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Effect of extrusion process on antioxidant and ACE inhibition properties from bovine haemoglobin concentrate hydrolysates incorporated into expanded maize products

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Abstract
Extrusion process has been widely used for the development of many functional foods. The aim of this study was to assess the effect of extrusion process on antioxidant and angiotensin-converting enzyme (ACE) inhibition properties from bovine haemoglobin concentrate (BHC) hydrolysates (P, FC, PF and FCF). Extrusion was carried out with a Brabender single screw extruder. The ACE inhibition and the antioxidant capacity (AC) were estimated by the inhibition of the ACE and ABTS + · radical cation expressed as Trolox equivalent antioxidant capacity (TEAC), respectively. The ACE inhibition and TEAC values from hydrolysates were significantly higher than that from BHC. The highest ACE inhibition corresponded to P hydrolysate and the highest TEAC corresponded to PF and FCF hydrolysates. The ACE inhibition and AC from extruded products with added hydrolysates were higher than that from maize control; however, the extrusion process modified both ACE inhibition and AC formerly present in hydrolysates.

Keywords: *extrusion, antihypertensive properties, antioxidant capacity, haemoglobin*

Introduction

In recent years, protein hydrolysates are also considered a source of bioactive peptides. Bioactive peptides are inactive amino acid sequences within the precursor protein, but exert biological activity when released by enzymatic hydrolysis or chemical *in vitro* digestion or fermentation. These peptides have different activities including immunomodulatory, antioxidant, antimicrobial, antithrombotic and antihypertensive activities (Clare and Swaisgood 2000). Antihypertensive peptides inhibit the angiotensin-converting enzyme (ACE). This enzyme hydrolyzes the decapeptide angiotensin I to yield the vasoconstrictor octapeptide angiotensin II, and in addition hydrolyzes the vasodilator peptide bradikinin. Angiotensin II is involved in the release of aldosterone, a sodium-retaining steroid, which increases blood pressure. Thus, the activity of ACE increases blood pressure by raising vascular resistance and fluid volume. High blood pressure is associated with a higher risk of cardiovascular disease and stroke. ACE inhibitors are used in the treatment of hypertension and have been studied for the treatment of chronic heart failure and myocardial infarction (Pedroche et al. 2002).

Peptides that contain hydrophobic amino acids, valine or leucine, at the *N*-terminal positions, and proline, histidine or tyrosine in the sequence are reported to have antioxidant activities. Also, studies with peptides that contain histidine have demonstrated that these peptides can act as metal-ion chelators, active-oxygen quencher and hydroxyradical scavenger. The ability of protein hydrolysates to inhibit deleterious changes caused by lipid oxidation appears to be related to the nature and composition of the different

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peptide fractions produced, depending on the protease specificity (Pihlanto 2006).

Antihypertensive and antioxidant peptides can be isolated from diverse protein sources, ranging from vegetable to animal proteins (Vioque et al. 2006). Animal blood produced during slaughtering is a valuable protein source, which has been used to obtain several bioactive peptides, such as opioid, having bacterial growth-stimulating, ACE-inhibiting and antioxidant activities (Chang-Kee and Heuyn-Kil 2000). Such hydrolysates with bio-functional properties may be incorporated into food vehicle. A functional food is any food or food ingredient that provides a beneficial physiological effect on health, other than the nutrition provided by the foods (Yust et al. 2003).

The extrusion process has been widely used for the development of many cereal-based foods and could be an interesting alternative to design functional foods (Poltronieri et al. 2000; Moreira-Araújo et al. 2008). Extrusion is considered an appropriate technology for the processing of cereals, pulses and oilseeds because it precooks starchy material and inactive enzymes, homogenizes the mixture and generates different textures (Harper 1981).

The aim of this study was to assess the effect of extrusion process on antihypertensive and antioxidant properties from bovine haemoglobin concentrate (BHC) hydrolysates incorporated into expanded maize products.

Materials and methods

Chemicals and reagents

Dithiotreitol, *o*-phthaldialdehyde (OPA), sodium dodecyl sulphate (SDS), bovine serum albumin, L-serine, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt, potassium persulphate hippuryl-L-histidyl-L-leucine (HHL), captopril, 2,4,6-trichloro-*s*-triazine (TT) and phenylmethylsulphonyl fluoride (PMSF) and flavourzyme (F) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was purchased from Aldrich Chemical Co. (Gillingham, UK). All reagents were analytical grade. The other enzymes protex 6L (P) and fungal protease concentrate (FC) were provided by Genencor SA (Arroyito, Argentina).

