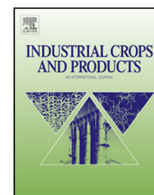
Industrial Crops and Products 85 (2016) 353–360

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop



Improving bioactivities of *Jatropha curcas* protein hydrolysates by optimizing with response surface methodology the extrusion cooking process

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A R T I C L E I N F O

Article history:
Received 15 October 2015
Received in revised form
28 December 2015
Accepted 30 December 2015
Available online 11 February 2016

Keywords:
Extrusion
Protein hydrolysates
Antioxidant capacity
Antihypertensive activity
Response surface methodology

A B S T R A C T

Bioactive peptides, as product of hydrolysis of diverse food proteins, exert various biological roles of great interest in health care. Several pre-hydrolysis treatments of food proteins are commonly used to improve specific bioactivities of protein hydrolysates. In this study, we employed an optimized extrusion cooking process before applying an enzymatic hydrolysis in order to increase bioactivities of *Jatropha* protein hydrolysates (JPH). Response surface methodology (RSM) was used to study the influence of two main independent variables of the single-screw extruder operation conditions: extrusion temperature (ET) and screw speed (SS). Bioactivities such as antioxidant capacity (AOXC) and antihypertensive activity (ACEI-activity) were selected as response variables. The model was statistically appropriate to describe both responses. Results revealed that both ET and SS significantly influence on AOXC, while only the ET showed influence on ACEI-activity. According to the numerical method used of RSM, the optimum operation conditions were ET = 160 °C and SS = 200 rpm. Optimized JPH exhibited a significant increase in both responses AOXC and ACEI-activity. Along with this increase, optimized JPH presented greater protein degree of hydrolysis indicating the presence of short biologically active peptides. Additionally, extrusion cooking process decreased the levels of the major antinutritional factors commonly present in raw *Jatropha* cake. Overall, this study might contribute to appraise *Jatropha* as a suitable source of bioactive compounds for the development of nutraceutical products highlighting the economic importance of this crop, and shows extrusion cooking process as appropriate method to improve food quality and increase protein digestibility.

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1. Introduction

Jatropha (*Jatropha curcas* Linn.), commonly known as physic nut, is a member of the Euphorbiaceae family native to Central America and widespread to most tropical regions (Heller, 1996). This plant has drawn the attention of biofuel entrepreneurs because of its oil-rich seeds with favorable properties for biodiesel production (Kabbashi et al., 2015). However, in order to turn *Jatropha* biodiesel production into a more profitable process, extra processing routes considering the by-product are needed.

In this context, *Jatropha* cake obtained after oil extraction normally presents up to 40% of protein content although the toxicity of *Jatropha* is well documented (Goffer et al., 2015). Previous studies attribute this toxicity to the presence of high levels of phorbol esters and antinutritional factors (Johnson et al., 2015). Makkar et al. (1997) found that nontoxic *Jatropha* varieties in Mexico exhibit

Abbreviations: JPH, *Jatropha* protein hydrolysates; DH, degree of hydrolysis; ET, extrusion temperature; SS, screw speed; RSM, response surface methodology; AOXC, antioxidant capacity; ACEI-activity, angiotensin converting enzyme inhibitory activity; TEAC, trolox equivalents antioxidant coefficients.

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http://dx.doi.org/10.1016/j.indcrop.2015.12.084
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negligible amounts of phorbol esters promoting their nutritional use. Furthermore, the extrusion cooking process has been proposed as an effective thermic method to increase the nutritional value of diverse foods. This effect is attributed to a decrease/inactivation of antinutritional factors as well as an improvement in protein digestibility (Guerrero et al., 2012; Fapojuwo et al., 2006).

Protein hydrolysates, commonly generated by food protein digestion, present certain bioactivities that can be used in health care. For example, protein hydrolysates with protection against cellular damage and arterial hypertension have been reported (Medina-Godoy et al., 2012; Valdez-Ortiz et al., 2012). Cellular damage, resulting from a disruption in the dynamic equilibrium between prooxidants and antioxidants, triggers an onset of numerous chronic degenerative diseases including atherosclerosis, diabetes, and cancer (Luna-Vita et al., 2015). Proteins and peptides with antioxidant capacity (AOXC) inhibit cellular oxidation either by biologically designed mechanisms or by nonspecific mechanisms including inactivation of reactive oxygen species, scavenging free radicals, and chelation of prooxidative transition metals (Elias et al., 2008).

Protein hydrolysates have been also shown to reduce arterial hypertension through the inhibition of the modular blood pressure control enzyme called Angiotensin Converting Enzyme (ACE). Therefore, ACE-inhibitory (ACEI) protein hydrolysates are extensively studied in the past decades as important natural sources for the management of arterial hypertension (Valdez-Ortiz et al., 2012; Megías et al., 2009).

AOXC and ACEI-activity of protein hydrolysates are reported to vary depending on the type of amino acids composition and the length of the peptides released during hydrolysis. Indeed, Gallegos-Tintoré et al. (2015) and Luna-Vital et al. (2015) reported that short bioactive peptides exert better bioactivities that long bioactive peptides in *Jatropha* and common beans, respectively. These studies suggest that the effect of extrusion cooking process on the increase in protein digestibility might trigger a positive impact on the release of short bioactive peptides and would therefore increase bioactivities of protein hydrolysates. Based on this assumption, the presented study aimed to potentiate bioactivities of *Jatropha* protein hydrolysate (JPH) by applying the extrusion cooking process at optimal operation conditions. For this purpose, the extrusion cooking variables: extrusion temperature (ET) and screw speed (SS) were varied to increase AOXC and ACEI-activity. Additionally, we determined the effect of the extrusion cooking process on the nutritional value of *Jatropha* cake by measuring the levels of antinutritional factors before and after the process.

