Effects of Papaya Leaves Crude Extract on the Physicochemical and Sensory Characteristics of Marinated Chicken Meat

Normah Ismail,[1] and Rosliana Rosman[1]

[1]Department of Food Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan

[1]Email: norismel@uitm.edu.my

Received: 23 October 2018
Accepted: 30 October 2018

ABSTRACT

The purpose of this study was to investigate the effect of papaya leaves crude extract on the physicochemical properties of marinated chicken meat. Papaya leaves was extracted with sodium acetate (CH_3COONa) buffer (pH 7.2) at room temperature. Protein concentration and activity of enzyme in the crude extract were determined by using UV-Spectrophotometer. The crude extract was mixed with marinated ingredients and then coated onto chicken meat which was subsequently kept for overnight in refrigerator at chill temperature. Protein concentration of the enzyme was identified as 166.36 µg/µl and enzyme activity was 1.28 CDU/ml. Results also showed that lightness (L), cooking loss and shrinkage of the marinated chicken were higher than control. Redness (a*), yellowness (b*), protein content, water-holding capacity, shear force, and texture profile analysis were lower than control. Microstructure analysis showed that the chicken meat muscle was destructed in the presence of papaya crude extract. Sensory acceptability evaluation of marinated chicken which was carried out by using 9 point hedonic scale suggested that chicken meat treated with papaya crude extract gave significant effect on texture, juiciness, flavour and overall acceptability compared to commercial bromelain. Additionally, the use of papaya crude extract gave a significant effect on the physicochemical properties of marinated chicken.*

Keywords: *papaya, crude extract, enzyme, marinade, marinated chicken meat*

INTRODUCTION

Marinades are traditional method widely used in meat to improve meat quality before thermal processing [1]. Meat consists of muscle and connective tissues that are made up of proteins which contain amino acids linked together in chains to make large molecules [1]. Thus, marinades are used to tenderise the meat by breaking apart the amino acids, making it softer and less chewy [2]. Marinade ingredients include vinegar, lemon juice or wine, phosphates, oils, herbs, spices, dairy products, fruits and vegetables [3]. Marinades can increase product yield, reduce water loss during cooking and improve meat tenderness.

Carica papaya is a medical plant which is being used as medicine to treat various diseases such as warts, corns, constipation, blood pressure and cancer [4]. The leaves and fruit are rich in vitamins, phenols, proteolytic enzymes which act as a good antioxidant and an excellent antimicrobial agent [5]. Papaya leaves are used in marination since it contains papain that breaks down protein and allows flavour to penetrate deeper [6]. Papain is an active endolytic cysteine protease which has a broad range of specificity among proteolytic enzymes and relatively heat stable. It is usually used in marination to tenderise the meat and improve meat quality [6].

Some of the chemicals used in food may impose health problem to consumer especially when use in long term. Chemical additives are widely used to preserve and tenderise meat, enhance flavour, inhibit bacterial growth and extend shelf life. However, these chemicals can turn to toxic and give adverse effect to health. Nowadays, consumers prefer natural foods with fewer additives. Thus, industries are looking for solutions to reduce dependency on chemical additive and at the same time increasing the usage of natural ingredients as well as reducing agricultural wastage. Therefore, papaya leave may be the solution to this problem. The objectives of this study are to determine the effect of papaya leaves crude extract on the physicochemical and sensory characteristics of marinated chicken meat.

MATERIALS AND METHOD

Several young papaya leaves (Carica papaya) from Eksotika variety were selected with width and length approximately 55 and 40 cm, respectively. The shoots were chosen from the top branch of the trunk. The leaves were obtained from Kalumpang Agriculture Department, Hulu Selangor, Selangor. The chicken was obtained from a slaughterhouse in Klang. All chemicals used were of analytical grade.

Preparation of the meat

The skin, external fat and connective tissues were initially removed from the meat. The meat was then cut into approximately 3 cm^3, washed and wiped with a paper towel.

Preparation of papaya leaves crude extract

Initially, papaya leaves were washed in 0.1% hydrogen peroxide (H2O2) solution and then cut into small pieces. The leaves were then homogenised in sodium acetate (CH3COONa) buffer in a blender followed by filtration and centrifugation for ten minutes to obtain the crude extract. The extraction process was performed in cold temperature (4°C).

