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Effects of myofibril-palatinose conjugate as a phosphate substitute on meat emulsion quality

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ABSTRACT

The objective of this study was to investigate a replacement for phosphate in meat products. Protein structural modification was employed in this study, and grafted myofibrillar protein (MP) with palatinose was added to meat emulsion without phosphate. Here, 0.15% of sodium polyphosphate (SPP) was replaced by the same (0.15%) concentration and double (0.3%) the concentration of grafted MP. Although the thermal stability was decreased, the addition of transglutaminase could increase stability. The rheological properties and pH also increased with the addition of grafted MP and transglutaminase. The addition of grafted protein could be perceived by the naked eye by observing a color difference before cooking, but it was not easy to detect after cooking. The cooking loss, emulsion stability, water holding capacity, lipid oxidation, and textural properties improved with the addition of grafted MP. However, the excessive addition of grafted MP and transglutaminase was not recommended to produce a high quality of phosphate replaced meat emulsion, and 0.15% was identified as a suitable addition ratio of grafted MP.

1. Introduction

Meat products are eaten as major sources of protein and various nutrients [1]. However, the health risks associated with additives in meat products have limited their use in recent years [2]. Among various additives, due to increasing concern regarding the negative health effects of phosphates, their use and addition have been limited or regulated [3]. However, phosphate serves a major role as an emulsifier in processed meat and can be used to regulate several characteristics such as water-holding capacity (WHC), emulsifying capacity, texture, color, lipid oxidation, and cooking loss [3,4]. There are two main strategies to reduce phosphates. The first type of approach involves the use of novel processing technologies such as ultrasound, high pressure, plasma, and pulsed electric fields to unfold meat proteins and enhance protein functionality [5,6]. These kinds of non-thermal technologies can be employed to eliminate microbial threats with high nutritional and sensory value [6]. However, the high installation cost and limited capacity of equipment will obstruct the utilization of these technologies. The other means of reducing phosphates in meat products entails the use of another ingredient that has high potential as a phosphate replacement, including modified starch, proteins, edible fiber, hydrocolloids, carbonate salt, and alkaline ingredient [3].

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Abbreviations

LVE	linear viscoelastic
MP	myofibrillar protein
SPP	sodium polyphosphate
TBARS	thiobarbituric acid reactive substance
TG	transglutaminase
TPA	texture profile analysis
WHC	water-holding capacity

Among the various strategies considered, the modification of structural characteristics and use of grafting technologies to enhance the quality characteristics of protein have been studied [7,8]. Reducing sugars can be grafted with protein, improving the surface functional properties such as emulsifying capacity and WHC [9]. This reaction can be explained by the Maillard reaction and the fact that the modified protein called "glycated protein" or "grafted protein" have increased hydrophilic residue due to sugars and the protein molecular weight [10]. Myofibrillar protein (MP) reacted with dextran has improved characteristics in terms of emulsifying capacity [7]. We compared the impact of various reducing sugars on grafted MP, and we observed that palatinose had increased water binding capacity and emulsion stability [11]. Therefore, we demonstrated the impact of a grafted MP with palatinose and for use alternative to phosphate to produce quality meat products.

Isomatulose, also called palatinose, has a positive effect on health because it has a low glycemic index and high potential as a prebiotic and anticariogenic sweetener [12]. Therefore, this sugar not only has become popular as a sucrose substitute, but also is suitable for children [13]. Because excessive occurrence of the Maillard reaction can be a reason for carcinogen generation during heating, producing grafted MP in the initial step is important [14]. Palatinose also results in less occurrence of the Maillard reaction, and it could be suitable for the production of grafted protein.

Additionally, transglutaminase (TG) has been used to improve meat product or other protein based foods [15]. Combination of modified protein and TG can improve quality of meat products when reducing phosphate [16]. Even, this enzyme has been used to improve water holding capacity and emulsion stability in meat products optionally [3]. Therefore, this enzyme was used to prevent the degradation of quality characteristics of meat emulsions that can occur when modified proteins are added.

In this study, the grafted MP was applied to meat emulsion without the addition of phosphates and the quality characteristics of the resulting meat emulsion were investigated to replace sodium polyphosphate (SPP).

2. Materials and methods

2.1. Materials and chemicals

Table 1

Forty-eight-hours post mortem pork ham and back fat from castrated boars (M. semitendinosus, M. semimembranosus, and biceps femoris) was obtained from A market (Wanju, Korea). Each cuts were divided from different 3 carcasses and excessive connective tissue was removed. Palatinose, SPP, and transglutaminase (47 U/g) were acquired from ES Food (Gunpo, Korea). All chemicals used in the experiments were obtained from Thermo-Fisher Scientific (Cleveland, OH, USA).

