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Optimization of the protein concentration process from residual peanut oil-cake

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RESUMEN

Optimización del proceso de preparación de concentrado de proteína de la torta residual de maní

El objetivo de este trabajo fue encontrar las mejores condiciones para obtener un concentrado de proteínas a partir de la torta residual de maní (POC). El estudio se llevó a cabo en POC provenientes de la extracción industrial de aceite de maní. Se utilizaron distintas condiciones para la extracción y precipitación de proteínas: relación agua / harina (10:1, 20:1 y 30:1), pH de extracción (8, 9 y 10), concentración de NaCl (0 y 0,5 M), tiempo de extracción (30, 60 y 120 min), temperatura (25, 40 y 60 °C), número de etapas de extracción (1, 2 y 3), y el pH de precipitación (4, 4,5 y 5). Las condiciones de extracción y de precipitación que mostraron mayor rendimiento de proteína fueron: relación de 10:1 en agua / harina, pH de extracción de 9, en ausencia de NaCl, 2 etapas de extracción de 30 min cada una a 40 °C y el pH de precipitación de 4,5. En estas condiciones, el concentrado de proteína de maní (PC) fue de 86,22%, mientras que el porcentaje de proteínas de la POC inicial fue de 38,04%. Las POC son una fuente alternativa de proteínas que pueden ser utilizadas para el consumo humano o la alimentación animal. De esta manera, se le puede dar un valor agregado extra a un residuo de la industria del aceite del maní.

PALABRAS CLAVE: Concentrado – Maní – Proteína – Torta Residual.

SUMMARY

Optimization of the protein concentration process from residual peanut oil-cake

The objective of this study was to find the best process conditions for preparing protein concentrate from residual peanut oil-cake (POC). The study was carried out on POC from industrial peanut oil extraction. Different protein extraction and precipitation conditions were used: water/flour ratio (10:1, 20:1 and 30:1), pH (8, 9 and 10), NaCl concentration (0 and 0.5 M), extraction time (30, 60 and 120 min), temperature (25, 40 and 60 °C), extraction stages (1, 2 and 3), and precipitation pH (4, 4.5 and 5). The extraction and precipitation conditions which showed the highest protein yield were 10:1 water/flour ratio, extraction at pH 9, no NaCl, 2 extraction stages of 30 min at 40 °C and precipitation at pH 4.5. Under these conditions, the peanut protein concentrate (PC) contained 86.22% protein, while the initial POC had 38.04% . POC is an alternative source of protein that can be used for human consumption or animal nutrition. Therefore, it adds value to an industry residue.

KEY-WORDS: Concentrate – Oil-cake – Peanut – Protein.

1. INTRODUCTION

Peanuts are characterized by high oil and protein content and by low percentages of carbohydrates and ash. Peanut seed contains approximately 47-52% oil and 25-30% protein (Grosso and Guzmán, 1995).

Previous research has shown that peanut seeds are a potential source of food-grade protein for the fortification of food products. Such protein could be concentrated from residual cakes and flours through industrially applicable techniques (Rhee *et al.,* 1972, 1973; Quinn and Beuchat, 1975; Kim *et al*., 1992; Yu *et al*., 2007).

The amino acid profile of peanut residual flours showed that it could be an ingredient for protein fortification (Yu *et al*., 2007). Peanut protein concentrates were obtained using raw and roasted, fermented and unfermented peanut flours (Yu *et al.,* 2007). These authors obtained peanut protein concentrates with 85% protein versus 50% protein in the defatted peanut flour.

Partially defatted peanut flour is an inexpensive and underutilized by-product from the peanut oil industry which is rich in protein and offers the same health and dietary benefits of peanuts but with less fat. Thus, in countries like Argentina, where peanut protein is available in abundance, it could replace animal proteins for product formulation. In Argentina, the annual peanut production is about 900,000 Tns, and nearly 30% of the total peanut production is used for oil extraction, leaving a large amount of residue in the form of peanut oil cake (Fiant *et al*., 2012).

There is limited information available in the literature on the peanut protein concentration process from residual oil-cake. The extraction and precipitation pH, extraction temperature, time and

number of extraction stages, and concentration of NaCl are important factors to be considered in the protein extraction process (Kim *et al*., 1992; Johnson and Kikuchi, 2004; Lopes Barbosa *et al*., 2006; Yu *et al*., 2007).

