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Obtaining Oligopeptides from Whey: Use of Subtilisin and Pancreatin

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Abstract: The whey hydrolysis was optimized aiming the production of oligopeptides. The use of a subtilisin and a pancreatin as well as the effect of the substrate concentration (SC) and enzyme:substrate ratio (E:S) were tested. The whey hydrolysates were fractionated by size-exclusion-HPLC and the rapid Correct Fraction Area method was used for quantifying peptides and free amino acids. Both enzymes were efficient for producing oligopeptides and the values reached 41.42 and 37.12%, for subtilisin and pancreatin, respectively. For subtilisin, the best peptide profile was obtained for a SC of 15% with an E:S of 1:100, while for pancreatin this same result was observed for a SC of 10% with E:S of either 1:100 or 2:100, as well as for a SC of 15% with E:S of 1:100, 2:100 and 4:100.

Key words: Whey, enzymes, hydrolysates, peptide profile, oligopeptides

INTRODUCTION

The introduction into a diet of enzymatic hydrolysates rich in oligopeptides, especially di-and tripeptides, represents a way of improving protein utilization (González-Tello *et al*., 1994). These preparations have been used in some countries for individuals presenting special nutritional or physiological necessities uncovered by conventional feeding, such as malabsorption disorders, protein intolerance or allergy, phenylketonuria, cystic fibrosis, chron disease (Clemente, 2000; Mira and Marquez, 2000). Moreover, these hydrolysates may be useful in the dietetic supplementation for old people, premature babies, children with diarrhea, athletes as well as for weight control diets (Lee *et al*., 2001; Trusek-Holownia, 2008).

This wide use of protein hydrolysates is associated to the fact that the amino acids provided by protein hydrolysates are more quickly and completely absorbed than intact protein and free amino acids (Frenhani and Burini, 1999). The study of absorption mechanisms revealed that in the case of small peptides the competition for the transport system is lower than that observed for free amino acids (Grimble *et al*., 1986; Frenhani and Burini, 1999; Clemente, 2000).

Considering that in Brazil the products normally used as dietetic supplements are not found in the market and consequently must be imported and show high price, our interest turned to the preparation of these products, containing protein hydrolysates as the main source of amino acids in a highly available form, that is, in oligopeptide form, especially di-and tripeptides. This is the reason we have been preparing several protein hydrolysates and testing different hydrolytic conditions for obtaining peptide profiles appropriate for nutritional purposes (Silvestre *et al*., 1994a, b; Morato *et al*., 2000; Morais *et al*., 2004, 2005; Lopes *et al*., 2005; Soares *et al*., 2007; Silva *et al*., 2007).

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Among several protein sources that may be used for preparing protein hydrolysates for dietary purpose and the choice in most cases, is isolated casein, the main milk protein (Outinen *et al*., 1996; Shimamura *et al*., 1999). However, in underdeveloped countries, this protein needs to be imported which will cause a considerable increase in production costs. Thus, the use of less expensive alternative sources, such as whey, must be investigated since its proteins are readily assimilated by the organism and show a high protein efficiency ratio (Nicolau *et al*., 2005). Moreover, the use of whey, a waste of milk industries in some countries, may contribute to reduce the environment pollution.

The hydrolysis of proteins can be made by enzymes or the use of an acid or a base. However, enzymatic treatment shows several advantages over the chemical one, like the possibility of controlling the degree of hydrolysis, the milder conditions used, the lower cost and salt content in the final product as well as the minimum formation of by-products (Lee *et al*., 2001). Moreover, the removal of the enzymes from the reaction system is, generally, not needed and if so it is easier than for other catalysers, since they are used in low concentrations (Tucker and Woods, 1995). Another advantage of the use of enzymes is associated with the formation of oligopeptides that are nutritionally beneficial and required in dietary supplements (Trusek-Holownia, 2008).

Several proteolytic enzymes have been used for preparing protein hydrolysates with dietary applications (Clemente, 2000; Morato *et al*., 2000; Carreira *et al*., 2004; Lopes *et al*., 2005; Soares *et al*., 2006). After having tested some enzymes for this purpose, we decided to use a subtilisin and a pancreatin in this research for the first time.