2.2. Extraction of MP and grafting reaction with palatinose

MP was extracted according to the method of Jia, Nirasawa [17]. The sarcoplasmic protein was removed from the pork ham using 0.1 M potassium chloride in 0.01 M phosphate buffer (pH 7.4), and the residue was considered to be MP. The protein concentration of MP was held constant at 40 mg/mL using saline solution (0.49 M NaCl, 17.8 mM Na₅P₃O₁₀, and 1 mM NaN₃, pH 8.4), and 20 mg/mL of palatinose was added. Well homogenate was reacted at 37 °C for 8 h [7]. After the reaction, the remaining salt and sugar were removed by dialysis using 3.5 K molecular weight cut-off snake skin tubing (Thermo-Fisher Scientific, Cleveland, OH, USA) and lyophilized to store the grafted MP.

he formulation of meat emulsion added grafted myofibrillar protein with palatinose as a phosphate replacer.								
Traits	Control (–)	Control (+)	0.15	0.15TG	0.30	0.30TG		
Meat	50	50	50	50	50	50		
Ice	25	25	25	25	25	25		
Fat	25	25	25	25	25	25		
Grafted myofibril	0	0	0.15	0.15	0.3	0.3		
Salt	1.5	1.5	1.5	1.5	1.5	1.5		
Phosphate	0	0.15	0	0	0	0		
Transglutaminase	0	0	0	1	0	1		

2.3. Preparation of meat emulsion

The 15 kg of pork ham and 10 kg of fat were ground through 8 mm and 3 mm plates using meat grinder (SMC-22A, SL Company, Incheon, Korea) and used to form a meat emulsion. The detailed information of meat emulsion was given in Table 1. The sodium chloride and SPP concentrations were 1.5% and 0.15% of the basic emulsion, respectively. Grafted MP was added at the same concentration (0.15%) and double the concentration (0.3%) as SPP concentration to compare effect of SPP and grafted MP. Transglutaminase (TG) was added at 1% to increase protein-protein interaction according to commercial recommendation of the company where it was purchased. A negative control (Control (–)) was manufactured without SPP, and a positive control (Control (+)) was produced with 0.15% SPP. The treatments were called 0.15, 0.15TG, 0.30, and 0.30TG according to the addition of grafted MP and TG without addition of SPP.

Approximately 3 kg of each batter was prepared in a batch, and there were three different batches. The manufacturing processing was conducted following the method of Kim, Yong [18]. The meat and salts were mixed using a silent cutter (C4VV, Sirman, Marsango, Italy), and flaked ice was added to ensure that the temperature of the meat batter remained below 3 °C. After that, the prepared pork back fat was homogenized until the meat batter temperature reached 10 °C. The silent cutter had two knives (plain knives), and knife speed was held constant at 2600 cut/min. The emulsified meat batter was stuffed into 25-mm-diameter collagen casing (#240, NIPPI Inc., Tokyo, Japan).

The meat batter was stored in a refrigerator for further experiments, and the stuffed meat batter was cooked until the core temperature of the meat emulsion had been raised to 75 °C using water bath (JSSB-30T, JS Research Inc., Korea). The cooked meat emulsion was cooled using ice water until the core temperature was less than 20 °C.

2.4. Quality characteristics of emulsified meat batter

2.4.1. Thermal stability

The thermal stability of the emulsified meat batter was estimated by differential scanning calorimetry [19]. An empty aluminum pan was used as a reference pan, and an aluminum pan contained 30 mg of the meat batter was used as the sample pan. Each pan was heated from 20 to 100 °C at 10 °C/min, and their changes in state were estimated by utilizing a DSC 4000 (PerkinElmer, Waltham, MA, USA). The collected data were calculated using Pyris data analysis software (PerkinElmer).

2.4.2. Rheological properties

The rheological properties (both viscosity and viscoelastic properties) were determined by using a Physica MCR 102 rheometer (Anton paar GmbH, Graz, Australia). All measurements were performed using a parallel plate with a diameter of 25 mm and a gap of 1 mm after temperature equilibration for 3 min. The apparent viscosity was measured in the shear rate range from 0.1 to 100 s^{-1} , and the temperature was maintained at 15 °C. Two tests were performed to measure the viscoelastic properties. For the amplitude sweep tests to identify the linear viscoelastic (LVE) region, the shear strain was swept from 0.1% to 100% at a constant angular frequency of 10 rad/s. The temperature sweep test was performed by increasing the temperature from 10 to 95 °C at 2 °C/min and a constant shear strain of 0.5%. The apparent viscosity, storage modulus (G'), and loss modulus (G'') were determined using RheoCompass v.1.26 software (Anton paar GmbH, Graz, Australia).

