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ARTICLE

Effects of Soy Protein Hydrolysates Prepared by Varying Subcritical Media on the Physicochemical Properties of Pork Patties

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Abstract

This study investigated the effect of soy protein hydrolysates (SPH) prepared by varying subcritical media on the physicochemical properties of pork patties. For resource of SPH, two different soybean species (*Glycine max* Merr.) of Daewonkong (DWK) and Saedanbaek (SDB) were selected. SPH was prepared by subcritical processing at 190ºC and 25 MPa under three different of media (water, 20% ethanol and 50% ethanol). Solubility and free amino group content revealed that water was better to yield larger amount of SPH than ethanol/water mixtures, regardless of species. Molecular weight (Mw) distribution of SPH was also similar between two species, while slightly different Mw distribution was obtained by subcritical media. For pork patty application, 50% ethanol treatment showed clear red color comparing to control after 14 d of storage. In addition, ethanol treatment had better oxidative stability than control and water treatment based on thiobarbituric acid-reactive substances (TBARS) analysis. For eating quality, although 20% ethanol treatment in SDB showed slightly higher cooking loss than control, generally addition of SPH did not affect the water-binding properties and hardness of pork patties. Consequently, the present study indicated that 50% ethanol was the best subcritical media to produce SPH possessing antioxidant activity, and the SPH produced from DWK exhibited better antioxidant activity than that produced SDB.

Keywords: soybean, hydrolysates, subcritical water, ethanol, antioxidant activity

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Introduction

Soybean (*Glycine max* Merr.) is a good source of proteins and soy protein consists of most essential amino acids. Recent literatures have dealt with various physiological function of soy protein hydrolysates (SPH) including the prevention of cancer, osteoporosis as well as cardiovascular disease (Omoni and Aluko, 2005). Most of all, anti-oxidative activity of SPH triggers the application of SPH in meat product formulations (Peña-Ramos and Xiong, 2001). SPH is usually prepared by enzymatic hydrolysis, however, high price of the proteases limited the mass production of SPH in food industry (Sarmadi and Ismail, 2010). To obtain soybean protein hydrolysates, acid hydrolysis using hydrochloric acid was the most simple and easy technique. However, the acid hydrolysis involved in the formation of toxic chlorine compounds such as 3-monochloropropane-1,2-diol (Ariseto *et al*., 2013). Alternately, novel hydrolysis technique such as subcritical water process (or referred to hydrothermal process) has been introduced. Lee *et al*. (2015) indicated that SPH prepared by subcritical water process provided high antioxidant activity and the addition of the SPH suppressed lipid oxidation thereby delaying the discoloration of pork patty during chilled storage. As SPH source, various domestic soybean species are available. Among them, new species of Saedanbaek (SDB) had been recently developed and this species contains high amount of protein (~50%) comparing to normal domestic species of Daewonkong (DWK) of which protein content is around 35%. Use of high protein source would provide better advantage in yield aspect. In addition, it was expected that SPH obtained from different species would show different functional properties.

In subcritical water process, processing temperature is a key factor regulating the degree of hydrolysis of protein. The degree of protein hydrolysis is depending on the types of proteins. However, excessively high temperature also manifested degradation of amino acids, thus the yield of protein hydrolysates was reduced. Alternate parameter regulating the degree of hydrolysis is changing medium,

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and ethanol is likely better solvent for subcritical water process. Ethanol is consisted of C, O and H, and has the critical point of 241ºC and 6.14 MPa (Marcus, 2012). Mixture of water and ethanol shifts the critical point toward to lower temperature and pressure than those of water alone, hence it is possible that the changes in the composition of medium will affect the hydrolysis efficiency of protein.

The effect of temperature of subcritical water process on the protein hydrolysis efficiency has been reported (Lee *et al*., 2013; Rogalinski *et al*., 2008; Watchararuji *et al*., 2008), nevertheless, no information regarding the changes in composition of medium in subcritical fluid process on the protein hydrolysis is available. Therefore, this study evaluated the effect of subcritical medium compositions (0, 20, and 50% ethanol) on the soy protein hydrolysis efficiency of two domestic species and their effects on the physicochemical properties of pork patty.

