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Quality enhancement of frozen chicken meat marinated with phosphate alternatives

Abstract

The effects of phosphate alternatives on meat quality in marinated chicken were investigated with the application of chilling and freezing. Breast muscles were injected with solution of the green weight containing 1.5% NaCl and 2% sodium tripolyphosphate (STPP) or phosphate alternatives. Treatment variables consisted of control (-) (no phosphate), control (+) (0.3% STPP), 0.3% prune juice (PJ), 0.3% oyster shell (OS), 0.3% nano-oyster shell (N-OS), and 0.3% yeast and lemon extract (YLE) powder. One-third of the meat samples were stored at 4℃ for 1 d, and the rest of the meats were kept at -18℃ for 7 d. In chilled meat, a lower drip loss was noted for control (+) and YLE, whereas higher cooking yield in YLE compared to all tested groups. Compared with control (+), the other treatments except PJ showed higher pH, water holding capacity, moisture content, lower thawing and cooking loss, and shear force. Natural phosphate alternatives except for PJ, improved the lightness (L*) value compared to control (-), and upregulated total protein solubility. However, phosphate alternatives showed similar or higher oxidative stability and impedance measurement compared to control (+), and an extensive effect on myofibrillar fragmentation index. A limited effect was observed for C*, h°, and free amino acids in treated meat. Eventually, the texture profile attributes in cooked of phosphate alternatives improved except for PJ. The results indicate the high potential use of natural additives could be promising and effective methods for replacing synthetic phosphate in chilled and frozen chicken with quality enhancement.

Keywords: chicken meat quality, marination, phosphate, natural phosphate alternatives

Introduction

Freezing meat is a wonderful way to preserve its quality and keep it fresh for a long period and is frequently applied owing to the quality of meat supply in the market (Ali et al., 2016). Moreover, this preservation technique affords a great logistical benefit required for the export of meat (Fagan et al., 2003). In general, however, the quality of frozen meat is closely linked with the freezing and thawing processes. Because of the high amounts of oxidation catalysts (such as myoglobin and iron) and lipids in poultry meats, they are vulnerable to oxidative processes as well as protein oxidation which affects flavor, texture, nutritional value, and color (Asghar, 1988; Xiong, 2000). It is well defined that freezing and thawing rates communally have a crucial effect on tissue damage and water loss resulting due to the formation of small ice crystals (freezing) and drip loss (thawing). During the thawing of meat or products, it undergoes damage by a series of physical and chemical changes (Kalichevsky et al., 1995). The decline of water-holding capacity (WHC), which manifests as loss of exudate (drip loss) during thawing, is a severe hazard to the quality of frozen meat. The formation of ice crystals draws water from intracellular spaces into intercellular spaces, resulting in excessive moisture loss upon thawing, which affects the sensory profile and tenderness of meat (Ngapo et al., 1999).

However, studies have demonstrated that the alkali-aided process is substantially effective in processed meat since oxidation and color problems are reduced and protein functionality is superior (Abdollahi et al., 2020). Alkaline phosphate is also used as a general meat enhancer because it enhances texture by dissociating actomyosin and increasing water holding capacity, as well as inhibiting lipid oxidation and microbiological growth by chelating metal ions in meat (Sebranek, 2009).

Notably, at the same time, it is also important to establish the techniques for the long-term preservation of meat without impairing the quality by using marinades (e.g., sodium chloride, phosphate, calcium chloride, etc.) in meat. They are used in meat products to increase freeze-thaw stability, keep water holding capacity, limit lipid oxidation, reduce cooking loss, and maintain color (Alvarado and McKee, 2007, Abdollahi et al., 2020, Sebranek, 2015).

The salt and phosphates in the marinade solution improve the water holding capacity of meat, meat tenderness, juiciness, and enhance the raw and cooked product yield (Alvarado and McKee, 2007; Hamm, 1961). Adding phosphates and salts to meat products has been shown to boost ionic strength, which improves protein functioning and helps to bind moisture to meat proteins, preventing weight loss during cooking and storage (Sebranek, 2015). This leads to an alteration in pH and extraction of myofibrillar proteins consenting to bind the phosphate (Offer and Trinick, 1983). Based on the ingredients unified into the meat, marination can also be used to improve flavor and extend product shelf-life (Smith and Acton, 2000). Although the maximum acceptable level of phosphate in the final processed meat and poultry products is 0.5% (US Department of Agriculture and Service, 2017), it is typically used at lower levels (0.3-0.4%) in the meat industry (Sebranek, 2009).

New trends, on the other hand, are demanding meats with more natural ingredients as clean label processed meat, citing superior taste, nutritional content, long-term health advantages, and product freshness as reasons (Sloan, 2003). This consumer demand for more natural meats is especially noticeable in chicken, which currently accounts for the majority of the organic meat market in the United States (O'Bryan et al., 2012). To meet consumer demand, unlike phosphate, several ingredients have been investigated as potential natural alternatives of synthetic phosphate (plum powder, herbs, winter mushroom, oat fiber, dried vinegar, whey protein, whey protein concentrate, oyster shell calcium, milk calcium powder, marine algae calcium powder, yeast extract, etc.) in meat and meat products due to consumer negative perception (Choe et al., 2018; Morris et al., 2019).

However, as a candidate of phosphate alternatives, nano-oyster shell calcium powder and yeast, and lemon extract are quite new candidates for synthetic phosphate in our marination study. Unlike oyster shell, and nano-oyster shell improved meat and meat products with its alkaline characteristics. Yeast extract is a natural substance that is abundant in high-quality proteins and contains a variety of amino acids, carbohydrates, vitamins, and minerals a common flavor enhancer such as monosodium glutamate (MGA) only a single substrate additive (Vidal et al., 2020). It is not thoroughly invested that how freezing and thawing affect the quality of the chicken meat treated with prune juice, oyster shell,

nano-oyster shell, and yeast and plant extract powder by injection marination in chicken meat. Thus far, however, there have been no reported studies regards to frozen/thawed meat with the abovementioned marinades ingredients in terms of meat quality and functionality. Hence, the main objective of this research is to determine the quality characteristics of clean label marinades treated meat as a replacement of phosphate under the storage conditions of chilling and freezing. Therefore, this study aimed to determine the optimal and superior methods to prolong the storage quality of frozen meat by using natural phosphate alternatives.

Materials and Methods

Sample collection and treatments

Broiler breast meat (120 to 220 g per fillet) was obtained from a local poultry processor 24 h after deboning. Samples were stored at 4℃ and marinated within 24 h after arrival. Immediately after marination, hanged the marinated sample at 20℃ for 20 min for good uptake of marinades. Marinade formulations were targeted to include NaCl (Sodium chloride, Beksul, Korea) and STPP (Sodium tripolyphosphate, Esfood, Co. Ltd, Korea) and phosphate alternatives treatment and water on a finished product basis (FPB). As a natural source of phosphate alternatives used in this study were prune juice contained 17% sorbitol (powder form, Saeyang FL, Co. Ltd, Korea), oyster shell calcium powder contained 39% calcium and magnesium, sodium, iron, and potassium <0.1% (JK Biochem Co. Ltd.), nano-oyster shell calcium powder contained 35% calcium, 60% magnesium oxide, 0.25% Vit-D3 and natural and functional ingredients 0.2% (Apexel Co. Ltd, Korea), yeast and lemon extract contained 95.1% yeast extraction powder and 4.9% lemon extraction powder (PRS-PHR, Spain). Unlike STPP treated marinade, all the natural phosphate alternatives were maintained with a same ratio in the brine while NaCl was common to all treatments in the brine solution. Treatment variables consisted of control (-) (no phosphate), control (+) (0.3% STPP), 0.3% prune juice (PJ), 0.3% oyster shell (OS), 0.3% nano-oyster shell (N-OS), and 0.3% yeast and lemon extract (YLE) powder based on the finished products. Formulations for each marinated chicken treatment are included in Table 1. Three independent

conditions (chilled at 24 h, frozen/thawed at 1 and 3 d respectively after 7 d of freezing) were considered. The experiment was conducted on 6 separate occasions, such that there were 6 independent replications of the 6 treatments. For each treatment a total of 10 breast fillets were marinated, subsequently, 60 of the total breast fillets were marinated in each condition. A total of 180 breast fillets were marinated for the 6 treatments. One-third of the samples were kept at a cool condition at 4℃ for 1 d for chilling and the rest two-third were stored in a freezer at -18℃ for seven d for freezing. Before freezing, the samples were covered with plastic film and were held in a cold room (4℃) for 24 h to allow for equilibration of the solution. Before analyzing the parameters of the frozen meat, it was thawed at 4℃ in a cold room overnight. The experiments were replicated three times.

Drip loss

Marinated chilled meats were used to determine drip loss by individually weighed, packed, and storing at 4 ± 1 ℃ for 24 h. Then, the difference in meat weight before (W1) and after 24 h storage (W2) was recorded and expressed as drip loss percentage. Drip loss (%) = [(W1 –W2)/W1] × 100.