2.4.3. pH

Five grams of sample was homogenized with 20 mL of distilled water, and the pH of the homogenate was estimated by employing a pH meter (360, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). Different pH calibration buffers with pH 4, 7, and 10 were used to calibrate the pH meter.

2.4.4. Instrument color

The instrument color values of the samples were estimated based on their CIE $L^*a^*b^*$ color values. The meat batter and cut cooked meat emulsion were bloomed in an oxygen-containing refrigerator at 2 °C for 30 min. A chroma meter (CR-410, Minolta Ltd., Osaka, Japan) was used to detect the color values. The observer degree was two, and D₆₅ was employed as the light source. The calibration conditions were Y = 87.1, x = 0.3166, and y = 0.3338.

2.5. Physicochemical properties of cooked meat emulsion

2.5.1. Cooking loss

The weight loss after cooking was considered to be the cooking loss. After stuffing, 10 stuffed meat batter (50 g per each sample) was prepared to determine cooking loss and total 30 samples are prepared in a batch. The initial weight of the meat batter was recorded prior to heating. After cooking at 75 °C until core temperature reached at 75 °C, the cooked sample was cooled using chilled water, and the water on the surface after cooling was wiped off. Further, the weight of the cooled sample was recorded, and the change in weight was presented as a percentage (1).

Cooking loss (%) = [weight of raw sample (g) - weight of cooked sample (g)] / weight of raw sample (g) \times 100

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(4)

2.5.2. Emulsion stability

The emulsion stability of meat batter was estimated using exudative fluids after cooking [20]. After 20 g of meat batter had been stuffed into the graduated tubes, the tubes were heated at 75 °C until the core temperature reached 75 °C. After cooling at 2 °C in a refrigerator, the exudative fluid was recorded. The total expressible fluid and separated fat were calculated as percentages (2 and 3).

Total expressible fluid (%) = weight of total expressible fluid (g) / weight of raw sample (g)
$$\times$$
 100 (2)
Separated fat (%) = weight of fat laver (g) / weight of raw sample (g) \times 100 (3)

2.5.3. Water holding capacity (WHC)

The WHC of the cooked meat emulsion was estimated by centrifugation [21]. After centrifugation 3g of sample at 1000 g for 10 min at 4 °C, the released moisture content was compared with the initial moisture content of the cooked meat emulsion. The compared data were expressed as percentages.

2.5.4. Thiobarbituric acid reactive substance (TBARS)

The lipid oxidation of the cooked meat emulsion was estimated based on the TBARS value [22]. After 10 g of the sample had been homogenized with 100 mL 0.1 N HCl, the distillate was collected and reacted with 0.02 M 2-thoibarbituric acid in 90% acetic acid at 100 °C for 30 min. After cooling, the absorbance of the reacted sample at 538 nm was recorded using a UV/VIS spectrophotometer (Optizen 2120 UV Plus, Mecasys Co. Ltd., Daejeon, Korea). The TBARS values was calculated according to Tarladgis, Watts [22] formula (4).

TBARS value (mg/kg) = absorbance at 538 nm \times K

$$K(distillation) = \frac{\text{concentration in moles/5 mL of distillate}}{\text{optical density}} \times \text{molecular weight of malondialdehyde} \times \frac{10^7}{10 \text{ g of sample}} \times \frac{100}{68.5 \% \text{ recovery}}$$
$$= 7.74$$

2.5.5. Texture profile analysis (TPA)

The TPA of the cooked meat emulsion was estimated by utilizing a textural analyzer (TA-XTplus, Stable Micro Systems Ltd., Surrey, England). The cooled sample was cut to 2 cm diameter and 2 cm height from the central portion and replicated technically 10 times. The distance between the 25-mm-diameter probe and sample was 1 cm, and the maximum load and threshold were 2 and 0.005 kg, respectively. The pre-test, test, and post-test were 2, 5, and 2 mm/s [23,24]. The obtained data were calculated by employing Exponent software (Stable Micro System Ltd.).

2.6. Statistical analysis

Table 2

The statistical analysis was conducted using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). With three different batches (P > 0.05), the experiments were repeated three times in each batch, and the significant differences among 2 of the controls and 4 of treatments were assessed by performing a one-way analysis of variance (P < 0.05). The concentrations of the phosphate, grafted MP, and TG were considered as fixed terms, and replicates were considered as random terms. When statistical analysis was run, the specific difference in means was calculated by performing Duncan's multiple range test, and the data were represented as mean \pm standard error.

