Application of bromelain powder produced from pineapple crowns in tenderising beef round cuts

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Abstract

The purpose of this study is to determine the effect of bromelain produced from pineapple crowns on the quality of beef round cuts. The beef was treated with bromelain at the following condition; the pH of beef was 5.6, the immersion temperature was 60ºC, the concentration of bromelain solution was 0.17% and the immersion time was 10 minutes. Bromelain decreased the hardness, water holding capacity (WHC), moisture content and a^* value of beef. On the other hand, bromelain increased the pH, cooking loss, and L^* and b^* values of beef. Bromelain treatment to beef cut samples significantly ($P < 0.05$) increased the essential and non-essential amino acids content of beef. This indicated that beef treated with bromelain had a desirable effect on the beef quality.

Introduction

Bromelain is a proteolytic enzyme found naturally in pineapple plant (Hebbar *et al.*, 2008). It has been used widely as a meat tenderiser (Omojasola *et al.*, 2008). For instance, bromelain had been used to tenderise beef (Ketnawa and Rawdkuen, 2011), mutton (Bille and Taapopi, 2008), chicken meat (Koide *et al.*, 2010) and pork (Ieowsakulrat *et al.*, 2011). Bromelain, the plant thiol proteases affects the structure of myosin and actin filaments of myofibrillar proteins. Gerelt *et al.* (2000) reported that proteolytic enzymes not only accelerate the fragmentation of myofibrils but also may disrupt the structure of intramuscular connective tissue in meat. According to Godfrey and Reichelt (1983), bromelain cleaves peptide bonds at the carbonyl end of lysine, alanine, tyrosine and glycine. In addition, bromelain is recognised by the United States federal agencies as generally recognised as safe (GRAS) to improve the meat tenderness (Sullivan and Calkins, 2010).

Brahman is a tropically adapted Bos indicus breed developed from the cattle of Indian origin. It is one of the numerous cattle breeds in South Africa adapted to tropical and subtropical conditions. The Brahman breed is well known for its rusticity and maternal ability (Soria *et al.*, 2010). Besides, it is able to withstand extreme climates (adaptability) and excels in crossbreeding programs (Pico *et al.*, 2004). There are many advantages of using Brahman crossbreed cattle in different parts of the world. Unfortunately, there are some widely known undesirable palatability attributes which reduce the value of cattle with Brahman background. Among the most important of these undesirable attributes is the reputation for inadequate tenderness (Riley *et al*., 2005). According to Maiti *et al.* (2008), Brahman breed is tougher than Hereford breed.

Tenderisation process could alter the physico–chemical properties of beef. Li and Zan (2011) reported that there is a need to study the physico–chemical properties of beef to correlate with its nutritional values. Amino acids are the building blocks of protein which function as the major structural components of body cells. There are two different types of amino acids which are essential and non–essential amino acids. Non–essential or dispensable amino acids are the amino acids that the body can create out of other chemicals found in the body. Meanwhile, essential or indispensable amino acids are the amino acids that cannot be created and therefore, the only way to get them is through food (Muhammad–Lawal and Balogun, 2007). Peraza–Mercado *et al.* (2010) stated that failure to get enough essential amino acids can cause detrimental health effects. This study was undertaken to determine the effect of bromelain on the quality of beef round cuts. The beef was treated with bromelain at the following condition; the pH of

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beef was 5.6, the immersion temperature was 60ºC, the concentration of bromelain solution was 0.17% and the immersion time was 10 minutes.

Materials and Methods

Materials

The crowns of pineapple variety N36 from maturity index 2 (all scales green with tinge of yellow between the scales at the base, the bracts are dry and whitish) were obtained from Peninsula Plantation Sdn Bhd, Simpang Renggam, Johor, Malaysia. The beef round cuts from the 3–year–old bull of Brahman species were obtained from MZR Livestock (M) Sdn Bhd, Rantau Panjang, Selangor, Malaysia. Food grade chemicals and analytical grade chemicals were purchased from Merck Chemical Company and Sigma Chemical Company. Custom–made cation exchange resin column and continuous diafiltrator were purchased from Agilent and Isetake, respectively.

