several starting points from which to begin searching for the optimal factor settings, being displayed as the global solution, which corresponds to the "best" combination of factor settings for achieving the desired responses. In more detail, the optimization is accomplished by:

- obtaining the individual desirability for each response. The individual desirability will be closer to one, if the response is closer to the defined target (in the present work, our goal was to maximize water loss and minimize solids gain and ΔE^*);

- combining the individual desirabilities to obtain the combined or composite desirability. This measure is the weighted geometric mean of the individual desirabilities for the responses. In the present work, all individual desirabilities were equally important, so they had the same weight. In the present work the weight used was 1 (we placed equal emphasis on the target and the bounds). The composite desirability has a range of zero to one. One represents the ideal case, while zero indicates that one or more responses are not inside their acceptable limits;

- at the end, Minitab employs a reduced gradient algorithm with multiple starting points that maximizes the composite desirability to determine the numerical optimal solution. At the end, the optimal input variable settings were tested to confirm if the optimal response was observed.

3 Results and Discussion

The coefficients of the second-order response surface models relating response variables with sugar concentration, temperature and time are described in Table 3. For the volume ratio and a_w, the model results are not shown because the p-value of the lack of fit was lower than 0.05 (0.001 and 0.003, respectively), suggesting that the models developed did not represent accurately the observed results. A good fit between the experimental data and the predicted values by the model is obtained when high R^2 and adj-R^2 (near 1) are achieved together with a p-value for the lack of fit higher than 0.05, indicating that the variation between samples was only due to the factors selected for the model and

the pure error (Minitab® software). Nevertheless, $\frac{V}{V_0}$ varied between 0.841 and 1.39 (Table 2) showing that in some situations chestnuts (slices) submitted to OD may shrink or increase volume due to solution absorption. Concerning a_w (Table 2), this parameter varied between 0.849 and 0.935, a range that is normally encountered for dried foods or with high concentrations of solutes (Pereda et al., 2005; Moreira, Chenlo, Torres, & Vázquez, 2007).

3.1 Color variation (ΔE^*)

Values of color variation predicted by the mathematical model were similar to the experimental data (Table 2), yielding a good fit with a R^2 of 0.976 and an adj-R^2 of 0.954 (Table 3), meaning that the experimental data may be predicted with great accuracy. Moreover, the model was good because the lack of fit was non-significant (p=0.717). Concerning the linear model coefficients, temperature and time were found to be significant model terms on color variation of chestnut slices, whereas the sucrose concentration was not a significant model term. This can be a direct consequence of Maillard reactions taking place when temperature and time increase. Regarding the quadratic terms, the temperature was the only parameter that had a significant effect ($p<0.05$). The results also showed that the interaction between temperature and time was significant for ΔE^*, yielding the following recalculated model taking into account only the significant terms:

$$\Delta E^* = 10.2 + 4.49X_2 + 2.02X_3 + 1.27X_2^2 + 0.866X_2X_3 \quad (8)$$

Furthermore, temperature had a higher effect on ΔE^* than time due to its higher linear coefficient (4.49 *versus* 2.02). In fact, as shown in Table 2, the maximum of the color variation was 21.7 at experiment 13 performed at the highest temperature (70 °C). Fig. 1 shows the effect of temperature and time on chestnut slices color variation for a sucrose concentration of 70%. At low temperatures, time had little effect on color variation, which remained quite low. On contrary, the highest variation in ΔE^* occurred when the highest temperature and time were applied. These results showed that, in some situations, an OD process may change chestnut slices color, with ΔE^* values higher than 12, an indicative value referred to by Cecchini, Contini, Massantini, Monarca, and Moscetti (2011). Color variation might be a negative point because color is one of the most important parameters for consumers' acceptance (Andrés-Bello, Barreto-Palacios, García-Segovia, Mir-Bel, & Martínez-Monzó, 2013). These color changes should be mostly due to non-enzymatic browning reactions, namely Maillard and caramelization reactions that are favored by high temperatures (Ajandouz, Desseaux, Tazi, & Puigserver, 2008). On the other hand, enzymatic browning, due to polyphenoloxidase activity, might be difficult to develop due to the high ionic strength of the medium.