Effects of grafted myofibrillar protein with palatinose on thermal stability of meat emulsion

Traits	Control (–) ^a	Control (+)	0.15	0.15TG	0.30	0.30TG
Peak 1						
Peak temperature (°C)	$61.04\pm0.25^{\rm c}$	$62.79 \pm \mathbf{0.43^a}$	$61.32\pm0.18^{\rm bc}$	$62.94\pm0.23^{\rm a}$	$61.52\pm0.34^{\rm bc}$	62.22 ± 0.39^{ab}
ΔH (J/g)	0.012 ± 0.003	0.019 ± 0.002	0.009 ± 0.005	0.009 ± 0.004	0.015 ± 0.001	0.025 ± 0.008
Peak 2						
Peak temperature (°C)	76.32 ± 2.00	73.68 ± 0.18	74.09 ± 1.09	73.32 ± 0.14	75.07 ± 1.52	75.13 ± 0.43
ΔH (J/g)	0.176 ± 0.029^{bc}	0.084 ± 0.018^{c}	0.386 ± 0.006^a	0.219 ± 0.037^b	0.177 ± 0.031^{bc}	0.066 ± 0.013^{c}

All values are mean \pm standard error of three replicates (n = 3).

^{a-c} Means within a row with different letters are significantly different (P < 0.05).

^a Control (–): no phosphate/grafted protein added, control (+): 0.15% phosphate added, 0.15: 0.15% grafted protein added, 0.15TG: 0.15% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added, 0.30: 0.30% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added.



Fig. 1. Effects of grafted myofibrillar protein with palatinose on rheological properties of meat emulsion: apparent viscosity (a), and storage modulus G' (filled symbols) and loss modulus G' (open symbols) values for amplitude sweep test (b) and temperature sweep test (c). ¹⁾ Control (–): no phosphate/grafted protein added, control (+): 0.15% phosphate added, 0.15: 0.15% grafted protein added, 0.15TG: 0.15% grafted protein and 1% transglutaminase were added, 0.30: 0.30% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added. The represented result was shown (n = 3).

3. Results and discussion

3.1. Quality characteristics of emulsified meat batter

3.1.1. Thermal stability

Generally, myosin, sarcoplasmic protein, actin, and connective tissues are classified as major proteins in meat, and the denaturation temperature is 45-60 °C in myosin, 60-76 °C in sarcoplasmic, and 76-92 °C in actin and connective tissues [25,26]. MP has an important role in thermal induced gelation, and its thermal stability can be affected by the pH, ionic strength, and protein structural characteristics [25]. The thermal stability of the meat emulsion is shown in Table 2, where two kinds of peak temperatures are observed. Although the difference in peak temperature is significant (P < 0.05), the difference among treatments in ΔH is not significant (P > 0.05) in peak 1. Peak 1 could be a point at which myosin is denatured and myosin is known as a major protein in meat protein gelation [25,26]. Control (+) shows the highest peak temperature (P < 0.05), and Control (-) produces the lowest peak temperature (P < 0.05). Although the addition of grafted MP cannot increase the thermal stability of myosin, the addition of TG increases the thermal stability of the meat emulsion. The 0.15 and 0.30 treatments yield peak temperatures lower than that of Control (+) (P < 0.05), and the TG treatment produces a peak temperature higher than that of Control (-) (P < 0.05). Because higher thermal stability of myosin corresponds to a higher WHC in the meat emulsion, TG treatment might have high WHC in this study [27]. However, comparison with peak 2 reveals that only ΔH differs significantly (P < 0.05), not the peak temperature (P > 0.05). The control groups exhibit the lowest ΔH , and the 0.15 treatment produces the highest ΔH (P < 0.05). These findings demonstrate that higher energy is needed to denature sarcoplasmic protein when grafted MP is added. However, the addition of TG and more grafted MP slightly decreases ΔH of the sarcoplasmic protein (P < 0.05). Therefore, the addition of phosphate reduces the enthalpy of the sarcoplasmic protein, and more energy is needed to denature the sarcoplasmic protein in the meat emulsion with the 0.15 treatment. Phosphate can inhibit the hydrophobic interaction, and its addition results in a decrease in the enthalpy during heat denaturation [28]. In addition, the TG cross-linked between proteins and enthalpy can decrease because protein-protein networking already occurred [29]. However, when protein is glycated, the exposed surface hydrophobicity of the protein can be decrease because of glycated sugars with hydrophobic residue on protein [9]. The decreasing in exposed hydrophobic amino acid residue such as tryptophan might be a reason of increased enthalpy of 0.15 treatment [30].

3.1.2. Rheological characteristics

The apparent viscosity can be used as an index indicating the degree of internal friction or flow resistance of the meat emulsion [31]. Fig. 1(a) shows the apparent viscosity of the meat emulsions prepared with grafted MP with palatinose. The apparent viscosity tends to decrease with increasing shear rate, confirming that all meat emulsions undergo shear thinning behavior [32–34]. The apparent viscosities of Control (+), the phosphate-added meat emulsion, and the meat emulsions prepared with grafted MP with grafted MP with grafted to the WHC [33,35]. Crosslinking between proteins by phosphate and transglutaminase might increase water retention capacity and viscosity [36,37]. The increased in the apparent viscosity of grafted MP seems to be that the carbohydrate component increases the solubility and water retention of the conjugate [38].