Bovine haemoglobin concentrate

Hydrolysates were prepared with commercial BHC supplied by Yeruvá SA (Esperanza, Argentina). BHC composition was determined using AOAC (1995) procedures. Total iron was measured by atomic absorption spectroscopy after dry mineralization. Ash was removed with 20% HCl (v/v). An atomic absorption spectrum (IL 551 device, Instrumentation Laboratory, Lexington, Massachusetts, USA) was used.

Hydrolysates preparation

Hydrolysates were obtained using 800-ml batch thermostatized reactor. The reaction pH was continuously measured using a pH meter (IQ Scientific Instruments, Loveland, Colorado, USA) and adjusted by adding base (NaOH) or acid (HCl) with a burette. Substrate concentration was $80\,g\,kg^{-1}$ in every case. Working conditions for the enzymes were *T*, 60°C; pH, 9.5; enzyme/substrate (E/S) ratio, $1\,g\,kg^{-1}$; *T*, 55°C; pH, 4.3; E/S ratio; $5\,g\,kg^{-1}$ and *T*, 55°C; pH, 7.0; E/S ratio, $10\,g\,kg^{-1}$ for P, FC and F, respectively.

Once the hydrolysis was complete, the enzyme was inactivated by thermal treatment following the manufacturer guidelines. BHC hydrolysates were prepared as follows:

- Simple hydrolysis system
  - (a) Hydrolysis P: P enzyme (2 h).
  - (b) Hydrolysis FC: FC enzyme (2 h).
- Sequential hydrolysis system
  - (c) Hydrolysis PF: P enzyme (2 h) + F enzyme (4 h). Total reaction time; 6 h.
  - (d) Hydrolysis FCF: FC enzyme (2 h) + F enzyme (4 h). Total reaction time; 6 h.

The hydrolysates were frozen and lyophilized.

Free amino groups were measured using OPA, according to Nielsen et al. (2001), and the degree of hydrolysis (DH) was calculated as follows:

$$DH = \frac{(h - h_0)}{h_{tot}} \times 100\%, \quad (1)$$

where h_{tot} is the total number of peptide bonds in the protein substrate ($8.3\,mEq\,g^{-1}$ protein), h is the number of peptide bonds cleaved during hydrolysis and h_0 is the content of free amino groups in the substrate.

Extruded products obtention

Extruded products were obtained using commercial maize grits in which the composition in dry base was protein; $69\,g\,kg^{-1}$, fat, $4.4\,g\,kg^{-1}$; ash, $2.7\,g\,kg^{-1}$; carbohydrates, $802.4\,g\,kg^{-1}$ and moisture, $121\,g\,kg^{-1}$. The composition was determined using AOAC (1995) procedures. BHC and each hydrolysate were added to commercial maize grits at $5\,g\,kg^{-1}$ ratio. Maize grits were conditioned to the corresponding moisture 1 h before each run. BHC and its hydrolysates were added to the water of hydration. Extrusion was carried out with a 20 DN Brabender (South Hackensack, NJ, USA) single screw extruder in the following conditions: 4:1 compression ratio screw, 150 rpm, $155\,g\,kg^{-1}$ grits moisture and 170°C barrel temperature. The feeding rate of the extruder was at full capacity. The following samples were obtained: extruded maize (M), extruded maize + P hydrolysate (MP), extruded maize + FC hydrolysate (MFC),

extruded maize + PF hydrolysate (MPF) and extruded maize + FCF hydrolysate (MFCF).

Water solubility of extruded products

Water solubility was calculated as soluble solids per 100 g of extruded products (d.b.). This was done by dispersing 2.5 g of flour in 50 ml water, by shaking for 30 min and by centrifuging at 2000*g*. Soluble solids were determined after water evaporation in an oven at 105°C (Gonzalez et al. 2004).

ACE inhibition properties

Preparation of ACE. ACE was prepared according to Hayakari et al. (1978) with modifications. Rabbit lungs were used as starting material. Tissue samples (1 g) were diced and homogenized in 5 ml of ice-cold 0.1 M potassium phosphate buffer, pH 8.3, containing 0.25 M sucrose and 0.1 mM PMSF. The homogenate was centrifuged at 12,000 *g* for 5 min, and the resulting supernatant was used as the source of ACE.

