ANTIOXIDATIVE ACTIVITY OF PHOSVITIN SEPARATED FROM EGG YOLK MIXED WITH GROUND BEEF

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***Abstract*-The antioxidative activity of phosvitin separated from egg yolk mixed with ground beef were investigated at concentrations 100 and 500 ppm levels. Reducing power of the raw ground beef mixed with phosvitin increased at day 0 and 7 when compared to that of control. The activity was maintained at storage day 0 and 3 day after cooking at a 500 ppm level. Consequently, 2-thiobarbituric acid-reactive substances (TBARS) value of the raw ground beef added phosvitin with both concentrations were significantly lower than that of control during stored at 4℃. The increase of TBARS value was also significantly retarded in cooked ground beef added with phosvitin at a 500 ppm level during whole storage while significant difference was found only at day 7 when 100 ppm of phosvitin was mixed. However, no effect on the inhibition of the growth of inoculated *Escherichia coli* and *Listeria monocytogenes* was observed. Results suggested that phosvitin can be used for the ingredient of muscle food as an antioxidant. Furthermore, it can be more attractive antioxidant than other synthetic or plant-derived antioxidants because meat products may be more harmonized with a natural protein derived from animal product such as phosvitin.**

***Index Terms*: phosvitin; ground beef; antioxidant activity; lipid oxidation**

I. INTRODUCTION

Phosvitin derived from egg yolk is a very specific protein which is composed about 30~50% serine in total amino acid composition and exists as phosphoserines. This special feature makes phosvitin to binding many bivalent metal ions, especially iron ions (Yoshinori, 2008). Iron ion is very important because it has a strong prooxidant role in muscles foods, thereby accelerating lipid oxidation (Han & Lee, 2000). Lipid oxidation in muscles food is a serious problem since it leads to deterioration of quality such as flavor, color, texture, and nutritional value (Mielnik et al., 2006). For this reason, phosvitin has been studied as a natural animal-derived antioxidative agent and reports suggested that it could be an effective antioxidant for the inhibition of lipid oxidation generated by iron ion (Lee at al., 2002). Phosvitin is also known to have an antimicrobial effect against the growth of pathogenic Gram-negative bacteria (Khan et al., 2000). Therefore, phosvitin has a great potential as a natural animal-derived antioxidaitve and antimicrobial material for muscles foods. However, the separation and purification of phosvitin form egg yolk is not easy, thus there is still limited information available about the biological activity of phosvitin. Therefore, this study was conducted to investigate the antioxidative and antimicrobial activity of phosvitin separated from egg yolk when mixed into ground beef.

II. MATERIALS AND METHODS

A. Separation of phosvitin

Egg yolk was homogenized with two volumes of distilled water, and then egg yolk membranes was removed by filtering through a cheese cloth. The diluted egg yolk solution was centrifuged at 9,000 x g for 30 min. The supernatant was removed and lipids of egg yolk were removed by using 85% ethyl alcohol from the precipitate. To extract phosvitin, the fat-free residue of egg yolk was homogenized with 9 volumes of 10% sodium chloride solution (pH 4). Sodium chloride was removed from phosvitin solution, and lyophilized.

B. Sample preparation

Beef loin was purchased from a local market in Daejeon, Korea. Beef loin was minced by a grinder to

obtain homogenate sample. The ground beef was mixed with the prepared phosvitin with concentration of 100 and 500 ppm (w/w). Since phosvitin was not soluble, it was dissolved in 1% sodium chloride solution and mixed with the ground beef. The sample was packed in a polyethylene bag. Half of the samples were heated to an internal temperature of 72℃ in a boiling water bath for cooked meat study. After cooling, drip was removed and stored at 4℃. The ground beef added with 1% sodium chloride solution was used as a control.

***C*. Antioxidative and antimicrobial activity**

The reducing power and TBARS value were determined according to the method of Oyaizu (1986) and Ahn et al. (1999), respectively. Reducing power was expressed as absorbance at 700 nm using spectrophotometer and TBARS value was expressed as mg malondialdehyde per kg meat.

Escherichia coli (KCTC 41682) and *Listeria monocytogenes* (KCTC 3569) were obtained from Korean Collection for Type Culture (KCTC, Daejeon, Korea). The strains were cultivated as the same method of Song et al. (2009). The sample was sub-divided into portions each have 5 g in sterile polyethylene pouch bag (8 × 10 mm), was inoculated with *L. monocytogenes* and *E. coli*. The test culture suspension (50 μL) was uniformly and aseptically inoculated in different areas on the samples and mixed to achieve uniform dispersal at the desired concentration throughout the sample for 5 min in enclosed polyethylene bag. The bags were sealed and the inoculated samples were stored at 4 °C. After storage, samples were blended with sterile saline using a stomacher (BagMixer ® 400, Interscience Ind., St. Nom, France) for 2 min. Then, a series of decimal dilution was prepared with sterile saline and each diluent (0.1 mL) was spread in tryptic soy agar (Difco Laboratories). The plates were incubated at 37 °C for 48 h and microbial counts were expressed as log CFU/g.

