Elimination of phosphate in restructured turkey steaks by the addition of eggshell calcium powder and low methoxyl pectin

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Abstract: This study was carried out to assess the effects of eggshell calcium powder (ESCP) and/or low methoxyl pectin (LMP) as phosphate replacers on the quality parameters of restructured turkey steaks. ESCP, 0.25% or 0.50%, was added to formulation alone or in combination with 0.25% LMP in powder and gel forms. The pH increased with the addition of ESCP. Soluble protein content, water holding capacity, and cooking yield were higher in steaks formulated with ESCP+LMP gel compared to control steaks containing phosphate. Hardness of steaks was decreased by the addition of ESCP and pectin. Pectin in powder form negatively affected the preference of panelists. Oxidation in phosphate-free steaks was more pronounced than in other treatments. The results showed that the binding properties of phosphate could be achieved by using ESCP or ESCP+LMP gel.

1. Introduction

Phosphate salts of sodium or potassium in meat products are used to increase space between meat proteins due to an increase in pH, which results in an increase of the binding ability of proteins to hold more fat and moisture. This, in turn, results in swelling of the muscle fibers and activation of proteins. Swollen and active proteins trap and immobilize water added to the meat system [1]. Hence, the processing yield, tenderness, juiciness, and water-binding capacity of meat products increase [2]. Also, phosphates can bind the Mg²⁺ and Ca²⁺, then lead to the breakdown of the calcium bridges of actomyosin complex and improve the functionality and solubility of meat proteins. Water holding, gelling, and emulsifying capacities are related quality properties of meat products such as texture, cooking losses, and flavor. These are the properties that could be improved with the changes in meat proteins stimulated by phosphates and the antioxidative effect of phosphates [2,3].

Although phosphate salts have multiple functions in meat products, studies demonstrated a good correlation between the high intake of phosphates and health problems such as reduced calcium absorption and cardiovascular and kidney diseases [4]. All these facts drew the attention of health authorities to re-evaluate the safety of phosphates as food ingredients [5]. However, salts also exist in our daily diet, and a relatively major part of the population has kidney diseases, which means these people cannot tolerate the proposed quantity of phosphates [6]. Taken all together, searching for natural ingredients as phosphate replacer has shined out as a hot research topic to meet the demands of consumers who prefer clean label meat products. Unfortunately, it is a challenge to find a complete phosphate replacer, since the related water and fat binding properties of meat products deteriorate when phosphate is removed from the formulation. Natural phosphate alternatives should be able to compensate...
for all the functions of phosphates. For this purpose, the application of blends of citrus flour and sodium carbonate [7], inulin and carbonate [8], and natural calcium powders [9,10,11] as phosphate replacers have been investigated. The use of natural calcium powders was found to successfully replace phosphate salts in meat products in terms of cooking yield and water holding capacity (WHC) [9,10]. Cho et al. [12] stated that the addition of 0.2% oyster shell calcium and 0.3% eggshell calcium to pork products resulted in less desirable textural properties. Accordingly, the combination of natural calcium powders with binding ingredients could be a more effective solution for improving quality of meat products [13]. Eggshell calcium powder (ESCP) is a byproduct obtained from calcination of eggshells through the processing of egg products [14]. Studies regarding the use of ESCP in meat products indicated that calcium powder showed ionic strength raising effects similar to phosphates [10,12].

Another functional ingredient, pectin, is used in formulations due to its gelling and stabilizing capacities. Neighboring pectin chains create a network by the connection of junction zones, and thus, high amounts of water could be entrapped in the network [15]. To the best of our knowledge, no study has been conducted to examine the combined effect of ESCP with pectin in powder form or with pectin gels in phosphate free restructured meat products. In this research, planned in the light of all aforementioned data, the effects of using two different levels of ESCP combined with low methoxyl pectin (LMP) in powder and gelled forms as phosphate replacers in restructured turkey steaks was investigated.

2. Materials and methods
Sodium tripolyphosphate (STPP), ESCP, and LMP were supplied from Pacovis Food Co. (Izmir, Turkey), Essentron Co. (Korea) and Sigma-Aldrich Co. (Istanbul, Turkey) respectively. For preparation of LMP gel, 1 gram of LMP was mixed with 10 ml of boiled distilled water and stirred until all solid matter was dissolved [16]. Control treatment (C) was prepared with 0.5% STPP. Remaining six treatments contained added 0.25% ESCP (E25), 0.50% ESCP (E50), 0.25% ESCP+ 0.25% LMP powder (EP25), 0.50% ESCP+ 0.25% LMP powder (EP50), 0.25% ESCP+0.25% LMP gel (EGP25), 0.50% ESCP+0.25% LMP gel (EGP50) as phosphate replacers. The amount of ingredients and additives were based on total amount of turkey breast meat plus water (Table 1). Breast cuts (2*3*3 cm) mixed with NaCl, STTP, ESCP, or ESCP+LMP by using a metal-tipped mixer operated with a rotation speed of 50 rpm for 5 min mixing - 5 min rest - 5 min mixing. Then, the meat mixtures were shaped in stainless steel ham molds (25x8x10 cm) and cooked at 85°C until core temperature reached 75°C. Lastly, steaks were cooled down and sliced. Sliced steaks were stored at 4°C for 7 days.

