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Antioxidant activity and inhibition of meat lipid oxidation by soy protein hydrolysates obtained with a microbial protease

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Abstract

Enzymatic proteolysis is helpful to release bioactive peptides encrypted in food proteins. The antioxidant capability of soy protein isolate (SPI) hydrolyzed with a novel protease preparation from *Chryseobacterium* sp. kr6 was investigated. The antioxidant capacity of the hydrolysates was evaluated using radical-scavenging and metal-chelating assays. Maximum values for scavenging of 2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid and 2,2-diphenyl-1-picrylhydrazyl radicals reached 88% and 78%, respectively. The Fe^{2+}-chelating ability of SPI hydrolysates reached higher values at 1 h hydrolysis (61%), decreasing thereafter; and the maximum reducing power was observed after 6 h hydrolysis. The SPI hydrolysates (10 mg/mL) were able to inhibit the formation of thiobarbituric acid reactive substances in pork (62% inhibition) and salmon (65% inhibition) as model systems. These hydrolysates might be applied as natural antioxidants delaying the lipid oxidation and therefore improving quality and increasing the shelf-life of food products.

Introduction

Lipid oxidation is a serious problem faced by the food industry since it produces off-flavors and also decreases the nutritional quality, safety and shelf-life of foods (Min and Ahn, 2005). Therefore, the control of lipid oxidation in food products is desirable and beneficial during food storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have been commercially used in various food products with the aim of increasing shelf-life and quality. However, there is a great concern about the use of such synthetic products due to their toxicity and carcinogenicity (Gharavi *et al*., 2007). In this regard, there is an increasing interest in the identification and development of low cost natural antioxidants.

Some plant proteins are considered as potential dietary sources of antioxidants. Among them, it is possible to emphasize the free radical-scavenging ability attributed to peptides encrypted in soybean proteins, which are released during gastrointestinal digestion. Similarly, hydrolysis of soy proteins using proteolytic enzymes from different sources might result in increased antioxidant capacity due to the release of such peptides (Chen *et al.*, 1995; Wang and Mejia, 2010). Several applications in food science and technology are postulated for the obtained antioxidant peptides, such as the production of functional foods, nutraceuticals, and the increase of shelf-life and quality of food products (Saramadi and Ismail, 2010).

Microbial proteases are regarded as robust biocatalysts for several purposes, including applications in food and feed industries. Some microbial proteases have been successfully used to modify proteins, resulting in hydrolysates with improved nutritional and/or functional properties (Sinha *et al.*, 2007; Santos *et al*., 2011), and also to release bioactive peptides from diverse proteins (Klompong *et al*., 2007; Correa *et al*., 2011; Pan *et al*., 2011). Although soy protein-derived peptides might present interesting biological activities (Wang and Mejia, 2005), relatively few information is available on the use of microbial proteases to produce such bioactive molecules. Additionally, microbial bioprospection opens the possibility of finding proteolytic enzymes well-suited for specific applications. Therefore, this study aimed to evaluate the antioxidant activity of soybean protein isolate (SPI) hydrolysates produced with a novel protease obtained from the bacterium *Chryseobacterium* sp. kr6 (Riffel *et al*., 2007), and also to evaluate the potential application of the hydrolysates to control lipid oxidation in meat systems.

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Materials and Methods

Microorganism and protease production

The bacterium *Chryseobacterium* sp. kr6 (LBM 9006), isolated from the effluent of a poultry processing industry, was kept in feather meal agar plates (Riffel *et al*., 2007). Feather meal (FM; Bunge, Esteio, Brazil) broth, employed as culture medium for extracellular protease production, contained the following components (g/L): NaCl (0.5), KH_2PO_4 (0.4), $CaCl_2$ (0.015), and feather meal (10.0). Initial pH of the medium was adjusted to 8.0. The cultures were performed in 250 mL Erlenmeyer flasks (working volume of 50 mL) for 48 h at 30°C in a rotary shaker (150 rpm). After cultivation, the cultures were centrifuged (10,000 × g for 20 min at 4°C), and the supernatants were collected as the source of proteolytic enzymes. All salts utilized throughout this study were from Merck (Darmstadt, Germany).

