Optimization of antioxidants extraction from peanut skin to prevent oxidative processes during soybean oil storage

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Abstract

The aim of this study was to determine the antioxidant efficacy of peanut skin extracts (PSE) to prevent the oxidative processes of soybean oil during storage and to compare the results with those obtained after using a positive control with butylated hydroxytoluene (BHT) under accelerated oxidation conditions (16 d at 60 °C). Progress in lipid oxidation of soybean oil was followed by chemical indices (peroxide value, p-anisidine value, and conjugated dienes) at the end of the storage. A second goal was to achieve the optimal conditions for the extraction of antioxidant molecules from peanut skin using response surface methodology. At level of 750 mg/kg of PSE, primary and secondary oxidation inhibition was equivalent to the obtained with BHT, hence our results revealed PSE as an effective antioxidant for the stabilization of soybean oil. The best conditions for the recovery of antioxidant compounds were dependent on the variables measured but, in general, concentration of ethanol (73.9%) and temperature of 66.5 °C maximized the responses and the recovery of activities was not significantly influenced by extracting time.

Keywords: soybean oil, ethanol solvent, polyphenols, primary and secondary oxidation, *in vitro* antioxidant methods

1. Introduction

Oxidative processes in food during storage lead to the degradation of proteins and lipids, that could affect the deterioration in flavour, colour, texture and nutritional food value (Lorenzo, González-Rodríguez, Sánchez, Amado, & Franco, 2013). Therefore, a great interest have been shown by both food retailers and consumers to improve food products' quality through preventing lipid oxidation processes (Pateiro, Lorenzo, Amado, & Franco, 2014). For instance, one useful strategy to reduce food deterioration is based on the utilization of synthetic antioxidants, but their use is restricted due to possible toxicity effects (Babbar, Oberoi, Uppal, & Patil, 2011).

Therefore, there has been increasing interest in alternative antioxidants from natural sources (Putnik, Kovačević, Penić, & Dragović-Uzelac, 2015; Putnik, Kovačević, Penić, Fegeš, & Dragović-Uzelac, 2016) which gradually provided impetus to eliminate synthetic preservatives in food (Barba, Galanakis, Esteve, Frijola, & Vorobiev, 2015). Food industry is actively exploring new solutions to reduce oxidative rancidity and increase the shelf-life of products in response to the recent consumers' demand for natural products. Recent studies have been reported about the use of natural plant extracts (eg. extracts from grape seeds or leaves from chesnut and green tea) (Lorenzo, Sineiro, Amado, & Franco, 2014, Lorenzo et al., 2013; Pateiro et al., 2014) in several meat products. However, not always the results are better than those obtained with synthetic antioxidants. Thus, over the last years, the antioxidant potential of many plant extracts and food by-products from different origin has been investigated (Barba, Zhu, Koubaa, Sant'Ana, & Orlie, 2016; Koubaa et al., 2017).

Specifically, the use of residual biomass as a source of antioxidant compounds can be a attractive and vaible alternative because it will reduce disposal costs and adds value to the by-product (Puértolas, Koubaa, & Barba, 2016; Putnik, Kovačević,

Jambrak, Barba, Cravotto, Binello, Lorenzo, & Shpigelman, 2017a; Putnik, Kovačević, Ježek, Šustić, Zorić, & Dragović-Uzelac, 2017b). In this sense, peanuts are an important crop in many parts of the world, thus accounting peanut world's production for 43 million tonnes a year (FAO, 2014). Peanuts by-products production represents 3% of the total weight of the fruit, which is mainly composed of peanuts skins (PS) (testae or seed coat) of a peanut seed from blanched process of peanut kernels (Hathorn, & Sanders, 2012).

Recent studies have confirmed that peanut skin extracts (PSE) contain many natural polyphenols such as phenolic acids, flavonols and tocopherols (Francisco, & Resurreccion, 2012). Moreover, previous works have reported PSE antioxidant activity in food products such as dry-cured sausages (Larrauri et al., 2013), sheep patties (Munekata, Fernandes, De Melo, Trindade, Lorenzo, 2016), vegetable oils (Nepote, Grosso, & Guzman, 2002) and honey roasted peanuts (Nepote, Mestrallet, & Grosso, 2004).