Determination protein concentration

About 10 µl crude extract and commercial bromelain solution were filled in two different test tubes. Bovine serum albumin (BSA) (10-200 µg) was pipetted into a separate test tube. To each test tube, 3 ml of complex forming reagent was added. This mixture was then incubated for 30 minutes at room temperature in the dark. About 0.3 ml of Folin Ciocalteu phenol reagent was added to all the test tubes and incubated for ten minutes. The absorbance was measured using a UV-spectrophotometer at 660 nm. The readings were tabulated and a graph of protein (µg) on x-axis and absorbance on y-axis was plotted. From this graph the unknown concentration of protein in the crude extract and commercial bromelain solution were determined.

Determination of the enzyme activity

About 5 ml of casein substrate solution was pipetted into a tube which was then inserted in a water bath (37°C) for approximately ten minutes. Then, 1.0 ml enzyme (crude extract or bromelain) was added to each test tube, vortexed and immediately returned to the water bath for incubation at 37°C for exactly ten minutes. Then, 5 ml trichloroacetic acid (TCA) stopping reagent was added to the tubes. The tubes were vortexed vigorously and allowed to cool at room temperature. The contents of each test tube were filtered twice through Whatman #1 filter paper. Then, the absorbance of the clear filtrate from all tubes was measured.

Sample marination

For every 1 kg diced chicken meat, the marinate ingredients consisting of 16 g papaya leave extract or commercial bromelain, 2.5 g salt, 2.5 g minced garlic, 2.5 g ground black pepper, 2.5 g ground cumin and 2.5 g ground white cumin were mixed. The control contains all these ingredients except for papaya crude extract or bromelain. After coating, the diced chicken meat was placed in a container which was then left in the refrigerator for overnight. After marination, the samples were analysed.

Texture profile analysis

The texture profile in terms of hardness, springiness, gumminess and chewiness were measured using a texture analyser (TA-XT2 Texture Analyser, UK) fitted with a 5-mm-diameter P/5 stainless steel cylindrical probe set a compression speed at 0.5 cm s-1 and 75% strain. The texture profile was calculated from the resultant force-deformation curves [1].

Shrinkage diameter

Shrinkage was calculated as the difference in diameter between the unbaked and baked marinated chicken meat divided by the diameter of the unbaked marinated chicken meat [7]. The diameter of the meat was measured by using a vernier caliper.

Shrinkage diameter =

$$\frac{\text{diameter of unbaked marinated chicken meat (mm)} - \text{diameter of baked marinated chicken meat (mm)}}{\text{diameter of unbaked marinated chicken meat (mm)}}$$

Microstructure

The microstructure was determined using a scanning electron microscope (SEM). The control and marinated chicken meat were cut into pieces of approximately 1.0 x 1.0 x 0.5 cm in size. Sample was fixed by soaking in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for two hours at room temperature. The sample then was rinsed with distilled water and dehydrated in a graded ethanol solution with series of 50, 70, 80, 90% and twice in absolute ethanol for one hour. The sample was dipped in liquid nitrogen and immediately cut with a razor blade. Dried specimen was attached to aluminium stubs, coated with gold and then examined and photographed with a scanning electron microscope using an accelerating voltage of 10 kV. Micrograph and video print of transverse section were taken at 200x magnification [1].

Colour analysis

The colour properties such as L* (lightness), a* (redness), and b* (yellowness) were measured by using a Chroma Meter CR-400.

Water-holding capacity

Water holding capacity (WHC) was determined by using the method described by Rasli and Sarbon [8]. Approximately 5 g samples were homogenised in 5 mL distilled water and vortexed for about 15 minutes followed by centrifugation at 3000 g for 20 minutes. The volume of the meat layer after centrifuge was read. Water holding capacity was calculated as follows:

$$\text{WHC (\%)} = \frac{\text{volume before centrifuge (mL)} - \text{volume of meat layer after centrifuge (mL)}}{\text{volume before centrifuge (mL)}} \times 100$$

Cooking loss

Cooking loss was calculated as the difference in weight between the unbaked and baked marinated chicken meat divided by the weight of the unbaked marinated chicken meat [7].

$$\text{Cooking loss} = \frac{\text{weight of unbaked marinated chicken meat (g)} - \text{weight of baked marinated chicken meat (g)}}{\text{weight of unbaked marinated chicken meat (g)}}$$

Protein determination

Crude protein content of the samples was determined by the Kjeldhal method [9].

Shear force

Shear force was determined using a texture analyser (TA-xT2i Texture Analyser, UK) equipped with a Warner-Bratzler shear apparatus. The cross-head speed was 2 mm/s and a distance between blade bottom and base plate was 1.5 cm. The highest peak of the shear force profile is expressed as shear force value [1].

