Indonesian Food Science and Technology Journal
IFSTJ : Vol 5 no 1 December 2021
ISSN : 2615-367X

Utilization of Papain Extracted from Chaya and Papaya Stalks as Halal Beef Tenderiser

Aekkalak Phothiwicha[1], Kiatisak Thamsaad [1], Kanjapach Boontranurak[1], Panlop Sintuya[2], and Naksit Panyoyai[1]
[1]Department of Agro-Industry, Chiang Mai Rajabhat University, Mae Rim Campus, Chiang Mai, 50330, Thailand
[2]Department of Agriculture Technology and Development, Chiang Mai Rajabhat University, Mae Rim Campus, Chiang Mai, 50330, Thailand
E-mail: naksit_pan@cmru.ac.th

Submission date: 04 July 2021 Published date: 31 December 2021

Abstract— **Papain is a proteolytic enzyme extracted from plant latex. The protease has been used widely in the meat industry and culinary work for a pre-tenderization step before further meat processing. The objective of this research was to evaluate the bioactivity of papain extracted from Chaya and papaya. The different levels of papain concentration, casein substrate, temperature, and pH were carried out in *vitro.* Then, the fresh papain was used to digest raw beef, and the beef dried under solar process. The sun-dried beef analyzed yield, moisture content, water activity, and tensile force. It was found that proteolytic activity from both plants accelerated at a high level of papain or casein up to 2.0 (w/v). The optimal proteolytic activity was recorded at 60oC and pH5. Later, the tenderized beef at pH 6.5 was dried by a solar dryer, where an inside tunnel temperature was approximately 40oC, 40% relative humidity, and 187 Btu/ft2. hr solar radiation. The sun-dried beef had a 75% yield, a 32% moisture content, and 0.90 Aw. Even though the drying temperature and pH of the beef were not optimal as given from the previous results, the tensile force and the work of tension of papain-treated beef in both moist and dried states were lower than the non-treated beef.**
***Keywords*— papain; Chaya; papaya; tenderiser; halal beef**

I. Introduction

Papain is a cysteine protease enzyme obtained from the endolytic plant. The enzyme has a proteolytic function as a biocatalyst to cleave peptide bonds of protein-containing arginine, lysine, and phenylalanine residues resulting in amino acid esters, and amide links. The structure of papain is globular with a molecular weight of 23,406 Da, a single-chain protein consisting of 212 amino acids in total, and four disulfide bridges. The enzyme has a potential mechanism supported with specific amino acids, namely Cys-25, His-159, and Asparagine-175 working together in the active site to bind the protein substrate at a backbone of the protein chain having a free amino end. The enzyme is stable at high temperatures, a wide pH range (3-9), hydrophobic solvents, and low moisture conditions [1,2].

The stems of papaya (*Carica papaya*) and Chaya (or tree spinach, *Cnidoscolus chayamansa*) are sources of papain. Cutting the stems will exclude a milky liquid. Active papain enzyme can be extracted and purified from the latex of the papaya stem. The pretreatment with ammonium sulfate and sodium chloride was carried out for the papain precipitation. The ultrasonication method at 60^{o}C for 1 hour was resulting in more active enzyme activity than the maceration method [3,4]. In the case of Chaya, the plant is originally grown in Mexico used as food, medicine, and ornament. It is called Mayan spinach due to its consumption manner like spinach. Chaya leaves are abundant in protein, beta-carotene, vitamins, calcium, potassium, and iron. Chaya leaves contain a toxic chemical, linamarin, or cyanogenic glycoside. The residue glycoside can eliminate by boiling for 5 minutes [5]. This thermal treatment is sufficient for the safe consumption of the leaves. The literature reports that proteolytic activity from Chaya leaves were best extracted using 0.04 M phosphate buffer (pH8). Then partially purified by isoelectric and precipitation with ammonium sulfate. The proteolytic activity to hydrolyze urea-denature haemoglobin, casein, and soybean protein was considerable, while the lesser activity found in bovine serum albumin, gelatine, and fish protein isolate [6].