2. Materials and methods

2.1. Materials

Nontoxic *Jatropha* seeds, variety Puebla, Mexico, were obtained from the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), located in Guasave Sinaloa, Mexico. ACE (from rabbit lung, CAS No. 9015-82-1) hippuryl-histidy-leucine (HHL, CAS No. 207386-83-2), and alcalase (CAS No. 9014-01-1) were purchased from Sigma Aldrich, (Sigma Chemicals, St. Louis, Mo., U.S.A.). Other chemicals were of analytical grade purity.

2.2. *Jatropha* cake

Jatropha seeds were manually dehulling and submitted to cold pressing to obtain partially defatted screw-pressed cake. The residual oil was removed by stirring with hexane for 24 h, and *Jatropha* cake was then milled with a Cyclone Sample Mill (UD Corp, Boulder, CO, USA) to pass through a 10-US mesh (2 mm) screen, packed in plastic bags, and stored at 4 °C.

Table 1
Proximate composition of *Jatropha* cake.

Component	% On dry weight
Protein (N x 6.26)	51.0 ± 0.4
Fat	2.8 ± 0.1
Ash	9.5 ± 0.2
Carbohydrates	36.7 ± 0.9

Values are means and SD of triplicate determinations.

2.3. Proximate composition

The following AOAC (1999) official methods were used to determine the proximate composition of *Jatropha* cake: moisture (method 925.09B); ashes (method 923.03); lipids (method 920.39), and protein (method 960.52). All measurements were performed in triplicate and results were expressed in percentage (%) on dry weight.

2.4. Extrusion conditions

The extrusion cooking process was carried out with a single-screw laboratory extruder Model 20 DN (CW Brabender Instruments, Inc., NJ, USA) presenting the following characteristics: a screw-diameter of 12 mm, lengh/diameter ratio of 20:1, nominal compression ratio of 2:1, and a die opening of 3 mm. The extrusion process was done according to the methodology of Milán-Carrillo et al. (2002). The extrusion temperature (ET, temperature at die end of barrel) and screw speed (SS) were selected as independent variables in the design. Other extrusion conditions were maintained constant including the screw-operated hopper fed the extruder at 30 rpm, and the feed moisture content at 28%. Extrudates were cooled and dried at RT for 2 days, then milled with a Cyclone Sample Mill (UD Corp, Boulder, CO, USA) to pass through a 100-US mesh (0.15 mm) screen, packed in plastic bags, and stored at 4 °C.

2.5. Experimental design

A central composite design of response surface methodology (RSM) was used to optimize the extrusion cooking process for the maximum AOXC and ACEI-activity of JPH. The independent variables, ET (X_1, 50–160 °C) and SS (X_2, 50–240 rpm) were defined as factors. Literature data and preliminary experiments were taken into account to determine the levels of the independent parameters. CDD conducted 13 experimental points including five replicates at the central point and four ($\lambda = 1.414$) axial points for a full factorial design. Individual experiments were carried out in random order. Table 2 presents the coded and actual values of the experimental matrices for the design. Experimental data were fitted to the quadratic polynomial regression models expressing the antioxidant capacity (Y_{AOXC}) and antihypertensive activity ($Y_{ACEI\text{-}activity}$). The following model was developed to describe the two response (Y) surfaces:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 \quad (1)$$

Y is the value of the considered experimental predicted response variable (AOXC or ACEI-activity); X_1 and ${X_1}^2$ are the values of linear and quadratic coefficients for ET, X_2 and ${X_2}^2$ are the values of linear and quadratic coefficients for SS, β_0 is the constant value, β_1 and β_2 are linear coefficients, β_{12} is the interaction coefficient, β_{11} and β_{22} are quadratic coefficients. Applying the stepwise regression procedure, non-significant terms ($p \geq 0.1$) were deleted from the second order polynomial and a new polynomial was recalculated

to obtain a predictive model for each variable (Khuri and Cornell, 1987). Design-Expert software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA) was used for multiple regression analysis (R^2) and analysis of variance.

2.6. Optimization analysis

The numerical method of RSM was used to calculate desirability for the optimization analysis. This approach is a multicriteria methodology often applied when various responses have to be considered at the same time and it is necessary to find optimal comprises between the total numbers of responses taken into account. The Derringer function or desirability (*D*) function is the most important and most currently used multicriteria methodology in the optimization of analytical procedures (Bezerra et al., 2008). The global *D* value was analyzed base on individual desirabilities. Statistical analyses were performed operating the Design-Expert software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA).

2.7. Antinutritional factors

Jatropha cake was extruded according to the statistic design, and analyzed for tannins (Broadhurst and Jones, 1978), trypsin inhibitors (Kakade et al., 1974), phytic acid (Makkar and Becker, 1997), and lectin. The latter was determined by the hemagglutination test using rabbit erythrocytes after trypsinization (Makkar and Becker, 1997). Lectin activity was expressed as the reciprocal of minimum amount of sample in mg required to show agglutination after dilution in 1 mL of final assay medium.

2.8. Characterization of JPH

2.8.1. Enzymatic hydrolysis

Protein hydrolysis was carried out with the alcalase enzyme according to the methodology of Humiski and Aluko (2007). Briefly, 3 g from each sample were dissolved in 30 mL of deionized water to prepare 10% (w/v) solutions. The hydrolysis reaction were carried out at optimal condition for the enzyme; 4% (v/w) enzyme:substrate ratio, pH 9.0 at 50 °C, for 1 h, with the thermostated hotplate stirrer (IKA™ C-MAG HS-7, IKA Works Inc. Wilmington, USA) and a Triator T-50 (Metter-Toledo Inc. Las Vegas, NV, USA). After the hydrolysis, the enzyme was inactivated and samples were centrifuged at 10,000 × *g* at RT for 30 min. The supernatant containing JPH was collected, lyophilized, and preserved at −20 °C until assays.

