Agr. Nat. Resour. 55 (2021) 219–228

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effects of chitosan and phosphate on quality characteristics and shelf life extension of pork meatballs

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Article Info

Article history:
Received 10 August 2019
Revised 18 January 2021
Accepted 29 January 2021
Available online 19 April 2021

Keywords:
Chitosan,
Meatballs,
Phosphate,
Shelf life,
Storage

Abstract

The effects of the chitosan (0%, 0.25%, 0.50%) and phosphate (0%, 0.10%, 0.20%) contents in pork meatballs were studied on the textural properties and their ability to extend product shelf life stored at 4°C for up to 28 d. The physical (texture), and chemical (pH, purge loss, 2-thiobarbituric acid or TBA) properties and microbial growth of pork meatballs were investigated. The results indicated that chitosan could be used as an alternative natural additive to improve both the chemical and physical properties and to extend the shelf life of meatballs. The texture of the meatballs also improved with added chitosan at 0.50% but there was no difference between phosphate added at 0.10% or 0.20%. The TBA value of the meatballs significantly ($p < 0.05$) decreased with the addition of chitosan, resulting in lower purge loss than for the control. The addition of 0.50% chitosan significantly extended the shelf life by inhibiting microbial growth. At 28 d the total viable count of meatballs with chitosan 0.50% and phosphate 0.20% was 2.51±0.08 log cfu/g while the control sample was 7.70±0.43 log cfu/g. The sensory evaluation determined that the overall acceptance of the chitosan-treated meatballs was higher than for the control.

Introduction

Pork meatballs are one of the most popular boiled, fried or grilled edible meat products in Thailand. The specific qualities of meatballs include good texture, reduced purge loss during storage and long shelf life without bad odor (Gomez et al., 2020). When untreated, meatballs have a short shelf life, high moisture and protein at a neutral pH that can cause contamination through the growth of spoilage pathogenic microorganisms and lipid oxidation so that the maximum storage life is normally 15 d at 4°C (Lekjing, 2016). Chemical degradation in food occurs from lipid oxidation, producing a rancid flavor and decreasing sensory scores and nutrition value (Sharma et al., 2017). Neto et al. (2017) reported on health impairment due to consumption of synthetic food additives which cause cell damage by oxidation and reduce human immunity. Inflammation, metabolic disorders, irregular cell aging, reperfusion damage, atherosclerosis and carcinogenesis are caused by free radical reactive oxygen species and reactive nitrogen in synthetic additives (Arivizhivendhan et al., 2018). The meat processing industry is striving to find suitable alternatives to synthetic additives including nitrites, phosphates, and ascorbic acids, where the phosphates are used as a food preservative to improve the texture of meat products through pH adjustment of the tissues and water holding capacity (Roldan et al., 2014). However, nowadays, it appears that consumers are becoming increasingly concerned regarding the safety of synthetic chemical preservatives and antioxidants as food additives. This has resulted in intensive investigation of alternative natural preservatives and recent research revealed that consumers will pay 200% or more extra for natural

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https://doi.org/10.34044/j.anres.2021.55.2.09

food ingredients, and that they choose natural sources of functional ingredients rather than chemical synthetic additives (Choe et al., 2018). This has encouraged researchers to explore the applicability and efficiency of natural compounds to substitute synthetic additives in food products.

Chitosan is a hydrocolloid polysaccharide prepared by the deacetylation of chitin and occurs naturally in the shells of crabs, shrimps and the cell walls of fungi as polymeric 1,4-linked 2-amino-2-deoxy-b-D-glucose (Latou et al., 2014). Chitosan is used as a food preservative as it has potential as an alternative preservative to reduce microbial growth of meat products by reducing total bacteria, coliform and Enterobacteriaceae counts compared with sulfur dioxide (SO_2) (Mathenjwa et al., 2012). Chitosan functions as an antioxidant in combination with either rosemary or α-tocopherol (Georgantelis et al., 2007). In addition, chitosan can be applied as a film coating to increase the shelf life of meat and meat products (Kuzgun and Inanli, 2018). Color stability during storage increased and exhibited a darker, more intense red color when chitosan was applied as a film coating (Cardoso et al., 2016). Chitosan also acts as a chelating agent by selectively binding trace metals and inhibiting the production of toxins and microbial growth, and as a water-binding agent that inhibits enzymes by blocking their active centers (Latou et al., 2014). Chitosan is classified as Generally Recognized as Safe (GRAS) by US Food and Drug Administration (2012) and has been proved to be non-toxic, biodegradable and biocompatible. Chitosan at a concentration of 0.1–1.0% inhibits the growth of spoilage bacteria and also acts as an antioxidant (No et al., 2007).

However, to date there has been no report on the effects of chitosan in combination with phosphate on the physical and chemical properties and shelf life of Thai meatballs. Thus, the main objective of this study was to determine the effect of chitosan in combination with phosphate on the characteristics of pork meatballs (texture, pH, 2-thiobarbituric acid (TBA), purge loss and microbial growth) during storage at 4°C for up to 28 d.