Production of bromelain powder

The production of bromelain powder was carried out following method as described by Nadzirah et al. (2012). Initially, the pineapple crowns were crushed in a food processor with the addition of purified water at a ratio of 1:1 to produce pineapple crown extract. The extract was filtered through a double layered muslin cloth to remove the solid parts. The filtrate was then collected and purified by a preparative High Performance Liquid Chromatography (HPLC) (Agilent 1200 Series, USA) using a cation exchange resin column of 21.2 mm internal diameter and 250 mm length. The eluents used were acetate buffer (25 mM, pH 4.0) and 1M NaCl solution and the flow rate used was 6 ml/min. Bromelain was detected at 280 nm wavelength. The purified bromelain was dialysed by a continuous diafiltrator using a hollow fiber membrane having a molecular weight cut-off of 10 kDa to remove salt as a result of the purification process. Purified water was used for buffer exchange. Finally, the bromelain solution was dried using a freeze dryer (Christ alpha 1-4LD Plus model, Belgium). The drying process which was carried out at -55ºC took about a week to produce a completely dried bromelain powder.

Tenderisation of beef using bromelain solution

Beef round was cut into cubes of approximately 2 cm^3. The beef cubes were randomly divided into two groups. One group was treated with bromelain solution according to the feasible optimum condition obtained by Zainal et al. (2013) whereby the pH of beef was 5.6, the immersion temperature was 60ºC, the concentration of bromelain solution was 0.17% and the immersion time was 10 minutes. Meanwhile, the other one group was not treated and served as control. Analysis on the beef cubes was performed in triplicate.

Determination of hardness

Beef hardness was determined following method as described by Zainal et al. (2013). The beef hardness was measured by a texture analyser (model TAX–T2i Stable Micro Systems, England) using a P2N needle probe with a load cell of 10 kg applied at a cross head speed of 60 mm/min. The immersion depth of beef cube was 5 mm. The beef hardness was expressed in gram (g).

Determination of water holding capacity

The water holding capacity (WHC) was determined following method as described by Özalp and Karakaya (2009). 8 g of grinded beef sample was put into a tube. Then, 12 ml of 0.6M NaCl solution was added into the tube. The tube was subsequently placed into a chiller at 5°C for 15 minutes. After that, the tube was centrifuged at 1977 x g for 15 minutes at 5°C. The supernatant was poured into a measuring cylinder and the volume was recorded. The WHC was calculated according to Ketnawa and Rawdkuen (2011) and expressed in percentage (%) as the following equation:

$$WHC\ (\%) = \frac{Vol.\ of\ NaCl\ before\ centrifuge - Vol.\ of\ NaCl\ after\ centrifuge}{Vol.\ of\ NaCl\ before\ centrifuge} \times 100$$

Determination of moisture content

The moisture content was determined using oven method as described by Ruiz (2001). The moisture content was calculated as the percent loss in weight after drying.

Determination of pH

The pH was determined by a pH meter (Model HI 110 series, Hanna devices, USA) at room temperature (27ºC). The pH meter was initially calibrated with pH 7 and pH 4 buffers before being used in pH determination (Wardy et al., 2009).

Determination of cooking loss

The beef samples were weighed accurately just before cooking. After cooking, the samples were cooled and weighed immediately. The cooking loss was calculated according to Sultana et al. (2009)

and expressed in percentage (%) as the following equation:

Cooking loss (%) = Weight (before cooking) – Weight (after cooking) x 100

Beef weight before cooking

Determination of colour

Colour were determined by the L^*, a^*, b^* colour space (also referred to as CIELAB) using a portable chromameter (CR–400 Minolta, Osaka, Japan) with D65 illuminant and 8 mm aperture size according to the method by Ergezer and Gokce (2011). It was calibrated using a white standard tile. The expression of colour was characterised as L^* (lightness) and a^*, b^* (chromaticity coordinates). The chromaticity coordinates represent colour directions as follows: $+a^*$ (red direction), $-a^*$ (green direction), $+b^*$ (yellow direction), $-b^*$ (blue direction).

Determination of amino acids content

The amino acids content was determined using method as described by Gam *et al.* (2005). The amino acids analysis was carried out by an analytical HPLC with a fluorescence detector. The chromatography was run using an AccQ tag column of 3.9 mm internal diameter and 150 mm length (Waters) at a flow rate of 1 ml/min. The eluent A and eluent B used were AccQ•Tag concentrate and 60% acetonitrile, respectively. The injection volume was 10 µl. The period of analysis was 50 minutes. The amino acids content in sample was determined from the standard curve of standard amino acids. The results were expressed as g/100g.