One of the goals of the food industry to produce antioxidants from wastes and by-products have to rest in develop an efficient, economically viable and environmental respectful extraction process (Putnik, Kovačević, Ježek, Šustić, Zorić, & Dragović-Uzelac, 2017b Putnik, Kovačević, Radojčin, & Dragović-Uzelac, 2017c). To obtain these objectives, operational conditions for extraction have to determined in order to maximize both phenolic yields and antioxidant activities of the extracts (Putnik, Bursać Kovačević, & Dragović-Uzelac, 2016). To determine the optimal extraction conditions from PS at a laboratory scale, thinking in a further development at industry level, the best methodology is based on response surface methodology (Wardhani, Vázquez, & Pandiella, 2010). The purpose of this work was to optimise the extration conditions of antioxidant compounds from PSE, using ethanol-water mixtures and to assess its

possibilities to reduce soybean oil oxidation as an alternative to commercial synthetic antioxidants.

2. Materials and methods

2.1. Raw material

Raw peanuts (Virginia variety) were purchased in a local market. Raw peanuts were manually pelled to eliminate the shell and the skin was also manually separated from the seed. Skins were collected, grounded, cleaned and placed in a polyethylene bags and stored in vacuum conditions until extraction treatment.

A sample of refined soybean oil provided by Aceites Abril (San Cibrao das Viñas, Ourense, Spain) was used to follow the oxidation tests. The composition of the soybean oil according to CODEX Stan 210 normative was acidity (0.04%), peroxide index (<1.2 meq O₂/kg), moisture (<0.01%) and impurities (<0.01%). The fatty acid profile in percentage was myristic (0.09), palmitic (10.8), palmitoleic (0.1), stearic (5.1), oleic (19.5), linoleic (48.2), linolenic (4.6), arachidic (0.4), eicosenoic (0.2) behenic (0.6) and lignoceric (0.3).

2.2. Extraction of polyphenolic compounds

2.2.1. Experimental design for antioxidants extraction

The effect of time-processing (*t*), temperature (*T*) and ethanol concentration (*E*) in the extraction of antioxidants from skin of peanut was evaluated by a rotatable second order design with 6 experiments in the centre of the experimental domain. The experimental conditions were: *t* in the range (5-150 min), *T* in the range (25-90 °C) and *E* in the range (20-100%). The next equations were used for the codification of the variables: $V_c = (V_n - V_0) / \Delta V_n$ for codification and $V_n = V_0 + (\Delta V_n \times V_c)$ for decodification.

where: V_n = natural value of the variable to codify; V_c = codified value of the variable; V_0 = natural value in the centre of the domain; ΔV_n = increment of V_n for unit of V_c

Table 1 summarizes the codified and natural values for each experimental run. The extractions of antioxidant compounds from peanut samples (0.5 g of peanut skin in each experiment) were performed at a solid to liquid ratio of 1:20 (w/v) in a controlled water bath under high agitation conditions. After the time of extraction defined for each assayed condition, samples were filtrated through Whatman N°1 filter paper and final extracts (filtrates) were lyophilised for analysis. Then, orthogonal least-squares calculation on factorial design data were used to obtain the empirical equations describing the different antioxidant activities or dependent variables assessed (R) in function of the independent variables (t , T and E):

$$R = b_0 + \sum_{i=1}^n b_i X_i + \sum_{i=1}^{n-1} \sum_{j=2}^n b_{ij} X_i X_j + \sum_{i=1}^n b_{ii} X_i^2 \quad (1)$$

where: R is the antioxidant response; b_0 is the constant coefficient, b_i is the coefficient of linear effect, b_{ij} is the coefficient of interaction effect, b_{ii} the coefficients of quadratic effect, n is the number of variables and X_i and X_j are the independent variables (T , E and t). The Student t-test ($\alpha=0.05$) was employed to determine the statistical significance of the coefficients. The coefficients of determination (R^2) were employed to establish the goodness-of-fit and the following mean squares ratios from Fisher F test ($\alpha=0.05$) were calculated to define the model consistency: $F1 = \text{Model} / \text{Total error}$, being the model acceptable when $F1 \geq F_{den}^{num}$ and $F2 = (\text{Model} + \text{Lack of fitting}) / \text{Model}$, being the model acceptable when $F2 \leq F_{den}^{num}$

where: F_{den}^{num} are the theoretical values to $\alpha=0.05$ with the corresponding degrees of freedom for numerator (num) and denominator (den). A Microsoft Excel spreadsheet was employed for the procedures of numerical fittings, coefficient estimates and statistical evaluations. Inhibition of oil oxidation data were adjusted to the hyperbolic

equation by non-linear least-squares method (quasi-Newton), using the Solver complement and ‘SolverAid’ macro present in a Microsoft Excel spreadsheet.