Materials and Methods

Raw material and chemical ingredients

Pork, back fat and tapioca starch were purchased from a local market in Bangkok, Thailand. Garlic powder and white pepper were purchased from Fatisco Co., Ltd. (Bangkok, Thailand). Sodium tripolyphosphate was purchased from Aditya Berla Co., Ltd. (Bangkok, Thailand). Chitosan powder (food grade) from shrimp shell was purchased from Bonafides Marketing Co., Ltd. (Bangkok, Thailand). The moisture content of chitosan was less than 10%, with a molecular weight of 500 kDa and degree of deacetylation at 95%. HCl, TBA, trichoroacetic (TCA) and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of chitosan solution

Chitosan solution at 3% (weight per volume) was prepared following the method of Cao et al. (2013) with some modifications. An amount (3 g) of dissolved chitosan was mixed with 90 mL of distilled water and stirred for 10 min. Then, 1 mL of glacial acetic acid was added to the mixture and the solution was made up to 100 mL with distilled water. The chitosan solution was stirred overnight at room temperature to ensure complete dissolution. Chitosan stock solution was prepared for use at 3% (weight per weight; w/w), with the chitosan concentration calculated from the total batch size as w/w.

Preparation of meatballs

The pork was ground twice using a meat grinder (Savioli 32 Classic, Mantova, Italy) with a 3 mm plate. The back fat was ground once using a meat grinder with a 3 mm plate. The ground meat was blended using a food processor (Ronic, Vitry-en-Charollais, France) with salt, phosphate and half of the ice for 3 min. The back fat and other half of the ice were then added and the mixture was blended for a further 3 min. Finally, white pepper powder, garlic powder, sugar and tapioca starch were added and blended for 2 min. The emulsion was shaped by hand to produce uniformly shaped meatballs weighing 20±2 g which were boiled at 60°C for 10 min, followed by boiling at 80°C for 10 min. The meatballs were then cooled in water, packed in plastic bags (nylon/linear low-density polyethylene) and stored at 4°C. The chitosan content was varied at three levels (0%, 0.25%, 0.50%) with phosphate also at three levels (0%, 0.10%, 0.20%). Experiments were replicated on different days at similar production levels for statistical analysis.

Microbiological analyses

Samples of 25 g were transferred aseptically into individual stomacher bags with 225 mL of sterile buffered peptone water and homogenized in the stomacher. Aerobic plate counts were determined following Chapter 3 in the FDA Bacteriological Analytical Manual (Tallent et al., 2001). *Staphylococcus aureus* was determined following Chapter 12 in Maturin and Peeler (2001). *Escherichia coli* was determined following Chapter 4 in Feng et al. (2002). Microbiological analyses of the samples were conducted at 0 d, 7 d, 14 d, 21 d and 28 d of storage. The results were expressed as log colony forming units (CFU) per gram of sample.

Chemical and physical analyses during the shelf life study

pH

The pH of the meatballs was measured using a pH meter as a suspension resulting from blending a 10 g sample with 90 g of deionized water for 60 s (Thermo Scientific Orion 210; Massachusetts, USA). Measurements were taken three times with the average calculated for each treatment.

Lipid oxidation analysis

Lipid oxidation of meatballs was determined using the 2-thiobarbituric acid (TBA) method (Akcan et al., 2017) at 0 d, 7 d, 14 d, 21 d and 28 d of storage. A sample of the meatballs was homogenized with 9 mL of 7.5% TCA solution and mixed with 50 µL of 7.2% BHT in ethanol. The solution was centrifuged at 15,000 rpm for 15 min and passed through filter paper. The MDA standard was diluted with 0.1 M HCl. Then, 1 mL of filtrate sample was added to 1 mL of 20 mM TBA solution and heated in a water bath at 90°C for 30 min before cooling for 10 min. A spectrophotometer was used to measure the absorption at 532 nm and the concentration of MDA in the meatball products was expressed in milligrams of MDA per kilogram of meatballs sample. All analyses were performed in triplicate.

Texture analysis

Ten samples for each treatment batch were determined for texture profile analysis (TPA) using a Texture Analyzer TA-XT2 with a 50 mm cylinder probe (P/50) (Stable Micro Systems; Godalming, UK). Before analysis, the samples were equilibrated at room temperature for approximately 3 hr. Samples were cut into cubes (1.5 cm ×1.5 cm ×1.5 cm), with a pre-test speed of 2 mm/s, a test speed of 2 mm/s and a post-test speed of 5 mm/s, compressed to 60% of their original height and double compression tested to determine their textural properties. The texture profile parameters were determined following Hsu and Sun (2006) and interpreted as follows. Hardness was the maximum force (peak force occurring during the first compression) required to compress the sample; Cohesiveness was the area of work during the second compression divided by the area of work during the first compression. Springiness was the distance of the detected height during the second compression divided by the original compression distance. Gumminess was the force to disintegrate a semisolid sample for swallowing applied only to semi-solid products. Chewiness was the energy used in chewing the food.