Sensory evaluation

The samples were placed in a plate with three coded labels and evaluated for their acceptability in terms of appearance, texture, juiciness, bitterness and flavour by indicating their acceptability level using nine points hedonic scale (1- extremely dislike to 9- extremely like).

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Duncan's multiple range tests were used to determine the significant difference between the mean. Statistical analysis was performed using the Statistical Analysis System (SAS) Version 9.2 for Windows [10].

RESULTS AND DISCUSSION

Protein concentration and enzyme activity

Protein concentration for commercial bromelain and papaya crude extract are shown in Table 1. Papaya crude extract has significantly higher ($w<0.05$) protein concentration than commercial bromelain. According to previous study, protein concentration of bromelain was 19.51 µg/µl [11]. However, commercial bromelain showed significantly ($p<0.05$) higher enzyme activity than papaya crude extract.

Table 1: Protein concentration and enzyme activity for commercial bromelain and papaya leaves crude extract

Sample	Concentration (µg/µl)	Enzyme activity (CDU/ml)
Commercial bromelain	12.73±0.04b	4.29±0.01a
Papaya leaves crude extract	166.36±0.02a	1.28±0.03b

Values are means ±standard deviation
Means within each row with different superscripts are significantly different at $p<0.05$.

Physicochemical properties of marinated chicken meat

The physichochemical properties of marinated chicken meat such as colour, protein, water-holding capacity, shear force and cooking loss are shown in the Table 2. Signifiicantly ($p<0.05$) lighter colour was observed for chicken meat treated with commercial bromelain. However, control shows significantly higher ($p<0.05$) values for redness (a*) and yellowness (b*). A dull brown colour of meat is due to the presence of metmyoglobin which indicated that the meat was in an oxidised form [12].

Protein content in the marinated chicken meat was significantly reduced (p<0.05) after applying the enzymes compared to the control. This may due to proteolysis effect of the enzyme. The finding shows that reduction of the protein content in the enzyme-treated samples might be due to increasing solubility of the protein that lead to increase in permeability of myofibrils, which will disintegrate easily [13]. This result agreed with Rawdkuen *et al.* [14] who suggested that the enzyme exist had hydrolytic activity, thereby degrading the protein.

Table 2: Physicochemical properties of chicken meat marinated with commercial bromelain and papaya leaves crude extract

	Treatment		
Parameters	Control	Commercial bromelain	Papaya leaves crude extract
Colour			
L*	44.65±0.27c	48.60±0.36a	47.26±0.005b
a*	3.88±0.21a	2.67±0.07b	1.76±0.02c
b*	15.86±0.14a	11.36±0.04c	15.03±0.02b
Protein	19.13±0.04a	16.25±0.19c	17.15±0.30b
Water- holding capacity (%)	49.67±0.31a	38.52±0.64b	21.05±0.39c
Shear force (N)	2.64±0.26a	1.53±0.09b	1.20±0.06c
Cooking loss (%)	46.12±3.37c	59.44±2.74a	51.67±1.86b

Values are means ±standard deviation
Means within each row with different superscripts are significantly different at p<0.05.

Water-holding capacity which is defined as the ability of meat to retain its own water is one of the characteristics that defined the quality of the meat [15]. Many attributes such as colour, texture, firmness, juiciness and flavour partially depend on water-holding capacity. Samples treated with commercial bromelain and papaya crude extract showed significantly lower (p<0.05) water-holding capacity compared to control. The reduced amount of water bound to the enzymatically tenderised meats can be explained by the changes of myofibrillar protein structure as a result of exogenous proteolytic enzyme action [16]. It is also supported by Rawdkuen *et al.* [14] who stated that reduced water-holding capacity is a result of myofibrillar shrinkage as well as the movement of water from the myofilament space to the extra-cellular space.

Enzymatic treatment results in significantly lower (p<0.05) shear force than the control. This is because control sample is less tender than sample treated with enzyme. Enzyme treatment denatures the protein and can be observed by increase in the protein solubilisation and water retention [1]. Fibre density decreased when meat was marinated [1]. The result is also supported by the study of Naveena *et al.* [17] who observed a decreasing in shear force value when ginger rhizome was added into buffalo meat. They suggested that this was due to extensive muscle fibre and connective tissue degradation.