For characterizing peptide profiles of protein hydrolysates, we developed a technique which consists in the fractionation of peptides lower than 1000 Da according to their chain size, followed by a quantification of these peptides by a rapid method based on the estimation of the corrected fraction area, after the removal the influence of aromatic amino acids (Silvestre *et al*., 1994a, b). The fractionation of whey hydrolysates was carried out by Size-Exclusion HPLC (SE-HPLC) on a PHEA column, according to the method developed by our group (Silvestre *et al*., 1994a, b). The rapid method of Correct Fraction Area (CFA) was used for quantifying peptides and free amino acids in SE-HPLC fractions of whey hydrolysates. The samples were fractionated and the CFA values calculated with aid of a standard curve, prepared by using whey as the substrate (Silvestre *et al*., 1994a, b).

Since then, we have been using this method for analysing several protein hydrolysates prepared in our laboratory (Morato *et al*., 2000; Barbosa *et al*., 2004; Carreira *et al*., 2004; Lopes *et al*., 2005; Morais *et al*., 2005; Soares *et al*., 2007).

The goal of the present research was to optimize the whey hydrolysis, using a subtilisin and a pancreatin, for obtaining high oligopeptide content.

MATERIALS AND METHODS

Materials

Whey (Kerrylac 700, demineralized), in a powder form, was kindly furnished by Kerry of Brazil Ltda. (Três corações, Minas Gerais, Brazil). A subtilisin (Protemax N200, from *B. subtillis*, Activity = 12.4 U mL^{-1}, where one unit of activity is defined as the amount of enzyme that liberates 1 μg of tyrosine in one min at 37°C) was kindly furnished by Prozyn (São Paulo, SP, Brazil). A pancreatin (Corolase PP, from porcine pancreas, Activity = 18.9 U mL^{-1}, where one unit of activity is defined as the amount of enzyme that liberates 1 μg of tyrosine in 1 min at 37°C) was kindly furnished by AB Enzymes® (Barueri, SP, Brasil). The HPLC system consisted of one pump (HP 1100 Series), an UV-VIS detector, coupled to a computer (HPchemstation HP1100, Germany). A poly (2-hydroxyethylaspartamide)-silica (PHEA) column, 250×9.4 mm, 5 μm, 200 Åpore size (PolylC, Columbia, MD), was used for HPLC. For HPLC, water was purified by passage through a water purification system (Aries-Vaponics, Rockland, EUA). All solvents used for the HPLC were carefully

degassed by sonication for 10 min before use. The freeze dryer was from Labconco (77500 model, Kansas City, MI, USA) and the stirrer from Fisatom (São Paulo, SP, Brazil). This work was conducted in Belo Horizonte, Minas Gerais, Brazil, during the year of 2007.

Methods

Determination of the Chemical Composition of Whey

The contents of moisture, protein, lipid, minerals were determined according to the AOAC International (1995). The carbohydrates were calculated by difference. The conversion factor of nitrogen to protein was 6.38 (Nielsen, 1998).

Preparation of Whey Hydrolysates

Twelve hydrolysates were prepared, 6 hydrolysates with subtilisin and 6 hydrolysates with pancreatin. The pH of whey solutions (10 and 15%, w/v) was adjusted to 7.0 with a 3 mol L^{-1} Na_2CO_3 solution. The solutions were heated in an oil-bath at 55°C, under continuous stirring and the enzyme was added in a concentration to attain the desired enzyme:substrate ratios. The total reaction time was 5 h for all samples and the hydrolytic reactions were stopped by heating at 85°C for 20 min. The hydrolysates were freeze-dried and labelled by different names (Table 1).

Characterization of Peptide Profiles of Whey Hydrolysates

This characterization was performed in two stages: fractionation of the peptides, according to their size, followed by their quantification. The fractionation of whey hydrolysates was carried out by Size-Exclusion HPLC (SE-HPLC) on a PHEA column, according to the method developed by our group (Silvestre *et al*., 1994a, b), using 0.05 mol L^{-1} formic acid as the mobile phase at a flow rate of 0.5 mL min^{-1}. Twenty microliters of 0.4% hydrolysate solutions were injected into the column. Peptides were detected at three wavelengths: 230, 280 and 300 nm. The fractions were separated according to the elution time: F1, from 13.2 to 18.2 min (large peptides with more than 7 amino acid residues); F2, from 18.2 to 21.7 min (medium peptides, with 4 to 7 amino acid residues); F3, from 21.7 to 22.7 min (di-and tripeptides); and F4, from 22.7 to 32 min (free amino acids).