Analysis for amino acids content

The amino acids were grouped according to its chemical structure: monoamino carboxylic acids (glycine, alanine, valine); monoamino dicarboxylic acids or acidic amino acids (aspartic and glutamic acids); ß–hydroxyamino carboxylic acids (serine and threonine); basic acids (lysine, histidine, arginine); sulphur–containing amino acids (methionine and cysteine); ring–containing amino acids (phenylalanine, tyrosine, proline) and leucines (leucine and isoleucine) (Bivolarski *et al.*, 2011). The amino acids were also grouped into essential, non–essential, flavour and medicinal amino acids. The amino acids content of bromelain-treated beef and untreated beef was calculated and analysed (refer to Table 1).

Determination of beef quality

The nutritional quality of beef was determined using amino acid score method (Ismail *et al.*, 2013) and fuzzy recognition method (Yu *et al.*, 2014). The

Table 1. Analysis for amino acids content

Group	Amino acids content
Total monoamino carboxylic acids	Glycine + alanine + valine
Total monoamino dicarboxylic acids (Acidic amino acids)	Aspartic acid + glutamic acid
Total ß–hydroxyamino carboxylic acids	Serine + threonine
Total basic acids	Lysine + histidine + arginine
Total sulphur–containing amino acids	Methionine + cysteine
Total ring–containing amino acids (Aromatic amino acids)	Phenylalanine + tyrosine + proline
Total leucines	Leucine + isoleucine
Total amino acids	Monoamino carboxylic acids + monoamino dicarboxylic acids + ß–hydroxyamino carboxylic acids + basic acids + sulphur–containing amino acids + ring–containing amino acids + leucines
Total essential amino acids	Methionine + valine + isoleucine + leucine + phenylalanine + histidine + lysine + threonine
Total non–essential amino acids	Glycine + cysteine + tyrosine + alanine + aspartic acid + glutamic acid + serine + arginine + proline
Total flavour amino acids	Glycine + alanine + aspartic acid + glutamic acid + serine + proline
Total medicinal amino acids	Aspartic acid + glutamic acid + glycine + leucine + tyrosine + phenylalanine + lysine + arginine + methionine

quality was determined based on the essential amino acids content of untreated beef and bromelain–treated beef.

Determination of amino acid score

The amino acid score was calculated according to Ismail *et al.* (2013) as follows:

Amino acids score (%) = $\dfrac{\text{Amount of amino acid per sample protein x 100}}{\text{Amount of amino acid per protein in reference protein}}$

The reference values used were based upon the essential amino acids requirements of 2- to 5-year-old children (refer to Table 4).

Determination of proximate extent

The proximate extent denoted the extent between test protein and reference pattern protein, namely the similarity of the composition of amino acid. It was determined using fuzzy pattern recognition method as described by Yu *et al.* (2014) and calculated according to the following equation:

$$\mu(a,\mu i) = 1 - 0.09 \sum_{k=1}^{n} \frac{[\alpha k - \mu ik]}{\alpha k + \mu ik}$$

Where μik is the amount of certain essential amino acids of test protein and αk is the amount of certain essential amino acids of reference pattern protein.

Statistical analysis

Data were analysed by Statistical Package for the Social Sciences (SPSS) version 15 using one–way ANOVA. Duncan's multiple–range test was used to determine the difference between means. A significant difference was considered at the level of $P < 0.05$ (5%).

Results and Discussion

It was observed that the hardness, WHC, moisture content and a^* of untreated beef decreased significantly from 187.23% to 19.73%, 14.44% to 12.22%, 70.48% to 65.75% and 7.20 to 5.61, respectively after the beef was treated with 0.17% concentration of bromelain solution in a water bath at 60ºC for 10 minutes. On the other hand, the pH, cooking loss and L^* and b^* of untreated beef increased significantly from 5.60 to 5.69, 8.77% to 15.89%, 41.19 to 42.95 and 11.86 to 13.15, respectively after the beef was treated with 0.17% concentration of bromelain solution in a water bath at 60ºC for 10 minutes (refer to Table 2).

