IRON BINDING ABILITY OF PHOSVITIN IN GROUND BEEF

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Abstract— **A chicken egg yolk was used to extract phosvitin and the antioxidative activity of the extracted phosvitin was investigated by mixing with ground beef at phosvitin concentrations of 100 and 500 mg/kg ground beef. The electrophoretic pattern of the extracted phosvitin on the SDS-PAGE was confirmed as identical to that of standard phosvitin. The extracted phosvitin at a 1,000 ppm level showed around 65% iron binding ability at 3 mM iron solution. The development of lipid oxidation was retarded in ground beef with extracted phosvitin at a 500 mg/kg level during storage at 4□ when compared to that of control ($P < 0.05$). Results suggest that the extracted phosvitin from egg yolk could be used in industry as an excellent antioxidant agent. In particular, it would be more harmonized with meat products because phosvitin is a natural protein derived from animal product.**

Keywords— **phosvitin, iron binding, beef, antioxidative**

Ⅰ. INTRODUCTION

Lipid oxidation is a serious problem in meat products because the oxidation products reduce the shelf life of food and lead to the deterioration of food quality factors such as flavor, color, texture, and nutritional value [1].

Iron is an essential dietary metal ion required by animals since it is needed for heme proteins that are involved in oxygen transport and storage in the body [2]. However, iron is released from heme during the meat production processes as free iron (non-heme iron) which promotes lipid oxidation in meat products through Fenton reactions (Fe^{2+} or Fe^{3+}/H_2O_2) [2,3]. Therefore, the elimination of free iron in meat products can be a way to inhibit lipid oxidation.

Phosvitin is a phosphoglycoprotein to be found in chicken egg yolk [4]. It is known to have a specific amino acid composition that is approximately 50% serine, and 90% of these serine residues are phosphorylated [5]. This specific structure makes phosvitin a potent metal chelator. Previous studies suggested that phosvitin could inhibit lipid oxidation promoted by iron in meat [6]. In addition, phosvitin could be more suitable for use in meat products than natural antioxidants derived from grains, oilseeds, honey, fruits, and vegetables because it is a natural protein derived from animal product. Previous studies reported that changes in the color or flavor in cooked beef, chicken, and goat meat patties resulted from adding natural antioxidants such as ones derived from tea, kinnow, pomegranate, or grapes [7,8].

Nevertheless, phosvitin is not used as antioxidant in meat products because of the complicated procedures and cost to produce. Recently, a simple and cost-effective method was established [9] for extracting phosvitin from chicken egg yolk using NaCl and alcohol. The aim of this study was to evaulate antioxidant activity of the extracted phosvitin when present in ground beef.

Ⅱ. MATERIAS AND METHODS

A. Purification of phosvitin from egg yolks

Phosvitin was extracted from chicken eggs according to the method by Ko et al. [9] with modifications. Egg yolk was separated from egg white, and the egg yolk membranes and chalaza were removed by filtering through a testing sieve (1000 μm). The filtered egg yolk was centrifuged at 4,070 x g for 30 min after homogenization with two volumes of

distilled water. The precipitate was homogenized with four volumes of 85% ethanol, and centrifuged at 4,070 x g for 30 min to remove phospholipids. The precipitate was then homogenized with five volumes of 10% NaCl solution and centrifuged at 4,070 x g for 30 min to extract the phosvitin. The supernatant was collected, filtered by an ultrafiltration system to remove NaCl (Quixstand Benchtop System using a membrane column with a 10 kDa molecular weight cut-off, GE Healthcare, Waukesha, USA). At the end of ultrafiltration, the solution without NaCl was centrifuged at 4,070 x g for 30 min after the pH was adjusted to 4.0. The supernatant was lyophilized. The identification of extracted phosvitin was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

B. Iron binding capacity of phosvitin

A sample solution was made using the following procedure. A mixture of 1.66 mL distilled water, 20 μL of 1.0 to 5.0 mM $FeCl_3$, and 20 μL of 1,000 μg/mL phosvitin in distilled water was transferred to conical tubes (15 mL). The mixture was left at room temperature for 1 min. Next, 0.2 mL of 5 mM ferrozine was blended with 0.1 mL of 1% ascorbic acid in distilled water and added to the tubes. After 5 min at room temperature, the final color change was monitored using a spectrophotometer at 532 nm using distilled water as a blank. The iron binding capacity was calculated with the following equation:

$$\text{Iron binding capacity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 indicates the absorbance of the sample solution without the phosvitin solution, and A_1 indicates the absorbance of the sample solution with the phosvitin solution.

C. Preparation of ground beef samples

The ground beef was divided into three treatment groups as follows: 1) the control, ground beef with 1% distilled water (v/w) without phosvitin, 2) GBP 100, ground beef with 1% distilled water containing phosvitin (100 mg/kg ground beef), and 3) GBP 500, ground beef with 1% distilled water containing phosvitin (500 mg/kg ground beef). The samples were aerobically stored at 4□ for 7 days.

D. Lipid oxidation

Lipid oxidation was determined as the TBARS value [10]. The TBARS value was reported as mg of malondialdehyde per kg of meat.

F. Statistical Analysis

This study was performed in triplicate. Analysis of variance was performed using the raw data, and the mean values and standard errors of the means (SEM) were calculated by the Statistical Analysis System (SAS, 2000). Differences among the means were determined by Duncan's multiple range test with $P < 0.05$ indicating statistical significance.

