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# Augmentation of Meat Quality Attributes of Spent Hen Breast Muscle (*Pectoralis Major)* by Marination with Lemon Juice vis-a-vis Ginger Extract

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**ABSTRACT**

A study was conducted with the aim of augmentation of post harvest quality attributes of spent hen breast meat with lemon juice (LM) vis-a-vis ginger extract (GM) marination. Moisture content and water holding capacity (WHC) significantly (P<0.05) increased whereas, protein, fat, ash, cholesterol, muscle fibre diameter, firmness and toughness values significantly (P<0.05) decreased. Percent marinade absorption and pH values were significantly (P<0.05) higher in GM and significantly (P<0.05) lower in LM compared to Con. LM showed significant (P<0.05) reduction in Na and increase in S, Si and Cl whereas GM showed increase in S, Ca, K, Zn, Cu and Fe along with reduction in Na and Cl content when compared to Con. Marination improved firmness and toughness and was corroborated by endomysium degradation seen in Scanning Electron Microscopy (SEM) studies. Hence, in light of improving the quality attributes of spent hen breast meat; LM and GM could be effectively used.

**Keywords:** Cholesterol, collagen, fat, sodium, toughness

Spent hen meat is obtained from old and culled chicken, well past their productive and reproductive phase of life, as a by-product of egg industry. It is high in fat and cholesterol content, less tender, less juicy and poor in functional characteristics because of increased cross-linking in collagen, resulting in lower remunerative prices (Kumar *et al*., 2015). Muscles from spent hen are less tender than those from broiler chicken and hence are classified as low value meat with due reason of at maturity; collagen develops the cross links that results in the formation of rigid three dimensionally architecture collagen fibers of considerable tensile strength. Such structure probably prevent the unfolding of peptide chain at the protein water interface within the collagen fibers, thus reduce the number of hydrophilic protein interaction. Meat with this type of collagen, which has retained its integrity, when cooked contributes to the exudation through its shrinkage as it compress the muscle fibers. Therefore, there is extensive interest of meat technologists in developing strategies to improve quality attributes of low value spent hen meat. The methods used for improving quality attributes of tough meat are through mechanical interventions (Pietrasik and Shand, 2005), heating (Christensen *et al*., 2004), high pressure (Macfarlane, 1985), marination (Sheard *et al*., 2005), addition of proteolytic enzymes (Naveena and Mendiratta, 2004) and citric acid (Ke *et al*., 2009) in formulation.

Marination is a traditional process used to improve the quality attributes of meat; which involves the immersion of meat in marinade solution. It is the process of soaking or injecting meat with a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids for flavouring and tenderize meat. Marinades with a tenderizing capacity are important in applications

involving tough muscles, which often render the cheaper meat cuts obtained from older animals more tender and thus tenderizing effect of marinating offers a commercially important tool of upgrading them. Widespread ingredients in marinating solutions are sodium chloride, phosphate, organic acid and enzymes. Acid marination affects tenderness of tough meat in many ways such as pH-induced swelling of muscle fibers and/ or connective tissue; accelerating or additional proteolytic weakening of muscle structure and increasing solubilization of collagen upon heating. Enzymatic proteolysis enlarges the space between actin and myosin filaments, which allows more water to be retained in the muscle (Lawrie, 1991).

Lemon (*Citrus limon*) is a small evergreen tree native to Asia, with ellipsoidal yellow fruits. The fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice, though the pulp and rind/zest are also used in cooking and baking. The juice of the lemon contains about 5-10% citric acid, which plays a very important role to increase tenderness and juiciness of meat and meat products. Previous studies reported that citric acid as a food acidulant is often used in meat marination to improve the water holding capacity and tenderness of beef muscle (Ke *et al*., 2009). The mechanism of the tenderising action of acidic marinades is revealed to involve in the weakening of structures due to swelling of the meat and increased conversion of collagen to gelatin at low pH during cooking (Berge *et al*., 2001). The acid breaks the transversal bounds of collagen, leading to the unstable structure loss of this connective tissue protein. Many studies bespoke out that the low meat pH after the marination has positive effects on the texture, increasing the water holding capacity and the moisture content.