Protease preparation

Solid ammonium sulfate was added to the culture supernatants, under stirring, to reach 50% saturation. The mixture was centrifuged (10,000 × g, for 20 min at 4°C), the precipitate was dissolved in 50 mmol/L Tris-HCl buffer (pH 8.0), and centrifuged again to remove insoluble materials. The concentrated sample was applied to a Sephadex G-100 gel-permeation column (30 × 0.8 cm), equilibrated and eluted with 50 mmol/L Tris-HCl (pH 8.0). Fractions showing proteolytic activity were pooled and used as protease preparation in the hydrolysis of SPI.

Determination of enzyme activity

The proteolytic activity was determined using azocasein (Merck, Darmstadt, Germany) as substrate, as described elsewhere (Thys *et al*., 2004). One unit of enzyme activity was defined as the amount of enzyme required to produce a change in absorbance of 0.01 at 420 nm under the assay conditions (40 min at 45°C, pH 8.0).

Enzymatic hydrolysis of soy protein

Soy protein isolate (SPI; Bunge, Rio Grande do Sul, Brazil) was dissolved in Tris-HCl buffer (50 mmol/L, pH 8.0), and the hydrolysis was initiated by adding the protease preparation using a ratio of 0.2 mL of enzyme (816.67 U /mL) to 0.1 g protein substrate. The reaction was carried out at 45°C, under constant shaking (150 rpm), for up to 6 hr. During hydrolysis, aliquots of 1 mL were withdrawn at defined intervals (ti; i = 0, 0.5, 1, 2, 4, and 6 h), and the reaction was stopped by boiling in a water bath. Hydrolysates were then centrifuged (10,000 × g for 20 min) to remove insoluble materials, and the supernatants were frozen and stored at -18°C until further analysis (Rossini *et al.*, 2009).

Determination of soluble protein and free amino acids concentration

The concentration of soluble protein on the supernatant of the hydrolysates was determined by the Folin phenol reagent method (Lowry *et al*., 1951), using bovine serum albumin (BSA) as standard. Concentration of amino acids was determined by the ninhydrin method (Moore and Stein, 1957), using glycine as standard. All measurements were performed using a Shimadzu UV mini-1240 spectrophotometer.

ABTS radical antioxidant activity

The ABTS (2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) cation radical was used for evaluation of antioxidant capacity, using the method described by Re *et al.* (1999). To obtain the ABTS cation radical ($ABTS^{\bullet+}$), a 7 mmol/L ABTS stock solution was reacted with 140 mmol/L potassium persulfate (final concentration), and this mixture was allowed to stand in the dark for at least 12 h at room temperature before use. Just before the assays, the $ABTS^{\bullet+}$ solution was diluted with phosphate-buffered saline pH (5 mmol/L, pH 7.0) to achieve an absorbance of 0.7 (± 0.02) at 734 nm. Then, 10 μL of sample (50 mg/mL) were mixed with 1 mL of diluted $ABTS^{\bullet+}$ solution and, after 6 min, the absorbance at 734 nm was measured. Results were expressed as the percentage of absorbance decrease at 734 nm, calculated from negative controls.

DPPH radical-scavenging assay

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams *et al*., 1995), which is based on the capture of the DPPH radical by antioxidants, producing a decrease in absorbance at 515 nm. Samples of 0.1 mL (200 mg/mL) were added to test tubes containing 3.9 mL of the radical DPPH (60 μmol/L DPPH solution in methyl alcohol), and homogenized by shaking. After 45 min, the scavenging activity was measured by the decrease in absorbance at 515 nm. Methyl alcohol was used as a blank. Control tests were performed by adding distilled water (0.1 mL) to the DPPH solution instead of the samples. DPPH concentrations from 0 to 60 μmol/L were utilized to construct a standard curve. Results were expressed as scavenging rate (%) = $[1-(A/A_0)]\times100$, where A is the absorbance of the test and A_0 is the absorbance of the controls.

Iron (II) chelating activity assay

The chelating activity of Fe^{2+} was measured

using the method described by Chang *et al.* (2007) with slight modifications. One milliliter of sample (3.5 mg/mL) was mixed with 3.7 mL distilled water and then the mixture was reacted with 0.1 mL of 2 mmol/L $FeSO_4$ (Fe^{2+}) and 0.2 mL of 5 mmol/L ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine). After 10 min, the absorbance was read at 562 nm. One milliliter of distilled water, instead of sample, was used as a control. Ethylenediaminetetraacetic acid (EDTA; 20 mg/mL) was used as standard. The results were expressed as chelating activity (%) = $[1-(A/A_0)]\times100$, where A is the absorbance of the test and A_0 is the absorbance of the control.

