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Effect of soy flour and whey protein concentrate on cookie color

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ABSTRACT

The objective of this work was to investigate the effect of soy flour and whey protein concentrate (WPC) addition on the extent of Maillard reaction and caramelization during cookie baking. Wheat flour from a rotary molded cookie formulation was partially replaced by full fat soy flour and whey protein concentrate. A central composite design was used and second order models were employed to generate response surfaces for loss of available lysine and for color development. Diffuse reflectance measurement of cookies was used to obtain the k/s coefficients of the Kubelka–Munk equation where k is the absorption coefficient and s is the scattering coefficient at 450, 557 and 680 nm. Luminance, dominant wavelength and excitation purity were also calculated. The addition of WPC produced an important increase in available lysine loss, k/s values and excitation purity, and a decrease in luminance and dominant wavelength. These results indicate that the addition of WPC favors the development of cookie color probably because of its high lactose content. On the contrary, the increment of water content produces a delay of Maillard reaction and caramelization. Soy flour had no significant effect on loss of available lysine and its effect on color parameter was much less significant than that of WPC.

1. Introduction

Cookies prepared from composite flours have been extensively used as protein fortification vehicles due to their long shelf-life and high acceptability (Tsen, Peters, Schaffer, & Hoover, 1973; Warren, Hnat, & Michnowski, 1983). The aim of protein fortification is to develop products with both enhanced protein content and quality, and good sensory acceptance. An important step in the development of composite flour products is the evaluation of the influence of composite flour ingredients on the product's nutritional, sensory and technological attributes (Chevallier, Della Valle, Colonia, Broyart, & Trysman, 2002; Soto-Mendivil & Vidal-Quintanar, 2001). Maillard reaction plays a major role in the cookie manufacturing process. On the one hand, the color and flavors developed during the last steps of Maillard reaction contribute to the acceptability of cookies and other baked products. On the other hand, it has been established that the condensation reaction between reducing sugars and the amino side-chain of lysine during Maillard reaction leads to a severe loss of lysine availability. Color development during cookie baking can be quantified using optical parameters obtained by measuring the diffuse reflectance (Broyart, Trysman, & Duquenoy, 1998; Gómez, Ruiz-París, & Oliete, 2011; Hadiyanto, van Straten, Boom, van Boxtel, & Esveld, 2008; Kane, Lyon, Swanson, & Savage, 2003; Yang, Song, Chen, & Zou, 2011).

The diffuse reflectance of an "infinitely thick" sample can be related to the absorption-scattering ratio coefficient k/s through the Kubelka–Munk equation: $k/s = R\infty/(1 - R\infty)^2$ where k is the absorption coefficient and s is the scattering coefficient. The k coefficient is related to the presence of chromophores that absorb light at the measured wavelength, while s depends on certain physical properties of the sample. The self-backing reflectance $R\infty$ can be obtained by increasing the sample thickness until no changes are appreciated in reflectance measures. Under this condition, the backing is negligible and the incident light is not transmitted but internally reflected by the sample (Pauletti, Matta, & Rozycki, 1999). If the scattering coefficient s is assumed to remain constant, the k/s coefficient is approximately linear with respect to the chromophore concentration. Diffuse reflectance measures also permit to calculate lightness (closely equivalent to luminance), dominant wavelength and excitation purity.

Chevallier et al. (2002) found that the lightness of cookies increases during the first part of the baking process and decreases in a following step. Broyart et al. (1998) showed that lightness variation during baking follows a second-order kinetics influenced by temperature and moisture. Singh and Mohamed (2007) found that the addition of soy protein isolate and vital gluten produced cookies with lower lightness and hue angle values and increased saturation.

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In a previous study, Pérez, Osella, de la Torre, and Sánchez (2008) recommended that an optimal quality of the cookies was achieved when they were made with 13 g/100 g soybean flour, 3 g/100 g whey protein concentrate and 23 g/100 g water in the formulation. Therefore, the objective of this study was to develop statistical models to evaluate the effect of the addition of soy flour and whey protein concentrate in protein-fortified cookies, and measuring the loss of available lysine and the parameters associated to color development.