A protease enzyme "Zingibain" isolated from *Zingiber officinale roscoe* (Ginger rhizome) has been reported to have proteolytic activity. Its proteolytic activity on collagen was found to be many folds greater than on actomyosin, and the combined proteolysis of these two muscle proteins improved the tenderness of meat (Abdeldaiem and Hoda, 2013). They have also pointed that "Zingibain" is the main protease associated with the tenderization effect of ginger extract on camel meat. GP-I and GP-II are the two main proteases in ginger rhizome. GP-I and GP-II have homologies with actinidin, glycyl endo-peptidase, omega, and papain (Choi and Laursen, 2000). The tenderization effect of ginger extract mainly results from its degradation of myofibrillar proteins such as myosin heavy chain (MHC), troponin-T, α-actinin, and desmin of duck breast muscle (Tsai *et al*., 2012).

Insight of above facts, the present study was conducted with the aim of development and standardization of household technological intervention for improvement of quality attributes of spent hen by utilization of vegetative extracts from lemon and ginger.

## MATERIALS AND METHODS

### Raw materials and preparations

The spent/culled White Leghorn hens of same age, sex and feeding group above 52 weeks of age were procured from Instructional Poultry Farm, GBPUA & T, Pantnagar, India. Spent hens were slaughtered and dressed as per methods approved by College Ethics Committee. The dressed carcasses were kept in a refrigerator at 4 ±1° C for 4 hours. Thereafter, the filleting of breast was carried out; meat was cut into one inch cubes and marinated in concern marinade solution. Lemon and ginger were procured from local market and used. All the chemicals used in the study were of analytical grade and purchased from standard firms (Hi media® and Merck®).

### Preparation of marinade solution

200 ml fresh lemon juice (Citric acid concentration 10.64±0.672%, determined as per procedures described by AOAC, 1995) was diluted with 800 ml chilled distilled water (4±1° C) to prepare 20% concentration of lemon juice marinade (pH 3.28±0.368).

Fresh ginger rhizome was washed, peeled, sliced and immediately blended with equal quantity of chilled distilled water (4±1° C) for 1-2 min, then filtered through two layers of muslin cloth. The filtrate was centrifuged for 10 minutes at 3000 rpm at 4±1° C and the supernatant (pH 6.78±0.564) was collected as the ginger extract.

### Marinating and ageing

Boneless lean meat cubes of one inch size were randomly assigned to three treatments viz. Con, LM and GM. The meat cubes were immersed in marinade solution in a plastic bowl in 2:1 ratio (w/v), covered with a lid and

kept for 16 hours at 4±1°C in refrigerator. Chilled distilled water (4±1ºC) @ 1:2 ratio (v/w) was used to immerse meat sample of control group.

**Physico-chemical properties estimation**

**Marinade absorption**

To determine the marinade absorption, control and marinated meat samples were weighed before and after marination. The marinade absorption was calculated as:

$$\text{Marinade absorption (\%)} = \frac{\text{Weight of sample after marination (g)} - \text{Weight of sample before marination (g)}}{\text{Weight of sample before marination (g)}} \times 100$$

**pH**

The pH of homogenized representative samples was recorded by using a digital pH meter (WTW®, Germany, Model 330i fitted with Sen Tix sp electrode) by immersing the electrode of pH meter into aliquot of the sample.

**Proximate composition**

Moisture, fat, protein and ash content of meat samples were determined by as per procedures of Association of Official Analytical Chemists (AOAC, 1995) using Hot air oven, Soxhlet apparatus, Micro-Kjeldhal apparatus and Muffle furnace, respectively.

**Cholesterol content**

Total cholesterol was determined as per procedure described by Rajkumar *et al.* (2004).

**Water holding capacity (WHC)**

Water holding capacity (WHC) was determined as per procedure described by Wardlaw *et al.* (1973).

