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EFFICACY OF DIFFERENT MARINADES ON TENDERNESS AND OVERALL QUALITY OF BUFFALO MEAT

(With 2 Tables and One Figure)

By

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تأثير طرق التمليح المختلفة علي جودة اللحم الجاموسي

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تم عمل هذه الدراسة لتحسين جودة اللحم الجاموسي باستخدام طرق التمليح المختلفة. حيث تمت التجربة على قطع من عرق التليبينكو لذكور الجاموس عمر 2-3 سنوات. تم تحضير محلول التمليح من الماء المقطر 20% وملح كلويد الصوديوم 1.5% وتم تقسيمه الى ثلاثة اجزاء، اضيفت عصارة الجنزبيل (1.5%) الى الجزء الاول، وعصير الليمون (3%) الى الجزء الثانى، اما الجزء الثالث فقد اضيف اليه فوسفات الصوديوم الثلاثى (0.4%) وتم نقع قطع اللحم الجاموسى فى هذه المحاليل الثلاثة بالاضافة الى المجموعة الضابطة حيث تم نقع اللحم فى الماء المقطر 20%. وحفظت لمدة 24 ساعة في الثلاجة (5° م). وتعرضت كل المجموعات للفحص الحسى، الكيميائى، الفيزيقى والميكروبى، اظهرت النتائج ان العد الميكروبى(10^2>) ودرجة تركيز ايون الهيدروجين(4.62) كانت اقل معنويا فى العينات اللتى اضيف لها عصير الليمون عن باقى المجموعات. وعند قياس مدى طراوة انسجة العضلات باستخدام جهاز قوة القطع فقد اظهرت كل العينات المملحة مقاومة اقل معنويا عن المجموعة الضابطة وكانت العينات المعاملة بفوسفات الصوديوم الثلاثى هى اقلهم مقاومة مما يدل على طراوة هذه العينات اكثر من باقى المعملات. وبقياس التحليل الكهربائى للبروتين اللحم دلت النتائج عن حدوث تكسير فى البروتينات المكونة للحم بشكل واضح فى العينات اللتى اضيف لها عصارة الجنزبيل وعصير الليمون وذلك يعزى الى التأثير الحامضى لعصير الليمون وللانزيمات الهاضمة للبروتين الموجودة فى عصارة الجنزبيل. بينما ظلت عينات مجموعة فوسفات الصوديوم الثلاثى وبالمجموعة الضابطة بدون حدوث تكسير ملحوظ فى بروتين العضلات. كما اظهر الفحص الحسى تحسينا معنويا فى طراوة وعصيرية كافة العينات

المعاملة بينما كانت العينات المعاملة بعصارة الجنزبيل وعصير الليمون اكثر المجموعات تفضيلا من حيث النكهه. لذلك استخلصت الدراسة الى ان استخدام كلا من عصارة الجنزبيل وعصير الليمون والفوسفات فى تمليح اللحم الجاموسى كان ذا اثر فعال فى تحسين طراوة والنكهة وايضا تقليل الحمل الميكروبى. وتعتبر عصارة الجنزبيل وعصير الليمون اكثر امنا فى الاستخدام سواء على مستوى التمليح فى المنازل والمصانع.

SUMMARY

This study was conducted to improve tenderness and overall quality of less tender buffalo meat using different marinades. Semitendenosus muscles from 2-3 years old bull buffaloes were cut into chunks 3 x 3 x 3 cm and marinated with either ginger extract (GE) 1.5% and NaCl 1.5% or lemon juice (LJ) 3% and NaCl 1.5% or Sodium tri-polyphosphate (STPP) 0.4% and NaCl 1.5% or distilled water 20% as control (C) for 24 hours at refrigerator shelf (5ºC). All groups were subjected to various physico-chemical and sensory evaluations. The sensory evaluation showed marked ($p< 0.05$) increase in flavour scores for GE and LJ marinated samples, while higher scores of tenderness and juiciness were noticed in GE and STPP marinated samples. Shear force values indicated significant reduction ($p< 0.05$) in the values of GE (10.28), LJ (10.57) and STPP (9.67) marinated samples. Cooking loss of the STPP marinated samples reduced significantly ($p< 0.05$) compared with control, GE and LJ marinated samples. A marked decrease ($p< 0.05$) in the aerobic plate count, psychrotrophic bacterial count and pH of LJ marinated samples were recoded. Electrophoretic pattern of muscle proteins revealed extensive proteolysis and reduction in the number of protein bands in GE and LJ marinated samples. TBARS decreased significantly ($p<0.05$) in GE and STPP marinated samples. The results obtained during this study pointed out that the use of GE, LJ or STPP marination solution could be an effective method of alleviating toughness associated with buffalo meat and improving the overall acceptability of buffalo meat eating quality.