2. Materials and methods

2.1. Materials

Wheat flour, suitable for industrial breadmaking and with the following characteristics: moisture 13.3 g/100 g, protein 12.1 g/100 g (N × 5.7) and available lysine 2.74 g/100 g protein, was provided by Molinos Matilde Santa Fe (Argentina). Physical properties at Brabender farinograph were: water absorption 59.5 g/100 g, development 2 min, stability 5.2 min and softening 50 Brabender units (BU); and physical properties at Chopin alveograph were: deformation energy $(W) = 230\ \mathrm{J} \times 10^{-4}$ and tensile strength/extensibility (P/L) ratio = 1.13. Soy flour with moisture 8.9 g/100 g, protein 35.1 g/100 g (N × 6.25), fat 17.6 g/100 g and available lysine 6.38 g/100 g protein was from Atilio Betella y Cía, Santa Fe, Argentina. Whey protein concentrate (WPC) with moisture 4.1 g/100 g, protein 41.2 g/100 g (N × 6.38), lactose 45 g/100 g and available lysine 8.17 g/100 g protein was from Milkaut, Santa Fe, Argentina. The fat used in the recipe was OPTIMA oleomargarine (melting point 36 °C) from CALSA S.A., Buenos Aires, Argentina, and the dried whole egg, from Compañía Avícola S.A., Santa Fe, Argentina. Sodium bicarbonate and ammonium bicarbonate, both supplied by Nutring S.A, Buenos Aires, were used as additives.

2.2. Manufacture of cookies

Cookies were manufactured according to the rotary-molded formula proposed by Gaines and Tsen (1980), with minor modifications to adapt it to a pilot plant conditions. The base formulation was: wheat flour (200 g), sucrose (68 g), oleomargarine (45 g), dried whole egg (10 g), sodium bicarbonate (1 g), ammonium bicarbonate (1 g) and variable water. The wheat flour was partially replaced by soy flour and whey protein concentrate.

All solid ingredients were placed in a Do-Coder Brabender farinograph and mixed during 5 min at 30 rpm. Ammonium bicarbonate and sodium bicarbonate were previously dissolved in water. After mixing, the dough was rolled on a wood table with two 2 mm aluminum strips at both sides and then it was allowed to rest for 1 min. The dough was cut with a 6 cm diameter mold. The pieces were then placed on a cookie sheet lubricated with shortening and baked in a rotary oven without steam at 220 °C during 8 min. After baked, cookies were allowed to reach room temperature, removed from the baking sheet, packaged in polypropylene bags with a moisture of 5–6 g/100 g, and heat-sealed. All samples were then stored at room temperature and protected from light.

2.3. Loss of available lysine determination

Protein content of cookies and ingredients was determined using a LECO FP-328 nitrogen analyzer. Available lysine was analyzed following Carpenter's method modified by Booth (1971). Loss of available lysine was calculated according to the following formula: $\mathrm{ALL} = 100 \times (\mathrm{Li} - \mathrm{Lf})/\mathrm{Li}$, where ALL is the available lysine loss expressed as percentage, Lf is the final content of available lysine for every 16 g of nitrogen and Li is the initial available lysine content for 16 g of nitrogen calculated from the available lysine contribution of each ingredient.

2.4. Color evaluation

The diffuse reflectance of cookies was evaluated using a Karl Zeiss Elrephomat DFC 5 reflectometer with diffused illumination, 10° geometry and D65 illuminant (CIE 1964 standard). The instrument was calibrated with a Zeiss dark standard and Merck barium sulfate for 450, 557 and 680 nm wavelengths. Ultraviolet radiation emitted by the illuminant was blocked using a UV cut filter in order to prevent interference from fluorescent compounds. Each 16 cookie sample was divided into 4 sub-samples of 4 cookies. Four measures were taken from each sub-sample by changing the position of the cookies. Diffuse reflectance was measured with three filters, corresponding to the three wavelengths mentioned before. The results were used to calculate the k/s coefficient for the three wavelengths using the Kubelka–Munk relation as well as the Luminance (L), Excitation Purity (EP) and Dominant Wavelength (DWL). EP and DWL were calculated using an algorithm based on the approximately linear behavior of the Spectrum Locus CIE in the range of 540–600 nm.

