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Limited Hydrolysis of Two Soybean Protein Products with Trypsin or Neutrase and the Impacts on their Solubility, Gelation and Fat Absorption Capacity

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Abstract: Soybean Protein Concentrates (SPC) and Soybean Protein Isolates (SPI) were hydrolyzed with trypsin or neutrase to a degree of hydrolysis of 1 and 2%, respectively, to reveal the impacts of limited hydrolysis on their functional properties. Sodium dodecylsulfate-polyacrylamide gel electrophoresis analysis showed that the hydrolysates prepared were hydrolyzed proteins but with different peptide profiles. The evaluation results indicated that the hydrolysates had an increased solubility in pH range of 3-7 over the original SPC or SPI, especially at pH of 4.5. When the hydrolysates were used to prepare heat-induced gels, limited hydrolysis of SPC or SPI led to a decreased or an enhanced hardness of the gels prepared. The SPC hydrolysates had an increased fat absorption capacity about 17-29% while the SPI hydrolysates had a decreased fat absorption capacity about 17-23%. The present study showed that limited hydrolysis of SPC and SPI by neutrase or trypsin could be applied to improve some functional properties of them intended.

Key words: Soybean protein, concentrates, soybean protein isolates, neutrase, trypsin, limited hydrolysis, functional property

INTRODUCTION

Soybean is a particularly valuable source of food proteins in the world characterized by its higher biological value and relatively lower cost. Soybean proteins now are widely used as desired functional and nutritional ingredient in processing foods and are believed to have several physiological functions such as cholesterol-lowering and body-fat reducing effects (Anderson *et al*., 1995; Aoyama *et al*., 2000). FDA approved the health claim concerning the role of soybean proteins in reducing the risk of coronary heart disease (FDA, 1999) which leads the customers increased interests in soybean protein-based foods. Meanwhile, a significant growth of interest in new food ingredients is observed in food science in recent years. Improvement on the functional properties of soybean protein products may increase their applications in processing foods and offer food producers more choice in production. To achieve desirable functional properties, physical, chemical and enzymatic approaches were applied to modify soybean protein products. Application of Maillard reaction to modify the properties of food proteins including soybean proteins has become an area of considerable research interests. However, Maillard reaction could form undesired browning color (Guerra-Hernandez *et al*., 2002) and some mutagenic compounds (Brands *et al*., 2000) which consequently affect sensory quality of final products badly or give rise to safety issue. Fortunately, an enzymatic approach to modify the functional properties of food proteins has some advantages over the chemical approach because of its high specificity, mild reaction conditions and higher food safety.

Among the enzymatic approaches used, limited proteolysis of soybean protein products offered a possibility to obtain the hydrolysates with better functional properties, as many studies have demonstrated that the enzymatic hydrolysis of soybean proteins improved its functional properties, including solubility, emulsification and foaming characteristics (Ortiz and Wanger, 2002; Tsumura, 2009). Lamsal *et al*. (2007) had studied the limited enzymatic hydrolysis of soybean proteins and measured the peptide profiles, water solubility and rheological properties of the products prepared. The effect of limited enzymatic hydrolysis on the interfacial and foaming characteristics of β-conglycinin (fraction 7S of soybean proteins) was investigated (Ruiz-Henestrosa *et al*., 2007). The final result obtained showed that the interfacial characteristics of β-conglycinin were much improved by the enzymatic treatment. Endo-protease treatment of soybean protein

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concentrates or isolates to a low Degree of Hydrolysis (DH) could improve some functional properties including solubility, emulsification capacity and emulsion stability (Jung *et al*., 2005). Also, enzymatic hydrolysis was confirmed to be an effective means to expand the cold gelation conditions (Kuipers *et al*., 2005) fat absorption (Yust *et al*., 2010) and water holding capacity (Yin *et al*., 2008) of some food proteins.