**Firmness and Toughness value**

Firmness and toughness values were determined as force required for shearing 2 × 1 × 1 $cm^3$ meat sample measured on Warner-Bratzler Shear force (Hot dog shearing probe SAU1, WBB, PRJ, using Stable Micro System, Model TA.XT2i/25, UK).

**Histological Study**

Histological study was conducted by a scanning electron microscope (JEOL JSM-6610LV). Spent hen breast muscle (Pectoralis major) samples (1×1 × 0.5 $cm^3$) were cut and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 2 h at room temperature. The specimens were rinsed with 0.1 M phosphate buffer and dehydrated in graded ethanol solutions with a serial concentration of 25%, 50%, 75%, 95% and 100% (v/v). The dehydration was conducted for 1 hour in each solution and dried with liquid $CO_2$ at the critical point for 60-90 minutes by using EMITECH K850**.** Dried samples were mounted on a bronze stub. Finally, they were coated with gold by using JEOL JFC-1600 auto fine coater and observed under a scanning electron microscope. The specimens were observed for cross sectional area and degradation of endomysial collagen of the muscle fibre at an acceleration voltage of 25 kV and a magnification of 500X.

**Mineral analysis**

Mineral analysis of control and marinated spent hen breast muscle (Pectoralis major) samples was done by energy dispersive X-ray spectra using a scanning electron microscope (JEOL JSM-6610LV). Muscle samples (1×1×0.5 $cm^3$) were cut and dried with liquid $CO_2$ at the critical point for 60-90 minutes by using EMITECH K850. After that samples were placed in vaccum chamber (BRUKER127eV) and high density electron beams were passed through them, which produced specific X-ray for specific element. The specific X-ray was detected by attached semiconductor probe, which analyses the concentration of minerals present in the samples.

**Statistical analysis**

The statistical design of the study was 3(treatment) × 6(replication) completely randomized design. Samples were analysed in duplicate leading to total observations 12 (n = 6 × 2 = 12) for various physico-chemical attributes of spent hen breast meat. Ten observations were recorded to firmness and toughness, histological study and mineral

analysis in each replication leading to total observations 60 (n = 10 × 6 = 60). To analyse the effect of marination with lemon juice vis-a-vis ginger extract on meat quality attributes of spent hen breast considered in this study, all variables were submitted to an analysis of variance considering one fixed factor (2 Marinated groups × 1 Control). Duncan's multiple range test and critical difference were determined at the 5 per cent significance level (Snedecor and Cochran, 1995).

## RESULTS AND DISCUSSION

The mean values of physico-chemical attributes of control and treatment groups are expressed in Table 1. The per cent marinade absorption significantly (P<0.05) differed among treatments. Marinade absorption was observed to be highest for GM and lowest for LM among all the treatments and the difference might be due to differences in osmotic pressure exerted by different marinade solutions. The mean values of pH differed significantly (P<0.05) among the treatments. pH was observed to be highest in GM among all the treatments. These results of pH were in agreement with Ke *et al*. (2009) and Naveena and Mendiratta (2004).

Marination significantly (P<0.05) increased moisture content compared to Con. However, no significant (P>0.05) difference was observed in moisture content of spent hen breast meat between LM and GM. Increase in the moisture content might be attributed to marinade absorption. Naveena and Mendiratta (2001) also reported similar finding and they found that moisture content tended to be higher in 3% ginger extract treated spent hen meat chunks compared to control. The protein content differed significantly (P<0.05) among treatments. Protein content was found to be highest in Con and lowest in GM. Decrease in the protein content could be due to increased moisture content. Further, it could also be attributed to degradation of collagen and myofibrillar proteins by the action of citric acid and Zingibain enzyme. The fat content significantly (P<0.05) differed among treatments. The decrease in fat content in LM and GM treatment groups might be due to absorption of marinade solution. Significant (P<0.05) differences were observed in the ash content among treatments. The ash content of Con group was found lowest and highest in LM among all treatments.

