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Correlations between the peptide profile and the antioxidant and the ACE inhibitory activities of whey protein concentrate hydrolysates

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Abstract. The peptide profile, the angiotensin converting enzyme-inhibitory activity (ACE-IA) and the antioxidant property are important characteristics of protein hydrolysates and this was the first time the correlation among these parameters was investigated. Twenty-four hydrolysates from whey protein concentrate were prepared using four enzymes. With respect to the antioxidant activity, when using the *B. licheniformis* protease, a positive and significant correlation of strong intensity was observed with the contents of di- and tripeptides and medium peptides. Also, a negative and significant correlation of strong intensity was observed with the large peptide content. For the *A. sojae* protease and pancreatin, a positive and significant correlation of strong intensity was observed with the contents of di-and tripeptides. For the *A. sojae* protease, a negative and significant correlation of strong intensity was observed with the large peptide content. Concerning the ACE-IA, for the *B. licheniformis* and *A. sojae* proteases and pancreatin, a positive and significant correlation of strong intensity was observed with the contents of di-and tripeptides and medium peptides. For the A. *sojae* protease and pancreatin, this same kind of correlation was observed with the free amino acid content. For the *B. licheniformis*, A. s*ojae* and A. o*ryzae* proteases and pancreatin, a negative and significant correlation of strong intensity was observed with the large peptide content.
Keywords: Peptide profile, Antioxidant, WPC hydrolysates.

Introduction

Protein hydrolysates may have several applications in special foods, such as those designed to provide nutritional support to individuals with particular physiological and nutritional needs not covered by conventional diets (Pacheco et al., 2002). Protein hydrolysates have been used as main ingredients of geriatric products, high-energy supplements, hypoallergenic formulas, as well as parenteral and enteral solutions. Additionally, these hydrolysates are used in formulas designed for premature newborns and children with acute or chronic diarrhea, food intolerances, or inborn errors of metabolism, such as phenylketonuria and cystic fibrosis (Silvestre et al., 2011).

Therefore, considering that protein hydrolysates show several important applications, it is evident that their characterization is required. Aiming at using a protein hydrolysate for dietetic purpose, a characterization regarding the size distribution of peptides (peptide profile) is needed, because the length of the peptide chain influences the rate of absorption (Frenhani and Burini 1999).

The evaluation of the ACE-IA represents an important feature of protein hydrolysates destinated to clinical nutrition. This enzyme plays a crucial role in the regulation of blood pressure and the intake of protein hydrolysates able to inhibit ACE could be used as an alternative and non-pharmacological approach to prevent and treat arterial hypertension (Costa et al., 2007; Jiang et al., 2007; Miguel et al., 2007; Otte et al., 2007a; Morais et al., 2014).

There is a growing interest in identifying dietary antioxidants, which are essential to minimizing the effects of the oxidative stress (Huang et al., 2005; Silvestre et al., 2014; Silvestre et al., 2013). Much attention has been directed toward the bioactive peptides obtained by enzymatic hydrolysis of various protein sources, which may have different functions for example, anti-inflammatory, anti-hypertensive and antioxidant functions (Dryáková et al., 2010; Liu et al., 2010; Park et al., 2010; Peng et al., 2010; Silvestre et al., 2013; Silvestre et al., 2014).

The establishment of a correlation between the peptide profile and the antioxidant property and ACE-IA is of interest because it shows what size of peptides found in protein hydrolysates is responsible for these activities. Therefore, initiatives may be taken aiming at optimizing the hydrolytic process of

proteins in order to obtain the desired peptide size and maximize the bioactive activities of these products.

To the authors knowledge, this was the first time a study was conducted regarding the correlation between the peptide profile and the antioxidant property and ACE-IA of WPC hydrolysates.

This work was conducted aiming at preparing several WPC hydrolysates using four different proteolytic enzymes and to evaluate the correlation between the peptide profile and the antioxidant property and ACE-IA.