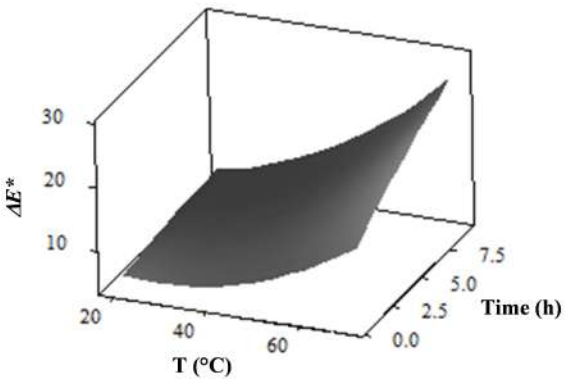

Figure 1: Response Surface plot for ΔE^* as a function of T (°C) and time (h) at sucrose concentration of 70%

3.2 Moisture content

By analyzing Table 2, the values of moisture content predicted by the model were also in good agreement with the experimental data, yielding a good R^2 (0.872), an adj-R^2 of 0.756 and a non-significant lack of fit (p=0.094) (Table 3). The minimum and maximum moisture contents on osmotic dehydrated product were 11.4 and 36.3 g of water 100 g^{-1}, obtained with a sugar concentration of 80% (w/v), 60 °C and 7.5 h, and 60% of sucrose, 30 °C and 2.5 h, respectively. The

Table 2: CCD with experimental and predicted values for the studied properties

Experiments	Levels of Coded variables[a]			Experimental values[b,c,d]										Predicted values						
	X1	X_2	X_3	V/V_0	a_w	ΔE^*	***Moisture***	***Ash***	***Fat***	***WR***	***SG***	***WL***	***NMC***	ΔE^*	***Moisture***	***Ash***	***Fat***	***WR***	***SG***	***WL***	***NMC***
1	0	0	0	0.905	0.897	11.0	18.7	1.42	1.70	0.062	0.131	0.193	0.508	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
2	-1	1	-1	0.997	0.909	13.5	27.4	0.95	1.82	-0.009	0.101	0.092	0.744	13.2	27.2	1.07	1.62	-0.010	0.125	0.113	0.737
3	1	-1	-1	0.875	0.918	5.97	16.8	1.56	2.07	0.052	0.157	0.209	0.457	5.92	19.1	1.69	1.96	0.069	0.092	0.227	0.518
4	-1	-1	-1	0.900	0.933	6.27	36.3	1.47	2.15	0.005	0.002	0.007	0.987	5.92	33.7	1.52	1.96	0.026	0.034	0.058	0.914
5	0	0	0	0.845	0.896	11.0	21.2	1.04	1.80	0.061	0.109	0.170	0.575	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
6	0	0	-1.68	1.06	0.921	6.10	33.1	1.59	1.84	0.032	0.017	0.049	0.898	6.91	25.0	1.51	1.88	0.030	0.086	0.170	0.677
7	1	1	1	0.976	0.856	17.1	11.4	0.78	1.17	0.090	0.175	0.265	0.308	18.9	7.13	0.842	1.36	0.102	0.248	0.283	0.192
8	1	-1	1	0.841	0.886	8.83	14.8	1.17	1.83	0.090	0.142	0.232	0.403	8.22	13.7	1.30	1.70	0.138	0.157	0.227	0.369
9	-1.68	0	0	1.39	0.935	10.1	34.9	1.15	1.54	-0.082	0.073	-0.010	0.948	10.3	32.6	1.04	1.66	-0.064	0.093	0.028	0.886
10	-1	-1	1	0.946	0.928	7.90	30.0	1.12	1.97	-0.015	0.079	0.064	0.814	8.22	28.2	1.13	1.70	-0.043	0.099	0.058	0.765
11	0	-1.68	0	0.922	0.908	5.63	33.4	1.63	1.89	0.005	0.031	0.036	0.908	6.31	25.9	1.57	1.95	0.060	0.065	0.123	0.702
12	0	0	1.68	0.959	0.878	13.1	17.0	0.89	1.55	0.006	0.193	0.199	0.462	13.7	15.8	0.855	1.44	0.030	0.196	0.170	0.429
13	0	1.68	0	0.992	0.872	21.7	16.3	0.84	1.64	-0.029	0.230	0.201	0.442	21.4	14.9	0.798	1.37	-0.0003	0.217	0.217	0.404
14	0	0	0	0.848	0.889	11.6	23.8	1.13	1.76	-0.020	0.145	0.125	0.647	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
15	0	0	0	0.895	0.892	8.89	16.4	1.21	1.59	0.017	0.189	0.207	0.447	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
16	1	1	-1	0.935	0.849	12.4	14.7	1.20	1.64	0.038	0.189	0.226	0.400	13.2	12.6	1.23	1.62	0.034	0.183	0.283	0.341
17	1.68	0	0	0.971	0.921	10.3	14.3	1.50	1.81	0.099	0.141	0.239	0.388	10.3	8.17	1.32	1.66	0.124	0.189	0.312	0.220
18	-1	1	1	1.17	0.857	20.2	28.8	0.61	1.46	-0.171	0.202	0.031	0.783	18.9	21.7	0.676	1.36	-0.078	0.190	0.113	0.588
19	0	0	0	0.900	0.889	9.99	19.9	1.09	1.65	0.021	0.153	0.174	0.539	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553
20	0	0	0	0.867	0.888	9.28	21.7	1.17	1.47	0.038	0.121	0.159	0.590	10.3	20.4	1.18	1.66	0.030	0.141	0.170	0.553