The cholesterol content of Con was revealed highest among all the treatments. The cholesterol content significantly (P<0.05) differed among the treatments. The cholesterol content of LM group was found to be lowest among all the treatments. The reduction in cholesterol content could be due to decreased fat content of LM and GM groups. There was no significant (P<0.05) difference observed in WHC of spent hen breast meat between LM and GM treatment groups but they were significantly (P<0.05) higher than the Con group.

The mean values of firmness and toughness for control and treatment groups are presented in Table 1. The firmness

**Table 1:** Effect of marinaion with lemon juice and ginger exract on various physico-chemical attributes of spent hen breast meat

| Parameter | Con | LM | GM |
|---|---|---|---|
| Marinade Absorption (%) | 10.87±0.09$^{b}$ | 10.24±0.42$^{c}$ | 15.67±0.11$^{a}$ |
| pH | 6.21±0.06$^{b}$ | 4.59±0.10$^{c}$ | 6.51±0.07$^{a}$ |
| Moisture (%) | 71.18±0.90$^{b}$ | 75.38±0.70$^{a}$ | 75.57±0.61$^{a}$ |
| Protein (%) | 68.21±0.09$^{a}$ | 66.27±0.11$^{b}$ | 65.73±0.20$^{c}$ |
| Fat (%) | 25.24±0.06$^{a}$ | 24.13±0.49$^{c}$ | 24.93±0.26$^{b}$ |
| Ash (%) | 7.53±0.08$^{b}$ | 8.92±0.26$^{a}$ | 8.86±0.13$^{a}$ |
| Cholesterol (mg %) | 109.61±0.76$^{a}$ | 97.93±0.35$^{c}$ | 106.43±0.37$^{b}$ |
| WHC (%) | 41.49±0.24$^{b}$ | 42.88±0.55$^{a}$ | 42.96±0.59$^{a}$ |
| Firmness (N) | 3.93±0.14$^{a}$ | 1.60±0.67$^{c}$ | 2.29±0.53$^{b}$ |
| Toughness (N.s) | 11.49±0.61$^{a}$ | 3.61±0.38$^{c}$ | 5.12±0.19$^{b}$ |
| Muscle Fibre Diameter (µm) | 81.27±1.74$^{a}$ | 69.38±0.76$^{b}$ | 69.50±1.45$^{b}$ |

n= 12; Values bearing different superscripts in same row differ significantly (P<0.05).

**Table 2:** Effect of marinaion with lemon juice and ginger exract on mineral contents of spent hen breast meat

| Mineral | Con | LM | GM |
|---|---|---|---|
| Na (%) | 2.05±0.19$^{a}$ | 1.39±0.17$^{b}$ | 1.07±0.11$^{c}$ |
| S (%) | 0.74±0.10$^{c}$ | 0.95±0.07$^{a}$ | 0.84±0.08$^{b}$ |
| Ca (%) | 0.17±0.05$^{b}$ | 0.16±0.04$^{b}$ | 0.27±0.06$^{a}$ |
| K (%) | 0.17±0.03$^{b}$ | 0.19±0.06$^{ab}$ | 0.20±0.05$^{a}$ |
| Cl (%) | 0.08±0.02$^{b}$ | 0.20±0.07$^{a}$ | ND |
| P (%) | 0.06±0.02$^{b}$ | 0.08±0.03$^{b}$ | 0.12±0.05$^{a}$ |
| Si (%) | ND | 0.06±0.02$^{a}$ | 0.04±0.01$^{b}$ |
| Al (%) | ND | 0.03±0.01 | ND |
| Zn (%) | 0.04±0.01$^{b}$ | 0.06±0.02$^{b}$ | 0.57±0.11$^{a}$ |
| Cu (%) | 0.08±0.02$^{b}$ | 0.07±0.02$^{b}$ | 0.47±0.08$^{a}$ |
| Fe (%) | 0.07±0.02$^{b}$ | 0.09±0.03$^{b}$ | 0.33±0.05$^{a}$ |

n= 12; Values bearing different superscripts in same row differ significantly (P<0.05);

ND- Not Detected.

significantly (P<0.05) differed among treatments. The values were found to be highest for Con and lowest for LM. Significant (P<0.05) difference was observed in the toughness among treatments. The highest toughness was observed in Con at 11.49±0.61 N.s and the lowest toughness was observed in LM at 3.61±0.13 N.s. Naveena and Mendiratta (2001) reported decrease in shear force values in ginger extract treated spent hen cooked chunks and attributed to increased collagen solubility and protein extractability. Similar findings were reported by Burke and Monahan (2003), who found that marination with the citrus juice marinade led to a reduction in WBSF values of shin beef.