Determination of reducing power

Reducing power of the hydrolysates was measured as previously described (Zhu *et al*., 2006). Samples (15 mg/mL) from each hydrolysis period were mixed with 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL potassium ferricyanide (10 mg/mL), and then the mixture was incubated at 50°C for 20 min. After this period, 2.5 mL TCA (10%, w/v) were added and this mixture was centrifuged (3,000 × g for 10 min). The supernatant (1 mL) was mixed with 2.5 mL distilled water and 0.2 mL ferric chloride (1 mg/mL), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a greater reducing power. Butylated hydroxytoluene (BHT) at the same concentration of samples was used as a positive control.

Antioxidant activity in meat homogenates

Antioxidant activity of the 6-h SPI hydrolysate in meat models systems was determined by measuring thiobarbituric acid reactive substances (TBARS) as described by Ohkawa *et al.* (1979). For this experiment, pork (2.27% fat, dry weight basis) and salmon (4.27% fat, dry weight basis) meat were employed. Meat samples (20 g) were homogenized in 100 mL of 0.1 mol/L Tris-HCl buffer (pH 7.4) in a blender for 1 min at room temperature. The test medium, containing 100 µL of meat homogenate, 30 µL of Tris-HCl buffer (pH 7.4), 30 µL of ferrous sulfate (10 µmol/L) and 100 µL of the 6-h SPI hydrolysate, was incubated at 100°C for 120 min in screw top tube. The mixture was then tested for the formation of TBARS, by adding 200 µL of 8.1% sodium lauryl sulfate (SDS), 500 µL of acetic acid buffer (pH 3.44), and 500 µL of 0.6% thiobarbituric acid (TBA). After additional incubation at 100°C for 60 min, the reaction products were determined by measuring absorbance at 532 nm in a spectrophotometer. The TBARS concentration was calculated using a standard curve, and results were expressed in nmol/Ll of malondialdehyde (MDA).

Statistical analysis

Results were compared using ANOVA and Tukey post-hoc test ($P < 0.05$). Statistical analysis of the data was performed using the Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA).

Results and Discussion

Hydrolysis of soybean protein

Enzymatic hydrolysis of soybean protein isolate (SPI) using a protease preparation from the bacterium Chryseobacterium sp. kr6 was monitored for up to 6 h through the determination of soluble protein. Under the experimental conditions (45°C, pH 8 and 150 rpm), an increased concentration of soluble proteins was observed as a function of hydrolysis time (Figure 1), reaching maximum values after 4 h of hydrolysis, with no significant increment on soluble protein observed after this period. The free amino acids concentration increased up to 2 h of hydrolysis, remaining almost constant until the 4th hour, and a further increase was observed at 6 h (Figure 1). The results indicate that the protease preparation is efficient to form smaller peptides by hydrolysis of SPI. This pattern is in agreement to that observed for casein hydrolysis by Alcalase, a commercial protease from *Bacillus licheniformis* (Rossini *et al.*, 2009) and soybean protein hydrolysis by neutral protease from *Bacillus subtilis* and validase from *Aspergillus oryze* (Zhang *et al*., 2010). SPI, usually obtained through alkali extraction of the soybean protein, removal of fiber by centrifugation, followed by reprecipitation and drying, contains >90% protein (Lusas and Riaz, 1995). As SPI is highly available and commonly employed by the food and feed industries, it could represent a promising protein source to obtain bioactive peptides.

The soluble protein profile indicates the decreased availability of peptide bonds as the hydrolysis reaction proceeds (Figure 1). Such a phenomenon is commonly observed, and is directly related to both enzyme specificity and the protein substrate (Klompong *et al.*, 2007). As observed from the increase in free amino acids content during hydrolysis (Figure 1), the generated peptides might also serve as substrates for the proteases, which often results in competition for the available enzyme catalytic sites, and a consequent decline in the overall rate of peptide release from the precursor protein.