Cooking yield cooking loss

The cooking loss for treated chilled and frozen/thawed meat was determined as the percentage weight loss after cooking in an electric grill with double pans (Nova EMG-533, 1,400 W, Evergreen enterprise, Yongin, Korea) for 60 s until it reached the internal temperature of the meat sample at 72℃ with the standardized of cuts sample (30×50×10 mm). Shortly, for cooking loss, samples with an average weight of 100 ± 5 g covered with polypropylene bags were heated for 30 min in a water bath at 95℃ and cooled for 30 min with ice-cool water. Recorded the weight before and after heating and cooling and calculated the yield percentage. Yield (%) = (weight after heating and cooling/initial weight) × 100.

pH

The pH values of marinated chilled and frozen/thawed meat were measured by blending 2 g of the meat sample and was mixed with 18 mL of distilled water then homogenized at 15,000 rpm for 30

s using a homogenizer (Polytron PT 10-35 GT, Kinematica., Switzerland). Then the samples were filtrated by filter paper (110 mm HM filter paper, Korea) and the pH value of filtrated samples was measured at room temperature using a pH meter (Seven Excellence™, METTLER TOLEDO, Switzerland).

Water holding capacity (WHC)

The water holding capacity (WHC) of marinated meat at chilled and frozen/thawed conditions was measured following the method described by Uttaro et al. (1993) with minor modifications. In short, 5 g of the meat sample from each treatment was centrifuged at 4℃ for 10 min at 1,000 rpm using a centrifuge (Combi 514-R, HANIL, Korea) and the weight of the meat sample was measured.

Moisture content

The moisture content of marinated meat under three different conditions was measured by the methods of AOAC, (2000) and, 3 g of minced meat sample was dried in a dry oven at 104℃ for 24 h. The difference in mass between before and after drying was measured.

Thawing loss

Thawing loss was calculated as a percentage of weight loss before and after thawing processes. The thawing loss of the samples was calculated according to the formula described by Ersoy and Ö zeren (2009). Thawing loss % = frozen sample weight - thawed sample weight/frozen sample weight × 100.

Meat color

Color values like lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*) of treated meats were determined utilizing a colorimeter (Konica Minolta CR-410, Tokyo, Japan). The standard white plate (Y=86.8; x=0.3156; y=0.3225) was employed for calibrating the colorimeter, and each patty was measured twice. The measurement for chroma (C*) value and hue angle (h°) value was carried out utilizing two equations of {(a* + b*)1/2} and {$\tan^{-1}$(b*/a*)}, respectively. At least six scans were taken per treatment on the cut after blooming (25℃ for 30 min) developed.

2-thiobarbituric acid reactive substances (TBARS) analysis

The TBARS value of the marinated chilled and frozen/thawed meat samples was analyzed following the procedure of Ahn et al. (1998). In brief, 5 g of minced meat sample was homogenized by adding 50 μL of butylated hydroxytoluene (7.2 % in ethanol, w/v) and 15 mL of distilled water in a 50 mL test tube. After homogenization, 2 mL of homogenized meat sample was transferred to a disposable test tube, and added 4 mL of thiobarbituric acid/trichloroacetic acid (TCA) solution (20 mM TBA/15 %, w/v). After the mixture was thoroughly shaken, the mixture was allowed to stand for 15 min in a constant temperature water bath at 90℃ for the development of color and cooled for 15 min. Then the supernatant was centrifuged at 3,000 rpm for 15 minutes at 4℃ using a centrifuge and the absorbance was measured at 531 nm using a spectrophotometer (T60 UV VIS Spectrophotometer, Oasis Scientific Inc, USA). 1 mL of distilled water and 2 mL of TBA/TCA solution were mixed as blank. The amount of TBARS is expressed in mg of malondialdehyde (MDA) per kg of the meat sample.

Protein solubility

The solubility of the sarcoplasmic and total (sarcoplasmic + myofibrillar) proteins from chilled and frozen/thawed marinated meat were determined according to the method as described by Joo et al. (1999) with slight modifications. Sarcoplasmic proteins were extracted from 1 g muscle from each treatment using 20 mL of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). The samples were minced, homogenized, and then left on a shaker at 4℃ overnight. Samples were centrifuged at 3,000 g for 15 min and protein concentration in the supernatants was determined by the Biuret method. Total protein from marinated meat was extracted excising 1 g of muscle using 20 mL of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2). The same events for homogenization, shaking, centrifugation, and protein determination were used as mentioned above. Myofibrillar protein concentrations were obtained by the distinction between total and sarcoplasmic protein solubility. The protein solubility was expressed as mg of protein per g of meat.

Myofibrillar fragmentation index

The myofibrillar fragmentation index (MFI) was determined by a modification of the method by

Hou et al. (2014). Briefly, 2 g samples from each treatment were homogenized with a homogenizer (Polytron PT 10-35 GT, Kinematica., Switzerland) at 15,000 rpm for 30 s at 4 ± 2℃ in 20 mL ice-cold buffer (100 mM KCl, 20 mM K_2HPO_4, 1 mM EGTA, 1 mM $MgCl_2$, and 1 mM NaN_3, pH 7.0). The homogenates were centrifuged using a centrifuge (Combi 514-R, HANIL, Korea at 1000 g for 15 min at 4 ℃ and the supernatant was discarded. The pellets were homogenized in 20 mL of homogenizing buffer and centrifuged, and the supernatant was discarded again. The resulting pellets were then resuspended in 5 mL of homogenizing buffer and filtered through a polyethylene strainer (200-mesh) to remove the fat and connective tissue. Then, 5 mL buffer was used to promote the passage of myofibrils through the strainer. The protein concentration of the suspension was determined by the biuret method (Gornall et al., 1949). The protein concentration was diluted to 0.5 mg/mL and measured spectrophotometrically at 540 nm using a spectrophotometer (T60 UV VIS Spectrophotometer, Oasis Scientific Inc, USA). MFI was calculated by multiplying A540 by 200.

Impedance measurements (Z)

The impedance of the samples was measured with an RCL electric bridge (630A automatic RCL meter with an adaptor PM 9542A, Philips, Germany). The distance between the rows was 3 cm while the distance was 1 cm between the pins in the same row. Proves were inserted at 2 cm in the breast meat with triplicate replications.

Warner-Bratzler shear force

The shear force values of the marinated breast meat from each treatment (cooked meat sample) were measured in a cubic form (30×50×10 mm). Subsequently, they were cut perpendicular to the longitudinal orientation of the muscle fiber using a Warner-Bratzler shear attachment on a texture analyzer (TA-XT2, Stable Micro System Ltd., Surrey, UK.). The maximum shear force value (kg.f) was taken for each sample. The test and pre-test speeds were set to 2.0 mm/s, and post-test speeds were set to 5.0 mm/s.

Texture profile analysis (TPA)

Marinated chilled and frozen/thawed meat samples with an average weight of 100 ± 5 g were cooked in a water bath at 95℃ with placed in a polypropylene bag. The internal meat temperature was monitored throughout the cooking process with a thermocouple inserted into the geometric center of the breast meat. The treatment was discontinued when the internal temperature of the sample reached 80 ± 2℃ (approximately 45 to 60 min). After cooking, samples were cooled in iced water until the internal temperature was lowered to 30℃. The cooked chicken meat was cut at a manner of 15 mm length and width 10 mm in a ridge following the distance of 20 mm from the probe and was finally stored at 4℃ until further texture analyses by using a cylindrical aluminum probe with a texture profile analyzer (TA-XT2, Stable Micro System Ltd., Surrey, UK.). The TPA parameters including hardness, cohesiveness, chewiness, gumminess, and springiness were calculated from the force-time curves recorded for each sample using the same machine mentioned above.

Fatty acid composition

The fatty acids composition of 7 d marinated frozen/thawed meat (1 d of thawing) was determined by using a slightly modified method described by O'fallon et al. (2007). After the separation of fatty acid methyl esters, the fatty acid analysis was performed using the Gas Chromatograph-Flame Ionization Detector (Agilent, 7890 series, USA) under the following conditions. The injector was split mode with a split ratio of 25:1, the temperature was 250℃, and the detector was Flame Ionization Detector (FID). High purity air, high purity H_2, and helium was used as the carrier gas. The flow rate was 40 mL/min for H_2 and 400 mL/min for air. HP-88 column (60 m × 250 μm × 0.2 mm) was used for the analysis. Fatty acids composition is expressed as a percent of meat.