2.8.2. Determination of degree of hydrolysis

Degree of hydrolysis percentage (DH%) was determined based on the precipitation method described by Ling et al. (2013) with modifications. This value was estimated by measuring soluble nitrogen content in 10 g/100 g of trichloroacetic acid (TCA) and determining its proportion versus total nitrogen concentration in the protein concentrate suspension according to Eq. (2):

$$\mathrm{DH\%} = \left[\frac{10\ \mathrm{g\ TCA/100\ mL\ soluble\ -N}}{\mathrm{total\ N\ in\ sample}}\right] \times 100 \quad (2)$$

2.8.3. Determination of AOXC

AOXC was determined with the oxygen radical absorbance capacity (ORAC) assay, as described by Ou et al. (2001). In this method, the antioxidant action of hydrogen atom transfer as well as single electron transfer is measured. Samples were evaluated against a standard of Trolox with fluorescein as a probe. Peroxyl radicals were generated by 2-2′-azobis (2-amidinopropane) dihydrochloride, and fluorescent loss was monitored in a Synergy microplate reader (Dynergy™ HT Multidetection, BioTek, Inc, Winoosgki, VT, USA). The absorbance of excitation and emission was set at 485 nm and 538 nm, respectively. Results were expressed as millimoles of Trolox equivalents antioxidant coefficients per mg of protein sample (mmol TEAC/mg).

Table 2
Optimization of extrusion cooking process of *Jatropha* cake using Central Composite Design.

Analytical parameters	Unit	Code	Levels				
			−1.414	−1	0	+1	+1.414
Extrusion temperature (ET)	°C	X_1	50	66	105	244	160
Screw speed (SS)	rpm	X_2	50	78	145	212	240

2.8.4. Determination of ACEI-activity

ACEI-activity was determined according to the reported by Miguel et al. (2006). The basis of the method is that ACE hydrolyses the substrate Hippuryl-Histidyl-Leucine (HHL) into hippuric acid and releases the peptide His-Leu. The ACE-activity was measured by determining the residual concentration of hippuric acid. The reaction mixture, containing HHL and the sample, was prepared in the buffer ACE (50 mM sodium borate, 0.5 M sodium chloride, pH 8.3). The mixture was pre-incubated at 37 °C for 5 min, and the ACE was added to a final concentration of 2.5 mU/mL. The reaction was stopped by the addition of 150 μL of 1 N HCl, followed by the addition of 1 mL of analytical grade ethyl acetate. The mixture was centrifuged at 5000 × *g* at RT for 10 min. Seven hundred fifty microliters of the organic phase was collected, transferred into a test tube, and incubated at 37 °C until complete evaporation. The residue (hippuric acid) was dissolved in 600 μL of MilliQ-water and the concentration of hippuric acid was determined at 228 nm. Results were expressed as IC_{50} (concentration needed to inhibit 50% of the enzymatic activity). The IC_{50} value of each sample was obtained adjusting data to a nonlinear regression model using the Hill's equation (Weiss, 1997).

3. Results and discussion

3.1. Proximate composition of Jatropha cake

The proximal composition of *Jatropha* cake indicated in Table 1 shows it to be rich in protein (51%) and compares favorably with other meals as a potential non-conventional source of proteins (Young et al., 2014; Ayerza and Coates, 2011). Many metabolic studies have confirmed the capacity of *Jatropha* as a novel source of protein. Our data were similar to those results previously reported in the literature for Capoverde and Nicaragua varieties (Waraporn et al., 2009).

3.2. Influence of the extrusion cooking process on antinutritional factors of Jatropha cake

The present study showed that the extrusion cooking process alters the antinutritional factors levels from those normally reported in raw *Jatropha* cake (Table 3). Whereas thermolabile antinutritional factors such as lectin and trypsin inhibitors were totally or partially removed depending on the extrusion operation conditions, the phytic acid (PA) exhibited a more thermostable activity. Similar results were found by Abd El-Hady and Habiba (2003), in which the inactivation of PA by thermic methods was only possible using extreme temperature. Our study showed an increase in PA content at higher ETs analyzed; effect that can be attributed to the negative charge of this compound that leads to a greater interaction with macromolecules (proteins and lipids), forming complex structures and thus affecting its analytical detection by the applied method (Makkar et al., 2007). We hypothesize

Table 3
Effect of CCD used for optimization of extrusion cooking process over response variables.

[a]Assay no	Actual variables		Antinutritional factors			Response variables (*Y*)		
	[b]ET	[c]SS	[d]TI	Lectin	Phytic acid	[e]DH%	[f]AOXC	[g]ACEI-activity
1	66(−1)	78(−1)	19.40	2.88	5.4	42.7	110.6	$0.80E^{-2}$
2	144(+1)	78(−1)	0.17	ND	7.0	53.6	112.7	$0.26E^{-2}$
3	66(−1)	212(+1)	17.97	2.88	5.9	46.9	169.3	$0.85E^{-2}$
4	144(+1)	212(+1)	0.25	ND	7.2	53.8	156.7	$0.54E^{-2}$
5	50(−1.414)	145(0)	19.64	2.88	4.5	42.3	145.0	$0.69E^{-2}$
6	160(+1.414)	145(0)	0.26	ND	7.9	54.2	151.0	$0.08E^{-2}$
7	105(0)	50(−1.414)	0.44	ND	7.2	51.7	99.5	$1.14E^{-2}$
8	105(0)	240(+1.414)	2.24	ND	7.1	51.9	164.8	$1.57E^{-2}$
9	105(0)	145(0)	1.83	ND	7.0	55.3	110.3	$1.52E^{-2}$
10	105(0)	145(0)	2.29	ND	7.2	51.2	120.0	$1.25E^{-2}$
11	105(0)	145(0)	1.68	ND	7.2	55.0	130.0	$1.34E^{-2}$
12	105(0)	145(0)	1.91	ND	6.9	50.8	105.6	$1.55E^{-2}$
13	105(0)	145(0)	1.93	ND	7.0	53.1	123.7	$1.45E^{-2}$
Raw *Jatropha* cake	–	–	20.20	2.88	5.9	33.6	98.7	$9.80E^{-2}$