The tenderness of untreated beef round cuts increased significantly by 89.46% after the beef was treated with 0.17% concentration of bromelain solution in a water bath at 60ºC for 10 minutes. Results from the present study were higher than Ketnawa and Rawdkuen (2011) and Huffman et al. (1967). Ketnawa and Rawdkuen (2011) had used bromelain powder produced from pineapple peel to tenderise beef. They found that the tenderness of untreated beef increased significantly by 20% after the beef was treated with 3% concentration of bromelain solution and left at room temperature for 60 minutes. Huffman *et al.* (1967) found that the tenderness of untreated beef round and loin cuts increased significantly by 16.09 and 33.18%, respectively after the beef was treated with papain and heated in an oven at 182ºC for 2.5 hours.

The increase in the tenderness of bromelain–treated beef (89.46%) was due to the proteolysis of muscle protein by bromelain. This is based on Ketnawa and Rawdkuen (2011) who found that bromelain fragmented the myosin heavy chain. Besides, the action of bromelain by denaturing protein and by breaking down the collagen, muscle fibers and tissues that connect it also contributed to the increase of beef tenderness (Bille and Taapopi, 2008). Rawdkuen and Benjakul (2012) reported that enzymes increased the collagen solubility and promoted the structural alterations through the action on collagens cross–link.

Heating process would accelerate the beef

Table 2. Physico–chemical properties of untreated beef and bromelain–treated beef

Properties	Untreated beef	Bromelain–treated beef
Hardness (g)	187.23 ± 1.25[a]	19.73 ± 2.59[b]
WHC (%)	14.44 ± 1.93[a]	12.22 ± 1.92[b]
Moisture content (%)	70.48 ± 0.16[a]	65.75 ± 0.07[b]
pH	5.60 ± 0.01[b]	5.69 ± 0.00[a]
Cooking loss (%)	8.77 ± 0.17[b]	15.89 ± 0.09[a]
Colour		
L*	41.19 ± 0.04[b]	42.75 ± 0.07[a]
a*	7.20 ± 0.01[a]	5.61 ± 0.03[b]
b*	11.86 ± 0.03[b]	13.15 ± 0.09[a]

[abc] Mean values ± standard deviation (n = 3 replicates) within each column with different superscripts differ significantly at $P < 0.05$.

tenderisation process. This is because, during heating of meat, the sarcoplasmic, myofibrillar and connective proteins undergo denaturation (Kołczak *et al.*, 2008). Chang *et al.* (2011) observed perimysium and endomysium in the raw meat samples were arranged clearly and closely, while collagen fibers were arranged regularly and orderly. The collagen fibers (thick bundles) in the raw meat samples showed a complex structure where the fibers of various striations crossed each other. When an internal endpoint temperature was up to 50ºC, the endomysium became slightly loose and the perimysium began to granulate. At 60ºC, the collagen began to solubilise which increased the beef tenderness.

The decrease in the WHC of bromelain–treated beef was in agreement with Ketnawa and Rawdkuen (2011). Ketnawa and Rawdkuen (2011) found that the 3% concentration of bromelain solution decreased the WHC of beef significantly from 40.01% to 27.97% after the bromelain–treated beef was left at room temperature for 60 minutes. The decrease of WHC was due to the action of bromelain in the denaturation of myofibrillar proteins which play a role in water retention (Murphy and Marks, 2000). Besides, the decrease of WHC was due to the myofibrillar shrinkage as well as the movement of water from the myofilament space into the extracellular space (Ketnawa and Rawdkuen, 2011).

The decrease in the moisture content of bromelain–treated beef was in agreement with Sultana *et al.* (2009). They found that tasty kit solution containing 1.2M NaCl, 0.25M sodium bicarbonate and 0.1% ascorbic acid decreased the moisture content of beef chuck cuts significantly from 75.80% to 68.77% after the tasty kit–treated beef was heated in a water bath at 80ºC for 30 minutes. The majority of water in meat is held within the structure of muscle and muscle cells. Therefore, the decrease of moisture content was caused by the destruction of the structure of muscle