Methods

WPC (Kerrylac 750) in powder form was kindly furnished by Kerry of Brazil Ltda. (Três Corações, MG, Brazil). Pancreatin (Corolase PP® from porcine pancreas, activity = 5.97 U mL^{-1}) and proteases from *Aspergillus sojae* (Corolase LAP®, activity = 0.63 U mL^{-1}), *Bacillus licheniformis* (Alcalase®, activity = 6.22 U mL^{-1}) and *Aspergillus oryzae* (Flavourzyme®, activity = 0.69 U mL^{-1}) were kindly furnished by AB Enzymes (Barueri, SP, Brazil). In this study, one unit of protease (U mL^{-1}) activity was defined as the activity that liberated 1 µg of tyrosine per minute (µg Tyr x mL^{-1} min^{-1}) under the described conditions (Dias et al., 2008).

Preparation of WPC hydrolysates

Twenty-four hydrolysates from WPC were prepared with (A) Protease from *Bacillus licheniformis*, pH 8, 60 °C (H1–H6), (B) Protease from *Aspergillus oryzae*, pH 7, 50 °C (H7–H12), (C) Protease from *Aspergillus sojae*, pH 7, 50 °C (H13–H18) and (D) Pancreatin, pH 7, 50 °C (H19–H24). The E:S ratios were 0.5, 1, 2, 3, 4 and 8:100 for all enzymes. WPC solutions (10 %, w/v) were prepared in distilled water, which corresponded to 3.42 % protein (w/v), and the pH was adjusted to 7.0 or 8.0 with a 3 mol L^{-1} NaOH solution. Then, the WPC solutions were heated in an oil-bath with continuous stirring (stirrer 752A model from Fisatom, São Paulo, SP, Brazil), and the enzymes were added in such a concentration to attain the desired enzyme:substrate ratios. The total reaction time was 5 h for all samples, and the hydrolytic reaction was stopped by heating at 75 °C for 15 s, followed by immediately cooling on ice bath until the temperature of 25 °C. The hydrolysates were freeze-dried (Freeze Dry System/FreeZone 4.5, model 77500, LABCONCO, Kansas City, MO, USA) and stored in the freezer (-4 °C) until analysis.

Characterization of peptide profiles from WPC hydrolysates

Characterization of peptide profiles was performed in two stages, which included the fractionation of peptides according to chain size (size exclusion-HPLC) and their subsequent quantification (rapid method of Correct Fraction Area) (Morais et al., 2015).

Evaluation of the antioxidant activity of protein hydrolysates

The evaluation of the antioxidant activity of WPC hydrolysates was performed by three methods (DPPH, deoxyribose and pyrogallol), as described before by our group (Silvestre et al., 2013, Silvestre et al., 2014).

Evaluation of ACE inhibitory activity

Evaluation of the ACE-IA of the WPC hydrolysates was performed according to the method developed by Wu et al. (2002) using RP-HPLC, as described before by our group (Morais et al., 2014).

Calculation of the correlations and Statistical analysis

All experiments and measurements were performed in triplicate. The correlations between the peptide profile with the antioxidant and ACE inhibitory activities of WPC hydrolysates were obtained by Pearson's correlation coefficient (r), which measures the degree of association between two variables, and p was calculated with t-tests, using the software BioStat for data analysis (Ayres et al., 2007).

Results and Discussion

The WPC hydrolysates were fractionated in four: F1 containing the large peptides (more than 7 amino acids residues); F2, containing the medium peptides (4 to 7 amino acids residues); F3, containing the di- and tripeptides and F4, containing the free amino acids fractions (Morais et al., 2013). The peptide and free amino acid contents of chromatographic fractions are shown in Table 1.

The antioxidant activity and the ACE-IA of WPC hydrolysates were evaluated before by our group (Morais et al., 2014), and the results are shown in Table 2.