2.5. Experimental design

Eight responses were measured: available lysine loss (ALL), k/s coefficient for three color filters (k/s 680, k/s 557, k/s 450), Luminance (L), Dominant Wavelength (DWL) and Excitation Purity (EP). Soy flour (SF), whey protein concentrate (WPC) and water (W) were chosen as variables, considering the mix wheat flour–soy flour–WPC as the 100% base. Table 1 shows the variables and their levels. The values of variables were established according to a central composite design composed of a complete factorial design 2^3, six axial points and six replicates of the central point (Ramandi, Najafi, Raofie, & Ghasemi, 2011). The range for soy flour and WPC was 0–15 g/100 g, while the one for water content was 17.45 g/100 g–27.55 g/100 g. The three factors were coded according to the equation (1):

$$\begin{aligned} X_1 &= 2 \times (\mathrm{SF} - 7.5)/8.92 \quad X_2 = 2 \times (\mathrm{WPC} - 7.5)/8.92 \\ X_3 &= 2 \times (\mathrm{W} - 22.5)/6 \end{aligned} \tag{1}$$

2.6. Statistical analysis

A software package (STATGRAPHICS) was used to fit second order models and generate response surface plots. The model proposed for each response is given by the equation (2).

$$\begin{aligned} Y = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \\ & + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \end{aligned} \tag{2}$$

where b_0 is the value of the fitted response at the central point of the design [point (0,0)]; b_1, b_2 and b_3 are linear regression terms; b_{11}, b_{22} and b_{33} are quadratic regression terms; b_{12}, b_{13} and b_{23} are the cross-product regression terms.

Table 1
Variables and their levels for central composite design.

Variable g/100 g	Symbol	Coded variable levels				
		1.68179	1	0	−1	−1.68179
Soy flour	X_1	15	11.96	7.5	3.04	0
WPC	X_2	15	11.96	7.5	3.04	0
Water	X_3	27.55	25.5	22.5	19.5	17.45

Table 2
Central composite design-arrangement and responses.

Coded variable levels			Responses						
X_1	X_2	X_3	ALL (%)	k/s 680	k/s 557	k/s 450	L (%)	DWL (nm)	EP (%)
−1	1	1	47.37	0.274	0.489	2.695	38.59	573.4	54.37
0	0	1.682	19.46	0.195	0.323	1.543	45.70	569.6	43.75
0	0	0	31.85	0.255	0.429	2.138	40.82	571.5	49.18
−1	−1	1	15.38	0.165	0.258	1.129	49.47	566.9	37.54
0	0	0	26.98	0.250	0.424	2.175	41.03	571.6	49.95
−1	1	1	35.09	0.218	0.380	2.007	42.89	571.6	49.40
0	1.682	0	40.40	0.283	0.507	2.753	37.96	573.8	54.46
0	0	0	30.22	0.260	0.457	2.447	39.74	572.8	52.49
−1.682	0	0	28.78	0.149	0.243	1.146	50.48	567.6	39.16
1.682	0	0	27.80	0.261	0.440	2.258	40.37	571.7	50.41
0	−1.682	0	06.69	0.192	0.296	1.310	47.16	567.4	39.73
0	0	0	35.50	0.243	0.425	2.451	40.99	572.3	53.60
1	1	1	39.61	0.319	0.568	3.064	36.04	574.3	55.77
1	−1	−1	20.72	0.247	0.419	2.035	41.23	571.6	48.13
0	0	0	32.25	0.251	0.438	2.341	40.45	572.5	51.79
1	1	−1	42.56	0.367	0.644	3.358	33.91	574.7	56.32
0	0	−1.682	37.52	0.340	0.600	3.044	35.11	574.5	54.63
0	0	0	29.82	0.231	0.400	2.063	42.00	571.7	60.29
−1	−1	−1	29.76	0.209	0.339	1.557	44.83	569.4	43.12
1	−1	1	14.38	0.217	0.362	1.793	43.71	570.4	46.50

ALL = available lysine loss; k/s = coefficient from Kubelka–Munk equation at 680, 557, 450 wavelength; L = Luminance; DWL = Dominant Wavelength; EP = Exitation Purity.