2.2.2. Total polyphenol quantification

The total phenolic concentration of peanut ethanolic extracts was quantified according the method of Singleton, Orthofer, and Lamuela-Raventós (1999), using the Folin–Ciocalteu Reagent (FCR) and gallic acid as standard. Results of total phenolics were calculated as weight of gallic acid equivalent per g of freeze dried extract (g GAE/g).

2.2.3. Determination of total flavonoid content

The total flavonoid concentration was quantified based on the protocol of Zhishen, Mengcheng, and Jianming (1999), with slight modifications (Amado, Franco, Sánchez, Zapata, & Vázquez, 2014). Total flavonoid concentration was calculated as weight of catechin equivalent per g of freeze dried extract (g CTE/g).

2.3. Determination of antioxidant capacity

2.3.1. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging capacity

DPPH radical-scavenging capacity was determined according to the protocol reported by Prieto, Curran, Gowen, and Vázquez. (2015a). The radical-scavenging activity (RSA) was calculated by means of the following expression:

$$RSA = \frac{(A_{control})_{t=60min} - (A_{sample})_{t=60min}}{(A_{control})_{t=60min}} \times 100 \quad (2)$$

2.3.2. ABTS and Crocin bleaching method

The ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) radical scavenging activities were assessed according the protocol developed by Prieto et al. (2015a) using BHT as control.

The crocin bleaching method was based on the protocol reported by Prieto, Vázquez, and Murado (2015b) for the kinetic evaluation of crocin bleaching affected by PSE, measured in microplate wells with Trolox as control. In both methods, the reversed curves from the kinetic reactions were generated by subtracting the absorbance at time t from the absorbance value at time 0. The area under the curves (AUC) was calculated as follows (Amado, González, Murado, & Vázquez, 2016):

$$AUC = (y_0 + 2y_1 + 2y_2 + \dots + 2y_{n-2} + 2y_{n-1} + 2y_n) \frac{\Delta t}{2} \quad (3)$$

where: y_0 to y_n are the $n+1$ y-values defining the curve, and Δt is the interval of sampling (min).

Calculated areas of controls concentrations were fitted by linear regression. Calculated areas of extracts dilutions were plotted against controls (equivalents) and the antioxidant activities were defined by means of the EC_{50} values obtained by fitting the data of equivalents versus extracts concentrations to the Weibull equation (Amado et al., 2016). Thus, the higher the value of EC_{50} (concentration of extract) less is its antioxidant capacity.

2.4. Evaluation of lipid oxidation

2.4.1. Oxidation stability of soybean oil under accelerated conditions

This test was assessed to evaluate the effectiveness of PSE against lipid oxidation. It was performed in soybean oil with six dosage levels for the PSE (50, 100, 250, 500, 750 and 1000 mg/kg), a control without extract, and BHT (200 mg/kg). This concentration of BHT was the maximum level accepted for safety reasons. Before

introducing, PSE and BHT were separately mixed with 1.5 mL of ethanol 96% (v/v) to ensure adequate dispersion in oil, using ultrasonic water bath during 10 min. Then it was added and mixed to 20 mL of soybean oil for 10 min and vacuum evaporated. Soybean oil samples were stored in glass containers at 60 °C and agitated at 100 rpm in an orbital shaker, and analyzed in triplicate after 16 d of storage. Due to the fast hydroperoxide degradation at high temperatures, this time could correspond to 4 months at room temperature (Warner, Frankel, & Mounts, 1989).