However, the apparent viscosity with the 0.30 treatment is lower than that with the 0.15 treatment, which may be due to the biochemical complexity of the protein. Protein and a limited degree of hydrophilic sugar residue can bind via the Maillard reaction, reducing the protein self-association tendency and increasing solubility [10]. On the other hand, excessive cross-linking can lead to protein reduction and reduced solubility, which will decrease the viscosity [10,39].

Fig. 1 (b) presents the results of amplitude sweep tests conducted using an oscillatory rheometer to investigate the effects of grafted MP with palatinose on the viscoelastic properties of the meat emulsions. Through the point at which G' decreases, the limit of the LVE regions of all samples is between the shear strain values of 1% and 10%. The fact that G' is higher than G'' in the LVE region confirm that all samples have gel-like structures, and similar results were reported following previous studies using meat emulsions [32]. G' of the meat emulsions containing grafted MP with palatinose or treated with transglutaminase is higher than that with untreated MP. In general, high-viscosity gels are known to have high water retention, elasticity, and emulsification abilities [32,39], and these characteristics were confirmed to be consistent with the previous results of our study.

Dynamic viscoelasticity measurement can reveal gel matrix formation in a non-destructive manner [31], and in this study, the characteristic G' and G" patterns according to temperature change (10–95 °C) were recorded at 0.5% shear strain (within the LVE region). G' and G" started to rise rapidly from approximately 32–48 °C in all meat emulsions. The initiation of such gelation and protein network structure formation may be due to the unfolding and cross-linking of heavy meromyosin in myosin filaments, and similar results have been reported previously [31]. At 48–52 °C, the rate of increase in G' temporarily decreased due to the increased mobility resulting from the modification and disruption of bonds between proteins [40]. Subsequently, second increases in G' and G" occurred at approximately 60 °C. The maximum G' value was observed near 68–76 °C, and it was confirmed that the G' value resulting from glycation treatment with palatinose or transglutaminase was higher than that of Control (–). At temperatures above 72 °C, most of the myosin was unfolded and aggregation could occur due to an increase in hydrophobic interbonding between proteins [41]. Moreover, glycosylation and enzymatic treatment would have further enhanced the interactions between the cross-linked proteins, enabling the aggregation of unfolded protein molecules to form an ordered elastic gel network [31,37].

3.1.3. pH and instrument color

The pH of meat products has an critical impact on quality characteristics of meat products and it is that changes in pH of meat

proteins by phosphate could improve quality characteristics of meat product [25]. The significant effect of phosphate and grafted MP was shown in Table 3. Control (–) had the lowest value and Control (+) and TG groups had the highest value (P < 0.05). These increase might be due to role of phosphate and changes in protein structure with protein-protein interaction induced by TG [42,43]. When added grafted MP, pH value of raw meat batter increased slightly when compared with Control (–) (P < 0.05). Therefore, the addition of grafted MP also helps enhance quality characteristics of meat products.

The instrument color value of raw meat batter was shown in Table 3. When compared CIE L^* value, addition of phosphate and 0.15% of grafted MP had no significant effect (P > 0.05). However, 0.30% of grafted MP had lower value in raw meat batter (P < 0.05). The CIE L^* value showed the lightness of meat products and light scattering and own color characteristics of ingredients and additives has significant effect on lightness of meat products [44]. In CIE a^* , significant difference was observed by phosphate addition and grafted MP concentration (P < 0.05). The highest value was observed in Control (+) and the lowest value observed in 0.30 treatment included TG treatment (P < 0.05). When the ΔE is less than 2, color difference can be recognized through close observation by human eye and the different samples which had ΔE among 2–10 can be perceptible at a glance [45]. In conclusion, the color difference with Control (+) may be more evident compared to 0.30% of grafted MP addition before cooking.

3.2. Physicochemical properties of cooked meat emulsion

3.2.1. pH and instrument color

The pH value of cooked meat emulsion was shown in Table 3. The heat disconnected ionic bonds and hydrogen bonds and hydrophobic interaction included disulfide bonds was increased with exposure of hydrophobic residue [41,46]. These heat induced changes in protein structure could change the pH value of general protein [47]. Similar with raw meat batter, pH of Control (–) was the lowest (P < 0.05) and that of Control (+) and TG groups was the highest (P < 0.05). However, 0.30 treatment had lower pH value than 0.15 treatment (P < 0.05). The grafted MP was already modified and changes in their pH characteristics could be less than raw meat after cooking. Therefore, the addition of excessive grafted MP could decrease pH of meat emulsion.