[a] X_1 – Sugar concentration (%); X_2 – Temperature (°C); X_3 – Time (hours).
[b] Average of two values after performing osmotic dehydrations regarding *WR, SG, WL, NMC* and Moisture content.
[c] Average of four values after performing two osmotic dehydrations regarding a_w, ash and fat contents.
[d] V/V_0 – Volume ratio; ΔE^* - Total color difference; *WR* – Weight reduction; *SG* – Solids gain; *WL* – Water loss; *NMC* – Normalized moisture content.

Table 3: Coefficients of the second-order polynomial models (with all terms) and model statistical parameters

Term	ΔE^*		Moisture		Ash		Fat		*WR*		*SG*		*WL*		*NMC*	
	Coefficient	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***	**Coefficient**	***p***
Constant	10.3	0.000	20.4	0.000	1.183	0.000	1.66	0.000	0.0296	0.032	0.141	0.000	0.170	0.000	0.553	0.000
X_1	-0.234	0.386	-7.28	0.000	0.0834	0.045	-0.0172	0.711	0.0560	0.000	0.0288	0.010	0.0848	0.000	-0.198	0.000
X_2	4.49	0.000	-3.26	0.011	-0.229	0.000	-0.172	0.003	-0.0178	0.049	0.0455	0.001	0.0277	0.037	-0.0885	0.011
X_3	2.02	0.000	-2.73	0.026	-0.195	0.000	-0.128	0.018	-0.00985	0.242	0.0326	0.005	0.0227	0.077	-0.0741	0.026
X_1^2	0.0381	0.883	0.984	0.357	0.0188	0.608	0.0147	0.745	-0.00622	0.439	-0.00844	0.365	-0.0146	0.221	0.0267	0.357
X_2^2	1.26	0.001	1.07	0.318	-0.0149	0.683	0.0478	0.303	-0.0134	0.112	-0.000023	0.998	-0.0134	0.259	0.0291	0.318
X_3^2	-0.185	0.479	1.14	0.291	-0.0129	0.724	0.0221	0.627	-0.00255	0.748	-0.00896	0.338	-0.0115	0.330	0.0308	0.291
X_1X_2	-0.595	0.109	0.562	0.690	0.0357	0.471	-0.0320	0.600	0.0195	0.089	-0.0196	0.131	-0.000169	0.991	0.0153	0.690
X_1X_3	-0.108	0.757	-0.0582	0.967	-0.0143	0.771	-0.0200	0.742	0.0341	0.008	-0.0258	0.056	0.00827	0.596	-0.00158	0.967
X_2X_3	0.866	0.028	0.798	0.573	-0.00249	0.959	-0.0534	0.386	-0.0160	0.152	0.00310	0.801	-0.0129	0.411	0.0217	0.573
Lack of fit		0.717		0.094		0.477		0.146		0.589		0.260		0.094		0.094
R^2	0.976		0.872		0.882		0.716		0.885		0.882		0.872		0.872	
Adj-R^2	0.954		0.756		0.775		0.460		0.781		0.775		0.757		0.756	

