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Enzymatic hydrolysis of soybean protein using lactic acid bacteria

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ABSTRACT

The proteolytic activity of 12 lactic acid bacteria (LAB) strains, assayed on soy protein extract at a temperature of 37 °C for 6 h, was evaluated by SDS–PAGE, reverse-phase HPLC and free-amino acid analyses. The results indicated that α- and α′-subunits of β-conglycinin were the preferred substrates for the majority of the LAB. Only a few strains exerted some action against the basic polypeptides of glycinin, this fraction was the least degraded of all soy protein fractions. Whole-cell suspensions of LAB used in this study generated hydrophilic and hydrophobic peptides from mainly soy protein fractions. RP-HPLC analyses indicated differences in the profiles of the hydrolysates, with several peaks decreasing in size and new peaks being formed. Three of the selected strains assayed increased the level of total free amino acids in the soy protein extract (SPE) and hydrolyzed principally essential amino acids and flavour precursor amino acids.

1. Introduction

Soybeans are an abundant and relatively inexpensive source of proteins that are widely recognized for their high nutritional value and excellent functional properties. The major soy proteins (greater than 85%) are β-conglycinin and glycinin. The latter does not contain any carbohydrates, whereas conglycinin is a glycoprotein containing approximately 4% carbohydrate (mainly mannose moieties). Acidic hydrolysis and enzymatic hydrolysis have been extensively applied to improve the solubility and other functional properties of soy protein products (Puski, 1975; Wang & Gonzalez de Mejia, 2005). The protein hydrolysates are physiologically better than the intact proteins because their intestinal absorption appears to be more effective (Ziegler et al., 1998). The enzymatic process involves commercial enzymes or enzymes from microorganism such as *Mucor* sp., *Apergillus oryzae*, *Rhizopus* sp., *Bacillus* (*B.*) *natto* and *B. subtilis* (Gibbs, Zougman, Masse, & Mulligan, 2004; Wang & Gonzalez de Mejia, 2005). One of the simplest ways of producing food-grade hydrolyzed proteins is to use lactic acid bacteria (LAB), which are generally recognized as safe and are traditionally used to ferment raw materials of vegetable and animal origin. In this manner, protein breakdown occurs during the food manufacture with LAB. These proteolytic events have been thoroughly investigated, not only because of their physiological significance, but also because of their technological importance in texture and flavour development (Visser, 1993). Several studies were undertaken to explain the main pathways involved in the industrial production and excellent reviews have covered these aspects of the most extensively utilized dairy starters (Kok & de Vos, 1994; Poolman, Kunji, Hagting, Juillard, & Konings, 1995; Savijoki, Ingmer, & Varmanen, 2006). The use of LAB in fermented soy products has received much attention, with studies on bacterial growth, end-product formation and taste (Garro, de Valdez, & de Giori, 2004; Garro, de Valdez, Oliver, & de Giori, 1998; Liu, 1997). Several studies on metabolism of α-galactosyl oligosaccharides by LAB and *Bifidobacterium* strains have been reported (Garro, Aguirre, & Savoy de Giori, 2006; Garro, Font de Valdez, Oliver, & Savoy de Giori, 1996; LeBlanc, Garro, & Savoy de Giori, 2004; Mital & Steinkraus, 1975; Shimakawa, Matsubara, Yuki, Ikeda, & Ishikawa, 2003) but there is a lack of detailed information in the literature about the proteolytic activity of LAB on soybean proteins. The aim of this study was to evaluate the action of whole cells of LAB on a soy protein extract as an attempt to gain better knowledge of their hydrolytic ability and the nature of the generated products in relation to soy food.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus (*L.*) *paracasei* subsp. *paracasei* CRL 207, *L. fermentum* CRL 251 and CRL 722, *L. delbruecki* subsp. *lactis* CRL 581 and CRL 654, *L. plantarum* CRL 759, CRL 785 and CRL 794, *L. helveticus* CRL 1062, *L. reuteri* CRL 1099 and CRL 1101 and *Pediococcus pentosaceus* CRL 761, were obtained from the culture collection (CRL) of the Centro de Referencia para Lactobacilos (CERELA). Working

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cultures of LAB were routinely grown in sterile MRS broth medium (De Man, Rogosa, & Sharpe, 1960) and incubated at 37 °C for 16 h without agitation.

2.2. Preparation of cell suspensions

The proteolytic activity on soy protein extracts was assayed in whole-cell suspensions. Cells at the exponential growth phase (OD_{560} = 0.5–0.6) were harvested by centrifugation (10,000*g* for 10 min at 4 °C), washed twice in 0.085% (w/v) NaCl containing 5 mM $CaCl_2$ (pH 7.0), and resuspended in 50 mM phosphate buffer (pH 7.0) (20% of initial volume).

