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Effect of Soy Protein Hydrolysates Prepared by Subcritical Water Processing on the Physicochemical Properties of Pork Patty during Chilled Storage

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Abstract

The present study was carried out to investigate the effects of soy protein hydrolysates (SPHs) addition on the quality characteristics of pork patties. The SPHs was prepared by subcritical water process (SWP) at 180°C without holding time and mixed with the pork patty components at varying concentrations (0-3%), and the patties were stored at 4°C for 14 d. As quality parameters, instrumental color, thiobarbituric acid-reactive substances (TBARS), pH, water holding capacity (WHC) and shear force were measured at the end of storage. Regardless of SPHs concentration, the addition of SPHs significantly manifested low L* and high a* values compared to those of untreated control ($p<0.05$). For b* value, addition of SPHs in the 0.5-1.5% was unaffected, while >2.0% of SPHs caused significantly lower b* than control ($p<0.05$). The color changes in pork patties with and without SPHs were also identified in visual appearance where the pork patties containing 0.5-2.0% showed bright red color which was comparable to brownish color of control and patties containing >2.5% SPHs. Lipid oxidation was delayed by the addition of 0.5-1.5% SPHs, while it was accelerated by the addition of 3% SPHs. The pH of patties increased with increasing concentration of SPHs, whereas there were no significant differences in WHC and shear force of patties. Consequently, the results indicated that the addition of 0.5-1.5% SPHs had a potential advantage in suppressing oxidative deterioration of fat-containing meat products during chilled storage.

Keywords: soy hydrolysates, antioxidants, pork patty, subcritical water, storage

Introduction

Soy protein has been recognized as a complete plant protein since it contains most of the essential amino acids. It was well identified that the consumption of soy protein provided protective effects against cancer, cardiovascular disease and osteoporosis (Omoni and Aluko, 2005). Furthermore, it has been reported that soy peptides have anti-diabetes and anti-irritants effects of the gastrointestinal tract, and are a protective effect on renal disease (Friedman and Brandon, 2001). In the meat processing aspect, Peña-Ramos and Xiong (2003) investigated the antioxidant activity of soy hydrolysates (SPHs) in meat patties and reported that SPHs suppressed lipid oxidation effectively.

Enzymatic hydrolysis is the simplest way to produce SPHs, while this method has not been practically adopted in mass production because of high price of the enzymes and bitterness of the hydrolysates (Sarmadi and Ismail, 2010). Subcritical water process (SWP) which has been occasionally called as hydrothermal reaction has been applied to hazardous organic compound destruction (Barner *et al.*, 1992). Water is most environmentally friendly solvent, and the critical point of water is reported to be 374 °C and 22.1 MPa. For industrial application, organic compound is completely destructed to CO_2 and H_2O within 10 s at the processing condition of 620°C and 23.5 MPa (Barner *et al.*, 1992). Despite the destructive efficiency of SWP, little information regarding the application of SWP process on the hydrolysis of food and bio-polymers is available. Rogalinski *et al.* (2008) demonstrated that the reaction rate constants for starch or cellulose hydrolysis were proportionally increased with the increasing of the reaction temperature. For protein hydrolysis, Zhu *et al.* (2008) tried to hydrolyze fish proteins using SWP process

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and reported that the free amino acids yielded maximum at 240-260°C. At above temperatures of 260°C, however, the destruction of amino acids with substantially less yield of free amino acids was manifested.

Based on a review of Sarmadi and Ismail (2010), bio-active peptides are composed of 2-20 amino acids (< 6,000 Da). In this case, excessively high temperature caused a negative effect on yielding the bio-active peptides. Watchararuji et al. (2008) proposed that the suitable processing condition to obtain soy peptides with antioxidant activity was 170-230°C, although the authors tested the efficiency of SWP process in a narrow range of temperature (200-220°C). Based on our previous study (Lee et al., 2013), processing at 180°C yielded the maximum peptide fraction within the molecular weight of 100-20,000 Da, reflecting that the best condition was depending on the type of processing conditions. Consequently, the SWP process has a potential application to produce oligo-peptides from various protein resources.

In meat processing, lipid oxidation is a spontaneous phenomenon caused during storage of fat-containing product. In particular, lipid oxidation causes quality deteriorations such as warmed-over flavor, discoloration, protein oxidation as well as loss of water-holding capacity. There have been numerous explorations regarding anti-oxidative effect of SPHs prepared by proteases (Omoni and Aluko, 2005; Sarmadi and Ismail, 2010). Although, some reports suggested the possibility of protein hydrolysis using SWP (Lee et al., 2013; Rogalinski et al., 2008), actual functionality of the hydrolysates obtained from the SWP was not evaluated. In the present study, therefore, the effect of SPHs produced by SWP on the physico-chemical properties of pork patties under aerobic storage condition was explored.