Free amino acids (FAA) analysis

The FAA concentrations of marinated frozen/thawed (1 d of thawing) were determined with a slightly modified method ascribed by Hughes et al. (2002). Removing visual fat, 3 g of minced meat samples were weighed from each treatment which was mixed with 27 mL of 2 % TCA solution. The mixture was then homogenized for 1 min at 13,500 rpm/min. After homogenization, centrifuged for 15 min and filtrated by 0.45 μM membrane filter. The HPLC condition was equipped with

cation separation column (LCAK07/li), 4.6 × 150 mm; buffer change (A: pH 2.90, B: pH 4.20, C: pH 8.00); (Lithium citrate buffer solution), buffer flow rate: 0.45 mL/min, Ninhydrin flow rate: 0.25 mL/min, Column temp.:37℃ during performing the analysis. FAA content is expressed as mg/100 g of meat.

Statistical analysis

This experiment had a completely randomized design with 6 treatments with 3 different freezing/thawing conditions. All analyses were replicated three times. Analysis of variance was performed on all the variables measured using the General Linear Model procedure of SAS (2003). Data were analyzed using two-way ANOVA whereas Duncan's multiple range tests were performed to calculate significant differences between means (p<0.05). The means values and the standard error of the means (SEM) were noted.

Result and discussions

Drip loss

Drip loss of marinated chilled meats is shown in Fig. 1. The result incorporated that, among all the tested groups, compared to control (-), phosphate and phosphate alternatives tested groups had the lower drip loss. However, among the tested groups of phosphate alternatives, YLE showed a similar result to phosphate treated treatment or control (+) likely lower than all other phosphate candidates might be attenuated due to action of citric acid in YLE and its synergistic impact with yeast, which exhibited moisture barrier properties/or reduced moisture loss, resulted in lower drip losses (Khare et al., 2016). A higher drip loss was observed in PJ but was lower than control (+). Drip loss indicates a drop in water-holding capacity during thawing. Phosphate replacement treatments with oyster shell and nan-oyster shell might be attributed to protein breakdown to a greater extent, which causes water to be ejected from the intermyofibrillar gaps, causing drips (Lesiak et al., 1996). Another possible reason for having lower drip loss in control (+), and YLE was higher marination uptake during injection marination

(data are not shown) and less loss of absorbed water at storage indicated that additives added in marination for yeast and lemon extract as well as phosphate increased the muscle water absorption. Thus, in terms of synthetic phosphate yeast and lemon extract could be altered like the typical mimicking role of phosphate in marinated chilled meat.

Cooking yield

The cooking yield of marinated chilled meats is presented in Fig. 1. In comparison with phosphate-treated treatment, YLE performed with significantly higher cooking yield than other and important measurements related to WHC, and cooking loss. And even, the result demonstrates that among all the tested groups, control (-) and PJ had the lower cooking yield compared to other treatments. Apart from this, except for PJ, OS, and N-OS treatment showed a similar result to phosphate-treated treatment or control (+). Furthermore, the result of marinated cooking yields could be associated with the high WHC and water absorbed ability (Choe et al., 2009). As a result, the components in the brine may be linked to the marinating and cooking yields of chicken breast as the most effective approach for enhancing brine dispersion. The higher cooking yield replacements with yeast and lemon extract may be due to their strong protein-water interaction created during cooking as well as improved carbohydrate content (Choe et al., 2009). Thus, from this study, among the phosphate alternatives tested groups, YLE treatment is quite effective to increase the cooking yield and could be an effective yield enhancer in ready-to-cook meat.

pH measurement

The pH of marinated meat at chilled and frozen/thawed conditions is listed in Table 2. Results reveal that a higher pH value was noted in OS and N-OS compared to all treatments ($p<0.05$) in chilled and frozen/thawed meat whereas PJ led to a significantly lower pH compared to all tested groups ($p<0.05$). This finding indicates that, unlike phosphate, oyster shell and nano-oyster shell was able to shift meat pH further away from its isoelectric point, thus increasing the ionic strength in the muscles deemed an important trait in meat quality (Glorieux et al., 2017). A lower pH in JP was due to the higher content of malic acid in the prune juice power (Buchanan and Golden, 1998). Phosphate replacement

with yeast and lemon extract treatment had a higher pH than in PJ and control (-) but lower than control (+), OS, and N-OS reason might be sought in the content of a small amount of malic acid (Buchanan and Golden, 1998). The overall variation of meat pH is attributed to the ionic strength of the marinade solution that we measured (Fig. 1). Regarding the thawing period, the pH of meat tended to increase as the d of thawing increased. Generally, however, at freezing and subsequent exudates release as well as loss of water from the meat may cause an elevate in the concentration of solute that could be the decline of pH of thawed meat (Leygonie et al., 2012). But in our study thawed meat led with higher pH might be attenuated due to marinades used in the marination with different ionic strengths.

Water holding capacity (WHC)

The ability of postmortem muscle (meat) to retain water despite external forces (e.g. gravity, heating) is defined as WHC. The results found for the WHC among the tested groups are listed in Table 2. Regardless of the chilling and thawing time, except for PJ, all phosphate alternatives treated meat led with higher water holding capacity than control groups. The addition of alkaline phosphate additives during the marination of meat increased the pH and resulted in electrostatic repulsion between the or within the muscle proteins, resulting in water-holding capacity (Glorieux et al., 2017). Alkaline marinade increases the solubility of the meat protein and its ability to bind and retain water (Choi et al., 2014). Apart from the pH effect, WHC can also be increased due to a change in ionic strength. Unlike the phosphates oyster shell, nano-oyster shell, and yeast and lemon extract powder affect ionic strength by forming polyelectrolytes in water, causing electrostatic repulsion between the meat proteins, which allows more space for binding water and hence, increased WHC (Glorieux et al., 2017). However, in situ, divalent cations, notably Ca^{2+} from oyster shell sources play an important role in the interaction of muscle proteins and calcium binds to meat, reducing myofibrillar swelling and increasing extracellular space (Xiong, 1999). The yeast and lemon extract led higher WHC likely to having chelating divalent cations enable the muscle and muscle protein in hydrate resulting more interaction of proteins with extracellular space as well as humectants. A similar result of WHC observed in PJ and control might be attributed to the prune juice powder containing some pectin and sorbitol works as humectants in

retaining moisture (Decker, 1999). However, compared to thawing time 3 d thawed meats from phosphate alternatives treated meat tended to increase than 1 d of thawing meat. However, literature evidenced with loss of WHC at thawing time due to mechanically damaging the cell membrane with frequent melting during thawing in untreated meat (Ali et al., 2016). But in our study, a higher WHC was noted with the thawing time increased resulted due to the increase of pH of the treated marinades aforementioned. In addition, lipid oxidation is thought to produce alterations in protein structures, affecting the muscle's ability to store water (Lagerstedt et al., 2008). Thus, phosphate alternatives treated meat from OS, N-OS, and YLE have a profound effect in WHC and resembles as an important commercial trait.

Moisture content

A limited effect on moisture content was noted in the chilled and frozen/thawed meat (Table 2.). Apart from this, at 3 d of thawing the control (-) and PJ had lower moisture content compared to all tested groups. The differences in moisture content with phosphate alternatives were partially due to the variation in cooking loss (Choi and Chin, 2020). The lowest moisture at thawing was noted at 3 d in PJ was noted in this experiment compared to 1 d thawing and even with chilled meat was due to higher cooking loss (Choi and Chin, 2020).

Cooking loss

Cooking loss is one of the important traits in the meat processing industry. Our result demonstrates that phosphate and phosphate alternatives treatments had an extensive effect on cooking loss except for PJ (Table 2.). Results implied that, in chilled meat, N-OS and YLE had significantly lower cooking loss compared to other tested groups. The increase in ionic strength caused by the formation of polyelectrolytes in water, generating electrostatic repulsion between the meat proteins and raising WHC, can explain the decrease in cooking loss in OS, N-OS, YLE (Glorieux et al., 2017). Cooking loss was considerable in PJ preparations, most likely due to the inability to hold water because the actomyosin complex was still intact which has been noted for MFI value in this experiment (Table

6.). Regarding the d of thawing, 3 d thawed meat led to less cooking loss in this study might be due to result in more leakage of immobilizing water in the muscle surface at the greater extent of thawing.

Thawing loss

The thawing loss of the marinated chilled and frozen/thawed meat can be described as changes in the WHC of meat and is manifested in Table 2. It has been observed that thawing loss was significantly lower in all tested groups than in the control (-). As compared to control (+), YLE showed a mimicking character like phosphate treated-treatment in both d of thawing. Regarding the thawing time, thawing loss increased significantly in all tested groups whereas the highest value was noted in PJ at both d of thawing compared to phosphate and phosphate alternatives treated groups. The production of ice crystals during freezing may be the cause of water loss in frozen meat. Freeze-thawed induced the melting of ice crystals with damaging of the muscle and reduces the protein functionality related to the loss of ability to entrap water of protein result in an increase in water loss (Leygonie et al., 2012). A higher thawing loss might be attenuated with low pH having lower WHC in PJ and control (-). Thus, the application of certain phosphate alternatives led to similar (YLE) or slightly higher (OS, N-OS) but lower than control (-) resulting in frozen meat quality improved owing to processed meat processors.