2.4.2. Determination of chemical indices (peroxide value, *p*-anisidine value, conjugated dienes and total oxidation value)

The chemical indices were quantified according to Agregán et al. (2016). The total oxidation of soybean oil was calculated using the next equation:

$$TOTOX = AV + 2PV .$$

2.4.3. Inhibition of oil oxidation

For all indices employed to monitor the oxidation process, the inhibition of oil oxidation (*I*) was calculated using the following equation:

$$I(\%) = \left(1 - \frac{\text{index in sample}}{\text{index in control}} \right) \times 100 \quad (4)$$

In order to establish the relation between oil oxidation inhibition and antioxidant concentration, dose response curves were adjusted using a hyperbolic equation, according to the next expression:

$$I = \frac{I_m C}{CI_{50} + C} \quad (5)$$

where: *I* is the inhibition of oil oxidation (%); *C* is the concentration of extract (ppm); *I_m* is the asymptotic maximum inhibition (%) and *CI₅₀* is the concentration for semi-maximum inhibition (mg/kg).

2.5. Statistical analysis

For the analysis of the antioxidant capacity of PS in soybean oil under accelerated conditions, it was used the IBM SPSS Statistics 22 software. After verification of normal distribution and constant variance of data, significant differences were determined using one-way analysis of variance. A Duncan's test was carried out to compare the mean values at the end of oxidation for the different concentration extract at a significance level of 95% ($P<0.05$). Correlations between variables were determined using the Pearson's linear correlation coefficient.

3. Results and Discussion

3.1. Optimization of variables that maximize the antioxidant activity of PS using surface response methodology.

The use of alcoholic solutions (mainly methanol and ethanol) is the conventional preferable procedure for the extraction of antioxidant compounds from agri-food wastes of plants (Amado et al., 2014) compared to other organic solvents (hexane, acetone, etc.) with more restrictions for food applications (Samarin, Poorazarang, Hematyar, & Elhamirad, 2012). In the present work, a 3-factor rotatable design has been applied to investigate the effect of three independent variables (temperature, time and ethanol concentration) in the maximization of antioxidants from skin by-products of the peanut. Experimental data were modelled using second-order polynomial equations and statistical analysis by response surface methodology was subsequently performed (Table 1). The outcomes of the multivariate analysis indicated that the statistical signification of coefficients, evaluated by Student t-test ($\alpha=0.05$), was different in each one of the responses quantified (yields, antioxidant activities, etc.). The only common trend was that in any responses the quadratic effect of time was significant. Furthermore, the quadratic effect of ethanol was almost always statistically significant.

This variability in the responses is common when the quantification of antioxidant properties is evaluated with different methodologies. Previous reports working with extracts isolated from other substrates observed similar discrepancies (Amado et al., 2014; Pinela et al., 2016).

The values of the coefficients of determination were acceptable in some cases ($R^2 > 0.61$) and remarkable in another ones ($R^2 > 0.83$) revealed, in general, a good correlation between experimental and theoretical data (Tables 1 and 2). Figure 1 displayed a representation of the predicted surfaces generated by the second order equations summarized in Table 2 for the different responses quantified as a function of the variables assessed. Based on these equations the optimal conditions were calculated by numerical derivations (Wardhani et al., 2010) (Table 3). The process of TP recovery from peanut showed a saddle response surface with higher levels of TP at low and high temperatures and an optimal condition of E was established (74%) at any time of extraction. The theoretical concentration of TP produced at these conditions was 0.39 g GAE/g of extract. For flavonoids only ethanol affected its extraction with a similar optimum value of 75.2% and 0.26 g CTE/g. The yield of obtaining solid extract from skin wastes was significantly affected by linear and quadratic coefficient of E and linear of T (not dependence with t was observed). Nevertheless, higher yields were executed at low ethanol concentration and high processing temperature.

The results of antioxidant activities were dependent on the protocol of evaluation employed. The values of Crocin and ABTS were determined as EC_{50} , therefore lower concentrations of extracts mean higher capacity of inhibiting oxidant kinetics. The convex surfaces generated (Figure 1) were a proof of the mentioned behaviour, higher recovery of trolox equivalents in the minima of the responses (69.5% of E when T is high, and 93.3% when T is low). Meanwhile, the best conditions for ABTS (as

equivalents of BHT) were reached at 27 °C and 27% of ethanol. As a compromise option (average of the different optimal values), the production of the peanut skin extract was established in the experimental conditions: 66.5°C, 73.9% of ethanol and 77.5 min of extraction processing.

