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Research Article

Pumpkin Peel Flour Extracts Obtained by an Ultrasound-Assisted System as a Rich Source of Bioactive Compounds with Antioxidant Properties

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Abstract: This study evaluated an Ultrasound-Assisted solid-liquid Extraction (UAE) aided by Response Surface Methodology (RSM) to obtain bioactive-rich extracts from pumpkin (*Cucurbita moschata*) dried peel. Total Phenolic Compounds (TPC), Total Flavonoids Content (TFC), as well as the *in vitro* Antioxidant Activity (AA), were assessed. The physicochemical analysis revealed that Pumpkin Peel Flour (PPF) has an excellent nutritional profile, comprised by lipids (11.80%), proteins (6.02%) and total fibre (1.96%), in addition to valuable quantity of minerals such as P, Mg, Ca, Fe, Na, Zn and Mn. A Box-Behnken design was applied to analyse the effect of the concentration of solvent, the extraction time and the solid-liquid ratio as independent variables and the TPC, TFC and AA in response. PPF is a rich source of TPC (145.51-479.05 mg GAE/L) and TFC (44.08-89.68 mg CTE/L), which contribute to a high antioxidant activity (653.90-1698.20 μmol TE/L). Using the RSM, a simultaneous optimisation was performed by the desirability function and the optimum condition to maximise the obtaining of target compounds was with 80% ethanol in the proportion of 10 mL/g (solvent vs raw material) during 45 min. External validation was subsequently conducted on the proposed optimal point and all results were within ±95% of the prediction intervals proposed by the models. Thus, the UAE aided by RSM was shown to be an adequate approach to model the recovery of bioactive compounds with antioxidant properties from a by-product of the pumpkin industry.

Keywords: Antioxidant capacity, box-behnken design, by-products, composition, *Cucurbita moschata*, desirability function

INTRODUCTION

Pumpkin from America has varieties which are classified into *Cucurbita maxima, Cucurbita pepo, Cucurbita moschata* and *Cucurbita mixta*. The peel is a source of carotene, pectin, minerals, vitamins and other compounds beneficial to the human health (de Carvalho *et al*., 2012). There is a wide diversity of applications for pumpkin and it is also essential to valorise its residues regarding the development of products, which contributes to maximising available resources while reducing problems related to environmental impact (Shi *et al*., 2013).

Cucurbita moschata has received considerable attention in recent years due to the nutritional and health benefits of its bioactive compounds used for nutritional enrichment in functional foods, as well as the oil obtained from its seeds and fruits (Abou-Elella and Mourad, 2015; Rezig *et al.*, 2012). The function of natural antioxidants, which are found in many compounds classified as secondary plant metabolites in the form of polyphenols (phenolic acids and flavonoids) and terpenoids (carotenoids), is to protect the living tissues against damage caused by oxidative stress and they are directly related in prevention of various diseases (Krishnaiah *et al*., 2011; Sarikurkcu *et al*., 2009).

Extraction processes comprise the fundamental methods in the phytochemical recovery of antioxidants from plant by-products because its objective is to

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release these composites from the structures in which they are found (González-Montelongo *et al*., 2010). The different conventional methods mostly employed for the extraction of bioactive compounds, including phenolics, are Soxhlet extraction, maceration and hydrodistillation (Azmir *et al*., 2013). These conventional procedures are now being replaced or modified as a result of the advent of alternative techniques, such as the ultrasound system (Maran *et al*., 2013, 2017) which can provide optimised extraction conditions in short periods of time, improving the removal of bioactive compounds. Some of these methods are related to 'green' or sustainable, according to the EPA (the United States Environmental Protection Agency), which means that they use less harmful chemicals and renewable raw materials. Furthermore, they are more energy efficient and use less derived compounds, among other advantages (Azmir *et al*., 2013).