Purge loss

A 200–250 g vacuum-packed sample was used to determine purge loss. Before packing, the sample skin was dried using tissue paper. Then, the sample and packaging were weighed (initial weight; Wo). After storage at 4℃ for 0 d, 7 d 14 d, 21 d or 28 d, the samples were again dried with tissue paper and weighed (storage weight; Wt). The purge loss was determined from the difference in weights between the two measurements expressed as a percentage of the initial weight as shown in Equation 1 (Henning et al., 2016):

$$\% \text{ Purge loss} = \frac{Wo - Wt}{Wo} \times 100 \quad (1)$$

where *Wo* is the initial weight before storage and *Wt* is the weight after storage.

Determinations were conducted in triplicate for each replication.

Sensory evaluation

Four treatments (Control, C0.25+P0.10, C0.50+P0.10 and C0.50+P0.20) were cooked in boiling water for 5 min. Sixty panelists recruited from the staff and students of Kasetsart University (Bangkok, Thailand) evaluated the samples. During evaluation, the panelists were situated in private booths. Testing was initiated after the panelists agreed on the specifications. Drinking water was provided between samples to cleanse the palate. A 9-point hedonic scale was used to rate attributes such as appearance, color, odor, texture and overall acceptance (1 = dislike extremely and 9 = like extremely).

Statistical analysis

The effects of chitosan and phosphate variation were evaluated using one-way analysis of variance. Duncan's multiple range test was used to determine the significance of mean values on the results of microbiological, physical and chemical, and sensory analyses with SPSS statistical software (version 12; SPSS Inc.; Chicago, IL, USA). A confidence interval at the 95% level ($p < 0.05$) was considered in all cases.

Results and Discussion

Effects of chitosan and phosphate content on meatball textural properties

The effects of chitosan and phosphate on the textural properties of meatballs were investigated, producing the results shown in Table 1. The textural properties (hardness, cohesiveness, springiness, chewiness, gumminess) for the nine treatments were compared with commercial meatballs. The results indicated there were no significant differences in textural properties between the commercial meatballs and the samples with 0.50% chitosan and 0.10% phosphate, and with 0.50% chitosan and 0.20% phosphate. Adding chitosan at the high level of 0.50% produced no significant difference in the textural properties between high (0.02%) and low (0.01%) levels of phosphate. Therefore, chitosan influenced the textural properties similar to phosphate. This result was supported by Huang and Tsai (2020) and Omara et al. (2019) who determined the water binding capacity (WBC) of chitosan as 4.90% and 3.12%, respectively. Han et al. (2018) observed the effects of chitosan on the textural properties of model meat products. Hardness, springiness and chewiness were higher with chitosan than the control (without chitosan). The phosphate concentration at 0.10% was chosen as the lower commercial dose with no significant difference from commercial samples combined with chitosan, while 0.20% was selected as the regular dose for meatball products.

Relationships between textural properties (hardness, cohesiveness, springiness, chewiness, gumminess), the phosphate content and the chitosan content were determined. The results are described below.

Table 1 Texture profile analysis of meatballs with three levels of phosphate and chitosan contents

Phosphate (%)	Chitosan (%)	Hardness (g)	Cohesiveness	Springiness (mm)	Chewiness (g.mm)	Gumminess (g)
0.00	0.00	2317.95±191.98^{b}	0.74±0.02^{b}	0.91±0.03^{ab}	1851.86±452.45^{a}	1705.24±159.59^{b}
	0.25	2008.80±521.09^{c}	0.66±0.07^{c}	0.88±0.02^{b}	1200.34±414 .88^{c}	1354.28±460.82^{d}
	0.50	1290.73±337.99^{e}	0.56±0.08^{d}	0.82±0.08^{c}	603.64±239.72^{d}	732.19±274.11^{e}
0.10	0.00	1830.68±180.00^{d}	0.77±0.01^{a}	0.92±0.02^{a}	1292.81±130.29^{bc}	1406.09±134.10^{d}
	0.25	2290.65±189.59^{b}	0.76±0.01^{a}	0.90±0.02^{ab}	1581.91±144.25^{ab}	1749.95±139.42^{b}
	0.50	2630.50±203.39^{a}	0.76±0.01^{a}	0.91±0.02^{ab}	1815.54±154.43^{a}	2003.35±149.39^{a}
0.20	0.00	1801.47±142.29^{d}	0.76±0.01^{a}	0.92±0.02^{a}	1260.97±103.19^{bc}	1373.19±100.63^{d}
	0.25	2072.99±116.88^{c}	0.76±0.01^{ab}	0.90±0.02^{ab}	1410.39±94.40^{bc}	1567.67±89.61^{c}
	0.50	2596.82±274.80^{a}	0.76±0.01^{a}	0.92±0.04^{a}	1815.52±174.62^{a}	1981.66±197.95^{a}

Mean±SD in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Hardness

The relationship between chitosan, phosphate and hardness is presented as Equation 2:

$$\text{Hardness} = 2261 + 283\ P - 522\ C - 17233\ P*P - 2086\ C*C + 17964\ P*C \quad (2)$$

where P is the percentage phosphate content and C is the percentage chitosan content.

