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Effect of Proteolysis on the Functionalities of 7S and 11S Soy Proteins

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大豆 7S 및 11S 蛋白質의 機能性에 대한 酵素的 加水분해의 效果

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Abstract

Proteolysis of 7S and 11S soy protein-rich fraction(PRF) with commercial proteases(alcalase and pronase) apperently increased protein solubility at pH 5, heat coagulation and calcium tolerence, while decreasing emulsifying capacity and foam stability regardless of the kind of protein and protease used. However, the proteolysis decreased the protein solubility of 7S PRF at pH 6 and 11S PRF at pH 4. The proteolysis of 11S PRF increased oil absorption and foam expansion, while slight decrease or almost no change was noted on 7S PRF. Heat stabilities of the emulsion and kinetic viscosities changed very little by the proteolysis.
Key words: soy proteins, protein functionalities, hydrolysates

Introduction

Functionalities are very important in food applications of soy proteins, because they are, at present, used as ingredients Both chemical and enzymatic modifications have been used to alter functionalities of proteins. However, enzymatic methods are preferred since they are generally considered safer than chemical methods[1].

Alteration of proteins by proteolytic enzymes has been one of the most commonly utilized enzymatic modification methods. Also, proteolytic modifications are assuming greater importance in improving the functionalities of soy proteins[2]. Actually, a number of authors have studied with many useful results on functionality changes of soy proteins upon proteolysis and the applications of the hydrolysates in various food systems[3-9]. However, not much studies have been carried out on the proteolysis of the main soy protein components such as 7S and 11S proteins. Only a few studies were done on the hydrolysis of glycinin(11S soy protein) and its subunits by pepsin and trypsin[10-13]. Futhermore, studies on the hydrolysis of 7S soy protein are very scarce.

Therefore, the objective of the present study was to measure the functionality changes of 7S and 11S soy protein-rich fractions(PRF), which are practically more important than the purified 7S and 11S soy proteins, resulting from the hydrolysis by two commercial proteases(alcalase and

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pronase).

Materials and Methods

Materials

Soybeans and the procedured used to fractionate 7S and 11S PRF were the same as descried elsewhere[14]. Proteases were purchased from Novo(alcalase 0.6L, solid type, Denmark) and Calibiochem(pronase, Los Angeles, CA).

Hydrolysis of proteins

The hydrolysis was carried out in batches as decribed previously[8]. The DH(degree of hydrolysis) of each hydrolysate was reported previously[14].

Functionality measurements of hydrolysates

Protein solubility profiles, foam expansion and stability, and emulsifying activity and heat stability of emulsion were determined in deionized water[15]. Heat coagulability[16], oil absorption[17] and calcium-precipitability[18] were also determined in deionized water. Kinetic viscosity was measured using a Ubbelohde Viscometer No. 1(Cannon Instrument Co., PA) on 1% protein solutions in deionized water at 25°C. Additional detials of the methods used in the functionality measurement were reported previously[19].

All experiments was carried out in duplicates or triplicates and the data presented were the means of these replicate analyses.

Results and Discussion

pH-solubility profiles

The pH-dependent solubility profiles of both soy proteins and their hydrolysates are shown in Fig. 1 and 2, respectively(Fig. 1,2). The protein solubilities at alkaline pH regions were almost the same, about 90% or higher for all hydrolysates. Generally, the 7S PRF with enzyme treatment

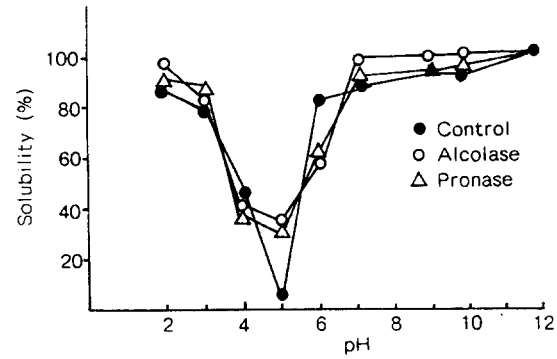

Fig. 1. Solubility profiles of 7S PRF and its hydrolysates.