2.3. Activity on soy protein extracts

2.3.1. Preparation of soy protein extracts (SPE)

Soy proteins were extracted according to the method described by Iwabuchi and Yamauchi (1987). Briefly, defatted soybean meal (20 g), obtained from a commercial source, was extracted once with 400 ml of 0.03 M Tris–HCl buffer (pH 8.0) containing 10 mM 2-mercaptoethanol (ME) at room temperature with agitation for 1 h and centrifuged at 15,000*g* for 20 min at 4 °C. The resulting supernatant (SPE), a glycinin- and β-conglycinin-rich fraction with high yield, was sterilized through 0.22 μm filters (Millipore, Bedford, MA). The protein concentration of this SPE was 10.6 mg/ml. The SPE was kept at −20 °C in aliquots until used.

2.3.2. Enzymatic mixtures

Three independent assays were carried out for SPE by using as an enzymatic sample of each whole-cell suspensions. A cell suspension of each microorganism was added to the SPE (2:1 ratio). The mixtures were incubated at 37 °C in a water bath. Samples were taken initially and after 6 h of incubation, centrifuged at 10,000*g* during 10 min and stored at −20 °C for further analysis. In every case, control samples without the addition of whole-cell suspensions and a control of whole-cell suspension without the addition of SPE were analyzed simultaneously.

2.3.3. Determination of protein concentration

The protein concentration in the different samples was determined according to Bradford (1976) using bovine serum albumin as standard.

2.3.4. Gel electrophoresis

The hydrolysis of soy protein extract was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), using 5% stacking gel and 12% separating gels according to the methods of Laemmli (1970). A vertical gel electrophoresis unit (Mini-Protean II; Bio-Rad Laboratories, Richmond, CA) was used. Aliquots of 20 μg of protein sample and 5 μg of a molecular weight (MW) marker were loaded into the lanes. Gel electrophoresis was carried out at 70 mV. Gels were stained with 0.1% (w/v) Coomassie brilliant blue R-250 at room temperature. MW values of protein fractions were estimated by using Broad range Protein Marker (2–212 kDa) from Biolabs (New England BioLabs Inc., P7702L). The protein-stained bands were quantified using a Quanti-Scan software version 2.1 (Biosoft, UK). A linear relationship between the stain intensity and the protein concentration was observed with each band, the relative ratios among the protein bands of a sample being calculated from these stain intensity values.

2.3.5. Peptide analysis

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to separate soy peptide fractions on the basis of the hydrophobicity (Riblett, Herald, Schmidt, & Tilley, 2001) using an ISCO (ISCO, Lincoln; NE, USA) liquid chromatograph equipped with a UV–visible detector. A Peak Simple II software data acquisition system was used. Samples of 25 μl were applied onto a Spherisorb ODS2 column (C_{18}, 5 μm particle size, 250 × 4.6 mm I.D. ISCO, Lincoln; NE, USA.). The binary solvent system consisted of solvent A containing 0.1% (v/v) trifluoroacetic acid (TFA) in water and solvent B containing 0.1% (v/v) TFA in acetonitrile at a flow rate of 1.0 ml/min. The multistep linear gradient elution was continuous from 0% solvent B at time 0 min to 40% of solvent B at 30 min, held at 40% of solvent B for 15 min, and then returned to initial conditions for 0% solvent B. Peptides were detected at 280 nm.

2.3.6. Amino acid analysis

Concentration of free amino acids present in the hydrolysates (supernatants) was also determined. These supernatants were derivatized to *o*-phthaldialdehyde (OPA) derivatives, and the amino acids concentration was determined by RP-HPLC. The chromatographic separation was carried out using an ISCO system (ISCO, Lincoln, NE) on a Microsorb 100 Å column, 4.6 × 250 mm, with an elution flow rate of 1.3 ml/min and a fluorometer detector (excitation 340 nm–emission 425 nm). The binary solvent system consisted of solvent A: methanol, 10 mM sodium phosphate buffer (pH 7.3), tetra hydrofurane (THF) (19:80:1) and solvent B: methanol, 10 mM sodium phosphate buffer (pH 7.3) (80:20). Identification and quantification of amino acids were carried out by comparison with a standard mixture of amino acids (Sigma Chemical).

2.4. Reproducibility

All results presented in this paper are averages of three independent assays. The variations among results were less than 10%. Results were expressed as means ± standard deviation, and their significance was analyzed using the Student's *t*-test.