X_1 – Sugar concentration (%); X_2 – Temperature (°C); X_3 – Time (hours). When $p<0.05$ the coefficient is significant.
ΔE^* - Total color difference; *WR* – Weight reduction; *SG* – Solids gain; *WL* – Water loss; *NMC* – Normalized moisture content.

only significant terms of the model were the linear ones of all factors, namely sucrose concentration, temperature and time. Considering these significant terms, the recalculated model was as follows:

$$Moisture\ content = 20.5 - 7.28X_1 - 3.26X_2 - 2.73X_3 \quad (9)$$

Our results showed that temperature and time had a significant negative effect on moisture content (Fig. 2A). Indeed, these are the main determinants on any drying procedure, being in agreement with Noshad, Mohebbi, Shahidi, and Mortazavi (2012), who reported that an increase of temperature and time promoted a decrease of moisture content in quince. Chenlo et al. (2007) also observed that an increase in the temperature made the dehydration of chestnuts more intense, reaching lower values of moisture content. Furthermore, sucrose concentration also had an important role on moisture content (Fig. 2B), with the high sucrose concentrations yielding the lowest moisture contents. Indeed, sucrose concentration had a higher role than temperature and time, as observed by the highest coefficient of the former (-7.28 *versus* -3.26 and -2.73); however, as the temperature increased (Fig. 2B), moisture content also decreased slightly.

3.3 Ash content

The total ash values varied between 0.61 and 1.63 g 100 g^{-1} of dry matter (Table 2). The predicted values were in agreement with experimental data, yielding a good R^2 (0.882), an adj-R^2 of 0.775 and a non-significant lack of fit (p=0.477). Again, just the linear terms were significant, yielding the following recalculated model:

$$Ash\ content = 1.176 + 0.0834X_1 - 0.229X_2 - 0.195X_3 \quad (10)$$

Due to the highest absolute values of the coefficients, temperature and time had a higher effect on ash content than sucrose concentration. By analyzing Fig. 2C, we can observe an inverse behavior between ash content and temperature, with the same being verified with time. So, the highest ash content was found at the lowest temperature and time. When comparing the osmotically treated samples (0.61 to 1.63 g ash 100 g^{-1} of dry matter) with fresh chestnuts (1.76 g ash 100 g^{-1} of dry matter), the ash content decreased after the osmotic treatment. This could be due to the diffusion of sucrose to the interior of chestnut or to the output of water from the fruit to the osmotic medium, increasing the dry matter. Furthermore, other possible explanations are as follows: the removal of the shell and pellicle when slicing chestnuts may greatly increase the mass transfer rate of minerals due to the disappearance of adhesive substances and other components in the endocarp that protect the fruit (Moreira, Chenlo, Chaguri, & Oliveira, 2007) and can also be due to the high osmotic pressure at high sucrose concentrations that may break the cellular walls (Sacchetti, Gianotti, & Dalla Rosa, 2001), promoting the transfer of some minerals to the osmotic medium.

3.4 Crude Fat

The crude fat of chestnut slices contents varied between 1.17 and 2.15 g 100 g^{-1} of dry matter (Table 2). A reasonable R^2 of 0.716 and an adj-R^2 of 0.460 were obtained, showing that this model can reasonably predict the experimental data. As desired, the lack of fit was not significant (p=0.146) (Table 3). Only temperature and time were significant factors, yielding the following recalculated model:

$$Fat\ content = 1.72 - 0.172X_2 - 0.128X_3 \quad (11)$$

By the analysis of the response surface plot (Fig. 2D), we can predict that crude fat decreases as temperature and time increase. This decrease on fat content could be related with the output of fat to the osmotic medium due to the breakage of cell walls due to the high osmotic pressure (Sacchetti et al., 2001) and/or high temperatures and time. Diffusion of sucrose to the interior of chestnut or the output of water from the fruit to the osmotic medium might also have increased the dry matter, decreasing the fat content in dry basis. In fact, all OD products presented lower crude fat contents than fresh chestnut (3.3%, dry basis), supporting the hypotheses described above.