Peanut oil-cake is a by-product from the oil extraction industry. The process consists of the following steps: a) lamination, b) cooking at 110 °C, c) pressing, d) solvent extraction (hexane), and e) solvent elimination process (Cheftel and Cheftel, 2000). This raw material is very hard to be treated and has never been used to study different extraction conditions for producing a high quality protein concentrate. The concentration of proteins from this material could increase its value; and it could become a source of new protein with applications in different industries and processes. Therefore, the objective of this study was to find the best processing conditions for the preparation of protein concentrate from residual peanut oil-cake.

2. MATERIALS AND METHODS

2.1. Materials

Peanut oil-cake (POC) is a residue obtained from industrial peanut oil production after pressing and solvent extraction of peanut kernels. POC was provided by the company Lorenzati- Ruescht y Cia from Ticino, Córdoba, Argentina in 2011.

2.2. Methods

2.2.1. Peanut oil-cake pre-treatment

POC was ground to flour until a homogenous particle size was obtained (sieve Mesh 0,75 mm). The flour was divided into two samples: peanut oil-cake flour (P) and peanut oil-cake flour extracted with 70% ethanol (EP). The EP sample was obtained by two stages of solid-liquid extraction of 50 g P with 150 mL 70% ethanol (in water, v/v) in order to eliminate soluble carbohydrates (Conkerton and Ory, 1976). This sample was dried in the oven at 60 °C for 2 h.

2.2.2. Protein extraction and precipitation

Proteins from P and EP samples (5 g) were extracted at different conditions with distilled aqueous solutions at different pH using a magnetic shaker. The different extraction conditions were: water/flour ratio (10:1, 20:1 and 30:1, v/w respectively), extraction pH (8, 9 and 10, adjusted with 1 M NaOH), NaCl concentration (0 and 0.5 M), extraction time (30, 60 and 120 min), temperature (25, 40 and 60 °C) and number of extraction stages (1, 2 and 3). The obtained slurries were centrifuged at 12,000 *g* for 20 min at 25 °C in a Beckman Coulter Allegra™ 25R centrifuge (Germany) in order to eliminate residual flour.

Proteins were precipitated from the obtained suspensions at different pH: 4, 4.5, and 5 and adjusted with 1 M HCl. Then, the extract was centrifuged at 12,000 *g* for 20 min (25 °C). The precipitate was analyzed for concentrate yield (g dry weight of the concentrate per 100 g flour), protein content in the concentrate (g protein per 100 g concentrate) and protein yield (g protein in the concentrate per 100 g flour).

2.2.3. Chemical composition of peanut oil-cake and protein isolates

Moisture, lipids, ashes and protein contents in POC and PC were determined by the AOAC methods (AOAC, 1995). The nitrogen content was converted to protein percentage using the conversion factor 5.46. The carbohydrate content was estimated by the difference of the other components using the following formula: carbohydrate content = 100% - (% protein + % oil + % ash) (Gayol *et al*., 2010).

2.2.4. Amino acid profile

The amino acid composition was determined by high performance liquid chromatography (Alonso *et al*., 1994) using a chromatograph Perkin Elmer (Waltham, Massachusetts, USA), series 200, equipped with a UV-visible detector and a Microsorb-mv 100-5 C18 (250 × 4.6 mm) column.

2.2.5. Statistical analyses

The experiment was carried out in three repetitions. Data were analyzed using the InfoStat software, version 2011p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Means and standard deviations were calculated. Simple and factorial analysis of variance and the LSD test were used to detect significant differences ($\alpha = 0.05$).

3. RESULTS AND DISCUSSION

3.1. Protein extraction and precipitation conditions

3.1.1. Water/flour ratio

Three water/flour ratios (10:1, 20:1 and 30:1, v/w) were tested for protein extraction from P and EP under the following conditions: pH = 9, 25 °C, shaking for 60 min. There were no significant differences in protein yields among the water/flour ratios tested. Under these conditions, protein yields were between 12-14 g protein $100g^{-1}$ peanut flour. Therefore, the lower water/flour ratio (10:1) was chosen in successive tests. The same water/flour ratio was used by Kim *et al*. (1992) to obtain protein isolates from nine peanut cultivars. Yu *et*

al. (2007) working on fermented and unfermented peanut flour and using a 10:1 – 100:1 water/flour ratio range (pH = 10, shaking for 60 min at room temperature), found a water/flour ratio of 20:1 for the optimum peanut protein recovery. The water/flour ratio of 50:1 yielded about the same amount of protein as the ratio 20:1; however, to remove excess water the 20:1 ratio is more cost effective.