Materials and Methods

Materials

Two soybean species of DWK (39.4% crude protein, 19.3% crude fat, and 10.4% moisture) and SDB (48.7% crude protein, 16.3% crude fat, and 9.8% moisture) harvested in 2013 were kindly donated by the National Institute of Crop Science (Rural development Administration, Korea). The soybean was milled 10 times using a commercial miller (Duksan Machinery Co., Korea) and stored in vacuum package prior to use (within 7 d).

SPH preparation

The Soybean powder was hydrolyzed using a lab-scale subcritical water processor as described in our previous study (Lee *et al*., 2013). As the reacting media, distilled water, 20% (v/v) and 50% (v/v) ethanol were prepared. The soybean powder was suspended into each media as the mass ratio of 1:15. The suspension was filled into reactor and heated from ambient temperature to 190ºC at 1.5ºC/min under controlled pressure of 25 MPa. When the inside temperature of reactor was reached to 190ºC, the reactor was cooled down to 40ºC at 1.7ºC/min. Finally, the suspension was centrifuged at 10,000 *g* for 10 min and the supernatant (SPH solution) was transferred to test tube. The SPH solution was kept at 4ºC prior to use (within 6 h).

Characteristics of SPH

SPH was characterized according to an operation procedure as described in our previous study (Lee *et al*., 2013). Total crude protein of the SPH solution was determined by kjeldahl methods (%N×6.25), and solubility was calculated by percentage of solubilized nitrogen over initial crude protein. Free amino group content was measured by the method of Benjakul and Morrissey (1997) and expressed in terms of L-leucine (Nagarajan *et al*., 2012). Molecular weight (Mw) distribution of SPH was estimated using a YL 9100 Gel permeation chromatograph (Younglin Instrument Co., Ltd., Korea) equipped with a YL 9100 refractive index detector and an Ultrahydrogel^{TM} 120 column (Waters, USA) by the method of Gu *et al*. (2011).

Pork patty preparation

Pork loin and back-fat were purchased in 24 h post-mortem from a local market (Seoul). The meat and fat were separately ground using 3 mm plate, and kept at 4ºC prior to use (within 1 h). Model pork patty was formulated based on our previous study (Lee *et al*., 2015). Ground pork loin and backfat was mixed by mass ratio of 8:2 and patty was formulated by 95.5% meat/fat mixture, 1.5% NaCl and 0.5% SPH (solution with 0.4% crude protein). For control, SPH was substituted to 0.5% water. Finally, the batch was adjusted to 100% by adding water (500 g batch for each treatment). The mixture and ingredient was mixed using a KMX51 food mixer (Kenwood Co., England) for 10 min. Approximately 80 g of the mixture was formed using a petri dish (90 mm in diameter and 15 mm in height) and each two patties was packed in a plastic container (3 containers of each treatment) and wrapped. The patties were stored at 4ºC for 14 d.

Physicochemical properties

Quality characteristics of the chilled stored pork patties were analyzed as described in our previous study (Lee *et al*., 2015). From 3 containers of each treatment, 2 containers were randomly selected. Patties were carefully removed and each patty was put into a plastic bag separately. The samples were cooked in 75ºC water bath for 30 min, and cooled down to ambient for 1 h. The surface exudate was gently wiped out and weighed. Cooking loss of patty was expressed as percent weight loss over initial weight. For texture analysis, the cylindrical forms of cooked patties were sampled using a cork borer (4 cm in diameter). The sample cylinder was punctured to 50% of sample height by 1 mm/s using a CT3 texture analyzer (Brookfield Engineering Labs Inc., USA) equipped with plunge (5 cm in diameter). From the remaining containers, patty was removed from the package and color of the patty was measured 10 times from the random surface using a CR-

10 color reader (Konica Minolta Sensing Inc., Japan). One gram of pork patties were taken and water holding capacity (WHC) of pork patty was determined by centrifuging method as described in Hong *et al*. (2008). Five grams of pork patties was mixed with 20 mL distilled water and pH was measured using a FF20-Five Easy pH meter (Mettler Toledo GmbH, Switzerland). From the remaining portion, 5 g of sample was taken to determine lipid oxidation. Thiobarbituric acid-reactive substances (TBARS) was conducted based on the method of Hoyland and Taylor (1991).