Table 1. Formulation of restructured turkey steak

| Additive/Ingredients | C | E25 | E50 | EP25 | EP50 | EGP25 | EGP50 |
|----------------------|---|-----|-----|------|------|-------|-------|
| Additive/Ingredients | NaCl | 2 | 2 | 2 | 2 | 2 | 2 |
| STTP | 0.5 | - | - | - | - | - | - |
| ESCP | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 | 0.25 | 0.25 |
| LMP powder/gel | - | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |

Moisture and ash contents of samples were determined according to AOAC [17] procedures. Fat content was analyzed according to Flynn and Bramblet [18]. Protein content of samples was determined using DUMAS method with LECO nitrogen analyzer (FP-528, USA), pH values were measured in triplicate using a pH-meter (WTW pH 3110 set 2, Germany) Color parameters of steaks were measured using a digital colorimeter (Chromameter CR 400, Minolta, Japan) to obtain the color coordinates lightness (L*), redness (a*) and yellowness (b*). Cooking yield was determined by calculating weight differences for samples before and after cooking. Fat and moisture retentions were calculated according to Murphy et al. [19] and El-Magoli et al. [20]. WHC of uncooked turkey steak was determined according to weight differences after heat treatment and centrifugation [21]. Soluble protein content (SPC) analysis was based on the reaction between dye reactive (Coomassie Brilliant Blue G-250) and
extracted proteins. SPC was expressed as mg protein/g [22]. Texture profile analysis (TPA) was performed five times for each treatment using a texture analyzer (TA-XT2, Stable Micro Systems, UK). Turkey steaks were subjected to sensory evaluation for appearance, color, juiciness, texture, flavor, and overall acceptability. Lipid oxidation was followed with Thiobarbituric Acid Reactive Substances (TBARS) analysis [23]. Purge loss was analyzed from the percentage of total weight difference between storage days. The effects of ESCP, LMP and storage on turkey steaks were determined by analysis of variance (ANOVA) and Duncan’s Post-Hoc tests in SPSS software.

3. Results and Discussion

The chemical composition of the turkey steaks is set out in Table 1. E25 had the highest moisture content (P<0.05) while C, E50, EP25, EGP25, and EGP50 had similar (lower) moisture contents. Higher moisture content was also obtained by Cho et al. [12] in restructured pork where phosphate was replaced with natural calcium powders. Steaks formulated with ESCP and LMP gel had similar protein contents as the C group. Fat, protein, and ash contents of steaks were increased by the cooking. Moisture contents of cooked steaks were similar, except E25. Similar results were previously obtained by Bae et al. [10], who showed the increasing effect of calcium powders on moisture content individually or combined using with binding ingredients. Lower fat contents were obtained in E50 and EP25. All steaks had protein content higher than 20%. Increasing ESCP level increased the ash contents of cooked steaks.

Table 2. Chemical composition of uncooked and cooked turkey steaks

| Sample | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Moisture (%) | Fat (%) | Protein (%) |
|--------|--------------|---------|-------------|---------|--------------|---------|-------------|
| C      | 75.4±0.18a   | 1.1±0.20a | 20.3±0.18a  | 3.47±0.00b| 73.39±0.41a  | 1.59±0.26a| 21.34±0.30a |
| E25    | 76.3±0.26a   | 1.17±0.12a| 19.78±0.27a | 3.07±0.01a| 72.36±0.58a  | 1.55±0.18a| 22.54±0.21a |
| E50    | 75.9±0.12a   | 1.02±0.03a| 20.34±0.18a | 3.72±0.18a| 73.14±0.16a  | 1.36±0.06a| 20.71±0.22a |
| EP25   | 75.4±0.26a   | 1.08±0.16a| 19.48±0.13a | 3.04±0.14a| 73.64±0.32a  | 1.31±0.29a| 20.87±0.08a |
| EP50   | 75.8±0.10a   | 1.29±0.00a| 19.74±0.25a | 3.17±0.11a| 72.51±0.25a  | 1.83±0.05a| 20.19±0.22a |
| EGP25  | 75.4±0.17a   | 1.18±0.15a| 20.00±0.26a | 3.03±0.01a| 73.68±0.71a  | 1.51±0.02a| 21.37±0.49a |
| EGP50  | 75.2±0.18a   | 1.16±0.05a| 20.16±0.05a | 3.11±0.03a| 73.71±0.25a  | 1.56±0.19a| 21.16±0.06a |

Means within a row with different letters are significantly different (P<0.05).