The results of the correlation between the peptide profile and the antioxidant activity of WPC hydrolysates are shown in Table 3. According to Sampaio (2002), *r* values greater than 0.7 with $p < 0.05$ indicate a strong association between the data. Therefore, it can be inferred that using the *B. licheniformis* protease and the pyrogallol method, a positive and significant correlation of strong intensity was observed between the antioxidant activity and the contents of two fractions: di-and tripeptides ($r = 0.8822$, $p < 0.0001$) and medium peptides ($r = 0.8966$, $p < 0.0001$). Additionally, for this same enzyme and the same method, a negative and significant correlation of strong intensity was observed between the antioxidant activity and the large peptide content ($r = -0.9172$, $p < 0.0001$).

Concerning the correlation between the peptide profile and the ACE-IA, similar results were observed for the the *B. licheniformis* protease concerning the correlation between the peptide profile and the antioxidant activity. In this way, a positive and significant correlation of strong intensity was also observed between the ACE-IA and the di-

and tripeptide content (r = 0.8418 p < 0.0001) as well as between the ACE-IA and the medium peptide content (r = 0.8061, p < 0.0001). Additionally, a negative and significant correlation of strong intensity was also observed between the ACE-IA and the large peptide content (r = -0.8485, p = 0.0001). When using the *A. sojae* protease and the DPPH method, a positive and significant correlation of strong intensity was observed between the antioxidant activity and the di-and tripeptide content (r = 0.7677 p = 0.0002), and a negative and significant correlation of strong intensity was observed between the antioxidant activity and the large peptide content (r = -0.7166 and p = 0.0008).

Although the same kind of correlation (negative and significant of strong intensity) for the *A. sojae* protease was observed between the ACE-IA and the large peptide content (r = -0.8887, p <0.001), contrarily to the antioxidant activity, the positive and significant correlation of strong intensity was observed between the ACE-IA and the free amino acid content (r = 0.9268, p < 0.001) and not with the the di-and tripeptide contents.

Table 1. Peptides and free amino acid contents of WPC hydrolysates.