Table 1. Antioxidant activities of soy protein hydrolysates

Hydrolysis time (hr)	ABTS radical scavenging activity (%)	DPPH radical scavenging activity (%)	Fe^{2+}-chelating ability (%)	Reducing power (Abs at 700 nm)
0	33.57 ± 0.20 [a]	66.70 ± 2.53 [a]	23.83 ± 0.13 [a]	0.089 ± 0.004 [a]
0.5	67.36 ± 0.30 [b]	78.59 ± 019 [b]	25.58 ± 0.34 [b]	0.194 ± 0.001 [b]
1	72.29 ± 0.81 [b,c]	78.02 ± 1.08 [b]	61.03 ± 0.28 [c]	0.228 ± 0.003 [c]
2	77.36 ± 4.34 [c]	78.06 ± 1.14 [b]	15.43 ± 0.14 [d]	0.213 ± 0.004 [d]
4	81.07 ± 2.12 [c, d]	78.78 ± 0.13 [b]	22.46 ± 0.34 [e]	0.249 ± 0.002 [e]
6	88.21 ± 3.94 [d]	78.96 ± 0.13 [b]	19.33 ± 0.35 [f]	0.379 ± 0.004 [f]

The results represent the average of triplicates ± standard deviation of three independent assays. Values followed by different small letters in columns indicate significant difference ($P < 0.05$) by Tukey-test.

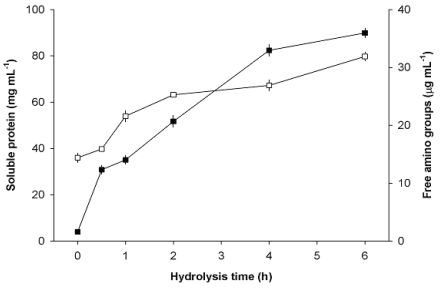

Figure 1. Soluble protein (■) and free amino acid (□) concentration during the hydrolysis of soy protein isolate with the protease preparation from *Chryseobacterium* sp. kr6. Values are the means ± s.e.m. of three independent experiments.

Antioxidant activity of soy protein hydrolysates

The antioxidant activity of the hydrolysates was assessed by two methods, based on the scavenging of the ABTS radical and the DPPH radical. The results showed that the hydrolysates of SPI were able to scavenge both radicals (Table 1). An increase in the ability to capture the ABTS radical was observed as the hydrolysis time increased and maximum values were achieved in 6-h hydrolysates (Table 1). The increase of $ABTS^{\bullet+}$ scavenging activity was also demonstrated for casein hydrolysates (Rossini *et al*., 2009) and whey protein hydrolysates (Dryákowá *et al*., 2010) obtained with microbial proteases, when compared to the non-hydrolyzed counterparts. Particularly, the ability of casein hydrolysates, obtained with a protease preparation from *Bacillus* sp. P7, to quench the $ABTS^{\bullet+}$ radical was observed to increse with hydrolysis time up to 2 h, remaining almost constant thereafter (Correa *et al*., 2011). Although hydrolysis with Flavourzyme showed a beneficial effect on the $ABTS^{\bullet+}$ scavenging capacity of fractions of soluble soy proteins (recovered from the effluents of a soy processing plant), increasing hydrolysis periods were not clearly associated with increments on $ABTS^{\bullet+}$ quenching (Moure *et al*., 2006). It was also demonstrated that fermentation could improve the $ABTS^{\bullet+}$ quenching capability of soybean (Zhu *et al*., 2008).

In the DPPH assays, non-hydrolyzed SPI showed a scavenging activity of 66%, and the SPI hydrolysates presented values around 78% (Table 1). Enzymatic proteolysis usually shows a beneficial effect on DPPH-scavenging activity of distinct proteins, including milk proteins (Mao *et al*., 2011), wheat germ proteins (Zhu *et al*., 2006), and porcine myofibrillar proteins (Saiga *et al*., 2003). For bovine sodium caseinate hydrolysates obtained with a protease preparation from *Bacillus* sp. P45, DPPH scavenging tended to increase with hydrolysis time (Hidalgo *et al*., 2012). Particularly, SPI hydrolysis with pepsin for up to 4 hours was reported to increase the DPPH-scavening activity, which gradually decreased at longer reaction periods, indicating that peptide structure is relevant to the radical-scavenging activity (Fan *et al*., 2005). In this sense, Zhang *et al*. (2010) performed the hydrolysis of SPI with three different microbial proteases, and observed distinct profiles of DPPH-scavenging activities in different fractions of SPI hydrolysates separated by ultrafiltration. Hydrolysis of soy sauce lees protein (SSLP) with Alcalase was also showed to increase the DPPH-scavenging ability when compared to native SSLP (Yang *et al*., 2011). Nevertheless, it is difficult to make direct comparisons with other studies due to the different specificity of the employed enzymes, which could generate different peptides. From Table 1, it could be observed that no major fluctuations on the DPPH scavenging occurred after 30 min of hydrolysis, which is in contrast to the antioxidant activity evaluated through the ABTS assay. As previously reported, this could be related to the different stereoselectivity of the radicals, different peptides able to react with and quench the distinct radicals, as well as the distinct solubility of ABTS radical (water-soluble) and DPPH radical (oil-soluble) in aqueous environments (Zhu *et al*., 2008).