Key words: *Marination, buffalo meat, tenderness, ginger extract, lemon juice, Sodium tri-polyphosphate*

INTRODUCTION

The world buffalo population is estimated to be approximately 130-150 million widely distributed throughout the world (FAO, 2004). Although buffalo meat has the advantage of low cholesterol content than

beef, its meat is not preferred because of its less tender texture (Naveena *et al.,* 2004). Tenderness has been identified as the most important factor affecting consumer satisfaction and perception of taste (Rust, 1998 and Robbins *et al.,* 2003).

Marination with phosphate salts, sodium chloride, citrus juice and proteolytic enzymes are the most popular methods for meat tenderization (Vote *et al.,* 2000; McGee *et al.,* 2003; Naveena *et al.,* 2004 and Baublits *et al.,* 2005). Historically, the purpose of marinating meat was to preserve it. Marination is commonly used to increase yields by increasing water retention and improve meat quality characteristics, particularly meat tenderness (Brooks, 2007). The key ingredients for meat marinades are NaCl and phosphate (Northcutt, 1999 and Davis *et al.,* 2004). Seasonings, sugar, oils, and organic acids such as vinegar or citric acid (lemon juice) are also incorporated in marination process (Wicker, *et al.,* 1999 and Bauermeister and Mckee, 2005). Also, natural flavorings such as rosemary, ginger and thyme spice extract are added because of their flavoring attributes and strong antioxidative properties. Water is used to dissolve the non-meat ingredients and contributes to the tenderness and juiciness of the meat product and also increases yield. Salt is added at low levels to improve flavor and to increase the uniformity of solution distribution inside the meat product, and alkaline phosphates interact with meat proteins and increase their ability to hold moisture inside the products during cooking, which increases the juiciness of the cooked meat (Brooks, 2007). However, the complexity of the process along with ingredient composition and subsequent interaction often results in variability in product quality (Wicker *et al.,* 1999).

Ginger has been investigated as a source of plant proteolytic enzyme (Lee *et al.,* 1986; Syed Ziauddin *et al.,* 1995 and Pawar *et al.,* 2007). The ginger protease is a thiol proteinase with an optimum activity at 60^{o}C (Naveena and Mendiratta, 2001). Thompson *et al.* (1973) and Naveena and Mendiratta (2001) pointed out that ginger extract is an effective meat tenderizer and the tenderization is achieved through its action on both myofibrillar and connective tissue components of meat.

The objectives of the present study are to evaluate the effectiveness of different marinades to overcome the toughness associated with buffalo's meat. Moreover, their effect on other meat quality parameters (pH, color, cooking loss, sensory attributes, lipid oxidation and microbial load) and protein degradation profile of meat were also studied.

MATERIALS and METHODS

Preparation and marination of samples

Semitendenosus muscles from 2-3 years old bull buffaloes were collected within 10 hours post slaughter from local butchers. They were stored in refrigerator for 24 hour at 5ºC. After chilling, muscles were cut into chunks of approximately 3x3x3 cm size and divided into four groups of equal weight. The four groups were randomly allotted for marination.

The first group was soaked in 20% distilled water as control(C). While the other three groups were soaked in a marinade solution consisting of 1.5% sodium chloride, 20% distilled water in addition to either 1.5% ginger extract (Ginger extract (GE) was prepared from manual grinding and squeezing 100gm of peeled and sliced local fresh ginger, the resulted extract was approximately 50% of the original weight Lee *et al.,* 1986) or 3% lemon juice (LJ) or 0.4% Sodium tripolyphosphate (STPP) for the 2^{nd} 3^{rd} and 4^{th} group, respectively. After thorough mixing, buffalo chunks were kept on refrigerator shelf (5ºC) for 24 hours..

Raw samples were investigated for moisture content, pH value, color components (a, b and L), lipid oxidation, microbial load (aerobic plate count (APC) and psychrotrophic bacterial count (PBC)) and electrophoretic pattern. While cooked samples (at 180^{o}C for 20 min. to attain an internal temperature of 70^{o}C) were examined for cooking loss, lipid oxidation, shear force and sensory attributes.