The chitosan and phosphate content did not significantly affect hardness. However, there was a significant interaction between the chitosan and phosphate. Fig. 1A shows that meat hardness increased as the phosphate content increased but decreased with increasing chitosan content. Phosphate worked together with salt to improve protein solubility and extraction of the protein improves meat hardness (Hsu and Sun, 2006).

Cohesiveness

The relationship between chitosan, phosphate and cohesiveness is presented in Equation 3:

$$\text{Cohesiveness} = 0.736 + 1.09\ P - 0.182\ C - 5.57\ P^2 - 0.305\ C^2 + 2.08\ P \times C \quad (3)$$

where P is the percentage phosphate content and C is the percentage chitosan content.

The regression equation indicated that phosphate and chitosan significantly affected the cohesiveness which increased as the phosphate content increased but decreased as the chitosan content increased, as shown in Fig. 1B. The addition of phosphate altered the pH value of the proteins in the meat, increasing dissolution and forming a stronger protein network (Villamonte et al., 2013). Chitosan solution is also slightly acidic which favors a denatured protein network (Ye and Chen 2019).

Springiness

The relationship between chitosan, phosphate and springiness is presented in Equation 4 and shown as a contour plot in Fig. 1C.

$$\text{Springiness} = 0.912 + 0.393\ P - 0.190\ C - 1.95\ P^2 + 0.107\ C^2 + 0.824\ P \times C \quad (4)$$

where P is the percentage phosphate content and C is the percentage chitosan content.

The regression equation showed that chitosan and phosphate had a significant effect on springiness which increased as the phosphate content increased but decreased as the chitosan content increased. After the proteins were denatured, the protein network was no longer tight and the springiness of the product decreased (He et al., 2018).

Chewiness

The relationship between chitosan, phosphate and chewiness is presented in Equation 5:

$$\text{Chewiness} = 1480 + 3178\ P - 770\ C - 25255\ P^2 - 1287\ C^2 + 14520\ P \times C \quad (5)$$

where P is the percentage phosphate content and C is the percentage chitosan content.

The regression equation indicated that phosphate had a significantly different effect on chewiness. From Fig. 1D, chewiness increased as the phosphate content increased but decreased as the chitosan content increased due to the phosphate function in the meat system that increased the solubilized myofibrillar protein, providing a chewy texture (Pinton et al., 2019).

Gumminess

The relationship among chitosan, phosphate and gumminess is presented as Equation 6.

$$\text{Gumminess} = 1639 + 2318\ P - 1307\ C - 22173\ P^2 - 497\ C^2 + 17003\ P \times C \quad (6)$$

where P is the percentage phosphate content and C is the percentage chitosan content.

The regression equation indicated that phosphate significantly affected gumminess. Fig. 1E shows that gumminess increased as the phosphate content increased but decreased as the chitosan content increased. Phosphate is one of the ingredients that promote the extraction of protein in meat products and improves textural characteristics (Pinton et al., 2019).

Chitosan at 0.25% and 0.50% were chosen as low and high dose additions. A preliminary experiment (data not shown) indicated that meatballs had an unacceptable taste at a chitosan content higher than 0.50%. A control without added chitosan and phosphate was also included. In the current study four treatments were selected to conduct a shelf life study: control (chitosan 0% and phosphate 0%), chitosan 0.25% combined with phosphate 0.10% (C0.25+P0.10), chitosan 0.50% combined with phosphate 0.10% (C0.50+P0.10) and chitosan 0.50% combined with phosphate 0.20% (C0.50+P0.20).

Shelf life study

Microbiological results

The results of the microbiological analysis of the meatballs during storage at 4°C for 28 d are shown in Table 2. For safety reasons, the total viable count of each product was lower than the acceptable total microbial quality standard for meat products at 5 log colony forming units (cfu)/g (Department of Livestock Development, 2008). The results indicated that every sample had a total viable count (TVC) on initial storage (day 0) lower than the standard and was therefore safe to consume. Samples containing chitosan with phosphate had a TVC of approximately 2 log cfu/g, while the control sample had 3.5 log cfu/g. Samples treated using chitosan with phosphate had lower TVC values than the control at initial storage.