There is a little number of studies in the literature regarding the extraction of bioactive components from pumpkin using Response Surface Methodology (RSM) and they are focused on the extraction of phenolic compounds from pulp (Altemimi *et al*., 2016), polysaccharides from peel by ultrasound (Maran *et al*., 2013) and assisted by microwave (Zheng *et al*., 2011; Košťálová *et al*., 2016). As we are aware, no study regarding maximising the level of extracted polyphenols and antioxidant activity of *Cucurbita moschata* peel was found in the literature. Thus, in this study, we present the assessment of an ultrasound-assisted extraction aided by RSM to obtain bioactive-rich extracts from pumpkin peel and evaluation of the total phenolic compounds, total flavonoids content and *in vitro* antioxidant capacity.

MATERIALS AND METHODS

Chemicals and sample collection: Fresh pumpkin peel with pulp residue was obtained from "Q-Sabor", a company specialised in confectionery in the city of Ponta Grossa (Paraná, Brazil). All solvents and chemicals utilised in this study were of analytical grade.

Processing of pumpkin peel: The peel from the fresh pumpkins was manually removed from the pulp, cut into pieces of about 3 cm^2 and subjected to drying (65°C) in a circulation oven and hot air renewal (MA 035 model, Marconi, São Paulo Brazil). The sample was then ground and sieved using 60 mesh (particle size up to 250 µm) to obtain the pumpkin peel flour (PPF, Fig. 1). A quantity of 750 g of raw peel resulted in 500 g of pumpkin peel flour (66.67% yield). This flour was stored in airtight containers and kept under-refrigeration until further analysis.

Physicochemical analysis: The contents of moisture, protein, ash, lipids and total dietary fibre of the PPF were determined according to AOAC (2008). For the mineral analysis which included calcium (Ca), copper (Cu), cobalt (Co), iron (Fe), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn) and manganese (Mn) the readings were taken with the aid of a flame atomic absorption spectrometer (Varian, model 240FS), using as an accessory an automatic SIPS diluter system equipped with deuterium lamp as background correction and multi-element hollow cathode lamps.

For the pectin extraction, it was used boiling nitric acid solution (55 mmol) which was added on the raw-material in a solid-liquid ratio of 1:25 (w/v), maintained for 10 min. The extract was filtered in synthetic fabric, cooled to 25±2°C and added ethanol (1:2 v/v) at room temperature (30 min) (Fertonani *et al*., 2009). The pectin was dried in an oven with air circulation (40°C) until constant weight. The yield was determined by the ratio between the mass of the dry pectin and the mass of PPF multiplied by 100 (%).

Bioactive compounds and *in vitro* antioxidant activity analysis: Total Phenolic Compounds (TPC) were assessed using the Folin-Ciocalteu reagent and the results expressed as milligrams of gallic acid equivalents per litre (mg GAE/L) (Singleton and Rossi, 1965). The Total Flavonoid Content (TFC) was expressed as milligrams of catechin equivalents per litre (mg CTE/L) (Zhishen *et al*., 1999). The *in vitro* Antioxidant Activity (AA) was performed by the FRAP assay as described by Benzie and Strain (1996) and expressed in µmol of Trolox equivalents per litre (µmol TE/L).

Optimisation of extraction of phenolic compounds by UAE: In order to optimise the conditions for the recovery of phenolic compounds from the PPF, a Box-Behnken design with 15 combinations and three repetitions of the central point. The following factors (independent variables) were studied: ethanol

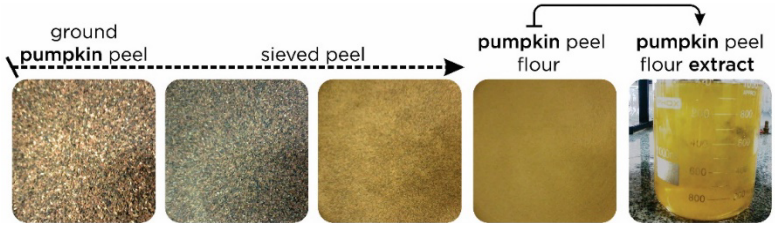


Fig. 1: Pumpkin peel flour and the extract obtained by the ultrasound-assisted system