3. Results and discussion

Table 2 shows the results corresponding to each treatment of the experimental design.

ANOVA (Table 3) shows the significant changes produced in some responses by the addition of SF (X_1) and WPC (X_2) and also with the variation of water content (X_3). All variables produce significant effects on all responses except X_1 on ALL. Regarding the lack of fit test, the p-value greater than 0.05 allows to accept the model as appropriate in four cases. The polynomial coefficients for the second-order equation (Table 4) show the relative importance of each variable.

3.1. Available lysine loss

The ANOVA shows that the incorporation of WPC and water content are highly significant ($p < 0.001$) while the addition of soy flour has no significant effect on this response. The loss of available lysine increases linearly with the incorporation of WPC and decreases significantly with the increment of water in the formulation (Fig. 1). The positive correlation between loss of available lysine and WPC was expected since the WPC used in this experience contains high levels of lactose. It can be supposed that the addition of this reducing sugar contributes to the development of Maillard reaction during cookie baking.

On the other hand, the negative correlation between water content and available lysine loss indicates that the addition of water to the formulation produces a delay in the development of Maillard reaction.

3.2. Coefficients k/s

According to ANOVA results, the models chosen for k/s at the three different wavelengths are highly significant ($p < 0.001$). The lack of fit test was significant for k/s 557 nm and k/s 680 nm, indicating that the proposed second-order model is not the best fit for those responses but gives a tendency. The k/s value presents a similar behavior for the three wavelengths analyzed. In all cases, the increment of WPC produces a linear increment of k/s all over the experimental domain. The increment of soy flour also leads to an increment of the response but the effect decreases for the higher levels of the variable (Fig. 2A). Finally, the incorporation of water reduces the k/s values for the three wavelengths in a similar way, as it can be seen for 450 nm in Fig. 2B. The Z450 nm wavelength presents the higher k/s absolute values and variations. If scattering is considered to remain constant in all samples, it can be assumed that k/s represents the absorption of the samples at the analyzed wavelength. The variation of absorption in the range 420–450 nm has been related to the increment in the concentration of chromophores

Table 3
F values from analysis of variance.

	d.f	ALL	k/s 680	k/s 557	k/s 450	L	DWL	EP
X_1	1	10.51ns	0.016^{***}	0.054^{***}	1.64^{***}	105.14^{***}	20.17^{***}	121.5^{***}
X_2	1	1457^{***}	0.018^{***}	0.082^{***}	3.62^{***}	137.18^{***}	51.28^{***}	311.2^{***}
X_3	1	322.1^{***}	0.013^{***}	0.046^{***}	1.28^{***}	72.01^{***}	14.64^{***}	68.71^{**}
X_1^2	1	0.244ns	0.002^{*}	0.008^{*}	0.350^{*}	23.90^{**}	6.57^{**}	37.92^{**}
X_2^2	1	47.10ns	0.0003ns	0.0001ns	0.022ns	1.09ns	1.66ns	9.43ns
X_3^2	1	0.051ns	0.0015ns	0.005ns	0.041ns	3.42ns	0.434ns	0.102ns
$X_1 X_2$	1	11.88ns	0.001ns	0.003ns	0.0412ns	0.589ns	0.361ns	3.51ns
$X_1 X_3$	1	37.71ns	0.0006ns	0.0004ns	0.042ns	2.34ns	0.911ns	8.20ns
$X_2 X_3$	1	3.77ns	0.0001ns	0.0003ns	0.012ns	0.060ns	0.281ns	0.211ns
Model	9	1890^{***}	0.052^{***}	0.199^{***}	6.81^{***}	345.7^{***}	96.30^{***}	560.8^{***}
Lack of fit	5	60.57ns	0.003^{*}	0.012^{*}	0.413ns	17.34^{*}	4.03ns	26.88ns

ALL = available lysine loss; k/s = coefficient from Kubelka–Munk equation at 680, 557, 450 wavelength; L = Luminance; DWL = Dominant Wavelength; EP = Exitation Purity.
$^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$.