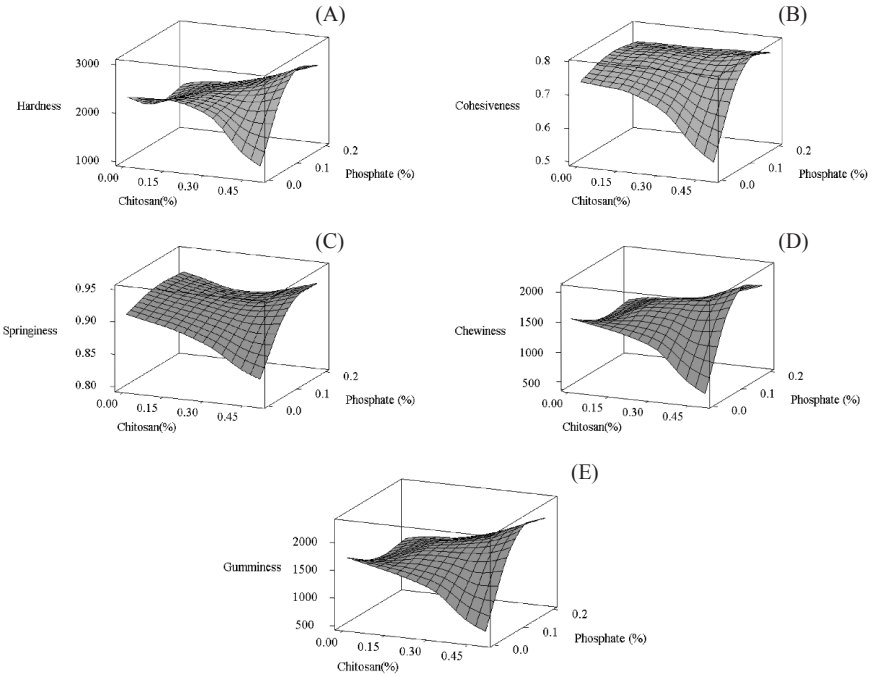

Fig. 1 Surface plot showing textural properties of meatballs with incorporation of different concentrations of chitosan and phosphate: (A) hardness; (B) cohesiveness; (C) springiness; (D) chewiness; (E) gumminess

Table 2 Total viable count (TVC; log colony forming units/g) of meatballs with diverse phosphate and chitosan contents during storage for 28 days at 4°C

Treatment	Storage time (d)				
	0	7	14	21	28
Control	3.51 ± 0.51^{de}	4.28 ± 0.92^{cd}	5.70 ± 1.02^{b}	7.03 ± 0.59^{a}	7.70 ± 0.43^{a}
C0.25+P0.10	2.23 ± 0.13^{fg}	2.61 ± 0.11^{efg}	4.93 ± 1.73^{bc}	5.61 ± 1.58^{b}	6.77 ± 0.21^{a}
C0.50+P0.10	2.14 ± 0.17^{fg}	2.66 ± 0.04^{efg}	2.67 ± 0.73^{efg}	2.23 ± 0.35^{fg}	3.35 ± 0.13^{def}
C0.50+P0.20	1.78 ± 0.52^{g}	2.69 ± 0.36^{efg}	2.91 ± 0.91^{efg}	2.83 ± 0.75^{efg}	2.51 ± 0.08^{efg}

Mean±SD in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.
C = chitosan; P = phosphate; numbers following C and P are the concentration in percent weight per weight

When the control sample was kept for 14 d, the TVC was higher than the standard. After storage for 7 d, there were no significant differences in the TVC values for all treated samples (C0.25+P0.01, C0.50+P0.10, and C0.50+P0.20) and they could be kept longer than the control. Comparison of treated samples with the same level of phosphate (C0.25+P0.01 and C0.50+P0.10) showed that using a higher chitosan content resulted in longer shelf life and significantly lower TVC values after 14 d of storage. The TVC values of the C0.50+P0.10 and C0.50+P0.20 samples, with the same chitosan concentration at 0.50% w/w, were not significantly different and could be safely kept for a storage time of 28 d. Furthermore, *Escherichia coli* and *Staphylococcus aureus* were not found in any of the samples stored for 28 d at 4°C.

These results highlighted that chitosan had a significant effect on the microbial growth of meatballs during storage. Chitosan is known to exhibit antimicrobial activity through its water binding capacity, inhibition of enzymes and ability to increase the permeability of cell membranes and disrupt their barrier properties (Helander et al., 2001). The mechanism of chitosan on shelf life extension involves interaction between positively charged chitosan molecules and negatively charged microbial cell membranes. This electrostatic interaction promotes changes in the properties of membrane wall permeability, resulting in an internal osmotic imbalance and leakage of intercellular electrolytes (Latou et al., 2014). Similarly, Mathenjwa et al. (2012) found that chitosan and chitosan in combination with other preservatives had a significant effect on reducing total bacteria, coliform and Enterobacteriaceae counts compared to SO_2 at 1 d, 3 d, 6 d and 9 d of storage in boerewors (a South African fresh sausage). Soultos et al. (2008) observed the effect of chitosan at 0.5% and 1.0% added individually or in combination with nitrite (150 parts per million) on microbes (TVC, lactic acid bacteria, *Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteriaceae, yeast and molds) in Greek style fresh pork sausage. They determined that chitosan addition resulted in significant ($p < 0.05$) inhibition of microbial growth, while nitrites did not appear to protect the sausage from microbial spoilage. Pandey et al. (2020) used chitosan-based composite nano-layers to develop a novel food packaging material with good antimicrobial properties, while Xiong et al. (2020) observed that 1% chitosan coating of fresh pork effectively extended shelf-life by minimizing any pH change, preventing lipid and protein oxidation and inhibiting microbial growth during 20 d at 4°C.