Assay of ACE activity inhibition of BHC and its hydrolysates. ACE activity was determined according to Hayakari et al. (1978) with modifications. This method relies on the colorimetric reaction of hippuric acid with TT, developed in a 1.995 ml incubation mixture containing 175 µl potassium phosphate buffer (0.1 M), pH 8.3, 15 µl sodium chloride (5 M), 20 µl of HHL ($3.3\,g\,l^{-1}$) and 20 µl of enzyme extract. Incubation was carried out for 45 min at 37°C. The reaction was terminated by the addition of 665 µl of TT ($30\,g\,l^{-1}$) in dioxane, followed by 1.1 ml of 0.1 M phosphate buffer, pH 8.3. After centrifugation of the reaction mixtures at 12,000 *g* for 10 min, the absorbance was measured at 382 nm. The ACE inhibition from BHC and its hydrolysates was evaluated in 20 µl of $2.3\,g\,l^{-1}$ of proteins. ACE inhibition activity expressed as ACE inhibition (%) was calculated as

$$\mathrm{ACE_{inhibition}}(\%) = 100 - \left[\left(\frac{\mathrm{AS} - \mathrm{ABS}}{\mathrm{AE} - \mathrm{ABE}}\right) \times 100\right], \tag{2}$$

where AS is the optical density of ACE with sample and substrate, ABS is the optical density of ACE and sample, AE is the optical density of ACE with substrate and ABE is the optical density of substrate without ACE or sample.

Assay of ACE activity inhibition of extruded products containing BHC and its hydrolysates. To 125 mg of flour from extruded samples, 1 ml of distilled water was added. The mixture was shaken with vortex for 10 s, allowed to stand for 10 min, shaken again and centrifuged at 10,000 *g* for 5 min. The ACE activity was determined in 20 µl of the supernatant.

Antioxidant capacity

To estimate the antioxidant capacity (AC), ABTS + · decolorization assay proposed by Pukalskas et al. (2002) was used. ABTS + · radical cation was produced by reacting 10 ml of $2\,mmol\,l^{-1}$ ABTS + · solution in $0.01\,mmol\,l^{-1}$ phosphate buffered saline (PBS, pH 7.4) with 40 µl of $70\,mmol\,l^{-1}$ potassium persulphate solution and allowing the mixture to stand in the dark at room temperature for 16–17 h before use. Then, the ABTS + · solution was diluted with the same buffer to an absorbance of 0.800 ± 0.030 at 734 nm.

An aliquot of sample (see below) was mixed with 990 µl ABTS + · diluted solution and the absorbance was read at 734 nm.

The inhibition rate of ABTS + · was calculated as follows:

$$I = \frac{A_{\mathrm{ABTS}\,(t_0)} - A_{\mathrm{ABTS}\,(t_n)}}{A_{\mathrm{ABTS}\,(t_0)}}, \tag{3}$$

where I is the inhibition rate, A_{ABTS} (t_0) is the absorbance at zero time and A_{ABTS} (t_n) is the absorbance at n time. All experiments were carried out in triplicates.

To estimate the Trolox equivalent antioxidant capacity (TEAC), a concentration-response curve for the absorbance at 734 nm for ABTS + · as a function of concentration of standard Trolox solution (0–$2.5\,mmol\,l^{-1}$) in $0.01\,mmol\,l^{-1}$ (PBS, pH 7.4) was plotted. The absorbance reading was taken at 6 min after initial mixing.

The AC from BHC hydrolysates (P, FC, PF and FCF), native BHC and expanded maize products was determined as described below.

AC of BHC and its hydrolysates. Lyophilized samples were dispersed at $50\,g\,kg^{-1}$ in PBS (pH 7.4) and then centrifuged at room temperature. The protein content of the supernatant was determined by Lowry et al. (1951). The samples were diluted to $5\,g\,l^{-1}$ of protein and an aliquot of 10 µl was evaluated for the AC. Also, TEAC was calculated.

To study the reaction kinetics, the absorbance at 734 nm was taken for every 1 min after the start of reaction until 6 min. The data were fitted with the following equation:

$$y = a \times \left(1 - \mathrm{e}^{-bt}\right), \tag{4}$$

where y is the inhibition rate, a and b are the regression parameters and t is the time (min).