Table 3. Amino acids content of untreated beef and bromelain–treated beef

Amino acids group	Untreated beef (g/100g)	Bromelain–treated beef (g/100g)
Monoamino carboxylic acids		
Glycine[2,3]	0.47 ± 0.01[Bi]	0.64 ± 0.02[Ai]
Alanine[2]	0.59 ± 0.00[Bg]	0.68 ± 0.01[Ah]
Valine[1]	0.55 ± 0.00[Bh]	0.60 ± 0.00[Ajk]
Monoamino dicarboxylic acids (Acidic amino acids)		
Aspartic acid[2,3]	0.92 ± 0.00[Bd]	1.18 ± 0.01[Ab]
Glutamic acid[2,3]	1.46 ± 0.01[Ba]	1.54 ± 0.04[Aa]
β–hydroxyamino carboxylic acids		
Serine[2]	0.44 ± 0.00[Bi]	0.51 ± 0.01[Ai]
Threonine[1]	0.58 ± 0.07[Bg]	0.87 ± 0.03[Af]
Basic acids		
Lysine[1,3]	0.94 ± 0.01[Bc]	0.98 ± 0.00[Ad]
Histidine[1]	0.44 ± 0.01[Bi]	0.77 ± 0.01[Ah]
Arginine[3]	0.74 ± 0.00[Bf]	0.80 ± 0.00[Ag]
Sulphur–containing amino acids		
Methionine[1,3]	0.99 ± 0.00[Bb]	1.02 ± 0.02[Ac]
Cysteine `	0.33 ± 0.00[Bj]	0.44 ± 0.07[Am]
Ring–containing amino acids (Aromatic amino acids)		
Phenylalanine[1,3]	0.46 ± 0.00[Bi]	0.58 ± 0.01[Ak]
Tyrosine[3]	0.48 ± 0.00[Bi]	0.62 ± 0.01[Aij]
Proline[2]	0.45 ± 0.01[Bi]	0.54 ± 0.01[Al]
Leucines		
Leucine[1,3]	0.84 ± 0.00[Be]	0.92 ± 0.01[Ae]
Isoleucine[1]	0.53 ± 0.01[Bh]	0.65 ± 0.00[Ai]
Total monoamino carboxylic acids	1.61	1.92
Total monoamino dicarboxylic acids (Acidic amino acids)	2.38	2.72
Total β–hydroxyamino carboxylic acids	1.02	1.38
Total basic acids	2.12	2.55
Total sulphur–containing amino acids	1.32	1.46
Total ring–containing amino acids (Aromatic amino acids)	1.39	1.74
Total leucines	1.37	1.57
Total amino acids	11.21	13.34
Total essential amino acids	5.33	6.39
Total non–essential amino acids	5.88	6.96
Total flavour amino acids	4.33	5.09
Total medicinal amino acids	7.30	8.28

[1] = essential amino acids, [2] = flavour amino acids, [3] = medicinal amino acids. [abc] Mean values ± standard deviation (n = 3 replicates) within each row with different superscripts differ significantly at P <0.05. Lower case indicates the significant different among amino acids content for both untreated beef and bromelain–treated beef. Capital letter indicates the significant different for each amino acids content between untreated beef and bromelain–treated beef.

cells due to the denaturation of myofibrillar proteins by the action of heat and meat tenderiser (Huang *et al.*, 2011).

The increase in the pH of bromelain–treated beef was in agreement with Naveena and Mendiratta (2004). They found that the pH of buffalo meat treated with ginger extract increased significantly from 5.76 to 5.87 after the ginger–treated buffalo meat was heated in an oven at 180ºC to an internal temperature of 75 ± 1ºC for 20 minutes. The increase of pH was due to the increase loss of free acidic groups (Huang *et al.*, 2011).

The increase in the cooking loss of bromelain–treated beef was in agreement with Klinhom *et al.* (2011). They found the cooking loss of untreated beef increased significantly from 36.55% to 42.90% after the beef was treated with 0.05M citric acid and heated to an internal temperature of 70ºC. Murphy and Marks, (2000) 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.

