Physicochemical properties and tenderness of meat samples using proteolytic extract from *Calotropis procera* latex

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**A B S T R A C T**

This study was conducted in order to tenderise muscle foods (pork, beef and chicken) by using crude enzyme extract from *Calotropis procera* latex. Chunks of knuckle muscle from pork and beef as well as of breast muscle from chicken were marinated with distilled water (control) and 0.05%, 0.1%, 0.2%, 0.3% and 0.5% (w/w) of crude enzyme extract powder for 60 min at 4°C. The marinated samples were then subjected to various physical and chemical property determinations. A decrease in moisture content was observed when the crude enzyme extract was added. Firmness and toughness of the muscle samples significantly decreased with the increased addition of crude enzyme extract (p < 0.05). The water holding capacity and cooking yield of the treated samples showed no significant difference throughout the crude enzyme extract addition (p > 0.05). Crude enzyme extract had no effect on the pH of the pork sample, but it slightly increased the pH in the beef and chicken. An increase in protein solubility and TCA-soluble peptides content was observed in all of the treated samples. The electrophoresis pattern of the muscle treated samples also revealed extensive proteolysis occurring in each muscle type. From the results, it is determined that latex from *Calotropis procera* can be used as an alternative source of proteolytic enzymes for the effective tenderising of meat.

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1. Introduction

Meat tenderness is generally considered one of the most important determinants of meat quality. Meat tenderness depends on the amount of intramuscular connective tissue, the length of the sarcomere and also the proteolytic potential of the muscle (Kemp & Parr, 2012). On the other hand, meat toughness is one of the most undesirable meat qualities for consumers (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010). Actomyosin and background toughness are 2 main types of classified meat toughness. The former is attributable to changes in myofibrillar proteins, whereas the latter is due to connective tissues or stromal proteins (Chen, He, Jiao, & Ni, 2006). A number of attempts have been made to improve meat tenderness and the overall quality of muscle foods. Normally, chemicals and physical treatments are used to do this. All methods focus on reducing or disrupting the myofibrillar proteins and connective tissues. Marinating muscle foods in acidic solutions such as acetic or lactic acid has been traditionally applied as a means of softening and flavouring meats (Berge et al., 2001). However, an extended period of time is needed for full marinat

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2. Materials and methods

2.1. Materials

L-Cysteine, sodium dodecyl sulphate (SDS) and bovine serum albumin (BSA) were obtained from Fluka (Buchs, Switzerland). Beta-mercaptoethanol (βME), Coomassie Brilliant Blue G-250, and casein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). N,N,N,N-Tetramethyl ethylene diamine (TEMED) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Trichloroacetic acid (TCA), hydrochloric acid, tris-(hydroxymethyl)-aminomethane, Folin phenol reagent, and other chemicals with analytical grade were obtained from Merck (Darmstadt, Germany).

Latex of *C. procera* was collected from Nayong, Trang Province, Thailand. Beef, pork and chicken muscles were purchased from Bandoo Market, Chiang Rai Province, Thailand.

2.2. Latex preparation

Latex was collected in a clean tube by breaking the *C. procera* stem. The collected latex was diluted with distilled water (1:1, v/v). It was mixed well and then centrifuged at 15,000 gs for 10 min according to the method previously reported by Rawdkuen et al. (2011). The supernatant obtained was filtered through a Whatman paper No. 1 and then freeze dried. This sample was thereafter referred to as the “crude enzyme extract” and was used for further study.

2.3. Caseinolytic activity assay

An enzyme sample of 0.1 ml was mixed with 1.1 ml of 1% (w/v) casein in 0.1 M Tris–HCl, pH 8.0, containing 12 mM cysteine. The mixture was incubated at 37 °C for 20 min. After that, the reaction was stopped by adding 1.8 ml of 5% TCA. After centrifugation at 3,000 g for 15 min, the absorbance of the supernatant was measured at 280 nm. One caseinolytic unit is defined as the amount of enzyme needed to produce an increment of 0.01 absorbance units per minute in an assayed condition (Rawdkuen et al., 2011).

