COMPARATIVE ANALYSIS ON THE EFFECT OF AGRO-CONOMIC MEAT TENDERIZER: BROMELAIN AND PAPAINE ENZYME ON INDIAN BEEF QUALITY

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Abstract— This present experiment was conducted to compare the final quality of Indian beef tenderness by applying two organic tenderizers which are papaya and pineapple. The papain and bromelin outcome through tenderization process on tough meat shows significant difference with (p<0.05) for L value color, which imparts good appearance for the meat. The enzyme treatments improve the water holding capacity (WHC) by retaining the texture, juiciness taste and higher cooking yield. All samples have soft, tender texture and more chewable, after cooking process. However, findings proved that papain enzyme is more effective in tenderising meat and it seems to be more palatable. It can be suggested that papain as valuable, low cost mechanism and safety way for household in having organic process of meat tenderization.

Keywords— papain, bromelin, meat, tenderize.

I. INTRODUCTION

Issue of meat quality among consumers is becoming a great concern globally. Currently, the worldwide demand for meat is forecasted to increase rapidly, as the number of populations keep on rising. In Malaysia perspective, the mushrooming of food-related industries such as fast food restaurants has transformed Malaysian peoples’ lifestyle, as they feed more on meat-based product. As [1] contends, one fourth from the total protein consumed in Malaysians’ daily diet are based on meat. Nonetheless, local meat supplies are not enough to feed the locals need. Therefore, multiple strategies has been taken into consideration, such as importing beef, poultry from outside countries [2] such as Australia, India and Thailand. Furthermore, each year, Malaysian government has allocated RM 300 million purposely in order to support this brilliant effort [1]. Meat could be generally categorized into beef, pork and poultry. In this study, Indian beef meat is used as the sample in the experiment since beef from India are exported rigorously in 2012 with no exception to Malaysia [3]. There are various factors that might contribute to meat quality. From consumerisms’ perspective, the quality assessment of meat could be determined by the degree of palatability such as sensory evaluation that makes them look more appealing. Consumers are aware of the meat appearance in expressing their positive purchasing decision [4]. However, the palatability in term of tenderness, juiciness and texture of meat is perceivably important after the meat is cooked via the appropriate technique of cooking method. This is supported by [5], [4], a good quality of cooked meat is expected to be juicy, less fat, redness colour of meat, and tenderness is the primary meat attribute while consuming a good quality of meat [6].

Basically, the tenderness of meat is much linked with the toughness of meat as a consequence of interconnectedness between connective tissues and myofibrils that enclosed the muscle protein. The utilization of enzymatic treatment, synthetic chemicals products, hand tools are available nowadays as an effort to reduce the toughness of meat during cooking process. The use of synthetic, non-natural products might lead to health problems and has long term side effects. Hence, the needs of organic meat tenderizer from Malaysian local fruits, papaya pineapple are the best way to substitute the existing artificial chemical products in the market while getting indirect benefits from the fruits itself. Both enzymes that resides in papaya (papain) and pineapple (bromelain) are well-known proteinase enzymes aided in various biological process, and significantly embraces its role in cooking appliances such as tenderizing meat. Also, the essential amino acid that released into human metabolic system is aided by proteolytic enzyme during the protein is cleavage into its smaller unit (amino acid) [7]. Despite their popularity, research comparing the effectiveness between papain and bromelain enzyme as organic meat tenderizer still has few gaps and need further investigations. Therefore, this research is conducted to reveal the significant of this research by technologically and scientifically examining the final cooking quality of Indian beef protein using organic
products derived from natural resources as well as answering the objectives of this study. **Study Objectives:** (1) To examine the healthier, practical, faster and natural way to tenderize the meat, (2) To test the possibility of tenderize the meat using papaya and pineapple, (3) To investigate among these two variables that will give better result in term of tenderness, appearance, pH and cooking yield, and (4) To discover the similarities and differences between two tenderizers.