Statistical analysis

The completely randomized block design was adopted to estimate the effect of hydrolysis media and soybean species on the physicochemical properties of pork patties. Means were analyzed by one-way analysis of variance using SAS 9.1 (SAS Institute Inc., USA). When the main effects (subcritical media and soybean species) were significant (p<0.05), the means were separated by Duncan's multiple range test.

Results and Discussion

Characteristics of SPH

The amount of soluble proteins and peptides was mainly affected by types of subcritical media (Table 1). Subcritical water processed SPH showed 80-81% solubility which was significantly higher than those prepared by subcritical water/ethanol mixture (p<0.05). When 20% ethanol was used as subcritical medium, solubility tended to decrease down to 73-78%, and the lowest solubility of SPH (67-69%) was obtained by usage of 50% ethanol (p<0.05). For species, SDB showed slightly lower solubility than that of DWK, excluding 20% ethanol subcritical process where the solubility of SDB was higher than that of DWK (p<0.05). The similar result was also found in free amino group content. The free amino group content of SPH prepared by subcritical water was higher than that prepared by ethanol (p<0.05). DWK prepared by subcritical water showed higher free amino group content than SDB (p< 0.05). Neither species nor ethanol concentration affected the free amino group content of SPH, however, increasing ethanol concentration in subcritical media tended to decrease the free amino group content of SPH.

Table 1. Effect of subcritical media on the solubility and free amino group content of soy protein hydrolysates

Media	Solubility (%)	Free amino group (mM)
Daewonkong		
Water	80.9±0.20$^{a,1)}$	6.07±0.09^{a}
20% Ethanol	73.3±0.43^{d}	5.17±0.07^{c}
50% Ethanol	69.4±0.53^{e}	5.07±0.11^{c}
Saedanbaek		
Water	80.0±0.36^{b}	5.46±0.04^{b}
20% Ethanol	78.4±1.12^{c}	5.15±0.11^{c}
50% Ethanol	66.8±0.55^{f}	4.98±0.09^{c}

[1)]Means with different superscript within same column are significantly different (p<0.05).

For Mw distribution of SPH, DWK prepared by subcritical water showed two major peaks at ~200 Da and ~600 Da (Fig. 1). In addition, minor Mw peak at 1,400 Da was also shown. As indicated by Pihlanto-Leppälä, (2001), physiologically active peptides were consisted of 3-20 amino acids, hence the obtained Mw distribution was expected to possess various beneficial functionalities. The SHP prepared by subcritical water/ethanol media also showed similar Mw distribution, while the peak at ~300 Da was more predominant than that at ~600 Da. Although, there was no comparative investigation, one could expect that the difference in Mw distribution manifests different functionalities. It was reported that dielectric constant of ethanol was higher than that of pure water under high temperature, indicating that polarity of ethanol was less than water (Curren and King, 2001). Therefore, ethanol/water mixture was favorable to interact with organic substances comparing to pure water, thereby producing different types of SPH. In the case of SDB, pattern was similar to DWK, indicated that hydrolysis pattern was mostly affected by processing media rather than soybean species. The results reflected that subcritical process did not hydrolyze protein randomly, but the breakage of peptide bonds would have regularity under same processing condition. Eventu-

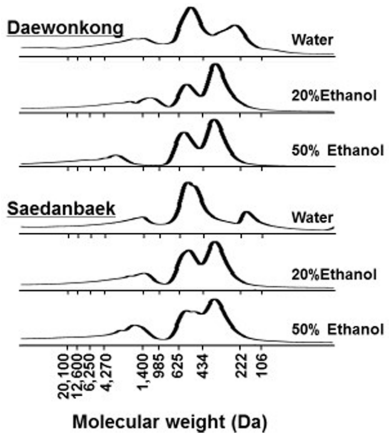

Fig. 1. Molecular weight distribution of soy protein hydrolysates prepared by varying subcritical media.

ally, processing media appeared to be potential factor involving in the antioxidant activity of SPH.