The maximum value of inhibition ($I_{\max}$) and the initial velocity of reaction ($V_{(0)}$) for each sample were obtained as described below.

When t tends to infinity, y is the maximum inhibition ($y_{(t\to\infty)} = a = I_{\max}$). The first derivative of Equation (4) respect to the time is

$$\frac{dy}{dt} = a \times b \times e^{-bt} \quad (5)$$

and for $t = 0$ the product of a by b is the initial velocity, i.e.

$$\frac{dy}{dt}(t = 0) = a \times b = V_{(0)}. \quad (6)$$

To determine the concentration causing an inhibition of 50% (IC_{50}), serial dilutions of BHC and its hydrolysates from 1 to 15 g l^{-1} protein were made. The IC_{50} value was obtained from the regression described above.

AC of extruded products containing BHC and its hydrolysates. To 20 mg of the sample, 10 µl of PBS (pH 7.4) and then 990 µl of diluted radical cation ABTS + · solution were added. The mixture was shaken with vortex and centrifuged at 8000 *g* for 3 min. The absorbance of supernatant was read at 734 nm and the TEAC was calculated.

Statistical analysis

The data were analysed by one-way analysis of variance, using the software Statgraphics Plus 3.0. Least significant difference test was used to determine statistical differences between samples. The significance was established at $p < 0.05$.

Results and discussion

BHC composition

BHC composition in dry base was protein, 923 g kg^{-1}; fat, 0.7 g kg^{-1}; ash, 27.7 g kg^{-1}; iron, 1.793 g kg^{-1} and moisture: 33.6 g kg^{-1}. The results are similar to those reported in the bibliography (Duarte et al. 1999), but iron content was lower than that reported for bovine haemoglobin (Hurrell et al. 1988). This indicates that the substrate was contaminated with plasma proteins, as was seen in BHC SDS-PAGE electrophoresis (results not shown).

ACE inhibition

ACE inhibition of BHC and its hydrolysates. Figure 1 shows the ACE inhibition results corresponding to BHC and its different hydrolysates (FC, FCF, P and PF). Hydrolysates showed good ACE inhibition (higher than 60%), whereas BHC is rather low (around 10%). The highest inhibition corresponded to P and PF hydrolysates. These values were very similar to those published by Yang et al. (2007) for corn gluten protein hydrolysates, using alcalase as enzyme.

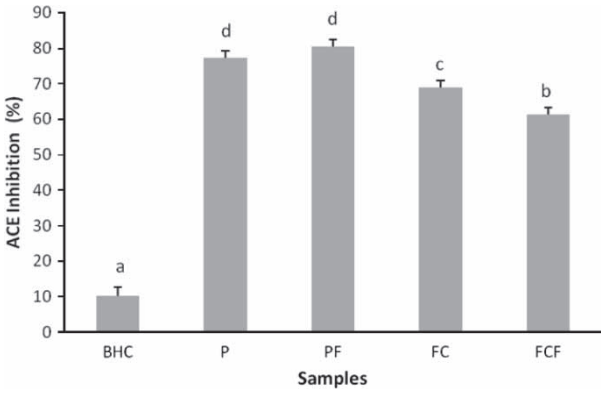

Figure 1. ACE inhibition (%) corresponding to BHC and its different hydrolysates (FC, FCF, P and PF). Different letters mean significant differences among samples ($p < 0.05$).

ACE inhibition of extruded products containing BHC and its hydrolysates. Figure 2 shows the ACE inhibition values corresponding to extruded maize grits (M) and extruded maize grits with the addition of BHC (MBHC) and its hydrolysates. It can be observed that the extruded maize grits showed some degree of inhibition of the enzyme ACE.

When BHC was added, a 96% reduction in ACE inhibition was observed in relation to the extruded maize grits sample. However, the extruded samples containing hydrolysates showed higher ACE inhibition values than that of extruded maize grits, being the sample containing FC hydrolysates, which showed the highest value.