Higher water-holding capacity can reduce the cooking loss of marinate meat in which an increase in water absorption resulted in high water-holding capacity and less cooking loss [1, 13, 18]. Chicken meat marinated with commercial bromelain and papaya crude extract showed significantly higher (p<0.05) cooking loss compared to control. The study of Murphy and Marks [19] reported that during heating, the water content within the myofibrils in the narrow channels between the filaments undergoes changes due to the shrinkage of tissue matrices and thus causing the cooking loss of meat to increase. The increasing of cooking loss is in agreement with Klinhom *et al.* [20]. However, other studies reported that enzyme can decrease the cooking loss [16, 17].

Texture is an important attribute in determining the quality of the meat. The textural chararcteristics that were measured in this study were hardness, springiness, gumminess and chewiness. Chicken marinated with commercial bromelain and papaya leaves crude extract had lower hardness, springiness, gumminess and chewiness values than the control whereas the shrinkage values were higher (Table 3). The study of Gokoglu *et al.* [13] also showed lower values when meat or squid were treated with bromelain and papain and this cause tenderness in the muscle. As reported by Sullivan & Calkins [21], there are significant increases in tenderness of papain and bromelain injected beef steaks with decreasing of hardness, springiness, gumminess, and chewiness. As mentioned by Ramadhan *et al.* [7], the marinated chicken meat shrunk due to meat protein denaturation and fluid loss while diameter reduction reflects volume reduction and it was not reflected by reduction of thickness.

The microstructures of the marinated chicken meat observed using scanning electron micrograph are shown in Figure 1. Destruction of the muscle structure were observed in commercial bromelain and papaya leaves crude extract compared with control. Previous study stated that the disorganisation effects of papain was larger than those of controls [22]. Destruction of the structure of intramuscular connective tissue is the reason for meat tenderisation by the proteolytic enzymes [23]. The muscle Fibres of the control sample was slightly bound to each other.

Table 3: Texture profile analysis (TPA) and shrinkage diameter for marinated chicken meat treated with commercial bromelain and papaya leaves crude extract

	Treatment		
Parameters	Control	Commercial bromelain	Papaya leaves crude extract
Hardness	56.70±0.69a	52.34±1.08b	46.77±2.96c
Springiness	0.76±0.04a	0.66±0.02b	0.55±0.05c
Gumminess	57.12±0.58a	49.08±0.80b	37.46±0.55 c
Chewiness	56.54±0.07a	32.69±0.21b	23.26±0.23c
Shrinkage diameter	0.22±0.01c	0.36±0.03b	0.47±0.02a

Values are means ±standard deviation
Means within each row with different superscripts are significantly different at $p<0.05$.

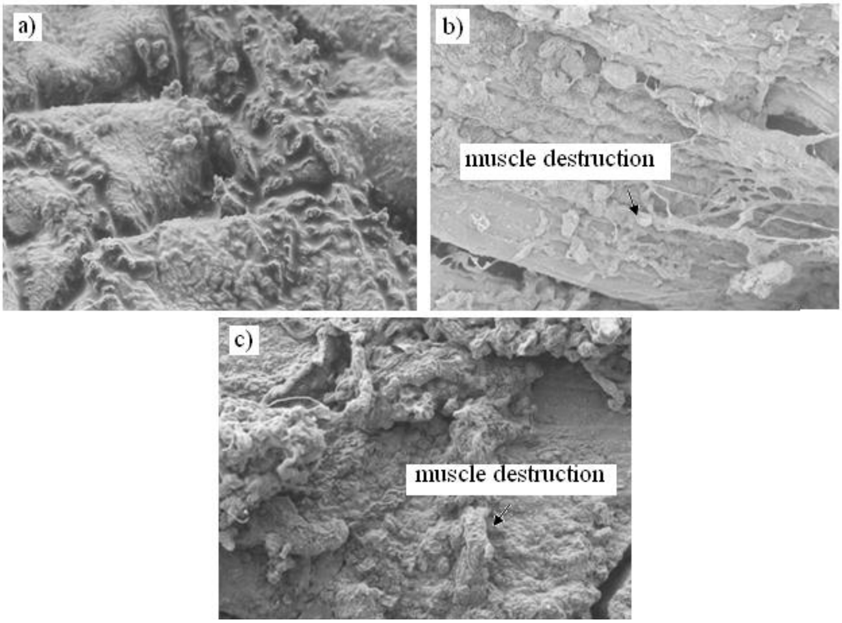

Figure 1: Microstructure of marinated chicken meat a) control, b) treated with commercial bromelain and c) treated with papaya leaves crude extract as observed at 200x magnification

