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## Effect of Partially Purified Ginger Enzyme and Commercially Available Papain on Quality of Spent Hen Meat

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### ABSTRACT

#### Keywords

Ginger enzyme, Meat quality, Microbial quality, Papain, Spent hen meat

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A study has been carried out to determine the partially purified ginger enzyme (GE) and commercially available papain in combination with curing agent on qualities of spent hen meat. The meat chunks were marinated for 3 hrs at room temperature and analysed for physico-chemical and microbial qualities. The pH values did not show any significant difference between the treatments. Water holding capacity and cooking yield were significantly ( $p < 0.01$ ) affected by the treatments. The collagen solubility was significantly ( $p < 0.01$ ) affected by treatment. The shear force value of GE and papain treated samples were lower ( $p < 0.01$ ) than control spent hen meat. There was also more reduction in the number of protein bands in the samples treated with GE and papain. Microbial counts were lower ( $p < 0.01$ ) in GE and papain treated samples. It is concluded that GE has the potential commercial applications to explore in the meat processing industry to improve the qualities of spent hen meat.

### Introduction

Meat tenderness is the most important palatability attribute affecting consumer's overall eating experience (Lawrie, 1991; Dikeman, 1987). Poultry meat production has increased all over the world.

Layer chickens after about 72 weeks of laying, are sacrificed to produce meat which is available at a cheaper rate. As the animal matures, fiber hypertrophy is accompanied by maturation of the endomysium, perimysial thickness, and the formation of nonreducible cross-links between the collagen molecules

(Robins *et al.*, 1973). The inferior quality, such as toughness in spent hen meat, is primarily due to increased cross-linking in the connective tissue of older animals (Bailey and Light, 1989). Many attempts have been made to tenderize spent hen meat (Kondaiah and Panda, 1992; Woods *et al.*, 1997; Naveena and Mendiratta., 2001; Bhaskar *et al.*, 2006; Vaithyanathan *et al.*, 2008).

Ginger is a rhizome widely used as spice in a variety of food products in general, and particularly in meat based foods. Ginger helps to enhance the flavour of the product. Ginger also has antimicrobial (Sazler, 1982) property

that helps to extend the shelf life of a product (Kim and Lee, 1995; Syed Ziauddin *et al.*, 1996) and also has antioxidant (Lee *et al.*, 1986 a; Mendiratta *et al.*, 2000) property. Ginger has also been shown to have a powerful proteolytic activity (Choi *et al.*, 1999) and this property is useful in improving the tenderness of tough meat (Lee *et al.*, 1986b; Naveena *et al.*, 2004). Recently two cysteine proteases have been isolated, purified and characterized from ginger rhizome (Kim *et al.*, 2007; Choi *et al.*, 2000). Commercial applications of these enzymes in meat processing industry need further investigations.

Many authors have held ginger extract treated muscles for periods of 24 h (Mendiratta *et al.*, 2000), 48 h (Naveena *et al.*, 2004) or 5 days (Syed Ziauddin *et al.*, 1995) at a low temperature of around 4°C and such a long period were needed as the enzyme activity is lower at refrigerated temperature and also only the crude extract was used. Bhaskar *et al.*, (2006) used ginger powder for treatment of spent hen muscles held for 3 h at ambient temperature. The tenderizing effect of a protease can be relatively hastened at an ambient temperature of  $29 \pm 2$ °C requiring shorter period of time provided the enzyme preparation is relatively pure enough to specifically act upon the protein to give the desired effect. The reports of Mendiratta *et al.*, (2000); Naveena *et al.*, (2004) and Syed Ziauddin *et al.*, (1995) have also suggested that ginger extracts showed a greater proteolytic activity towards collagen than actomyocin and showed increased collagen solubility and reduced shear values which resulted in improved tenderness of ginger marinated meat.

Devitre and Cunningham (1985) reported that the fillets soaked in solutions of 1% sodium chloride and phosphate plus either papain, bromelin, or ficin were significantly more

tender than all other treatments. Also mentioned those treated with sodium chloride plus phosphate plus papain were the most tender. Therefore the aim of the present work has been to determine combination of partially purified ginger enzyme with a curing agent on qualities of spent hen meat. The ability of papain on qualities of spent hen meat was also compared with these enzymes.