Table 1: Total phenolic compounds (TPC), total flavonoids content (TFC) and antioxidant activity (FRAP) in the extracts made with the ethanol solution

Run	Ethanol (%) (X_1)	Proportion of sample × solvent (mL/g) (X_2)	Time (min) (X_3)	TPC (mg GAE/L)	TFC (mg CTE/L)	FRAP (µmol TE/L)
1	50	20	30	291.33±25.46^{c}	61.20±2.50^{def}	855.50±246.98^{cde}
2	50	20	30	284.54±29.41^{cd}	57.71±4.82^{efg}	904.30±182.84^{cde}
3	50	20	30	288.34±22.06^{c}	56.80±1.39^{efg}	789.90±81.2^{de}
4	50	10	15	437.32±36.05^{ab}	81.65±5.48^{ab}	1811.90±283.74^{a}
5	50	10	45	409.00±46.05^{b}	77.41±3.94^{bc}	1638.30±136.7^{a}
6	50	30	15	219.26±30.62^{e}	47.71±2.50^{hi}	661.90±347.1^{e}
7	50	30	45	196.38±24.23^{ef}	44.08±3.53^{i}	683.40±328.03^{e}
8	20	10	30	479.05±48.7^{ab}	69.38±2.66^{cd}	1622.80±161.97^{ab}
9	20	30	30	206.91±24.50^{e}	51.95±2.53^{ghi}	653.90±190.69^{e}
10	20	20	15	293.56±39.97^{c}	62.26±3.09^{def}	1112.00±188.3^{cd}
11	20	20	45	296.15±30.45^{c}	59.83±2.89^{efg}	812.30±247.49^{cde}
12	80	10	30	395.61±60.41^{b}	89.68±11.76^{a}	1698.20±291.82^{a}
13	80	20	15	177.47±32.81^{ef}	53.92±9.36^{fgh}	778.40±281.2^{de}
14	80	20	45	229.01±16.52^{de}	71.35±1.72^{c}	1216.10±132.61^{bc}
15	80	30	30	145.51±26.43^{f}	44.68±3.64^{i}	776.50±350.88^{de}
p (Normality)				0.060	0.161	0.052
p (Hartley)				0.976	0.203	0.945
p (ANOVA)				<0.0001	<0.0001	<0.0001

Results represent the mean±standard deviation. Different letters in the same column signify statistically different results ($p<0.05$).

concentration (%, X_1); solid-liquid ratio (mL/g, X_2); and time of extraction (min, X_3), at three levels of variation (Table 1). The extractions were performed at a controlled temperature (25°C) using an ultrasound device (47 kHz, 130 W, Ultrasonic Cleaners, Vernon Hills, USA) equipped with a digital time controller, in airtight jars with the liquid part entirely submerged. After the extraction, the samples were submitted to centrifugation during 20 min at 3400 g and the supernatant was vacuum filtered through a 0.45 µm Millipore membrane.

Statistical analysis: All the variables were tested for normality using the Shapiro-Wilk test and homogeneity of variances checked by Hartley test. The data followed a normal distribution with $p>0.05$. Differences between groups were evaluated by one-way ANOVA, followed by Fisher's test when discrimination was necessary between samples. A p-value≤0.05 was considered significant. RSM was used to model the recovery of TPC and the AA of the pumpkin peel. A second-order polynomial model was applied to analyse the experimental data. The generalised model used in the analysis of RSM is shown in Eq. (1):

$$Y=\beta_0+\sum_{i=1}^{3}\beta iXi+\sum_{i=1}^{3}\beta iiX_1^2+\sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j \quad (1)$$

where,

Y = The predicted response

β_0 = Constant

β_i, β_{ii} and β_{ij} = The coefficients of linear, quadratic and interaction regression