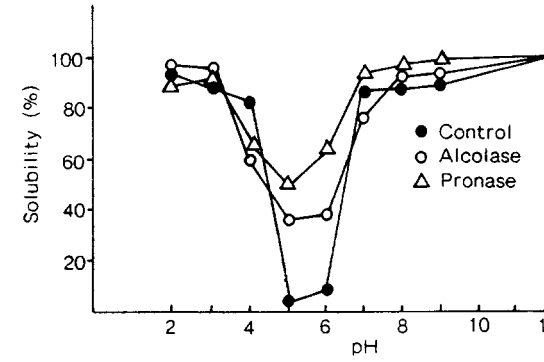

Fig. 2. Solubility profiles of 11S PRF and its hydrolysates.

showed about 30-40% increase in protein solubilities at pH 5, whereas at pH 6, both alcalase and pronase treatments decreased the solubilities by about 30% (Fig. 1). Likewise, enzyme treatment on 11S PRF resulted in increased solubilities of hydrolysates by about 30-50% at pH 5 and pH 6, but at pH 4, the solubilities decreased by 20-30% (Fig. 2). According to a previous work[5], the amount of soluble nitrogen in soy isolate increased at neutral and isoelectric pH values with increased enzyme treatment. Also, the acid solubility of protein increased with the increase in DH(degree of hydrolysis) of alcalase hydrolysis in soy isolate[21]. Enzyme treatment of soy isolate caused the solubility to increase at pH 5 and decrease at

pH 6[8].

A review[22] revealed that the breaking of proteins resulted in three major modifications; 1) an increase in the number of polar groups; 2) a decrease in molecular weight of proteins; and 3) a possible alteration in molecular configuration. Thus, the increase in the number of polar groups in hydrolysates may have been responsible for the increased solubilities in this study. However, the decrease in solubilities at pH 6 for 7S PRF and at pH 4 for 11S PRF hydrolsates may have been due to changes in molecular configuration. Consequently, isoelectric points of the proteins may have been changed. It was also reported that the isoelectric region of the glycinin(11S soy protein) shifted to the acidic side by a tryptic digestion[23].

Heat coagulation and calcium precipitations

Enzymatic hydrolysis of the soy proteins increased heat coagulability(Fig. 3). The coagulablity of the hydrolysates increased by about 10% for 7S PRF and 20% for 11S PRF. Generally, heat treatment, especially moist heat, of soy protein rapidly insolubilized native soy proteins[24]. However, in this study, the control(no enzyme treatment) showed relatively low values of heat coagulability due probably to the effect of buffer(sodium phosphate-citric acid, pH 7.0) included in sample preparation since the unhydrolyzed or hydrolyzed proteins were directly lyophilized after suspension and/or hydrolysis. Protein solubilities are influenced by various factors, the nature of the solvent(pH, ionic strength), heating temperature and disulfide bond content, etc. Especially, the relatively high stability of 11S soy protein to heat has generally been interpreted to the result of extensive disulfide bridging within the 11S soy protein[25].

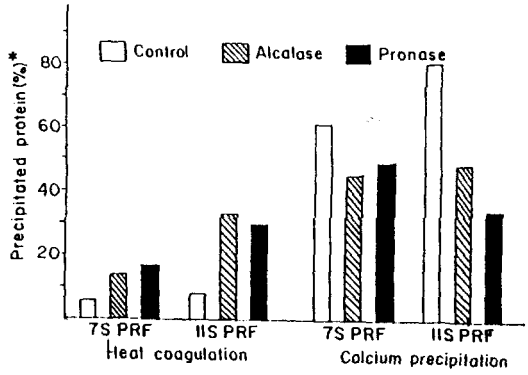

Fig. 3. Properties of heat coagulation and calcium precipitation on the soy proteins and their hydrolysates.
* %: [precipitated protein from protein solution(1%)/total protein] × 100.

The amount of calcium precipitated 7S PRF decreased by about 15% with alcalase and pronase hydrolyses(Fig. 3). For 11S PRF the reduction was about 30% with alcalase and 40% with pronase hydrolyses. As a whole, enzyme hydrolysis considerablely increased calcium tolerance of soy proteins. Calcium usually tends to precipitate preferentially the 11S soy protein[26]. However, enzymatic modification of proteins generally increased calcium tolerance, water absorption, and foaming properties[2]. The calcium tolerance of soy protein is important when additional calcium is needed for nutritional improvement of calcium products, i.e., imitation dairy products[5].

Emulsification and oil absorption properties

Table 1 shows the results of emulsification and heat stability studied. Enzymatic hydrolyses decreased emulifying activity of 7S PRF by about 20% and of 11S PRF by 10%. However, the hydrolysis does not seem to have any significant effect on the heat stability of the emulsions. A previous work[5] indicated that limited enzyme treatment of soy protein may not have a detrimental effect on emulsification properties. However, it also reported that enzyme treatment decreased emulsifying activity, and heat treatment of the emulsions reduced the stability only slightly. Emulsifying properties of soy proteins were improved by tryptic hydrolysis but the low molecular weight digests showed poorer emulsifying properties[23].

Table 1. Relative emulsifying properties of soy proteins and their hydrolysates

	Emulsifying activity[a](%)		Emulsion heat stability[b] (%)	
	7S PRF	11S PRF	7S PRF	11S PRF
Control (no enzyme)	73	61	73	63
Alcalase Treatment	50	56	74	63
Pronase treatment	52	51	68	67

a%: emulsion volume/total volume x 100.
b:Heated at 80°C for 30min.