The rapid method of Correct Fraction Area (CFA) developed by our group (Silvestre *et al*., 1994a, b) was used for quantifying peptides and free amino acids in SE-HPLC fractions of whey hydrolysates. The samples were fractionated and the CFA values calculated with aid of a standard curve, prepared by using whey as the substrate. Briefly, five whey standard hydrolysates (two using trypsin and three using pancreatin) were prepared and then fractionated in four fractions by SE-HPLC, as described above. The four fractions were collected and submitted to an amino acid analysis. The calculation of CFA was performed using the formulas described by Silvestre *et al*. (1994b). A standard

Table 1: Hydrolytic conditions employed for preparing whey protein hydrolysates

Hydrolysates	Substrate concentration (w/v)	E:S[a]
S1[b]	10	1:100
S2[b]	10	2:100
S3[b]	10	4:100
S4[b]	15	1:100
S5[b]	15	2:100
S6[b]	15	4:100
P1[c]	10	1:100
P2[c]	10	2:100
P3[c]	10	4:100
P4[c]	15	1:100
P5[c]	15	2:100
P6[c]	15	4:100

[a]E:S: Enzyme:substrate ratio; [b]S: Hydrolysates prepared with subtilisin; [c]P = Hydrolysates prepared with pancreatin

curve was drawn correlating the CFA with the amino acid contents of the fractions. The amino acid contents of the whey hydrolysates prepared by using the subtilisin and the pancreatin described below were determined based on the CFA values obtained using the standard curve as a reference.

Statistical Analysis

All experiments were replicated three times and all measurements were carried out in triplicate. Differences between means of areas were evaluated by analysis of variance (ANOVA) and Duncan test (Pimentel-Gomes, 2000). Differences were considered to be significant at $p<0.05$ throughout this study. The least square method was used to fit the standard curve and the adequacy of the linear model ($y = ax + b$) was tested at $p<0.05$. The factorial analysis was used to evaluate peptide and free amino acid contents of chromatographic fractions. The analysis of variance was performed for each condition, in order to investigate the presence of significant effects among treatments ($p<0.05$) and in these cases the Duncan test was applied to establish the differences among the means (Pimentel-Gomes, 2000).

RESULTS AND DISCUSSION

Chemical Composition of Whey

In general, the values found here are close to those of the literature, although several factors such as the animal, the feed and the climatic conditions may affect the milk and therefore whey composition (EMBRAPA, 2006). Moreover, the analytical method used may also influence the results. The results of the analysis of some components of whey are shown in Table 2. The ash content is lower than those already reported in literature because the whey used in this study was a demineralized one. (Silva *et al*., 2007; USDA, 2006).

Chromatographic Patterns of Whey Hydrolysates

The SE-HPLC technique used here showed to be efficient in fractionating whey hydrolysates, especially peptides of molecular mass lower than 1,000 Da (Fig. 1). These results were previously reported by our group using different protein sources such as fluid whey (Delvivo *et al*., 2006; Silva *et al*., 2007), casein (Morato *et al*., 2000; Carreira *et al*., 2004; Morais *et al*., 2005; Barbosa *et al*., 2004), milk (Lopes *et al*., 2005; Soares *et al*., 2006) and rice (Bizzotto *et al*., 2006). In these works using whey, the enzyme was different (papain and pancreatin P-1500 from Sigma Chemical, St. Louis, MO, EUA) from those used here and were previously immobilized in two supports (activated carbon and alumina).

The fractionation of protein hydrolysates based on peptide chain length has been described by several researchers. However, most of the described techniques are concerned with the separation of peptides of high molecular mass (> 1,000 Da). The main reported methods are sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE (Perea *et al*., 1993); size-exclusion chromatography, SEC (Parrado *et al*., 1993); capillar HPLC (Davis and Lee, 1992); ligand exchange-HPLC, LE-HPLC (Aubry *et al*., 1992) and SE-HPLC (Visser *et al*., 1992). These techniques showed some inconvenience,

Table 2: Chemical composition of whey obtained in this study compared with other data available in the literature

Nutrients	Values found[a]	Silva *et al*. (2007)[b]	USDA (2006)[c]
Moisture	2.63	3.51	3.19
Protein	13.54	11.82	12.93
Lipids	0.75	0.85	1.07
Total ash	5.11	8.72	8.35
Total sugars	77.97	67.47	74.46

All the values are in g/100 g. [a]Values (g/100 g) found in the present work; [b]Values (g/100 g) found in whey from Prolácteos Indústria e Comércio Ltda (Contagem, MG, Brazil); [c]Values (g/100 g) from USDA National Nutrient Database for Standard Reference, Release 19, 2006