Materials and Methods

Materials

Pork loins (crossbreed of Landrace × Yorkshire × Duroc, 6 mon old hogs) and back-fat were purchased in 24 h post-mortem from a local market (Majang meat market, Seoul). The loins and fat were separately ground through 3 mm plate and vacuum-packaged using a poly-nylon bag. Soybean (Glycine max Merr. Daewonkong) was kindly donated by the National Institute of Crop Science (Rural Development Administration, Korea). The soybean was milled using a commercial miller (Duksan Machinery Co., Korea) and vacuum-packaged for storage at 4°C. All chemicals used in this study were of analytical grade.

SPHs preparation

The milled soy powder was suspended into 9 volume of distilled water, and aliquots 100 mL was transferred to sample bottles. For SWP process, lab-scale processor used in this study was consisted of reactor (working volume of 300 mL), ohmic heater and cooler as described in our previous study (Lee et al., 2013). Pressure and temperature inside the reactor were monitored throughout reaction, and pressure was built by increasing temperature inside the reactor. Immediately after the temperature in the reactor was reached to 180°C, the reactor was cooled down to 40°C by circulating cold water. The sample bottle was removed from the reactor and the SPHs suspension was filtered using a 0.45 µm syringe filter (DISMIC-13CP, Advantec Co., Japan) to remove any precipitate. The filtrate (2.0% protein and 96.6% moisture) was kept in 4°C refrigerator prior to use (within 12 h) without any other treatment.

Preparation of pork patties

Ground meat and back-fat were initially mixed together by mass ratio of 8:2. All sample patties were formulated by 95.5% meat/fat mixture, 1.5% NaCl and varying concentrations of SPHs (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%, w/w based on weight of the filtrate). Finally, all batches were adjusted to 100% by adding water. The ingredients were entirely mixed for 10 min using a food mixer (KMX51, Kenwood Co., England). Approximately 80 g of the mixture was formed using a petri dish (Internal dimension: 90 ×15 mm, SPL, Korea) to result in a uniform and reproducible sample shape and volume. Each of the two pork patties was packed in a plastic container and wrapped to prevent evaporative loss. Finally, the patties were kept at 4°C for 14 d.

Color

Color values were measured from the surface of pork patty with a color reader (CR-10, Konica Minolta Sensing Inc., Japan) which was calibrated using a standard white plate (X=97.83, Y=81.58, Z=91.51). The CIE L*, a* and b* values were designated as indicators of lightness, redness and yellowness, respectively. Color of the sample was taken 10 times from the random surface.

Thiobarbituric acid-reactive substances (TBARSs)

The TBARS of samples were determined by the method of Hoyland and Taylor (1991). Thiobarbituric acid (TBA) reagent was prepared by dissolving 0.69 g 2-TBA in 100 mL distilled water followed by mixing with the same amount of acetic acid. A sample of approximately 5 g was

homogenized with 20 mL distilled water in a filter stomacher bag using a stomacher (WS400, Shanghai Zhisun Equipment Co., Ltd., China). 0.75 mL aliquot of the filtrate was mixed with 2.5 mL benzene and 2.5 mL TBA reagent, and centrifuged at 1,500 g for 10 min. After phase separation, the lower phase was filtered using a 0.45 μm syringe filter (DISMIC, Advantec MFS Inc., Japan) and boiled for 30 min. After cooling at an ambient temperature, absorbance of the sample was measured at 530 nm. The TBARS level was determined using a standard curve pre-estimated using tetraethoxypropane and calculated by the amount of malonaldhyde (MA) equivalents (mg MA/ kg sample).

pH

Five grams of pork patties was mixed with 20 mL of distilled water and placed in a bag-filter (Interscience, France). The samples were blended using a stomacher (WS 400, Shanghai Zhisun Equipment Co., Ltd., China) for 3 min. The pH of the filtrated solution was determined using a pH meter (Model FE20-Five Easy, Mettler Toledo GmbH, Switzerland). All samples were measured five times.