3.1.2. pH and NaCl concentration for protein extraction

The concentrate and protein yield results after protein extraction from flour samples (P and EP) at different pH and NaCl conditions are presented in Table 1. Samples were processed in one extraction stage with a 10 water/flour ratio by shaking for 60 min at 25 °C. After extraction, the protein was precipitated at pH 4.5.

Data were analyzed by factorial ANOVA (factors: sample, extraction pH, and NaCl concentration). Significant effects on the "concentration yield" variable after protein extraction were found for all of these factors and in the interactions "sample × pH", "sample × NaCl", and "pH × NaCl". The maximum concentrate yields (between 16.04-16.98%) were observed when P and EP where extracted at either pH 9 or 10, in the absence of NaCl.

Another variable included in Table 1 was the protein content in the concentrates. Sample and NaCl were the only factors with significant effects on this variable. In general, the average protein contents were higher in the concentrates from EP with 0.5 M NaCl than from P with no NaCl. The pH factor did not show a significant effect on the protein content in the concentrate. The concentrate with the highest protein content (88.38%) was obtained from EP at pH 9 and 0.5 M NaCl. However, significant differences were not found for the protein content with respect to the concentrate obtained from P under the same conditions (pH 9 and 0.5 M NaCl).

The last variable studied (Table 1) was the protein yield after the flour sample extractions. The sample factors, pH, and NaCl and the interactions "sample × NaCl", and "pH × NaCl" showed significant effects on the protein yield variable. The maximum protein yield were obtained when the P and EP samples were extracted at pH 9 or 10, in the absence of NaCl, with no significant differences between them.

Considering the obtained results, a washing in the peanut flour with 70% ethanol may not be necessary since no significant increase has been obtained in the final protein yield. In addition, the extraction yield of P with 70% ethanol was 7.31%. P and EP had 38.04% and 36.26%, respectively, and the ethanolic extract had 10.57% proteins, which indicates that washing peanut flour samples with 70% ethanol not only extracted soluble compounds such as carbohydrates but also could have extracted some soluble proteins.

The extraction of P at pH 9 and 10 showed higher concentrations and protein yields than at pH 8. The percentages of proteins recovered from POC were 35.12% and 32.75% (g of protein in the

Table 1
Concentrate and protein yields of protein extracts obtained from peanut flour samples (P and EP) extracted at different pH and NaCl concentrations. Protein extraction conditions: 10:1 water/flour ratio, one extraction stage, shaking for 60 min, 25 °C, and pH 4.5 for protein precipitation

Flour sample[a]	Extraction pH	NaCl (M)	Concentrate yield[b]*	Protein content in the concentrate[c]*	Protein yield[d]*
P	8	0.5	8.03 ± 0.44 d	78.43 ± 6.03 bc	6.48 ± 0.98 d
P	8	0	10.64 ± 0.45 c	78.44 ± 5.77 bc	9.16 ± 1.43 bc
P	9	0.5	8.53 ± 0.43 d	**82.03 ± 4.10 abc**	7.01 ± 0.35 cd
P	9	0	**16.66 ± 0.83 a**	**80.23 ± 3.81 bc**	**13.36 ± 1.83 a**
P	10	0.5	9.52 ± 0.48 cd	79.53 ± 5.63 bc	7.57 ± 0.38 bcd
P	10	0	**16.08 ± 0.80 a**	77.50 ± 2.25 c	**12.46 ± 1.28 a**
EP	8	0.5	2.99 ± 0.15 f	**84.21 ± 3.13 ab**	2.52 ± 0.13 f
EP	8	0	12.62 ± 0.05 b	78.96 ± 5.03 bc	9.44 ± 0.52 b
EP	9	0.5	4.03 ± 0.20 f	**88.38 ± 3.42 a**	3.56 ± 0.18 ef
EP	9	0	**16.04 ± 0.80 a**	80.98 ± 4.02 bc	**12.89 ± 0.90 a**
EP	10	0.5	6.33 ± 0.37 e	**84.08 ± 4.20 ab**	5.32 ± 0.31 de
EP	10	0	**16.98 ± 0.85 a**	80.98 ± 4.05 bc	**13.75 ± 1.71 a**