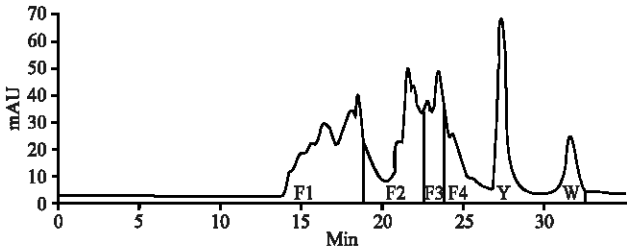

Fig. 1: Chromatographic pattern of a whey hydrolysate (S1) at 230 nm. F1: Large peptides (>7 aminoacid residues); F2: Medium peptides (4-7 aminoacid residues); F3: Di-and tripeptides; F4: Free amino acids. Y = tyrosine peak, W = tryptophan peak. Hydrolysate S1: Substrate concentration = 10%, E:S = 1%

Table 3: Peptide and free aminoacid contents of chromatographic fractions of whey hydrolysates

Hydrolysates	F1 (>7 AA[a] residues)	F2 (4-7 AA[a] residues)	F3 (2-3 AA[a] residues)	F4 (Free AA[a])	F2+F3 (2-7 AA[a] residues)
S1[b]	45.91$^{b/1}$	18.38$^{d/3}$	7.15$^{c,d/4}$	28.54$^{c/2}$	25.53^{e}
S2[b]	46.84$^{b/1}$	17.72$^{d/3}$	7.88$^{c,d/4}$	27.54$^{c/2}$	25.60^{e}
S3[b]	43.65^{c1}	19.97$^{c/3}$	7.96$^{c,d/4}$	28.39$^{c/2}$	27.96^{d}
S4[b]	43.21$^{c/1}$	30.53$^{a/2}$	10.89$^{a/4}$	15.37$^{e/3}$	41.42^{a}
S5[b]	55.17$^{a/1}$	17.49$^{d/3}$	6.86$^{d,e/4}$	20.40$^{d/2}$	24.34^{e,f}
S6[b]	54.84$^{a/1}$	17.70$^{d/3}$	6.22$^{e/4}$	21.19$^{d/2}$	23.92^{f}
P1[c]	22.44$^{d/3}$	26.26$^{b/2}$	10.24$^{a/4}$	41.04$^{b/1}$	36.50^{b}
P2[c]	23.34$^{d/3}$	26.52$^{b/2}$	10.45$^{a/4}$	39.68$^{b/1}$	36.97^{b}
P3[c]	8.69$^{e/3}$	21.51$^{c/2}$	8.26$^{b,c/3}$	61.52$^{a/1}$	29.77^{d}
P4[c]	23.85$^{d/3}$	28.50$^{a/2}$	8.62$^{b,c/4}$	39.01$^{b/1}$	37.12^{b}
P5[c]	23.15$^{d/2}$	24.83$^{b/2}$	9.35$^{a,b/3}$	42.64$^{b/1}$	34.18^{c}
P6[c]	22.12$^{d/3}$	25.47$^{b/2}$	10.20$^{a/4}$	42.19$^{b/1}$	35.67^{b,c}

The values are in % of nmols of the four fractions and represent the means of triple repetition. Different numbers are significantly different ($p<0.05$) for different fractions of the same hydrolysate. Different letter(s) are significantly different ($p<0.05$) for the same fraction of different hydrolysates. AA[a] = Amino acid; S[b] = Hydrolysates prepared with subtilisin; P[c] = Hydrolysates prepared with pancreatin

like hydrophobic and electrostatic interactions between solute molecules and the matrix (Golovchenko *et al*., 1992) and the inefficiency in separating small peptides (Lemieux *et al*., 1991). The use of SEC and LE-HPLC appeared to be able to separate only peptides from a mixture of amino acids. The SE-HPLC and capillar HPLC failed to separate peptides based on their chain length and several molecular weight overlaps have been reported (Davis and Lee, 1992).

Peptide and Aminoacid Contents of Hydrolysates

According to Frenhani and Burini (1999), during the metabolism of proteins, the first stage of their hydrolysis leads to the formation of oligopeptides containing 2 to 6 amino acid residues and free amino acids. Then, these peptides are broken to di-and tripeptides which will be absorbed and so will free amino acids. The absorption of the formers is quicker. González-Tello *et al*. (1994) also reported the advantage of the di-and tripeptides over the free amino acids, in relation to the rate of absorption.