In the present study, two soybean protein products, Soybean Protein Concentrates and Isolates (SPC and SPI), were hydrolyzed by two proteases neutrase and trypsin under controlled conditions to a DH of 1 and 2%, respectively. The peptide profiles of the hydrolysates were studied with Sodium Dodecylsulfate-polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. The solubility, gelation and fat absorption capacity of the hydrolysates were evaluated and compared to that of the original SPC and SPI, in order to reveal the impacts of limited enzymatic hydrolysis of SPC and SPI on some functional properties.

MATERIALS AND METHODS

Materials: Both SPC and SPI were obtained from a local manufacture (Harbin Hi-tech Soybean Food Co., Ltd., Harbin, Heilongjiang, China) with crude protein content (on dry basis) about 64.93 and 89.24%, respectively. Neutrase (70 U mg^{-1}) was purchased from Aoboxing Chemical Co. (Beijing, China) while trypsin (300 U mg^{-1}) was purchased from Amresco Co. (USA). All reagents used were of analytical grade. Water used was distilled water.

Limited enzymatic hydrolysis of SPC and SPI: The whole study began at Jan, 2007 and lasted to Dec, 2008. The SPC was hydrolyzed at a fixed concentration of 10% (w/w, on protein basis). After the pH of the SPC suspension was adjusted to 6.8, the hydrolysis was carried out at a temperature of 25 or 35°C for neutrase or trypsin with continuous agitation. The enzyme addition level used was 400 or 1700 U g^{-1} proteins for neutrase or trypsin. The SPC then was hydrolyzed for 60 or 240 min by two proteases, respectively. The four hydrolysates prepared were heated at 95°C for 30 min to inactivate the protease, freeze-dried and evaluated for their DH and some functional properties as below.

The SPI was also hydrolyzed by neutrase or trypsin, with a fixed substrate concentration of 7.5% (w/w, on protein basis), pH of 7.0 and an enzyme addition level of 250 or 600 U g^{-1} proteins for neutrase or trypsin. The hydrolysis was carried out at 25 or 35°C for neutrase or trypsin. When the SPI was hydrolyzed for 60 or 150 or 180 min by two proteases, the four hydrolysates prepared were heated at 95°C for 30 min to inactivate protease, freeze-dried and also evaluated for their DH and some functional properties as below.

SDS-PAGE analysis: SDS-PAGE analysis of the original SPC or SPI and the hydrolysates prepared followed the method of Laemmli (1970).

Determination of protein content and degree of hydrolysis: Protein content was measured in triplicate by the Kjeldahl method as described in Method 920.123 (AOAC, 2000) on a Kjeltec 2300 Analyzer (Foss, Sweden) and a conversion factor of 6.25 was used. The content of free amino groups of the original SPC or SPI and the hydrolysates was determined in triplicate by a formaldehyde titration method described in the study of Zhang *et al*. (2007).

Evaluation of some functional properties: The evaluation of solubility was carried out as the method of Babiker (2000) with some modifications. Protein samples of 2.0 g were dispersed in water of 30 mL, adjusted to different pHs with diluted HCl or NaOH solution and made to a final volume of 50 mL. The solutions were continuous agitated for 30 min and centrifuged at 5000 rpm for 15 min. The soluble nitrogen in the supernatants was measured by the Kjeldahl method. The solubility of the protein samples were expressed as nitrogen solubility index (NSI%) which is a ratio of the soluble nitrogen to total nitrogen.

The hardness of the heat-induced gels of the protein samples was determined as per the method of Batista *et al*. (2005) with some modifications. The sample suspension (20% w/v, pH 7.0) was heated to 90°C and lasted for 30 min to assure protein unfolding. The suspension was poured into 6 cm diameter cylindrical containers, filled up to 4.5 cm height. The gels were allowed to set at a temperature of 4°C in a refrigerator. The measurement was carried out 24 h after preparation of the gels. Before performing any measurement, the gels were allowed to equilibrate at ambient temperature for approximately 3 h. Then, the hardness was determined using a TA-XT2 Texture Analyzer (Stable Micro Systems, UK) with operating software Texture Expert. Penetration test was performed by using a 5 mm diameter cylindrical probe (p = 0.5) in gels (0.02 N preload force, 10 mm penetration, 5 sec waiting time and 2 mm sec^{-1} crosshead speed).

