Journal of Science and Technology, Vol. 10 No. 2 (2018) p. 77-84

Potential Effect of *Averrhoa bilimbi* (belimbing buluh) Marinades on Tenderizing the Buffalo Meat Compared to *Actinidia chinensis* (kiwifruit), *Citrus limon* (lemon) and Commercial Bromelain

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Received 30 September 2017; accepted 10 April; available online 1 August 2018
DOI: https://10.30880/jst.2018.10.02.013

Abstract: This study was conducted to analyze the effect of *Averrhoa bilimbi* (belimbing buluh) marinades versus other meat tenderizers on the physicochemical properties of buffalo meat. The buffalo meat chunks were marinated with 40% *Averrhoa bilimbi*, 40% *Citrus limon*, 40% *Actinidia chinensis*, 5% commercial bromelain meat tenderizer (positive control) and distilled water (negative control) for 24 hours at 4°C. The treated samples were cooked at 100°C for 20 minutes. Both raw and cooked samples were subjected to physicochemical analyses. There was significantly lower pH ($p<0.05$) for raw and cooked meat chunks observed in all treated samples compared to control. *Citrus limon* and *Averrhoa bilimbi* showed the lowest pH at 5.04±0.06 and 5.06±0.03, respectively, indicated that the meat chunks were well tenderized. *Citrus limon*-treated sample recorded the highest ($p<0.05$) expressible water compared to others. The moisture content of cooked sample and the cooking yield increased significantly ($p<0.05$) in all treated samples compared to control. The hardness from TPA decreased significantly ($p<0.05$) for all treated samples compared to control. It can be suggested that *Averrhoa bilimbi* has the potential to be used as meat tenderizer with the ability to retain the moisture content as compared to other well-known and commercial meat tenderizers.

Keyword: Food technology; marinades; *Averrhoa bilimbi*; meat tenderizers; buffalo meats.

1. Introduction

According to FAO, the world food consumption of meat in the year 2015 are 41.3 kg per capita, and these projected to be 45.3 kg per capita for the year 2030. Increasing consumption of meats had generated high demands on meat quality. The most important quality attribute of meats is tenderness which affecting consumer satisfaction and eating quality [1]. Meat tenderness is due to the major components which are myofibrillar proteins, connective tissue proteins and collagen [2, 3].

Buffalo meat is a vital source of red meat and popular in many parts of the world. Buffalo meat is much more superior than beef as it has a high amount of lean meat, high in protein, low in cholesterol and calories as compared to beef [4]. Besides that, buffalo meat consumption does not possess a restriction by any religion. Buffalo meats are commonly utilize in meat industry to produce numerous value-added meat products such as patties, meatballs and sausages [5]. However, buffalo meat perceives tough texture as the old animal usually being slaughter [6]. Improving the tenderness of tough meats could potentially profitable for the meat industry and consumers itself.

Tenderization is the process of improving the tenderness of tough meats by utilizing either physical forces, chemical or enzymatic treatments [7, 8]. Physical treatment involves the mechanical process such as blade/needle tenderization. Enzymatic treatments is a method of applying exogenous protease into the meat via infusion, marinating and injection techniques [9]. The application of proteolytic enzyme such as papain, bromelain and ficin as meat tenderizers had been extensively investigated by previous researchers [10, 11]. These proteolytic enzymes are mostly derived from plants and some of them are commercially available in the retails.

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e-ISSN: 2600-7924/penerbit.uthm.edu.my/ojs/index.php/jst

Method of tenderizing is important to increase the acceptability of tough meat by reducing its toughness. Hence, the application of exogenous enzyme as meat tenderizers are gaining popularity among researchers and industry. Several studies attempt to investigate the potential use of another plant as sources of exogenous proteases.

Actinidia chinensis is commonly known as kiwifruit. This fruit rich in Vitamin C and suitable to be consumed fresh or juice. Fresh kiwifruit juice reported being effective in solubilizing connective tissues [12]. Previous study also found that Actinidin exhibit tenderizing effects toward lamb meat [13].

Citrus limon is also known as lemon normally grown in tropical and warm temperate countries. This fruit widely use in beverages industry as this fruit contains high in Vitamin C. *Citrus limon* is highly acidic and this is beneficial for its application as a meat tenderizers. The previous study has shown that Citrus juice marinades proved to be effective on tenderizing shin beef and solubilize collagen [14].