Hydrolysates	F1 (> 7 AA residues)	F2 (4-7 AA residues)	F3 (2-3 AA residues)	F4 (free AA)
		Bacillus licheniformis protease		
H1	71.37 ± 3.78^{I1}	26.26 ± 3.66^{E2}	1.47 ± 0.09^{FG3}	0.90 ± 0.08^{GHI4}
H2	68.04 ± 1.87^{J1}	29.21 ± 1.71^{E2}	1.69 ± 0.17^{F3}	1.06 ± 0.13^{FGH3}
H3	63.97 ± 1.75^{K1}	33.18 ± 1.64^{D2}	1.70 ± 0.21^{F3}	1.15 ± 0.14^{FGH3}
H4	67.70 ± 1.43^{J1}	29.12 ± 1.56^{E2}	1.41 ± 0.09^{FG3}	1.77 ± 0.07^{F3}
H5	62.56 ± 1.23^{K1}	34.18 ± 1.26^{D2}	1.44 ± 0.03^{FG3}	1.82 ± 0.13^{F3}
H6	44.61 ± 1.63^{M1}	45.40 ± 1.83^{B1}	8.79 ± 0.44^{A2}	1.20 ± 0.15^{FGH3}
		Aspergillus oryzae protease		
H7	90.86 ± 0.07^{E1}	7.45 ± 0.03^{HI2}	0.30 ± 0.02^{IJ4}	1.38 ± 0.06^{FGH3}
H8	92.10 ± 1.10^{DE1}	6.97 ± 1.02^{HI2}	0.10 ± 0.01^{J4}	0.83 ± 0.10^{HIJ3}
H9	82.70 ± 1.21^{G1}	11.73 ± 1.46^{G2}	1.38 ± 0.16^{FG}	4.19 ± 0.39^{CD}
H10	86.87 ± 0.33^{F1}	9.38 ± 0.27^{GH2}	2.39 ± 0.37^{E3}	1.35 ± 0.22^{FGH4}
H11	75.76 ± 0.79^{H1}	15.69 ± 1.01^{F2}	4.13 ± 0.53^{D3}	4.42 ± 0.14^{CD3}
H12	72.73 ± 1.19^{I1}	16.74 ± 1.66^{F2}	5.66 ± 0.35^{C3}	4.87 ± 0.40^{C4}
		Aspergillus sojae protease		
H13	97.89 ± 0.19^{AB1}	1.68 ± 0.17^{KLM2}	0.25 ± 0.04^{J3}	0.18 ± 0.03^{IJ3}
H14	95.23 ± 0.25^{BC1}	3.60 ± 0.22^{JKL2}	1.05 ± 0.07^{GH3}	0.12 ± 0.01^{J4}
H15	97.35 ± 0.09^{AB1}	1.30 ± 0.06^{LM2}	0.35 ± 0.00^{IJ3}	1.03 ± 0.15^{FGH2}
H16	98.21 ± 0.13^{A1}	0.26 ± 0.02^{M3}	0.50 ± 0.02^{IJ3}	1.03 ± 0.10^{FGH2}
H17	94.39 ± 0.29^{CD1}	3.51 ± 0.27^{JKL2}	0.38 ± 0.03^{IJ4}	1.73 ± 0.04^{FG3}
H18	89.73 ± 0.31^{E1}	4.62 ± 0.08^{IJK2}	0.83 ± 0.06^{HI3}	4.81 ± 0.45^{C2}
		Pancreatin		
H19	92.38 ± 0.34^{DE1}	5.85 ± 0.42^{IJ2}	0.34 ± 0.03^{IJ4}	1.44 ± 0.21^{FGH3}
H20	66.72 ± 0.34^{J1}	27.46 ± 0.85^{E2}	2.45 ± 0.16^{E4}	3.37 ± 0.51^{E3}
H21	49.61 ± 0.40^{L1}	42.75 ± 0.65^{BC2}	3.67 ± 0.29^{D3}	3.97 ± 0.42^{DE3}
H22	44.89 ± 1.93^{M1}	42.29 ± 1.92^{C1}	6.52 ± 0.64^{B2}	6.30 ± 0.85^{B2}
H23	40.90 ± 0.92^{N2}	49.12 ± 0.51^{A1}	3.78 ± 0.14^{D4}	6.20 ± 0.57^{B3}
H24	41.13 ± 2.09^{N1}	44.18 ± 2.78^{BC1}	6.46 ± 0.12^{B3}	8.22 ± 0.88^{A2}

WPC = Whey Protein Concentrate.Values are in % of nmol of the four fractions and represent the means of triplicate experiments ± standard deviation. Different numbers represent significantly different (p<0.05) values for different fractions of the same hydrolysate. Different letters represent significantly different (p<0.05) values for the same fraction of different hydrolysates. AA = amino acid. F1: large peptides (> 7 amino acid residues); F2: medium peptides (4 to 7 amino acid residues); F3: di- and tripeptides; F4: free amino acids. E:S ratio = Enzyme:Substrate ratio.

Table 2. Antioxidant property and ace inhibitory activity of WPC hydrolysates.