Color and TBARS of pork patties

Addition of SPH hydrolyzed by subcritical water did not affect the L* of pork patties comparing to control (Table 2). Meanwhile, the L* of pork patties tended to decrease by adding SPH hydrolyzed under high ethanol concentration and significantly lower L* value was obtained when the added SPH was prepared under subcritical 50% ethanol (p<0.05). The types of SPH also attributed to a* of pork patties. In particular, SPH-treated pork patty showed higher a* than control, when the SPH was hydrolyzed by 50% ethanol of subcritical media. Alternately, b* of all treatments was not significantly different with control. In comparison of soybean species, all color parameters were not differ each other excluding a* of 50% ethanol treatment which was higher in DWK than SDB (p <0.05). In visual appearance, control showed locally brown discoloration after 14 d of storage which was comparable to SPH treatments (Fig. 2). All SPH treatments showed redder and local discoloration was less noticeable than control. In particular, 50% ethanol treatment showed the uniform red color regardless of applied soybean species.

Discoloration during chilled storage is an indicator of freshness of pork patty for consumers. In our previous study (Lee *et al*., 2015), SPH prepared by subcritical water at 190ºC showed effective antioxidant activity in pork patties, showing high color stability during chilled storage. Meanwhile, less color stabilizing effect of subcritical water treatment would be explained by different amount of SPH addition. Despite small amount of SPH addition, maintaining stable color of ethanol treatments reflected that the SPH hydrolyzed by subcritical water/ethanol had better antioxidant activity than that applied in water alone, which was also confirmed by TBARS (Table 2). In SDB

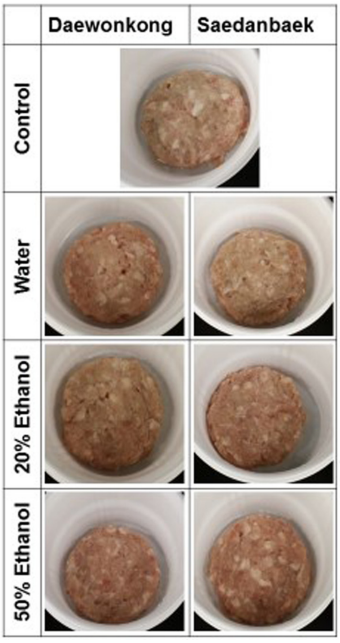

Fig. 2. Effect of subcritical media on the visual appearance of pork patties.

treatments, although, TBARS of water treatment was not differ from control, all SPH treatments showed significantly lower TBARS than 0.73 mg/kg of control (p<0.05). For comparison of supercritical media, ethanol treatments showed better stability against lipid oxidation than water treatments with irrespective of species (p<0.05), while the TBARS between 20% and 50% ethanol treatments was not different. In TBARS, still DWK showed lower TBARS than those counterpart of SDB (p<0.05).

From the results, it was concluded that the anti-oxidative activity of SPH was manifested by subcritical media. However, it should be noted that anti-oxidative substances obtained from soybean was not limited to peptides, since raw material of this study was not isolated soy protein but

Table 2. Effects of soy protein hydrolysates prepared by varying subcritical media on the CIE color and thiobarbituric acid-reactive substances (TBARS) of pork patties