Instrumental color

The color value (L*, a*, and b*), Chroma (C*), and Hue angle (h°) of the marinated chilled and frozen/thawed meat are presented in Table 3. The result indicates that compared to control (-), all treated groups improved the lightness value (L*) except for PJ in chilled meat. As a perspective of the phosphate alteration issue, unlike control (+), the OS, N-OS, and YLE also improved the lightness value due to low protein denaturation with higher pH and subsequently lightest color in PJ for lower pH leading with higher protein denaturation (Janz et al., 2005). However, 1 d thawing, L* value presented no variation among the treatments except PJ. And Also, meat from 3 d thawing had no variation among the treatments. For the redness value (a*), OS, N-OS, and YLE tended to be higher values than other treatments in both conditions was due to less myoglobin reduction with higher pH (Janz et al., 2005).

In terms of yellowness (b*), certain alternatives like OS and YLE played a mimicking role in phosphate-treated meat or control (+). Regardless of the chilling and or frozen/thawed meat, a higher yellowness (b*) was noted in PJ and was due to the color of prune juice powder used as marinade during marination. The meat from frozen/thawed conditions had a limited effect of yellowness at d of thawing in progress. However, the Chroma (C*) value of N-OS and YLE had similar like phosphate-treated group or control (+) in chilled meat. For the frozen/thawed meat, the intensity of red color or saturation index tended to be higher at 1 d thawed meat compared to 3 d thawed meats attributed as lower myoglobin denaturation as well as lower lipid oxidation. A higher (C*) value in PJ was due to the ingredient color added during the marination. However, A lower h° value has been connected to a slower red color fade (Yousuf and Srivastava, 2017) and the discoloration (h°) value was lower in OS, N-OS, and YLE compared to control (+) in chilled meat indicates lower color decline. In 3 d frozen/thawed meat, phosphate, and phosphate alternatives treatments had lower h° value indicated that additives added in marination had preventing effects on discoloring for marinated meats during frozen storage time. This might be related to lower lipid oxidation since oxidation of lipid can cause reduced discoloration (Zahid et al., 2020). The previous studies demonstrated that lipid oxidization for meat products resulted in redness degradation (Jung et al., 2012). Furthermore, the antioxidative action of phenolic compounds was demonstrated to have a protective effect for natural plant extract on discoloration for meats and meat-based products (Falowo et al., 2014). However, the higher h° value in control (-), control (+), and prune juice powder treated groups might be due to the metmyoglobin formation which is the oxidized form of myoglobin causing reduction in redness in this study (Renerre, 1990). For 1 d thawed meat, no variation was found among the treatments. But for 3 d thawed meat, compared to control (-), all treated treatments had a lower discoloration trend was observed. Regarding the thawing time, all phosphate candidate treatments tended to a reduction of discoloration in frozen meats might be a beneficial trait to the consumers result of adding natural additives with discoloration protective effects that lead to the improvement of overall meat color in meat (Falowo et al., 2014).

Lipid oxidation

Malondialdehyde (MDA) is one of the most abundant aldehydes in meat that is used as an oxidation marker and content in the meat was quantified by using the thiobarbituric acid reactive substances (TBARS) assay. The TBARS assay measures the secondary oxidation products responsible for oxidative rancidity (Turgut et al., 2016). The result demonstrates that, compared to control (-), all tested groups marinade with phosphate and phosphate alternatives led to a lower MDA value in both chilled and frozen/thawed meat. Once the alkaline phosphates had the potential to sequester metal ions, lowering oxidative rancidity, this result was expected (Feiner, 2006). However, compared to the phosphate-treated group, all phosphate alternatives treated groups showed similar or lower MDA in both chilling and frozen/thawed meat. Even, in chilled meat, PJ, and YLE led the meat with lower MDA production than in control (+) or OS, and N-OS. However, injection marination in chicken meat with OS, and N-OS containing Ca^{2+} decreased the lipid oxidation in this study. The lowered TBARS value with the OS and N-OS was due to the higher concentration of the Ca^{2+} reduce the release of Fe^{2+} bond to negatively charged lipid groups, decreasing the catalytically active Fe^{2+}, thereby, reducing the stimulating of the Fenton reaction (Van Hecke et al., 2017). Prune juice powder, mainly made from plum contained phenolic compounds but also contains some carotenoids and α-tocopherol, as well as water-soluble ascorbic acid that reduces the lipid oxidation in marinated chilled and frozen/thawed meat (Stacewicz-Sapuntzakis et al., 2001).

In relation to oxidative stability, studies by Bao et al. (2008) demonstrated an increase in pH and a decrease in oxidation meats thus improving the retail display characteristics. Overall, the pH effect on lipid oxidation in heated muscle systems appeared to be via its influence on the catalytic activities of haem and metal ions. The YLE made from yeast and citrus extract has many bioactivities compounds like phenolics, flavonoids having antioxidant properties resulting in a reduction of lipid oxidation (Ejaz et al., 2006). Unlike phosphate, yeast and lemon extract powder has chelating divalent cations that can bind with particular ions and reduce lipid oxidation. Light, pH, oxygen, oxidation duration, water activity, substrate shape, and the presence of unsaturated fatty acids are all elements that influence oxidation and their concentrations during processing or storage (Kim and Nawar, 1993). Thawed

enhanced lipid oxidation but quietly reduced the oxidation rate than control (-). Thus, thawing increased the lipid oxidation rate but unlike phosphate, oxidation the rate can be reduced by adding any of the phosphate alternatives and found effective in this study. As a result, substances with antioxidative action may help to reduce lipid oxidation in meat.

Protein solubility

The solubility of the proteins in various ionic strengths was employed as a criterion to assess meat protein functioning. Protein solubility was used in this investigation to indicate the amount of proteins that were solubilized from the samples. The solubility of the protein from the marinated meats is shown in Table 5. Result demonstrates that a replacement of phosphate with OS and N-OS treatment resulting in higher total protein solubility compared to control (+) in chilled meat. The activity of calpains is thought to be regulated by calcium-specific ions, with tropomodulin protein acting as a possible substrate for protein degradation (Li et al., 2017). This study confirmed a previous study by (Sams, 1997), which found an increase in total soluble protein, as well as myofibrillar protein solubility, was likely attributed to calcium specific effect, the ionic strength of oyster/nano-oyster shell could promote a higher protein extractability and faster tenderization effect considering the other treatments. For frozen/thawed meat, 1 d and 3 d showed a similar trend of chilled meat solubility in the treatments whereas N-OS performed better than other OS and YLE. However, regardless of the chilling and freezing conditions, PJ had the lowest solubility. In low pH with PJ, protein solubility is lower than in alkaline pH because of having lower electrostatic force (Nahar et al., 2017). After thawing, total protein solubility increased with the thawing time increased. In terms of sarcoplasmic protein solubility, a limited effect was noted in the meat where mostly N-OS implied a higher trend in both chilling and freezing conditions. The myofibrillar protein solubility in frozen/thawed meat had an extensive effect in all candidates of phosphate alternatives except for PJ for both thawing times. Phosphate dissociates actomyosin, while salt solubilizes myosin, allowing myosin to engage in protein-protein interactions (Siegel and Schmidt, 1979). Unlike phosphate, it has been demonstrated that yeast and lemon extract have a synergistic effect in actomyosin degradation and solubilizing the myosin. Due to adding

phosphate and phosphate alternatives with the combination of salt, frozen meat improved the overall protein solubility which is most important for meat emulsion, gelation as well as meat functionality.

Myofibril fragmentation index (MFI)

Myofibrillar fragmentation index (MFI) value of marinated chilled and frozen/thawed meat is manifested in Table 6. The MFI is associated with the degradation of myofibrils in the vicinity of the Z-disc throughout aging or ripening. Furthermore, endogenous proteinases influence variations in meat quality post-mortem in relation to myofibrillar protein degradation, and myofibrillar protein degradation is a significant determinant in meat softness, including sarcomere length, ionic strength, and animal characteristics. It has been demonstrated that, OS, N-OS, and YLE had higher MFI values than control (-) for chilled and frozen/thawed meat. And even OS, N-OS, and YLE performed similar or higher values compared to control (+). MFI values of thawing meat increased significantly as the thawing time increased. A complicated interaction between myofibrillar protein thick filament termed myosin and actin causes a decrease in meat tenderness. Sequestration of metal ions such as $Ca^{2+,}$ $Mg^{2+,}$ $Fe^{2+,}$ $Fe^{3+,}$ etc., which are present in meat, by condensing phosphates to form a complex is an important function of phosphates in food applications (Lampila and Godber, 2002). The binding of phosphates with Ca^{2+} and Mg^{2+} (cross-bridges in actomyosin complex) is thrown into separate actin and myosin after rigor mortis. Hence, the above-mentioned process will improve the degree of meat tenderness. The calcium ion has been shown to influence the activity of calpains (Sams, 1997), as a result, a higher calcium ion concentration promotes more calpains activity, which causes myofibrillar protein fragmentation and muscle integrity degradation. Moreover, the binding of metal ions could reduce oxidative rancidity (Feiner, 2006). In the meat proteolytic system, pH plays an important role in meat tenderization. Many researchers demonstrate that high pH meat is consistently more tender than low and intermediate of pH meat (Yu and Lee, 1986). Our data suggest that the low pH of PJ led to the higher WBSF value resulted in tough meat. The MFI result supports the shear force (kg.f) data in this study (Obanor, 2002). Our measurements revealed that the high pH group had a higher postmortem proteolytic activity compared

to others. To some extent, these results may explain why the PJ from the low pH had a higher WBSF value.