The amount of oil absorbed by 7S PRF was not changed by enzymatic hydrolysis, but the hydrolysis increased the emulsion capacity(oil absorption property) of 11S PRF(Fig. 4). Oil absorption of soy protein has been studied less extensively than other functional properties. Consequently, the mechanism of oil absorption has yet to be explained in depth; however, oil absorption is attributed mainly to the physical entrapment of oil, and also affected temperature, size of ingredient particles, and degree of denaturation of the protein[25].

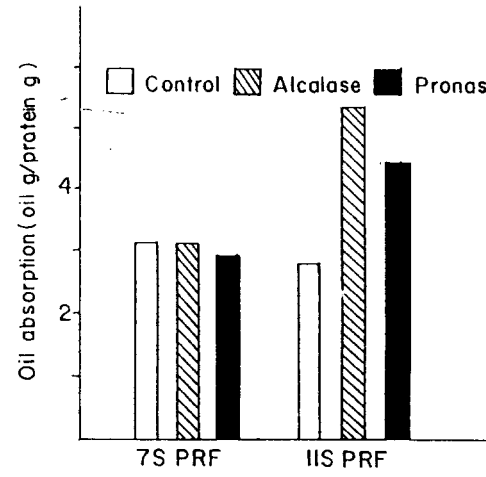

Fig. 4. Properties of oil absorption on the soy proteins and their hydrolysates.

Foaming properties and viscosity

The foam expansion showed some increase for 11S PRF hydrolysates, but decrease for 7S PRF hydrolysates over the controls(Table 2). However, the foam stabilities of soy proteins were apparently decreased by enzymatic hydrolysis. The decreased foam stabilities were about 30-40% over their controls. Propeolytic modifications generally produce great volumes of foam than unmodified proteins, but most proteins treated with enzymes have inferior stabilities to the untreated controls[21]. Protein derivatives exhibiting aerating and whipping properties were produced by the action of a proteolytic enzyme on a suitable protein substrate[27]. The factors principally involved in foam formation were surface tension, viscosity and the character of the liquid.

Table 2. Relative foaming properties of soy proteins and their hydrolysates

	Foam expansion (ml)		Foamstability[a] (ml)	
	7S PRF	11S PRF	7S PRF	11S PRF
Control (no enzyme)	99	78	87	74
Alcalase treatment	88	110	57	54
Pronase treatment	77	89	52	51

a: Measured at 1 hr after foam expansion measurements

Generally, the viscosities of hydrolyzed proteins were slightly lower than unhydrolyzed proteins(Fig. 5). It was reported that the viscosities of several soy hydrolysates seemed generally to decrease gradually with increasing DH(degree of hydrolysis)[21].

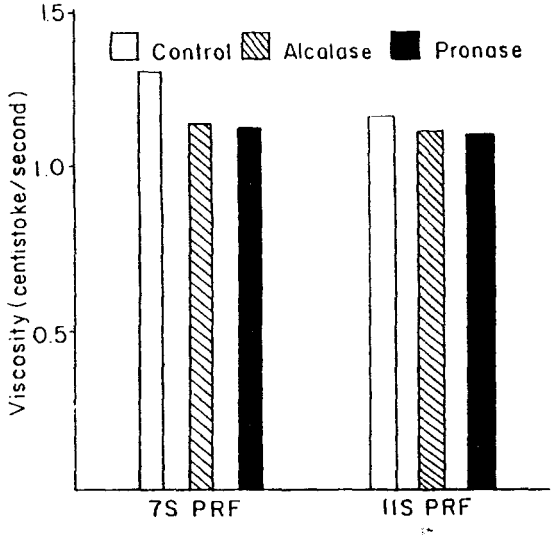

Fig. 5. Viscosities of the soy proteins and their hydrolysates.

요 약

分劃된 大豆 7S 및 11S 蛋白質은 蛋白加水分解 酵素(alcalase 및 pronase)로 1時間동안 加水分解하였을 때 使用된 酵素 및 蛋白質 種類에 관계없이 pH 5에서 溶解度, 熱凝固性 및 Ca^{++}에 대한 耐沈澱性은 상당히 增加, 에멀젼 活性 및 거품 安定性은 減少, 에멀젼 熱安定性 및 動粘度는 거의 變化되지 않았다. 그러나 溶解度에서 7S 蛋白質은 pH 6에서 11S 蛋白質은 pH 4에서 減少하였으며 또한 11S 蛋白質은 油吸收性 및 거품 形成能에서 加水分解에 의하여 增加하였으나 7S 蛋白質은 거의 變化하지 않았다.

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