II. LITERATURE REVIEW

### II. Muscle protein muscular structure

Structural muscle protein is build up of thousands myofibrils, stromal protein and sarcoplasmic protein. [5] defined stromal protein as undissolved, fibrous connective tissues consisted up to 50% of collagen and 47% mixture of reticulin and other proteins, while 3% of reticulin are the remaining constituent. Sarcoplasmic on the other hands has poor ionic strength dissolved easily in water and almost negligible after cooking process. Collagen connective tissue contribute to the tenderness of beef due to the bonding that scaffolding the meat [8]. Apart from that, myofibrillars are salt-soluble proteins and play a major role in the muscle contraction process by orderly transverse the muscular fiber and lessen the tenderness. A myofibril consisted of actin, myosin and titin filament. Both actin and myosin are term as sarcomere. The complexity of meat tenderness of meat is an effect of the contraction process of sarcomeres - actomyosin effect [9][10]. There are two alternating bands, thick (A-bands) and thin (I-bands) in contraction striation. Primarily, actin filament acts as the backbone of the I-bands whereas myosin filaments build up the A-bands, both are split by two Z-lines, namely sarcomere [11]. Tenderness of a meat protein is depending on the sarcomeres length. The shorter the sarcomere, the more tender the meat. The formation of actomyosin complex is catalyzed by Adenosine TriPhosphate, ATP and Adenosine DwiPhosphate, ADP nucleotide binding. [12] Contraction of actin-myosin occurs when ATP attached to myosin filament, and dissociated, releasing hydrolysis products after the depletion of ATP in a post-rigor muscle cyclically.

### JJ. Perceived meat tenderness

Beef tenderness is the most desired attribute in consumer palatability [13][4][9] asserted that culinary satisfaction quality in grading red meat or beef must consider the sensory assessment such as chewable aspect [14], tenderness [6], and texture. Toughness of a meat has lead to dissatisfaction and low acceptance among consumer. An approach to lessen the toughness is by degrading the connective tissue that surrounds the muscle fibre [15] without any rapid detrimental to the proteins [13].

**KK. Papain and Bromelin**

As a matter of fact, the improvement of beef tenderness could be aided by the utilization of enzymatic treatment. In the early 1940’s, papain is proven to have a prominent effect on tenderizing beef by breaking the peptide bonds of connective tissues and fragmentation [16]. According to [17], papain enzymes are derived from the unripe *Carica papaya* L. species. The researcher claimed that, the most powerful, dynamic papain enzyme could be obtained from the immature, green papaya fruit. As it is ripen, the enzymes naturally degraded and malfunction.

Papain is regarded as natural proteolytic enzymes which could break off the lysine, phenylalanine and arginine peptide bond [18] in the muscle protein upon tenderization mechanism and supported by [6], the cleavage of amino acid peptidase linkages are as consequences of tenderization process of via proteolytic enzymatic treatment. The enzymes lose their strength as the fruit ripens and are inactive when it is fully ripe. Long ago natives learned that papaya latex is also a very effective meat tenderizer. Tough meat was wrapped in fresh leaves for several hours to make it tender [19]. As a cystein proteinase enzyme, the stability of papain is not rigorous. Papain actively react between its optimal temperature is 65-80°C,[10] with pH optimum about pH 3.0-9.0[16]. However, It starts to reactive at 60 °C, slows down at 77 °C, and dies at 85 °C. Arguably, [10] stated that the optimal pH for papain reaction is between pH 4.0 –pH 6.0. The enzyme will denature if exposed to high cooking temperature above its optimal level.

Bromelin is a complex mixture of substances that can be extracted from the stem and core fruit of the Bromeliaceae or pineapple family, mostly from *Ananas comosus* Merr., *sp.* [19] Also, this protease enzyme is seems to be quite impressed in tenderizing meat. Similar to papain, bromelin enzyme soften the toughness of meat to by solubilised the sarcolemnic collagen up to 40% before attack the myofibrils of the protein [10]. The active temperature range for Bromelin is 50-80°C and its optimal temperature is 65-75°C. Meanwhile, the active pH range is 4.5-9.8 [19].

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III. METHODOLOGY

II. Materials and methods

Indian beef meat (brisket part) is purchase from Butcher shop in Bandar Baru Bangi, Selangor Malaysia. The meat is cleaned and cut into pieces with dimension of 5cm x 5cm x 3cm. Papaya and pineapple fruits are purchased from Econsave Hypermaket, Kajang Selangor. The skin of papaya and pineapple is removed, washed and cut into even pieces. An electrical blender is used to obtain the puree. After obtaining the puree, the meat cuts are immersed into separate containers labelled: a) Papaya, b) Pineapple, c.) Papaya + Pineapple (ratio 1:1), d) control. The ratio for the puree to the meat is 1:1. They are left for 60 minutes at 60°C in oven. After that the meats are ready, meats are cooked in microwave at high power for 5 minutes. All treatments are replicated three times and the average is accumulated to maintain the validity of the result.