Impedance (Z) measurements

The impedance module of marinated chilled and frozen/thawed meat is presented in Table 6. It is well known that the impedance module values decreased in thawing meat as the d of thawing increased. The result showed that the impedance module of marinated chilled and frozen/thawed meat was significantly higher in control (+), OS, N-OS, and YLE than control (-). The OS, N-OS, and YLE showed similar functions to phosphate-treated treatment. The impedance of frozen-thawed grass carp reduced throughout a 10-d frozen storage period, according to previous fish investigations (Wei et al., 2017). At low frequencies, the cell membrane behaves as an insulator, similar to a capacitor (Pliquett, 2010). Fresh meat has intact cell membranes, but frozen-thawed samples have destroyed cell membranes (Leygonie et al., 2012). The disintegration of cell membranes lowers the capacitance component of biological tissues and raises the number of free electrolytes in the tissue, which enhances conductivity and lowers the impedance module (Fuentes et al., 2013). So the impedance module of marinated meat with phosphate and phosphate alternatives (OS, N-OS, and YLE) is much higher than in control (-) and the reason might be attributed to lower oxidation, less leakage of fluid during storage and processing (Table 2.), and low protein denaturation having higher pH in OS, N-OS, and YLE (Wei et al., 2017). The PJ had a similar result to control (-) caused to low pH that leading more protein denaturation as well as higher drip loss (Fig. 1.). The impedance of living tissues, on the other hand, changes significantly more slowly during frozen storage (Damez et al., 2008). During frozen storage, ice crystal development, protein denaturation, lipid oxidation, and fluid leakage from beef tissue could all contribute to the impedance module decrease Thus, the application of certain phosphate alternatives like OS, N-OS, and YLE could be used as synthetic phosphate replacers to marinated chilled or frozen/thawed meat in terms of impedance module quality enhancement.

Warner-Bratzler shear force (WBSF) and Texture profile analysis (TPA)

WBSF values, which characterize meat tenderness, depending on the structure of two main protein components of a muscle, i.e., proteins of intramuscular connective tissue and myofibrillar proteins. Table 7. lists the shear force values of marinated chilled and frozen/thawed meat. Result demonstrates that, regardless of the chilling and or frozen/thawed conditions, except PJ, OS, N-OS, and YLE greatly influenced by lowering the shear force value that is subjected to tender meat deemed an important trait for consumer preference. It was suggested by Koohmaraie et al. (1990) that this improved tenderness may be due to increased proteolysis by calpains, because calpastatin is not as active in previously frozen muscle, whereas calpains remain fully active (Koohmaraie et al., 1990). Therefore, improvement in tenderness by Ca-rich marination apparently derives from increased calpain proteolysis, because the addition of exogenous Ca^{2+} activates the calpain present, which is reflected by a decrease in calpastatin activity (Koohmaraie et al., 1990). The decrease in calpastatin activity seemed to allow greater proteolysis by the calpains with the application of Ca^{2+}. In addition, calpastatin activity decreased with freezing, which enhanced the effects of marination on tenderness. It is also possible that freezing ruptured cell membranes, allowing more Ca^{2+} to enter the muscle cell (Koohmaraie et al., 1990). Unlike phosphate, YLE has a synergistic effect on the solubilization capacity of actomyosin resulting in more degradation of protein and also which boosts the water retention of meat in led to lower shear force or tender meat (Vidal et al., 2020). In short, thawing decreased the shear force value as the d of thawing increased but increased tenderness was noted like phosphate-treated meat (Hergenreder et al., 2013).

Similarly, texture profile parameters (hardness, cohesiveness, chewiness, and gumminess) of marinated meats exhibited a similar trend to the shear force and presented in Table 7. This decreasing trend was similar to that of shear force as moisture permeability increased. Result reveals that all of the TPA attributes intensively improved in phosphate and phosphate alternatives treated tested groups compared to control (-) in chilling and freezing/thawing conditions except PJ. The interior myofibrillar structure was disrupted, resulting in a decrease in binding force between internal molecules, which could explain why cohesiveness was decreasing as moisture permeability increased. Chewiness relates

to the amount of energy required to chew solid samples, and it encompasses the samples ongoing resistance to chewing (Lepper-Blilie et al., 2016). The chewiness was found to be connected to hardness and cohesiveness, with chewiness decreasing as hardness and cohesiveness decreased in our study. Shear force, hardness, cohesion, and chewiness all had a strong link (Caine et al., 2003). Springiness is a mechanical textural property that refers to the speed and extent to which a material recovers from a deforming force (Di Monaco et al., 2008). Springiness had no significant relationship with shear force or hardness (Di Monaco et al., 2008). However, springiness in meat treated with phosphate and phosphate alternatives demonstrate good quality enhancing by adding additives in marination during processing. Regarding thawing time, 1 d thawed meat tended to be higher with springiness all treatments except OS caused more fiber swelling resulted in more intracellular space between myofilaments which perceived the juiciness of cooked meat (Smith and Acton, 2000). In general, hardness, cohesiveness, and chewiness were all linked to shear force when they had a comparable fluctuation trend, according to the findings. The disruption of the linkages between myofibrils and collagen was likely responsible for the improved springiness (Pietrasik and Shand, 2004). These results could indicate the feasibility of phosphate replacement by the oyster shell, nao-oyster shell as well as yeast and lemon extract powder.

Fatty acid composition

Fatty acid compositions of marinated meat from 7 d of frozen and at 1 d of thawing are presented in Table 8. No variation was noted among the treatments owing to total fatty acid, SFA, UFA, MUFA, DFA, UFA/SFA, AI, TI, and P/S in this study indicates that lower promoting of fat oxidation during storage time (Kim et al., 2020). A limited effect was noted for n-6, and n-3 and n-6/n-3 could be attributed as the among the treatments that may have manifested due to the composition of additives used in the marination and influence of acetyl-CoA carboxylase which catalyzes the malonyl-Co-A, which is the regulatory enzyme in fatty acid synthesis (Ohlrogge and Jaworski, 1997). Thus, we infer that treated meat with phosphate alternatives does not negatively affect the fatty acid composition of the frozen/thawed meat quality that can be stored for a long time.

Free amino acid composition

Free amino acid compositions of marinated 7 d frozen and 1 d of thawing meat are manifested in Table 9. The result from this study indicates that a limited effect was noted for free amino acid composition in treated meats. A higher total free amino acid was noted for control (-), OS, and N-OS compared to control (+), PJ, and YLE. The addition of Ca source additives (OS and N-OS) in marinated frozen meat led to increasing total free amino acids and subsequently, however, lower non-bitter/bitter amino acids compared to control (+), PJ, and YLE could be attenuated due to the proteolytic mechanism in cell regulated ATP dependent and ca-activated protease enzymes (Jurkovitz et al., 1992). The variation of total free amino acids might be accomplished with the proteolytic enzyme activities towards the specific amino acid synthesis. A lower amino acid content in phosphate-treated and yeast and plant extract-treated treatments might be saught in water absorption acts as a barrier to amino acids synthesis. Apart from this, prune juice treated treatment had lower amino acids resulted in lower pH meat resulted in a reduction of amino acid synthesis as we observed the lower myofibril fragmentation herein (Jurkovitz et al., 1992).

Conclusion

The result evidenced that the performance of natural phosphate alternative such as OS, N-OS, and YLE was effective in lowering lipid oxidation, cooking loss, shear force, and L* values, increasing pH and WHC, and providing adequate textural properties in marinated frozen chicken meat compared to control (+). In chilled meat without freezing, certain phosphate alternatives in marinated chicken meat showed superior cooking yield to control (+). OS, N-OS, and YLE evidenced similar or higher protein solubility to control (+). In terms of myofibrillar fragmentation index and impedance value, the natural phosphate alternatives performed similarly to phosphate. Such additives may be effective as an independent alternative to phosphate in the preparation of clean labels or no-artificial phosphate meat in terms of extending storage life. In contrast, the use of YLE had the most positive effects on cooking yield, drip loss, color, and texture properties that generally mimic phosphate. Therefore, the use of OS, N-OS, and YLE in refrigerated meat as well as frozen/thawed can contribute to the functional properties as a supplementary replacement for synthetic phosphates that extend the shelf life of frozen meat. Future

research should explore the effects of combinations of marinade ingredients at various levels and ratios to produce functional and high-quality meat.