Among the hydrolysates prepared with subtilisin, S4 showed the best peptide profile. Although the amount of most of its peptides as well as that of free amino acids were near those of the other samples, its oligopeptide content (41.42%) was much higher and its di-and tripeptide content (10.89%) were also higher than those of the other samples (Table 3).

Concerning the use of pancreatin, no significant difference was observed among the peptide profiles of five hydrolysates. Thus, P1, P2, P4, P5 and P6 showed similar peptide profiles from the nutritional point of view, since they contain low large peptide content (F1 = 22.98%, in average) as

well as high amount of oligopeptides (F2+F3 = 36.09% in average), especially of di-and tripeptides (F3 = 9.77%, in average) and of free amino acids (F4 = 40.91%, in average).

Also, we can observe that although P3 showed much lower amount of large peptide than the other hydrolysates (P1, P2, P4, P5 and P6), its extremely high amount of free amino acids (61.52%) may be inconvenient, producing high hyperosmolarity and, consequently, causing diarrhea (González-Tello *et al*., 1994).

To date, no information was available on the action of subtilisin on the peptide profile of whey hydrolysates. Our group have evaluated this effect for casein hydrolysates, using a subtilisin (Carlsberg, Sigma, from *B. Licheniformis*) different from that was used here and obtained a much higher di-and tripeptide contents (36%) and a much lower amount of large peptides (14%). However, the substrate concentration used in the study of Morato *et al*. (2000) was 80-120 times lower (0.125% of casein) than the one used here (10 and 15% of whey), which would then increase the costs associated to the higher time required for the drying stage, notably in a scaling-up process (Morato *et al*., 2000).

To date, no information is available on the use of pancreatin for hydrolysing whey. However, we have studied before the effect of a pancreatin, purchased from a different company (Sigma Chemical, St. Louis, MO, EUA), on the peptide profile of whey hydrolysates, using different hydrolytic conditions. In this study (Silva *et al*., 2007), the pancreatin was previously immobilized on activated carbon and the hydrolysis was performed at a lower temperature (30°C) and E:S ratio (1:10000) than those used here. The peptide profile obtained for the whey hydrolysates in the study of Silva *et al*. (2007) was nutritionally inferior from those of the present research for P1, P2, P10 and P12, considering the higher large peptide (58%) and the much lower free amino acid contents (2%). However, with respect to the oligopeptide content, no significant difference between the two works was shown (40%), but the amount of di-and tripeptides was higher (15%) in the study of Silva *et al*. (2007), which is an advantage from the nutritional point of view.

Comparison between Different Enzymatic Treatments

No report was found in the literature concerning the effect of hydrolytic parameters of a subtilisin on peptide profile of whey hydrolysates. Thus, the results obtained in this study were compared with those obtained for this enzyme but using casein as substrate (Morato *et al*., 2000). In case of pancreatin, some works in the literature were performed with whey, but the reaction conditions were different from those used here (Silva *et al*., 2007). Also, for these two enzymes, no mention concerning the effect of substrate concentration was made in all reports found in the literature.

Effect of Enzyme Type

Comparing the best peptide profiles obtained by the use of the two enzymes (S4 and the average values of P1, P2, P4, P5 and P6), it can be inferred that pancreatin was more advantageous than subtilisin. Thus, although no significant differences were observed among their oligopeptides (36.09% in average and 41.42 %, respectively) as well as di-and tripeptides (9.77%, in average and 10.89%, respectively) contents obtained by these two enzymes, the use of pancreatin produced lower amount of large peptides (22.98%, in average and 43.21, respectively) and higher free amino acid contents (40.91%, in average and 15.37, respectively). The other advantage of pancreatin is related to the number of hydrolysates that presented the best peptide profiles. Thus, among six hydrolysates prepared with each enzyme, five showed the best peptide profiles for pancreatin and only one for subtilisin.

Effect of Substrate Concentration

The evaluation of the effect of substrate concentration (SC) in the action of subtilisin on whey hydrolysis, can be done by comparing the following hydrolysates: S1 with S4 (E:S = 1:100), S2 with

S5 (E:S = 2:100), S3 with S6 (E:S = 4:100) As shown in Table 3, the concentration of 10% was more advantageous, from the nutritional point of view than 15% in two of the three cases studied (E:S = 2:100 and 4:100), since it produced lower large peptide and higher free amino acid contents as well as higher amount of oligopeptides, especially di-and tripeptides (E:S = 4:100). When comparing S1 with S4 (E:S = 1:100), the best peptide profile was obtained for the concentration of 15%, in relation to its lower large peptide and higher oligopeptide production, especially di-and tripeptides. The only advantage, from the nutritional point of view of 10% over 15% was its higher free amino acid content.