## **Materials and Methods**

Fresh meat was obtained from spent hens slaughtered by the traditional halal method. The carcasses were deboned manually in a commercial poultry processing unit and brought to the lab within half an hour. Meat samples were stored at 4°C for approximately 4 h before treatments. Fresh meat samples were obtained separately for each of the five replications.

### **Enzyme purification**

Ginger enzyme was purified as described by Kim *et al.*, (2007). Briefly, fresh ginger rhizome was blended in 0.1 M sodium phosphate buffer (pH 7.0) containing 5 mM EDTA (Ethylene Diamine Tetra Acetic acid) and STT or 10 mM cysteine for 3 min. The pH was adjusted with 2M NaOH to 7.0 and the mixture was stirred for 30 min followed by filtering through two layers of cheese cloth.

The solid residue was precipitated by ammonium sulfate (enzyme grade). After 1 h standing, the mixture was centrifuged at 1000xg for 30 min at 4°C and supernatant was filtered through celite (Diatomaceous earth as SiO<sub>2</sub>, Sigma) to remove suspended materials. Solid ammonium sulfate was added again to the filtrate to bring its concentrate to 60 % saturation. After centrifugation, the resulting precipitate was suspended in a minimum amount of buffer A (30 mM sodium phosphate buffer, pH 7.0 containing 5 mM STT and 1

mm EDTA) and dialysed against 4 changes of 41 of 0 mm sodium phosphate buffer, pH 7.0 containing 1 mM STT and 1 mM EDTA for 16 h. The dialysed solution was centrifuged at 10000xg for 45 min at 4o C. The supernatant was lyophilized. The lyophilized powder was used in the experiment after ascertaining its proteolytic activity.

### **Proteolytic activity assay**

Assay was performed by incubating 2 ml of 1 % casein solution in 0.1 acetate buffer (pH 5.5) containing 2 mM EDTA with purified enzyme containing 2 mM DTT. After 15-min incubation at 50°C, 2ml of 5% (w/v) Trichloro Acetic Acid (TCA) was added to terminate the reaction followed by centrifugation at 2500 x g for 10 min. The absorbance of TCA –soluble product was measured at 280 nm and one unit of caseolytic activity is defined as the amount of enzyme causing an increase in absorbance by 1 unit.

### **Experimental design**

The experimental design is presented in Table 1. Fresh meat samples were obtained separately for each of the five replications. After preparation of marinating solution the meat chunks were marinated with this solution in the ratio of 3:1 (Meat: Marinating solution) and then kept for 3 hrs at room temperature.

### **Analysis of meat samples**

Evaluation of meat quality parameters were made on breast muscle: pH, Water Holding Capacity (WHC), hydroxyl proline content, collagen solubility, muscle fibre diameter, proximate composition, electrophoretic pattern of muscle and microbial quality were evaluated in raw meat after 3 h treatment at ambient temperature. Cooked samples were evaluated for cooking yield, pH, shear force values and sensory attributes.

### **pH**

The pH of the samples was determined by blending 10 g of sample with 50 mL of distilled water for 60 s in a homogenizer (MICCRA D8-Si, ART-moderne Labortechnik, Mullheim, Germany). The pH values were measured using a standardized electrode attached to a digital pH meter (Thermo Orion model 420A+, Beverly, MA).

### **Shear Force Value**

The cooked samples were chilled at refrigerator temperature overnight and used for objective determination of tenderness (after equilibration at room temperature).

Shear Force Value (SFV) were estimated in triplicate with a Warner-Brazler blade attached to a texture analyzer. The crosshead speed was 2mm/s. The Warner–Brazler Shear Force (WBSF) was measured in 21 cores of 1-cm<sup>3</sup> sizes with fibres perpendicular to the direction of the blade (Model no. 81031307, GR Elect. Mfg. Co., USA). The force required to shear the samples was recorded (N/cm<sup>2</sup>).

### **Water holding capacity**

Water Holding Capacity (WHC) was determined according to Wardlaw, Maccaskill, and Acton (1973). Minced meat (20 g) was placed in a centrifuge tube containing 30 ml of 0.6 M NaCl and was stirred with glass rod for 1 min. The tube was then kept at 4 ± 1 °C for 15 min, stirred again and centrifuged at 3000g (R-24, Remi Instruments, India) for 25 min. The supernatant was measured and WHC was expressed in percentage.