After cooking, the color difference value of meat emulsion was less than raw meat batter. 0.15 treatment groups showed the lowest color difference (P < 0.05) and their value was under 1; not perceptible by the human eye [45]. In addition, Control (-) and 0.30 treatment groups had similar value (P > 0.05) and their value was under 2. Therefore, 0.15% addition of grafted MP had similar effect on color value with phosphate. Color changes in processed meat products can be caused by a variety of factors. Oxidation of meat pigments can be one of these causes, as can non-enzymatic browning reactions during heating. In this study, it is likely that the protein and sugar present in the meat during heating caused a non-enzymatic browning reaction, which almost eliminated the difference in color between the treatment and control groups [48].

3.2.2. Cooking loss, emulsion stability, and WHC

The changes in protein structure and their interaction induced moisture loss during heating [3]. Addition of phosphate improve immobilization of the water and fat in meat products and cooking loss could be decreased [4]. According to Xu, Dong [7], surface hydrophobicity of glycated MP was higher than native MP and emulsifying capacity of MP was increased after glycation. In addition, attachment of hydrophilic ingredient could enhance WHC of final meat products [49]. The effect of grafted MP as phosphate replacement on cooking loss, emulsion stability, and WHC was given in Table 4. The highest cooking loss was observed in Control (-) and 0.15TG treatment (P < 0.05). Although the addition of TG could enhance protein-protein interaction, the excessive linked protein-protein could lose their WHC because of the reduced space between protein by protein-protein linking [16]. However, the addition of grafted MP showed positive effect on decreasing of cooking loss. Although Control (+) had the lowest cooking loss (P <

Table 3

Effects of grafted myofibrillar protein with palatinose on pH and instrument color value of meat emulsion.

Traits	Control (–) ^a	Control (+)	0.15	0.15TG	0.30	0.30TG
Raw meat bo	utter					
pН	$5.72\pm0.03^{\rm c}$	$5.93\pm0.03^{\rm a}$	$5.75\pm0.02^{\rm b}$	$5.92\pm0.01^{\rm a}$	$5.76\pm0.01^{\rm b}$	$5.91\pm0.01^{\rm a}$
CIE L*	79.65 ± 1.40^{a}	$78.09 \pm \mathbf{2.38^a}$	78.79 ± 1.85^{a}	$80.02\pm0.67^{\rm a}$	$75.77\pm0.84^{\rm b}$	75.99 ± 1.39^{b}
CIE a*	$3.58\pm0.17^{\rm b}$	$3.88 \pm \mathbf{0.43^a}$	$3.43\pm0.18^{\rm bc}$	$3.56\pm0.24^{\rm b}$	$3.21\pm0.10^{\rm cd}$	$2.99\pm0.21^{\rm d}$
CIE b^*	$12.46\pm0.48^{\rm a}$	$11.79\pm0.44^{\rm b}$	$11.87\pm0.23^{\rm b}$	$10.95\pm0.17^{\rm c}$	$10.96 \pm 0.26^{\rm c}$	$10.55\pm0.34^{\rm c}$
ΔE	$1.39\pm0.23^{\rm b}$	b	$1.19\pm0.34^{\rm b}$	$1.79\pm0.55^{\rm b}$	$3.06\pm0.77^{\rm a}$	3.28 ± 0.64^{a}
Cooked mea	t emulsion					
pН	$5.85\pm0.02^{\rm d}$	$6.06\pm0.01^{\rm a}$	$6.00\pm0.06^{\rm b}$	$6.10\pm0.01^{\rm a}$	$5.94\pm0.02^{\rm c}$	$6.09\pm0.01^{\rm a}$
CIE L*	$80.63\pm0.45^{\rm d}$	$81.75\pm0.52^{\rm c}$	82.84 ± 0.61^{a}	$81.96\pm0.48^{\rm bc}$	82.35 ± 0.40^{ab}	$82.78\pm0.36^{\rm a}$
CIE a*	$1.35\pm0.07^{\rm c}$	$1.63\pm0.13^{\rm a}$	$1.19\pm0.14^{\rm d}$	$1.52\pm0.08^{\rm ab}$	$1.43\pm0.07^{\rm bc}$	$1.48\pm0.10^{\rm b}$
CIE b^*	11.05 ± 0.15	10.95 ± 0.12	11.08 ± 0.42	11.26 ± 0.13	11.12 ± 0.15	11.03 ± 0.16
ΔE	1.22 ± 0.16^{a}	-	0.61 ± 0.14^{b}	$0.42\pm0.06^{\rm b}$	1.08 ± 0.26^{a}	1.04 ± 0.07^{a}

All values are mean \pm standard error of three replicates (n = 3).