Respectively

X_i and X_j = The independent variables

The statistical significance of the terms in the regression equations was evaluated by ANOVA for each response. The terms with statistically insignificant results were excluded from the initial model and the experimental data were tested again only for the significant parameters ($p<0.05$). After the models were built, a simultaneous optimisation to maximise the extraction of TPC, TFC and AA was obtained using the desirability function proposed by Derringer and Suich (1980). In order to check the predictive value of the models, by comparing the predictive values with the experimental data, experimental procedures were performed and the results were analysed using the prediction intervals at 95%. The STATISTICA v. 13.2 software (StatSoft Inc., USA) was used to perform all the statistical analyses.

RESULTS AND DISCUSSION

Proximate composition of Pumpkin Peel Flour (PPF): The flour obtained from *Cucurbita moschata* showed 11.80±0.31% lipids, 8.20±0.13% moisture, 7.36±0.09% ash, 6.02±0.12% protein and 1.96±0.02% total dietary fibre. These values may vary depending on the part of the fruit that is used and how it is processed. Mirhosseini *et al*. (2015) studied the pumpkin flour from Malaysia and they obtained 1.17 g/100 g lipids, 10.54 g/100 g moisture, 6.01 g/100 g ash and 9.63 g/100 g protein, different from the results found in this study.

Potassium was the element found in the most significant quantity (223.47 mg/100 g of PPF), followed by 24.93 mg magnesium, 11.68 mg calcium, 0.39 mg iron, 0.38 mg sodium, 0.23 mg zinc and 0.10 mg manganese. Potassium was the highest mineral found by Ponka *et al*. (2015) in a study about pumpkin. According to Akwaowo *et al*. (2000), the evaluation of the mineral content of edible parts of pumpkin, as well

as its non-conventionally edible parts, showed that parts such as seeds, roots and stems have significant concentrations of minerals which may be used for the enrichment of foods. Thus, the same could be assumed for the peel.

The pectin extracted from the raw pumpkin peel presented a yield of 3.1±0.83%. Other works also evaluated the pectin extraction from pumpkin and they found a yield between 3.1 to 7.1% (Košťálová *et al*., 2016) and a yield of 14% (Ptichkina *et al*., 2008).

Bioactive compounds and antioxidant activity of pumpkin peel flour extracts: Pumpkin peel flour has an excellent potential for the extraction of phenolic compounds such as other by-products that have already been studied aiming to obtain bioactive-enriched extracts, including those from peanut skin (Elsorady and Ali, 2018), beer by-products (Barbosa-Pereira *et al*., 2014), wine by-products (Casazza *et al*., 2010), potato peels (Wu *et al*., 2012), olive oil (Rubio-Senent *et al*., 2013), jabuticaba peel (Santos and Meireles, 2011) and apple pomace flour (Ito *et al*., 2016).

The mean values for the extraction of phenolic compounds, flavonoids and antioxidant activity in PPF extracts are shown in Table 1. The mean values of the coordinates relating to the central point of the dependent variables in this experiment were: 288.07 mg GAE/L for phenolic compounds (CV = 1.2%); 58.57 mg CTE/L for total flavonoids (CV = 4%) and 849.9 µmol TE/L (CV = 6.8%) for FRAP. The TPC from pumpkin peel extracts varied significantly (p<0.05), from 145.41 to 479.05 mg/L. The highest value for TPC was observed in experiment 8 under the following conditions: Concentration of 20% ethanol, in the proportion of 10 mL/g, for 30 min. The model for the extraction of phenolics was significant (p<0.001), but it showed a lack-of-fit (p = 0.017). However, the model that was built was able to explain 95.64% of total variance (R^2_{adj} = 0.9446).