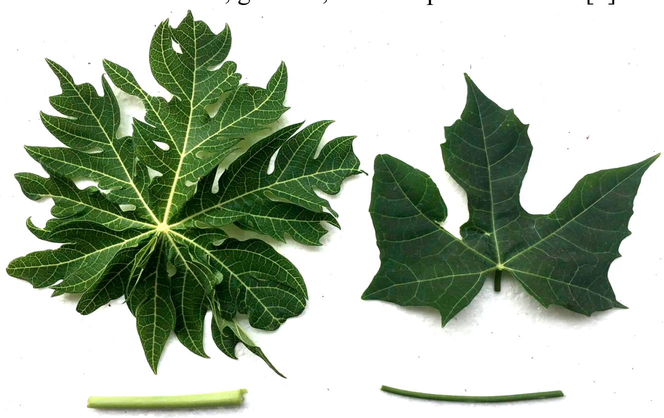

Fig. 1 a Papaya leaf (left) and a Chaya leaf (right) and their stalks

Papain (EC.3.4.22.2) is a natural meat tenderiser at a commercial level for meat tenderisation. The source of papain comes from the latex of papaya fruit and some from stalks and leaves [7,8]. Similar to papaya, Chaya leaves are a much popular plant protein for vegetarians. However, stalks considered as waste contains papain. In order to implement the zero-waste concept, the utilization of Chaya green stalks as a new source of papain will carry out by extracting papain. Test its tenderisation potential on halal raw meat and halal meat product, e.g. sun-dried beef with Thai seasoning.

This research aimed to study the effects of papain concentration, substrate concentration, pH, and temperature on the enzyme activity and further application of the papain in tenderisation on the sun-dried beef product.

II. Materials and Methods

A. Materials

The Chaya and papaya stalk organically grown in Chiang Mai, Thailand used as a papain source. The fresh stalks cut from Chaya and papaya trees around Chiang Mai Rajabhat University, Mae Rim Campus. The length of the stalks was 5 cm. apart from the under a leaf to the correct position. Then the stalks were conducted on papain extraction in Food Science and Technology laboratory. The chemicals, disodium hydrogen phosphate, sodium hydrogen phosphate, acetic acid, sodium acetate used for buffer preparation, casein used for papain substrate, and trichloroacetic acid used for terminate enzyme activity were analytical grade with purity more than 98% and used without further purification. Distilled water was used as a solvent in the buffer preparation, enzyme extraction and its activity studies.

B. Extraction crude papain from Chaya and papaya stalks

The fresh Chaya and papaya stalks were washed with distilled water. Twenty grams of each stalk blended with 100 ml. of 0.01 M phosphate buffer pH 7 for a minute [3,4]. The mixture was then transferred to centrifugal tubes and capped, following by centrifugation (Ohaus, China) at 10,000 rpm/min at an ambient temperature. The blended solid was filter using filter paper, and the filtrate was collected to study the effects of enzyme concentration, substrate concentration, pH and temperature on papain activity.

C. Effects of enzyme concentration, substrate concentration, pH and temperature on papain activity.

Enzyme concentration- Crude papain extracted from Chaya or papaya was diluted to different concentrations (0.5%, 1.0%, 1.5%, 2.0%) using phosphate buffer pH 7. Pipette 1.8 ml. of 2% casein solution at a concentration to a test tube and then capped the tube. Incubated the casein substrate at 40^{o}C for 3 min. The 0.2 ml crude enzyme was added to react with the substrate. The 0.2 ml pure phosphate buffer was a control solution. Mixed the substrate and the enzyme before further incubation at 40^{o}C 10 min. To terminate the enzyme activity, 6 ml of 5% trichloroacetic acid was mixed with the enzyme/substrate solution. The final solution was read the absorbance using a UV spectrophotometer (Cintra, Australia) at 270 nm. The absorbance results of each treatment were expressed as an average of five readings.