Lipid oxidation

Changes in the TBA values of all samples during storage for 28 d at 4 °C are shown in Fig. 2.

As observed in Fig. 2, the TBA values of all treated meatballs with chitosan and phosphate at every concentration level were significantly lower than the control sample throughout the storage period. The TBA value increased during 28 d of storage at 4°C for all samples, and the TBA values of samples treated with chitosan and phosphate at every concentration level were not significantly different after storage for 28 d.

A comparison of the C0.25+P0.10 and C0.50+P0.10 samples at the same phosphate concentration of 0.10% w/w showed that the higher chitosan content resulted in a lower TBA value. However, comparing the C0.50+P0.10 and C0.50+P0.20 samples with the same chitosan concentration at 0.50% w/w showed that different phosphate contents did not affect the TBA value. Thus, chitosan lowered the TBA value in meatballs. This result concurred with Kim and Thomas (2006) who found that chitosan addition decreased the thiobarbituric acid reaction substance value during storage at 4°C for 15 d in salmon (*Salmo salar*). Georgantelis et al. (2007) observed the effect of rosemary extract, chitosan and α-tocopherol on lipid oxidation of beef burgers during storage at -18°C for 180 d. They found that chitosan alone and in combination with either rosemary or α-tocopherol showed improved antioxidant effect ($p < 0.05$) compared to individual use of rosemary or α-tocopherol. In addition, Duran and Kahve (2020) found that application of chitosan with vacuum packed beef during storage at 4°C for 45 d was significantly ($p < 0.05$) more effective at reducing the TBA value compared to vacuum packing over a long storage period.

pH

The pH of meatballs was determined during storage for 28 d at 4°C. The initial pH of the meatballs in all sample treatments was in the range 5.7–5.9. The initial pH values of the control and C0.25+P0.10 samples were significantly higher than for the C0.50+P0.10 and C0.50+P0.20 samples. The pH changed in the meatball samples during storage for 28 d at 4°C. The pH of the control and C0.25+P0.10 samples decreased during storage for 28 d, while there was no significant difference between the pH levels of the C0.50+P0.10 and C0.50+P0.20 samples during storage. Both the C0.50+P0.20 and C0.50+P0.10 samples had a higher level of chitosan (0.05%). The chitosan was dissolved in acetic acid; therefore, samples with more chitosan had lower pH values. With increased storage time the control sample decreased in pH value due to the microbial increase that produced lactic acid in the product. These results were in agreement with Lavieri and Williams (2014) who reported that microbial

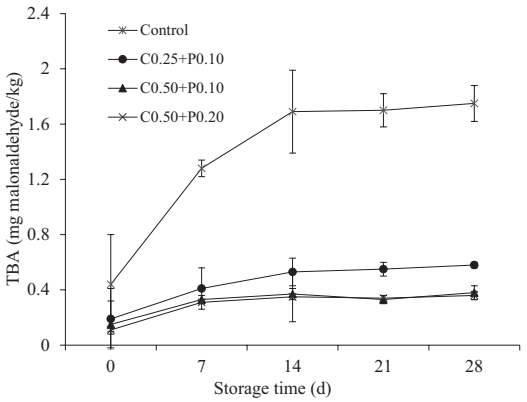

Fig. 2 Change in 2-thiobarbituric acid (TBA) values of meatballs with diverse phosphate and chitosan contents during storage for 28 d at 4°C, where P is phosphate, C is chitosan, numbers following P and C are the concentration as percent weight per weight and error bars = ±SD

growth produced lactic acid with increased storage time, resulting in decreasing pH values in meat products. This finding concurred with the microbiological results in section 3.2 where the C0.50+P0.20 and C0.50+P0.10 samples did not show microbial growth, and lactic acid was not produced; thus, pH did not change.

Purge loss

Fig. 3 shows the purge loss of the meatball samples during storage for 28 d at 4°C. The control sample showed increased purge loss during storage, which at 21 days was significantly ($p < 0.05$) higher than for the samples treated with chitosan and phosphate. Samples treated with different chitosan and phosphate contents were not significantly ($p > 0.05$) different during storage to 28 d. These results were supported by Amiza and Kang (2013) who studied the effect of chitosan on the gelling properties of surimi gels made from African catfish (*Clarias gariepinus*). The addition of 1.5% chitosan improved the gel strength by 58.92% and the water holding capacity by 36.8%. Liu et al. (2013) found that applying chitosan with acetylated gellan gum improved the hydrogel properties and water holding capacity. Roldan et al. (2014) studied the effect of phosphate addition on the physicochemical and sensory features of cooked lamb. Their results indicated that phosphates led to lower cooking loss, increased hardness and shear force value, with improved juiciness and sensory texture. They concluded that samples treated with chitosan and phosphate showed decreased purge loss due to improvement in the water holding capacity of the meat.