[a] Peanut flour samples: P = peanut oil-cake flour and EP = peanut oil cake flour extracted with 70% ethanol solution.
[b] Concentrate yield: g isolate 100 g^{-1} flour.
[c] Protein content in the concentrate: g proteins 100 g^{-1} concentrate.
[d] Protein yield: g proteins 100 g^{-1} flour.
* Means and standard deviations followed by different letters in each column indicate significant differences (ANOVA, LSD test, $\alpha = 0.05$).

concentrate per 100 g of protein in the flour) for different pH extraction (pH 9 and 10, respectively).

The addition of 0.5 M NaCl had a slightly positive effect on the protein purity of concentrates, but negatively affected protein yield.

Rhee *et al.* (1973) and Basha and Cherry (1976) reported that the largest amounts of soluble proteins extracted in water and sodium phosphate buffer were at pH 9 and 10. At pH values above 10, protein levels in different extraction media decreased slightly as a result of alkaline denaturation. Natarajan *et al*. (1975) reported that peanut proteins are known to be soluble at pH < 2 and pH > 7, and are very stable to heat treatment. Yu *et al*. (2007) found that the optimum peanut protein recovery was achieved at pH 10. The use of pH higher than 10 was not advisable because of undesirable changes such as protein denaturation and discoloration, which could affect the functionality and sensory quality of peanut protein concentrate. Liu *et al* (2012) obtained a concentrate (89% protein) from defatted peanut flour at pH 8 extraction.

In this study, the extraction and protein yields did not differ significantly at either pH 9 or 10. The conditions chosen for preparing protein concentrates from peanut oil- cake were those using peanut flour without washing with 70% ethanol, and extracting the protein at pH 9 with no NaCl.

3.1.3. pH of protein precipitation

The concentrate and protein yields after the peanut oil-cake flour (P) extraction and precipitation at different pH (4.0, 4.5 and 5.0) are shown in Table 2. The protein extraction conditions were 10:1 water/flour ratio, one extraction stage, shaking for 60 min, pH 9, and 25 °C. The precipitate obtained at pH 4.5 showed the highest values in concentrate and protein yields in comparison with the other pH conditions. The concentrates at pH 4.5 and 5.0 had higher protein contents than that obtained at pH 4.0. The percentages of protein recovered from POC were 23.34%, 35.12%, and 28.55% (g of protein in the concentrate per 100 g of protein in POC) for different protein precipitation pH (pH 4, 4.5, and 5, respectively).

Yu *et al*. (2007) worked at pH 4.0 to separate the peanut protein from the supernatant by isoelectric precipitation because the peanut proteins appear to be less soluble at this pH. Wu *et al.* (2009) found that 4.5 was the isoelectric pH for precipitation of peanut protein for preparing protein concentrates.

3.1.4. Extraction time and number of extraction stages of proteins

Protein content in the concentrate and protein yield after the extraction of peanut oil-cake flour (P) using different extraction stages and shaking during different times are shown in Figure 1. The protein extraction conditions were: 10:1 water/flour ratio, pH 9, with no NaCl, 25 °C, and pH 4.5 for protein precipitation.

The data were analyzed by two-way ANOVA (factors: number of extraction stages and extraction time). The number of stages factor was the only one that showed a significant effect on the variables studied, protein content in the concentrate and protein yield. The protein yield was higher in the first stage (between 10.34 and 11.66%) than in the other stages. About 90% of total proteins were extracted in the first and second stages. The extraction time did not show a significant effect on the variables studied. As a consequence of these results, the extraction process chosen consisted of two extraction stages of 30 min each. Lopes Barbosa *et al.* (2006) reported that an increase in extraction time (1 and 3 h) in defatted soy flour did not result in a higher protein yield.