Note: ND Not detected levels.
[a] Does not correspond to order of processing.
[b] Extrusion temperature (X_1, °C).
[c] Screw speed (X_2, rpm).
[d] Trypsin inhibitors (mg trypsin inhibited/g of DM); Lectin (mg/mL); Phytic acid (%).
[e] Degree of hydrolysis (%).
[f] Antioxidant capacity (mmol TEAC/mg).
[g] Angiotensin corverting enzyme inhibitory activity [IC_{50} (μg/ml)]; Tannis were no detected.

that the impact of extrusion cooking process caused the rupture of these complex structures and the release of PA causing a better analytical detection. Although the antinutritional effect of PA is widely reported, recent previous studies have shown several beneficial effects including control of blood glucose and triglycerides levels (Omoruyi et al., 2013) as well as the inhibition of colon carcinogenesis (Okazaki and Katayama 2014). On the other hand, the presence of low levels of tannins in raw *Jatropha* cake have been previously reported (Johnson et al., 2015), however, we did not detect this antinutritional factor in the variety analyzed.

In summary, this study indicated that indeed extrusion cooking process improves *Jatropha* cake quality by decreasing the main antinutritional factors reported in raw material such as lectin and trypsin inhibitors, two antinutritional factors highly related to protein digestibility.

3.3. Influence of the extrusion cooking process on the DH% of JPHs

Results revealed that the DH% increase by the effect of extrusion cooking process in a range from 42.3% to 55.3% in comparison to 33.6% presented by raw JPH (Table 3). Although the DH% was analyzed in all extrusion treatments, this was not included in the optimization analysis as an extra response variable. The analysis of variance of independent variables showed that DH% was significantly ($p < 0.01$) dependent on the linear and quadratic terms of ET, while SS, and the interaction between variables yielded no significant effect (Table 4). The mathematical regression model for DH% (Y) presenting only the significant terms ($p \leq 0.05$) in coded factors was the following:

$$(Y_{DH\%}) = +53.08 + 4.33X1 - 2.61X1^2 \quad (3)$$

With actual factors:

$$(Y_{DH\%}) = +11.51792 + 0.52667ET - 1.7151(ET)^2 \quad (4)$$

The regression model showed a good fit with the experimental data with an R^2 value of 0.9082, and the lack of fit was not significant relative to the pure error (Table 4). This model was suitable to generate the response surface. Surface plot in Fig. 1 (A and B) displays the effect of extrusion cooking process on DH% showing an increase in all extrusion conditions. The greatest point of DH% was outside of the experimental region, finding the highest value (55.3%) at the maximum ET used (160 °C).

The increase in DH% observed can be attributed to the fact that in extruded samples, proteins were most likely denatured and might have exhibited more cleavage sites for the enzymatic action increasing the protein hydrolysis (Benjakul and Morrissey, 1997). Besides this, the decrease in antinutritional factors, as discussed above, might have contributed to improve the enzyme catalytic action (Abd El-Hady and Habiba 2003).

3.4. Influence of the extrusion cooking process on bioactivities of JPHs

3.4.1. Predictive models for response variables

The study used a central composite design to develop prediction models for optimizing the extrusion conditions of a single-screw extruder (Table 3). The design conducted 13 experimental conditions to find the optimal values of ET and SS in order to maximize the response variables, AOXC and ACEI-activity.

3.4.2. Influence of the extrusion cooking process on AOXC

The influence of extrusion cooking process on AOXC of JPHs was analyzed using the ORAC. As shown in Table 3, raw JPH presented AOXC value of 98.7 mmol TEAC/mg. This data was slightly higher than the results reported previously by Medina-Godoy et al. (2012), in which authors presented AOXC values ranging from 18.17 to 95.62 mmol TEAC/mg for hard-to-cook chickpea protein hydrolysates. By applying the extrusion cooking process before the enzymatic hydrolysis AOXC values of JPHs increased ranging from 99.5 to 169.3 mmol TEAC/mg. The analysis of variance of the independent extrusion variables showed that AOXC was significantly ($p < 0.01$) dependent on the quadratic term of ET and the linear term of SS ($p < 0.01$). Moreover, interaction between variables showed no significance (Table 4). The mathematical regression model for AOXC (Y) showing only the significant terms ($p \leq 0.05$) in coded factors was the following:

$$(Y_{AOXC}) = +117.92 + 24.38X2 + 14.35X1^2 \quad (5)$$

With actual factors:

$$(Y_{AOXC}) = +178.555894 + 0.096339SS + 9.4362E - 003(ET)^2 \quad (6)$$

Table 4
Regression coefficients and analyses of variance of the second order polynomial models showing in coded factors the relationship among process variables (X) and response variables (Y_k).

Coefficient	Degree of hydrolysis Y (DH%)	Antioxidant capacity Y (AOXC)	Angiotensin converting enzyme inhibitory activity Y (ACEI-activity)
Intercept			
β_0	53.08	117.92	0.014
Linear			
β_1	+4.33*	−0.25^{NS}	−2.141E-003*
β_2	+0.59^{NS}	+24.38*	+1.173E-003^{NS}
Quadratic			
β_{11}	−2.61*	+14.35*	−5.829E-003*
β_{22}	−0.83^{NS}	+6.43	−9.787E-004^{NS}
Interaction			
β_{12}	−1.00^{NS}	−3.67^{NS}	+5.750E-004^{NS}
Lack of fit	0.7395^{NS}	0.8585^{NS}	0.1576^{NS}
R^2	0.9082	0.9319	0.9329
R^2adj	0.8427	0.8832	0.885
P	0.0016	0.0006	0.0006

Note: ^{NS}Non significant.
*Significant at 1%.