Sánchez Del Pulgar *et al.* (2012) reported that the increase in the cooking loss of heated meat is caused by three main processes. First, water evaporates with the increase of heating temperature. Second, the increased temperatures during heating would cause the myofibrillar proteins to shrink. It is a process that starts at 40ºC and becomes more intense with the increase of heating temperature. As a result, a parallel decrease occurs in the interfibrillar volume and thus leads to a reduction in the myofibril's ability to hold water. As a consequence, a part of water retained by capillarity is lost. Finally, at temperatures between 56 and 62ºC, a contraction of perimysial connective tissue seems to take place causing the compression of muscle fiber bundles and thus encourages the water to be released from the beef muscle.

The increase in the colour L^* and b^* values and the decrease in the colour a^* value of bromelain–treated beef were in agreement with Ergezer and Gokce (2011). They found the L^* and the b^* values of marinated turkey breast meat treated with lactic

Table 4. Amino acid score and proximate extent of untreated beef and bromelain–treated beef

Essential amino acids	Untreated beef (g/100g)	Amino acid score of untreated beef (%)	Bromelain –treated beef (g/100g)	Amino acid score of bromelain –treated beef (%)	Reference pattern protein recommended by WHO (g/100g)
Methionine + cysteine	1.32	52.80	1.46	58.40	2.50
Valine	0.55	15.71	0.60	17.14	3.50
Isoleucine	0.53	18.93	0.65	23.21	2.80
Leucine	0.84	12.73	0.92	13.94	6.60
Phenylalanine + tyrosine	1.32	20.95	1.20	19.05	6.30
Histidine	0.44	23.16	0.77	40.52	1.90
Lysine	0.94	16.21	0.98	16.90	5.80
Threonine	0.58	17.06	0.87	25.59	3.40
Total	6.52		7.45		32.80
Limiting amino acid		12.73 (Leucine)		13.94 (Leucine)	
Proximate extent	0.53		0.57		1.00

acid solution increased significantly from 44.90 to 58.45 and 8.38 to 18.64, respectively after the treated turkey breast meat was heated in an oven at 180ºC to an internal temperature of 80ºC for 35 minutes. Meanwhile, the a^* value of marinated turkey breast meat treated with lactic acid solution decreased significantly from 3.09 to 2.18 after the treated turkey breast meat was heated in an oven at 180ºC to an internal temperature of 80ºC for 35 minutes. They reported that heat treatment contributed to the colour changes. This had also been proved by Fletcher *et al.* (2000) and Chueachuaychoo *et al.* (2011). They found that the heat treatment tended to produce meat with a lighter colour, less red and yellower colour. During heating, meat becomes progressively browner from its initial red colouration (Huang *et al.*, 2011). The increase in the core temperature of heating meat products intensifies the lightness L^* on the cross–section, and decreases the redness a^* (Wyrwisz *et al.*, 2012).

The changes of meat colour were due to the fact that the meat was in an oxidised form, metmyoglobin (metMb) which has a dull brown colour (Hernández *et al.*, 2006). The meat tended to be a lighter and also turned to a brown–grey hue with the increase in heating temperature. The lightening was due to an increase reflection of light, arising from light scattering by denatured proteins. The a^* value correlated with total pigment, Mb, and iron concentrations. Thus, the changes of a^* value were correlated with the content of Mb that might undergo oxidation to form MetMb, resulting in a more brownish colour (Chueachuaychoo *et al.*, 2011). When measuring the stability of beef colour (surface metMb formation) over time, a^* is probably more useful than b*. This is because the scale of a* value is from a red colour to a green colour. The formation of metMb was due to the changes in the colour of beef from a red colour to a

greenish–brown colour (Page *et al.*, 2001).

Beef is one of the widely consumed protein sources in the world (Muchenje *et al.*, 2009). The reason is that they contain all the amino acids required by the human body (Muhammad–Lawal and Balogun, 2007). Therefore, it is important that the amino acids content of beef is unaffected by the tenderisation process. The amino acids content of untreated beef and bromelain–treated beef was determined and the results are shown in Table 3.

The total amino acids content of raw round beef cuts (11.21 g/100g) from the present study was in agreement with Hall and Schönfeldt (2013) who discovered that the raw round beef cuts of South African cattle had the lowest total amino acids content with 13.44 g/100g. It was found that the bromelain–treated beef (13.34 g/100g) had higher total amino acids content compared to the untreated beef (11.21 g/100g). The increase in the total amino acids content of bromelain–treated beef was in agreement with Kuzelov *et al.* (2010). They found that the total amino acids content of untreated beef chuck cuts increased significantly from 21.25 g/100g to 22.92 g/100g after the beef was treated with the proteolytic enzyme of Streptomyces species 82 at 0ºC for 48 hours.