The evaluation of Fat Absorption Capacity (FAC) of the original SPC or SPI and the hydrolysates prepared followed the method of Moure *et al*. (2005).

Statistical analysis: All data were expressed as Mean±Standard deviation from at least three independent trials. Differences between the mean values of multiple

groups were analyzed by one-way Analysis of Variance (ANOVA) with Duncan's multiple range tests. The SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA) and MS Excel version 2000 program (Microsoft Corporation, Redmond, WA, USA) was used to analyzed or report the data.

RESULTS AND DISCUSSION

Limited hydrolysis of SPC and SPI: The SPC or SPI was hydrolyzed by neutrase or trypsin under controlled conditions to prepare corresponding hydrolysates with lower DH because extensive hydrolysis would destroy gelation or fat absorption capacity of the proteins mostly. Due to the different protein content of the substrate, enzyme activity and enzyme specificity to amino acid residues, different conditions were needed to hydrolyze the SPC and SPI. The practical conditions are listed in Table 1, together with the DH of the eight hydrolysates prepared. The DH of four SPC hydrolysates or four SPI hydrolysates was 1 or 2% and was lowered than that of the protein hydrolysates in the reported works.

SDS-PAGE was applied to show the peptide profiles of the eight hydrolysates prepared. The peptide profiles for the original SPC and the four SPC hydrolysates are presented in Fig. 1a while that for the original SPI and four SPI hydrolysates are presented in Fig. 1b. Figure 1a shows that the four SPC hydrolysates had different peptide profiles to the original SPC. The four SPC hydrolysates had less peptide fractions with Molecular Weight (MW) larger than 43 kDa, indicating the hydrolysis of soybean protein fractions. The SPC hydrolysates having higher DH had less peptide fractions with MW larger than 31 kDa, indicating much hydrolysis occurred. The SPC hydrolysates prepared by trypsin had much peptide fractions with MW larger than 31 kDa, compared to that prepared by neutrase with same DH, indicating neutrase had stronger hydrolysis on soybean proteins than trypsin. The results about the peptide profiles of the SPI and its four hydrolysates shown in Fig. 1b shared similarity to those shown in Fig. 1a. The four SPI hydrolysates had less peptide fractions with MW larger than 43 kDa and the SPI hydrolysates having higher DH had less peptide fractions with MW larger than 31 kDa. Also, the SPI hydrolysates prepared by trypsin had much peptide fractions with MW larger than 31 kDa, compared to that prepared by neutrase with same DH. All analysis results also declared that many peptide fractions existed in the eight hydrolysates with MW less than 20.1 kDa.

Neutrase prefers to catalyze the bonds formed by hydrophobic amino acids; however, trypsin only catalyzes these bonds formed by carboxyl group of Lys or Arg (Kunst, 2003). It can be inferred that neutrase can break protein molecules in many sites while trypsin can break protein molecules only in some sites, viz. the hydrolysates prepared by neutrase would have much smaller peptides as shown in Fig. 1a and b. The peptide fractions existed in the SPC or SPI hydrolysates had different profiles which accounted the different functional properties of the hydrolysates prepared.

Solubility of the SPC or SPI hydrolysates: Figure 2a and b give the NSI profiles for the original SPC, SPI and the eight hydrolysates prepared. The results in Fig. 2a show that the SPC and the four SPC hydrolysates all exhibited their NSI profiles like a V-shape and had the lowest NSI around a pH of 4.5. The SPC hydrolysates obtained had an improved NSI, especially when the pH of the dispersion was about 4.5, compared to the original SPC. It was also demonstrated that the SPC hydrolysates prepared by neutrase (e.g., N-1 and N-2) showed better solubility than that prepared by trypsin (T-1 and 2); if prepared by same protease, the hydrolysates with a higher DH had higher solubility (N-2 vs. N-1, or T-2 vs. T1). The NSI of the SPI and its hydrolysates shown in Fig. 2b also share similarity to those shown in Fig. 2a and are not to be described here.