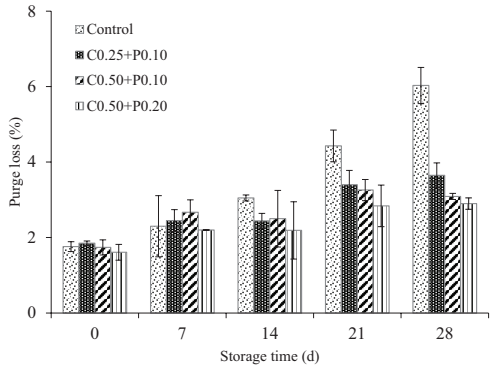

Fig. 3 Purge loss of meatball samples during storage for 28 d at 4°C, where P is phosphate, C is chitosan, numbers following P and C are the concentration as percent weight per weight and error bars = ± SD

Texture profile analysis

The texture of meatballs with diverse phosphate and chitosan contents during storage for 28 d at 4°C was evaluated using TPA as shown in Table 3. The addition of chitosan and phosphate affected the texture parameters of the meatballs. Samples C0.25+P0.10, C0.50+P0.10 and C0.50+P0.20 had significantly higher hardness, cohesiveness, gumminess and chewiness than the control; however,

Table 3 Texture profile analysis of meatballs with diverse phosphate and chitosan contents during storage for 28 d at 4°C

Storage time (d)	Treatment	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (g.mm)
0	Control	2039.92 ± 121.63^{h}	0.67 ± 0.06^{i}	0.91 ± 0.03^{e}	1385.88 ± 274.35^{h}	1255.70 ± 255.37^{i}
	C0.25+P0.10	2445.66 ± 30.82^{f}	0.77 ± 0.01^{de}	0.92 ± 0.02^{abc}	1938.96 ± 121.36^{f}	1780.79 ± 124.21^{g}
	C0.50+P0.10	3100.85 ± 137.54^{ab}	0.76 ± 0.01^{f}	0.92 ± 0.02^{bc}	2378.42 ± 203.36^{ab}	2202.12 ± 185.08^{ab}
	C0.50+P0.20	3046.80 ± 314.88^{ab}	0.76 ± 0.02^{ef}	0.92 ± 0.03^{cd}	2378.09 ± 313.95^{b}	2193.16 ± 348.88^{b}
7	Control	2025.28 ± 297.80^{gh}	0.72 ± 0.02^{h}	0.92 ± 0.02^{bc}	1469.48 ± 246.92^{h}	1355.96 ± 225.84^{i}
	C0.25+P0.10	2476.13 ± 174.88^{f}	0.79 ± 0.01^{a}	0.94 ± 0.02^{a}	1956.99 ± 139.37^{f}	1834.36 ± 131.85^{fg}
	C0.50+P0.10	3063.99 ± 256.30^{ab}	0.78 ± 0.01^{abcd}	0.92 ± 0.02^{bc}	2394.51 ± 195.62^{b}	2198.87 ± 189.57^{b}
	C0.50+P0.20	2890.99 ± 246.40^{cd}	0.78 ± 0.01^{bcd}	0.93 ± 0.02^{bc}	2253.53 ± 170.05^{cd}	2087.32 ± 147.22^{bcd}
14	Control	1878.76 ± 333.49^{i}	0.69 ± 0.02^{i}	0.89 ± 0.03^{e}	1307.18 ± 255.85^{i}	1172.88 ± 252.58^{j}
	C0.25+P0.10	2558.60 ± 179.56^{ef}	0.79 ± 0.00^{abc}	0.93 ± 0.01^{abc}	2014.43 ± 141.96^{ef}	1873.69 ± 138.41^{ef}
	C0.50+P0.10	2992.40 ± 202.64^{bc}	0.78 ± 0.01^{cde}	0.93 ± 0.02^{abc}	2326.51 ± 144.34^{bc}	2159.70 ± 163.87^{bc}
	C0.50+P0.20	2744.17 ± 215.15^{d}	0.79 ± 0.02^{abc}	0.94 ± 0.04^{ab}	2171.50 ± 174.84^{d}	2045.26 ± 190.39^{cd}
21	Control	2052.03 ± 249.98^{h}	0.73 ± 0.02^{h}	0.91 ± 0.02^{cd}	1505.14 ± 217.39^{h}	1369.80 ± 190.45^{i}
	C0.25+P0.10	2445.15 ± 214.03^{f}	0.79 ± 0.01^{a}	0.92 ± 0.02^{bc}	1930.30 ± 172.31^{f}	1769.68 ± 158.84^{fg}
	C0.50+P0.10	3049.58 ± 263.43^{ab}	0.78 ± 0.01^{abcd}	0.92 ± 0.01^{bc}	2386.55 ± 200.69^{ab}	2204.38 ± 191.28^{ab}
	C0.50+P0.20	2864.87 ± 187.70^{cd}	0.79 ± 0.01^{abc}	0.93 ± 0.01^{ab}	2257.38 ± 135.77^{bcd}	2109.04 ± 133.51^{bc}
28	Control	2263.77 ± 224.37^{g}	0.74 ± 0.02^{g}	0.91 ± 0.03^{cd}	1685.17 ± 184.68^{g}	1537.06 ± 172.30^{h}
	C0.25+P0.10	2687.69 ± 124.44^{de}	0.79 ± 0.01^{a}	0.92 ± 0.02^{abc}	2126.83 ± 104.08^{de}	1966.34 ± 105.65^{de}
	C0.50+P0.10	3246.76 ± 318.44^{a}	0.78 ± 0.01^{abcd}	0.92 ± 0.03^{abc}	2549.60 ± 239.13^{a}	2345.74 ± 274.03^{a}
	C0.50+P0.20	3073.16 ± 286.01^{bc}	0.79 ± 0.01^{ab}	0.93 ± 0.02^{bc}	2431.31 ± 221.17^{b}	2254.56 ± 244.06^{ab}