3. Results

3.1. Electrophoretic analysis

The protein profiles resulting from the hydrolysis of soy proteins by LAB strains on SDS–PAGE were analyzed by Quanti-Scan

Table 1
Degradation of soy protein extract by whole-cell suspension of lactic acid bacteria[a]

LAB	Soy protein extract (% hydrolyzed)					Total average
Strains	β-Conglycinin			Glycinin		
	α	α′	β	A	B	
CRL 207	69	82	56	83	60	70
CRL 251	0	0	0	5	4	2
CRL 581	68	89	0	47	52	51
CRL 654	64	43	37	21	0	33
CRL 722	15	12	52	0	8	17
CRL 761	9	14	11	11	0	9
CRL 759	35	53	22	11	5	25
CRL 785	83	78	53	45	0	52
CRL 794	28	0	2	20	0	10
CRL 1062	70	71	35	0	19	39
CRL 1099	61	9	22	31	1	25
CRL 1101	50	0	40	53	1	29
Total average	46	38	28	27	13	

Note:
α, α′, β: main fractions of β-conglycinin.
A: acidic fraction of glycinin.
B: basic fraction of glycinin.
LAB: lactic acid bacteria.
[a] % of hydrolysis of each main fraction of soy protein extract (SPE) treated with whole-cell suspensions of different LAB with respect to those of SPE without treatment.

software. Table 1 shows the results of these analyses. In control samples, lacking any bacterial enzyme, proteolytic changes were undetectable. Proteolytic activity of each whole-cell suspension in the enzymatic mixture varied considerably among strains belonging to the same species. The α- and α′-subunits of β-conglycinin were the preferred substrates for the majority of the lactic acid bacteria *L. plantarum* CRL 785 being the most efficient microorganism for the α-subunit (83% of proteolytic activity). *L. delbrueckii* subsp. *lactis* CRL 581 and *L. paracasei* subsp. *paracasei* CRL 207 exhibited major activity against the α′-subunit (89% and 82% of proteolytic activity, respectively). On the other hand, the proteolysis of the β-subunit of β-conglycinin was minor (*L. paracasei* subsp. *paracasei* CRL 207, *L. plantarum* CRL 785 and *L. fermentum* CRL 722 hydrolyzed 52–56%). Regarding to glycinin degradation by LAB strains, *L. paracasei* subsp. *paracasei* CRL 207 and *L. delbrueckii* subsp. *lactis* CRL 581 were able to hydrolysis both acid and basic subunits (83–60% and 47–52%, respectively), while *L. plantarum* CRL 785 and *L. reuteri* CRL 1101 only degraded the acid subunit. Only few strains exerted some action against the basic polypeptides of glycinin; this fraction was the least degraded of all the soybean protein fractions (only 13%). The hydrolytic actions of *L. fermentum* CRL 251, *L. plantarum* CRL 794 and *P. pentosaceus* CRL 761 on the soy protein extract were not clearly identified by electrophoresis.

It is noteworthy that, after incubation of SPE with some of the whole-cell extracts studied, new protein bands, with different molecular weights, appeared in these hydrolyzates (see conditions in Section 2) (Fig. 1). These bands could be derived from the action of each bacterial protease on the different soy protein fractions. After hydrolysis with *L. paracasei* subsp. *paracasei* CRL 207 (strain that showed major hydrolysis, 70%) (Table 1), the bands present in SPE (α-, α′- and β-subunits present for β-conglycinin, and acid and basic subunits present for glycinin) diminished in intensity and a low-intensity new band was generated at 52 kDa. On the other hand, the hydrolysates obtained with *L. reuteri* CRL 1099 or CRL 1101 showed similar profiles (in Fig. 1 only one shown). These strains generated an important new band with a MW of approximately 50 kDa. The action of strains of *L. plantarum* displayed several new bands corresponding to peptides of 63–25 kDa, *L. plantarum* CRL 759 being the strain that displayed the highest intensity in all new bands (Fig. 1, indicated by arrows).