3.2. Effect of addition of peanut skin extracts (PSE) on the oxidation stability of soybean oil

The antioxidant activity of PSE, obtained in the previous optimal conditions mentioned, on soybean oil was assessed. For this purpose, the PSE extract concentration (from 0 to 1000 mg/kg) over physicochemical lipid oxidation indices (PV, AV, CD and TV) was evaluated. There are several factors that can have a significant influence in the final antioxidant capacity. Such factors are: i) raw material composition (affected by variety, agricultural, seasonal and climatic conditions), ii) processing conditions (heat treatment for roasting process) and iii) extraction conditions (solvent polarity, particle size, relation solvent/solid, initial moisture, time, temperature and number of extraction stages) (Francisco, & Resurreccion, 2012).

The addition of PSE at different levels to soybean oil affected all lipid oxidation indices (Fig. 2A to D). The concentration of BHT of 200 mg/kg was the maximum level accepted for safety reasons. The test was performed at 60 °C during 16 d. Due to the fast hydroperoxide degradation at high temperatures, this time could correspond to 4 months at room temperature (Warner, Frankel, & Mounts, 1989). PV is an indicator for the primary oxidation, because hydroperoxides are the primary products of lipid oxidation. As expected, the thermal treatment generated strong oxidation in soybean oil but this effect was significantly diminished in oil samples with BHT or PSE above 100 mg/kg ($P<0.05$). The control oil samples reached a maximum PV of 179.1 meq O₂/kg oil after 16 d of storage, whereas the PV of the sample containing 1000 mg/kg of PSE

and 200 mg/kg of BHT was 92.4 and 61.4 meq O₂/kg oil, respectively (Figure 3A). These data point out a greater soybean oil stability treated with PSE addition, being the inhibitory effect of PSE against primary oxidation of lipid concentration-dependent as can be observed in Figure 3. This relation between data of I for PV and extract concentration was no-linear and described by means of a logarithmic equation: $I=12.83 \ln [C] -40.35$, $R^2=0.983$ or even in a better way by a hyperbolic equation as previously defined in material and methods (equation 5) with a R^2 value of 0.987. Moreover, the proposal equation permitted to establish two useful parameters for comparative purposes that define the maximum inhibition of oil oxidation executed by PSE (I_m) and the concentration of PSE for $I_m/2$ (CI_{50}). Such values for PV were $I_m= 58.3\%$ and $CI_{50}= 227$ mg/kg.

Our findings are in agreement with those reported by Nepote et al. (2002) who found antioxidant activity of PSE added at 0.05% in sunflower oil against control samples. These authors employed four solvents (methanol, ethanol, acetone and water) and they did not find significant differences in the antioxidant capacity among the four PSE extracts, according to peroxide values. Moreover, as in the present work, they used BHT at 0.02% and reported a higher antioxidant activity in samples with synthetic antioxidant regarding PSE samples. However, Yadav, Yogesh, &, Aswani, (2014) using a methanolic PSE at a level 10 times higher (0.5%) found similar activity that BHT during accelerated storage of soybean oil at 65 °C for seven days. Duh and Yen (1997) also reported similar antioxidant activity of methanolic extract from peanut hulls in soybean oil. Hence, our results showed that the antioxidant compounds of PSE play an important role in the inhibition of the initial reactions of lipid oxidation acting as an electron donor or as a free radicals scavenger, since we can affirm that PSE did not show pro-oxidative effects during heat oxidation process in the concentration range

assessed. At the end of the accelerated oxidation, PSE at 750 mg/kg had an inhibitory effect of primary lipid oxidation similar to BHT at 200 mg/kg (Figure 2A, $P<0.05$). Using potato peel extracts, Rehman, Habib, & Shah (2004) needed levels 8–12 times higher than those of synthetic antioxidants to control oil per-oxidation during display, whereas (Agregán et al., 2016) employed a concentration three times higher using *Bifurcaria bifurcata* aqueous extract.