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Table 1. Formulation of the "golden clean label" recipe for marination brine treated with phosphate and phosphate alternatives

Materials (%)	Treatments[1)]					
	Control (-)	Control (+)	PJ	OS	N-OS	YLE
Salt	1.50	1.50	1.50	1.50	1.50	1.50
Phosphate blend		2.00				
Prune juice			2.00			
Oyster shell				2.00		
Nano-oyster shell					2.00	
Yeast and lemon extract						2.00
Water	98.50	96.50	96.50	96.50	96.50	96.50
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1)]PJ = prune juice; OS = oyster shell; N-OS = nano-oyster shell; YLE = yeast and lemon extract

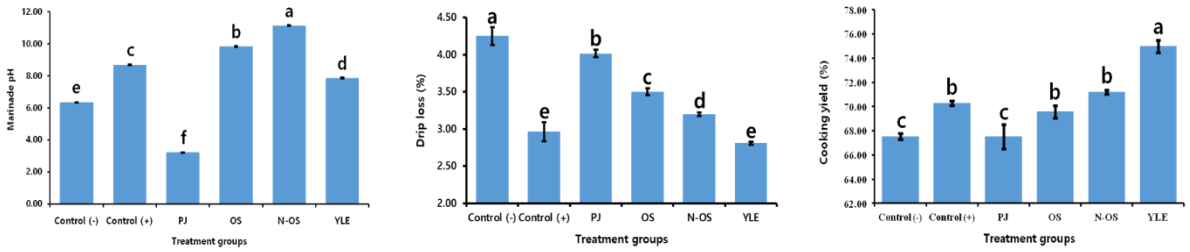

Fig. 1. Marinade pH, and drip loss and cooking yield of marinated chilled meat. Data are presented as SEM (n=36). [a-f]Mean values with different superscript letters in the different columns differ significantly ($p<0.05$). [1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YL = 0.3% yeast and lemon extract.

Table 2. Quality characteristics of chilled meats and 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Items	d of thawing	Treatments[1)]						SEM[2)]	*P*-value
		Control (-)	Control (+)	PJ	OS	N-OS	YLE		
pH	0*	5.94^{ey}	6.18^{cy}	5.87^{fy}	6.43^{bz}	6.75^{ay}	6.13^{dz}	0.011	.0001
	1	6.00^{ex}	6.19^{dy}	5.95^{fx}	6.54^{by}	6.71^{ay}	6.29^{cy}	0.016	.0001
	3	6.00^{ex}	6.32^{dx}	5.95^{fx}	6.71^{bx}	6.85^{ax}	6.39^{cx}	0.011	.0001
	SEM[3)]	0.005	0.009	0.010	0.013	0.022	0.013		
	P-value	.0001	.0001	.0001	.0001	0.0011	.0001		
WHC (%)	0*	88.92^{dz}	91.33^{cy}	88.68^{dz}	94.17^{by}	96.18^{ay}	95.59^{ay}	0.409	.0001
	1	91.34^{cy}	94.38^{bx}	90.88^{cy}	93.94^{by}	95.81^{ay}	94.96^{aby}	0.332	.0001
	3	93.23^{dx}	95.61^{bx}	94.34^{cx}	97.08^{ax}	97.13^{ax}	97.06^{ax}	0.279	.0001
	SEM[3)]	0.485	0.408	0.201	0.373	0.236	0.302		
	P-value	0.0002	.0001	.0001	.0001	0.0045	0.0008		
Moisture (%)	0*	78.06^{ax}	77.84^{ab}	77.94^{abx}	78.39^{ax}	77.89^{abx}	77.83^{abx}	0.171	0.0063
	1	77.33^{aby}	77.20^{ab}	76.73^{by}	77.27^{aby}	77.60^{ax}	77.33^{abx}	0.171	0.0417
	3	76.54^{bcx}	77.33^{a}	76.13^{cz}	76.73^{by}	77.06^{aby}	76.53^{bcy}	0.147	0.0001
	SEM[3)]	0.142	0.195	0.164	0.187	0.149	0.139		
	P-value	.0001	0.1023	.0001	.0001	0.0043	0.0014		
Cooking loss (%)	0*	23.95^{ax}	20.99^{bx}	23.28^{ax}	21.06^{bx}	19.23^{cx}	19.93^{cx}	0.264	.0001
	1	22.63^{ax}	19.86^{bx}	22.06^{ay}	19.80^{by}	18.34^{bx}	19.59^{bx}	0.431	.0001
	3	20.85^{ay}	18.45^{by}	21.11^{ay}	17.65^{bcz}	15.95^{dy}	16.96^{cy}	0.368	.0001
	SEM[3)]	0.456	0.382	0.355	0.260	0.309	0.313		
	P-value	0.0027	0.002	0.0034	.0001	.0001	.0001		
Thawing loss (%)	0*	-	-	-	-	-	-	-	-
	1	9.40^{ay}	4.43^{ey}	8.05^{by}	7.01^{cy}	5.72^{dy}	4.69^{ey}	0.140	.0001
	3	14.45^{ax}	7.87^{ex}	12.29^{bx}	11.01^{cx}	9.62^{dx}	7.33^{ex}	0.332	.0001

SEM[4)]	0.425	0.217	0.300	0.169	0.147	0.140
P- value	.0001	.0001	.0001	.0001	.0001	.0001

[a-f]Mean values with different superscripts letters within the same row differ significantly ($p<0.05$).

[x-z]Mean values with different superscript letters within the same column differ significantly ($p<0.05$).

Star (0*) indicates the 1 d chilled marinated meat.

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

[3)]SEM: standard error of the means (n=18).

[4)]SEM: standard error of the means (n=12).

Table 3. Instrumental color of chilled meats and 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Items	d of thawing	Treatments[1)]						SEM[2)]	*p*-value
		Control (-)	Control (+)	PJ	OS	N-OS	YLE		
L*	0*	58.97^{ax}	54.80^{d}	60.78^{ax}	57.15^{bc}	57.30^{bcx}	55.87^{cdx}	0.593	<.0001
	1	53.79^{by}	54.30^{b}	57.46^{ay}	55.05^{b}	53.51^{bz}	54.38^{bxy}	0.525	0.0001
	3	54.40^{y}	54.06	55.23^{z}	55.48	54.86^{y}	53.36^{y}	0.878	0.556
	SEM[3)]	0.656	1.160	0.432	0.580	0.441	0.550		
	p-value	.0001	0.8989	.0001	0.0513	.0001	0.0182		
a*	0*	1.82^{by}	1.64^{b}	1.55^{by}	2.27^{ax}	2.22^{ay}	1.69^{by}	0.121	0.0003
	1	2.82^{ax}	1.75^{b}	1.77^{y}	2.09^{bxy}	3.27^{ax}	2.68^{ax}	0.218	.0001
	3	1.08^{cz}	1.81^{b}	3.32^{ax}	1.89^{by}	1.87^{by}	1.91^{by}	0.178	.0001
	SEM[3)]	0.178	0.094	0.218	0.091	0.188	0.142		
	p-value	.0001	0.4246	.0001	0.0303	0.0003	0.0005		
b*	0*	8.25^{ab}	6.31^{cz}	8.72^{ay}	7.33^{bcy}	7.60^{by}	7.11^{bcy}	0.298	.0001
	1	9.33^{b}	8.78^{bx}	9.96^{ax}	8.93^{bx}	10.25^{ax}	8.67^{bx}	0.218	.0001
	3	8.37^{bc}	7.48^{cdy}	10.13^{ax}	7.80^{cdy}	7.30^{dy}	8.73^{bx}	0.258	.0001
	SEM[3)]	0.301	0.162	0.227	0.335	0.238	0.262		
	p-value	0.045	.0001	0.001	0.012	.0001	0.001		
Chroma (C*)	0*	8.01^{aby}	6.37^{cy}	8.81^{az}	8.74^{ax}	6.88^{bcz}	7.50^{bcz}	0.344	.0001
	1	12.40^{ax}	9.43^{cx}	11.42^{bx}	8.76^{cx}	10.98^{bx}	10.97^{bx}	0.314	.0001
	3	8.36^{by}	6.99^{cdy}	10.15^{ay}	6.43^{dy}	7.85^{bcy}	8.55^{by}	0.344	.0001
	SEM[3)]	0.377	0.289	0.302	0.491	0.279	0.277		
	p-value	.0001	.0001	.0001	0.008	.0001	.0001		
Hue angel (*h*°)	0*	80.52^{abxy}	84.47^{az}	83.97^{ax}	78.89^{bx}	74.68^{b}	75.82^{b}	1.795	0.0013
	1	75.96^{y}	78.14^{xy}	78.88^{xy}	84.25^{x}	75.84	76.45	2.292	0.1668
	3	85.87^{ax}	75.09^{bcy}	76.29^{bcy}	69.11^{cy}	76.91^{b}	76.00^{bc}	1.817	.0001

SEM[3)]	2.545	2.302	1.944	2.492	1.398	1.537
p-value	0.030	0.036	0.040	0.003	0.576	0.956

[a-f]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[x-z]Mean values with different superscript letters within the same column differ significantly ($p<0.05$).