Cooking yield, WHC, pH, SPC, moisture, and fat retention values of turkey steaks are given in Table 2. The pH of the uncooked steaks decreased with the addition of ESCP and LMP (P<0.05), but there was no difference between EP25 and C. However, the pH of steaks was increased by the effect of ESCP and LMP, except for EGP50. Like our results, when ESCP was added to ground pork, pH significantly increased [12]. While the treatment containing 0.50% ESCP and LMP gel had the highest protein solubility, E25 and E50 treatments showed similar protein solubility as was shown by the C group. SPC of restructured chicken meat increased by the addition of 4.5% gelled inulin and 0.2% carbonate as phosphate alternatives [8]. Samples formulated with ESCP+LMP gel had the highest WHC, and the addition of 0.50% ESCP showed a rising effect on WHC (P<0.05) due to the pH-increasing effect of ESCP. A high WHC was achieved with the addition of 3% LMP in squid meat [24]. In our study, E50, EP25, and EGP50 had higher cooking yields than other treatments (P<0.05). It was observed that the cooking yield was increased by using ESCP alone and/or combined with pectin. Treatments containing pectin gel had the highest moisture retention (P<0.05). Gelation is the most unique property of pectin, with gel formed in the presence of Ca\textsuperscript{2+} ions or sugar and acid. Also, pectin traps the liquid by forming a three-dimensional network due to merging or cross-linking of long polymer chains [25]. The highest fat retention values were observed in the E50 and EP25 groups. Bae et al. [10] reported that using ESCP increased pH values and cooking yield in ground pork.
Table 3. pH, soluble protein content, water holding capacity and cooking properties of turkey steaks

| Sample | pH (uncooked) | pH (cooked) | SPC (mg protein/ml) | WRC (%) | Cooking yield (%) | Moisture retention (%) | Fat retention (%) |
|--------|--------------|-------------|---------------------|---------|-------------------|------------------------|-------------------|
| C      | 6.00±0.02a1 | 5.40±0.03a2 | 933.5±7.79b         | 60.3±2.04b | 82.4±6.34c         | 0.7±3.14a9          | 8.3±4.48d        |
| E25    | 5.31±0.01b1 | 4.60±0.04b1 | 1000.2±4.13a6      | 59.6±4.34a | 84.8±2.34b         | 0.7±3.14a9          | 8.7±4.34c        |
| E50    | 5.78±0.01c1 | 4.90±0.05c  | 991.4±6.31a2       | 61.4±4.40b | 85.2±2.14b         | 0.7±3.14a9          | 9.2±4.34b        |
| EP25   | 5.99±0.01d1 | 5.30±0.05d  | 979.3±6.17d        | 59.2±4.88a | 87.3±2.14b         | 0.7±3.14a9          | 9.9±4.34b        |
| EP50   | 5.90±0.01e1 | 4.20±0.03e  | 790.7±3.37e         | 61.0±6.14a | 84.8±2.34b         | 0.7±3.14a9          | 8.7±4.34c        |
| EG25   | 5.71±0.01f1 | 4.40±0.03f  | 931.6±4.82f         | 64.0±1.74c | 84.2±4.03c         | 0.7±3.14a9          | 8.0±4.00e        |
| EGP20  | 5.82±0.01g1 | 5.80±0.03g  | 1000.3±1.54f        | 63.0±4.44a | 86.9±1.87f         | 0.7±3.14a9          | 8.2±1.32d        |

Means within a row with different letters are significantly different (P<0.05).

Color and textural parameters are shown in Table 4. The addition of ESCP at a level of 0.50% lowered lightness (P<0.05). Increasing the amount of ESCP did not affect a* values; on the other hand, the addition of natural alternatives for phosphate increased a* values (P<0.05). The highest value was found in EP25. These results were likely due to the elevated pH caused by ESCP treatment. At high pH values, a lesser amount of myoglobin is likely to be denatured during cooking, which could contribute to an increase of red color [26]. Using ESCP and/or LMP in powder increased b* values, and the highest b* value was registered EP25. b* values decreased when ESPC level increased in steaks containing LMP (P<0.05). A similar result was also stated by Bae et al. [10] in ground pork meat products. However, this is not consistent with results stated by Lee et al. [27] for sausages formulated with oyster shell powder. Regarding the texture parameters, addition of both pectin forms and ESCP to the formulation decreased the hardness and increased the springiness, except in EGP25 treatment (P<0.05). The addition of mushroom powder as a phosphate alternative in sausage formulation resulted in a soft structure [12]. EGP25 had similar springiness and cohesiveness as C group. The cohesiveness values of turkey steak formulated with ESCP increased compared to C steaks due to binding properties being improved by ESCP. Gumminess of steaks was decreased when LMP was added in powder form (P<0.05). Restructured pork products formulated with potato or rice starch to replace nitrite had decreased hardness, chewiness, and gumminess values [28]. Using ESCP alone improved the chewiness values. As a result, it could be said that even ESCP alone could compensate for textural properties provided by phosphate.