The mean values of muscle fibre diameter of control and treatment groups are expressed in Table 1. The muscle fibre diameter measured using SEM for Con, LM and GM were 81.27±1.74 µm, 69.38±0.74 µm and 69.50±1.45 µm, respectively. There was no significant (P<0.05) difference in muscle fibre diameter of spent hen breast meat between LM and GM treatment groups but they were significantly (P<0.05) lower than the Con group. Muscle fibre diameter results of GM group were similar to the findings of Naveena and Mendiratta (2004), who observed that muscle fibre diameter values of buffalo meat were slightly lower in 5% ginger extract marinated samples as compared to control group. Zochowska-Kujawska *et al*. (2012) reported a significant reduction in muscle fibre diameter of wild boar and deer longissimus muscle, when treated with 10% lemon juice marinade. The reduction of muscle fibre diameter in LM and GM might be due to degradation of endomysial collagen, sarcolemma and myofibrillar proteins by proteolytic activity of marinade solutions.

Disintegration of myofibrillar structure with lot of exudate and increased interfibrillar space or appearance of voids between muscle fibers was observed in higher magnification 500X (Microphotograph 1, 2 and 3). The degradation of endomysial collagen and sarcolemma surrounding muscle fibers could be observed much more in LM and GM samples as compared to Con. The results of endomysium degradation were in agreement with Zochowska-Kujawska *et al*. (2012). They reported a significant reduction in endomysium diameter surrounding wild boar and deer longissimus muscle, when treated with 10 % lemon juice marinade.

**Mineral analysis**

The mean values of mineral content of dried control and marinated groups are shown in Table 2. The sodium and sulphur content of spent hen breast meat significantly (P<0.05) differed among treatments. Calcium, phosphorus, zinc, copper and iron content were significantly (P<0.05) higher in GM than Con and LM. Significant (P<0.05) difference in potassium content was observed between Con and GM but LM did not differ significantly (P<0.05)

from Con and GM. Chlorine was not detected in GM treated samples of spent hen breast meat but a significant ($P<0.05$) difference was observed between Con and LM treatment groups. Silica content was not detected in Con, but a significant ($P<0.05$) difference in silica content was observed between LM and GM. The study did not find aluminium in Con and GM but LM was found to contain 0.02±0.003%. USDA (2014) detailed that mineral composition of ginger rhizome (per 100 g) as Ca 16 mg, Fe 0.60 mg, Mg 43 mg, P 34 mg, K 415 mg, Na 13 mg, Zn 0.34 mg, Cu 0.226 mg, Mn 0.229 mg and Se 0.7 µg and the mineral composition of lemon per 100 g as Ca 26 mg, Fe 0.60 mg, Mg 8 mg, P 16 mg, K 138 mg, Na 2 mg, Zn 0.06 mg, Cu 0.037 mg, Mn 0.030 mg and Se 0.4 µg. These differences in mineral composition might have contributed to the differences observed in mineral analysis in the present study.

## CONCLUSION

Results of this study showed that LM and GM can be used to improve tenderness and juiciness of spent hen breast meat. Mineral analysis showed an increase in iron and zinc content in GM and reduction of sodium in LM and GM treatment groups compared to Con. SEM studies revealed extensive endomysial degradation in LM and GM marination. Therefore, lemon juice and ginger extract as marinade can be exploited to improve of spent hen meat quality along with some nutritional benefits to consumers viz. lowering of fat, cholesterol and sodium content, improving the tenderness and juiciness.

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