Although the model presented a lack-of-fit, it can be considered proper to predict the content of phenolic compounds because the model generated by RSM presented a p-value lower than 0.05 and adjusted R^2>0.70, thus it is possible to assume that the model had good predictive characteristics (Khajeh, 2011). The ethanol concentration (X_1) and the solid-liquid ratio (X_2) significantly decreased the recovery of TPC and the quadratic regression coefficient was significant and positive. The model that predicted the extraction of phenolic compounds is given in Eq. (2):

$$TPC = 753.78 - 1.37\ X_1 - 30.05\ X_2 + 0.45\ X_2^2 \quad (2)$$

The response surface values and the predicted values compared with those that were observed, are shown in Fig. 2. The total flavonoids content ranged significantly, from 44.08 to 89.68 mg/L, with experiment 12 providing the highest values under the following conditions: 80% ethanol, in the proportion of 10 mL/g, for 30 min. The created model was significant (p<0.001), showed no lack-of-fit (p = 0.12) and was able to explain 81.69% of the variance, presenting an adjusted R^2 of 0.8028. The solid-liquid ratio significantly decreased the extraction of total flavonoids. The model that predicted the extraction of total flavonoids is presented in Eq. (3):

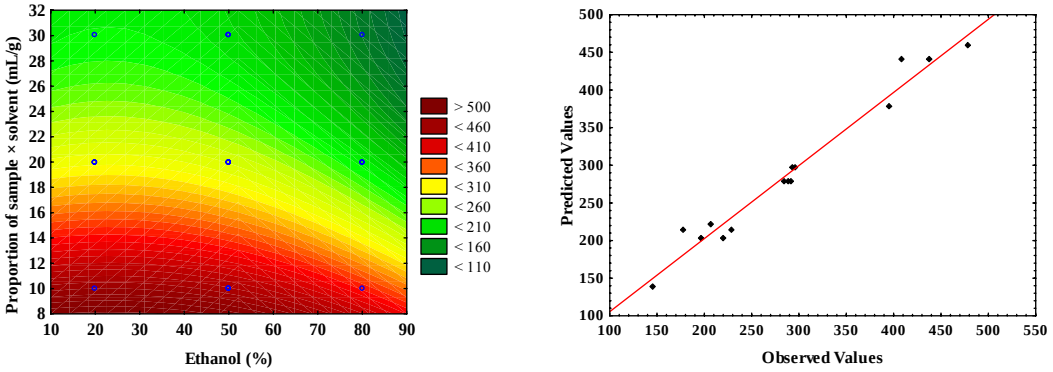
$$TFC = 94.39\text{-}1.62X_2 \quad (3)$$

The antioxidant activity measured by the FRAP method ranged from 653.90 to 1811.90 µmol/L and experiment 4 provided the highest AA under the following extraction conditions: 50% ethanol, at a ratio of 10 mL/g, for 15 min. A model built to predict the antioxidant activity of the pumpkin peel extracts is shown in Eq. (4):

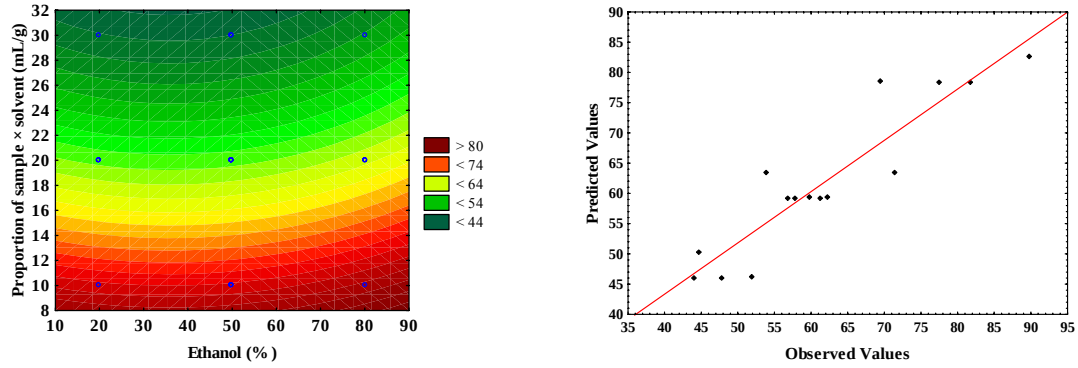
$$FRAP = 3000.11\text{-}157.66X_2\text{+}2.69X_2^2 \quad (4)$$

This model was significant (p<0.001), showed no lack of fit (p = 0.15) and was able to explain 91.56% of the variance of the data with an adjusted R^2 of 0.9015. The interaction between the ethanol concentration and time significantly increased the extraction of antioxidant activity. As in the other predictive models, the solid-liquid ratio was significant and negative.