2.4. Marination of muscle samples

Meat samples were purchased from Bandoo market and packed in low-density polyethylene (LDPE) bags, then cleaned and stored in a refrigerator at 4 ± 1 °C for 24 h. The samples were then removed from the refrigerator and cut into small 3 cm³ sized chunks.

The prepared samples were weighed and then marinated with the powder of *Calotropis procera* latex at concentrations of 0%, 0.05%, 0.1%, 0.2%, 0.3%, and 0.5% (w/w). After mixing the samples by hand, the chunks were placed in a bowl and covered with polyethylene bags and kept at 4 °C for 60 min according to the method previously reported by Naveena et al. (2004). After incubation, the meat chunks were evaluated for both their chemical and physical properties.

2.5. Physical properties determinations

2.5.1. Shear force value

Texture was analysed using a TAxT2 texture analyser (Stable Micro Systems, Surrey, UK), equipped with a Warner–Bratzler blade (square shape) in accordance with the method used in Ketnawa and Rawdkuen (2011). Seven rectangular shaped samples of raw beef, raw chicken and raw pork were prepared. Each sample was cut perpendicular to the longitudinal orientation of the muscle fibres. The blade was pressed and applied at a constant speed of 2 mm s⁻¹ through the sample. The maximum shear force (Firmness: N) and total work (Toughness: N·Sec) were recorded.

2.5.2. Water-holding capacities (WHC)

WHC was determined according to the method used in Wardlaw, Maccaskill, and Acton (1973). Minced meat (20 g) was placed in a centrifuge tube containing 30 ml of 0.6 M NaCl and was stirred with a glass rod for 1 min. The tube was then kept at 4 ± 1 °C for 15 min, stirred again, and then centrifuged at 3000 g (PLC-05, Industrial Corp., Taipei, Taiwan) for 25 min. The supernatant was measured, and the WHC was expressed as a percentage of initial volume.

2.5.3. Cooking yield

The treated samples (10 g) were steamed for 1 min and then cooled to room temperature. The cooked sample was surface-dried with a filter paper and re-weighed using an analytical balance (Sartorius, ED224S Sartorius AG, Goettingen, Germany). The cooking yield was calculated by the difference in weight when raw and then when cooked, as described in Ketnawa and Rawdkuen (2011).

2.6. Chemical properties determinations

2.6.1. Moisture content

The moisture content of the samples was determined according to the Association of Official Analytical Chemists (AOAC) method No. 950.46 (2000).

2.6.2. pH

To determine pH, 10 g of the sample were homogenised with 50 ml of chilled distilled water. The pH values were measured with a digital pH metre (Model pH 510, Eutech Instrument, Ayer Rajah Crescent, Singapore).

2.6.3. Protein solubility

Protein solubility was determined according to the procedures of Naveena et al. (2004). The proteins in the treated samples were extracted from 2 g of minced meat by using 40 ml of ice-cold 1.1 M potassium iodide in a 0.1 M potassium phosphate buffer (pH 7.2). The samples were homogenised and kept overnight at 4 °C with frequent shaking. The samples were then centrifuged at 1,500 g for 20 min and the protein concentration in the supernatant was determined by the Biuret method (Robinson & Hodgen, 1940). Solubility was expressed as the percentage of total protein in meat samples solubilised directly in 0.5 M NaOH.