Iron acts as a catalyst in the production of hydroxyl radicals through the Fenton reaction, initiating the chain reactions that lead to lipid peroxidation and, consequently, food rancidity (Min and Ahn, 2005). In SPI hydrolysates, the ability to chelate iron was dependent on hydrolysis time, increasing up to 1 h (61% chelation) and decreasing thereafter (Table 1). Pownall *et al*. (2010) reported 95% iron chelation by a pea protein hydrolysate obtained after 3 h of hydrolysis with thermolysin, indicating the importance of aromatic and hydrophobic amino acids for the observed bioactivity in peptide fractions. The role of carboxyl and amino groups in branches of acidic and basic amino acids, respectively, is also indicated (Saiga *et al*., 2003). As the hydrolysis proceeded, the iron-chelating ability decreased (Table 1), which might indicate that the peptides responsible for the observed activity were further hydrolyzed, and the products were less capable to chelate iron. In this sense, in fractions of soy protein isolate hydrolysates obtained through ultrafiltration, high-molecular mass fractions showed higher chelating abilities than that

of low-molecular mass fractions (Zhang *et al.*, 2010). On the other hand, hydrolysis of porcine hemoglobin with Flavourzyme or Alcalase resulted in decreased ferrous ion chelating ability when compared to the non-hydrolyzed protein (Chang *et al*., 2007).

Hydrolysis of SPI with *Chryseobacterium* sp. kr6 protease preparation resulted in higher reducing powers than that evaluated for native SPI (Table 1). The reducing power was also showed to increase during production of okara koji and soybean koji through fermentation by *Bacillus subtilis* B2 (Zhu *et al*., 2008), and during the hydrolysis of mackerel meat homogenates (Wu *et al*., 2003). In contrast, the reducing power of porcine hemoglobin was reported to be higher than the obtained hydrolysates (Chang *et al.*, 2007). Specifically, the reducing power of ovine caseinate hydrolysates obtained with a microbial protease preparation was reported to reach maximum values (1.094 absorbance units at 700 nm, representing an 80% increase in comparison to non-hydrolyzed caseinate) after 1 h of hydrolysis, decreasing thereafter (Correa *et al.*, 2011). An increasing trend on reducing power was observed (Table 1) until the maximum value was reached after 6 h of hydrolysis (0.379 absorbance units at 700 nm, characterizing a 325% increment in comparison to non-hydrolyzed SPI). Since the reducing power was evaluated through an electron-transfer assay based on the reduction of Fe^{3+} from the ferricyanide complex to the Fe^{2+} form (Pownall *et al*., 2010), the obtained results indicate that the SPI hydrolysates contain peptides able to donate electrons, and suggesting an application on the reduction of oxidized intermediates of lipid peroxidation in foods.

The bioactivities observed in protein hydrolysates are highly dependent on the enzyme employed, the protein substrate and hydrolysis conditions. Nevertheless, enzymatic proteolysis often results in the release of peptides with low molecular mass, increased number of ionizable groups, and exposure of hidden hydrophobic groups, which are related to antioxidant activities (Saramadi and Ismail, 2010). Particularly, from hydrolysates of soy β-conglycinin (7S protein) obtained with a microbial protease, six antioxidative peptides composed of 5-16 amino acid residues were identified, possessing hydrophobic amino acids (valine or leucine) at the N-terminal positions, and proline, histidine, or tyrosine in the sequences (Chen *et al*., 1995). Subsequently, histidine and proline-containing peptides were showed to play an important role in the observed antioxidant activities (Chen *et al*., 1998). Also, the presence of C-terminal tyrosine might be related to the antioxidant capability of peptides obtained through hydrolysis of soy protein isolate with pancreatic trypsin/chymotrypsin (Beermann *et al*., 2009).