Moisture% and pH value

Moisture content of the examined meat samples was determined as weight loss of two grams after drying in hot air oven for 18 hours at 102°C as recommended by AOAC (1990).

Twenty grams of the prepared sample were mixed with 20 ml distilled water, then a digital pH meter (EU TECH England) with a calibrated probe type combined electrode (ORION/KNI pHE) was introduced into prepared mixture and the constant pH value was recorded as recommended by Ronald and Ronald (1991).

Instrumental color measurement*

Instrumental color determinations were made on the surfaces of Control and marinated samples using a micro color unit attached to a data station (Brano Lange -Germany) using the standard CIE LAB color system as follows: a-value (redness/green), b-value (yellowness/blue) and L-value (lightness/darkness,). Six readings were taken at various points on each sample (CIE, 1978).

Lipid oxidation

Lipid oxidation was assessed on both control and marinated samples before and after cooking. Thiobarbituric acid values (TBA-value) were determined as recommended by Vynke (1970) using 7.5% trichloroacetic acid (TCA) and freshly prepared 0.02 M TBA solution. The absorbance (A) of the developed red color was measured at wave length 538 nm against blank. Thiobarbituric acid reactive substances (TBARS) were calculated and expressed as mg malonaldehyde (mal)/ kg sample.

Electrophoresis**

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the methods of Laemmli (1970) and See and Jackowski (1993) using electrophoresis apparatus (Model. protean Xi Cell, BioRad USA). Five grams of minced meat was mixed with 50 ml 0.01 N sodium phosphate buffer (pH 7.0) containing 1% SDS and 1% 2-mercaptoethanol and incubated at 37^{o}C for 2 hours. The mixture was centrifuged at 1500 rpm for 30 min. An aliquot of supernatant was dialyzed overnight at room temperature (25^{o}C) against 0.1 N Sodium phosphate buffer containing 0.1% 2-mercaptoethanol. About 50 µm of dialyzed solution was used for loading the gel. Electrophoresis was performed at a constant voltage mode of 100 V/slab at 30 mA for 5-6 hours or until the tracking dye reached the lower end of the gel. The gel was removed and stained with Coomassie blue for 4-5 hours. The gels were then de-stained and photographed.

APC and P BC

Twenty five grams of each sample were aseptically homogenized in 225 ml sterilized peptone water 0.01% using a stomacher (model 400, Seward laboratory, London) for one minute. The homogenate was serially diluted, inoculated onto standard plate count agar medium and incubated at 32^{o}C for 48 hours for APC and at 7^{o}C for 10 days for PBC (APHA, 1992).

*The test was preformed in National Center for Radiation Research and Technology (NCRRT).
**The test was preformed in Biotechnology Center. Fac. of Vet. Med. Cairo Univ.

Shear force* and Cooking loss**

The samples were oven cooked at 180°C for 20 minutes to attain an internal temperature of 70°C. Samples were chilled at refrigerator temperature over night and used for determination of tenderness (after equilibration at room temperature).

The shear force was measured with a blade (68 mm wide× 72 mm long× 3 mm thick) (Yoon, 2003) using Instron 1195 (England). The blade advanced 10mm/ min and the pick up strength of the measuring head was 50 kg with the muscle fibers parallel to the direction of the blade. The results were expressed as kg force (f) to shear.

Cooking loss of the examined samples were determined, each sample was weighed prior to cooking. Upon completion of cooking, a final weight was obtained and cooking loss % was determined as the difference between the fresh and cooked weight divided by the fresh weight.

Sensory evaluation

Meat chunks were cooked (as mentioned before) and served to the panel within 2 min after cooking. Samples were assessed for a number of sensory characteristics by seven panel members who are familiar with the characteristics to be evaluated (tenderness, juiciness, flavour intensity and overall acceptability). The sensory characteristics were evaluated using 8-point hedonic scale where one and eight were the extremes of each characteristic (8= extremely tender, extremely juicy, extremely good flavour and extremely acceptable) (Keeton, 1983).

Statistical analysis

The values given in each treatment category are the mean values for three replicates. The data generated were analyzed by statistical software package using standard procedures for analysis of variance and Duncan multiple range test to compare the means and determine the effect of treatments (SAS, 1990).