2.6.4. TCA-soluble peptides

The TCA-soluble peptide content of the samples was measured by the method used in Ketnawa and Rawdkuen (2011). Two grams of the samples were weighed and then homogenised with 18 ml of 5% (w/v) TCA for 1 min and kept at 4 °C for 1 h before they were centrifuged at 8000 g for 5 min. The soluble peptides in the supernatant were measured by using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). The content of TCA soluble peptides was calculated as the μmol of tyrosine/g of the samples.
2.6.5. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was carried out according to the method in Laemmli, Beguin, and Gujer-Kellenberger (1970). Samples (2 g) subjected to different treatment conditions were all mixed with 18 ml of 5% (w/v) SDS solution (85 °C). The mixture was then homogenised with an homogeniser (IKA Ultra Turrax, T25D, Germany). The homogenate was incubated at 85 °C in a water bath for 1 h to dissolve the protein. It was then centrifuged at 8000g for 5 min at room temperature using a centrifuge (PLC-05, Industrial Corp., Taipei, Taiwan) to remove the un-dissolved debris. The supernatants were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8 containing 4% SDS, 20% glycerol and 10% βME) and then boiled for 3 min. The samples (20 µg protein) were loaded into a poly-acrylamide gel (10% running and 4% stacking gels). Then, they were subjected to electrophoresis set at a constant current of 15 mA per gel using a Mini Protean Tetra Cell unit (Bio-Rad Laboratories, Richmond, CA, USA). After electrophoresis, the gels were stained overnight with staining solution (0.02% (w/v) Coomassie Brilliant Blue R-250) in 50% (v/v) methanol and 7.5% (v/v) acetic acid. The protein patterns were then made visible after de-staining the gel until a clear background was achieved.

2.7. Statistical analyses

The experiment was carried out in triplicate using different three lots of samples. A randomised design was used throughout the study. The data were analysed statistically with the SPSS program for Windows (SPSS version 11.5, SPSS Inc., Chicago, IL, USA). Duncan’s multiple-range test was used to compare the difference between means. The accepted level of significance for all comparisons was $p < 0.05$. Experiments were conducted in triplicate.

3. Results and discussion

3.1. Effect of crude enzyme extract on meat firmness and toughness

The texture properties of the muscle samples treated with the powder of crude enzyme extract at different concentrations are presented in Fig. 1. The firmness and toughness values significantly decreased in all of the treated samples when compared to the control (without crude enzyme extract) ($p < 0.05$). It was observed that the shear force values continuously decreased in all of the treated samples when the level of crude enzyme extract from $C. procera$ latex increased, especially when the concentration was >0.10% (w/w) (Fig. 1A). The lowest firmness value was found in all of the meat samples treated with 0.5% (w/w) of the crude enzyme extract, especially in the chicken sample. The firmness values for the starting pork beef, and chicken were 535, 650 and 302 N, respectively. The addition of 0.5% (w/w) crude enzyme extract to the muscle reduced the firmness of the samples by about 59, 65, and 52% when compared to the control for pork, beef and chicken, respectively. The reduction in meat firmness came as a result of the action of the proteolytic enzymes on the myofibrillar proteins or by disruption of the connective tissues. Post-mortem proteolysis by endogenous proteases causes a weakening of myofibril structures and associated proteins, which results in tenderisation (Kemp & Parr, 2012). Both myofibrillar proteins and collagen tissues fragment when treated with ammonium hydroxide, resulting in the tenderisation of buffalo meat (Naveena et al., 2011). When the breakdown of myofibrillar protein occurred, small peptides with low molecular weight were generated, thereby reducing the firmness of the meat samples.

The toughness values of the treated meat samples are presented in Fig. 1B. Beef muscle showed the highest toughness value while the chicken sample provided the lowest toughness value. Slightly decreased toughness was observed when the crude enzyme extract was added ($p > 0.05$) at the level of 0.05–0.20% (w/w). However, at the highest addition level, the lowest toughness was observed in all of the samples. The lowest toughness value was found in chicken for both the treated and untreated samples with crude enzyme extract. According to Koak et al. (2011), using kiwi fruit content (containing proteolytic enzymes), beef texture became softer when the kiwi was added. In the beef sample, when adding crude enzyme extract at the level of 0.50% (w/w), a decrease in toughness was observed of about 50%. Toughness is determined by the amount of intramuscular connective tissue, intramuscular fat and the length of the sarcomere (Kemp & Parr, 2012). In addition, older animals normally produce tougher meat than is produced by young animals. The tougher the meat, the more connective tissues that are present. Proteolytic enzymes, especially plant proteases, are widely used for meat tenderisation (Ha et al., 2012; Ketnawa & Rawdkuen, 2011; Sullivan & Calkins, 2010). Naveena et al. (2004) also observed that in buffalo meat with extensive muscle fibre and connective tissue degradation, shear force values decreased when ginger rhizome extract was added. Ha et al., 2012 reported that the actinidin protease was the most effective for hydrolysing beef myofibrillar proteins, while zingibain protease is most effective for degrading connective tissue.