Table 4
Regression coefficients of polynomials.

Coefficient	ALL	k/s 680	k/s 557	k/s 450	L	DWL	EP
b_0	30.97	0.248	0.428	2.26234	40.89	572.0	50.59
b_1	−0.877	0.035	0.063	0.346	−2.77	1.215	2.98
b_2	10.33	0.036	0.078	0.516	−3.17	1.938	4.77
b_3	−4.86	−0.031	−0.058	−0.306	2.30	−1.035	−2.243
b_{11}	−0.130	−0.012	−0.024	−0.156	1.29	−0.675	−1.622
b_{12}	1.22	0.013	0.020	0.0723	−0.271	−0.213	−0.663
b_{13}	2.17	0.0028	0.0071	0.0725	−0.541	0.338	1.012
b_{22}	−1.81	−0.0004	−0.0028	−0.039	0.275	−0.339	−0.809
b_{23}	0.686	−0.0038	−0.0059	−0.039	−0.086	0.188	0.163
b_{33}	−0.595	0.010	0.018	0.053	−0.487	0.174	50.59

ALL = available lysine loss; k/s = coefficient from Kubelka–Munk equation at 680, 557, 450 wavelength; L = Luminance; DWL = Dominant Wavelength; EP = Exitation Purity.

generated during non-enzymatic-browning reactions (Bates, Ames, MacDougall, & Taylor, 1998).

3.3. Luminance, dominant wavelength and excitation purity

The ANOVA results indicate that the linear components of the three factors and the quadratic component of soy flour have a significant effect on these responses. The increment of WPC produces a linear reduction of the luminance values (Fig. 3A) and a linear increment of the DWL (Fig. 4A). Excitation Purity has a behavior similar to DWL with the three variables used. The effect of soy flour is also similar, but in this case the variation is more pronounced at lower levels of the factor and diminishes at high levels to reach a stationary point within the experimental domain. Finally, the increase of water level produces a linear increment of luminance (Fig. 3B) and a linear reduction of DWL and EP responses (Fig. 4B). When comparing the response values, a reduction of luminance is seen to correspond to an increment of the excitation purity and to the displacement of the dominant wavelength from values of a greenish-yellow (566.9 nm) to an orange–yellow (574.7 nm) hue. The low luminance levels are responsible for the brown color of the 570–575 nm orange.

Luminosity decrease and saturation increment during cookie baking have been reported by Chevallier et al. (2002) and Broyart et al. (1998). Broyart et al. (1998) found a significant correlation between the decrease in reducing sugar concentration and the lightness value, indicating that the main cause of color development was the Maillard reaction, although sugar caramelization may have had some influence. Piazza and Masi (1997) reported the displacement of the hue angle from yellow to red colors during cookie baking. Bates et al. (1998) verified that the pH values that contribute to a diminution of luminosity as a consequence of non enzymatic browning also produced a reduction of the hue angle, displacing the dominant hue from yellow to red.

Singh and Mohamed (2007) showed that the replacement of wheat flour by blends of soybean protein concentrate and vital gluten in wire-cut formula cookies produced an increase of saturation, expressed as Chroma, as well as a decrease of the hue angle and lightness.

Color variations of cookies may be explained as the result of the development of colored compounds through the Maillard reaction between the WPC lactose and the free amino-groups from the lysine incorporated with the protein ingredients. These colored compounds absorb light in the region of 450 nm, which explains the higher levels of k/s for this wavelength.

As the original sample has some natural chromophores (it is not "white"), the k/s value for a certain wavelength can be considered as the addition of the contribution of the chromophores present in the dough before baking and that of the Maillard reaction products. During the baking process, the concentration of the original chromophores remains constant while that of Maillard products increases (Mundt & Wedzicha, 2007). The increase of the relative contribution of Maillard products to the total color of the cookies results in an increment of the excitation purity or saturation of the samples. At the same time, the variation of DWL toward higher values verified during the Maillard reaction confirms the fact that new colored products absorbing in lower wavelengths (420–450 nm) are produced.