^{a-d} Means within a row with different letters are significantly different (P < 0.05).

^a Control (–): no phosphate/grafted protein added, control (+): 0.15% phosphate added, 0.15: 0.15% grafted protein added, 0.15TG: 0.15% grafted protein and 1% transglutaminase were added, 0.30: 0.30% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added.

^b Color difference (ΔE) was calculated according to CIE 76 color difference calculation.

Table 4

Effects of grafted myofibrillar protein with palatinose on cooking loss, emulsion stability, and water holding capacity of meat emulsion.

Traits		Control (–) ^a	Control (+)	0.15	0.15TG	0.30	0.30TG
Cooking loss (%) Emulsion stability	Separated total fluid (%) Separated fat (%)	$\begin{array}{c} 24.92\pm 0.95^{a} \\ 20.52\pm 1.13^{b} \\ 1.12\pm 0.16 \end{array}$	$\begin{array}{c} 17.03 \pm 0.38^{e} \\ 16.18 \pm 1.10^{c} \\ 1.15 \pm 0.16 \end{array}$	$\begin{array}{c} 19.59 \pm 0.11^{d} \\ 18.78 \pm 0.49^{bc} \\ 1.02 \pm 0.01 \end{array}$	$\begin{array}{l} 24.54\pm 0.65^{ab}\\ 21.94\pm 0.68^{b}\\ 1.00\pm 0.01\end{array}$	$\begin{array}{c} 22.91 \pm 0.53^{bc} \\ 28.34 \pm 1.32^{a} \\ 1.00 \pm 0.03 \end{array}$	$\begin{array}{c} 22.70 \pm 0.27^c \\ 22.22 \pm 0.84^b \\ 1.00 \pm 0.01 \end{array}$
Water holding capacity (%)		66.14 ± 0.77^{d}	69.37 ± 0.31^c	71.31 ± 0.20^b	73.22 ± 0.49^a	70.28 ± 0.20^{bc}	$71.07 \pm 0.43^{\mathrm{b}}$

All values are mean \pm standard error of three replicates (n = 3).

 $^{a-e}$ Means within a row with different letters are significantly different (P < 0.05).

^a Control (–): no phosphate/grafted protein added, control (+): 0.15% phosphate added, 0.15: 0.15% grafted protein added, 0.15TG: 0.15% grafted protein and 1% transglutaminase were added, 0.30: 0.30% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added.

0.05), 0.15 treatment was followed and 0.30 treatment groups showed improved cooking loss value compared with Control (–) (P < 0.05). When compared emulsion stability, the level of exudative fat layer was not significantly different among controls and treatments (P > 0.05), even Control (+) and 0.15 treatment had no significant difference (P > 0.05). However, 0.30 treatment showed higher value in separated total fluid than Control (–) (P < 0.05). The lowest WHC was observed in the Control (–) and the highest WHC was observed in the 0.15TG treatment group (P < 0.05). Although the 0.30 treatment produced a value similar to that of the Control (+) (P > 0.05), the addition of grafted MP increased the WHC of the cooked meat emulsion. This result could be due to increased hydrophilic residue in the grafted MP. However, the excessive addition of grafted MP is not suitable for replacing phosphate in meat emulsion because it can induce high cooking loss and unstable meat emulsion.

3.2.3. TBARS value

Antioxidant action is also a major role of phosphate in meat products [4]. The TBARS value was measured in this study, and the results are shown in Fig. 2. The addition of grafted MP inhibits lipid oxidation of the meat emulsion, and the 0.15TG treatment produces the lowest TBARS value (P < 0.05). This finding could be due to the high water retention capacity of the grafted MP protein. The large amount of free water could accelerate lipid oxidation, and the high WHC of grafted MP could inhibit radical lipid oxidation [50]. However, the highest value was observed with the 0.30TG treatment, followed by Control (-) (P < 0.05). These findings could be due to the excessive interactions of proteins by TG because an unstable protein structure or an excessive amount of aggregated protein could result in high lipid oxidation during heating [51]. According to Fig. 1 (c), the storage and loss moduli with the 0.30TG treatment are higher than those corresponding to the treatments, even Control (+). Excessive protein interactions can reduce the moisture content between the proteins and increase lipid oxidation.