Table 2. Effects of 6 h soy protein hydrolysate on the lipid oxidation of meat homogenates, as evaluated by the TBARS assay

Meat homogenate	Treatment	MDA concentration (nmol/l)[a]
Pork	Control	4.39 ± 0.31[a]
	Hydrolysate (2 mg/mL)	2.34 ± 0.82[b]
	Hydrolysate (10 mg/mL)	1.64 ± 0.11[c]
Salmon	Control	12.52 ± 0.65[a]
	Hydrolysate (2 mg/mL)	11.05 ± 0.74[b]
	Hydrolysate (10 mg/mL)	4.37 ± 1.03[c]

[a] MDA, malondialdehyde. Results are presented as means ± standard deviations of three independent assays. Different uppercase letters within each meat homogenate group indicate significant differences ($P < 0.05$) by Tukey-test.

Antioxidant activity in meat homogenates

Since the antioxidant activity of peptides could be attributed to its radical scavenging capabilities, chelation of metal ions, and reducing power, such activities might potentially contribute to the inhibition of lipid peroxidation in foods (Wu *et al*., 2003; Saramadi and Ismail, 2010). Meats are lipid-rich products that are extensively utilized as food models to evaluate the application of antioxidant agents in inhibiting or reducing lipid oxidation (Hogan *et al*., 2009). Therefore, the 6-h SPI hydrolysate was added at 2 or 10 mg/mL to pork and salmon meat homogenates, and lipid oxidation was evaluated by measuring the TBARS. The results are presented in Table 2. The presence of SPI hydrolysates appeared to diminish lipid oxidation in both meat model systems when compared to controls where no hydrolysate was added. For pork homogenates, the presence of hydrolysates at 2 or 10 mg/mL decreased the formation of TBARS by 46 and 62%, respectively, whereas for salmon homogenates, the reductions were 12 and 65%.

Previously, casein hydrolysates at 2.5 and 10 mg/mL inhibited the TBARS formation in 23% and 94%, respectively, in ground beef homogenates; also, at the same concentrations, the lipid oxidation in mechanically deboned poultry meat was inhibited in 10% and 14% (Rossini *et al*., 2009). Also, casein calcium hydrolysates (20 mg/mL), obtained after 20 h hydrolysis with a protease from *Aspergillus* sp., inhibited by 70% the formation of TBARS in ground beef homogenates (Sakanaka *et al.*, 2005). Fractions of milk protein hydrolysates obtained through ultrafiltration, and selected due to their performance in antioxidant and metal-chelating assays, showed an inhibitory trend on the lipid oxidation of cooked beef homogenates at a concentration of 200 or 800 μg/g; however, only the 1-3 kDa fraction of hydrolysates obtained with validase, at 200 μg/g, was demonstrated to significantly inhibit (35%) the

meat lipid oxidation during 15 days at 4°C (Hogan *et al.*, 2009). In a similar study with ultrafiltration-fractionated soy protein hydrolysates, the >10 kDa fraction of hydrolysates obtained with a neutral protease (from *Bacillus subtilis*) and the 1-3 kDa fraction of hydrolysates obtained with alkaline protease (from *Bacillus licheniformis*), at 800 μg/g, were observed to inhibit lipid oxidation (13-26%) in cooked ground beef stored at 4°C for 15 days, when compared to controls prepared without hydrolysate addition (Zhang *et al.*, 2010). Also, the addition of SPI and SPI hydrolysates inhibited lipid oxidation in cooked pork patties (Peña-Ramos and Xiong, 2003).

Conclusion

In this study, a protease preparation from *Chryseobacterium* sp. kr6 was employed to hydrolyze SPI. As a general trend, hydrolysis was observed to positively affect the radical-scavenging capacity, metal-chelating abilities, and reducing power, when compared to native SPI, and 6-hhydrolysates were showed to inhibit lipid oxidation in meat model systems. In summary, this investigation demonstrated the utilization of a novel microbial protease preparation in the production of hydrolysates possessing antioxidant activities from soy protein. These hydrolysates might be potentially applied as alternative natural antioxidants in food science and technology, hindering/delaying the lipid oxidation that ultimately leads to food rancidity, thus improving quality and increasing the shelf-life of food products.

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