***The test was preformed in National Center for Radiation Research and Technology (NCRRT).

RESULTS and DISCUSSION

The results of physico-chemical tests are shown in Tables 1&2.

Table 1: Physico-chemical tests of raw samples

Groups / Parameters	Control	GE	LJ	STPP
Moisture%	78.47[a]	78.77 [a]	79.00 [a]	78.27 [a]
PH value	5.6 [b]	5.5 [b]	4.62 [c]	5.88 [a]
TBARS mg mal/kg	0.19 [a]	0.14 [b]	0.20 [a]	0.12 [b]
APC $\log_{10}$ CFU/g	3.94[b]	4.93 [a]	$<10^2$ [c]	3.47 [c]
PBC $\log_{10}$ CFU/g	3.72 [b]	5.03 [a]	$<10^2$ [d]	2.28 [c]
Color				
a-value	5.73 [a]	2.15 [c]	5.68 [a]	4.08 [b]
b-value	5.42 [b]	3.40 [c]	7.58 [a]	1.99 [d]
L-value	48.74 [b]	41.47 [c]	62.44 [a]	39.17 [c]

APC: Aerobic plate count PBC: Psychrotrophs bacterial count

Means in the same row with unlike superscripts are different ($p< 0.05$).

Table 2: Physico-chemical test and sensory attributes of cooked samples

Groups / Parameters	Control	GE	LJ	STPP
Cooking loss%	39.01 [a]	42.67 [a]	39.99 [a]	25.01 [b]
TBARS mg mal/kg	0.57 [a]	0.22 [b]	0.27 [b]	0.13 [c]
Shear force kg f	19.84 [a]	10.28 [b]	10.57 [b]	9.67 [b]
Sensory attributes				
Flavor	4.00 [c]	6.67 [a]	7.17 [a]	5.50 [b]
Tenderness	3.50 [b]	7.17 [a]	6.83 [a]	7.50 [a]
Juiciness	3.50 [c]	7.00 [a]	5.67 [b]	7.67 [a]
Overall acceptability	4.17 [c]	7.50 [a]	6.67 [b]	6.50 [b]

Means in the same row with unlike superscripts are different ($p< 0.05$).

Moisture and pH

The moisture content of different marinated samples (Table 1) did not differ significantly ($p< 0.05$) from that of control samples. Such results are in harmony with that obtained by Naveena *et al.* (2004). The interaction between marinades and meat chunks lead to a marked reduction in pH value of LJ marinated samples (4.62) this could be attributed to effect of high citric acid content in LJ marinade. Similar result was recorded by Burke and Monahan (2003), Aktas *et al.* (2004) and Rio *et al.* (2007). On the other hand STPP marinated samples showed the highest pH value (5.88), followed by GE marinated samples (5.5). These findings coincide with those of Pearson and Gillett (1996) and Rio *et al.* (2007) who emphasized the role of alkaline phosphate in the initial increase in pH of poultry and meat after treatment.

Color

It was clear from Table 1 that in terms of a-values (redness/green) GE (2.15) and STPP (4.08) marinated samples were less red ($p< 0.05$) than the control (5.73) and LJ (5.68) marinated samples. This finding was in agreement with Robbins *et al.* (2002) and Baublits *et al.* (2006) who reported a general decline for enhanced versus untreated beef muscles which may be a function of increased water retention. Although the b-values increased significantly ($p< 0.05$) in LJ marinated samples (7.58), it decreased significantly ($p< 0.05$) in STPP (1.99) and GE (3.40) marinated samples compared with the control (5.42). This finding was in agreement with that of Lee *et al.* (1998). As expected, L-values of LJ marinated samples (62.44) were lighter ($p< 0.05$) in color than all other samples. In this regard Swatland (1993) stated that the pale color of meat is influenced by the decline of pH and light scattering properties of the meat.

Although there were no significant difference ($p< 0.05$) in L-values between GE and STPP marinated samples (39.17 and 41.47, respectively), there was a significant ($p< 0.05$) decrease of their L-values compared with the control (48.74). In this regard, Bauermeister and Mckee (2005) indicated that lower L-values may be due to marination of meat, where lower L-values suggest that the meat was darker in color. Faustman and Cassens (1990) added that the general decline may be a function of the increasing ionic strength causing increased water retention which may cause a darker surface with elevated pH values and decreased oxygen penetration.