Hydrolysates	Antioxidant activity			Inhibitory activity	
	DPPH method (radical scavenging %)	Deoxyribose method (Inhibition %)	Pyrogallol method (Inhibition %)	ACE inhibition (%)	$IC_{50}{}^{c}$ (mg mL^{-1})
			Bacillus licheniformis protease		
H1	41.33 ± 3.05^{Db}	51.11 ± 2.10^{BCa}	48.70 ± 4.19^{FGHa}	82.66 ± 0.74 ^{cde}	0.78 ± 0.007 ^{e}
H2	13.80 ± 0.05^{Nc}	33.23 ± 2.84^{HIb}	50.91 ± 0.65^{EFGa}	83.69 ± 0.21 ^{c}	0.77 ± 0.002 ^{e}
H3	12.01 ± 1.03^{Nc}	27.15 ± 2.67^{JKb}	68.78 ± 2.64^{Ca}	80.16 ± 0.93 ^{ef}	0.80 ± 0.009 ^{e}
H4	50.67 ± 1.57^{Ba}	27.78 ± 2.72^{Jb}	53.25 ± 0.21^{Ea}	83.11 ± 0.69 ^{cd}	0.78 ± 0.006 ^{e}
H5	25.20 ± 2.60^{IJc}	41.39 ± 2.37^{FGb}	59.56 ± 4.70^{Da}	89.47 ± 0.45 ^{b}	0.72 ± 0.004 ^{e}
H6	35.20 ± 1.10^{Fb}	30.99 ± 1.60^{Ib}	90.76 ± 0.67^{Aa}	96.66 ± 0.27 ^{a}	0.67 ± 0.002 ^{e}
			Aspergillus oryzae protease		
H7	38.93 ± 1.81^{Eb}	43.60 ± 2.03^{EFa}	22.05 ± 1.11^{Nc}	68.84 ± 0.57 ^{i}	0.94 ± 0.008 ^{e}
H8	22.59 ± 0.48^{Kb}	40.66 ± 0.14^{FGa}	16.95 ± 0.37^{Oc}	76.54 ± 0.19 ^{gh}	0.84 ± 0.002 ^{e}
H9	24.24 ± 0.32^{IJKb}	43.25 ± 1.06^{EFGa}	3.79 ± 0.33^{Pc}	74.64 ± 0.40 ^{h}	0.86 ± 0.005 ^{e}
H10	13.87 ± 0.63^{Nb}	36.14 ± 0.40^{Ha}	15.28 ± 1.09^{Ob}	70.28 ± 0.70 ^{i}	0.92 ± 0.009 ^{e}
H11	19.30 ± 0.54^{Lb}	45.13 ± 0.31^{DEa}	17.04 ± 0.96^{Ob}	78.20 ± 0.06 ^{fg}	0.83 ± 0.001 ^{e}
H12	30.19 ± 0.64^{Gb}	31.93 ± 2.45^{Ib}	84.26 ± 3.69^{Ba}	90.22 ± 0.65 ^{b}	0.72 ± 0.005 ^{e}
			Aspergillus sojae protease		
H13	34.31 ± 0.18^{Fb}	43.50 ± 1.09^{EFa}	15.48 ± 0.96^{Oc}	2.32 ± 0.20 ^{l}	27.97 ± 2.398 ^{b}
H14	47.85 ± 1.11^{Ca}	39.92 ± 3.01^{Gb}	34.94 ± 2.94^{Lc}	2.65 ± 0.24 ^{l}	24.48 ± 2.070 ^{c}
H15	26.10 ± 1.47^{HIc}	58.77 ± 0.39^{Aa}	47.18 ± 0.97^{GHb}	2.02 ± 0.20 ^{l}	32.22 ± 3.263 ^{a}
H16	28.15 ± 1.23^{GHb}	49.96 ± 4.40^{BCa}	45.85 ± 1.98^{HIa}	2.34 ± 0.07 ^{l}	27.58 ± 0.842 ^{b}
H17	21.98 ± 1.74^{Kb}	25.93 ± 1.96^{JKb}	49.78 ± 2.97^{EFGHa}	2.43 ± 0.18 ^{l}	26.63 ± 2.098 ^{b}
H18	59.70 ± 0.54^{Aa}	52.78 ± 2.68^{Bb}	30.79 ± 1.85^{Mc}	14.22 ± 0.26 ^{k}	4.40 ± 0.083 ^{d}
			Pancreatin		
H19	24.41 ± 2.57^{IJKc}	33.35 ± 0.97^{HIb}	42.05 ± 3.65^{IJa}	63.23 ± 0.04 ^{j}	1.02 ± 0.001 ^{e}
H20	16.89 ± 0.91^{Mc}	23.96 ± 0.59^{Kb}	37.81 ± 3.31^{KLa}	75.65 ± 0.27 ^{gh}	0.85 ± 0.003 ^{e}
H21	18.30 ± 0.83^{LMc}	35.58 ± 1.17^{Hb}	52.83 ± 2.94^{EFa}	80.29 ± 0.76 ^{cde}	0.80 ± 0.008 ^{e}
H22	19.46 ± 0.30^{Lc}	27.28 ± 0.80^{JKb}	52.46 ± 1.93^{EFa}	80.03 ± 1.04 ^{ef}	0.81 ± 0.010 ^{e}
H23	22.64 ± 0.53^{JKb}	35.35 ± 1.67^{HIa}	39.43 ± 1.99^{JKa}	82.41 ± 0.38 ^{cde}	0.78 ± 0.004 ^{e}
H24	49.44 ± 0.62^{BCb}	47.90 ± 0.45^{CDb}	60.67 ± 0.41^{Da}	88.98 ± 0.72 ^{b}	0.73 ± 0.006 ^{e}