Media	L*	a*	b*	TBARS (mg/kg)
Control	61.0±1.58$^{a,1)}$	1.96±0.07^{c}	10.5±1.09	0.73±0.03^{a}
		Daewonkong		
Water	60.4±0.28^{ab}	2.40±0.19^{bc}	11.0±0.40	0.68±0.01^{b}
20% Ethanol	60.4±1.66^{ab}	2.18±0.32^{bc}	11.3±1.19	0.44±0.03^{d}
50% Ethanol	59.4±0.16^{bc}	3.08±0.50^{a}	11.0±0.23	0.42±0.05^{d}
		Saedanbaek		
Water	61.1±1.11^{a}	1.55±0.05^{c}	11.2±0.66	0.76±0.01^{a}
20% Ethanol	58.8±0.22^{bc}	2.32±0.17^{bc}	10.8±0.19	0.59±0.03^{c}
50% Ethanol	58.4±0.08^{c}	2.57±0.33^{b}	10.6±0.23	0.54±0.02^{c}

[1)]Means with different superscript within same column are significantly different (p<0.05).

Table 3. Effects of soy protein hydrolysates prepared by varying subcritical media on the eating qualities of pork patties

Media	WHC (%)	Cooking loss (%)	Hardness (N)
Control	84.5±1.02	22.3±0.76$^{b,1)}$	20.7±1.01^{a}
		Daewonkong	
Water	84.0±0.48	21.6±1.06^{b}	18.1±0.22^{b}
20% Ethanol	83.9±3.89	25.4±2.25^{a}	19.2±1.25^{a}
50% Ethanol	80.9±3.13	21.5±1.82^{b}	17.6±1.00^{b}
		Saedanbaek	
Water	82.8±3.66	21.1±1.35^{b}	16.8±0.45^{bc}
20% Ethanol	82.9±3.66	22.5±1.44^{b}	17.3±0.10^{b}
50% Ethanol	84.2±0.85	20.2±0.48^{b}	15.8±0.53^{c}

[1)]Means with different superscript within same column are significantly different (p<0.05).

soybean powder including hull. Various phytochemicals were extracted from varying plants by subcritical water process (Ibañez *et al*., 2003) and these chemicals probably influenced on the oxidative stability of pork patties. Consequently, the reason why subcritical water/ethanol treatment showed better antioxidant activity was not understood in this study, nevertheless, the present study demonstrated that hat DWK had a potential to be used as an antioxidant in processed meat products.

Eating qualities of pork patties

The WHC of pork patties was affected neither by SPH treatment nor by soybean species and showed 81-85% (Table 3). Although, 20% ethanol treatment in DWK had 25.4% of significantly high cooking loss comparing to control (p<0.05), normally cooking loss of pork patties was not depending on the addition of SPH and ranged to 20-23%. Alternately, all treatments exhibited significantly lower hardness than control (p<0.05) with the exception of 20% ethanol treatment in DWK. It has been reported that lipid oxidation has a relationship with protein oxidation which was characterized by free carbonyl group formation (Faustman *et al*., 2010; Lund *et al*., 2011; Xiong and Decker, 1995). The protein oxidation was therefore manifest a modification of protein functionality such as water-binding properties or high shear force of muscle foods (Jia *et al*., 2012; Lund *et al*., 2011). However, no evidence of protein oxidation was found in our previous study (Lee *et al*., 2015) and it would be explained by short storage period (14 d). Therefore, addition of SPH did not affect the eating quality of pork patties. Finally, the results implicated that SPH prepared by subcritical ethanol/water was efficient to prevent or delay oxidative metabolisms in meat products without altering the physical qualities, and exhibited a potential application of the ethanol/water mediated SPH as an antioxidant of various fat-containing muscle foods.

Conclusion

This study demonstrated that ethanol/water mixture was better subcritical media to produce an antioxidant SPH than pure water media. SDB had a potential to produce larger amount of SPH, however, SPH obtained from DWK exhibited better antioxidant activity than that from SDB. Consequently, the SPH was an appropriate antioxidant for fat-containing meat products, and the selection in proper type of subcritical medium as well as soybean species should be required to obtain high physiologically active SPH. Still, it was obscure if the antioxidant activity of SPH come from entirely hydrolyzed peptides or other unknown phytochemicals, which warranted further research.

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