Sensory evaluation

Sensory evaluation is an important indicator for potential consumer preferences. Sensory was evaluated by 30 untrained panelists and the maximum score limit for each item was nine indicating that the sample was favoured extremely. Based on Table 4, the score ranged between 6.13 to 7.20 for all the attributes suggesting that the marinated chicken meats were preferred slightly and moderately, respectively. From the previous study, increasing sensory score with the application of bromelain and papain does not give any statistical significant [13]. Based on result, appearance and bitterness showed no significant different ($p>0.05$) among the samples. However, commercial bromelain gave significantly higher ($p<0.05$) acceptability score for texture, juiciness, flavour and overall acceptability compared to papaya crude extract. The result for the juiciness is supported by the finding of Sullivan & Calkins [21] who showed that papain treatment reduced juiciness. This showed that the enzyme improved sensory attributes of marinated chicken meat.

Table 4 :Sensory analysis for marinated chicken meat treated with commercial bromelain and papaya leaves crude extract

	Treatment		
	Control	Commercial bromelain	Papaya leaves crude extract
Sensory attributes			
Appearance	6.70±1.09a	6.67±1.18a	6.23±1.07a
Texture	6.27±1.05a,b	6.90±1.09a	6.23±1.25b
Juiciness	6.57±0.94a,b	6.80±1.13a	6.17±1.09b
Bitterness	6.27±0.94a	6.33±1.15a	6.13±1.11a
Flavour	6.77±1.10a,b	7.17±1.12a	6.30±1.18b
Overall acceptability	6.73±0.87a,b	7.20±1.16a	6.27±1.14b

Values are means ±standard deviation
Means within each row with different superscripts are significantly different at $p<0.05$

CONCLUSION

Application of papaya leaves crude extract as part of marinating ingredient affects the physicochemical properties of marinated chicken meat such as colour, protein content, water-holding capacity, cooking loss, shear force, texture and shrinkage. Acceptability for texture, juiciness and flavour were lower when papaya leaves crude extract was added compared to commercial bromelain. As for bitterness, papaya leaves crude extract and bromelain treated marinated chicken meat were equally acceptable. Both enzymes led to tenderisation of chicken meat as revealed by the destruction of muscle fibre. Thus, enzyme in papaya leaves extract assists in the marinating process of chicken meat.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor for providing the laboratory facilities.

REFERENCES

[1] P. Komoltri and P. Pakdeechanuan, 2012. Effects of marinating ingredients on physicochemical, microstructural and sensory properties of golek chicken, *International Food Research Journal, Vol. 19*(4), pp. 1449-1455.

[2] K.H. Choi and R.A. Laursen, 2000. Amino-acid sequence and glycan structures of cysteine proteases with proline specificity from ginger rhizome Zingiber officinale, *European Journal of Biochemistry, Vol. 267*(5), pp. 1516-1526. Doi: 10.1046/j.1432-1327.2000.01152.x.

[3] N. Aktas, M.I. Aksu and M. Kaya, 2003. The effect of organic acid marination on tenderness, cooking loss and bound water content of beef, *Journal of Muscle Foods, Vol. 14*(3), pp. 181-194. Doi: 10.1111/j.1745-4573.2003.tb00699.x.

[4] G. Aravind, B. Debjit, S. Duraivel and G. Harish, 2013. Traditional and medicinal uses of Carica papaya, *Journal of Medicinal Plant Studies, Vol. 1*(1), pp. 7-15.

[5] R.R. Banala, V.B. Nagati, and P.R. Karnati, 2015. Green synthesis and characterization of Carica papaya leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bactericidal properties, *Saudi Journal of Biological Sciences, Vol. 22*(5), 637-644. Doi: https://doi.org/10.1016/j.sjbs.2015.01.007.

[6] M.N. Islam and E.M. Molinar-Toribio, 2013. Development of a meat tenderiser based on papaya peel, *Universidad Tecnológica de Panamá, Vol. 9*(2), pp. 24-31.

[7] K. Ramadhan, N. Huda and R. Ahmad, 2012. Physicochemical and sensory characteristics of burger made from duck surimi-like material, *Poultry Science, Vol. 91*(9), pp. 2316-2323. Doi: https://doi.org/10.3382/ps.2011-01747.