Substrate concentration- Different casein substrate concentrations of 0%b 0.5%, 1.0%, 1.5%, 2.0% dissolved in phosphate buffer solution pH 7 was digested with 2% crude enzyme extract, then ended the digestive activity using 5% trichloroacetic acid, and read the absorbance in a similar manner of papain activity study as described before.

pH- acetate buffer solution was prepared in a series of pH (3-6) to extract crude papain from Chaya and papaya stalks. Each acidic buffer solutions was used to prepare 2% casein solution and 2% crude enzyme solution. The phosphate buffer pH 7 was a control solution. The enzyme activity was carried out on aromatic amino acids induced UV absorption 270 nm.

Temperature-crude enzyme extract (2% V/V) was incubated for 40^{o}C for 3 min, and the warm solution was mixed thoroughly with a casein solution (2% w/v). Then, a series of solution tubes were incubated in a water bath (Memmert, USA) at 40, 50, 60, 70, and 80^{o}C for 10 min. The proteolytic activity was finished with an addition of trichloroacetic acid. The reaction was determined by absorbance reading at 270 nm.

D. Halal beef tenderisation

Beef, striploin section, was purchased from the Halal butcher (Chiang Mai, Thailand). The halal ingredients such as fish sauce, soya sauce, oyster sauce, salt, and sugar, were obtained from the local supermarket. Drinking water used for crude papain extraction from Chaya and papaya stalks was qualified by The Thai Food and Drug Administration, Ministry of Public Health. The water quality regards Notification of the Ministry of Public Health No. 61 1981 drinking water in sealed containers. The extraction process was conducted as mentioned in section *B*.

The beef was removed fat and cut lengthways with a thickness of approximately 1 cm. The stripped-cut beef was randomly divided into three groups, beef treated with crude papain extract from Chaya stalk, beef treated with crude papain extract from papaya stalk, and non-enzyme treated beef. Each group was soaked in the crude enzyme extract or drinking water (as control) at a ratio of beef to enzyme extracted of 1:5 for 5 min. Then, the beef was set aside for 10 min. Marinated the beef with ingredients for 10 min, weight the fresh beer, and placed the marinated beef on stainless trays. The beef trays were put in a solar dryer for 7 hours of drying (9.00 am – 4.00 pm, January 2021). The temperature (^{o}C), relative humidity (%RH), and solar radiation (Btu/f^{t2}.hr) were recorded throughout the drying process. At the end of drying, the finished sun-dried beef was weighted, and vacuum packed for further analysis.

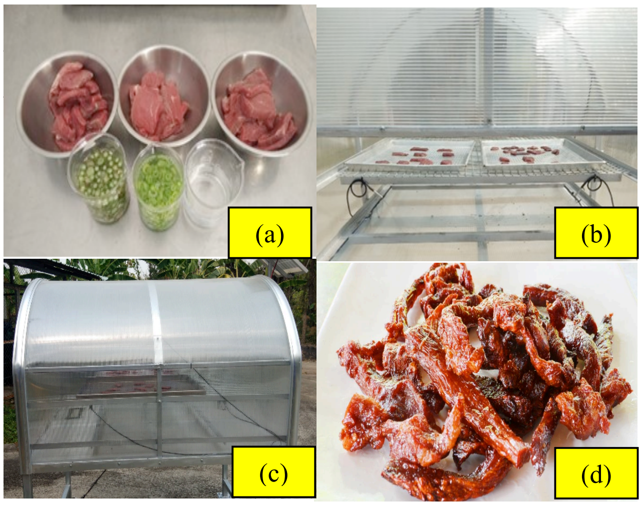

Fig. 2 Enzyme extraction for beef tenderization (a) Solar drying process (b) Solar dryer with a multivariable record (c) and halal sun-dried beef (d)

E. Sun-dried beef analysis

The yield of sun-dried beef was calculated by a ratio of sun-dried beef weight and fresh beef weight times 100. The moisture content of the sun-dried beef was determined by the gravimetric method. The water activity value was analyzed by a water activity meter (AquaLab, USA). The analysis results were expressed as an average of three measurements. Tensile testing of sun-dried beef was investigated using a texture analyser (TA. XT plus, Stable Micro System, UK). The tensile testing was observed meat tension at 27°C. The experiments were carried out using tensile grips suitable for holding stripped-cut beef. The lower grip was static at the base while the moving grips were lifted to the upper direction, resulting in a more strengthen beef muscle. The low-test speed was set at 0.1 mm/s. The typical TPA analysis results showed a relationship between force (g force) and time (second). The maximum tensile force is calculated as the peak force of the tension seen in Fig.3. The work of tension (g force x s) was the total area under the graph. The results were expressed as an average of five measurements.