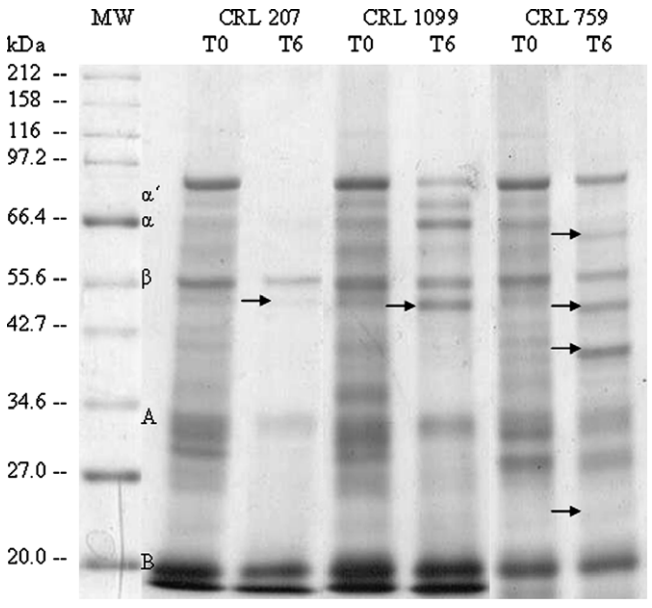

Fig. 1. SDS–PAGE patterns of soy protein extract (SPE) hydrolysates with lactic acid bacteria enzymes. (Lane 1) molecular weight standard (MW); (lanes 2–3) SPE treated with whole-cell suspensions of *Lactobacillus (L.) paracasei* subsp. *paracasei* CRL 207 at 0 h and 6 h of incubation, respectively; (lanes 4–5) SPE treated with whole-cell suspensions of *L. reuteri* CRL 1099 at 0 h and 6 h of incubation, respectively; (lanes 6–7) SPE treated with whole-cell suspensions of *L. plantarum* CRL 759 at 0 h and 6 h of incubation, respectively. α-, α′- and β- subunits of β-conglycinin; A and B: acidic and basic subunits of glycinin.

3.2. Peptide analysis

The reverse-phase HPLC profiles of the SPE and hydrolyzed SPE with whole-cell suspensions of LAB are shown in Figs. 2 and 3. The SPE chromatogram showed seven important peaks with different retention times (RT) (Fig. 2A and 3A): 7.03 min and 7.79 min (peaks 1–2) and RT of 13.65 min and 15.04 min (peaks 3–4), corresponding to the majority of β-conglycinin fractions. The other three peaks (5–7), had RTs of 25.87 min; 29.19 min and 33.12 min, respectively, corresponding to the majority of glycinin protein. Slight changes were observed in the RP-HPLC profiles when the SPE was mixed with different whole-cell suspensions at the initial time (Fig. 2B and 3B) with respect to SPE; this control was similar for each strain of LAB used (one representative pattern is shown in the figures). The treatment of the SPE with whole-cell suspensions of LAB after 6 h of incubation resulted in the hydrolysis of principal protein fractions (peaks 1–7), as revealed by the RP-HPLC elution pattern, with several peaks decreasing in size and new peaks being formed (Fig. 2C–F and Fig. 3C–E). The incubation of SPE with *L. paracasei* subsp. *paracasei* CRL 207 generated hydrophobic peptides with RT values of 34.40 and 39.60 min, respectively, and also peaks with a RT from 2.90 to 7.00 min (hydrophilic peptides) (Fig. 2C). The activity of *L. fermentum* CRL 251 (same effect CRL 722 and CRL 761 strains) resulted in a similar peptide pattern with more defined peaks in the hydrophilic fraction (two peaks with TR of 4.48 and 7.79 min) with respect to those of strain CRL 207 (Fig. 2D). However, different peptide mappings were observed with *L. delbruecki* subsp. *lactis* CRL 581, or CRL 654, or *L. helveticus* CRL 1062 (Fig. 3C–E). In this case, new peaks were observed only in the range from 2.90 to 7 min of retention time (hydrophilic peptides) and considerable variations in size were detected in the region from 7 to 33 min (corresponding to principal soy protein fractions). The action of CRL 581 and CRL 1062 were similar, the intensity of peptide peaks eluting between 13 and 33 min diminished while a high content of hydrophilic peptide increased, eluting at 4.60 and 7.59 min or 4.60 and 8.60 min, respectively (Fig. 3D–E). The activity of CRL 654 on the SPE was not as relevant but also contributed to release of new peptides in the hydrophilic area (in the region from 6.0 to 12.5 min (Fig. 3C).

Four strains (*L. paracasei* subsp. *paracasei* CRL 207, *L. delbrueckii* subsp. *lactis* CRL 581, *L. helveticus* CRL 1062, and *L. reuteri* CRL 1099) were selected for further study for their different abilities to hydrolyze the soy proteins evaluated on SDS–PAGE and RP-HPLC, in addition to particular characteristics of the strains. CRL 207 has great activity in soy milk (SM) and gave good taste and rheological characteristics in this fermented food. CRL 581 and CRL 1062 also had good proteolytic activity on other substrates, such as caseins and whey proteins. CRL 1099 had ability to hydrolyze the sugars present in SM and strong probiotic characteristics. Strains of *L. plantarum* species were not selected because, in SDS profiles, these strains released several peptides with high MW and in SM lead a bad taste.