The increase in the total amino acids content of bromelain–treated beef was due to the loss of water holding capacity of the proteins as they were denatured by heat (Wilkinson *et al.*, 2014). Kuzelov *et al.* (2010) reported that raw tough beef cut has low hydrolysing characteristics which would not digested thoroughly during human consumption. Based on those reported by Kuzelov *et al.* (2010), the bromelain–treated beef which contained high amino acids content indicated that the beef had high hydrolysing characteristics which would be digested thoroughly during human consumption.

The total acidic amino acids content of

bromelain–treated beef (2.72 g/100g) was higher than that of untreated beef (2.38 g/100g). Results from the present study were complied with Kuzelov *et al.* (2010). They found that the total acidic amino acids content of Streptomyces species 82 enzyme–treated beef (5.64 g/100g) was higher than that of untreated beef (5.44 g/100g).

Non–essential amino acids such as glutamic acid, aspartic acid, glycine, alanine, serine and proline are flavour amino acids. Glutamic acid and aspartic acid are the characteristic amino acids of palatable taste. Meanwhile, glycine, alanine, serine and proline are the characteristic amino acids of sweetish taste (Yu *et al.*, 2014). The flavour of raw fresh meat is bland, metallic and slightly salty and only a blood–like taste whereas the flavour of a desirable meat is apparent only after heating (Ahmed *et al.*, 2010). The total flavour amino acids content of bromelain–treated beef (5.09 g/100g) was higher than that of untreated beef (4.33 g/100g). Based on those reported by Yu *et al.* (2014), the bromelain–treated beef was more palatable and sweeter than the untreated beef.

Aspartic acid, glutamic acid, glycine, leucine, tyrosine, phenylalanine, lysine, arginine and methionine are medicinal amino acids. It was found that the total medicinal amino acids content of bromelain–treated beef (8.28 g/100g) was higher than that of untreated beef (7.30 g/100g). Glycine, which was one of the major components of human skin collagen together with other amino acids such as alanine, proline, arginine, serine, isoleucine and phenylalanine, formed a polypeptide that would promote regrowth and tissue healing (Yu *et al.*, 2014).

It was found that the bromelain–treated beef (1.46 g/100g) had higher total sulphur–containing amino acids content compared to the untreated beef (1.32 g/100g). The amount of cysteine in the untreated beef and the bromelain–treated beef was 0.33 and 0.44 g/100g, respectively. Meanwhile, the amount of methionine in the untreated beef and the bromelain–treated beef was 0.99 and 1.02 g/100g, respectively. These results were in agreement with Adeyeye and Adanlawo (2011). They reported that many animal proteins contain substantially more methionine than cysteine.

According to Ibegbulem *et al.* (2013), cysteine is required for the synthesis of glutathione, a redox buffer. Glutathione maintains the sulfhydryl groups of proteins in the reduced state whereby the iron of haem was in the ferrous ions state. It also acts as a reducing agent for glutaredoxin in deoxyribonucleotide synthesis. Under aerobic conditions, the redox function of glutathione is to remove toxic peroxides formed in the normal course of growth and

metabolism. The synthesis of glutathione is limited by the availability of cysteine. Since the bromelain–treated beef had higher cysteine compared to the untreated beef, therefore the bromelain–treated beef could provide higher cysteine for the synthesis of glutathione.

Both untreated beef and bromelain–treated beef had higher total acidic amino acids content with 2.38 and 2.72 g/100g, respectively compared to total basic amino acids content with 2.12 and 2.55 g/100g, respectively. The higher total acidic amino acids content in the untreated beef and the bromelain–treated beef indicated that the beef contained more negatively charged amino acids. This showed that the untreated beef and the bromelain–treated beef served as acids at physiological pH.

Essential amino acids are amino acids that cannot be manufactured in the human body but can be obtained from food. There are nine essential amino acids namely methionine, valine, isoleucine, leucine, phenylalanine, histidine, lysine, threonine and tryptophan (Ismail *et al.*, 2013). Non–essential amino acids like cysteine and tyrosine have been classified as semi–essential amino acids, meaning that they must be synthesized from the essential amino acids if insufficient amounts are eaten. Deficiency in these essential amino acids may lead to some health problems (Ibegbulem *et al.*, 2013) such as the deficiency of phenylalanine may result in stunted growth, heart damage, fatigue and lethargic (Ismail *et al.*, 2013).