3.2.4. TPA

The TPA of the meat emulsion containing grafted MP is shown in Table 5. The lowest hardness is observed for Control (–), and the highest value is observed with both TG treatment (P < 0.05). The springiness is also the highest in the case of TG treatment (P < 0.05). As mentioned above, the excessive addition of grafted MP is not suitable for stable meat emulsion. This conclusion can also be drawn from the present results. Specifically, the 0.30 treatment produces lower cohesiveness, gumminess, and chewiness than the 0.15 treatment (P < 0.05), and springiness and cohesiveness similar to those of Control (+) are also observed with the 0.15 treatment. The WHC enhancement in meat products due to fiber or whey protein could decrease the hardness and springiness by interrupting the formation of a stable three-dimensional network of MP [52]. When TG is added, the hardness, springiness, cohesiveness, gumminess, and chewiness increase, and the highest values are observed in 0.30TG. However, the high cooking loss and lipid oxidation could be obstacles to combining TG with grafted MP to obtain a phosphate replacement. Furthermore, if the difference in properties from Control (+) is large, it may not be a good quality material as it may have a different texture than existing foods [53]. Since the 0.15



Fig. 2. Effects of grafted myofibrillar protein with palatinose on lipid oxidation of meat emulsion.¹⁾ Control (–): no phosphate/grafted protein added, control (+): 0.15% phosphate added, 0.15: 0.15% grafted protein added, 0.15TG: 0.15% grafted protein and 1% transglutaminase were added, 0.30: 0.30% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added. ^{a-e} different letters on top of column meant significant difference among treatments (P < 0.05).

Table 5

Effects of grafted myofibrillar protein with palatinose on texture profile analysis of meat emulsion.

Traits	Control (–) ^a	Control (+)	0.15	0.15TG	0.30	0.30TG
Hardness (kg)	$1.61 \pm 0.09^{\rm d}$	$2.22\pm0.12^{\rm c}$	$2.63 \pm \mathbf{0.21^{b}}$	$\textbf{4.17} \pm \textbf{0.27}^{a}$	2.50 ± 0.16^{bc}	3.84 ± 0.10^{a}
Springiness	$0.58\pm0.03^{\rm c}$	$0.68\pm0.02^{\rm b}$	$0.66\pm0.02^{\rm b}$	$0.77\pm0.01^{\rm a}$	$0.64\pm0.02^{\rm b}$	$0.77\pm0.02^{\rm a}$
Cohesiveness	$0.50\pm0.02^{\rm e}$	$0.59\pm0.01^{ m c}$	$0.60\pm0.03^{\rm c}$	$0.64\pm0.02^{\rm b}$	$0.54\pm0.02^{\rm d}$	$0.68\pm0.01^{\text{a}}$
Gumminess (kg)	$0.79\pm0.06^{\rm d}$	$1.30\pm0.07^{\rm c}$	$1.54\pm0.10^{\rm b}$	$2.64\pm0.13^{\rm a}$	$1.34\pm0.08^{\rm c}$	$2.59\pm0.06^{\rm a}$
Chewiness (kg)	0.46 ± 0.05^{d}	0.88 ± 0.05^{bc}	1.01 ± 0.07^{b}	$\textbf{2.01} \pm \textbf{0.10}^{a}$	0.86 ± 0.08^{c}	1.99 ± 0.06^{a}

All values are mean \pm standard error of three replicates (n = 3).

^{a-e} Means within a row with different letters are significantly different (P < 0.05).

^a Control (-): no phosphate/grafted protein added, control (+): 0.15% phosphate added, 0.15: 0.15% grafted protein added, 0.15TG: 0.15% grafted protein and 1% transglutaminase were added, 0.30: 0.30% grafted protein added, 0.30TG: 0.30% grafted protein and 1% transglutaminase were added.

treatment had a similar texture to the Control (+), it is considered appropriate as a grafted MP addition for phosphate replaced sausage manufacturing based on texture.

4. Conclusion

This study was conducted to investigate the replacement of phosphate with grafted MP with palatinose. Replacing phosphate with denatured sarcoplasmic protein decreased the thermal stability of myosin but increased the enthalpy. The rheological properties were improved with the addition of grafted protein and transglutaminase. The pH increased with the addition of grafted protein after cooking, but no color difference could be perceived by the human eye. The cooking loss, emulsion stability, WHC, and TBARS value were improved compared with those of the phosphate-free meat emulsion. However, the excessive addition of grafted protein decreased the quality of the meat emulsion. In conclusion, 0.15% addition of grafted protein is more suitable than 0.30% when replacing phosphate, and the addition of transglutaminase is not recommended.

Data availability statement

Data will not be required for this article.

CRediT authorship contribution statement

Tae-Kyung Kim: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. Yun Jeong Kim: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. Min-Cheol Kang: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. Ji Yoon Cha: Formal analysis. Yea-Ji Kim: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. Yoo-Jeong Choi: Formal analysis, Data curation. Samooel Jung: Writing – original draft, Validation, Formal analysis, Data curation. Yun-Sang Choi: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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