***D*. Statistical analysis**

All experiments were duplicated with three observation numbers adapted for each experiment. Analysis of the variance was performed using the raw data, and the mean values and standard deviation were calculated by the Statistical Analysis System (SAS, 2000). Differences among the mean values were determined by the Duncan's multiple range test with a significance defined at $p<0.05$.

III. RESULTS AND DISCUSSION

The antioxidative activity of phosvitin mixed with ground beef was analyzed by monitoring reducing power, which is an indicator of antioxidative potential and based on chelating effect on ferrous ion (Fe^{3+} to Fe^{2+}) (Hsu et al., 2006). The reducing power of raw ground beef added with 100 ppm of phosvitin (GBP 100) was significantly higher than that of control at day 7 but no difference was found at days 0 and 3 (Table 1). When 500 ppm of phosvitin (GBP 500) was added, the reducing power was significantly higher at day 0 when compared to that of control. However, no difference was found at days 3 and 7. The cooked ground beef with GBP 500 had significantly higher reducing power at days 0 and 3 while the difference was only found at day 0 in cooked ground beef with GBP 100. Previous study reported that phosvitin had strong antioxidant activity and this activity originated iron binding capacity (Lu & Baker, 1986). Also, the iron binding capacity of phosvitin had not been changed by thermal treatment up to 90℃ for 1 hour (Castellani et al., 2004).

The effect of phosvitin on the inhibition of lipid oxidation was assured by TBARS method (Table 2). In raw ground beef, the TBARS values of GBP 100 and GBP 500 were lower than that of control during storage. After storage for 7 days, TBARS values of GBP 100 and GBP 500 were inhibited as much as 28% (1.96) and 25% (2.03) compared to that of control (2.74). In cooked state, the inhibition of lipid oxidation in ground beef was more certain. TBARS values increased as 45% after cooking. The cooked ground beef with GBP 500 had lower TBARS value than that of control during whole storage days, however, those of GBP 100 showed the difference at only day 7. Result was in agreement to a previous result that phosvitin inhibited lipid oxidation of ground pork (Lee et al., 2002).

Antimicrobial effect of phosvitin has been reported (Khan et al., 2000). They suggested that phosvitin caused damage to the cellular surface of bacteria through its chelating to Fe, Ca, and Mg in an outer cell membrane, and consequently ingenerating cell disruption. However, antimicrobial effect of phosvitin in inoculated GBP 100 and GBP 500 was not found (data not shown).

IV. CONCLUSION

Results suggested that phosvitin can be used for the ingredient of muscle food as an antioxidant. Furthermore, it can be more attractive antioxidant than other synthetic or plant-derived antioxidants because meat products may be more harmonized with a natural protein derived from animal product such as phosvitin.

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Table 1. Reducing power of ground beef added with phosvitin during storage at 4℃

Treatment	Storage day 0	3	7	SEM[1]
		Raw ground beef		
Control	0.34^{ay}	0.29^{b}	0.29^{by}	0.005
GBP 100[3]	0.34^{axy}	0.30^{b}	0.32^{cx}	0.004
GBP 500[4]	0.35^{ax}	0.30^{b}	0.30^{by}	0.004
SEM[2]	0.002	0.006	0.003	
		Cooked ground beef		
Control	0.27^{az}	0.21^{by}	0.20^{b}	0.003
GBP 100	0.28^{ay}	0.22^{by}	0.21^{b}	0.004
GBP 500	0.30^{ax}	0.24^{bx}	0.22^{c}	0.004
SEM[2]	0.002	0.003	0.005	

[1]Standard errors of mean (n=9), [2](n=9).
[3]Ground beef added with 100 ppm phosvitin (w/w).
[4]Ground beef added with 500 ppm phosvitin (w/w).
[x-z]Different letters within the same column differ significantly ($p < 0.05$).
[a,b]Different letters within the same column differ significantly ($p < 0.05$).

Table 2. 2-Thiobarbituric acid-reactive substances value of ground beef added with phosvitin during storage at 4℃

Treatment	Storage day 0	3	7	SEM[1]
		Raw ground beef		
Control	1.96^{bx}	2.23^{bx}	2.74^{ax}	0.099
GBP 100[3]	1.57^{xy}	1.74^{y}	1.96^{y}	0.144
GBP 500[4]	1.33^{by}	1.58^{by}	2.03^{ay}	0.104
SEM[2]	0.150	0.122	0.061	
		Cooked ground beef		
Control	3.50^{bx}	6.75^{ax}	6.74^{ax}	0.117
GBP 100	3.43^{bx}	6.30^{ax}	6.07^{ay}	0.146
GBP 500	2.50^{by}	5.39^{ay}	5.35^{az}	0.171
SEM[2]	0.126	0.186	0.115	

[1]Standard errors of mean (n=9), [2](n=9).
[3]Ground beef added with 100 ppm phosvitin (w/w).
[4]Ground beef added with 500 ppm phosvitin (w/w).
[x-z]Different letters within the same column differ significantly ($p < 0.05$).
[a,b]Different letters within the same column differ significantly ($p < 0.05$).