MM. Analysis

1) pH value
pH reading of the samples were measured using (Accumet Basic AB15, Fischer Scientific). The pH meter was calibrated with Solution buffer pH4 and pH 7 to maintain the accuracy of the reading.

2) Color analysis (L∗a∗b∗)
All the samples were analyzed via colorimeter (MINOLTA CR300, Konica Minolta Sensing America Inc, NJ) referred to [20]

3) Cooking Yield
Weights were recorded before and after cooking for determination of percentage of cooking loss [21].

4) Texture analysis
TPA ‘hardness’ measurements were performed via CT3 Texture Analyzer Brookfield to determine the hardness and energy used to cut the meat, as referred to [22] with some modifications.

IV. RESULT AND DISCUSSION

NN. Colour assay
The results of color analysis on different treatments are given in Table 1. The Hunter L, a, b color space is organized in cube form. The L axis runs from top to bottom. The maximum for L is 100 which would be a perfecting diffuser. The minimum for L would be zero, which would be black. The a and b axes have no specific numerical limits. Positive a is red, negative a is green. Positive b is yellow and negative b is blue. Before marinating, there is no significant difference among the control and treated meat for value L, a, b. After marinating, there were significant difference (p<0.05) for L value between control and all treated meats, but for a and b value, there were no significant difference between the treatment. Marination with bromelain as well as papain resulted in the darker in color if compared to control sample. This might be probably due to the presence of pineapple and papaya pulps on the meat surface. For redness and yellowness values there were no significant variations between the control and enzyme-treated samples. Our data were similar to results found by [23]. For cooked meat, the treated meat with bromelain and combination of bromelain and papain showed the L values had significant difference compared to control and papain. The treated meat with combination of bromelain and papain showed that there was significant difference with others for a and b values. The effect of cooking and enzyme-treated sample caused the decreased in the redness and increased in the yellowness color.

Table 1: Results of Hunter Lab Color Analysis on 4 Types of Treatment on Meat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before Marinating</th>
<th>After Marinating</th>
<th>After Cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control</td>
<td>53.40</td>
<td>-1.75</td>
<td>-0.32</td>
</tr>
<tr>
<td>Bromelain</td>
<td>48.80</td>
<td>-2.60</td>
<td>-0.67</td>
</tr>
<tr>
<td>Papain</td>
<td>49.60</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>50% Bromelain</td>
<td>50% Papain</td>
<td>50% Papain</td>
<td>50% Papain</td>
</tr>
</tbody>
</table>

OO. pH determination
The initial pH of meat before treatment was 5.79. This was supported by the fact that the pH of normal muscle at death if about 6.8 but this will decrease to a level of pH 5.6 [24]. In the same study, [24] examined the factors that greatly affected that pH, this included initial glycogen content of muscle, the type of muscle, the cooling rate of carcass and the stress susceptibility of the individual animal. The pH value of meat product is highly important because it has a major influence on water holding capacity, tenderness and juiciness [25]. For pH 5.79, it was just nice for a good water holding capacity. According to [23], the acidity of the muscle increases causing an increase in negative charges. This neutralizes the positive protein charges and releases the
water molecules. The meat has the poorest water holding capacity at about pH 5.4. The meat for the control was immersed in distilled water and the pH was slightly increased to pH 5.92. This was because the osmosis happened causing the distilled water to transfer into the meat. Whereas the pH for the meat treated with bromelin slightly decreased to pH 5.37. This was because the pineapple by itself was acidic, namely pH 3.71.

Meanwhile, the meat treated with papain was about pH 5.75. It did not fall dramatiscally as compared to the meat treated with bromelin. The pH of mature papaya was 5.8. The pH for the meat treated with the mixture of bromelin and papain was 5.65. Generally, as depicted in the graph (Figure 1), the pH for the treated meat after cooking had shown higher pH compared to before the cooking. This was because the structure of protein (myofibrillar) had been denatured and thus it lost the ability to hold water. For the control meat, the pH was 6.14. This pH was merely similar with meat treated with papain which was about 6.11, while the pH for the meat treated with the mixture of bromelin and papain was 6.01. Whereas, the pH of meat treated with bromelin was 5.74, which was the lowest among the meats. However, if compared to the pH before cooking, it slightly increased, namely from 5.37 to 5.74. It was believed that the meat treated with bromelin had the sour taste.