Water holding capacity (WHC)

Water holding capacity (WHC) of pork patty was determined by the method of Hong *et al*. (2008). One gram of patty (M_S) was weighed and placed in a centrifuge tube along with gauze as an absorbent. The samples were centrifuge for 15 min at 3,000 rpm and 4°C in a refrigerated centrifuge (1248R, Sorvall Co., USA). The pellet was carefully removed from the tube, and the tube was weighted (M_1). The tube was dried at 102°C for 24 h and weighed again (M_2). Measurement was done by triplicated determinations, and the WHC of the sample was calculated from the following equation:

$$WHC(\%) = \left(1 - \frac{M_1 - M_2}{M_S}\right) \times 100$$

Shear force

All sample patties were placed in a plastic bag, thermal treated in 75°C water-bath (DX9, Hanyoung Co., Korea) for 30 min, and cooled down to ambient for 60 min. The surface exudates were gently wiped out using a tissue, and all samples were cut into a strip (1 cm × 1 cm × 5 cm) and sheared using a texture analyzer (CT3, Brookfield Engineering Labs Inc., USA) equipped with a knife (TA-SBA, Brookfield Engineering Labs) under the conditions of 1.5 mm/s test speed and a trigger load of 1 g.

Statistical analysis

Complete randomized block design was adopted to estimate the effects of SPHs concentrations on the physicochemical properties of pork patties. Means collected from three entirely repeated experiments were analyzed by one-way analysis of variance (ANOVA) using SAS 9.1 (SAS Institute Inc., USA). When the main effect (SPHs concentration) was significant ($p < 0.05$), the means were separated by Duncan's multiple range test.

Results and Discussion

Color and appearance

The color parameters of pork patties after 14 d storage are given in Fig. 1. The L^* value of all SPHs-treated patties exhibited significantly lower L^* value than that of untreated control ($p < 0.05$). Among SPHs treatments, addition of 2% SPHs showed the lowest L^* value among patties. Meanwhile, a^* values of SPHs treatments were significantly higher than those of control ($p < 0.05$). However,
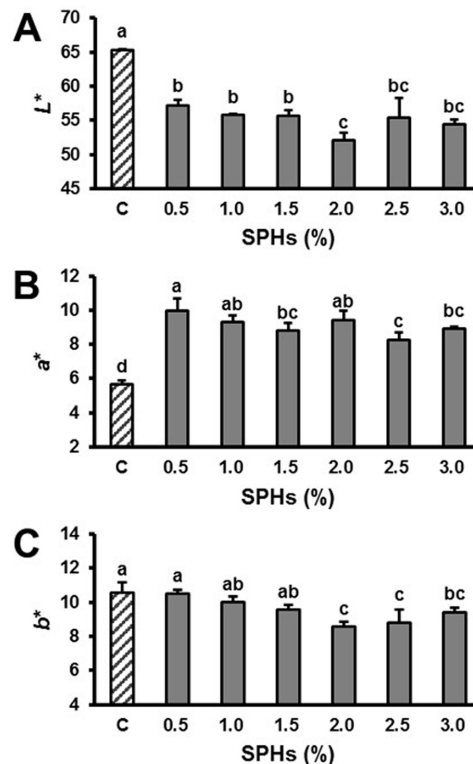

Fig. 1. Effects of concentrations of soy protein hydrolysates (SPHs) produced by subcritical water process on the CIE (A) L^*, (B) a^* and (C) b^* of pork patties. Vertical bars indicate standard deviations. Means with different letters were significantly different ($p < 0.05$).
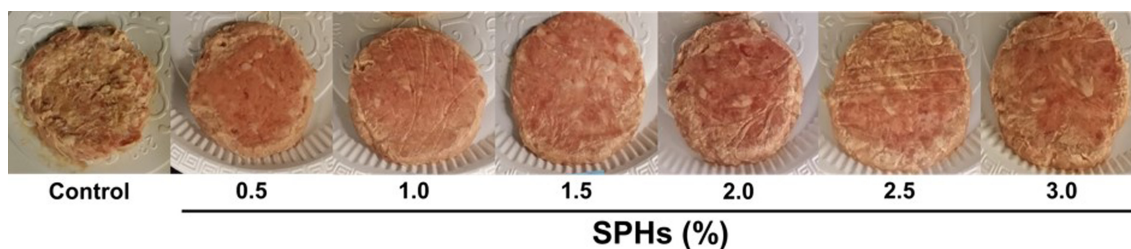
Fig. 2. Visual appearance of pork patties prepared with soy protein hydrolysates prepared by subcritical water process.