Lipid oxidation

Lipid oxidation, as determined by TBA measurements, revealed that TBARS decreased significantly ($p< 0.05$) in GE and STPP marinated samples (0.14 & 0.12 mg mal/ kg, respectively) compared to control and LJ marinated samples (0.19 & 0.20 mg mal/ kg, respectively) for raw samples (Table 1). These results were in harmony with that obtained by Lee *et al.* (1986) and Ke *et al.* (2009). Furthermore, Jimenez-Villarreal *et al.* (2003) hypothesized that pH of treated meat was related to TBA-value the higher the pH, the less lipid oxidation the samples had). On the other side, it was found that the TBARS of all cooked marinated samples (Table 2) were significantly lower ($p< 0.05$) than the control (0.57 mg mal/ kg). Moreover, STPP marinated samples (0.13 mg mal/ kg) showed marked reduction in TBARS compared with the GE and LJ marinated samples (0.23 & 0.27 mg mal/ kg, respectively), while no difference could be found between GE and LJ marinated samples. The increase in TBARS of GE cooked marinated samples may be referred to the time/temperature treatment used for cooking the samples (Lee *et al.*, 1986).

Elechtrophoretic pattern of muscle protein

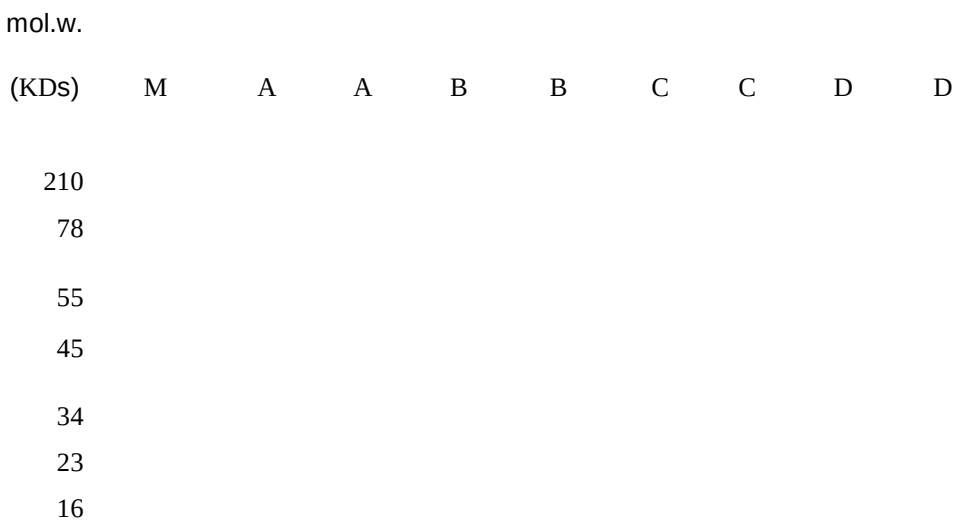

Fig. 1: Electrophoretic pattern of control (A), GE (B), LJ (C), STPP (D) marinated samples and protein marker (M).

A representative SDS-PAGE gel elechtrophorsis of the marinated and control samples (fig. 1) proved significant reduction ($p<0.05$) of

protein bands in GE and LJ marinated samples compared with the control and STPP marinated samples. This could be attributed to the effect of protease (zingibain) in ginger extract which degrades muscles collagen and myofibril (Lee *et al.*, 1986; Adulyatham and Apenten, 2004 and Pawar *et al.,* 2007). The obtained results were in harmony with that recoded by Sachindra *et al.* (2006) and Naveena *et al.* (2004). Regarding LJ marinated samples, Burke and Monahan (2003) indicated that the tenderization of beef samples using citrus juice marinade could be attributed to marinade uptake by muscle proteins and also to solubility of collagen. Moreover, acidic marinades denature proteins, as the bonds between protein bundles in the meat break and the proteins unwind and run into each other to form a loose mesh. STPP marinated and control samples showed no proteolytic effect evidently by no significant differences ($p<0.05$) in the electrophoretic pattern among these samples.