### **Cooking yield**

The weight of meat was recorded before and after cooking and the yield was expressed as percentage

Cooking yield = Weight of cooked meat / Weight of raw meat × 100

### **Muscle fibre diameter**

Muscle fibre diameter of meat samples were assessed according to the method outlined by Jeremiah and Martin (1982). Five grams of minced meat sample was homogenized in tissue homogenizer. At low speed for two 15 s periods interspaced with a 5 s resting interval in a 30 ml solution containing 0.25 M sucrose and a mM EDTA to produce a slurry. One drop of the slurry was then transferred on to a glass slide and covered with a cover slip and the suspension was examined directly under a light microscope with 10X objective and eye piece equipped with calibrated micrometer. Muscle fibre diameter was measured as mean diameter of the middle and two extremities of 25 randomly selected muscle fibres and expressed in micrometer.

### **Hydroxyproline estimation**

Hydroxyproline content of the meat sample was determined based on the procedure of Nueman and Logan (1950). Two grams meat samples were hydrolyzed with 40 ml of 6 N HCl for 18 h.

The hydrolysate was filtered and the volume adjusted to 50 ml with distilled water. An aliquot was used for hydroxyproline estimation. Absorbance was measured at 540 nm and the hydroxyproline content was determined by referring to a standard graph. Collagen content was calculated by multiplying by 7.14 and was expressed in mg/g tissue.

### **Proximate composition**

Moisture, crude protein, fat and ash content of meat samples were determined by the AOAC (1995) method.

### **Electrophoresis**

Protein samples were subjected to Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (Sreeramulu and Singh, 1995; Lamlli, 1970) for qualitative separation of polypeptides. Five grams of minced meat was mixed with 50 ml of 0.01 N sodium phosphate buffer containing 1 % SDS and 1% 2-mercaptoethanol and incubated at 37°C for 2 h and then centrifuged at 1500g for 30 min. An aliquot of supernatant was dialysed over night at room temperature (26°C) against 0.1 N sodium phosphate buffer containing 0.1% 2-mercaptoethanol.

About 50 µl of dialysed solution was mixed with equal volumes of sample buffer pH 7.4 (consisting of 15.14g Tris, 20g SDS, 200g glycerol and 0.02g bromophenol blue per litre buffer) after determining the protein concentration (Lowry *et al.*, 1951) for loading onto the stacking gel. Just before the use of sample buffer 50 µl 2-mercaptoethanol was added to each 0.95ml stock sample buffer. The gel (1.0 x 100 x 1000mm) consists of 4.54g Tris pH 8.8, 0.12g SDS, 29.2 g acrylamide, 0.8g bisacrylamide per 100 ml. Ammonium persulphate (0.0175g) and TEMED (40µl) was added for polymerization. The stacking gel was 5% and the separating gel was 12%. The samples were subjected to electrophoresis after keeping it in boiling waterbath for 3 min before loading onto the gels. Electrophoresis was run initially at 50v until the dye entered the stacking gel and then it was run at 80v until the dye reached 5mm before the bottom of gel.

After electrophoresis, the gels were stained overnight with a staining solution containing TCA 60g, acetic acid (60ml), methanol (180ml) and Coomassie brilliant blue R-250 (0.25%) in 1000ml distilled water and destained with 3% NaCl in distilled water until the bands are clearly visible.

### **Microbial quality evaluation**

Three replicates from each treatment were used in microbial quality evaluation. Ten grams of meat was homogenized with 90 ml, 0.1% sterile peptone water using a Stomacher (Seeward, 400, Circulator, UK) and 10 fold serial dilutions (using 0.1% peptone water) were made before inoculating (pour plate method) into plate count agar for total plate counts (APC). For each dilution, duplicate plates were incubated at 37 °C for 48 h for APC (APHA, 1984).

### **Statistical analysis**

Data on pH, water holding capacity, collagen content, collagen solubility (%), muscle fibre diameter, moisture, protein, fat, cooking yield, cooked pH, shear force value, total plate count, lactic acid bacteria, yeast and mould and enterobactor variables were analyzed with treatment as the main effect using the mathematical model given below in a 1-way ANOVA procedure of SPSS 10.0 (SPSS Inc., Chicago, IL). If a value of  $P < 0.05$  was detected, differences among means were tested separately with a Bonferroni test:

$$Y_{ijk} = \mu + D_i + e_{ijk}$$

Where  $\mu$  = general mean,  $D_i$  = effect of  $i$ th treatment and  $E_{ijk}$  = random error.