As it was mentioned before, the highest ACE inhibition corresponded to that obtained with P and PF enzymes (Figure 1), but after their addition to maize grits and then extruded, the highest ACE inhibition corresponded to those extruded samples

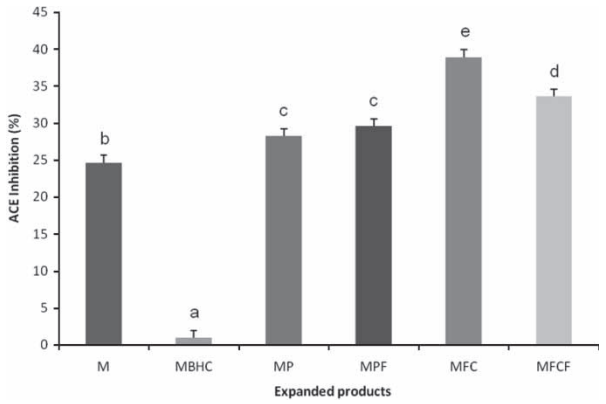

Figure 2. ACE inhibition (%) corresponds to expanded maize products (M) and expanded maize products containing BHC (MBHC) and its hydrolysates (MP, MPF, MFC and MFCF). Different letters mean significant differences between samples ($p < 0.05$).

containing hydrolysates obtained with FC enzyme. This confirms the importance of the evaluation of ACE inhibition in the final product, as both processing effects and interactions between food matrix and hydrolysates could affect the ACE inhibition properties.

Another fact to be pointed out is that although the ACE inhibition value decreased after extrusion, the dilution of peptides having this property contained in hydrolysates is very high, as they are added at a rate of $5\,g\,kg^{-1}$ with respect to maize grits.

On the other hand, to see if the higher ACE inhibition property of extruded samples containing hydrolysates was not due to solubility differences, a solubility assay was done with extruded samples. Results are showed in Table I. When $5\,g\,kg^{-1}$ of BHC or its hydrolysates was added, solubility decreased about 10–15% with respect to extruded maize sample and no significant differences were found between samples containing BHC and its hydrolysates.

Antioxidant capacity

AC of BHC and its hydrolysates. Table II shows TEAC obtained at 6-min reaction time and DH of each hydrolysate.

It is observed that TEAC corresponding to hydrolysates is three times higher than that of BHC. These suggest that enzymatic hydrolysis is a good alternative to increase AC of BHC. Similar results were also found by Dávalos et al. (2004) who worked with egg albumin hydrolyzed with pepsin for 3 h and by Chang-Kee and Heuyn-Kil (2000) who worked with porcine haemoglobin hydrolysates obtained by sequential enzyme system (alcalase + F) with a 13.14% DH and 6 h of reaction time. These works showed that the peptides produced by proteolysis have higher AC than native proteins.

The highest values of TEAC corresponded to the most hydrolyzed samples (PF and FCF). This suggests that a higher DH is associated with a higher TEAC. Dávalos et al. (2004) and Moosmann and Behl (2002) suggested that hydrolysates with high DH obtained with sequential protease systems have higher proportion of low molecular weight peptides which would access more easily to the oxidant system and lead to high values of TEAC.

However, there was not a lineal correlation between TEAC and DH. Although DH of PF is higher than FCF, there was no significant difference between them for their TEAC values. This could be explained by the fact that AC of hydrolysates is affected by the amino acid sequence of the produced peptides, which depends on protease specificity (Hurrell et al. 1988). Thus, DH can affect AC but it is not the only involved factor (Xie et al. 2008).

Table I. Solubility corresponding to expanded maize products (M) and expanded maize products containing BHC (MBHC) and its hydrolysates (MP, MPF, MFC and MFCF).

Samples	Solubility (%)
M	61.8^{B}
MBHC	53.7^{A}
MP	55.1^{A}
MPF	53.7^{A}
MFC	53.4^{A}
MFCF	52.4^{A}

Note: Different letters mean significant differences between samples ($p < 0.05$).

Table II. TEAC obtained at 6-min reaction time and DH of each hydrolysate.

Sample	TEAC (μmol Trolox g^{-1} of protein)	DH (%)
BHC	143.19^{A}	–
P	399.27^{B}	8.33^{A}
FC	402.94^{B}	8.43^{A}
PF	420.64^{B}	19.84^{C}
FCF	425.09^{B}	16.67^{B}

Note: Different letters mean significant differences ($p < 0.05$).

Kinetic of ABTS + · reaction. ABTS + · inhibition rates corresponding to different samples are shown in Figure 3.