Star (0*) indicates the 1 d chilled marinated meat.

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

[3)]SEM: standard error of the means (n=18).

Table 4. Lipid oxidation rate as TBARS value (mg MDA/kg) of chilled meats and 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Items	d of thawing	Treatments[1)]						SEM[2)]	*p*-value
		Control (-)	Control (+)	PJ	OS	N-OS	YLE		
TBARS (mg MDA/kg)	0*	0.18^{az}	0.14^{bz}	0.12^{cz}	0.15^{bz}	0.15^{bz}	0.13^{cz}	0.004	.0001
	1	0.23^{ay}	0.19^{cy}	0.18^{cy}	0.20^{by}	0.20^{by}	0.19^{cy}	0.003	.0001
	3	0.30^{ax}	0.24^{dx}	0.23^{dx}	0.25^{cx}	0.27^{bx}	0.24^{dx}	0.004	.0001
	SEM[3)]	0.003	0.004	0.004	0.004	0.003	0.003		
	P-value	.0001	.0001	.0001	.0001	.0001	.0001		

[a-d]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[x-z]Mean values with different superscript letters within the same column differ significantly ($p<0.05$).

Star (0*) indicates the 1 d chilled marinated meat.

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

[3)]SEM: standard error of the means (n=18).

Table 5. Protein solubility (mg/g) of chilled meats and 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Items	d of thawing	Treatments[1)]						SEM[2)]	*P*-value
		Control (-)	Control (+)	PJ	OS	N-OS	YLE		
Total protein (mg/g)	0*	771.60^{dz}	789.87^{cz}	774.14^{dz}	831.44^{bz}	839.71^{az}	793.35^{cz}	16.989	.0001
	1	841.72^{cy}	891.05^{by}	796.92^{dy}	890.67^{by}	943.18^{ay}	875.37^{by}	7.202	.0001
	3	875.93^{ex}	916.28^{bx}	870.20^{ex}	905.18^{dx}	995.36^{ax}	890.63^{dx}	2.603	.0001
	SEM[3)]	4.703	2.032	2.492	2.294	9.976	3.485		
	P-value	.0001	.0001	.0001	.0001	.0001	.0001		
Sarcoplasmic protein (mg/g)	0*	308.12^{bx}	282.59^{by}	310.65^{bx}	316.48^{b}	389.56^{a}	317.20^{bx}	16.989	0.0154
	1	305.25^{bx}	312.26^{abx}	268.32^{cz}	299.44^{b}	330.43^{a}	317.95^{abx}	5.932	0.0002
	3	272.63^{cy}	315.50^{ax}	293.57^{bcy}	297.98^{b}	305.46^{ab}	265.74^{cy}	4.068	.0001
	SEM[3)]	4.703	6.700	2.267	12.729	21.008	4.886		
	P-value	.0001	0.0245	.0001	0.554	0.0715	0.0004		
Myofibrillar protein (mg/g)	0*	463.48^{z}	507.28^{z}	463.50^{z}	514.96^{y}	450.14^{z}	476.15^{z}	15.778	0.0706
	1	536.47^{dy}	578.79^{bcy}	528.60^{dy}	591.24^{abx}	612.74^{ay}	557.42^{cdy}	8.428	.0001
	3	603.30^{cx}	600.78^{cx}	576.63^{dx}	607.20^{cx}	689.89^{ax}	624.89^{bx}	4.703	.0001
	SEM[3)]	4.703	5.762	3.269	12.067	20.709	7.637		
	P-value	.0001	.0001	.0001	0.0035	0.0005	.0001		

[a-f]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[x-z]Mean values with different superscript letters within the same column differ significantly ($p<0.05$).

Star (0*) indicates the 1 d chilled marinated meat.

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

[3)]SEM: standard error of the means (n=18).

Table 6. Myofibril fragmentation Index (MFI) and Impedance (Z) of chilled meats and 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Items	d of thawing	Treatments[1)]						SEM[2)]	*p*-value
		Control (-)	Control (+)	PJ	OS	N-OS	YLE		
MFI	0*	109.17^{cz}	116.39^{bz}	91.15^{dz}	116.66^{bz}	118.99^{bz}	123.18^{az}	0.698	.0001
	1	116.65^{cy}	136.57^{aby}	99.69^{dy}	134.77^{by}	135.63^{aby}	137.44^{ay}	0.544	.0001
	3	124.59^{bx}	144.58^{ax}	113.37^{cx}	146.40^{ax}	146.66^{ax}	145.53^{ax}	0.595	.0001
	SEM[3)]	0.562	0.634	0.540	0.579	0.543	0.797		
	p-value	.0001	.0001	.0001	.0001	.0001	.0001		
Z	0*	135.07^{bx}	141.07^{ax}	133.10^{bx}	138.24^{ax}	139.47^{ax}	140.42^{ax}	6.280	.0001
	1	115.27^{by}	125.83^{ay}	113.65^{by}	120.50^{ay}	122.67^{ay}	125.48^{ay}	4.355	.0001
	3	109.68^{bz}	117.25^{az}	107.8^{bz}	118.28^{az}	117.99^{az}	119.42^{az}	5.903	.0001
	SEM[3)]	5.102	5.403	4.374	3.698	4.341	6.702		
	p-value	0.001	.0001	.0001	.0001	.0001	.0001		

[a-d]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[x-z]Mean values with different superscript letters within the same column differ significantly ($p<0.05$).

Star (0*) indicates the 1 d chilled marinated meat.

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

[3)]SEM: standard error of the means (n=18).

Table 7. Shear force and texture profile analysis (TPA) of chilled meats and 7 d frozen/thawed cooked meats marinated with phosphate and phosphate alternatives

Items	d of thawing	Treatments[1)]						SEM[2)]	*p*-value
		Control (-)	Control (+)	PJ	OS	N-OS	YLE		
Shear force (kgf)	0*	1.26^{ax}	1.07^{bcx}	1.24^{ax}	1.13^{bx}	1.11^{bcx}	1.02^{cx}	0.028	.0001
	1	1.16^{ay}	0.90^{by}	1.13^{ay}	0.92^{by}	0.96^{by}	0.92^{bxy}	0.024	.0001
	3	0.99^{az}	0.84^{by}	0.99^{az}	0.84^{bz}	0.88^{bz}	0.85^{by}	0.023	0.0002
	SEM[3)]	0.022	0.036	0.024	0.012	0.011	0.034		
	p-value	.0001	0.003	0.000	.0001	.0001	0.023		
Hardness (kgf)	0*	2.52^{a}	1.48^{d}	2.66^{ax}	1.80^{bcx}	1.60^{dx}	1.73^{bc}	0.092	.0001
	1	2.21^{a}	1.50^{b}	1.80^{ay}	1.73^{by}	1.52^{by}	1.55^{b}	0.105	0.003
	3	2.18^{a}	1.37^{b}	1.78^{ay}	1.23^{bz}	1.45^{bz}	1.55^{b}	0.090	.0001
	SEM[3)]	0.101	0.107	0.074	0.154	0.035	0.054		
	p-value	0.077	0.0748	.0001	0.0194	.0001	0.0777		
Cohesiveness	0*	0.26^{ab}	0.24^{bc}	0.25^{ab}	0.24^{bc}	0.23^{bcx}	0.18^{c}	0.011	0.0016
	1	0.25^{a}	0.20^{bc}	0.24^{ab}	0.20^{bc}	0.20^{bcy}	0.19^{c}	0.011	0.0014
	3	0.23^{ab}	0.21^{b}	0.24^{a}	0.18^{b}	0.18^{by}	0.19^{b}	0.010	0.0293
	SEM[3)]	0.012	0.009	0.015	0.010	0.008	0.007		
	p-value	0.188	0.2347	0.8011	0.0878	0.0018	0.6599		
Chewiness (kgf)	0*	0.35^{a}	0.28^{bc}	0.26^{ab}	0.22^{bcy}	0.23^{bx}	0.17^{c}	0.021	0.003
	1	0.30	0.24	0.25	0.22^{x}	0.20^{x}	0.19	0.024	0.0749
	3	0.32^{a}	0.24^{b}	0.24^{b}	0.21^{by}	0.17^{by}	0.20^{b}	0.022	0.0038
	SEM[3)]	0.025	0.023	0.026	0.022	0.020	0.015		
	p-value	0.7534	0.4993	0.7038	0.032	0.0052	0.101		
Gumminess (kgf)	0*	0.51^{ax}	0.41^{ab}	0.40^{ab}	0.36^{abxy}	0.40^{abx}	0.27^{b}	0.035	0.0017
	1	0.52^{x}	0.39	0.38	0.35^{x}	0.39^{xy}	0.28	0.042	0.1104