### **Results and Discussion**

In the traditional meat preparation, the condiments are used to increase the flavor and appetite. One of the most important condiments is ginger rhizome which is made into paste along with other condiments and used in the marination of the meat. In addition to that, salts of chloride and phosphates are also used to improve the functional properties of meat during comminuted meat preparation. Results of various biochemical and

physicochemical parameters analyzed are presented in Table 2.

### **pH and WHC**

The pH values varied from 6.08 to 6.18. There was no significant difference in the pH of the treatments. It is in agreement with observation by Naveena and Mendiratta (2004) in buffalo meat treated with ginger extract, but in disagreement with result of Naveena and Mendiratta 2001 with ginger extract and Khanna and Panda (2007) with papain treated spent hen meat. They have observed that higher pH was in treated samples. This result may be due to tripoly phosphate content in all the treatments, although the value was slightly higher for ginger treated sample compared to control.

Water Holding Capacity (WHC) ranged from 38.70 to 47.00 (%) which were significantly ( $p < 0.01$ ) different between the samples (Table 2). GE treated sample had higher WHC than control which are in agreement with Naveena and Mendiratta (2001). WHC of papain treated sample was in between the broiler and ginger treated samples. Khanna and Panda (2007) reported increased WHC of spent hen meat treated with papain and Naveena *et al.*, (2004) reported similar results in buffalo meat treated with ginger extract. Compared to broiler meat, the treated sample had lower WHC, which may be due to meat from very old animals that have lower WHC (Syed Ziauddin, 1994).

### **Collagen content and solubility**

The total collagen content which is an important parameter in the spent hen meat varied from 2.42 to 5.73 (mg/g) (Table 2). There was highly significant ( $p < 0.001$ ) difference between the collagen content of broiler and spent hen meat. Spent hen meat having higher collagen content, Bailey (1984)

also reported that increased collagen content and cross linkages in spent hen meat. There were no significant difference between collagen content of control and treated spent hen meat samples. The collagen solubility (%) ranged from 17.10 to 21.37.

A highly significant ( $p < 0.01$ ) difference between the collagen solubility of spent hen meat, spent hen meat treated with proteolytic enzymes and broiler meat was observed. It is agreed with results of spent hen meat treated with Ginger Extract by Naveena and Mendiratta (2001). The Collagen solubility were higher ( $p < 0.01$ ) for GE and papain treated samples compared to untreated. Increase in collagen solubility by GE treatment was also observed by Thompson *et al.*, (1973) and Naveena and Mendiratta (2001).

They reported that proteolytic activity of ginger protease on collagen was many times greater than that on actomyosin and the combined proteolysis of these two muscle proteins resulted in significantly more tender meat. The solubility of connective tissue rather than total amount of connective tissue is more highly associated with sensory characteristics (Crouse *et al.*, 1985).

### **Proximate composition**

The moisture content of broiler chicken muscle was higher ( $p < 0.01$ ) than spent hen meat and the moisture varied from 77.62 to 76.51 (%). Chuaynukool *et al.*, (2007) also reported the same. But moisture content of the treated samples were higher ( $p < 0.01$ ) than control. The protein content of meat sample varied from 19.43 to 19.63 (%) and the protein content of the sample had no significant difference. However, the broiler samples had higher ( $p < 0.01$ ) fat content compared to both control and treated spent hen meat and the fat content varied from 2.70 to 1.72 (%). This

may possibly be due to feeding of broilers with high energy diet to attend higher growth rates, while the spent hen selected were almost at the end of their lifespan.

### **Muscle fibre diameter and electrophoretic pattern of muscle protein**

The muscle fibre diameter of untreated and treated samples varied 17.85 to 23.19  $\mu\text{m}$  (Table 2). The diameter of muscle fibres were slightly higher ( $p < 0.01$ ) for treated samples compared to controls. Higher values of muscle fibre diameter in GE treated samples compared to control were also reported by Naveena *et al.*, (2001) but were contrast to findings of Tuma *et al.*, (1962) who reported that fibre diameter increases and tenderness decreases with increasing animal age.