Inhibition rate increases with reaction time for all cases. This behaviour is typical for different antioxidants compounds that reduce ABTS + ·. As mentioned before, the AC from hydrolysates is significantly higher than that from BHC. The maximum inhibition (I_{max}) and the initial velocity (V_0) for each sample (Table III) were obtained from the inhibition of ABTS + · curves (Figure 3). I_{max} values follow a similar tendency to TEAC results. Relative differences among samples would be maintained for reaction times higher than 6 min.

Regarding V_0, the highest value corresponded to PF and the lowest one to BHC, indicating that peptides

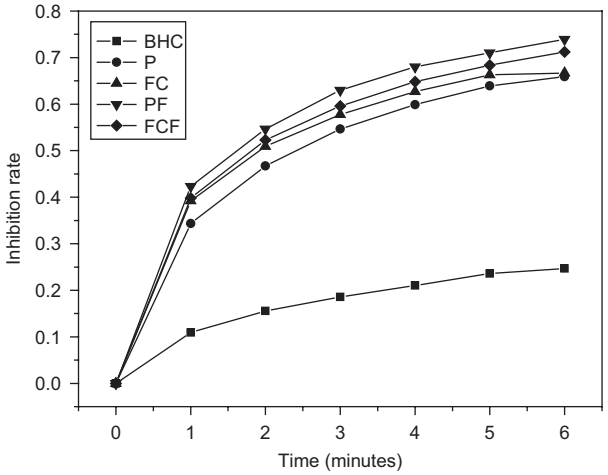

Figure 3. ABTS + · inhibition rate vs. time from BHC and its hydrolysates (P, PF, FC and FCF).

Table III. Maximum inhibition (I_{max}), initial velocity (V_0) and protein concentration required to produce 50% of inhibition of ABTS + · (IC_{50}) for BHC and its hydrolysates.

Sample	I_{max} (%)	V_0 (min^{-1})	IC_{50} ($g\,L^{-1}$ protein)
BHC	26.072^{A}	0.1164^{A}	24.65^{E}
P	65.815^{B}	0.4258^{B}	3.53^{C}
FC	65.732^{B}	0.5332^{C}	4.06^{D}
PF	68.301^{B}	0.6144^{D}	3.11^{B}
FCF	69.547^{B}	0.5187^{A}	2.52^{A}

Note: Different letters mean significant differences ($p < 0.05$).

from PF hydrolysates react faster with ABTS + · than those from others samples. On the other hand, V_0 values of FC and FCF did not differ significantly. The differences in V_0 could be attributed to the different composition of peptides from each sample, which determine their reaction time with ABTS + · (Moure et al. 2006).

Influence of protein concentration on AC. Figure 4 shows that as protein concentration increases, inhibition rate of BHC and its hydrolysates increases and then tends to a plateau when protein concentration is around $11\,g\,l^{-1}$ of protein. This behaviour was also found by Yang et al. (2008) for gelatine hydrolysates obtained with different proteases (papain, pancreatin, tripsin and bromelin). In this case, the inhibition of ABTS + · tended to a plateau when concentration reached $20\,g\,l^{-1}$ of protein. As in our case, the plateau reached an inhibitory level of 75–85%. Similar results have been found for other proteins; Wang et al. (2007) obtained gluten hydrolysates using papain and 6 h hydrolysis time. They reported an increase in anti-2,2-diphenyl-1-picrylhydrazyl (DPPH) activity as hydrolyzed wheat gluten concentration increased in the range of $0–2.4\,g\,l^{-1}$ of protein. Sakanaka and Tachibana (2006) found the same tendency, working with yolk protein hydrolysates, obtained by sequential system (orientase + protease); the anti-DPPH activity increased as protein concentration increased in the range $0.6–10\,g\,l^{-1}$ of protein. Similar results were found by Xie et al. (2008) working with alfalfa leaf protein hydrolysates obtained with alcalase and 4 h hydrolysis time in a concentration range of $0–2\,g\,l^{-1}$ of protein.

The parameter IC_{50} is shown in Table III. The lowest value corresponded to FCF sample. A similar IC_{50} value ($2.11 \pm 0.10\,g\,l^{-1}$ of protein) was reported by Ren et al. (2008) for grass muscle hydrolysates. In these works, the AC was measured by the anti-radical hidroxil.