3.1.5. Extraction temperature of proteins from peanut oil-cake flour

The results of the concentrate and protein yields after peanut oil-cake flour (P) extraction obtained at different temperatures (25, 40 and 60 °C) are shown in Table 3. Proteins were extracted and precipitated at the following conditions: 10:1 water/flour ratio, two extraction stages, shaking for 30 min, pH 9, with no NaCl, 25 °C, and pH 4.5 for protein precipitation. The percentages of proteins recovered from POC were 37.57%, 45.37%, and 45.61% (g of protein

Table 2
Concentrate and protein yields of protein extracts obtained from peanut oil-cake flour (P) extracted and precipitated at different pH. Protein extraction conditions: 10:1 water/flour ratio, one extraction stage, shaking for 60 min, pH 9, and 25 °C

Precipitation pH	Concentrate yield[a] *	Protein content in the concentrate[b] *	Protein yield[c] *
4.0	12.03 ± 0.20 b	73.82 ± 2.05 c	8.88 ± 0.10 c
4.5	16.66 ± 0.83 a	80.23 ± 3.81 ab	13.36 ± 1.83 a
5.0	12.83 ± 0.44 b	86.76 ± 1.88 a	10.86 ± 0.36 b

[a] Concentrate yield: g isolate $100g^{-1}$ flour.
[b] Protein content in the concentrate: g proteins$100g^{-1}$ concentrate.
[c] Protein yield: g proteins$100g^{-1}$ flour.
* Means and standard deviations followed by different letters in each column indicate significant differences (ANOVA, LSD test, $\alpha = 0.05$).

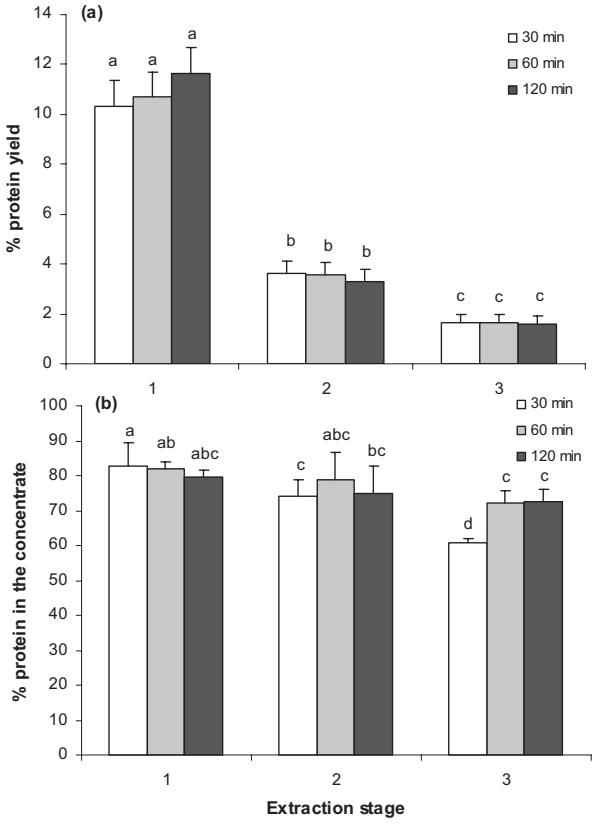

Figure 1
(a) Protein content in the concentrate and (b) protein yield of the protein extracts obtained from peanut oil-cake flour (P) using different extraction stages and shaking during different times. Protein extraction conditions: 10:1 water/flour ratio, pH 9, 25 °C, and pH 4.5 for protein precipitation.

in the concentrate per 100 g of protein in POC) for different extraction temperatures (25, 40 and 60 °C, respectively). The highest concentrate and protein yields were obtained at 40 and 60 °C without significant differences between them. The protein content in the concentrate was higher at 40 °C. Lopes Barbosa et al. (2006) reported an increase in the efficiency of protein extraction in defatted soy flour, measuring 64 and 88% protein extracted at 4 and 50 °C, respectively. Johnson and Kikuchi (2004) worked on temperatures between 27 and 66 °C for the protein extraction of soy flour observing that the proteins became progressively less soluble at temperatures higher than 70 °C. In the present study, 40 °C was the best extraction temperature for preparing peanut protein isolates from peanut oil-cake flour.

Ma *et al.* (2010), using response surface methodology, found the optimal parameter for the process of protein extraction from defatted peanut flour for primary and secondary extraction. They reported that the optimal values are 11.79:1 liquid/solid ratio (v/w), 36.35 °C extraction temperature, and 85% ethanol concentration for the primary protein extraction; and 8:1 liquid/solid ratio (v/w), 38.40 °C extraction temperature, and 97.5% ethanol concentration for the secondary protein extraction.