3.2. Effect of crude enzyme extract on water holding capacity and cooking yield

The water holding capacity of the meat samples when treated with different concentrations of crude enzyme extract is shown in Fig. 2A. A slightly decreased WHC was observed when the crude enzyme extract was added ($p > 0.05$). Pork showed the highest WHC while the lowest value was found in the treated chicken muscle. The higher WHC in the control sample may be due to the overall reduction in the protein reactive group, which is available for water binding (Forrest, Aberle, Hedrick, Judge, & Merkel, 1994). Slight denaturation of sarcoplasmic proteins, which has an important role in determining WHC, could be the reason for decreased WHC (Joo, Kauffman, Kim, & Park, 1999). Reduced WHC is a result of myofibrillar shrinkage, as well as the movement of water from the myofilament space to the extra-cellular space. One of the main factors involved in the shrinkage and/or swelling of the myofibrils is protein fragmentation (Huff-Lonergan & Lonergan, 2005). The WHC of meat is very important since many physical properties such as colour, texture and firmness are partially dependent on the WHC. The cooking yield of the meat samples treated with different concentrations of crude enzyme extract is shown in Fig. 2B. Decreased cooking yield was observed in all samples when the C. procera latex powder was added. The control beef sample showed the highest cooking yield while the chicken gave the lowest value. At the level of 0.5% (w/w) addition, pork muscles provided the highest cooking yield and chicken muscles gave the lowest. The addition of increasing levels of crude enzyme extract led to decreased cooking yield for all of the meat muscles. This result indicates that thermal treatment could remove more water from treated chicken samples than that of treated pork muscles. It can be implied that the enzymes in crude $C. procera$ latex could hydrolyse protein in chicken more than in beef and pork. Kumar and Berwal (1998) reported that spent hen meat treated with sulphate extracted Cucumis powder produced a significant reduction in cooking yield value. Degradation of sarcoplasmic and myofibrillar proteins may be caused by the reduction of cooking yield in the enzyme treated sample (Pawar, Mule, & Machewad, 2007).

3.3. Effect of crude enzyme extract on moisture content

No changes in the moisture content of the meat samples treated with 0.5% (w/w) of crude enzyme extract were observed, compared
with the control (p > 0.05) (Table 1). However, increased crude enzyme extract concentration could reduce some amount of moisture, especially in pork muscle. In general, the hydroscopic nature of the powder probably contributed to the moisture reduction when it was attached to the wet surface of the sample. The low moisture content of the treated meat samples is normally related to consumer preference. It also affects the yield of the final product. Naveena and Mendiratta (2001) reported moisture retention in spent-hen meats treated with ginger rhizome extract as compared with an untreated sample. Similar observations were found: 77.18% moisture in ginger rhizome extract was found in a treated buffalo meat sample as compared with 76.31% in an untreated one.

3.4. Effect of crude enzyme extract on muscle pH

The pH value of the meat samples treated with different concentrations of crude enzyme extract slightly increased when the concentration of crude enzyme extract increased (Table 2). Significantly increased pH value was found in beef and chicken muscles when compared with the control (p < 0.05). However, the pH value in the pork sample was not significantly different (p > 0.05), except at the 0.50% (w/w) addition level. The high pH of the crude enzyme extract (pH 6.3) was probably caused by the higher pH of the treated samples, especially when a high level of crude enzyme extract was applied. Moreover, enzymatic hydrolysis of the muscle may release amino acids that can increase the pH of the system. The
Fig. 2. Water holding capacity (A) and cooking yield (B) of meat samples treated with different concentrations of crude enzyme extract from *Calotropis procera* latex.