The study of Ortiz and Wanger (2002) showed that soybean protein hydrolysates by bromelain exhibited better protein solubility in a pH of 4.5 or in trichloroacetic acid. Radha *et al.* (2008) reported that partial hydrolysis of the autoclaved soybean flour increased its protein solubility at pH 4.5 from 17 to 56%. Lamsal *et al.* (2007) found that when soybean protein products were limited

Table 1: Hydrolysis conditions applied on soybean protein concentrates (SPC) or soybean protein isolates (SPI) and degree of hydrolysis (DH) of the hydrolysates prepared

Substrate	Product	Enzyme addition [a](U/g proteins)	Temperature (°C)	pH	Hydrolysis time (min)	DH (%)
SPC (10%, w/w)	N-1	N, 400	25	6.8	60	1
	N-2	N, 400	25		240	2
	T-1	T, 1700	35		60	1
	T-2	T, 1700	35		240	2
SPI (7.5%, w/w)	N-1	N, 250	25	7.0	60	1
	N-2	N, 250	25		150	2
	T-1	T, 600	35		60	1
	T-2	T, 600	35		180	2

N: Neutrase, T: Trypsin

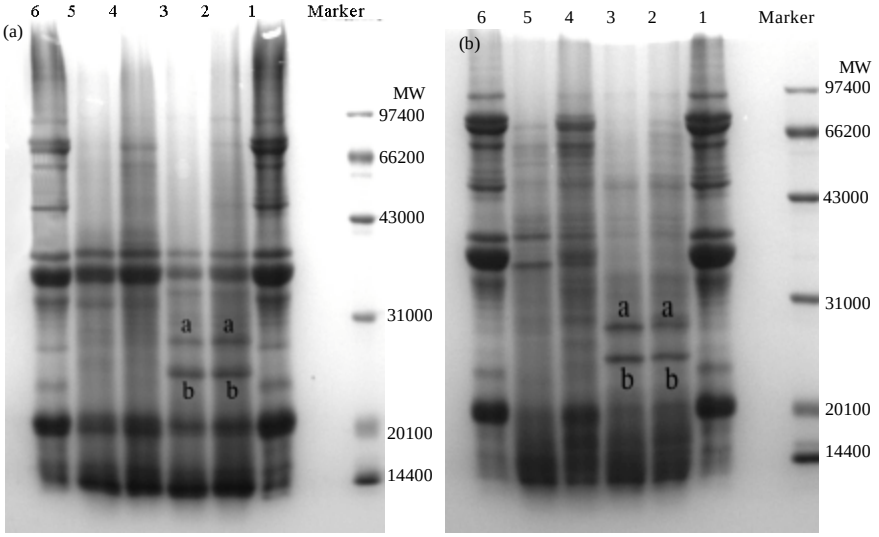

Fig. 1: SDS-PAGE profiles of Soybean Protein Concentrates (SPC) and four SPC hydrolysates (a) or Soybean Protein Isolates (SPI) and four SPI hydrolysates (b). Lane 1 and Lane 6, SPC or SPI; Lane 2 and 3, the hydrolysates prepared by neutrase with DH of 1 and 2%, respectively; Lane 4 and 5, the hydrolysates prepared by trypsin with DH of 1 and 2%, respectively. Symbol MW represents molecular weight