	3	0.32^{by}	0.30^{b}	0.36^{a}	0.32^{by}	0.29^{by}	0.29^{b}	0.035	0.0267
	SEM[3)]	0.036	0.047	0.037	0.034	0.037	0.033		
	p-value	0.0063	0.5413	0.7038	0.0509	0.0132	0.2099		
Springiness (%)	0*	57.60^{abx}	59.75^{aby}	54.92^{by}	62.43^{a}	58.33^{aby}	61.95^{ax}	1.553	0.0111
	1	59.20^{x}	64.83^{x}	66.0^{x}	66.75	66.89^{x}	62.89^{x}	1.980	0.0944
	3	47.88^{by}	55.50^{aby}	58.23^{ay}	58.79^{a}	57.33^{ay}	57.32^{ay}	1.873	0.0038
	SEM[3)]	1.567	1.685	1.462	2.426	2.180	1.268		
	p-value	0.0013	0.0015	0.0013	0.1209	0.024	0.0273		

[a-c]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[x-z]Mean values with different superscript letters within the same column differ significantly ($p<0.05$).

Star (0*) indicates the 1 d chilled marinated meat.

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

[3)]SEM: standard error of the means (n=18).

Table 8. Fatty acid composition (%) of 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Fatty acid	Treatments[1)]						SEM[2)]	*P*-value
	Control (-)	Control (+)	PJ	OS	N-OS	YLE		
14:0	0.71^{ab}	0.76^{ab}	0.67^{b}	0.70^{ab}	0.77^{a}	0.76^{ab}	0.021	0.022
16:0	21.39	21.68	20.79	20.82	21.62	21.15	0.201	0.030
16:1	4.01^{ab}	4.09^{a}	3.39^{b}	3.45^{ab}	3.95^{ab}	3.71^{ab}	0.146	0.019
18:0	8.62^{ab}	7.95^{b}	9.14^{a}	8.80^{a}	7.98^{b}	8.36^{ab}	0.188	0.005
18:1	36.53	35.18	34.24	35.02	35.67	34.93	0.578	0.189
18:2	17.31^{b}	18.57^{a}	17.56^{b}	17.98^{ab}	18.79^{a}	18.91^{a}	0.231	0.001
18:3	0.46^{ab}	0.49^{a}	0.50^{a}	0.49^{a}	0.42^{b}	0.45^{ab}	0.012	0.004
20:2	0.55^{b}	0.64^{ab}	0.69^{a}	0.68^{a}	0.56^{b}	0.53^{b}	0.031	0.009
20:3	1.25^{ab}	1.34^{ab}	1.50^{a}	1.47^{a}	1.16^{b}	1.26^{ab}	0.060	0.012
20:4	4.04	4.15	5.29	4.75	4.07	4.59	0.295	0.068
20:5	0.24	0.25	0.28	0.25	0.23	0.23	0.016	0.279
22:6	0.34^{b}	0.34^{b}	0.50^{a}	0.44^{ab}	0.37^{b}	0.36^{b}	0.025	0.003
24:1	1.11^{c}	1.16^{bc}	1.39^{a}	1.35^{ab}	1.15^{bc}	1.25^{abc}	0.052	0.010
Total F.A.	96.54	96.59	95.94	96.18	96.73	96.48	0.229	0.230
SFA	30.72	30.39	30.60	30.32	30.38	30.27	0.177	0.466
UFA	65.82	66.20	65.35	65.86	66.35	66.21	0.301	0.264
MUFA	41.65	40.43	39.03	39.82	40.76	39.89	0.673	0.189
PUFA	24.18	25.77	26.31	26.05	25.59	26.32	0.470	0.060
UFA/SFA	2.14	2.18	2.14	2.17	2.18	2.19	0.021	0.396
n-6	21.89^{b}	23.35^{ab}	23.54^{ab}	23.40^{ab}	23.41^{ab}	24.03^{a}	0.408	0.049
n-3	2.29^{bc}	2.42^{abc}	2.77^{a}	2.65^{ab}	2.17^{c}	2.29^{bc}	0.098	0.007
n-6/n-3	9.57^{ab}	9.69^{ab}	8.53^{b}	8.88^{b}	10.80^{a}	10.47^{a}	0.309	0.002

[a-c]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).

DFA: desirable fatty acids (C18: 0 + UFA).

Table 9. Free amino acid composition (mg/100g) of 7 d frozen/thawed meats marinated with phosphate and phosphate alternatives

Free amino acids	Treatments[1)]						SEM[2)]	*P*-value
	Control (-)	Control (+)	PJ	OS	N-OS	YLE		
Taurine	11.08^{a}	7.14^{cd}	6.36^{d}	8.86^{b}	7.67^{c}	8.13^{bc}	0.282	.0001
Aspartic Acid	19.58^{a}	11.91^{c}	10.76^{c}	19.90^{a}	19.70^{a}	16.25^{b}	0.618	.0001
Threonine	16.67^{a}	10.85^{b}	10.73^{b}	15.22^{a}	16.14^{a}	12.56^{b}	0.508	.0001
Serine	26.34^{a}	17.86^{bc}	16.64^{c}	24.66^{a}	24.69^{a}	20.11^{b}	0.849	.0001
Asparagine	1.53^{a}	1.09^{b}	1.20^{b}	1.07^{b}	1.56^{a}	1.49^{a}	0.0835	.0022
Glutamic acid	28.23^{a}	17.05^{c}	16.72^{c}	24.15^{b}	25.67^{b}	18.26^{c}	0.826	.0001
Glycine	28.87^{a}	22.05^{b}	20.17^{b}	26.22^{a}	27.97^{a}	20.72^{b}	0.812	.0001
Alanine	48.36^{a}	34.01^{b}	33.12^{b}	45.84^{a}	46.40^{a}	36.86^{b}	1.548	.0001
Valine	18.78^{a}	11.51^{b}	9.85^{b}	17.81^{a}	18.14^{a}	12.49^{b}	0.724	.0001
Methionine	10.16^{a}	8.15^{b}	7.79^{b}	9.72^{a}	10.43^{a}	6.99^{b}	0.370	.0001
lsoleucine	11.19^{a}	7.07^{b}	6.69^{b}	10.31^{a}	11.11^{a}	7.85^{b}	0.340	.0001
Leucine	21.26^{a}	13.10^{b}	13.93^{b}	19.17^{a}	19.93^{a}	14.85^{b}	0.635	.0001
Tyrosin	11.32^{a}	7.31^{b}	7.54^{b}	10.22^{a}	10.88^{a}	7.78^{b}	0.402	.0001
Phenyalanine	9.35^{a}	3.93^{c}	6.16^{b}	8.35^{a}	8.69^{a}	6.48^{b}	0.526	.0001
Histidine	9.43^{a}	5.53^{b}	5.35^{b}	9.25^{a}	9.68^{a}	6.43^{b}	0.378	.0001
Tryptophan	56.49^{a}	42.83^{b}	43.92^{b}	45.62^{b}	37.43^{b}	29.63^{c}	2.280	.0001
Carnorsine	47.87^{a}	41.76^{ab}	41.14^{ab}	43.57^{ab}	38.64^{b}	31.21^{c}	1.632	.0003
Lysine	22.84^{a}	13.79^{b}	13.98^{b}	21.74^{a}	22.09^{a}	14.90^{b}	0.863	.0001
Arginine	15.04^{a}	8.55^{c}	8.60^{c}	12.88^{b}	12.46^{b}	9.12^{c}	0.571	.0001
Total free amino acid	414.39^{a}	285.49^{b}	280.65^{b}	374.53^{a}	369.28^{a}	282.09^{b}	13.290	.0001
Non-bitter A.A.	192.41^{a}	128.61^{b}	123.30^{b}	178.79^{a}	184.23^{a}	141.14^{b}	5.932	.0001
Bitter A.A.	95.21^{a}	57.85^{b}	58.39^{b}	87.48^{a}	90.44^{a}	64.20^{b}	3.238	.0001
Non-bitter/Bitter A.A.	2.02^{c}	2.23^{a}	2.11^{bc}	2.04^{c}	2.04^{c}	2.20^{ab}	0.030	.0013

[a-d]Mean values with different superscript letters within the same row differ significantly ($p<0.05$).

[1)]Control (-) = no phosphate; Control (+) 0.3% sodium tripolyphosphate; PJ = 0.3% prune juice; OS = 0.3% oyster shell; N-OS = 0.3% nano-oyster shell; YLE = 0.3% yeast and lemon extract.

[2)]SEM: standard error of the means (n=36).