WPC = whey protein concentrate. E:S ratio = Enzyme:Substrate ratio. The values represent the means of triplicate experiments ± standard deviation. Different capital (column) and small (line) letters represent significantly different ($p<0.05$) values. H1 to H6: hydrolysates prepared using B. licheniformis protease. H7 to H12: hydrolysates prepared using A. oryzae protease. H13 to H18: hydrolysates prepared using A. sojae protease. H19 to H24: hydrolysates prepared using pancreatin.
ACE: angiotensin-converting enzyme. WPC: whey protein concentrate. IC_{50}: concentration of hyrolysates (mg mL^{-1}) required to inhibit 50% of enzymatic activity. Results are expressed as mean ± standard deviation. Different letters represent significantly different values ($p<0.05$) for different hydrolysates.

For the pancreatin and the pyrogallol method, a positive and significant correlation of strong intensity was only observed between the antioxidant activity and the di-and tripeptide content ($r = 0.7008$ and $p = 0.0012$). Contrarily to the antioxidant activity, for the pancreatin, a significant correlation of strong intensity was observed between the ACE-IA and the contents of the four fractions. This correlation was positive for the contents of di- and tripeptides ($r = 0.8458$, $p < 0.001$), medium peptides ($r = 0.9176$, $p < 0.001$) and free amino acids ($r = 0.9073$, $p < 0.001$). Additionally, this correlation was negative for the large peptide content ($r = -0.9450$, $p < 0.001$).

In case of the *A. Oryzae* protease, no significant correlation was observed between the antioxidant activity and the peptide profile, no matter the method used for evaluating this property. However, the results for this enzyme concerning the ACE-IA were superior and showed similar behavior to those of the *B. licheniformis* protease, *i.e.,* a positive and significant correlation of strong intensity was observed between the ACE-IA and the di-and tripeptide content ($r = 7684$ $p = 0.0002$), as well as between the ACE-IA and the medium peptide content ($r = 0.7380$, $p < 0.0005$). Additionally, a negative and significant correlation of strong intensity was observed between the ACE-IA and the large peptide content ($r = -0.7699$, $p = 0.0002$).

These results indicate that depending on the enzyme used for hydrolyzing the WPC and on the method used for evaluating the antioxidant activity it

was able to show that, in general, the greater the small peptide content (from 2 to 7 amino acid residues) as well as the lower the amount of large peptides (more than 7 amino acid residues) the higher is the antioxidant.

Table 3. Correlation between the peptide profile and the antioxidant activity and the ace inhibitory activity of WPC hydrolysates.