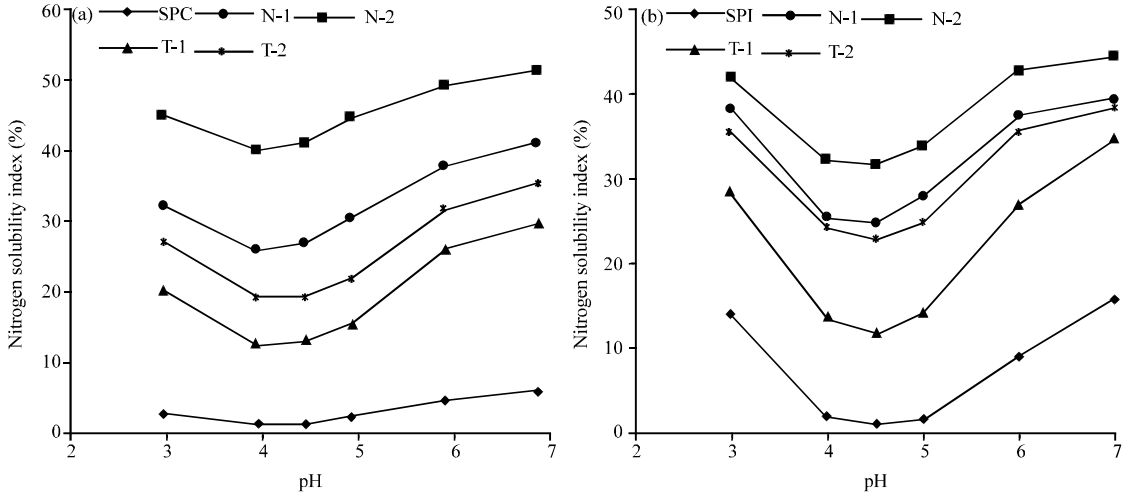

Fig. 2: Nitrogen solubility index of Soybean Protein Concentrates (a) (SPC) and four SPC hydrolysates or Soybean Protein Isolates (SPI) (b) and four SPI hydrolysates which is the ratio of the soluble nitrogen to total nitrogen. Symbol N or T represents neutrase or trypsin used in hydrolysis. The numbers after the symbol N or T represent the DH value of the hydrolysates evaluated

modified by bromelain to the DH of 2-4%, water solubility of all hydrolysates in the pH range of 3-7 were enhanced by hydrolysis. When endo-protease treatment was applied on SPC and SPI to achieve a low DH (2 and 4%), the solubility profile (pH 3 to 7) of the hydrolysates increased as DH increased (Jung *et al*., 2005). When a whey protein concentrate was partial hydrolyzed by Protamex to a DH from 5 to 20%, the solubility would increase from 75 to 77-86% (Gad and Sayed, 2009). Similarly, when exo-proteases were applied on treat soybean proteins to DH ranged from 2 to 10%, an increase in protein solubility was also achieved (Jung *et al*., 2004).

Our work shared same conclusion to these reported works, in spite of the hydrolysates we evaluated had a much lower DH (1-2%).

Gelation of the SPC or SPI hydrolysates: The original SPC, SPI and the eight hydrolysates obtained were used to prepare heat-induced gels. The gel hardness of the gels was evaluated and compared, as given in Fig. 3a and b. The hardness of the gels prepared by the original SPC or SPI was 4483 or 1200 g. Limited hydrolysis of the SPC by two proteases showed an impaired effect on its gelation because the gels prepared with the SPC hydrolysates gave much lower gel hardness ($p<0.05$). The decrease level of the gel harness ranged from 33.6 (T-1) to 72.5% (N-2). In contrast to the SPC hydrolysates, the SPI hydrolysates had improved gelation. The gels prepared with the SPI hydrolysates (except for N-2) had higher gel hardness than that prepared with the original SPI ($p<0.05$) with increase level ranging from 80 (T-2) to 234% (T-1). The results indicated that limited hydrolysis of SPI by trypsin to a DH of 1-2% or by neutrase to a DH of 1% would be a good selection to improve its gel hardness while limited hydrolysis of SPC by two proteases didn't improve it gel hardness. In the study of Kuipers *et al*. (2005) soybean proteins were hydrolyzed by subtilisin Carlsberg to a DH of 10%. The acid-induced cold gels formed by the hydrolysates had a softer texture (lowered gel hardness). When enzyme-hydrolyzed soybean protein (0.5-10 kDa ultrafiltration fraction) was added to myofibril protein isolates, the gels formed above 65°C had lower gel strength than the control gels (Huang *et al*., 2010) which meant an adverse effect occurred to the gelation property of SPI. If β-lactoglobulin B was hydrolyzed by an immobilized proteinase from *Bacillus licheniformis*, longer hydrolysis time before gelation led the gels a weaker texture than the controls (Otte *et al*., 2000). The behavior of the gel hardness of the SPC hydrolysates in our work was similar to these reported works while that of the SPI hydrolysates was different to these reported works.