Oxidation was more pronounced by the elimination of phosphate from the formulation (P<0.05). Initially the highest and the lowest TBARS values were recorded in EP25 and C, respectively (Table 5). Steaks with 0.50% ESCP individually and combined with LMP gel had TBARS values close to those of C at the beginning and end of the storage (P<0.05). All cooked steaks showed increased TBARS values when the cold storage increased (P<0.05). Cooking directly influences the extent of oxidation that develops in poultry muscle during storage. This fact shows that the incorporation of additives, except 0.50% ESCP, did not produce any antioxidant behavior during refrigerated storage. TBARS values of cooked chicken ground meat formulated without phosphate reached above 7 μmol/kg [29].

Table 4. Color and textural parameters of turkey steaks

| Sample | L*  | a*  | b*  | Hardness (N) | Springiness (mm) | Cohesiveness | Gumminess (N) | Chewiness (N/mm) |
|--------|-----|-----|-----|-------------|-----------------|-------------|--------------|-----------------|
| C      | 70.8±1.05ab | 1.69±0.29a | 8.99±0.38b | 27.1±1.09a | 0.20±0.02c | 0.19±0.01c | 5.4±2.04c | 1.3±0.08d |
| E25    | 71.79±1.49b1 | 3.32±0.41b2 | 9.58±0.23a2 | 27.8±0.78b | 0.26±0.04b | 0.24±0.02b | 6.19±0.19b | 1.60±0.33b |
| E50    | 69.82±0.58c1 | 3.01±0.26c2 | 9.36±0.21c4 | 24.8±1.30a | 0.31±0.03a | 0.28±0.02a | 6.9±0.48a | 2.1±0.04a |
| EP25   | 70.79±1.76b2 | 5.58±0.42a2 | 10.24±0.34d | 17.7±1.17d | 0.31±0.02b | 0.24±0.01b | 4.0±0.65d | 1.59±0.34a |
| EP50   | 71.45±1.13b2 | 3.36±0.36b2 | 9.47±0.34b2 | 20.8±2.00a | 0.28±0.03b | 0.24±0.02b | 4.4±0.59d | 1.4±0.12b |
| EGP25  | 71.13±0.34ab | 3.13±0.34bc | 9.54±0.26b2 | 24.7±2.81a | 0.21±0.03b | 0.19±0.02a | 4.7±0.16c | 1.0±0.06b |
| EGP50  | 71.08±0.68c2 | 2.83±0.36d2 | 8.97±0.14b2 | 20.7±2.08b | 0.29±0.03b | 0.26±0.03b | 5.9±0.94b | 1.7±0.38a |

Means within a row with different letters are significantly different (P<0.05).
The highest purge loss was found in E50 (Figure 1), while EP25 had the lowest on day 3 (P<0.05). Purge losses of steaks decreased then increased throughout the whole storage (P<0.05). On day 7, the lowest losses were observed in EP25 and EP50 that were formulated with ESCP+LMP powder (P<0.05). None of the reformulated steaks had higher purge losses than the C group. No statistical differences were found between treatments in terms of color or flavor (Figure 2). The addition of LMP in powder form resulted in a decrement in appearance and juiciness; however, LMP gel increased juiciness scores (P<0.05). EP25 and EP50 steaks had lower texture and appearance scores than other treatments (P<0.05). Tabak et al. [11] showed combined use of eggshell powder with pectin or carrageenan enhanced the technological and sensory qualities of phosphate free chicken patties. Utilization of ESCP alone or in combination with LMP gel did not negatively affect product quality. Consequently, panelists gave lower acceptability scores for EP25 and EP50 steaks (P<0.05).

4. Conclusion
The results of this study showed that using EGSP and pectin gel in combination is a promising natural additive that can be used in phosphate free meat product formulations. Protein solubility and juiciness increased with the use of ESCP in combination with pectin gel. Pectin in powder form resulted in lower sensory scores. However, none of the combinations were able to retard oxidation, and therefore, further studies should focus on the use of natural antioxidants in phosphate free restructured meat products.

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