It is also observed that IC_{50} corresponding to BHC is 10 times higher than that obtained for the FCF system, which indicates that the low capacity of native proteins inhibits ABTS + ·. Thus, the use of a sequential system for the protein hydrolysis is a good approach to increase AC for a particular protein substrate.

Evaluation of AC of extruded products containing BHC and its hydrolysates. Results of AC expressed as TEAC are shown in Figure 5. All hydrolysates showed higher TEAC values than BHC (Table II). However, only three of the four extruded samples containing hydrolysates (P, PF and FCF) showed higher TEAC than the food matrix (M). The higher TEAC corresponded to the extrudate with added FCF hydrolysate (MFCF). Besides that, in all cases the lowest TEAC value corresponded to the extruded maize, which is in agreement with the results obtained by Sakanaka and Tachibana (2006) who worked with yolk protein hydrolysates incorporated to either cow meat or tuna meat products. In this work, all samples containing hydrolysates showed higher antioxidant activity than the control. On the other hand, TEAC value obtained from the extrudate containing FC did not differ significantly from the extrudate containing BHC, indicating that the extrusion process affected AC. Even though extrusion is a high temperature short-time process, it caused a significant loss of the AC present in the hydrolyzed samples before they were

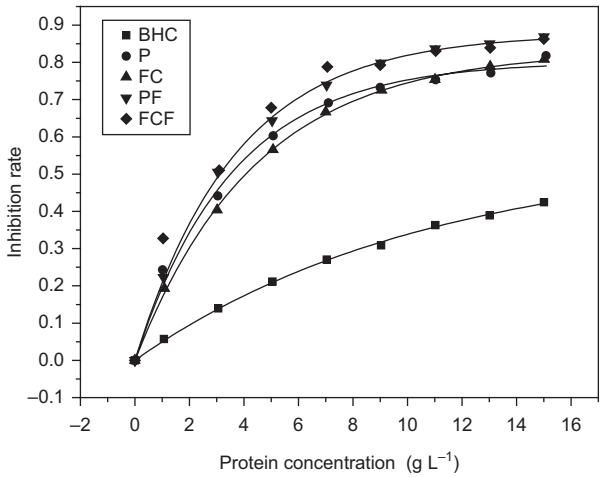

Figure 4. Inhibition rate of ABTS + · measured after 6-min reaction time as a function of protein concentration ($1–15\,g\,l^{-1}$ of protein) corresponding to BHC and its hydrolysates (P, PF, FC and FCF).

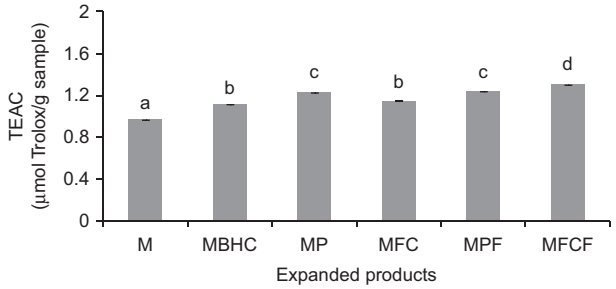

Figure 5. TEAC corresponds to expanded maize products (M) and expanded maize products containing BHC (MBHC) and its hydrolysates (MP, MPF, MFC and MFCF). Different letters means significant differences among samples ($p < 0.05$).

incorporated into the extruded product. This effect could be attributed to both possible interactions of peptides with the food matrix and possible amino acid degradation during extrusion (Sorbetti-Guerri and Denis 1998).

It is appropriate to mention that the physical properties of extruded samples (expansion, the degree of cooking and compressive strength; Cian et al. 2010) and sensorial quality (taste and sensorial hardness) were not affected by the addition of BHC or their hydrolysates (Caballero et al. 2009). However, the colour of the extruded maize samples including 0.5% of BHC or its hydrolysates was different from that of extruded maize grits. This does not mean a problem, as this kind of product usually includes flavouring and food colouring into the formulation.

Conclusion

The proteolysis is a good alternative to increase the ACE inhibition and AC properties of BHC. Thus, hydrolysis could be used as a means to obtain peptides with ACE inhibition and AC properties.

Addition of $5\,g\,kg^{-1}$ of BHC hydrolysates increases the ACE inhibition and TEAC of the maize matrix, although extrusion modified both ACE inhibition and AC properties formerly present in the hydrolysates, confirming the importance of ACE inhibition and AC evaluation in the final product.

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