In Figure 2B are depicted the changes recorded in AV during oxidation process. This index is related to secondary oxidation products, reflecting the magnitude of aldehyde formation in oils (Khan, & Shahidi, 2001). In control samples, the net carbonyls formation was greater than in samples treated with PSE reaching a maximum of 12.4 (Figure 2B). Addition of BHT and PSE resulted in significant decrease in AV with respect to samples without antioxidants at the end of oxidation process (reduction of 42% using PSE at highest level of 1000 mg/kg with respect to the control; $P<0.05$). Contrary to results previously indicated for PV, the lowest values did not reach for synthetic antioxidant; hence we can deduce that phenolic compounds presented in PSE had a strong inhibitory effect on the secondary lipid oxidation. At the end of oxidation process, oil samples treated with PSE at levels higher than 500 mg/kg and with BHT were not significantly affected. Once again, the observed correlation between AV and extract concentration was well-explained with a no-linear relation using the equation (8) (Figure 3). The coefficient of determination was in this case of 0.982 and the inhibitory parameters ($I_m= 54.9\%$ and $CI_{50}= 392$ mg/kg).

A strong relationship between CD and oil oxidation has been reported in the literature because presence of CD has been related with PUFA oxidation (Kim, Yeo, Kim, Kim, & Lee, 2013). For this, authors have used CD index to assess the antioxidant activity of different extracts as garlic (Chatha, Anwar, Manzoor, & Bajwa, 2006), potato

skin (Amado et al., 2014) or *Bifurcaria bifurcate* (Agregán et al., 2016). The accumulation of CD decreased significantly ($P<0.05$) employing 750 mg/kg of PSE (Figure 2C) and no differences between this amount and the highest PSE level were found. At a dose of 750 mg/kg of PSE it was obtained the same result in reducing CD values than adding BHT at 200 mg/kg. The values of lipid oxidation secondary indices (CD and AV) are indicative of lipid degradation due to double bonds by primary oxidation action, which is supported by a highly positive correlation between CD and AV with PV: $R = 0.848$, $P<0.001$ for $n = 48$ between CD and PV and $R = 0.881$, $P<0.001$ for $n = 48$ between AV and PV. A similar trend was noticed by other authors in soybean oil (Kim et al., 2013) or canola oil (Agregán et al., 2016). As it was described for PV and AV, the inhibitory effect of PSE on CD formation was concentration-dependent. Again logarithm equation provides a good fit, but hyperbolic adjusts is better $R^2 = 0.977$, although in this case the variability associated to the experimental data, expressed as confidence intervals, was higher than in the other indices (Figure 2). In this case, although the parameters were statistically significant their error was high because the asymptotic phase was not clearly defined and the numerical estimates therefore had a high degree of uncertainty (Figure 3).

Finally, in Figure 2D can be observed the changes in the total oxidation values for both peroxides and aldehydes (Shahidi, & Wanasundara, 2002). After the end of the oxidation period TV increased significantly ($P<0.05$) in samples without antioxidant than in samples added with BHT or PSE at levels of 250 mg/kg or higher. In general, the values of I_m for the different oxidative responses were quite similar and ranged among 53-58%; however, the values of CI_{50} were less homogeneous.

4. Conclusions

The results of this research reveal PSE could be a relevant source of antioxidant compounds, since a similar antioxidant capacity was found of PSE in the stabilization of soybean oil compared to a synthetic antioxidant when using only a 3.75 times higher amount of the natural compound. Besides in this study, second order factorial designs were performed with the purpose of improving the conditions for extraction of antioxidant activities from PS. The best conditions were dependent on the type of antioxidant method employed but the time of extraction did not affect the process of extraction. The optimal values of ethanol concentration and temperature were approximately 75% and higher than 60 °C, respectively. Our findings open a possibility for use PSE as a natural antioxidant in the oily industry or over a wider range of food matrices. As PS are primarily a by-product of low-value, this alternative it becomes even more attractive for the food industry.

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FIGURE CAPTIONS

Figure 1. Predicted response surfaces showing the combined effect of the independent variables studied on the different responses evaluated: Y, TP, Fv, DPPH, Cr and ABTS.

Figure 2. Effect of the addition of peanut skin extract (PSE) (50, 100, 250, 500, 750 and 1000 mg/kg) and BHT (200 mg/kg) on the evolution of peroxide value (A), p-anisidine value (B), conjugated dienes (C) and TOTOX value (D) in soybean oil stored at 60 °C.