However, the correlation between these two variables differed greatly with age of animals. The average diameter of chicken muscle white fiber has been reported to be 38-46  $\mu\text{m}$  (Smith and Fletcher, 1988) and 26-28  $\mu\text{m}$  (Wattanachant *et al.*, 2005). The differences in muscle fiber diameter were possibly due to the difference in age, rate of rigor on set and degree of sarcomere shortening (Wattanachant *et al.*, 2005). The fiber diameter of broiler chicken muscle was larger than spent hen muscles.

The electrophoretic pattern of control and treated samples after 3 h treatment presented in Figure 1 and Table 3. The treated muscles had fewer protein bands, i.e., 3 and 2 bands for GE and papain treated muscles respectively as compared to 7 bands for broiler meat and spent hen meat samples. The similar results was observed by Naveena and Mendiratta (2001) in ginger extract treated spent hen meat, but sample treatment was for 48h and Bhaskar *et al.*, (2006) in ginger powder treated spent hen sample treatment was for 3h.

**Table.1** Experimental design

Curing ingredients	Broiler meat	Control (spent hen meat)	Ginger enzyme (spent hen meat)	Papain (spent hen meat)
Water	1000 ml	1000 ml	1000 ml	1000 ml
Ginger enzyme powder (0.5%)	-	-	5 g	-
Papain powder (0.5%)	-	-	--	5 g
Sodium tripoly phosphate (0.5%)	5g	5g	5g	5g
Salt (5%)	50g	50g	50g	50g

**Table.2** Effect of partially purified ginger enzyme and commercially available papain treatment on physico-chemical parameters of raw and cooked spent hen meat

Parameter	A	B	C	D	Level of significance
<b>Raw meat</b>					
pH	6.09±0.01	6.08±0.05	6.18±0.01	6.12±0.04	ns
Water holding capacity (%)	47.00±0.17 <sup>c</sup>	38.70±0.04 <sup>a</sup>	43.06±0.07 <sup>bc</sup>	41.20±0.09 <sup>ab</sup>	**
Collagen content (mg/g)	2.42 ±0.23 <sup>a</sup>	5.12±0.44 <sup>b</sup>	5.73±0.15 <sup>b</sup>	5.16±0.13 <sup>b</sup>	***
Collagen solubility (%)	21.37±0.75 <sup>b</sup>	17.10±0.58 <sup>a</sup>	20.00±0.61 <sup>b</sup>	19.71±0.62 <sup>b</sup>	**
Muscle fibre diameter (µm)	23.19±0.37 <sup>c</sup>	17.85±0.48 <sup>a</sup>	20.88±0.46 <sup>b</sup>	20.08±0.76 <sup>b</sup>	**
Moisture	77.62±0.062 <sup>b</sup>	76.51±0.163 <sup>a</sup>	77.36±0.626 <sup>b</sup>	77.49±0.108 <sup>b</sup>	**
Protein	19.63±0.14	19.43±0.19	19.60±0.40	19.53±0.61	ns
Fat	2.70±0.12 <sup>b</sup>	1.72±0.02 <sup>a</sup>	1.80±0.09 <sup>a</sup>	1.73±0.03 <sup>a</sup>	**
<b>Cooked meat</b>					
Cooking yield (%)	75.74±0.68 <sup>b</sup>	70.83±0.49 <sup>a</sup>	73.63±1.51 <sup>ab</sup>	72.88±0.63 <sup>ab</sup>	*
Cooked meat pH	6.14±0.02 <sup>a</sup>	6.13±0.04 <sup>a</sup>	6.23±0.01 <sup>b</sup>	6.16±0.01 <sup>ab</sup>	*
Shear force value (N/cm <sup>2</sup> )	12.66±1.32 <sup>a</sup>	21.46±1.29 <sup>b</sup>	12.85±0.05 <sup>a</sup>	13.67±0.82 <sup>a</sup>	**

A=Broiler meat, B=Spent hen meat, C=Spent hen meat + ginger enzyme, D=Spent hen meat + papain

**Table.3** Effect of partially purified ginger enzyme and commercially available papain treatment on protein disintegration of spent hen meat