Fat absorption capacity of the SPC or SPI hydrolysates: FAC of food proteins is an important functional property to some food products. The data shown in Fig. 4a indicate that limited hydrolysis of SPC would improve its FAC significantly (increased from 0.69 to 0.81-0.89 g oil g^{-1} proteins) ($p<0.05$) with an increase level about 17-29%. In contrary, limited hydrolysis of SPI would impair its FAC clearly (decreased from 0.99 to 0.76-0.82 g oil g^{-1} proteins) ($p<0.05$), with a decrease level about 17-23% (Fig. 4b). The results stated that limited hydrolysis of SPC could be applied to enhance its FAC.

Yin *et al*. (2008) hydrolyzed hemp protein isolates in limited extent by trypsin and found the FAC of the hydrolysates to be impaired markedly. If soybean meal

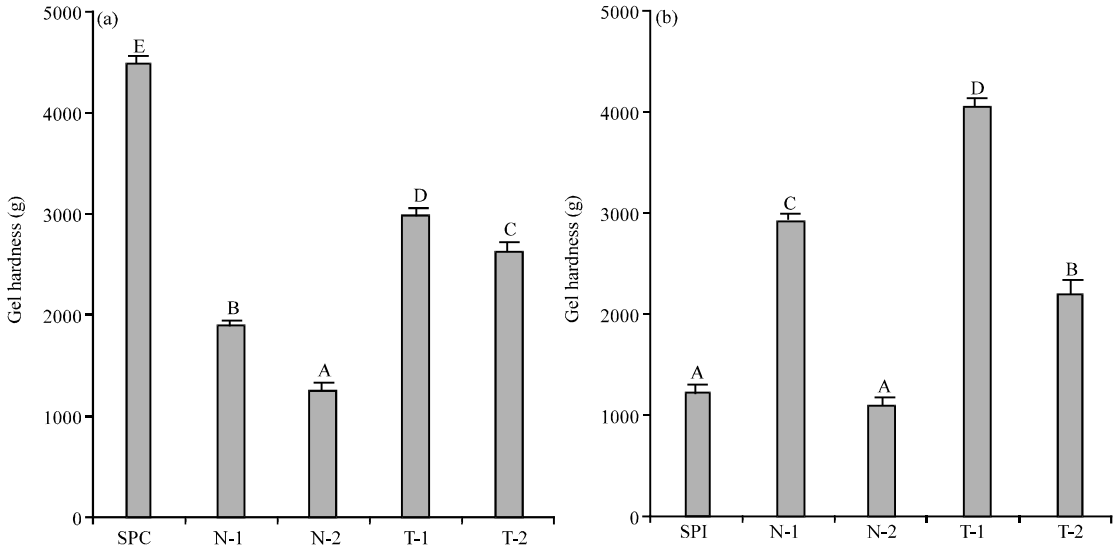

Fig. 3: Gel hardness of the heat-induced gels prepared by Soybean Protein Concentrates (a) (SPC) and four SPC hydrolysates or Soybean Protein Isolates (SPI) (b) and four SPI hydrolysates. Symbol N or T represents neutrase or trypsin used in hydrolysis. The numbers after the symbol N or T represent the DH value of the hydrolysates evaluated. Different capital letters above the bars indicate that one-way ANOVA of the means is significantly different ($p<0.05$)