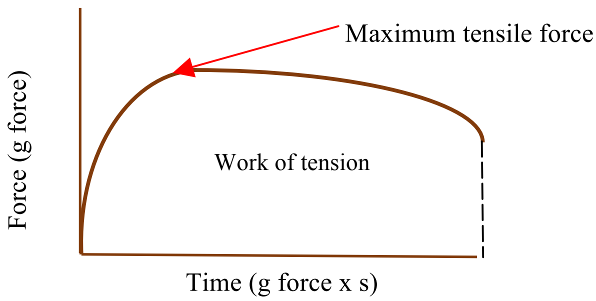

Fig. 3 Tensile testing of halal sun-dried beef shown the maximum tensile force and work of tension

F. Statistical analysis

The data from the meat tenderisation experiments were reported as the mean value and standard deviation (SD). The Completely Randomised Design (CRD) was applied to product yield, moisture content, water activity value, and textural results. The effects of crude papain addition to the sun-dried beef characteristics were evaluated using analysis of variance (ANOVA). Duncan's multiple range test (p<0.05) was used to describe the difference between the three treatments (crude papain from Chaya, crude papain from papaya, and no papain treatment as a control). The statistical analysis was performed using Statistix 10.

III. Results and Discussion

A. Effects of enzyme concentration, substrate concentration, pH and temperature on papain activity.

The crude enzyme from Chaya/papaya stalks in different concentrations or tested on 2% (w/v) casein as a common substrate of papain. Conversely, the various concentrations of casein digested by the activities of 2% (w/v) at 40oC for 10 min. The results of the two experiments presented in Table 1 and Table 2, respectively.

Table 1 UV absorbance of digestive activity between different casein concentrations and 2% (w/v) crude papain extract

Casein Concentration (%)	Absorbance Chaya	Absorbance Papaya
0	0.00 e ±0.00	0.00 e ±0.00
0.5	0.27 d ±0.02	0.35 d ±0.03
1.0	0.61 c ±0.03	0.60 c ±0.04
1.5	0.92 b ±0.01	0.83 b ±0.02
2.0	1.35 a ±0.03	1.15 a ±0.02

*a-e following the mean value (n=5) suggested a significant difference between treatments at p<0.05.

UV absorbance of free amino acids, e.g., tyrosine, tryptophan, and phenylalanine found in casein, increased by the proteolytic action of papain. As seen in Table 1, both papain extracted from Chaya and papaya had a pronounced effect on casein hydrolysis, where more substrate concentration could be digested by papain.

Table 2 UV absorbance of digestive activity between different enzyme concentrations and 2%(w/v) casein

Crude papain Concentration (%)	Absorbance Chaya	Absorbance Papaya

0	0.00 d ±0.00	0.00 d ±0.00
0.5	0.13 c ±0.01	0.18 c ±0.02
1.0	0.35 b ±0.02	0.48 c ±0.03
1.5	0.76 b ±0.02	0.82 b ±0.02
2.0	1.25 a ±0.03	1.11 a ±0.02

*a-d following the mean value (n=5) suggested a significant difference between treatments at $p<0.05$.

Conversely, a small amount of papain was not active to change the casein structure, except for the equal concentration of both enzyme and substrate (Table 2). Chinas and Lopez Munguia [6] have reported the moderate hydrolysis degree on a globular protein e.g., casein protein and soybean protein. That might be due to the tight binding of a globular structure and molecular weight of about 24,000 Da of both protein molecules.