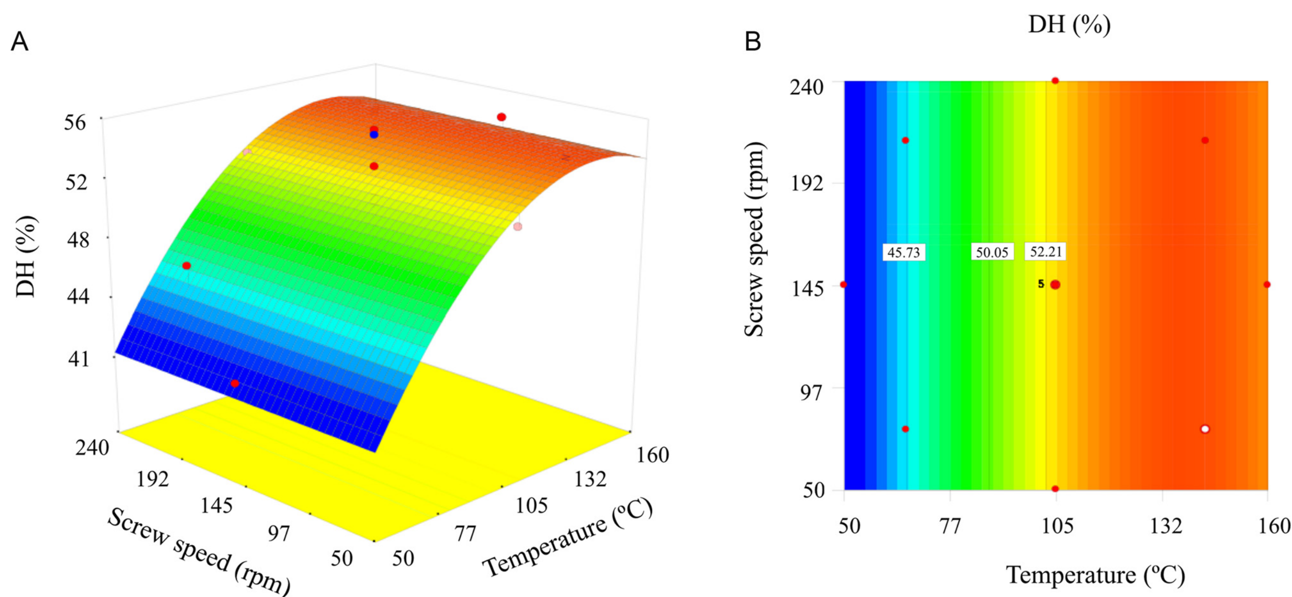

Fig. 1. Response surface plot (A) and the corresponding contour plot (B) showing the combined effect of ET and SS on the DH%.

The regression model showed a good fit with the experimental data, with an R^2 value of 0.9319. The lack of fit was not significant relative to the pure error indicating the adequacy and reproducibility of the model. Fig. 2 (A and B) displays the combined effect of ET and SS on AOXC. In this surface plot it is shown that the maximum point is outside the experimental region, finding the highest AOXC at the highest SS (240 rpm) combined with either low or high ET (50 °C or 160 °C).

The AOXC of protein hydrolysates can be altered by several factors. Chen et al. (1998) reported a correlation between AOXC of soybean hydrolysates with the peptide composition. Moreover, the operational conditions applied for protein isolation and the peptide concentration (Liu et al., 2005) as well as the DH% (Gallegos-Tintoré et al., 2011) have been reported to alter AOXC. In this study, high AOXC values with low ETs were associated with low DH%, suggesting that there are peptides with considerable size presenting a good AOXC. Additionally, high AOXC values were discovered at higher ETs, indicative of the release of another set of peptides. This could be due to the fact that a greater DH% was found, may constitute a large amount of small peptides with high AOXC. However, further studies are needed to confirm this hypothesis.

Overall, our results clearly showed that the effects of the extrusion process potentiates the AOXC of JPHs showing suitable values for the control of oxidative stress, which is a key step to slowing down pathology of diseases related to cellular damage. The exact mechanism underlying the antioxidant activity of protein hydrolysates is not fully understood, however it is suggested that this may be due to the activity of peptides for scavenging free radicals and chelating transition metal ions (Qian et al., 2008; Rajapakse et al., 2005). Besides the effect of the protein hydrolysates on AOXC, in our study the PA might have also influenced on increasing this activity since PA is reported to present antioxidant effect by chelating Fe^{2+} ions (Sakač et al., 2010).

3.4.3. Influence of the extrusion cooking process on ACEI-activity

The influence of the extrusion cooking process on the antihypertensive effect of JPHs was determined by measuring the ACEI-activity of raw and extruded JPHs. Raw JPH exhibited ACEI-activity of IC_{50} 9.80E^{-2} μg/mL. This activity was lower than the