3.3. Amino acids analysis

The generation of FAA in samples of the SPE hydrolyzed after 6 h of incubation with previously selected LAB strains, in respect to those obtained without enzymatic treatment (control sample), is shown in Table 2. Alanine, arginine, glutamine, cysteine, proline

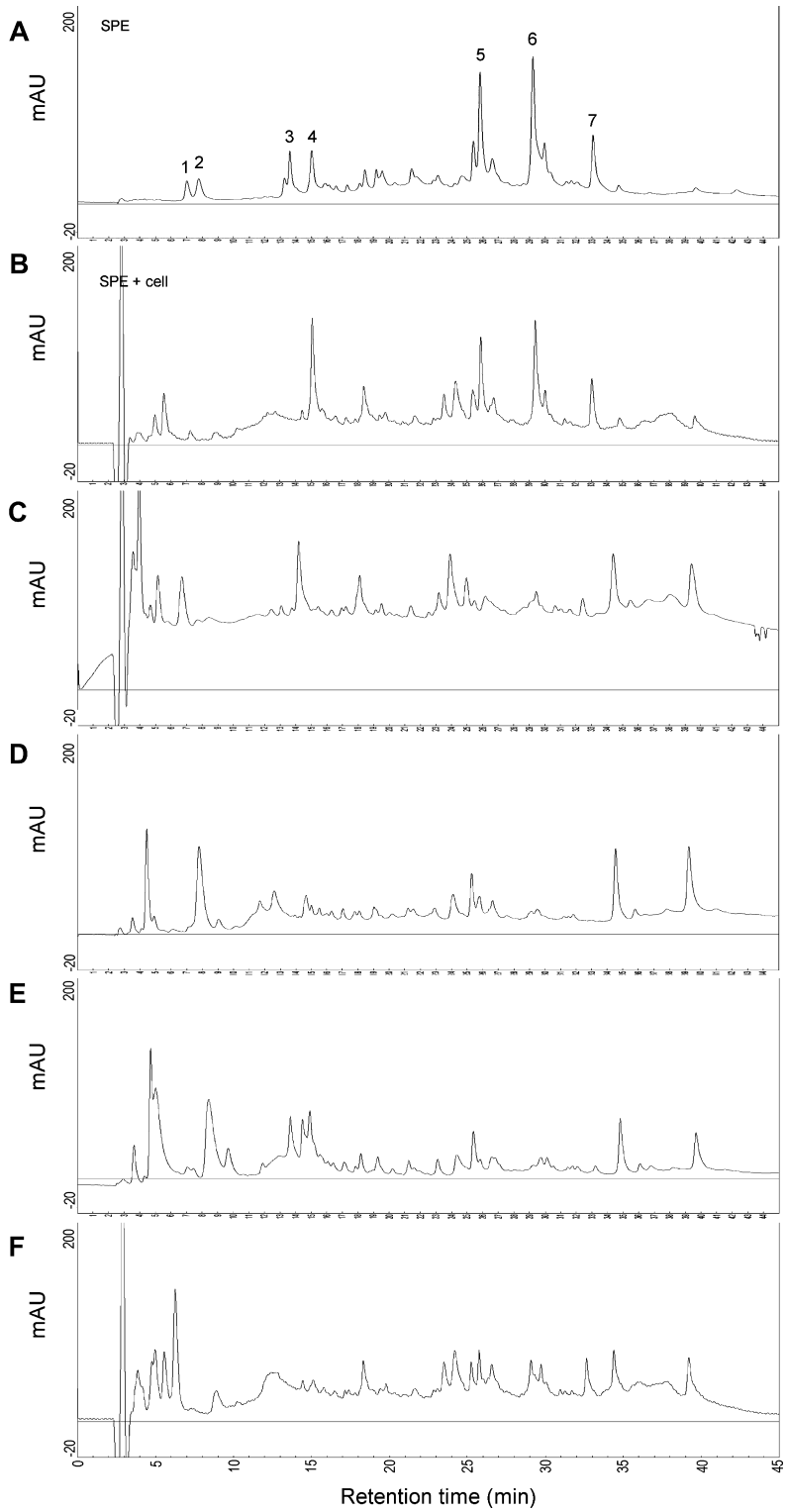

Fig. 2. Reverse-phase HPLC patterns of soluble peptides present in soy protein extract (SPE) treated with lactic acid bacteria (LAB) enzymes. Controls: (A) SPE; (B) SPE and whole-cell suspension of LAB at 0 h. Samples, SPE treated with whole-cell suspensions of LAB at 6 h; (C) *L. paracasei* subsp. *paracasei* CRL 207; (D) *L. fermentum* CRL 251 (same patterns of *L. fermentum* and CRL 722 and *Pediococcus pentosaceus* CRL 761); (E) *L. plantarum* CRL 785 (same patterns of *L. plantarum* CRL 759 and CRL 794); (F) *L. reuteri* CRL 1099 (same pattern of *L. reuteri* and CRL 1101).