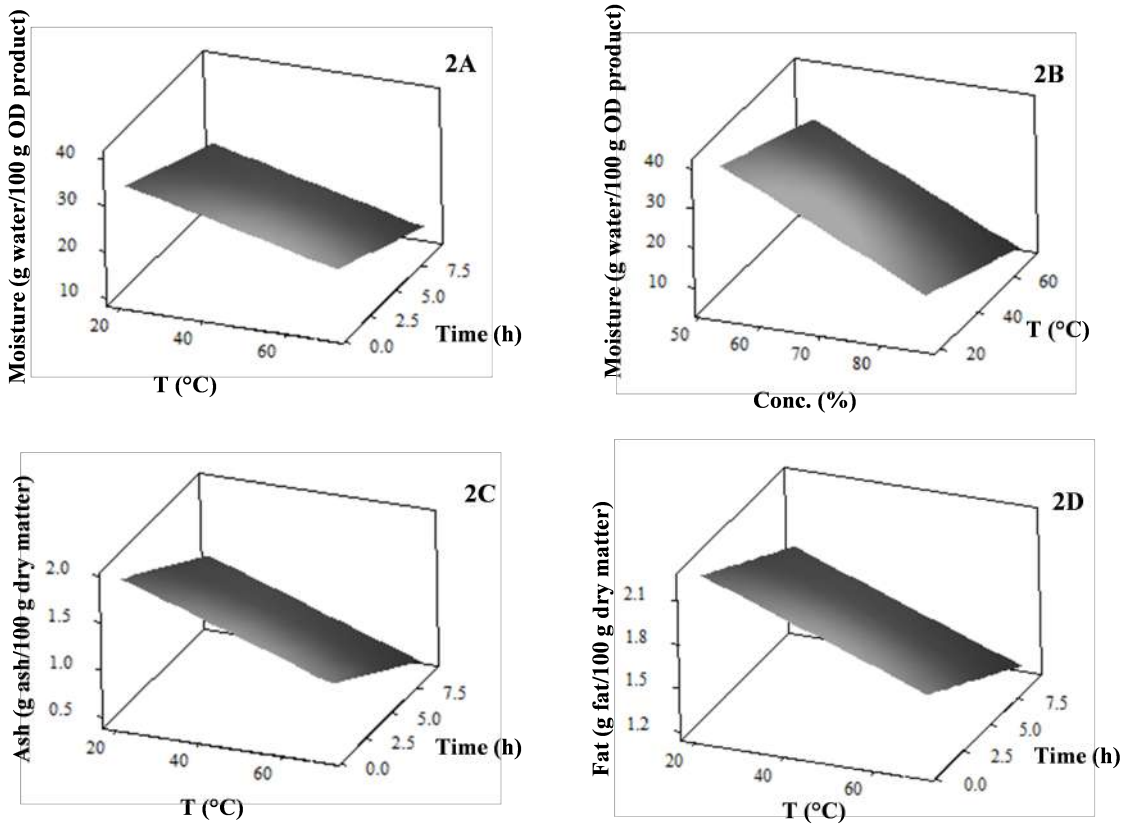

Figure 2: Response Surface plots: (A) Moisture content as a function of T (°C) and time (h) at sucrose concentration of 70%; (B) Moisture content as a function of sucrose conc. (%) and T (°C) at a contact time of 5.0 hours; (C) Ash content as a function of T (°C) and time (h) at sucrose concentration of 70%; (D) Fat content as a function of T (°C) and time (h) at sucrose concentration of 70%

3.5 Weight Reduction (*WR*)

For *WR*, the linear terms of sucrose concentration and temperature were found to be significant variables (Table 3), as well as the interaction between sucrose concentration and time. The R^2, adj- R^2 and p-value for the lack of fit of the predicted model were 0.885, 0.781 and 0.589, respectively, suggesting that the fitted model predicted well the experimental data. The recalculated model with only the significant terms is the following:

$$WR = 0.0145 + 0.0560X_1 - 0.0178X_2 + 0.0341X_1X_3 \quad (12)$$

The response surface plot of sugar concentration and temperature on *WR* is shown in Fig. 3A. The use of high temperature and high sucrose concentration would give the highest values of *WR*. When comparing the effect of the interaction of sugar concentration with time (Fig. 3B), we could conclude again that the highest *WR* were obtained at high sucrose concentrations and contact times. Chenlo et al. (2007) when performing osmotic dehydration of chestnut using glycerol solutions also stated that the *WR* increased with glycerol solution concentration. Moreover, our results are in agreement with previous studies performed on plums (Koocheki & Azarpazhooh, 2010) where an increase in WR

of the fruits is promoted by an increase of sucrose concentration and temperature. Nevertheless, at the highest sugar concentration, increasing the contact time from 2.5 to 7.5 h caused an increase of only 20% on *WR*. On the other hand, at the lowest sucrose concentration (60%), an increase of time did not cause an increase on *WR*. Furthermore, in some runs (ex. 2, 9, 10, 13, 14 and 18), negative *WR* values were observed due to the occurrence of a case-hardening effect that may induce some rigidity of the external cell layers and form a barrier to sucrose transfer, as suggested by Lee, Tham, and Wong (2014).