Mean±SD in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.
C = chitosan; P = phosphate; numbers following C and P are the concentration in percent weight per weight

the hardness, cohesiveness and chewiness of the C0.50+P0.10 and C0.50+P0.20 samples were not significantly different during storage at 4°C for 28 d. Thus, samples with added chitosan and phosphate did not show differences in texture with increased storage time. The exact mechanism of chitosan remains unclear but participation in hydrophobic interactions, hydrogen bonding and electrostatic interactions during the setting process have been proposed as possible methods by which chitosan can improve the formation of cross-linked myosin heavy chain components during their polymerization by endogenous enzymes (Alishahi and Aider, 2012). Phosphates fulfill various functions in meat products such as water-binding, buffering, emulsification and oxidation inhibition (Thangavelu et al., 2019). Chitosan applied in food products functions as a fat substitute and gelling agent with antimicrobial and antioxidant properties (Ozaki et al., 2020). The current study investigated the effects of chitosan combined with phosphate on the physical and chemical properties of meatballs. Chitosan was dispersed uniformly in meatballs and assimilated into the gel network to enhance the gelation ability of salt soluble meat protein by various chemical interactions (Li and Xia, 2010).

Sensory evaluation

The sensory scores of the meatball samples are shown in Table 4. The control sample scored significantly lower in the color, taste, texture and overall acceptance attributes than samples treated with chitosan and phosphate that scored slightly lower in the odor attribute. For the color and odor parameters, the samples treated with chitosan and phosphate were not significant different. The highest liking score for taste and texture was for the sample with C0.50+P0.20. The sample C0.50+P0.20 scored 7.40 for overall acceptance, while the control, C0.25+P0.10 and C0.50+P0.10 scored overall acceptance levels of 5.28, 6.70 and 6.77, respectively.

The current study evaluated the effect of chitosan on the physical, chemical and textural properties and the shelf life properties of meatballs. The results suggested that 0.50% chitosan and 0.10% phosphate improved texture and purge loss and reduced TBA during storage for 28 d. Samples with 0.50% of added chitosan extended the shelf life compared to the control and 0.25% chitosan. A 0.50% chitosan concentration may be suitable to improve the shelf life of meatballs. In addition, a 0.10% phosphate combination with chitosan at 0.50% showed results similar to 0.20% phosphate during storage. Thus, a combination of chitosan and phosphate could reduce the amount of phosphate in meat products. Furthermore, samples with added chitosan did not negatively impact the sensory attributes of texture, color, odor, taste and overall acceptance. Therefore, chitosan can be used to improve both the physical and chemical properties and to extend the shelf life of meatballs as an alternative to reduce phosphate content. Chitosan could be used as an alternative natural additive to improve the chemical and physical qualities as well as the shelf life of meatballs.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are grateful for financial support from the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand and the Institute of Food Research and Product Development, Kasetsart University (IFRPD).

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Table 4 Sensory evaluation scores using a 9-point hedonic scale for meatballs with diverse phosphate and chitosan content

Treatment	Attribute				
	Color	Odor	Taste	Texture	Overall acceptance
Control	6.25 + 1.51^{b}	6.05 + 1.67^{a}	5.63 + 1.97^{c}	4.75 + 1.91^{c}	5.28 + 1.85^{c}
C0.25+P0.10	6.80 + 1.28^{a}	6.72 + 1.46^{b}	6.28 + 1.51^{b}	6.33 + 1.49^{b}	6.70 + 1.47^{b}
C0.50+P0.10	6.77 + 1.18^{a}	6.48 + 1.49^{ab}	6.42 + 1.53^{ab}	6.67 + 1.32^{b}	6.77 + 1.21^{b}
C0.50+P0.20	7.08 + 1.37^{a}	6.68 + 1.42^{b}	6.93 + 1.38^{a}	7.47 + 1.20^{a}	7.40 + 0.92^{a}

Mean±SD in a column superscripted with different lowercase lettersare significantly ($p < 0.05$) different.

C = chitosan; P = phosphate; numbers following C and P are the concentration in percent weight per weight

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