and γ-aminobutiric could not be separated under the chromatographic conditions used in this study. Great increases of total FAA were observed in SPE treated with whole-cell suspension of *L. paracasei* subsp. *paracasei* CRL 207, *L. delbruecki* subsp. *lactis* CRL581 and *L. helveticus* CRL 1062 (more than three times). Whole cells of *L. reuteri* CRL 1099 consumed the majority of free amino acids present in the SPE, except for aspartic acid, glutamic acid, valine, and tryptophan, where slight releases were observed. After the effect of enzymes present in *L. delbruecki* subsp. *lactis* CRL581 and *L. helveticus* CRL 1062, some FAAs, such as aspartic acid,

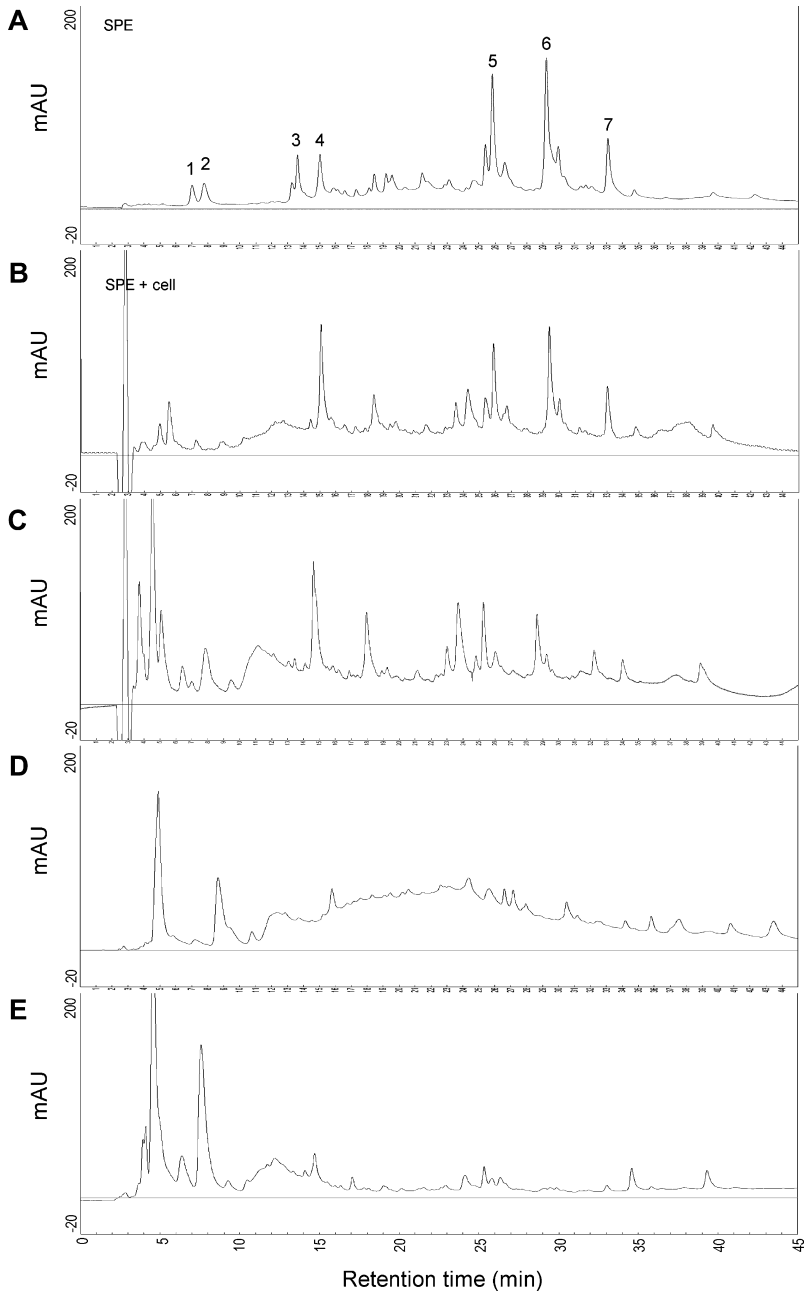

Fig. 3. Reverse-phase HPLC patterns of soluble peptides contained in soy protein extract (SPE) treated with lactic acid bacteria (LAB) enzymes. Controls: (A) SPE; (B) SPE and whole-cell suspensions of LAB at 0 h. Samples, SPE treated with whole-cell suspensions of LAB at 6 h; (C) *L. delbruecki* subsp. *lactis* CRL 654; (D) *L. delbruecki* subsp. *lactis* CRL 581; (E) *L. helveticus* CRL 1062.