3.6 Solids Gain (*SG*)

Solids gain is an important factor to consider in OD, since it is intended to be the minimum as possible. Nevertheless, solids gain should be enough for preservation but not so high to induce changes of sensorial and nutritional properties. Only the model's linear terms were significant, and the lack of fit of the model was not significant (p=0.260). The R^2 and adj-R^2 were 0.882 and 0.775, respectively, showing a good adjustment between the experimental data and the values predicted by the model (all terms). When considering only the significant terms, the model obtained was:

$$SG = 0.129 + 0.0288X_1 + 0.0455X_2 + 0.0326X_3 \quad (13)$$

Fig. 3C represents the response surface plot, showing the role of sucrose concentration and temperature on *SG*. Generally, increasing temperature always favored the *SG* increase. At high temperatures, the effect of sugar concentration was almost negligible. Thus, the lowest *SG* would be obtained at low sucrose concentrations and temperature.

Considering the sucrose concentration and time (Fig. 3D), the lowest *SG* was obtained when applying low concentrations of sucrose and contact times, in line with observations by Chenlo et al. (2006a) for osmotic dehydration of whole chestnuts using glucose solutions. Nevertheless, even when high sucrose concentrations and times were used, *SG* was always lower than 0.25. These results were in agreement with Koocheki and Azarpazhooh (2010), and Uddin, Ainsworth, and Ibanoglu (2004), who also observed an increase in *SG* of plums and carrots when temperature, time and sucrose concentration increased during osmotic dehydration. This could be attributed to the increased mass transfer of sugar molecules due to possible membrane swelling/plasticizing effect, enhanced by the effect of temperature and contact time, which might increase cell membrane permeability to sucrose molecules (Lazarides, Gekas, & Mavroudis, 1997).

3.7 Water Loss (*WL*)

Beyond the *SG*, another main mass flux that is taking place is *WL*. During an OD process the water removal must be greater than solute acquisition (Chenlo et al., 2006a, 2007). A good fit between experimental and predicted values was obtained (Table 3), with a R^2 of 0.872 and an adj-R^2 of 0.757. The lack of fit of the model (all terms) was not significant (p=0.094). In terms of *WL*, only sugar concentration and temperature (linear terms) were significant, yielding the following recalculated model:

$$WL = 0.143 + 0.0848X_1 + 0.0277X_2 \quad (14)$$

By analyzing the response surface plot (Fig. 3E), high temperatures and sugar concentrations promoted *WL* that was equal to 0.30. Moreover, the role of sucrose concentration was more significant than temperature, with a higher coefficient for the former. These results were in agreement with Park, Bin, Brod, and Park (2002) and Uddin et al. (2004) who also observed an increase in *WL* with the increase in sucrose concentration. In general, *WL* in osmotic dehydrated chestnuts was favored by increasing sugar concentration and temperature. These results were in agreement with Chenlo et al. (2006a), Cao et al. (2006), Eren and Kaymak-Ertekin (2007), Koocheki and Azarpazhooh (2010), Rodrigues and Fernandes (2007) and Uddin et al. (2004) for chestnuts, kiwifruit, potato, plums, melons and carrots, respectively. Indeed, when temperature increases the water diffusion rate might also increase (Kim, 1990) and it will promote faster *WL* through swelling and plasticizing of the cell membrane, as well as by the better transfer characteristics