Table 3 UV absorbance resulted from crude papain and casein reaction as influenced by temperature and pH

Temp (°C)	Absorbance		pH	Absorbance	
	Chaya	Papaya		Chaya	Papaya
40	0.92 c ±0.03	1.01 c ±0.03	3	0.60 b ±0.02	0.64 a ±0.01
50	1.02 b ±0.02	1.13 a ±0.02	4	0.66 a ±0.01	0.65 a ±0.02
60	1.10 a ±0.04	1.15 a ±0.01	5	0.66 a ±0.02	0.65 a ±0.03
70	1.04 b ±0.02	1.06 b ±0.04	6	0.61 b ±0.02	0.63 b ±0.01
80	1.03 b ±0.01	0.86 d ±0.05	7	0.62 b ±0.03	0.61 c ±0.02

*a-e following the mean value (n=5) suggested a significant difference between treatments at $p<0.05$.

Papain has an optimal temperature to hydrolyse casein substrate. It had an optimal temperature at 60°C shown in Table 3. Thus, papain is a mild heat-resistant enzyme. For pH, papain from Chaya and papaya had a broad pH (3-7), but the suitable pH value of papain found in the study was in the range of 4-5. However, a previous study has reported the optimal pH of the proteolytic activity of papain from Chaya at 7.5. The stability of Chya papain showed at pH10 with more than 70% of initial activity after a month [6].

B. Solar drying of beef treated with papain

Halal beef treated with papain from Chaya or papaya stalks then was marinated with food seasoning to tenderize the meat before the solar drying process. The drying conditions, inside dryer temperature (°C), relative humidity (% RH), and solar radiation (Btu/ft².hr) were monitored for seven hours seen in Fig. 4.

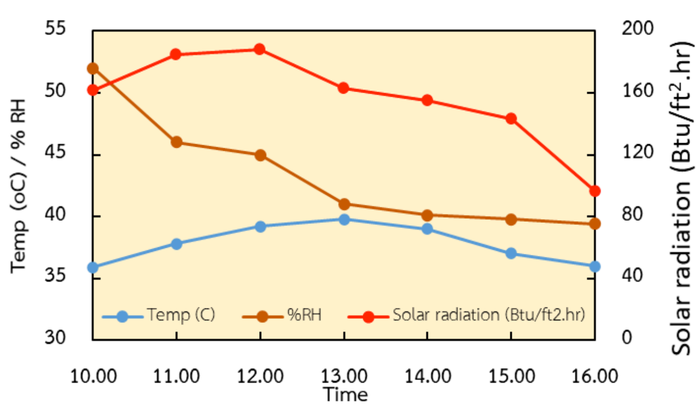

Fig. 4 Changes in tunnel temperature (°C) and relative humidity (%RH) (right Y-axis) and solar radiation (Btu/ft².hr) (left Y-axis) during sun-dried beef processing.

The highest solar radiation was between 11.00 am -12.00 pm due to a high temperature inside the solar dryer. The lower relative humidity of the air around the raw beef accelerated the water evaporation from inside the meat to the surface. The drying process had proceeded till the moisture content of the dried meat was lower than 30% (w/v). The water activity value was approximately 0.85. The yield percentage of sun-dried beef estimated to be 72%.