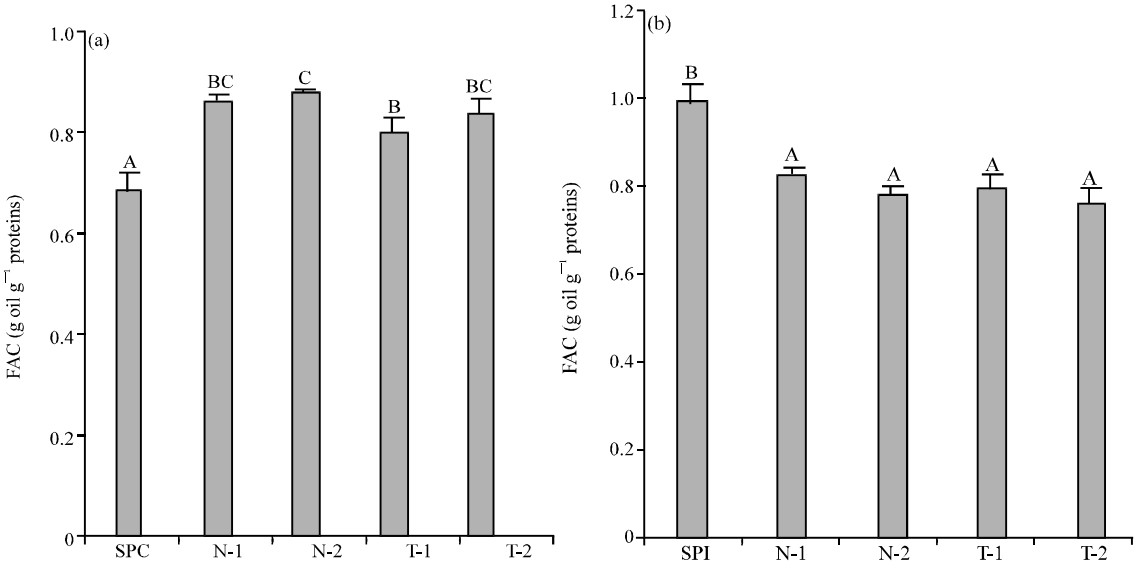

Fig. 4: Fat Absorption Capacity (FAC) of Soybean Protein Concentrates (a) (SPC) and four SPC hydrolysates or Soybean Protein Isolates (SPI) (b) and four SPI hydrolysates. Symbol N or T represents neutrase or trypsin used in hydrolysis. The numbers after the symbol N or T represent the DH value of the hydrolysates evaluated. Different capital letters above the bars indicate that one-way ANOVA of the means is significantly different ($p<0.05$)

was hydrolyzed with papain and bromelain for 5 min, the hydrolysates had nearly one-fold increase in FAC (3.6 or 3.7 vs. 1.9%); if the hydrolysis time was 30 min, the hydrolysates would have a decreased FAC (Taha and Ibrahim, 2002). Taha and Ibrahim also found that sesame hydrolysates exhibited a decreased FAC over the nonhydrolyzed meal. Yust *et al.* (2010) found that the chickpea protein hydrolysates having DH of 1-4.9% exhibited much improved FAC (the value increased from original 308 to 542-628) while the hydrolysates having DH of 10% exhibited less improved FAC (the value increased to 443). When rapeseed proteins were hydrolyzed to a DH of 3.1%, the hydrolysates showed a FAC about 1.55 oil g g^{-1} proteins while the original proteins had a FAC of 0.63 g oil g^{-1} proteins (Vioque *et al.*, 2000). These works indicated that enzymatic hydrolysis of some food proteins might have an impaired or enhanced effect on their FAC. Shared similarity to these results, the SPC hydrolysates prepared in our work had an improved FAC while the SPI hydrolysates prepared exhibited an impaired FAC.

CONCLUSION

Neutrase and trypsin could be intended applied to modify Soybean Protein Concentrates (SPC) and Soybean Protein Isolates (SPI) to enhance their some functional properties when SPC and SPI were hydrolyzed by two proteases to a limited hydrolysis extent, a degree of hydrolysis about 1-2%. Sodium dodecylsulfate-polyacrylamide gel electrophoresis analysis confirmed that both SPC and SPI were degraded Into small peptides and the hydrolysates prepared by trypsin had much larger peptides due to the specificity of the proteases used. The limited enzymatic hydrolysis applied impacted the solubility and other functional attributes of the SPC and SPI. The SPC hydrolysates or SPI hydrolysates all had better solubility over the original SPC or SPI, especially at a pH of 4.5. Compared to the original SPC or SPI, the SPC hydrolysates had impaired gel hardness but improved fat absorption capacity while the SPI hydrolysates had improved gel hardness but an impaired fat absorption capacity.

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