3.2. Chemical composition of peanut oil-cake and protein concentrate

The chemical composition of peanut oil-cake (POC) and protein concentrate (PC) from peanut oil-cake flour (P) obtained at the optimum extraction and precipitation conditions (10 water/flour ratio, two extraction stages, shaking for 30 min, pH 9, in the absence of NaCl, 40 °C, and pH 4.5 for protein precipitation) are shown in Table 4. PC had 86.22% protein content and POC showed 38.04% protein content. Lower ashes, carbohydrates, and moisture and higher lipids were observed in PC than in POC.

Yu *et al.* (2007) reported that the proximate composition of peanut protein concentrates is influenced by the type of peanut flour used. The peanut protein concentrate obtained from roasted peanut flour exhibits higher protein (85.67%) and lower fat contents (2.9%), moisture (2.73%), and other components (0.55%) than the concentrate obtained from raw peanut flour (77.8 % protein, 13% fat, 4.6% moisture, and 1.86% other components). The lower protein content (77%) of the protein concentrate prepared from raw peanut flour was probably due to a higher fat content exhibiting 17% fat in raw peanut flour and 12% fat in roasted peanut

Table 3
Concentrate and protein yields of protein extracts obtained from peanut oil-cake flour (P) extracted at different temperatures. Protein extraction conditions: 10:1 water/flour ratio, two extraction stages, shaking for 30 min, pH 9, 25 °C, and pH 4.5 for protein precipitation

Extraction temperature (°C)	Concentrate yield[a] *	Protein content in the concentrate[b] *	Protein yield[c] *
25	17.37 ± 0.47b	82.30 ± 0.91 b	14.29 ± 0.21 b
40	20.02 ± 0.33a	86.22 ± 0.92 a	17.26 ± 0,47 a
60	20.76 ± 0.45a	83.65 ± 0.31b	17.35 ± 0.30 a

[a] Concentrate yield: g isolate 100 g^{-1} flour.
[b] Protein content in the concentrate: g proteins/100g concentrate.
[c] Protein yield: g proteins 100 g^{-1} flour.
* Means and standard deviations followed by different letters in each column indicate significant differences (ANOVA, LSD test, $\alpha = 0.05$).

flour. The fat matter in raw peanut flour could have reduced the efficiency of protein extraction due to the formation of emulsion in conjunction with protein during extraction, resulting in higher fat and lower protein contents in the final product. The ash contents in both protein concentrates were about the same but much lower than those found in flours. Decreasing ash content is expected since most minerals should be discarded in the supernatant after protein precipitation (Yu *et al.,* 2007).

Wu *et al.* (2009) obtained peanut protein concentrates through different preparation processes precipitating the peanut proteins at isoelectric pH of 4.5. The peanut protein concentrates were called as follows: isoelectric precipitation peanut protein concentrate, alcohol precipitation peanut protein concentrate, isoelectric precipitation with alcohol precipitation peanut protein concentrate, and alkali solution with isoelectric precipitation, and showed different protein contents (72.35%, 69.54%, 71.49%, and 96.65%, respectively).

Jangchud and Chinnan (1999) reported 81.37% protein in peanut protein concentrated from defatted peanut flour with hexane (oil 2%). Liu *et al.* (2012) reported a concentrate with 89% protein from defatted peanut flour. Johnson and Kikuchi (2004) reported 88.3-91.8% protein, 4.9-7% moisture and 2.4 - 3.8% ash in soybean protein isolates.

Table 4
Chemical composition (% w/w on dry basis) of peanut oil cake (POC) and protein concentrates (PC) from peanut oil-cake (P) obtained at the optimum extraction and precipitation conditions (10:1 water/flour ratio, two extraction stages, shaking for 30 min, pH 9, with no NaCl, 40 °C, and pH 4.5 for protein precipitation)

Component (%)	POC*	PC*
Proteins	38.04 ± 0.32 b	86.22 ± 0.92 a
Moisture	5.99 ± 0.03 a	4.16 ± 0.18 b
Lipids	2.54 ± 0.23 b	7.76 ± 0.03 a
Ashes	8.32 ± 0.04 a	1.16 ± 0.01 b
Carbohydrates	51.10 ± 0.23 a	0.85 ± 0.22 b

* Means and standard deviations followed by different letters in each column indicate significant differences (ANOVA, LSD test, $\alpha = 0.05$).