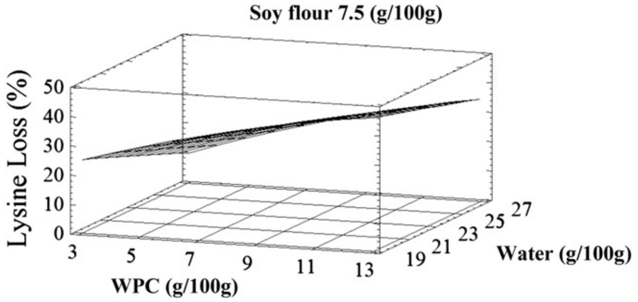

Fig. 1. Response surface for lysine loss as a function of whey protein concentrate and water added in the formulation of cookies, at 7.5 g/100 g soy flour.

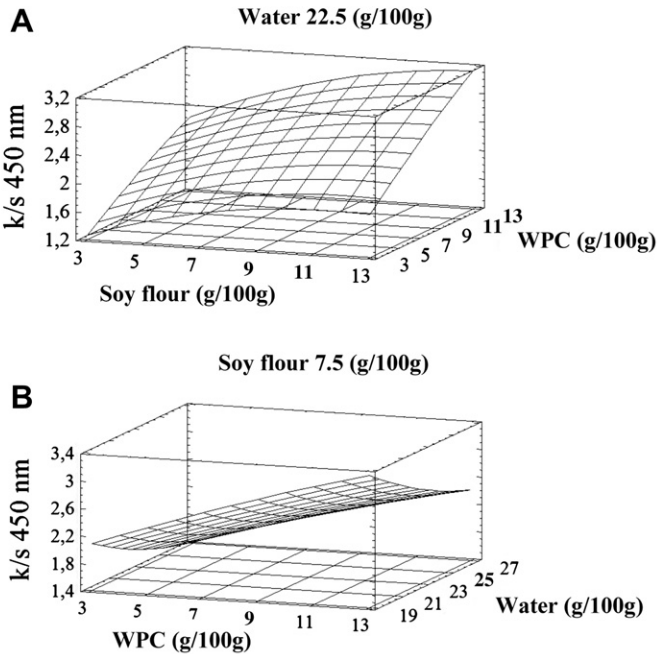

Fig. 2. Response surface for k/s coefficient at 450 nm (A) as a function of soy flour and whey protein concentrate, at 22.5 g/100 g water and (B) as a function of whey protein concentrate and water added in the formulation of cookies, at 7.5 g/100 g soy flour.

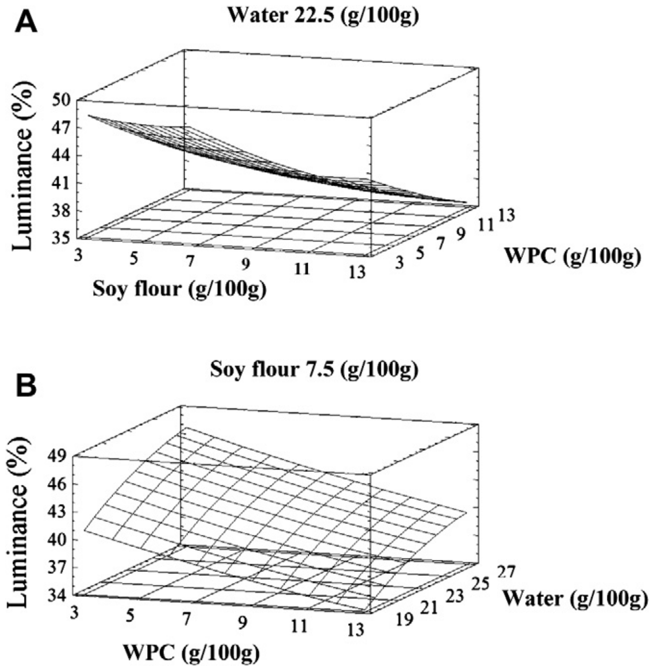

Fig. 3. Response surface for luminance (A) as a function of soy flour and whey protein concentrate, at 22.5 g/100 g water and (B) as a function of whey protein concentrate and water added in the formulation of cookies, at 7.5 g/100 g soy flour.