In Malaysia, there are variety of traditional fruits that also are used by locals to tenderize meats. Sometimes, the fruits are directly incorporated in the preparation of dishes. Besides act as a tenderizer, the addition this plant can enhance the flavour and increase the bulk. There are a lot of local fruits that can be investigated to explore their potentials. *Averrhoa bilimbi* (belimbing buluh) have been widely used in cooking by means to tender the meat. Although these have been practiced by locals for many years, there is lack of scientific literature exist to support this practices.

Thus, the present study was conducted to investigate the potential of used *Averrhoa bilimbi* as a tenderizer for buffalo meats as compared to *Actinidia chinensis*, *Citrus limon* and commercial bromelain.

2. Materials and Methods

2.1 Sample preparation

Buffalo meats were purchased from Pasar Borong Serdang, Selangor. Then, the samples were closely wrapped in polyethylene bags. The samples were stored in the freezer (≤ -18°C) for subsequent use in experiments after proper thawing at 4°C. The visible fats and connective tissue were discarded and the meat was cut into uniform size approximately 3 × 3 × 3 cm.

2.2 Fruit extracts preparation

Commercially available Bromelain Meat Tenderizers (McCormick) were purchased from local market. The commercial bromelain were dissolved in distilled water to obtain 5% concentration as recommended. Fresh *Averrhoa bilimbi*, *Citrus limon* and *Actinidia chinensis* were purchased from the local supermarket (Giant, Sri Kembangan). The fresh fruits were peeled, sliced and blended separately in a blender with distilled water at ratio 1:1 for 2 minutes. Then, the homogenate was filtered through cloth sieve and the filtrate collected was known as the concentrated fruit extract. The 40% concentration of fruit extracts were prepared by diluted with distilled water.

2.3 Plant extract treatment and marination

Buffalo meat chunks (3 x 3 x 3cm) were marinated with either *Averrhoa bilimbi* (40%), *Citrus limon* (40%), *Actinidia chinensis* (40%),
Commercial Bromelain Meat Tenderizers (5%), and distilled water (control). The meat chunks were marinated for 24 hours at 4±1°C.

The marinated samples were then placed in an oven at 100 ± 1°C for 20 min until it properly cooked. The cooked samples were subjected to analysis assessed for moisture content, pH, cooking yield, water holding capacity (WHC), texture and sensory properties. In addition, the raw treated samples were also evaluated to determine the physicochemical properties.

2.4 pH, moisture and cooking yield determination

The pH values of raw and cooked meat samples were determined based on method described by Suvanich et al. [15], 10g from each meat samples were mixed with 50 ml distilled water for 30 s. pH meter (Jenway 3505, England) was used to determine the pH of the homogenate. Moisture content of treated meat samples were determined according to AOAC [16] procedures by oven drying for 5 hours at 105°C. The cooking yield was calculated as the weight of cooked meat samples divided by weight of raw meat samples, and expressed as a percentage.

2.5 Water holding capacity (WHC)

WHC of meat samples were evaluated in triplicate using centrifugation method as described by previous study Ramirez [17]. Treated meat (5g) was weighed onto a Whatman No. 1 filter paper and placed in a 50 ml centrifuge tube. The samples were then centrifuged at 3500 g for 15 min. The meat sample was weighed before and after centrifugation. The expressible water was calculated based on the Eq. 1.

$$EW = IW - FW/IW \times 100 \quad (1)$$

Where EW, IW and FW are indicate expressible water, weight before centrifuge and weight after centrifuge respectively. WHC for each sample is inversely proportional to the amount of water expressed after centrifugation process.

2.6 Texture profile analysis (TPA)

The texture profile analysis (TPA) of cooked meat chunks were evaluated using a texture profile analyzer (TA-XT2i, Stable Micro System, UK). Six samples from each treatment were cut into cuboid form (3 × 3 × 1cm) and placed on the middle of the compression plate. The test samples were compressed to 50% of their original height through two continuous compressions by using cylindrical probe (diameter 75 mm). The instrument setting were set at 1.0 mm/s, 4.2 mm/s, 5.0 mm/s for Pre-test speed, Test speed and Post-test speed respectively. Five textural parameters involve hardness (N), springiness, cohesiveness, gumminess and chewiness were analyzed from the data obtained.

2.7 Sensory evaluation

Sensory analysis of cooked meat chunks were conducted among 40 panelist according to the 9-points hedonic scale, where 1 denoted dislike extremely and 9 denoted like extremely. Each treated buffalo meat chunks were cooked in an oven just before sensory evaluation and presented in a randomised order. The panelist were asked to evaluate the attributes of aroma, taste, colour, flavour, tenderness, juiciness, chewiness and overall acceptability. Tap water was provide for cleansing the mouth between samples.