The effect of SC in the action of pancreatin on whey hydrolysis, can be evaluated by comparing the following hydrolysates: P1 with P4 (E:S = 1:100), P2 with P5 (E:S = 2:100), P3 with P6 (E:S = 4:100). As shown in Table 3, for all these three cases, the peptide profiles obtained by the two substrate concentrations were similar and just few significant differences were observed. Thus, comparing P1 with P4 (E:S = 1:100), the only advantage showed by 10% over 15%, was related to its higher di-and tripeptide production. In the second and third cases (E:S = 2:100 and 4:100), 15% gave rise to higher amount of oligopeptides than 10%, as well as of di-and tripeptides for the third case (P3 e P6) (E:S = 4:100).

Effect of E:S Ratio

For assessing the influence of the E:S ratio of the subtilisin action on whey, the following comparisons must be made: 1.SC of 10% = S1 with S2 with S3 (E:S = 1, 2 and 4:100); 2. SC of 15% = S4 with S5 with S6 (E:S = 1, 2 and 4:100). The data in Table 3 show that, for a SC of 10%, no significant difference was observed between the peptide profiles obtained for E:S of 1 and 2%. However, the use of a higher amount of enzyme (E:S = 4:100) was more beneficial, since it increased the oligopeptide and reduced the large peptide contents. In the case of the more concentrated samples (SC = 15%), the best result was obtained at the lowest E:S ratio (1%), since it showed the lowest large peptide and the highest oligopeptide contents, especially di-and tripeptides than the two other E:S ratios (2 and 4:100), which presented similar contents of all fractions.

In a previous study of our group, the use of an E:S ratio of 4:100 was also more advantageous than 2:100, for the peptide profile of casein hydrolysates prepared by using another subtilisin (Carlsberg, from Sigma Co.), leading to a lower large peptide (30-14%) and higher oligopeptide contents (44- 82%) (Morato *et al*., 2000). To date, no data is available concerning the effect of E:S on peptide profiles of whey hydrolysates by the action of a subtilisin.

In the case of pancreatin, the effect of E:S ratio on whey hydrolysis may be estimated by comparing the following conditions: 1. SC of 10% = P1 with P2 with P3 (E:S = 1, 2 and 4:100); 2. SC of 15% = P4 with P5 with P6 (E:S = 1, 2 and 4:100). It can be seen in Table 3 that, for a SC = 10%, the use of an E:S of 4:100 was the more advantageous, leading to much lower large peptide and much higher free amino acid contents, although its amount of oligopeptides was a little lower than those obtained with E:S of 1 and 2:100. For a SC = 15%, it was observed that the peptide profiles for the three values of E:S used were similar.

No report from another author was found in the literature concerning the effect of E:S on the action of a pancreatin on whey hydrolysis. However, our group have analysed before the peptide profiles of whey hydrolysates, obtained by the action of a pancreatin, purchased from Sigma (Silva *et al*., 2007). In the previous study the whey hydrolysates were first submitted to a treatment by Activated Carbon (AC) for removing phenylalanine. The pancreatin was previously immobilized on AC and a SC of 10% and it was shown by Silva *et al*. (2007) that the use of a E:S of 1:10000 produced a better peptide profile than 1:100, notably concerning the oligopeptide content (8 and 40%, respectively). In another study, using this same enzyme in batch and a more diluted substrate solution (SC of 1%), Delvivo *et al*. (2006) reported that the E:S (1:10000 and 1:100) showed no effect on the peptide profiles of whey hydrolysates.

CONCLUSION

Pancreatin was more effective than subtilisin for hydrolysing whey, mainly because it produced a peptide profile containing lower large peptide and higher free amino acid contents. The effect of E:S and SC in the action of subilisin on whey hydrolysis showed that the best associations of these parameters were: SC = 10% with E:S = 4:100 as well as SC = 15% with E:S = 1:100. For pancreatin, the best results were obtained using the following associations: SC = 10% with E:S = 1 or 4:100 as well as SC = 15% with E:S = 2 or 4:100.

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