The effect of water content on color development and available lysine loss indicates that a water excess in the formulation reduces the extent of the Maillard reaction during cookie baking. This influence may be explained through two mechanisms:

1) Water activity has a direct influence on Maillard reaction development (Bates et al., 1998; Eskin, 1990, pp. 239–287). Jokinen, Reineccius, and Thompson (1976) showed that the relationship between water activity and loss of available lysine follows a quadratic function. The upper value of available lysine loss is produced at water values between 0.65 and 0.7. Piazza and Masi (1997) reported that browning during cookie baking is produced when water activity reaches approximately 0.7. According to these antecedents, the increase of water content in cookie dough makes the cookie take more time to reach the water activity value corresponding to the maximum Maillard reaction rate.
2) The water content of the dough formula may also modify the cookie temperature profile during the baking time. The higher water content means that more time is needed for the cookie's surface temperature to reach the value at which the browning reaction is faster. Broyart et al. (1998) analyzed the kinetics of browning during cookie baking. His results indicated that lightness reduction is not detected until the product temperature reaches a critical value between 100 °C and 110 °C. Chevallier et al. (2002) reported that reduction of the reducing sugar concentration at the surface of cookies during baking is produced when the product temperature reaches 95 °C.

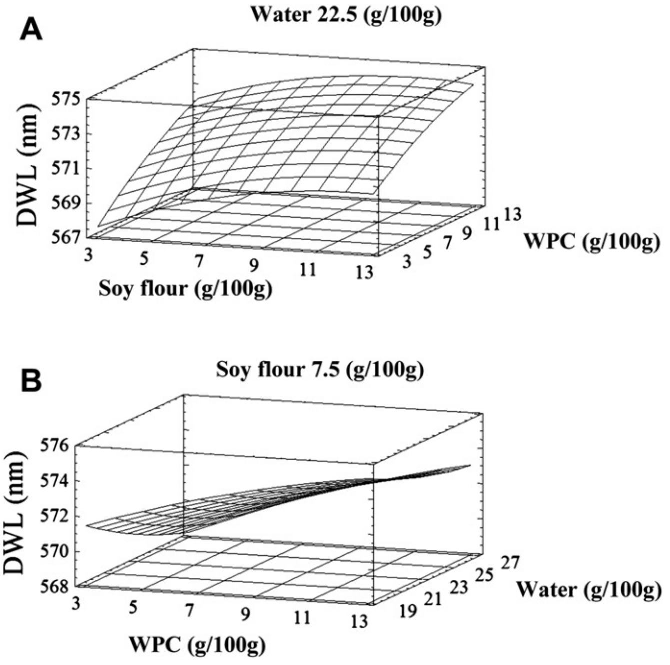

Fig. 4. Response surface for dominant wavelength (A) as a function of soy flour and whey protein concentrate, at 22.5 g/100 g water and (B) as a function of whey protein concentrate and water added in the formulation of cookies, at 7.5 g/100 g soy flour.

4. Conclusions

The replacement of wheat flour by soy flour and WPC affected the development of Maillard reaction. The addition of WPC was highly significant since it reduced lysine availability and increased color development. The addition of soy flour had a significant effect on color development but did not affect available lysine loss. Color development was characterized by the increase of k/s coefficient, dominant wavelength and excitation purity, while the luminance decreased. These modifications indicate the production of chromophores with a strong absorption in the region closely below 450 nm. Finally, the increment of water in the formulation produced a significant delay in the development of Maillard reaction, thus reducing color development and available lysine loss. The effect of ingredients in the development of Maillard reaction in cookies has to be considered when using composite-flours as it modifies sensory and nutritional attributes of the final product.

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