Meanwhile, there was a decrease for weight percentage after cooking in microwave oven. The microwaves heat and penetrate the food through water and fat. They were then converted to energy or heat. Thus, moisture would lost. For the cooked meat, sarcomere is fragmented and degraded by proteolytic enzyme and undergone proteolysis affected by heat and temperature. The meat treated with papain, pH and cooking yield increase which was found to be similar with [26] finding. This was due to the elimination of water and some soluble substance from the meat and that was because of the breaking of polypeptide chains, the weakening of collagen network and thermal denaturation of the proteins.

Table 2: Weight and Weight Percentage Lost Before Marinating, After Marinating and After Cooking

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight Before Marinating (g)</th>
<th>Weight After Marinating (g)</th>
<th>Weight After Cooking (g)</th>
<th>Weight Increase (%)</th>
<th>Cooking Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>192</td>
<td>194</td>
<td>90.35</td>
<td>1.84%</td>
<td>46.57%</td>
</tr>
<tr>
<td>Bromelin</td>
<td>201</td>
<td>203</td>
<td>92.04</td>
<td>1.90%</td>
<td>45.24%</td>
</tr>
<tr>
<td>Papain</td>
<td>233</td>
<td>234</td>
<td>116.63</td>
<td>8.86%</td>
<td>49.84%</td>
</tr>
<tr>
<td>Bromelin + Papain</td>
<td>265</td>
<td>267</td>
<td>83.05</td>
<td>0.98%</td>
<td>40.12%</td>
</tr>
</tbody>
</table>

QQ. Tenderness

From the table shown below (Table 2), it was obvious that the papain had the lowest in hardness, namely 62.42+4.84 N if compared to the meat treated with bromelin and mixture of bromelin and papain, which were 89.28+23.61 N and 82.46+12.25 N respectively. This showed that papain had a good property in tenderising meat if compared to bromelin. This was supported by [16] that papain is a highly aggressive, indiscriminate enzyme causing significant degradation to both myofibrillar and collagen proteins, yielding protein fragment of several sizes. According to [10], papain has the most excellent strength of hydrolysis of myofibrillar proteins and collagen followed by Bromelin, Ficin, Aspergillus, and Bacillus. Effect of Bromelin on tenderness of meat was supported by [27] that bromelin first degrade 40% of the collagen in the sarcolemma followed by degradation of myosin in the myofibrillar component. The comparison for our meat treatments also reflects the trend in the previous measure.

For energy analysis, the energy used for cutting each meat followed the trend of meat hardness. Logically, the graph trend of energy supposed to follow the graph trend of meat hardness. The harder the meat was, the more energy was needed to cut the meat. Based on the Table 3, the energy used for
control, meat treated with bromelin, papain and mixture of bromelin and papain were 347.67±98.74 Nmm, 292.42±18.11 Nmm, 219.45±44.07 Nmm and 270.00±34.21 Nmm, respectively. Papain has show the lowest energy consumption to penetrate the meat followed by mixture treatment and bromelin. The results revealed the effectiveness of papain in making the meat more tender compared to bromelain. Much energy is used to cut off the meat probably due to complexity and abundancy of tendons or fibers, which make the meat less tender and hard to be chewed [14].

Table 3: Harness and Energy For Beef Treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hardness</th>
<th>Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92.27 ± 7.85 N</td>
<td>347.67±98.74 Nmm</td>
</tr>
<tr>
<td>Bromelin</td>
<td>89.28±23.61 N</td>
<td>292.42±18.11 Nmm</td>
</tr>
<tr>
<td>Papain</td>
<td>62.42 ± 4.84 N</td>
<td>219.45±44.07 Nmm</td>
</tr>
<tr>
<td>Bromelin+Papain (1:1)</td>
<td>82.46±12.25 N</td>
<td>270.00±34.21 Nmm</td>
</tr>
</tbody>
</table>

V. CONCLUSION

The result obtained in this experiment clearly indicates the tenderizing effect of bromelin, papain and the mixture of both. Papain has shown the greatest proteolytic activity to meat followed by enzyme mixture and bromelin. However, factors such as raw material, holding time and temperature, other ingredients, handling and cooking procedure need to be evaluated to determine the tenderness. Therefore, cheaper and easily available pineapple and papaya can be effectively utilized at household or industrial level to tenderize the tough meat.

VI. REFERENCES