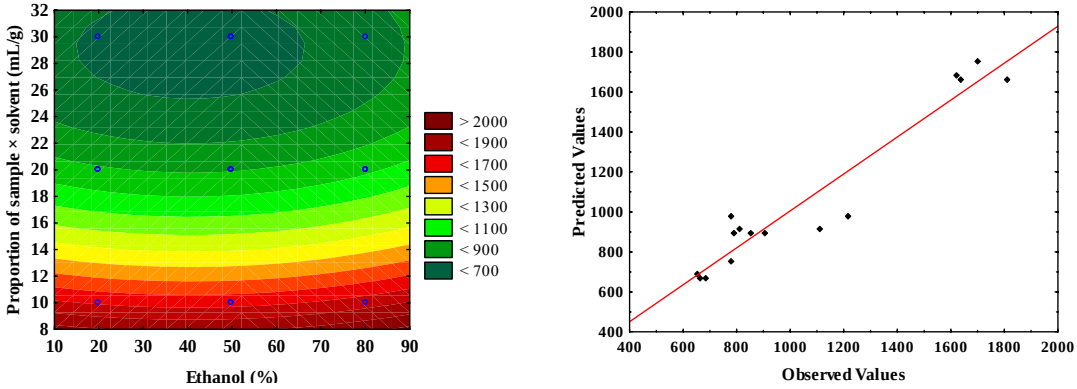
Morelli and Prado (2012) optimised the parameters of solvent concentration, time and temperature for the extraction of TPC and AA of red grape jelly using an ultrasound-assisted system with a central rotating composite model using RSM. Within the evaluated parameters they found that the use of ultrasound was more efficient when compared to other methods and that RSM was excellent in determining the best combination of parameters. Using RSM and the same extraction parameters as for pumpkin peel, Sarkis *et al*. (2014) analysing a sesame seed 'cake' (a by-product of the oil industry) they confirmed that the second-order polynomial model provided a satisfactory description of most of the experimental data. These approaches can be applied in the development of industrial processes, thereby improving the efficiency of large-scale systems. Figure 3 shows the positive correlation coefficients between all the analyses. The content of TPC correlated with antioxidant capacity (r = 0.87, p<0.001) and a significant correlation (p<0.001) was found between the content of total flavonoids and antioxidant capacity (r = 0.92) and between TPC and TFC (r = 0.81). A study by John *et al*. (2014) found that the high content of phenolic compounds in *Chukrasia tabularis* (17.2 mg GAE/g) showed a linear correlation between phenolic content and antioxidant activity. The results of a study by Maizura *et al*. (2011) also demonstrated a positive correlation between TPC and FRAP assay of kesum,



(a)



(b)



(c)

Fig. 2: Response surface and predicted values *vs* observed values of the proposed models for (a) total phenolic compounds, (b) total flavonoid content and (c) antioxidant activity measured by FRAP

ginger and turmeric extract (r = 0.91, $p<0.01$), demonstrating that phenolic compounds are the main contributors to antioxidant activity. Turumtay *et al.* (2014) studied tissues of Anzer tea and found a clear correlation (r = 0.93) between phenolic content and antioxidant activity. According to Chirinos *et al.* (2013), this same correlation also occurs in Andean plants in Peru.