increasing SPHs concentration tended to decrease the a^* of patties. The b^* value of patties indicated that >2.0% SPHs addition decreased the b^* of pork patties significantly compared to that of control ($p<0.05$). The result suggested that the addition of SPHs prevented or delayed the myoglobin oxidation by acting as an anti-oxidant which was also in accordance with other findings (Aida Peña-Ramos and Xiong, 2002, 2003). The differences in color parameters of patties manufactured with and without SPHs were also confirmed by visual appearance as depicted in Fig. 2. Control exhibited brownish color after storage of 14 d, while SPH treatments maintained their light red color during storage. However, some browning appearance was also evidenced in patties containing greater than 2.5% of SPHs. In general, the color changes of patties, which are easily recognized by consumers, were attributed to metmyoglobin formation (Byeon et al., 2009; Chun et al., 2013). Since the oxidative color defect of meat products were characterized by lower a^* with higher L^* and b^* values (Kim et al., 2012), this study demonstrated that the addition of SPHs successfully prevented the metmyoglobin formation in pork patties during refrigerated storage. Still, it was unclear why the color of patties containing 2.5-3% SPHs showed negative characteristics compared to those containing < 2.0%. In fact, SPHs prepared by SWP had a dark brownish color, and large addition of SPHs could have affected the color of final product. Alternately, soy protein also contains large amount of lipoxygenase which takes part in various oxidative metabolisms (Brash, 1999). It was possible that the lipoxygenase residue which was not completely hydrolyzed by SWP could affect the oxidative reaction in pork patties during storage, which warrants further exploration. Consequently, usage of < 2% of SPHs was favorable in stabilizing the color of pork patties for long chilled storage periods.

Thiobarbituric acid-reactive substances (TBARS)

Lipid oxidation is a major factor causing the quality loss of pork patties. The effect of the SPHs on TBARS of pork patties is shown in Fig. 3. Lipid oxidation of pork patties was suppressed or accelerated depending on the concentration of added SPHs. The TBARS of the patties was significantly lower than control, when low level of SPHs (0.5-1.5%) was added into the patties ($p<0.05$). At 2.0-2.5% SH addition, the TBARS of the patties was not different from control, while the patties manufactured with 3.0% SPHs had significantly higher TBARS than control ($p<0.05$). The former revealed that SPHs had an ability to suppress or delay lipid oxidation in meat products (Peña-Ramos and Xiong, 2003; Seo and Morr, 1984). Meanwhile, the latter was not consistent with the result of Park et al. (2010) where the authors reported that the anti-oxidative activity of SPHs was concentration-dependent. Different results estimated in this study and other literatures suggested were resulted from the hydrolysis method and degree of protein hydrolysis. Jung et al. (2014) compared the hydrolysis efficiency of collagen and noted that SWP (200°C, 37.5 MPa) produced peptides with high molecular weight compared to protease. Upon protease treatment, phenolic compounds in soybean were rarely affected, while proteins including lipoxygenase were completely hydrolyzed (Seo and Morr, 1984). Meanwhile, soy
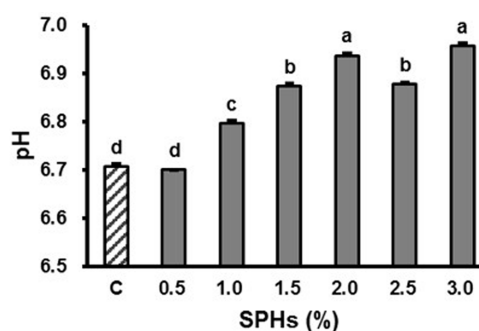

Fig. 3. Effects of concentrations of soy protein hydrolysates (SPHs) produced by subcritical water process on the thiobarbitric acid-reactive substances of pork patties. Vertical bars indicate standard deviations. Means with different letters were significantly different ($p<0.05$).

protein (globulins) has relatively high thermal stability, thus part of proteins could be retained after SWP treatment. Eventually, these unaffected protein portions would attribute to oxidative reaction as shown by high TBARS and discoloration of pork patties during storage.

pH

Effects of SPH concentrations on pH of pork patties are given in Fig. 4. Excluding 0.5% SH addition, all SPH treatments showed significantly higher pH than control ($p<$ 0.05), and increasing SPH concentration tended to increase the pH of the patties. The high pH of SPHs-treated patty was due to the high pH of SPHs (data not shown). Although, actual pH of SPHs was not determined in this study, it was believed that loss of free acidic group was responsible for higher pH of protein after thermal treatment (Lawrie, 1998). The higher pH of final product had a probability of rapid microbial growth, which should be studied in future works. Essentially, this study was focused on anti-oxidative effect of SPHs on meat products, while Cavalheiro *et al.* (2014) reported that the addition of hydrolyzed mechanically deboned chicken meat in mortadella-type sausage showed strong antimicrobial activity during 60 d of storage. Although there was no information regarding the antimicrobial activity of SPHs, it was likely that high pH mediated by SPH addition was not considered in the limitation of meat product shelf-life, due to general function of protein hydrolysates in preventing microbial growth. Conversely, the high pH was beneficial in improving color and WHC of the meat products (Chun *et al.*, 2013). Further evaluation has been warranted in this field.