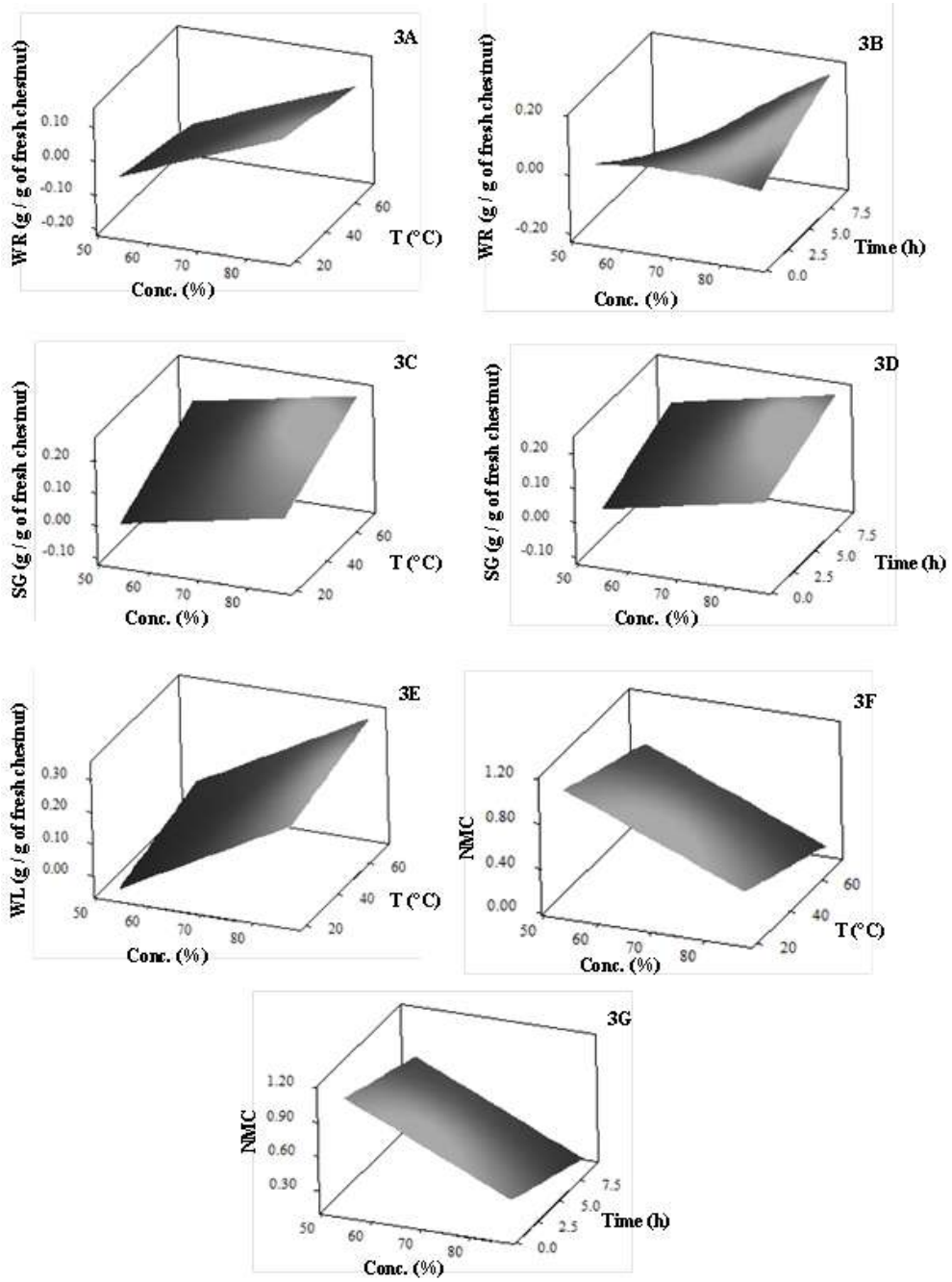

Figure 3: Response Surface plots: (A) *WR* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (B) *WR* as a function of sucrose concentration (%) and time (h) at 45 °C; (C) *SG* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (D) *SG* as a function of sucrose concentration (%) and time (h) at 45 °C; (E) *WL* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (F) *NMC* as a function of sucrose concentration (%) and T (°C) for a time of 5.0 hours; (G) *NMC* as a function of sucrose concentration (%) and time (h) at 45 °C

of the water on product surface that might be due to the lower viscosity of the osmotic medium (Contreras & Smyrl, 1981).