### Table 1
Moisture content of meat samples treated with different concentrations of crude enzyme extract from *Calotropis procera* latex.

<table>
<thead>
<tr>
<th>Crude enzyme extract (%)</th>
<th>Moisture content (%)</th>
<th>Pork</th>
<th>Beef</th>
<th>Chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>84.51 ± 1.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.18 ± 4.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.28 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>87.63 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85.24 ± 4.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.14 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>85.08 ± 2.97&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>82.16 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.53 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>84.76 ± 1.61&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>81.13 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.93 ± 1.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.30</td>
<td>81.83 ± 1.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>80.48 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.35 ± 1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>81.00 ± 2.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.23 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.46 ± 0.83&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts in the same column indicate significant differences (p < 0.05).

<sup>A</sup> Values are given as mean ± SD from triplicate determinations.

### Table 2
pH values of meat samples treated with different concentrations of crude enzyme extract from *Calotropis procera* latex.

<table>
<thead>
<tr>
<th>Proteolytic extract (%)</th>
<th>pH&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Pork</th>
<th>Beef</th>
<th>Chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.42 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.59 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.50 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>0.05</td>
<td>5.41 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.61 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.52 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>5.43 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.63 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.53 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>5.44 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.66 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.54 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.30</td>
<td>5.46 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.64 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.55 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>5.51 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.67 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.60 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts in the same column indicate significant differences (p < 0.05).

<sup>A</sup> Values are given as mean ± SD from triplicate determinations.
increases in pH of the treated sample (0.5%, w/w) ranged from 5.42 to 5.51, 5.59 to 5.67, and 5.50 to 5.60 when compared to the control for pork, beef and chicken, respectively. The pH value in meat products is highly important because it has a major influence on other physico-chemical and quality properties such as WHC, tenderness and juiciness. Changes in pH are caused by postmortem metabolism and also by the application of any added substances to the meat during the application of any technological processes.

3.5. Effect of crude enzyme extract on protein solubility and TCA-soluble peptides content

The protein solubility of meat samples treated with different concentrations of crude enzyme extract is shown in Fig. 3A. Protein solubility was significantly affected by the crude enzyme extract from *C. procera* latex. Significantly higher protein solubility values were observed in all enzyme treated samples compared to the control (*p* < 0.05). The control chicken meat (without added crude enzyme) showed the highest protein solubility, while the lowest value was found in the control beef sample. The regularly aligned filament of myofibrils in the control sample may have helped to prevent crude enzyme extract penetration, thus making the action seemingly resistant to extraction (Davey & Gilbert, 1968). Continuously increased protein solubility was clearly observed in the beef and pork muscles. At the level of 0.5% (w/w) crude enzyme extract addition, the solubility of protein in pork, chicken, and beef was 42%, 43% and 52%, respectively. More than a 50% percent increase of protein solubility was found in the pork and beef muscle when compared with the control. These results suggest that the protein solubility changes were due to myofibrillar protein degradation. An increase in solubility of enzyme-treated samples might be due to an increase in permeability of myofibrils, which will then disintegrate easily. Differences in protein solubility may be caused by the difference in structure of the meat muscle. An increase in

![Graph A](image1.png)

![Graph B](image2.png)