Several studies have reported the content of phenolic compounds and antioxidant activity present in pumpkin seeds. However, researches involving pumpkin peel flour are still scarce. Pumpkin is considered as a healthy and functional plant because it is rich in α-tocopherol, β-carotene, vitamin A, vitamin C, phenols, flavonoids, amino acids and carbohydrates (Guiné *et al*., 2011). Some varieties, such as *Cucurbita moschata, Cucurbita maxima* and *Cucurbita pepo*, have colours ranging from bright yellow to orange and they reveal elevated levels of carotenoids, mainly α and β-carotene, β-cryptoxanthin, lutein and zeaxanthin (de

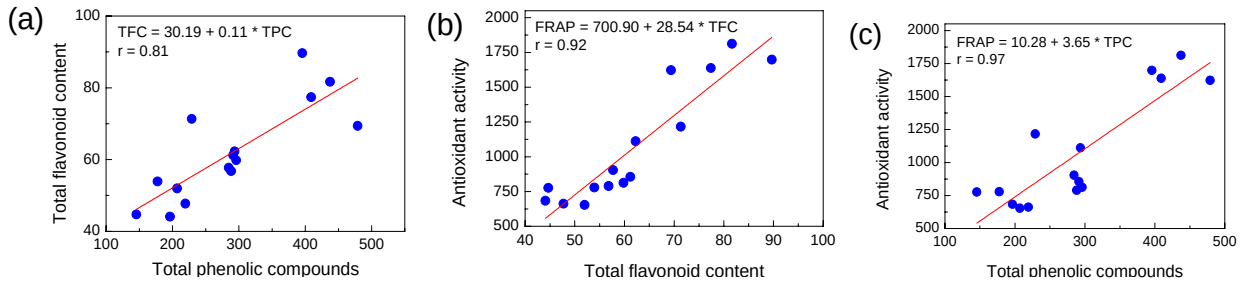


Fig. 3: Analysis of the correlation between the analyses; (a) TPC×FRAP, (b) TFC×FRAP, (c) TPC×TFC