Figure 3. Effect of the PSE concentration on the inhibition of oil oxidation for each response: peroxide value (PV), p-anisidine value (AV), conjugated dienes (CD) and TOTOX value (TV). Experimental data were fitted to the hyperbolic equation (8). Error bars are the confidence intervals for $n=2$ and $\alpha=0.05$.

Figure 2

Figure 3

Table 1. Independent variables in the experimental design performed together with the obtained experimental (R_{exp}) and predicted (R_p) data for the peanut extracts. X_1 : temperature (°C); X_2 : ethanol concentration (%) and X_3 : extraction time (min). Y: extraction yield (mg extract/g of solid sample); TP: total polyphenols (g of GAE/ g of extract); Fv: total flavonoids (g of CTE/ g of extract); DPPH: DPPH scavenging activity (%); Cr: crocin bleaching activity (as EC_{50} in $\mu\text{g/mL}$ of extract) and ABTS: ABTS radical activity (as EC_{50} in $\mu\text{g/mL}$ of extract). Natural values of experimental conditions are in brackets.

Independent variables			Y (mg/g)		TP (g GAE/g)		Fv (g CTE/g)		DPPH (%)		Cr ($\mu\text{g/mL}$)		ABTS ($\mu\text{g/mL}$)	
X_1 : T	X_2 : E	X_3 : t	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p
-1 (38.2)	-1 (36.2)	-1 (34.4)	34.37	37.88	0.339	0.333	0.218	0.200	56.23	53.54	54.53	48.74	0.97	0.98
1 (76.8)	-1 (36.2)	-1 (34.4)	43.50	41.81	0.287	0.297	0.184	0.200	57.49	55.02	34.01	33.80	2.98	3.78
-1 (38.2)	1 (83.8)	-1 (34.4)	21.54	26.68	0.432	0.422	0.285	0.259	86.42	78.45	16.34	16.90	1.32	1.77
1 (76.8)	1 (83.8)	-1 (34.4)	29.56	30.61	0.424	0.386	0.266	0.259	84.89	79.93	17.52	16.91	0.64	1.83
-1 (38.2)	-1 (36.2)	1 (120.6)	33.76	37.88	0.360	0.333	0.234	0.200	71.98	71.12	19.43	21.71	1.81	2.35
1 (76.8)	-1 (36.2)	1 (120.6)	38.48	41.81	0.321	0.297	0.217	0.200	64.11	58.73	20.60	21.70	1.66	2.41
-1 (38.2)	1 (83.8)	1 (120.6)	25.61	26.68	0.481	0.422	0.313	0.259	96.14	85.24	9.76	11.65	0.67	0.40
1 (76.8)	1 (83.8)	1 (120.6)	31.50	30.61	0.380	0.386	0.250	0.259	75.99	72.86	19.13	26.59	3.29	3.20
-1.68 (25)	0 (60)	0 (77.5)	32.73	28.73	0.397	0.450	0.230	0.252	79.39	82.26	19.93	19.65	1.84	1.67
1.68 (90)	0 (60)	0 (77.5)	32.18	35.34	0.370	0.390	0.242	0.252	74.06	73.09	22.26	19.65	5.40	4.08
0 (57.5)	-1.68 (20)	0 (77.5)	51.63	47.70	0.183	0.203	0.123	0.139	36.64	37.75	43.39	45.42	3.02	2.15
0 (57.5)	1.68 (100)	0 (77.5)	31.06	28.86	0.301	0.353	0.209	0.238	60.21	70.57	27.81	22.75	1.55	1.17
0 (57.5)	0 (60)	-1.68 (5)	39.81	32.03	0.327	0.385	0.225	0.252	65.46	73.25	23.60	26.94	2.57	1.66
0 (57.5)	0 (60)	1.68 (150)	36.84	32.03	0.330	0.385	0.221	0.252	73.00	82.09	20.18	12.35	3.04	1.66
0 (57.5)	0 (60)	0 (77.5)	30.39	32.03	0.380	0.385	0.260	0.252	78.25	77.67	23.28	19.65	2.00	1.66
0 (57.5)	0 (60)	0 (77.5)	33.47	32.03	0.373	0.385	0.239	0.252	74.61	77.67	18.35	19.65	1.14	1.66
0 (57.5)	0 (60)	0 (77.5)	29.41	32.03	0.412	0.385	0.268	0.252	81.00	77.67	18.20	19.65	1.09	1.66
0 (57.5)	0 (60)	0 (77.5)	33.78	32.03	0.376	0.385	0.242	0.252	77.52	77.67	18.25	19.65	1.89	1.66
0 (57.5)	0 (60)	0 (77.5)	30.90	32.03	0.408	0.385	0.278	0.252	81.51	77.67	19.14	19.65	1.92	1.66
0 (57.5)	0 (60)	0 (77.5)	33.21	32.03	0.375	0.385	0.235	0.252	75.09	77.67	20.14	19.65	1.67	1.66