glutamic acid, serine, glycine, threonine, tryptophan and lysine, showed a great increase, which suggests a strong effect of these enzymes on SPE. The effects of these strains were similar. The other strain evaluated, *L. paracasei* subsp. *paracasei* CRL 207, had a minor effect; glutamic acid, ornithine and lysine were the most abundant amino acids released, followed by histidine, tyrosine, valine, phenylalanine and leucine, together representing 80% of total FAA. When the SPE was subjected to the action of enzymes of *L. paracasei* subsp. *paracasei* CRL 207, *L. delbruecki* subsp. *lactis* CRL581 and *L. helveticus* CRL 1062, most of the essential FAA (histidine, threonine, valine, tryptophan, phenylalanine, isoleucine, leucine, lysine) increased moderately and only a few of them (methionine and threonine) suffered a decrease by action of CRL 207.

4. Discussion

Soy proteins are widely used as a nitrogen source in infant and adult formulas, both in the intact and hydrolyzed form. Protein hydrolysates possess properties that make them attractive as a protein source in human nutrition. In this study, 12 LAB strains were analyzed for their ability to hydrolyze soybean protein. Electrophoretic analyses demonstrated the suitability of soy protein extract as a substrate for proteolytic enzymes from LAB. Soy proteins consist mainly of β-conglycinin and glycinin, but also contain several minor proteins. β-Conglycinin was the fraction preferred by the LAB evaluated, while only three strains exerted some action against the basic subunit of glycinin (*L. paracasei* subsp. *paracasei* CRL 207, *L. delbrueckii* subsp. *lactis* CRL 581, *L. helveticus* CRL

Table 2
Generation of free amino acids (FAA, mg/ml) in hydrolyzed soy protein extracts (6 h at 37 °C) by whole-cell suspensions of LAB (*Lactobacillus* (*L.*) *paracasei* subsp. *paracasei* CRL 207, *L. delbruecki* subsp. *lactis* CRL 581, *L. helveticus* CRL 1062, *L. reuteri* CRL 1099)

FAA	Control[a]	CRL 207	CRL 581	CRL 1062	CRL 1099
Aspartic acid	3.28	2.96	37.8	38.3	6.32
Glutamic acid	6.70	29.3	46.8	60.1	8.28
Asparagine	0.97	0.68	5.12	5.40	0.29
Serine	2.15	2.15	18.4	18.4	1.15
Histidine	1.51	6.27	9.48	7.86	1.51
Glycine	1.35	1.35	15.0	15.0	0.65
Threonine	1.66	1,18	17.8	19.0	1.20
Tyrosine	1.49	6.05	4.00	4.13	0.47
Methionine	1.66	−1.77	3.11	6.43	1.06
Valine	2.56	10.1	8.49	1.31	2.89
Tryptophan	1.34	2.50	1.83	21.2	1.79
Phenylalanine	2.11	9.16	3.83	3.75	0.72
Isoleucine	2.26	4.20	6.69	6.94	1.10
Leucine	3.03	10.5	8.31	9.70	1.78
Ornithine	5.49	26.9	5.49	7.91	5.09
Lysine	6.18	33.8	27.8	27.7	5.08
Total	43.7	145	220	253	39.4

[a] Control SPE (SPE without enzymatic treatment). Expressed as mg/ml.

1062). These results indicate that this fraction of glycinin is more resistant to proteolytic attack by LAB enzymes than are the other soy protein fractions, probably due to its chemical and physical structure. This can be explained by the native conformation of the unmodified proteins of the extract (SPE) in which the proteolytic LAB enzymes cannot easily access cleavage sites. The results of studies by the method of electrophoresis, as presented in this work, are in agreement with the findings of other authors (Hrackova, Rusnakova, & Zemanovic, 2002; Marsman, Gruppen, Mul, & Voragen, 1997), who also reported an easier enzymatic degradation of the β-conglycinin than the glycinin fraction. Also, other authors have found marked resistance to glycinin breakdown when treated with chymotrypsin or different commercial enzymes (Peña-Ramos & Xiong, 2002). Previous reports by Kella, Barbeau, and Kinsella (1986) and Romagnolo, Polan, and Barbeau (1990) have indicated that basic subunits of glycinin are more resistant to *in vitro* enzymatic hydrolysis than are acidic polypeptides, suggesting that the reduction of disulfide bonds caused a change in conformational structure of basic polypeptides.