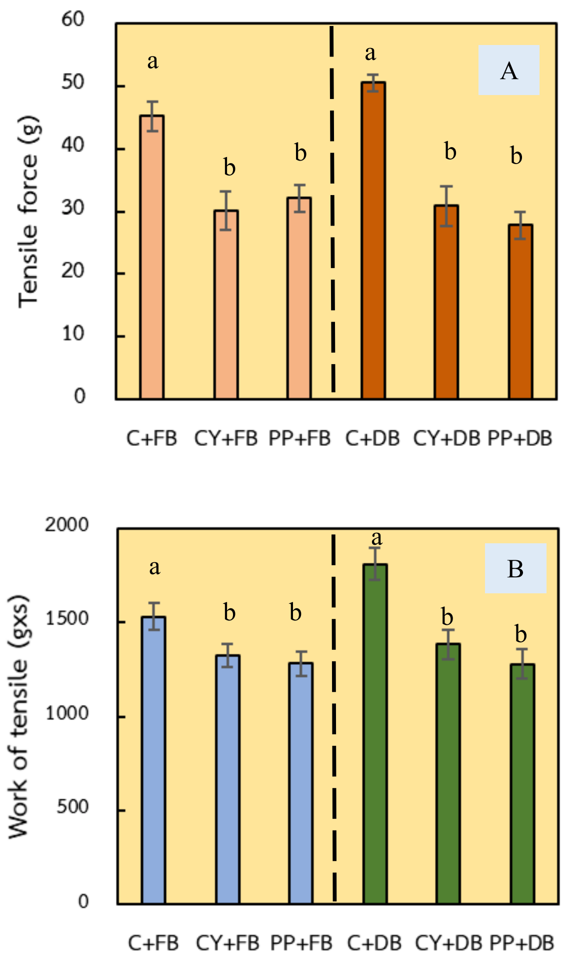

Fig. 5 Tensile force (A) and work of tensile (B) of fresh beef (C+FB), Chaya papain treated-fresh beef (CY+FB), papaya papain treated- fresh beef (PP+FB), dried beef (C+DB), Chaya papain treated- dried beef (CY+DB), and papaya papain treated- dried beef (PP+DB)

As seen in Fig 5, tensile forces of fresh beef treated with papain from Chaya or papaya were lower than the tensile forces of control. A similar trend was observed on sun-dried meat, which had a less tension force in the papain treated sample. It was suggested that papain could hydrolyze fibrilla protein of meat muscle [2,7,8]. In addition, beef samples treated with papain showed a reduction in work of tensile values after marination. That led to a softer texture and effortless mastication. Both papains extracted from Chaya and papaya similarly worked on tough beef.

CONCLUSION

In the present day of this study, we have found that papain extracted from Chaya has similar bioactivity as generally known in papaya. The protease has an optimal condition of mild temperature and slightly acidic conditions. The applications of Chaya papain are to tenderise fresh beef and sun-dried beef. The Chaya stalk can place as an alternative source of papain for a safe food additive.

ACKNOWLEDGMENT

We would thank Asian Development College for Community Economy and Technology (adiCET), Chiang Mai Rajabhat University for a solar dryer and data logger supports.

REFERENCES

[1] Amri, M. and Mamboya, F. (2012). Papain, a plant Enzyme of biological importance: a review. *American Journal of Biochemistry and Biotechnology.* 8(2), 99-104.

[2] Shouket, H. A., Ameen, I., Tursunor, O., Kholikova, Kh., Pirimor, O., M., Kubonov, N., Ibragimor, I., and Mukimor, B. (2020). Study on industrial applications of papain: a succinct review. IOP Conference: Earth and Environmental Science. 614(2020) 012271.

[3] Welde, Y. and Wakes, A. (2018). Identification and extraction of papain enzyme from papaya leaf in adigrat town, northern Ethiopia. *Journal of Medicinal Plants Studies.* 6(3): 127-130.

[4] Tigist, M., Getnet, B., Beza, K., Endalamau, M., Lulit, M., Tamirot, D., and Tinsae, M. (2016). Extraction and purification of papain enzyme from papaya leaf and phytochemical components of the laef. *Biotechnology International.* 9(8): 176-104.

[5] Kuri-garcia, Charez-Servin, J.L., Guzmain-Maldonado, S. H. (2017). Phenolic profile and antioxidant capacity of *Cnidpscolus chayamansa* and *Cnidoscilus aconitifolius*: A review. *Journal of Medicinal Plant Research.* 11(45): 713-727.

[6] Chinas, F. C. A., and Lopez Munguia, A. (2006). Proteolytic enzymes from *Cnidoscolus chayamansa* “Chaya”. *Journal of Food Science.* 51(1): 243-244.

[7] Wolnue, Y., Ayele, A. Hailu, M. and Chaurasiya, R. S. (2020). Comparison of different meat tenderization methods: a review. *Food Research*. 1-7.

[8] Maiti, A. K., Ahlawat, S. S., Sharma, D. R., and Khanna, N. (2008). Application of natural tenderizers in meat- a review. *Agricultural Review.* 29(3): 226-230.