*Fig. 3.* Protein solubility (A) and TCA-soluble peptides content (B) of meat samples treated with different concentrations of crude enzyme extract from *Calotropis procera* latex.
thus increasing meat tenderness. The degradation of muscle protein plays a major role in determining the tenderness and WHC of meat during the postmortem storage (Melody et al., 2004). Naveena et al. (2004) revealed that protease from Cucumis trigonus Roxb and Zingiber officinale Roscoe improved the tenderness and the overall quality of tough buffalo meat. Ketnawa and Rawdkuen (2011) also concluded that bromelain extract from pineapple peels could be used as an effective meat tenderiser for beef, chicken and squid muscles. The bromelain extract applied to the meats plays a role in collagen hydrolysis into small peptides (Ketnawa, Rawdkuen, & Chaiwut, 2010). Moreover, degradation of the AC from the treated sample was also observed on the SDS–PAGE, especially in chicken and pork muscles. According to Wada, Suzuki, Yaguti, and Hasegawa (2002), plant thiol proteases affect the structure of the MHC and the AC filaments of myofibrillar proteins. Furthermore, these enzymes have very broad specificities and, therefore, indiscriminately break down the major muscle proteins (connective tissue/collagen and myofibrillar proteins), resulting in over-tenderisation and a mushy-textured product. The degradation of muscle protein plays a major role in determining the tenderness and WHC of meat during postmortem storage. Based on these results, it is determined that a crude enzyme extract from C. procera latex potentially has high proteolytic activity and is able to be used to make meat more tender.

3.6. Effect of crude enzyme extract electrophoretic patterns

A representative protein pattern by SDS–PAGE for the muscle sample treated with different concentrations of crude enzyme extract can be seen in Fig. 4. Similar protein patterns in original beef, chicken and pork were observed (lane 1). The myosin heavy chain (MHC) and actin (AC) are the major proteins in all muscle types. There was increased proteolysis of the muscle proteins in all treated samples as evidenced by the reduction in number and intensity of the protein bands when the crude enzyme extract was added. The breakdown of proteins in high amounts was more visible in the pork sample than in the other samples. When comparing the treatment (crude enzyme treated) to the control, the MHC band was markedly degraded into lower molecular weight products as shown at the bottom part of the gel. In addition, comparing beef and chicken muscles, the MHC band of the former was markedly degraded into lower molecular weights than that found in the latter. Hydrolysis of these proteins has been shown to disrupt muscle fibre structures with an associated decrease in shear force and an improvement in meat tenderness (Kemp et al., 2010). Jorgova, Danchev, and Kostov (1989) reported that bacterial proteolytic enzyme treatment of muscle protein reduced the level of higher molecular weight fractions due to degradation of myosin, thus increasing meat tenderness. The degradation of muscle protein solubility with ginger and papain treatment was also reported by Naveena and Mendiratta (2001) in spent hen meat.

The TCA-soluble peptides content of the muscle samples treated with different concentrations of crude enzyme extract is shown in Fig. 3B. The highest content of TCA-soluble peptides was found in the pork, followed by the chicken and beef muscles treated with 0.5% (w/w) crude enzyme extract. When the concentration of crude enzyme extract was increased, the TCA-soluble peptides content in the entire treated sample increased (p < 0.05). The TCA-soluble peptides content was the lowest in the beef muscle. More than a 50% increase of TCA-soluble peptides content was found in the sample treated with 0.5% (w/w) crude enzyme extract as compared to the control. This result suggests that the enzyme that exists in the latex had hydrolytic activity, thereby degrading the protein. TCA-soluble peptides content indicated that the endogenous oligopeptides and/or free amino acids, as well as degradation products, all accumulated after being marinated with the crude enzyme extract. From these results, it is found that for high TCA-soluble peptides content, more muscle protein hydrolysis is generated by the proteolytic enzymes present in C. procera latex.

4. Conclusion

The results obtained in these experiments clearly indicate that the tenderness and other physicochemical properties of meat samples were improved through the use of a crude enzyme extract from Calotropis procera latex. By adding increased amounts of crude enzyme extract, the quality characteristics of the treated meat samples were improved. Technology for applying this enzyme is easily and cheaply available and can be exploited at the household or industrial level for tenderising tough meat, and it can be used as a better alternative to chemical tenderisers or other plant proteases.

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