2.8 Statistical analysis

The experimental data obtained were subjected to statistical analysis using Minitab version 17 (Minitab Statistical Software, USA). Analysis of variance (ANOVA) and Tukey's test for multiple comparisons were used to compare the means and determine the significant difference between treatments.

3. Results and Discussion

3.1 Physicochemical characteristics

The results of pH, moisture content, cooking yield and expressible water of the meat samples are presented in Table 1. Buffalo meat chunks marinated with *Avverhoa bilimbi* and *Citrus limon* recorded significantly ($p<0.05$) lowest pH at 5.06±0.03 and 5.04±0.06, respectively compared to control. It can be suggested that the lowest pH might be influenced by the low pH of *Averrhoa bilimbi* extract (2.50 – 2.70) and *Citrus limon* extract (2.90 - 3.00). The previous study also found that reduction in pH of buffalo meats treated *Cucumis trigonus Roxb* (Kachri) compared to control [18]. The pH of goat meat treated with 4% pomegranate seed powder was reduced as reported by Narsaiah et al. [19]. Burke and Monahan [14] also reported a significant reduction in pH of bovine muscle strips marinated with citrus juice marinade compared to the untreated sample. As can be seen, the treatment of various fruit extracts had altered the pH of both raw and cooked meat chunks. Commercial bromelain treated samples recorded highest pH value at 5.78 ± 0.08 as compared to others. Meanwhile, *Actinidia chinensis* treatment did not cause extensive change ($p>0.05$) on pH value of meat for both raw and cooked samples. It is vital to monitor the changes in pH value of meat as it would impact the physicochemical attributes of the meat involves water holding capacity (WHC), tenderness and juiciness [8].

Moisture content of cooked meat is normally contribute to the juiciness of meats. The higher moisture content is needed to produce a meat product that high in juiciness. Overall, the moisture content of cooked meat chunks treated with various fruit extracts showed an improvement ($p<0.05$) compared to control, except *Actinidia chinensis* treated samples. Abdel-Naeem and Mohamed [20] also reported the similar effect on increased on the moisture content of cooked camel burger patties added with 7% ginger extracts compared to control. Other studies also found that higher moisture content of cooked beef steak marinated with increase concentration organic acid as reported by Aktaş et al. [21]. The result of higher moisture content might be due to the improvement of hydrophilic characteristics.

Expressible water indicate the amount of water squeezed from muscle by the application of physical forces. WHC is inversely proportional to expressible water expressed by centrifugation. Thus, lower expressible water indicated that WHC is high due to the ability to retain water even undergoes physical forces [22]. From the result in Table 1, WHC of *Averrhoa bilimbi*, *Actinidia chinensis* and commercial bromelain treated sample did not vary notably (p>0.05) with control. This results are in contrast with Ketnawa and Rawdkuen [22] who found that WHC was reduced (p<0.05) as the concentration of bromelain used in beef, chicken, and squish increases. *Citrus limon* treated sample recorded the highest (p<0.05) expressible water in comparison with other treatments. The lower WHC in *Citrus limon* marinated samples are presumably due to the myofibrillar proteins denaturation, which is vital for water retention. Since most of the water found in muscle is present in the myofibril [1], the fragmentation of myofibrillar proteins might cause loss the capacity to bind water [23, 24]. Besides that, the lower pH value of *Citrus limon* treated samples resulted in decreased WHC. The drop in pH apparently caused the reduction in protein reactive groups that responsible for water holding properties. The possible movement of water from myofilament space into the extracellular space, the shrinkage of myofibrillar resulted in decrease WHC [21, 22].

Cooking yield is the important cooking attributes that most of the consumers are concern about. The experimental data showed that cooking yield for all treated meat samples were improved compare to control. *Averrhoa bilimbi* treated sample recorded the highest cooking yield (65.59 ± 4.55%). Naveena et al. [18] reported an improvement (p<0.05) in the cooking yield for buffalo meat chunks (*Biceps femoris* muscle) treated with ginger extract and papain. The previous study also found that bovine muscles marinated with a citrus juice marinade resulted in lower cooking loss compared to those control samples [14]. Other studies also found that cooked ground buffalo meat subjected to the 1.5% and 2% *Moringa oleifera* leaves extract treatment recorded low cooking loss as reported by Hazra et al. [25].