Table 5
Amino acid composition expressed as relative percentages (g 100g^{-1} amino acids) of peanut oil-cake (POC) and protein concentrate (PC) prepared at optimum extraction and precipitation conditions (10:1 water/flour ratio, two extraction stages, shaking for 30 min, pH 9, with no NaCl, 40 °C, and pH 4.5 for protein precipitation)

Amino acid	POC*	PC*
Ala (Alanine)	0.97 ± 0.09	1.20 ± 0.16
Arg (Arginine)	0.91 ± 0.16	0.39 ± 0.19
Asx (Asparagine or aspartic acid)	19.50 ± 0.36 b	34.41 ± 0.41 a
Glx (Glutamine or glutamic acid)	17.98 ± 0.18 b	24.13 ± 0.22 a
Gly (Glycine)	1.84 ± 0.06 a	0.50 ± 0.09 b
His [a] (Histidine)	8.78 ± 0.07	8.64 ± 0.06
Ile [a] + Leu (Isoleucine + Leucine)	6.46 ± 0.13 a	2.04 ± 0.15 b
Lys [a] (Lysine)	7.74 ± 0.03 a	2.08 ± 0.05 b
Phe [a] (Phenylalanine)	5.96 ± 0.04 a	2.15 ± 0.07 b
Pro [a] (Proline)	0.46 ± 0.06 b	2.58 ± 0.08 a
Ser (Serine)	2.04 ± 0.08 a	1.01 ± 0.11 b
Thr [a] (Threonine)	0.20 ± 0.03 b	0.90 ± 0.06 a
Tyr [a] (Tyrosine)	5.24 ± 0.05 a	4.17 ± 0.07 b
Cys + Val [a] + Met [a] (Cysteine + Valine + Methionine)	21.92 ± 0.16 a	15.77 ± 0.13 b

[a] Essential amino acids
* Means and standard deviations followed by different letters in each row indicate significant differences (ANOVA, LSD test, $\alpha = 0.05$).

3.3. Amino acid composition in peanut oil-cake and protein concentrate

Amino acid composition in peanut oil cake (POC) and in protein concentrate (PC) obtained at the optimum extraction and precipitation conditions (10:1 water/flour ratio, two extraction stages, shaking for 30 min, pH 9, 40 °C extraction temperature, and pH 4.5 for protein precipitation) are shown in Table 5. Aspartic acid - asparragine (Asx), glutamic acid - glutamine (Glx), and cysteine, valine and metionine (Cys + Val + Met) were the major amino acids in both samples (PCO and PC). PC had a higher percentage of Asx (34.41%), Glx (24.13%), proline (2.58%) and threonine (0.90%) with respect to POC. On the contrary, POC had a higher content of glycine (1.84%), isoleucine + leucine (6.46%), lysine (7.74%), phenylalanine (5.96%), serine (2.04%), tyrosine (5.24%) and Cys + Val + Met (21.92%) than PC.

Ferreyra et al. (2007) and Kim et al. (1992) also reported higher proportions in the contents of glutamic acid and amino acids in the peanut flour and peanut protein isolates, respectively. Other authors (Neucere, 1969; Dawson, 1971; Basha and Cherry, 1976; Kim *et al*., 1992) reported peanut seed proteins with high percentages of aspartic acid, glutamic acid, arginine and glycine and low percentages of cysteine and methionine. The contents of acid and basic amino acids correspond to 31-32 g and 15-16 g per 100 g peanut protein, respectively (Neucere, 1969; Dawson, 1971; Basha and Cherry, 1976; Kim *et al*., 1992).

4. CONCLUSION

The preparation of peanut protein concentrates was affected by changes in the extraction and precipitation conditions such as temperature, extraction pH, ionic strength, and number of extraction stages, and the precipitation pH. The extraction conditions which resulted in higher protein yield from peanut oil-cake were 10:1 water/flour ratio, pH 9 for extraction solution, with no NaCl, 2 extraction stages, shaking for 30 min, 40 °C, and pH 4.5 for protein precipitation.

The residual peanut oil-cake is a sub product of the oil extraction industry, and it could be extracted efficiently under the conditions described in this study in order to obtain a peanut protein concentrate with protein content higher than 85%. This kind of protein concentrate could be a potential source of vegetal proteins with applications in different industries and processes.

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