Therefore, it is important to determine the nutritional quality of beef in order to achieve and fulfill the day's need for the essential amino acids. The nutritional quality of untreated beef and bromelain–treated beef was determined and the results are shown in Table 4.

It was found that the total essential amino acids content of bromelain–treated beef (7.45 g/100g) was higher than that of untreated beef (6.52 g/100g). Results from the present study were complied with Kuzelov *et al.* (2010). They found that the total essential amino acids content of *Streptomyces* species 82 enzyme–treated beef (9.58 g/100g) was higher than that of untreated beef (8.32 g/100g).

Food protein quality is a key nutritional issue because it varies from one food protein to another, and it is important to consider in the dietary protein requirements. The main determinant of food protein quality is the content and the availability of essential amino acids. It can be used to calculate the amino acid score which provides a way to predict how efficiently the food protein will meet a person's amino acid need. This concept assumes that the tissue protein synthesis

is limited unless all the required amino acids are available at the same time and in appropriate amounts at the site of tissue protein synthesis (Caire-Juvera *et al.*, 2013).

The method is based on a comparison of concentration of the first limiting essential amino acid in a test protein with the concentration of that amino acid in a reference pattern protein (Caire-Juvera *et al.*, 2013). The amino acid score is expressed in percentage (%). The amino acid score of 100 is considered as a good quality protein. The amino acid that shows the lowest score is termed as the limiting amino acid that determines the overall score (Jiang *et al.*, 2008). The reference pattern protein is based on the recommended essential amino acids (g/100g) for 2- to 5-year-old children according to the World Health Organization (WHO) (Ismail *et al.*, 2013). Millward (2012) stated that if a food protein meets the amino acid need of children, then it should also meet the amino acid need of adults.

The highest amino acid score in both untreated beef and bromelain–treated beef was sulphur–containing amino acids (methionine and cysteine) and the respective values were 52.80 and 58.40%. This indicated the untreated beef and the bromelain–treated beef contained high protein food of sulphur–containing amino acids. Leucine was found as the limiting amino acid in the untreated beef and the bromelain–treated beef and the respective values were 12.58 and 13.94%. The main function of leucine is to release energy during muscle work and acts as a precursor of cholesterol to produce hormones and vitamin D (Ismail *et al.*, 2013). Therefore, the intake of leucine should be enough since it cannot be synthesized in the human body.

Theoretically, the consumption of 100 g of food protein would be enough to supply the daily human requirements (Nurhan, 2007). In order for the leucine to function properly and match the 100 g of beef samples with the recommended pattern protein of human requirements, thus 100/12.58 or 7.95 times of untreated beef and 100/13.94 or 7.17 times of bromelain–treated beef would need to be consumed if they serve as the sole sources of protein food. This means that the requirement of essential amino acids would be adequately met if 795 g of untreated beef and 717 g of bromelain–treated beef were consumed per day.

The proximate extent denoted the extent between the test protein and the reference pattern protein (Yu *et al.*, 2014). It was found that the proximate extent of bromelain–treated beef (0.57) inclined towards the reference pattern protein (1.00) compared to untreated beef (0.51). This indicated that the bromelain treatment to beef round cuts had a desirable effect on the nutritional quality of beef.

Conclusion

The hardness, WHC, moisture content and a^* of untreated beef decreased significantly (P <0.05) after the beef was treated with 0.17% concentration of bromelain solution in a water bath at 60ºC for 10 minutes. On the other hand, the pH, cooking loss and L^* and b^* of untreated beef increased significantly (P <0.05) after the beef was treated with 0.17% concentration of bromelain solution in a water bath at 60ºC for 10 minutes. Bromelain treatment to beef round cuts significantly (P <0.05) increased the essential and non-essential amino acids content of beef. Leucine was the limiting amino acid in both untreated and bromelain-treated beef. The bromelain–treated beef had better nutritional quality compared to the untreated beef due to its higher content of essential amino acids.

Acknowledgement

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