Table 1 Physicochemical characteristics of raw and cooked buffalo meat samples marinated with *Averrhoa bilimbi*, *Actinidia chinensis*, *Citrus limon* and Commercial Bromelain

Parameters	Samples				
	Control	*Averrhoa bilimbi*	*Actinidia chinensis*	*Citrus limon*	Commercial Bromelain
Raw meat chunks					
pH	5.54 ± 0.05^b	5.06 ± 0.03^c	5.52 ± 0.13^b	5.04 ± 0.06^c	5.78 ± 0.08^a
Moisture (%)	$73.95{\pm}0.86^{bc}$	$74.56{\pm}0.54^{bc}$	$75.99{\pm}0.98^{ab}$	$72.04{\pm}2.28^c$	$78.40{\pm}0.51^a$
Expressible water (%)	$19.65{\pm}0.26^b$	$20.21{\pm}2.81^b$	$19.18{\pm}1.20^b$	$28.51{\pm}2.30^a$	$22.20{\pm}1.36^b$
Cooked meat chunks					
pH	$6.04{\pm}0.03^b$	5.41 ± 0.07^c	6.06 ± 0.05^b	5.34 ± 0.10^c	6.27 ± 0.06^a
Moisture (%)	$60.08{\pm}0.30^c$	$64.78{\pm}0.51^a$	$60.56{\pm}0.47^c$	$62.40{\pm}0.80^b$	$65.42{\pm}0.14^a$
Cooking Yield (%)	52.57 ± 2.12^c	65.59 ± 4.55^a	$59.46{\pm}1.30^{ab}$	$58.34{\pm}3.58^{bc}$	$62.26{\pm}1.54^{ab}$

Means followed by different superscript letters (a-c) within the same row are statistically significant at the 5% level.

3.2 Texture profile analysis (TPA)

Tenderness is the most important palatability attribute that affects eating quality and perception. Tenderness can be quantified by measuring the hardness of the TPA. The treatment of various fruit extracts altered most of the cooked meat textural parameters as shown in Table 2. The results indicated that all treatment reduced the parameter of hardness for cooked meats significantly compared to control. The lower hardness value of all treated sample strongly suggested that marination treatment enhanced meat tenderization process. *Actinidia chinensis* treated sample recorded the lowest ($p<0.05$) hardness (18.68±3.98N) value among all samples. This is because actinidin protease presence in kiwifruit displayed enzymatic activity towards muscle proteins, as reported by Lewis and Luh [26] who stated that shear values of beef steaks treated with actinidin were reduced significantly compared to control. Han et al. [27] also reported that significantly lower shear force of lamb meats (*longissimus dorsi* muscle) infused with fresh kiwifruit juice compared to control. Christensen at al. [28] found that the injection of brine solution added with actinidin into porcine musle caused a significant reduction in the shear force values of porcine meats. Acitinidin normally extracted from the fresh fruit, and most of them found in kiwifruit [12, 13]. Another study also found that increased concentration of bromelain resulted in continuous decreased of shear force values in all beef, chicken and squid muscles [22]. The reduction in hardness following treatment with fruit extracts might be due to the cooler conditioning of meat at 4°C which enhance degradation of cytoskeleton myofibrillar protein by proteolytic enzymes. The action of weakening muscle structure by proteolytic enzymes would eventually lead to reduced muscle fibers physical integrity and change of structural cover membrane [2, 29]. The myofibrillar protein degradation favour the weakening of endomysium and perimysium structure [29]. The marination of meat chunks with fruit extracts at 40% concentration for 24 hours give more time for exogenous enzymes to act on meat which results in significant reduction of the hardness of TPA. The hardness of TPA is a measuring tool to predict sensory assessment of tenderness of meats. In general, all treatments resulted in better tenderness of buffalo meat as there was a reduction in hardness.

The result showed that treatment of fruit extracts significantly reduce the gumminess and chewiness of meats compared to control. However, the marination of buffalo meat chunks with *Averrhoa bilimbi*, *Actinidia chinensis*, *Citrus limon* and commercial bromelain had no influence on the springiness and cohesiveness prior to cooking.