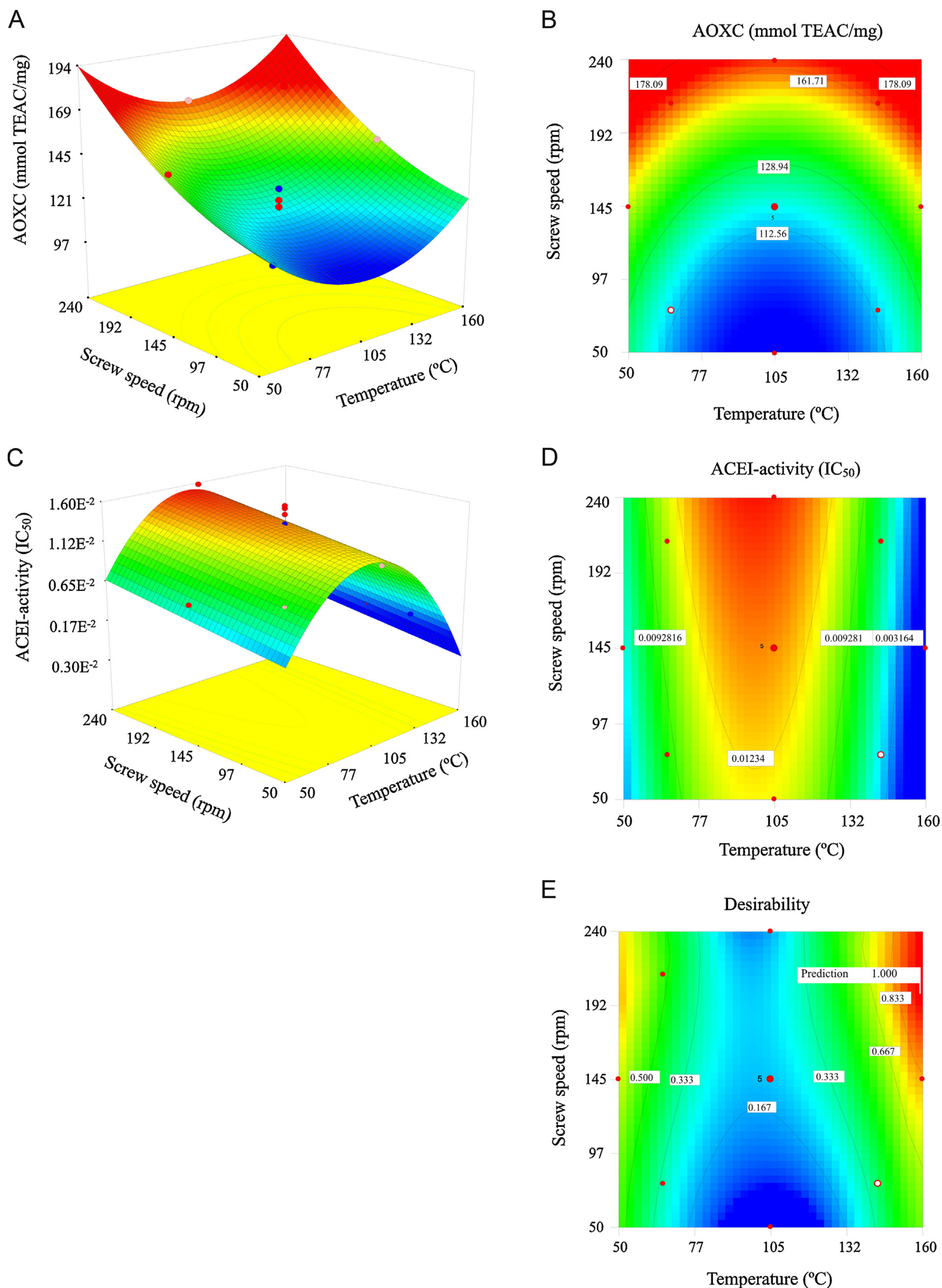

Fig. 2. Response surface plot and the corresponding contour plot showing the combined effect of ET and SS on AOXC (A and B), ACEI-activity (C and D), and contour plot of global desirability for the optimization of both variables (E).

Table 5
Characterization of raw/optimized *Jatropha*.

Parameter	Raw *Jatropha*	Optimized *Jatropha*
Trypsin inhibitors	20.20 mg/g	0.22 mg/g
Lectins	2.88 mg/ml	ND
Phytic acid	5.9%	7.1%
[a]DH% (JPH)	33.6%	57.3%
[b]AOXC (JPH)	98.7 mmol TEAC/mg	173.8 mmol TEAC/mg
[c]ACEI activity (JPH)	IC_{50} 9.80E^{-2} μg/ml	IC_{50} 0.08E^{-2} μg/ml

Note: ND No detected levels; JPH *Jatropha* protein hydrolysates.
[a] Degree of hydrolysis.
[b] Antioxidant capacity.
[c] Angiotensin converting enzyme ihibitory activity.

data reported previously by Valdez-Ortiz et al. (2012) in which authors report ACEI-activity values ranging from IC_{50} 0.10E^{-2} to 3.19E^{-2} μg/mL for protein hydrolysates of common beans. Extrusion cooking process incremented ACEI-activity ranging from IC_{50} 1.57E^{-2} to 0.08E^{-2} μg/mL (Table 2). The analysis of variance showed that ACEI-activity was significantly ($p < 0.01$) dependent on the linear and quadratic terms of ET, while SS, and the interaction between variables did not showed significant effect (Table 4). The mathematical regression model for ACEI-activity (Y) showing only the significant terms ($p \leq 0.05$) in coded factors was the following:

$$\left(Y_{\mathrm{ACEI-activity}}\right) = +0.014 - 2.141E - 003X1 - 5.829E - 003X1^2 \quad (7)$$

With actual factors:

$$\left(Y_{\mathrm{ACEI-activity}}\right) = -0.026038 + 7.17957E - 004ET - 3.83218E - 006(ET)^2 \quad (8)$$

The regression model showed a good fit with the experimental data, with an R^2 value of 0.9329, and the lack of fit was not significant relative to the pure error indicating the adequacy and reproducibility of the model. In plots corresponding to ACEI-activity (Fig. 2 (C and D)), a plateau in relation to the SS variable was observed suggesting that variation of its levels does not affect this system. In the same plots it was observed that the maximum point is outside the experimental region, the highest ACEI-activity (IC_{50} 0.08E^{-2} μg/mL) was found at the maximum ET used (160 °C) where the highest DH% was also found. Therefore, extruded JPHs with greater DH% and presumably containing peptides with low molecular weight did not only show good AOXC, but they also exhibited the highest ACEI-activity. This conclusion is supported by the study of Segura-Campos et al. (2013), in which it was determined that the ACEI-activity of chia protein hydrolysates with four different molecular weights fractions (1 kDa, 3 kDa, 5 kDa, and 10 kDa) had the highest activity at the lowest molecular weight fraction (1 kDa).