[8] H. I. Rasli, and N. M. Sarbon, 2014. Effects of different drying methods on the rheological, functional and structural properties of chicken skin gelatin compared to bovine gelatin, *International Food Research Journal, Vol. 22*(2), pp. 584-592.

[9] AOAC, 2005. Official Method of Analysis, 15th ed. Virginia, Association of Official Analytical Chemistry International.

[10] SAS Institute Inc., 2009. SAS user's guide to the statistical analysis system. SAS Institute Inc. Raleigh, NC.

[11] A. V. Krishnan and M. Gokulakrishnan, 2015. Extraction, purification of bromelain from pineapple and determination of its effect on bacteria causing periodontitis, *International Journal of Pharmaceutical Sciences and Research, Vol. 6*(12), pp. 5284. Doi: 10.13040/IJPSR.0975-8232.6(12).5284-94.

[12] B. Hernandez, G. Lizaso, A. Horcada, M. J. Beriain, and A. Purroy, 2006. Meat colour of fighting bulls versus beef colour, *Archivos Latinoamericanos de Producción Animal, Vol. 14*(3).

[13] N. Gokoglu, P. Yerlikaya, I. Ucak and H.A. Yatmaz, 2016. Effect of bromelain and papain enzymes addition on physicochemical and textural properties of squid (Loligo vulgaris), *Journal of Food Measurement and Characterization, Vol. 11*(1), pp. 1-7. Doi: https://doi.org/10.1007/s11694-016-9403-3.

[14] S. Rawdkuen, M. Jaimakreu, and S. Benjakul, 2013. Physicochemical properties and tenderness of meat samples using proteolytic extract from Calotropis procera latex, *Food Chemistry, Vol. 136*(2), pp. 909-916. Doi: 10.1016/j.foodchem.2012.08.077.

[15] M. Janhvi, R. Gayathri and V. Vishnupriya, 2016. Tenderisation of meat using bromelain from pineapple extract, *International Journal of Pharmaceutical Science and Research, Vol. 39*(1), pp. 81-85.

[16] D. Istrati, A. Ionescu, C. Vizireanu, A.M.S. Ciuciu and R. Dinica, 2012. The tenderization of bovine biceps femoris muscle using marinades on the basis of wine, *Romanian Biotechnological Letters, Vol. 17*(6), pp. 7787-7795.

[17] B.M. Naveena, S.K. Mendiratta and A.S.R. Anjaneyulu, 2004. Tenderization of buffalo meat using plant proteases from Cucumis trigonus Roxb (Kachri) and Zingiber officinale roscoe (Ginger rhizome), *Meat Science, Vol. 68*(3), pp. 363-369. Doi: 10.1016/j.meatsci.2004.04.004.

[18] S.S. Latif, 2011. Effect of marination on the quality characteristics and microstructure of chicken breast meat cooked by different methods, *Lucrări Stiinţifice, Vol. 54*(6), pp. 314-324.

[19] R.Y. Murphy and B.P. Marks, 2000. Effect of meat temperature on proteins, texture and cook loss for ground chicken breast patties, *Poultry Science, Vol. 79*(1), pp. 99-104. Doi: 10.1093/ps/79.1.99.

[20] P. Klinhom, J. Klinhom, J. Senapa, and S. Methawiwat, 2011. Influence of citric acid, lemon and porcupine fruit juice on the tenderness and sensory properties of bovine meat. In *Proceedings of the 49th Kasetsart University Annual Conference,* Kasetsart University, Thailand, 1-4 February, 2011. Volume 4. Subject: Agricultural Extension and Home Economics, pp. 1-10.

[21] G.A. Sullivan and C.R. Calkins, 2010. Application of exogenous enzymes to beef muscle of high and low-connective tissue, *Meat Science, Vol. 85*(4), pp. 730-734. Doi: 10.1016/j.meatsci.2010.03.033.

[22] M. Wada, T. Suzuki, Y. Yaguti and T. Hasegawa, 2002. The effects of pressure treatments with kiwi fruit protease on adult cattle semitendinosus muscle, *Food Chemistry, Vol. 78*(2), pp. 167-171. Doi: https://doi.org/10.1016/S0308-8146(01)00395-8.

[23] C. Qihe, H. Guoqing, J. Yingchun and N. Hui, 2006. Effects of elastase from a bacillus strain on the tenderization of beef meat, *Food Chemistry, Vol. 98*(4), pp. 624-629. Doi: https://doi.org/10.1016/j.foodchem.2005.06.043.