Y (mg of solid extracted/g of peanut skin); TP (g of total phenolics/g of extract); Fv (g of total flavonoids/g of extract)

Table 2. Polynomial equations modelling the effect of T , E and t (conditions in coded values according to the criteria defined in Table 1) on the extraction of antioxidants from peanut skin. The coefficients of determination (R^2) and F-values (F1 and F2) are also shown. S: significant; NS: non-significant.

Parameters	Y (mg/g)	TP (g/g)	Fv (g/g)	DPPH (%)	Cr (μ g/mL)	ABTS (μ g/mL)
b_0 (intercept)	32.03	0.39	0.25	77.67	19.65	1.66
b_1 (T)	1.97	-0.02	NS	-2.73	NS	0.72
b_2 (E)	-5.60	0.04	0.03	9.76	-6.74	-0.29
b_3 (t)	NS	NS	NS	2.63	-4.34	NS
b_{12} ($T \times E$)	NS	NS	NS	NS	3.74	NS
b_{13} ($T \times t$)	NS	NS	NS	-3.47	3.73	NS
b_{23} ($E \times t$)	NS	NS	NS	-2.69	5.44	NS
b_{123} ($T \times E \times t$)	NS	NS	NS	NS	NS	0.684
b_{11} (T^2)	NS	0.01	NS	NS	NS	0.430
b_{22} (E^2)	2.21	-0.04	-0.02	-8.31	5.11	NS
b_{33} (t^2)	NS	NS	NS	NS	NS	NS
R^2	0.716	0.731	0.646	0.826	0.883	0.609
$F1$	13.4 [$F_{16}^3 = 3.24$] $\Rightarrow$ S	10.2 [$F_{15}^4 = 3.06$] $\Rightarrow$ S	15.5 [$F_{17}^2 = 3.59$] $\Rightarrow$ S	10.3 [$F_{13}^6 = 2.92$] $\Rightarrow$ S	16.3 [$F_{13}^6 = 2.92$] $\Rightarrow$ S	5.85 [$F_{15}^4 = 3.06$] $\Rightarrow$ S
$F2$	0.315 [$F_3^{13} = 8.73$] $\Rightarrow$ S	0.412 [$F_4^{13} = 5.89$] $\Rightarrow$ S	0.226 [$F_2^{13} = 19.42$] $\Rightarrow$ S	0.552 [$F_6^{13} = 3.98$] $\Rightarrow$ S	0.518 [$F_6^{13} = 3.98$] $\Rightarrow$ S	0.488 [$F_4^{13} = 5.89$] $\Rightarrow$ S

Table 3. Experimental optimal conditions of temperature, ethanol and time (T_{opt} , E_{opt} , t_{opt}) in which the maximum responses are achieved. R_{max} are the responses obtained in each optimal value.

	Y	TP	Fv	DPPH	Cr	ABTS
T_{opt} (°C)	29.1*	71.6*	-	61.4	T ↑ / T ↓	26.6*
E_{opt} (%)	90*	74.0	75.2	76.9	69.5* / 93.3*	26.7*
t_{opt} (min)	-	-	-	43	-	-
R_{max}	25.6* (mg/g)	0.39 (g/g)	0.26 (g/g)	79.9 (%)	15.4* / 13.9* (µg/mL)	0.26* (µg/mL)

*minima values