3.4.4. Optimization and confirmation

The goal of the optimization process was to achieve the maximum value for AOXC and the minimum IC_{50} value for ACEI-activity. The optimal extrusion conditions given by the numerical method of RSM were ET = 160 °C and SS = 200 rpm. Fig. 2 (F) illustrates the contour plot of the optimization method presenting a global D value of 1.0. Results predicted by the design were 170 mmol TEAC/mg and IC_{50} 0.05E^{-2} μg/mL for AOXC and ACEI-activity, respectively. To confirm the predicted results by the design, a new extrusion experiment was performed at the determined optimal conditions. Table 5 presents the conformation results showing DH% values, AOXC, and ACEI-activity of optimized JPH in comparison to raw JPH. Table 5 also shows the effect of extrusion processing on antinutritional factors of *Jatropha* cake. The optimum extrusion process greatly decreased the concentration of trypsin inhibitors (20.20 mg/g to 0.22 mg/g) and lectins (2.88 mg/ml to no detected levels), however a slight increase in PA content (5.9% to 7.1%) was observed. The optimum extrusion process increased AOXC and ACEI-activity of JPHs. Raw and optimized JPHs exhibited AOXC values of 98.7 and 173.8 mmol TEAC/mg, respectively, and ACEI-activity values of IC_{50} 9.80E^{-2} and 0.80E^{-2} μg/mL. Optimized JPH presented the highest DH% (57.3%), which suggests a direct correlation between DH% and the increase in JPH bioactivities.

To our knowledge, this is the first report that includes optimization of extrusion cooking process based on bioactivities of protein hydrolysates. Our study clearly demonstrated that AOXC and ACEI-activity of JPH were potentiated by the effect of extrusion cooking process. This increase could be ascribed particularly to the different combinations of temperature and cutting force during processing. The extrusion cooking process uses high temperatures and short processing times that might produce changes in proteins making more available the peptide bond cleavage sites. This effect can trigger diverse enzymatic actions and in turn generate protein hydrolysates with different levels of DH% (Reyes-Moreno et al., 2003; Colonna et al., 1989). Our study suggests the presence of short biologically active peptides, however to validate this hypothesis, a comprehensive investigation of peptides must to be made, which would require techniques beyond the scope of this study. However, these findings support the conclusion of other studies that short peptides with low molecular weight exhibit better bioactivities than large peptides or their parent native proteins (Gallegos-Tintoré et al., 2015; Luna-Vita et al., 2015).

4. Conclusions

Response surface methodology was a useful tool for optimization of the extrusion cooking process, which subsequently potentiated AOXC and ACEI-activity of the JPH. The optimized JPH exhibited excellent bioactivities that could potentially be used for the control of degenerative diseases such as hypertension and those generated by oxidative cellular stress. The data generated herein contributes to evaluate *Jatropha* not only as a source of oil for biodiesel production, but also as a suitable source of bioactive compounds for the development of nutraceutical products.

Acknowledgements

The authors thank Sánchez-Magaña LM for his valuable technical support. This work was supported by grants of Universidad Autónoma de Sinaloa (PROFAPI2012/179). MAVF also thanks CONACYT-México for the scholarship granted.

References

Abd El-Hady, E.A., Habiba, R.A., 2003. Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. LWT-Food Sci. Technol. 36, 285–293.
AOAC, 1999. Official Methods of Analysis, 16th Edition. Association of Official Analytical Chemists, Washington, DC.
Ayerza, R., Coates, W., 2011. Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). Ind. Crops Prod. 34, 1366–1371.
Benjakul, S., Morrissey, M.T., 1997. Protein hydrolysates from pacific whiting solid wastes. J. Agric. Food Chem. 45 (9), 3423–3430.
Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76 (5), 965–977.
Broadhurst, R.B., Jones, W.T., 1978. Analysis of condensed tannins using acidified vanillin. J. Sci. Food Agric. 29, 788–794.
Chen, H.M., Muramoto, K., Yamauchi, F., Fujimoto, K., Nokihara, K., 1998. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J. Agric. Food Chem. 46, 49–53.

Colonna, P., Tayeb, J., Mercier, C., 1989. Extrusion cooking of starch and starchy products. In: Mercier, C., Linko, P., Harper, J.M. (Eds.), Extrusion Cooking. American Association of Cereal Chemists, Inc., St. Paul, MN, pp. 247–319.

Elias, R.J., Kellerly, S.S., Decker, E.A., 2008. Antioxidant activity of proteins and peptides. Crit. Rev. Food Sci. Nutr. 48 (5), 430–441.

Fapojuwo, O.O., Maga, J.A., Jansen, G.R., 2006. Effect of extrusion cooking on in vitro protein digestibility of sorghum. J. Food Sci. 52, 218–219.

Gallegos-Tintoré, S., Torres, C.F., Martínez, A.L., Solorza, J.F., Alaiz, M., Girón, J.C., 2011. Antioxidant and chelating activity of *Jatropha curcas* L. protein hydrolysates. J. Sci. Food Agric. 91, 1618–1624.

Gallegos-Tintoré, S., Torres, C.F., Solorza, J.F., Alaiz, M., Girón, J.C., Martínez, A.L., Guerrero, L.C., Vioque, J., 2015. Antioxidant and chelating activity of nontoxic *Jatropha curcas* L. protein hydrolysates produced by in vitro digestion using pepsin and pancreatin. J. Chem. 1, 1–9.

G. Goffer,M. Schmid,A. Stäbler.Characterization of Jatropha curcas L. protein cast films with respect to packaging relevant properties Int J Polym Sci. 2015 (In Press).

Guerrero, P., Beatty, E., Kerry, J.P., de la Caba, K., 2012. Extrusion of soy protein with gelatin and sugars at low moisture content. J. Food Eng. 110, 53–59.

Heller, J., 1996. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. In: Institute of Plant Genetics and Crop Plant Research. Gatersleben/International Plant Genetic Resources Institute, Rome.

Humiski, L.M., Aluko, R.E., 2007. Physicochemical and bitterness properties of enzymatic Pea protein hydrolysates. J. Food Sci. 72, 605–611.

Johnson, O.R., Samuel, S., Belema, S.S., Chiamaka, I.S., 2015. Proximate and toxicological analyses of detoxified *Jatropha Curcas* seeds. J. Pharm. Biol. Sci. 10, 2278–3008.

Kabbashi, N.A., Mohammed, N.I., Alam, Md Z., Mirghani, M.E.S., 2015. Effect of process parameters on yield and conversion of Jatropha biodiesel in a batch reactor. J. Fundam. Renew. Energy Appl. 5, 155.