Protein band number	A	B	C	D
1	86.64	86.64		
2	72.97	76.39		
3	49.05	55.89		
4	35.38	38.80		
5	11.46	18.29	28.55	25.13
6	4.62	4.62	4.62	25.13
7			Low mwt peptides	Low mwt peptides

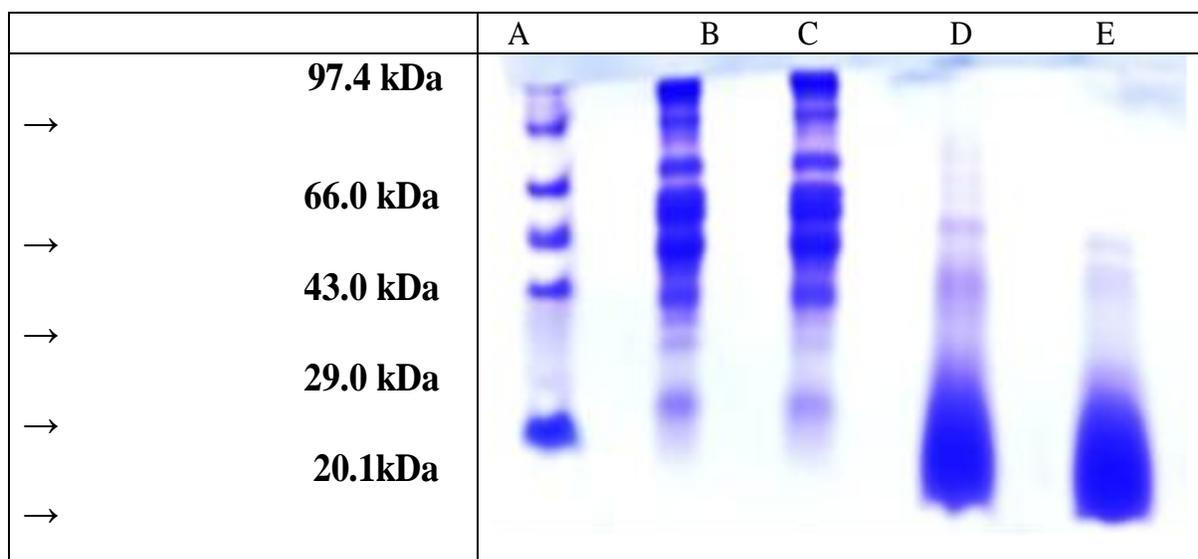
A=Broiler meat, B=Spent hen meat, C=Spent hen meat + ginger enzyme, D=Spent hen meat + papain

**Table.4** Effect of partially purified ginger enzyme and commercially available papain treatment on microbial qualities of spent hen meat

Parameter	A	B	C	D	Level of significance
Total plate count	4.91±0.00 <sup>b</sup>	4.49±0.01 <sup>b</sup>	3.61±0.00 <sup>a</sup>	3.88±0.01 <sup>a</sup>	**
Lactic acid bacteria	1.95±0.00 <sup>d</sup>	1.78±0.01 <sup>c</sup>	1.48±0.01 <sup>a</sup>	1.73±0.04 <sup>b</sup>	**
Yeast and mould	1.81±0.00 <sup>d</sup>	1.76±0.01 <sup>c</sup>	1.50±0.01 <sup>a</sup>	1.61±0.00 <sup>b</sup>	**
Enterobactor	1.73±0.04 <sup>a</sup>	2.12±0.03 <sup>c</sup>	1.94±0.01 <sup>b</sup>	2.07±0.03 <sup>c</sup>	**

A=Broiler meat, B=Spent hen meat, C=Spent hen meat + ginger enzyme, D=Spent hen meat + papain<sup>1</sup> = log<sub>10</sub>/g; \*P<0.05; \*\*P<0.01

**Fig.1** Effect of partially purified ginger enzyme and commercially available papain treatment on electrophoretic pattern of muscle proteins after holding for 3 h at room temperature



A: Protein marker B: Broiler meat C: Spent hen meat D: Spent hen meat + ginger E: Spent hen meat+ papain

The less number of bands in treated samples were not prominent as compared to control samples. It suggested that there was an increased proteolysis of muscle proteins in GE and papain treated samples, as shown by reduction in the number of protein bands. There was also more reduction in the number of bands in the samples treated with papain compared with GE samples, which may be due to high purity of papain. Reduction in the number of protein bands in GE and papain treated samples may also be due to proteolysis of high molecular weight proteins into a number of low-molecular weight peptides, which may overlap each other and get accumulated at the bottom of the gel.