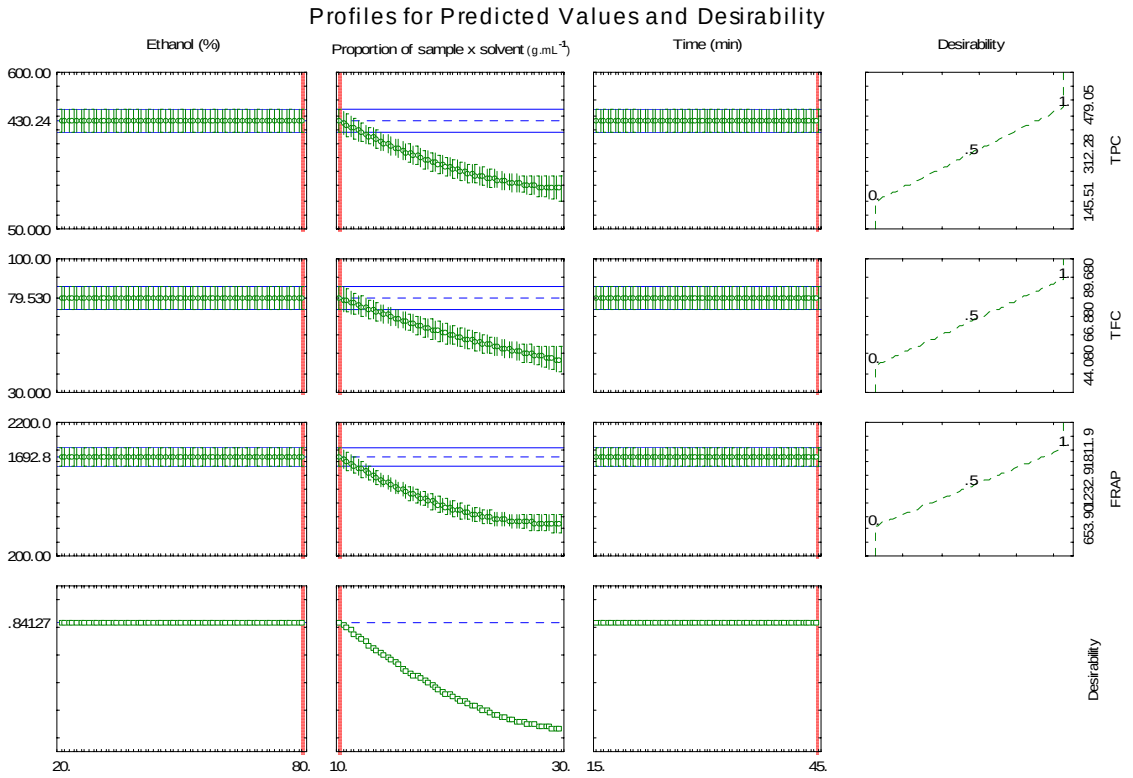


Fig. 4: Optimisation of bioactive compounds from pumpkin peel using the desirability function; TPC: Total Phenolic Compound; TFC: Total Flavonoids Content and FRAP: Ferric Reducing Antioxidant Power

Carvalho *et al*., 2012). The extracted phytochemicals from pumpkin have shown to present antioxidant, antimicrobial, antiproliferative and hypoglycemic effects (Peschel *et al*., 2006). Some other investigations showed that a diet rich in pumpkin has pharmacological activity in reducing blood glucose (Adams *et al*., 2011). After modelling the extraction of total phenolics, total flavonoids and antioxidant activity, a simultaneous optimisation was performed using the desirability function in order to maximise results, as shown in Fig. 4. The optimum values suggested for the extraction, with function desirability of 0.8531, were: 45 min, ratio of 10 mL/g and a solution of solvent containing 80% ethanol. The extraction conditions that were theoretically suggested were used and the content of TPC determined was 365.76±46.10 mg/L of extract and the model provided a value of 389.24 mg/L (absolute error = 23.48 mg/L). The value for antioxidant activity was 1607.07±32.89 mmol/L of extract and the model provided a value of 1692.80 mmol/L (absolute error = 85.73 mg/L). The TFC was 74.79±6.04 mg/L of extract and the model provided a value of 78.19 mg/L (absolute error = 3.40 mg/L).

All the results found in the external validation of the experiments were within ±95% of the prediction intervals proposed by the models. The low values of the absolute errors of the experimental results, compared to the predicted results, confirm that the models proposed by RSM are suitable for the prediction of phenolic compounds, total flavonoids and antioxidant activity of pumpkin peel prepared for the extraction of bioactive compounds.

Knowledge of the nutritional value and bioactive properties of by-products, especially from vegetables, is necessary in order to encourage increased consumption as well as their use as an ingredient in enriched nutritional applications in foods. Bearing in mind that after the removal of the pulp, pumpkin peel is either discarded or used as animal feed and considering its nutritional value, phenolic content and low cost of production, this by-product can be applied for industrial purposes in the food and pharmaceutical industries, for the extraction of target compounds and to complement and supplement human nutrition.

CONCLUSION

Pumpkin peel flour is a by-product comprising a significant source of bioactive compounds with antioxidant characteristics. The results of this study show that pumpkin peel is also rich in lipids, proteins, minerals, phenolics and flavonoids. RSM was useful in estimating the effect of three independent variables on the extraction of total phenolics, total flavonoids and *in vitro* antioxidant activity of PPF using ethanol as a green solvent. The TPC was influenced by the solvent used and the solid-liquid ratio of the extraction process, while total flavonoids and FRAP assay only by the solid-liquid ratio. The optimum combinations of the variables to maximise the yield of extraction of bioactive compounds was obtained with a time of 45 min, a ratio of 10 mL/g and a solvent solution containing 80% ethanol. These results reinforce the idea that dried pumpkin peel flour could potentially be a resource for food enrichment, thereby providing extra health benefits to consumers.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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