WHC and shear force
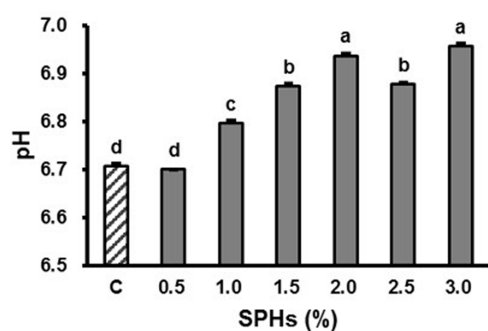
Lipid oxidation has been known to promote protein

Fig. 4. Effects of concentrations of soy protein hydrolysates (SPHs) produced by subcritical water process on pH of pork patties. Vertical bars indicate standard deviations. Means with different letters were significantly different ($p<0.05$).

oxidation or vice versa (Faustman *et al.*, 2010; Jia *et al.*, 2012); hence, it was expected that the WHC and shear force of pork patties would be changed by degree of lipid oxidation (Table 1). For WHC, no significant difference was observed among treatments, and the WHC of all patties was ranged to 65-75%. Although shear force of pork patties showed significant differences depending on SPHs concentration, while the shear force of SPH-treated pork patties was not different comparing to untreated control. Protein oxidation was characterized by formation of free carbonyl group formation which was reported to attribute either loss of WHC or high shear force (Jia *et al.*, 2012; Lund *et al.*, 2011). The results indicated that protein oxidation was not progressed compared to measured lipid oxidation. In the current study, there was no agent to progress protein oxidation, thus free radical produced by lipid oxidative metabolism was the only factor facilitating protein oxidation (Labuza and Dugan Jr., 1971). Kinetically, 14 d of chilled storage in this study was not enough to detect the evidences of protein oxidation; therefore, deteriorations in WHC or shear force of pork patties might not have been obtained. Consequently, the results indicated that the addition of SPHs in pork patty formulation did not modify the eating quality of the patty.

The above results demonstrated that the SPHs had an advantage in preventing or delaying oxidative metabolisms such as discoloration and high TBARS of meat products. The various functions of SPHs in meat products have been already proposed (Tsumura *et al.*, 2005). Meanwhile, this study applied SPHs prepared by SWP instead of enzymatically hydrolyzed peptides. As previously reported (Lee *et al.*, 2013), effective protein hydrolysis efficiency was processed in SWP by which oligopeptides was prepared. The SPHs prepared by SWP presented a strong anti-oxidative activity when optimal amount (0.5-1.5%) was added in the product formulation. Consequ-

Table 1. Effects of concentrations of soy protein hydrolysates (SPHs) produced by subcritical water process on water-holding capacity (WHC) and shear force of pork patties

SPHs (%)	WHC (%)	Shear force (kg)
Control	74.6±7.49	1.30±0.10[ab]
0.5	65.2±13.6	1.24±0.09[ab]
1.0	69.9±4.05	1.49±0.15[a]
1.5	73.5±7.69	1.31±0.16[ab]
2.0	69.5±3.21	1.14±0.04[b]
2.5	68.2±5.31	1.42±0.17[a]
3.0	74.5±3.98	1.39±0.05[ab]

[a,b]Means with different superscripts within same column are significantly different ($p<0.05$).

ently, these results demonstrated that SPHs prepared by SWP could be applied in meat product formulation to extend shelf-life or to delay oxidative deterioration.

Conclusion

In the present study, effects of SPHs prepared by SWP on the physicochemical properties of pork patties were evaluated. The addition of SPHs successfully reduced the risk involved in oxidative metabolisms (TBASR and discoloration) of meat products during chilled storage without deteriorations in eating qualities such as WHC and texture. In chilled pork patties, prevention of discoloration during storage had an advantage in providing freshness and wholesomeness of the products for consumers. Meanwhile, the addition of optimal amount of SPHs (0.5-1.5%) was required since oxidative factors were still involved in SWP-treated SH preparation. It was also required if the SPHs produced by SWP could provide other functional properties such as antimicrobial activity or not, which warrants further study.

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