Kakade, M.L., Rackis, J.J., McGhee, J.E., Pusk, G., 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51, 376–382.

Khuri, A.A., Cornell, J.A., 1987. Response Surfaces: Designs and Analyses, 254. Marcel Dekker Inc, New York, pp. 1–17.

Ling, K.N., Khan, M.A., Said, M., Osman, Ma., Ismail, A., 2013. Optimization of enzymatic hydrolysis of palm kernel cake protein (PKCP) for producing hydrolysates with antiradical capacity. Ind. Crops Prod. 43, 725–731.

Liu, J.R., Chen, M.J., Lin, C.W., 2005. Antimutagenic and antioxidant properties of milk- kefir and soymilk-kefir. J. Agric. Food Chem. 53, 2467–2474.

Luna-Vita, D.A., González de Mejía, E., Mendoza, S., Loarca-Pina, G., 2015. Peptides present in the non-digestible fraction of common beans (*Phaseolus vulgaris* L.) inhibit the angiotensin I-converting enzyme by interacting with its catalytic cavity independent of their antioxidant capacity. Food Funct. 6, 1470–1479.

Makkar, H.P.S., Becker, K., 1997. Potential of Jatropha seed cake as a protein supplement in livestock feed and constraints to its utilization. In: Proceedings of Jatropha 97: International Symposium on Biofuel and Industrial Products from Jatropha curcas and other Tropical Oil Seed Plants, February 23-27, Managua, Nicaragua.

Makkar, H.P.S., Becker, K., Sporer, F., Wink, M., 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J. Agric. Food Chem. 45, 3152–3157.

Makkar, H.P.S., Francis, G., Becker, K., 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 1 (9), 1371–1391.

Medina-Godoy, S., Ambríz-Pérez, D., Fuentes-Gutiérrez, C.I., Germán-Báez, L.J., Gutiérrez-Dorado, R., Reyes-Moreno, C., Valdez-Ortiz, A., 2012. Angiotensin-converting enzyme inhibitory and antioxidative of protein hydrolysates of hard-to-cook chickpeas. J. Sci. Food Agr. 92 (9), 1974–1981.

Megías, C., Pedroche, J., Yust, M.M., Alaiz, M., Girón, C.J., Millán, F., 2009. Purification of angiotensin converting enzyme inhibitory peptides from sunflower protein hydrolysates by reverse-phase chromatography following affinity purification. LWT-Food Sci. Technol. 42, 228–232.

Miguel, M., Aleixandre, M.A., Ramos, M., López, F.R., 2006. Effect of simulated gastrointestinal digestion on the antihypertensive properties of ACE-inhibitory peptides derived from ovalbumin. J. Agric. Food Chem. 54, 726–731.

Milán-Carrillo, J., Reyes-Moreno, C., Camacho, H.I.L., Rouzaud, S.O., 2002. Optimization of extrusion process to transform hardened chickpeas (*Cicer arietinum* L.) into a useful product. J. Sci. Food Agric. 82, 1718–1728.

Okazaki, Y., Katayama, T., 2014. Dietary phytic acid modulates characteristics of the colonic luminal environment and reduces serum levels of proinflammatory cytokines in rats fed a high-fat diet. Nutr. Res. 34 (12), 1085–1091.

Omoruyi, F.O., Budiaman, A., Eng, Y., Olumese, F.E., Hoesel, J.L., Ejilemele, A., Okorodudu, A.O., 2013. The potential benefits and adverse effects of phytic Acid supplement in streptozotocin-induced diabetic rats. Adv. Pharmacol. Sci., 172494.

Qian, Z.J., Jung, W.K., Kim, S.K., 2008. Free radical scavenging activity of a novel antiox- idative peptide purified from hydrolysate of bullfrog skin, Rana catesbeiana Shaw. Bioresour. Technol. 99, 1690–1698.

Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluo-rescent probe. J. Agric. Food Chem. 49 (10), 4619–4626.

Rajapakse, N., Mendis, E., Jung, W.K., Je, J.Y., Kim, S.K., 2005. Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. Food Res. Int. 38, 175–182.

Reyes-Moreno, C., Milán-Carrillo, J., Gutiérrez-Dorado, R., Paredes-López, O., Cuevas-Rodríguez, E.O., Garzón-Tiznado, J.A., 2003. Instant flour from quality protein maize (*Zea mays* L). Optimization of extrusion process. LWT-Food Sci. Technol. 36, 685–695.

Sakač, M., Čanadanovič-Brunet, J., Mišan, A., Tumbas, V., Medič, D., 2010. Antioxidant activity of phytic acid in lipid model system. Food Technol. Biotechnol. 48 (4), 524–529.

Segura-Campos, M.R., Peralta-González, F., Chel-Guerrero, L., Bentacur-Acona, D., 2013. Angiotensin I-converting enzyme inhibitory peptides of Chia (*Salvia hispanica*) produced by enzymatic hydrolysis. Int. J. Food Sci. 158482, 1–8.

Valdez-Ortiz, A., Fuentes-Gutiérrez, C.I., Germán-Báez, L.J., Gutiérrez-Dorado, R., Medina-Godoy, S., 2012. Protein hydrolysates obtained from Azufrado (sulphur yellow) beans (*Phaseolus vulgaris*): nutritional, ACE-inhibitory and antioxidative characterization. LWT-Food Sci. Technol. 46, 91–96.

Waraporn, A., Pilanee, V., Phanu, S., Taweesiri, M., 2009. Optimization of protein hydrolysate production process from *Jatropha curcas* Cake. World Acad. Sci. Eng. Technol. 53, 109–112.

Weiss, J.N., 1997. The hill equation revisited: uses and misuses. FASEB J. 11, 835–841.

Young, Y.S., Gui, B., Arnison, P.G., Wang, Y., Reaney, J.T., 2014. Flaxseed (*Linum usitatissimum* L.) bioactive compounds and peptide nomenclature: a review. Trends Food Sci. Technol. 38, 5–20.