3.8 Normalized Moisture Content (*NMC*)

In terms of *NMC*, the experimental and predicted values were similar (Table 2), were a R^2 of 0.872, an adj-R^2 of 0.756 and a non-significant lack of fit (p=0.094) were obtained (Table 3). The experimental values varied between 0.308 and 0.987 (Table 2), which were observed when applying simultaneously high and low sugar concentrations, temperature and time, respectively. After submitting the samples to 50% sugar concentration, at 30 °C for 2.5 h, the normalized moisture content of the samples (0.987) almost did not vary when compared to the beginning (1.0), suggesting a low water transfer of the samples to the osmotic medium and therefore ineffective drying. Again, only the linear terms were significant, with the sucrose concentration being the term with the highest negative effect. The recalculated model obtained was the following:

$$NMC = 0.612 - 0.198X_1 - 0.0885X_2 - 0.0741X_3 \quad (15)$$

By observing Fig. 3F, the lowest *NMC* values were obtained when applying the highest sucrose concentration and temperature. When considering the sucrose concentration with time (Fig. 3G), we could observe that increasing sugar concentration caused a more pronounced decrease on *NMC* than increasing contact time. By applying high sucrose concentrations, chestnut samples with only 30% of the moisture content of the beginning could be obtained.

Our results were in agreement with Chenlo et al. (2007) for osmotic dehydrated chestnut (whole fruits) with sucrose. These authors also reported that an increase in temperature caused lower values of *NMC* but the intensity of the effect was higher with the most concentrated sugar solutions. Furthermore, generally our results obtained for chestnut slices by RSM were in accordance with those observed for whole fruits (chestnuts) that were osmotically dehydrated with sucrose but where this optimization methodology was not followed, namely Chenlo et al. (2007) and Moreira, Chenlo, Chaguri, and Oliveira (2007). Furthermore, our study also showed that to obtain an osmotic dehydrated product we can apply a low energy cost process due to the low temperatures that might be involved.

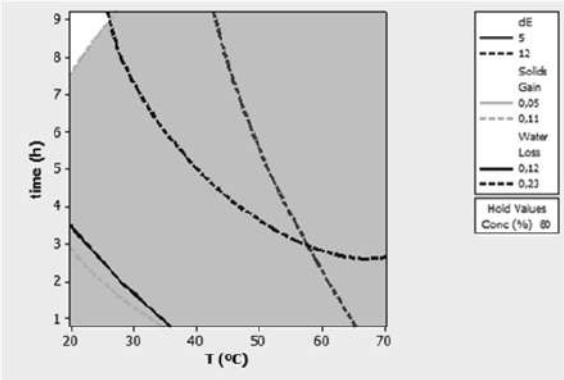

Figure 4: Combination of temperature and time to obtain ΔE^* between 5 and 12, *SG* between 0.05 and 0.11, and *WL* between 0.12 and 0.23

3.9 Optimization of solid gain, water loss and color variation

To evaluate the best osmotic dehydration conditions that optimized the responses of *WL, SG* and ΔE^* simultaneously, an optimization study was performed using the "Response Optimizer" option of Minitab® software. Our target was to obtain simultaneously high *WL* (0.12-0.23 g g^{-1} fresh matter), and low *SG* (0.05-0.11 g g^{-1} fresh matter) and ΔE^* (5-12) effects. The optimal osmotic dehydration conditions determined by the software were a sucrose concentration of 83% (w/v), a temperature of 20 °C and a duration of 9.2 hours. Fig. 4 represents the region (white area) where the values of ΔE^*, *SG* and *WL* mentioned above were obtained simultaneously. When the optimal conditions were applied, a ΔE^* equal to 7.53, a *SG* of 0.095 g g^{-1} of fresh matter and a *WL* of 0.23 g g^{-1} of fresh matter were obtained, showing that these results were within the previously defined ranges. Our results for chestnut slices were in accordance with Chenlo et al. (2006a), Chenlo et al. (2007) that

obtained the best results at low temperatures and high concentrations of the osmotic media when performing osmotic dehydration of whole fruits in glucose and sucrose solutions, respectively, and when studying osmotic dehydration kinetics without using any optimization software.

4 Conclusions

The optimal process parameters for the osmotic dehydration of chestnuts slices in sucrose solutions were determined by applying RSM. The developed models showed good correlation with the experimental data at 95% confidence level. The optimal osmotic dehydration conditions were 83% sucrose (w/v), 20 °C and 9.2 hours to achieve maximum WL and lower SG and ΔE^*. These results indicated that this process may be applied by the industry without high energy requirements and costs while not changing significantly the color of this nut, which is a characteristic valued by consumers.

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