Table 2 Textural characteritics of cooked buffalo meat samples marinated with *Averrhoa bilimbi*, *Actinidia chinensis*, *Citrus limon* and Commercial Bromelain

TPA Parameters	Samples				
	Control	*Averrhoa bilimbi*	*Actinidia chinensis*	*Citrus limon*	Commercial Bromelain
Hardness (N)	48.32±2.75^{a}	29.01±8.91^{bc}	18.68±3.98^{c}	27.22±4.32^{bc}	31.31±11.55^{b}
Springiness	0.63±0.11^{a}	0.65±0.17^{a}	0.61±0.07^{a}	0.50±0.11^{a}	0.59±0.08^{a}
Cohesiveness	0.62±0.08^{a}	0.60±0.02^{a}	0.57±0.09^{a}	0.56±0.10^{a}	0.58±0.08^{a}
Gumminess (N)	29.74±3.71^{a}	17.52±5.80^{b}	10.96±3.68^{b}	15.48±4.71^{b}	18.63±8.37^{b}
Chewiness (N)	19.01±5.33^{a}	11.26±4.57^{ab}	6.80±2.60^{b}	8.04±3.66^{b}	11.41±6.37^{ab}

Means followed by different superscript letters (a-c) within the same row are statistically significant at the 5% level.

3.3 Sensory attributes

The sensory attributes could be useful information to indicate the acceptability of the meat chunks treated with various fruit extracts. Table 3 indicated the scores for main attributes include flavour, colour, tenderness and overall acceptability obtained from sensory evaluation. Sensory characteristics of *Averrhoa bilimbi* treated samples did not differ significantly with control samples. This could be good findings to show that marination of meat chunks with *Averrhoa bilimbi* did not impact the natural taste, and flavour of the meats. The commercial bromelain treated samples received the highest ($p<0.05$) scores for aroma, taste, flavour, juiciness and overall acceptability among all samples. This results proved that panellists were preferred the taste of commercial products. A significant ($p<0.05$) higher scores for tenderness and chewiness were recorded for meat chunks treated with *Actinidia chinensis* and commercial bromelain. The previous study also found that higher scores for tenderness of broiled bovine *semitendinosus* steaks following treatment with actinidin [26]. Hazra et al. [25] reported that cooked ground buffalo meat treated with *Moringa oleifera* leaves extract recorded higher score for tenderness and juiciness. The higher tenderness score also corresponds with the result from the textural analysis that indicates lowest hardness for *Actinidia chinensis* treated samples. The tenderness of the meat was increased due to increases in moisture content within the treated meats [21].

However, the scores of colour for all treated samples were not differ significantly as compared to control. This results proved that the meat chunks treated with plant extracts did not change the colour of meats.

Table 3 Sensory properties of cooked buffalo meat samples marinated with *Averrhoa bilimbi, Actinidia chinensis, Citrus limon* and Commercial Bromelain

Sensory Attributes	Samples				
	Control	*Averrhoa bilimbi*	*Actinidia chinensis*	*Citrus limon*	Commercial Bromelain
Aroma	5.25±1.43^{ab}	5.25±1.61^{ab}	5.25±1.68^{ab}	4.92±1.64^{b}	5.98±1.62^{a}
Taste	4.68±1.83^{b}	4.80±1.86^{b}	4.35±1.66^{b}	4.63±1.94^{b}	6.23±1.75^{a}
Flavour	4.68±1.75^{b}	4.63±1.73^{b}	4.28±1.83^{b}	4.45±1.81^{b}	6.03±1.67^{a}
Colour	5.65±1.33^{a}	5.38±1.75^{a}	5.48±1.52^{a}	5.35±1.72^{a}	5.88±1.26^{a}
Tenderness	5.20±1.51^{bc}	4.88±2.04^{c}	5.98±1.73^{ab}	4.8±1.73^{c}	6.35±1.61^{a}
Juiciness	4.45±1.65^{b}	5.13±1.79^{b}	5.08±1.62^{b}	4.58±1.84^{b}	6.5±1.57^{a}
Chewiness	5.35±1.93^{bc}	4.53±2.04^{c}	5.70±1.88^{ab}	4.60±1.89^{bc}	6.70±1.62^{a}
Overall Acceptability	5.23±1.62^{b}	4.88±1.84^{b}	5.28±1.75^{b}	4.93±1.69^{b}	6.45±1.62^{a}

Means followed by different superscript letters (a-c) within the same row are statistically significant at the 5% level.

4. Conclusion

The result from this study shows that the buffalo meats marinated with *Averrhoa bilimbi* showed the best result according to the physicochemical properties and sensory evaluation. From this experiment, *Averrhoa bilimbi* treated samples resulted in lower pH value, higher moisture content for the cooked sample, higher cooking yield and a better result for texture. This indicates that *Averrhoa bilimbi* marinades can potentially act as meat tenderizer with the ability to improve the tenderness. Thus, tough meat can be tenderized. These findings might help the industry and consumers to improve the quality attributes of tough meats.

Acknowledgements

The authors would like to thank Universiti Putra Malaysia for the fund support.

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