APC and P BC

It was obvious that there was a significant reduction ($p< 0.05$) in the APC and PBC in LJ marinated samples ($<10^2$ CFU/g) followed by STPP marinated samples (3.47 & 2.28 $\log_{10}$ CFU/g, respectively) as compared with GE marinated samples (4.93 & 5.03 $\log_{10}$ CFU/g, respectively) and control samples(3.94 & 3.72 $\log_{10}$ CFU/g, respectively) (Table 1). This may be referred to the existence of high concentrations of citric acid in LJ up to 8% of their dry weight (Penniston *et al.,* 2008) which acidified marinated meat and reduced the risk of bacterial contamination. Antibacterial activity of citric acid is dependent on pH, concentration and anion effects (Mohamed *et al.*, 2008). Similar results were recorded by Mahrour *et al.* (2003) and Kanellos and Burried (2005) who stated that pH values observed after treatment with alkaline phosphate and citric acid are within ranges that inhibit the multiplication of most bacteria, thus adding to the bactericidal effect of both these compounds. On the other hand, the unexpectedly higher APC (4.93 $\log_{10}$ CFU/g) and PBC (5.03 $\log_{10}$ CFU/g) of GE marinated samples by one log cycle than those of the control (3.94 & 3.72 CFU/g for APC & PBC, respectively). This increase in bacterial count may be attributed to the degree of contamination of fresh ginger purchased from local market and manual preparation of the extract.

Shear force, Cooking loss and Sensory evaluation

Although the shear force values were significantly reduced ($p< 0.05$) in all marinated samples (10.28, 10.57 and 9.67 kg f for GE, LJ

and STPP marinated samples, respectively) compared with the control (19.84 kg f), no significant difference ($p< 0.05$) was seen among marinated samples (Table 2). Reduction in shear force values with GE marinated samples could be attributed to the effect of zingibain (protease) in GE, which was reported in buffalo by Syed Ziauddin *et al.* (1995) and Naveena *et al.* (2004) and in spent hen meat by Naveena and Mendiratta (2001) and Sachindra *et al.* (2006). Moreover, Davis *et al.* (2004) reported significantly lower shear force values for injected fresh pork loins than uninjected samples. Looking for cooking loss (Table 2), a significant reduction ($p< 0.05$) was observed in STPP marinated samples (25.01%) compared to the control (39.01) and other marinated samples (42.67 & 39.99% for GE & LJ marinated samples, respectively), similar results were recorded by Baublits *et al.* (2005). However, in samples marinated with GE and LJ the cooking loss didn't differ significantly ($p<0.05$) than that of the control samples.

Sensory evaluation

Results of the sensory taste panel analysis for different marinades are reported in Table (2). Sensory panelists found that tenderness, flavour, juiciness and overall acceptability were significantly improved ($p<0.05$) with marination. STPP and GE marinated samples recorded the highest tenderness scores (7.5 and 7.17, respectively), followed by LJ marinated samples (6.83). These results are in agreement with Brashear *et al.* (2002) and Smith *et al.* (2002) who found that the use of a NaCl/STPP solution increased tenderness in enhanced pork lion in comparison to the non-enhanced control. Regarding juiciness, STPP and GE marinated samples (7.67 & 7, respectively) were significantly higher ($p<0.05$) than that of control (3.5), and LJ marinated samples (5.67). This could be explained on the base that alkaline STPP interact with muscle proteins and increase their ability to hold moisture inside meat during cooking, which increases the juiciness of the cooked meat (Brooks, 2007). In this respect, Naveena *et al.* (2004) pointed out that the alkalinity of GE marinated samples help protein in keeping its water. While pH of LJ marinated samples (4.62) was near isoelectric point so that it's protein becomes unable to hold water. It's worth mentioning that the electrophoretic pattern emphasis the higher tenderness, juiciness score and lower shear force value in GE marinated samples. The flavour of both GE and LJ marinated samples (6.67& 7.17, respectively) had significant pleasant flavour ($p<0.05$) than that of control (4) and STPP marinated samples (5.5). This could be attributed to flavoring substances in both GE and LJ (ICMR, 2003 and Bauermeister and Mckee, 2005).

Taste panel proved that GE marinated samples obtained excellent overall acceptability followed by LJ, STPP marinated and control samples.

In conclusion, the results obtained in this study clearly indicate the tenderizing effect of GE, LJ and STPP (in combination with 1.5% NaCl) to improve tenderness of buffalo meat and overall quality characteristics. Therefore, GE and LJ provide a cheap, safe and easy method that can be used effectively to tenderize and improve quality of buffalo meat, at both industrial and household level.

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