Ⅲ. RESULTS AND DISCUSSION

A. Purification of phosvitin from egg yolks

Phosvitin consists of two polypeptides, α- and β-phosvitin, with different numbers of sub-units 3 or 4 of 35 to 40 kDa for α-phosvitin; 4 or 5 sub-units of 45 kDa for β-phosvitin) [4]. The electrophoretic patterns of the extracted phosvitin on SDS-PAGE are shown in Fig. 1. Bands of the extracted phosvitin were between 37.8 and 46.2 kDa in size and almost identical to those of the phosvitin standard. This result indicates that phosvitin was successfully purified by a simple extraction method using NaCl.

B. Iron binding capacity of phosvitin

Iron binding capacity of the extracted phosvitin was measured and compared to that of the phosvitin standard (Figure. 2). Both the extracted and standard phosvitin were shown to bind iron although the binding capacity of the extracted phosvitin was lower than that of the phosvitin standard.

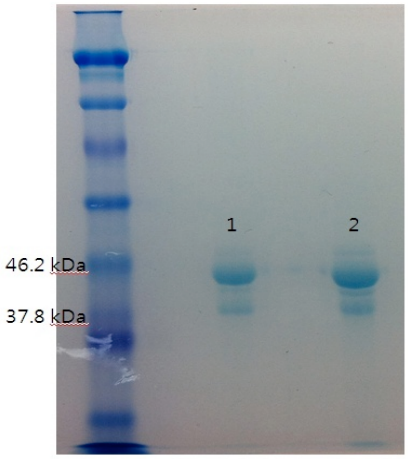

Fig. 1. Polyacrylamide-gel electrophoretic patterns of the phosvitin standard (1) and the phosvitin extracted from egg yolk (2).

Albright et al. [11] reported that unlike the phosvitin standard the extracted phosvitin bound almost 95% of irons present in egg. This could also be one of the reasons why the iron binding capacity of the extracted phosvitin was lower than that of the phosvitin standard. Castellani et al. [12] suggested that iron bound to phosvitin can be completely removed by anion exchange chromatography. However, this process is costly and therefore may not be applicable for industrial use. The results of our study show that the iron binding activity of the extracted phosvitin is still effective.

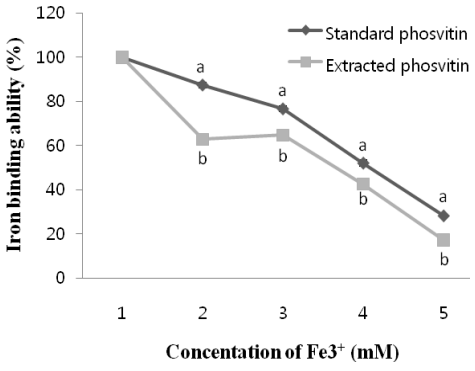

Fig 2. Iron binding ability of the phosvitin extracted from egg yolk and phosvitin standard.
[a,b]Points with different letters in the lines for the standard and extracted phosvitin differ significantly in value ($p < 0.05$).

C. TBARS values of the ground beef

Based on the iron binding capacity of the extracted phosvitin, it can be assumed that this reagent will inhibit lipid oxidation in meat products. Ishikawa et al. [13] reported that phosvitin decreases the generation of hydroxyl radical from Fenton reactions responsible for the catalysis of lipid oxidation. The TBARS values for the control ground beef increased from 0.65 to 1.49 mg of malondialdehyde kg^{-1} meat stored for 7 days at 4℃. However, the TBARS values of the GBP 500 stored under the same conditions increased from 0.62 to 1.14. These results indicate that adding 500 mg/kg of phosvitin to ground beef significantly decreased lipid oxidation ($P < 0.05$).

The antioxidant activity of phosvitin in pork was previously reported by Lee et al. [6]. They reported that phosvitin has no effect on the inhibition of lipid oxidation in raw ground pork but is significantly effective in cooked pork. In contrast, significant inhibition of lipid oxidation by the extracted phosvitin in raw ground beef in this study was probably due to the difference in free iron concentrations between beef and pork. Beef contains higher concentrations of free iron compared to pork [14]. Estévez and Cava [4] reported that the amount of free iron is increased by cooking and storage, and the highest concentrations are found in meat that is stored after being cooked. Importantly, Castellani et al. [15] found that phosvitin maintained a high iron binding capacity when it was treated by thermal (90 ℃) or high pressure processing (600 MPa). For these reasons, the extracted phosvitin from egg yolk could be used as a valuable antioxidative reagent in processed meat products which are subject to heat and storage before consumption.

Table 1. 2-Thiobarbituric acid-reactive substances values (mg malonedialdehyde/kg meat) in ground beef containing phosvitin during storage at 4□

	Storage (days)			
Treatment	0	3	7	SEM[1]
Control	0.65^{b}	1.44^{ax}	1.49^{ax}	0.025
GBP 100[3]	0.63^{c}	1.28^{by}	1.54^{ax}	0.008
GBP 500[4]	0.62^{c}	1.00^{bz}	1.14^{ay}	0.016
SEM[2]	0.008	0.024	0.017	

[1]Standard error of the means (n = 9), [2](n = 9).
[3]Ground beef with phosvitin100 mg/kg.
[4]Ground beef with phosvitin500 mg/kg.
[x-z]Figures with different letters within the same column differ significantly ($p < 0.05$).
[a-c]Figures with different letters within the same row differ significantly ($p < 0.05$).

Ⅳ. CONCLUSIONS

Phosvitin was prepared using a relatively easy and cost-effective method. The addition of the extracted phosvitin at concentrations of 100 and 500 mg/kg effectively reduced lipid oxidation in ground beef when compared to that of the untreated control. Based on the results of this study, it can be concluded that the extracted phosvitin could be used in manufacturing meat products as a highly effective antioxidant. Furthermore, the extracted phosvitin appears to be more amenable for use in meat products because it is a natural protein derived from animal sources.

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