The limited sensitivity of the electrophoretic analyses did not reveal the possible activity of enzymes from *L. fermentum* CRL 251, *L. plantarum* CRL 794 or *P. pentosaceus* CRL 761 in initiating the hydrolysis of soy protein extract. Nevertheless, the activity of these enzymes will be important only for peptides released and analyzed by RP-HPLC. Comparison of RP-HPLC profiles, for our SPE, with those reported by Riblett et al. (2001) reveals similar retention times for the main soy proteins present in soybean (RT: 7–15 for β-conglycinin and RT: 25–33 min for glycinin). The profiles of SPE obtained by RP-HPLC were modified after actions of LAB enzymes. Thus, a decrease in the overall concentration of the main soy protein fractions after treatment with whole-cell suspensions was demonstrated by the response area of the peaks. The hydrolysis of SPE led to generation of mainly hydrophilic peptides, which increased under the action of proteolytic enzymes from all the strains assayed. *L. lactis* CRL 581 and *L. helveticus* CRL 1062 were the strains that exerted the major effect over the main soy protein fractions and released only hydrophilic peptides (Fig. 3D–E). It is noteworthy that hydrophilic peptides are normally correlated with desirable fermented soy flavours (Smit, Smit, & Engels, 2005). By the action of enzymes of *L. paracasei* subsp. *paracasei, L. fermentum, L. plantarum* and *L. reuteri* on SPE numerous new peaks were generated as can be seen in the RP-HPLC profiles (Fig. 2C–F). These released peptides were hydrophobic in nature (RT: 25–35 min) as well as hydrophilic (RT: 7–15 min). The hydrophobic residues are associated with bitterness. Three of the four strains examined for FAA liberated some hydrophobic amino acids, such as valine, leucine, phenylalanine, tyrosine and isoleucine, from SPE. The hydrophobic amino acids are involved in the bitter taste of various peptides (Nishiwaki & Hayashi, 2001). Ishibashi et al. (1987a,b) reported that the bitter taste of peptides was more intense when the content of hydrophobic amino acids was high. For these reasons, the decrease in bitterness of the different protein hydrolysates may have occurred due to the release of such hydrophobic amino acids by enzymatic treatments (Nishiwaki, Yoshimizu, Furuta, & Hayashi, 2002). A more effective elimination of the bitterness in the SPE hydrolyzates may be achievable by treatment with the whole-cell suspensions of strain *L. paracasei* subsp. *paracasei* CRL 207 or *L. delbruecki* subsp. *lactis* CRL 581; probably, use of both enzymatic extracts, increased the amount of hydrophobic amino acids released from the peptides. The treatment of SPE with enzymes from *L. paracasei* subsp. *paracasei* CRL 207, *L. delbruecki* subsp. *lactis* CRl 581 or *L. helveticus* CRL 1062 released high levels of glutamic acid (22–53 mg/l). Glutamic acid and its salts are the principal agents which impart delicious taste to various Asiatic soybean fermented products, such as miso, koji and soy sauce (Fukushima, 1995; Liu, 1997). When an enzymatic suspension of *L. delbruecki* subsp. *lactis* CRL 581 on SPE was evaluated, increases of glutamic acid, valine, aspartic acid and glycine were found. Similar results were reported by Odunfa (1985) during the natural fermentation of African bean seeds. Like proteins of most leguminous plants, soy protein is low in sulfur-containing amino acids, with methionine being the most significant limiting amino acid (Eggum & Beames, 1983). However, soy protein contains sufficient lysine, which is deficient in most cereal proteins. This makes it particularly valuable when combined with cereal proteins, by their complementary lysine and methionine. Lysine, an essential amino acid that is limiting in most cereal flours, increased in SPE hydrolyzed by the action of proteolytic enzymes from CRL 207, CRL 581 or CRL 1062. In this sense, the addition of lysine-producing lactic acid bacteria to soy protein might be considered as an alternative way to increase this amino acid concentration in mixed cereal-soybean food. Although, low methionine content is a limiting factor in the nutritional quality of the final soy product, the enzymatic treatment of soy protein allowed improvement of the nutritional quality of the hydrolysates (Calderón de la Barca, Ruiz-Salazar, & Jara-Marini, 2000). In our study, *L. lactis* CRL 581 and *L. helveticus* CRL 1062 were able to increase this amino acid concentration; probably the methionine was released from the glycinin fraction of soy bean protein (Liu, 1997).

In conclusion, LAB strains were able to hydrolysis soy proteins. This novel study gives detailed information on the breakdown of the individual soy protein fractions by proteolytic enzymes from LAB. Further investigations to identify the peptides generated, and to find how the SPE and bacterial proteases interact, must be carried out, especially in relation to the hydrophobic peptides which may contribute to undesirable tastes. Studies, such as mass spectrometry and proteomics, are necessary to evaluate the structural and compositional properties of the peptides obtained by the proteolytic system of these LAB. Since sufficient amounts of essential amino acids and flavour precursor amino acids were released by the action of LAB, SPE hydrolyzed with these microorganisms could be used in fortified soluble formulas, in special diets or mixed with cereal proteins with their complementary lysine and methionine.

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