### **Cooking yield, cooked meat pH and shear force value**

The cooking yield did not vary much between the control and treated samples. But there was a significant ( $p < 0.05$ ) difference was observed in cooking yield between broiler meat and spent hen meat. However the cooked meat pH showed differences between the control and GE treated samples. The shear force values varied from 12.66 to 21.46 ( $N/cm^2$ ) (Table 2).

The shear force value of GE and papain treated samples were lower ( $p < 0.01$ ) than control spent hen. Thompson *et al.*, (1973); Lee *et al.*, (1986); Syed Ziauddin *et al.*, (1995) and Naveena and Mendiratta (2001) also observed the reduction ( $p < 0.05$ ) in shear force values in ginger extract-treated samples. Bhaskar *et al.*, (2006) also observed that in ginger powder treated samples the shear force value was less as compared to control. Khanna and Panda (2007) also reported that reduction ( $p < 0.05$ ) in shear force values of papain treated spent hen meat. The shear force value of GE and papain treated samples were almost closer to broiler meat. Devitre and Cunningham (1985) observed that fillets

treated with sodium chloride plus sodium tripolyphosphate plus papain were the most tender. The decrease in shear force values and the increase in collagen solubility in ginger extract treated samples were quite similar to papain treated sample.

### **Microbial quality**

The microbial quality of experimental samples showed somewhat a clear picture. The Total plate counts varied from 3.61 to 4.61 ( $\log_{10}/g$ ), Lactic acid bacterial counts varied from 1.48 to 1.95 ( $\log_{10}/g$ ), Yeast and mould counts varied from 1.50 to 1.81 ( $\log_{10}/g$ ) and Enterobactor counts varied from 1.73 to 2.12 ( $\log_{10}/g$ ) (Table 4).

### **Means bearing uncommon superscripts within rows differ significantly for all tables**

Total plate count, Lactic acid bacterial count, Yeast and mould and Enterobactor count were lower ( $p < 0.01$ ) in GE and papain treated samples. However, the difference in the counts was below 1 log unit, a minimum requirement or significant difference between samples (Gill and McGinnis, 1999) in the microbial counts except the total plate counts. The difference of 1 log unit was observed in the total plate counts of samples treated GE and papain.

However this difference of 1 log unit was not observed in the differential microbial counts. Mascolo *et al.*, (1989) reported that the hydro ethanolic extract of ginger have potent antimicrobial activity against Gram negative and Gram positive bacteria. Sazler (1982) reported that inhibition of *E.coli*, *E.faecalis*, *S.typhimurium*, *S.aureus*, *B.cerus* and *C.perfringes* by the use of ginger extracts in meat products. Mendiratta *et al.*, (2010) recommended ginger extract when meat curry is to be used after storage at refrigerated

temperature; the magnitudes of changes during storage were less in ginger-treated chunks. Sudharshan *et al.*, (2011) also reported that the essential oil of ginger significantly decreased the bacterial count, whereas aqueous extract of ginger had no much effect in chicken meat. This might be due to the fact that active principle of ginger di-allyl-di-sulphide and gingerols are insoluble in water and are extracted only during solvent extraction process (Shelef, 1983). Emeruwa (1982) and Seenivasan *et al.*, (2010) reported the antimicrobial effect of papain. In the present study also, the solvent extracted enzyme has shown the antimicrobial effect in the treated sample.

The use of condiments in marinating the meat to improve the eating quality is an important culinary art in the meat preparation. The commercial application of the purified form of chemical compound from the condiments requires authentication in the meat processing laboratory. The results from the present study have suggested that treatment with partially purified enzyme from ginger rhizome have increased the collagen solubility, the protein degradation (myofibrillar protein) and reduced the shear force value as similar to that of the treatment with commercially available papain of spent hen meat. Further the results have suggested that partially purified enzyme from ginger rhizome reduced the total plate (microbial) counts of spent hen meat as similar to that of the treatment with commercially available papain. It is concluded that ginger enzyme has the potential commercial applications to explore in the meat processing industry to improve the qualities of spent hen meat.

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