Antioxidant activity methods / ACE-IA results	Chromatographic fractions	*B. licheniformis* Protease		*Aspergillus sojae* protease		*Aspergillus oryzae* protease		Pancreatin	
		r	P	r	p	r	p	r	p
DPPH	F1	-0,0013	0,9959	-0,7166	0,0008	0,0916	0,7177	-0,2660	0,2859
	F2	-0,0703	0,7817	0,6536	0,0032	-0,0931	0,7132	0,1766	0,4832
	F3	0,1369	0,5881	0,7677	0,0002	-0,1656	0,5114	0,4351	0,0711
	F4	0,2770	0,2657	0,5442	0,0195	0,0217	0,9317	0,6460	0,0038
Deoxyribose	F1	0,3050	0,2184	0,0630	0,9056	0,2914	0,2406	-0,3444	0,1616
	F2	-0,3080	0,2137	-0,3454	0,5025	-0,2212	0,3776	0,2998	0,3273
	F3	-0,2375	0,3425	-0,2255	0,6675	-0,5223	0,0261	0,3404	0,1668
	F4	-0,2156	0,3882	0,3479	0,4992	-0,1153	0,6488	0,5644	0,0146
Pyrogalol	F1	-0,9172	< 0,0001	0,1401	0,7912	-0,5909	0,0098	-0,4871	0,0403
	F2	0,8966	< 0,0001	-0,0891	0,8666	0,5551	0,0167	0,4159	0,5279
	F3	0,8822	< 0,0001	-0,1002	0,8501	0,6983	0,0013	0,7008	0,0012
	F4	-0,0456	0,8576	0,0452	0,9323	0,4201	0,0825	0,6098	0,0072
ACE-IA	F1	-0,8485	< 0,0001	-0,8887	< 0,001	-0,7699	0,0002	-0,9450	< 0,001
	F2	0,8061	< 0,0001	0,6445	0,0039	0,7380	0,0005	0,9176	< 0,001
	F3	0,8418	< 0,0001	0,4465	0,0632	0,7684	0,0002	0,8458	< 0,001
	F4	0,1829	0,4676	0,9268	< 0,001	0,6825	0,0018	0,9073	< 0,001

ACE-IA Angiotensin-Converting-Enzyme-Inhibitory WPC= Whey Protein Concentrate. Activity F1: large peptides (> 7 amino acid residues); F2: medium peptides (4 to 7 amino acid residues); F3: di- and tripeptides; F4: free amino acids. DPPH = 2,2-diphenyl-1-picrylhydrazyl.

Some authors reported that for different protein hydrolysates, peptides showing antioxidant activity have from 5 to 16 amino acid residues and molecular mass from 500 to 1,800 Da (Sarmadi and Ismail, 2010; Samaranayaka and Chan, 2011). However, we showed in the present work that for WPC hydrolysates even peptides lower than 5 amino acid residues (< 500 Da) may have antioxidant activity and the ACE-IA of WPC hydrolysates.

With respect to the ACE-IA, some works in the literature where casein hydrolysates were studied *in vivo* reported that peptides lower than 3,000 Da, especially those containing between 4 and 7 amino acid residues could be considered as antihypertensive (Contreras et al., 2009; Jiang et al., 2010). Therefore, the results found in the present work for the WPC hydrolysates are similar to those from other authors obtained with casein hydrolysates.

No report was found in the literature on the correlation between the peptide size distribution with the antioxidant activity and the ACE-IA of WPC hydrolysates.

Conclusion

For some WPC hydrolysates, positive and significant correlations of strong intensity were observed between the peptide profile and the antioxidant property and ACE-IA, especially with the di- and tripeptide and medium peptide (4 to 6 amino acid residues) contents. Concerning the antioxidant property, the use of the *B. licheniformis* protease for preparing the WPC hydrolysates was the most advantageous whereas for the ACE-IA one can highlight the use of *A. Oryzae* protease and pancreatin as well.

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