Proceedings of the 9th International Symposium on Machinery and Mechatronics for Agriculture and Biosystems Engineering (ISMAB)
28-30 May 2018, Jeju, Korea

IMPROVING MEAT TENDERNESS BY USING PROTEASE EXTRACT FROM PADDY OATS (*Gnetum gnemon*) FRUIT PEEL

Cahyo Indarto[1,2], Shyang-Chwen Sheu[3], Pomin Li[4*]

[1] Department of Tropical Agriculture and International Cooperation, National Pingtung University Science and Technology, Taiwan
[2] Department of Agroindustrial Technology, Trunojoyo University, East Java, Indonesia
[3] Department of Food Science, National Pingtung University Science and Technology, Taiwan
[4] Department of Biomechatronics Engineering, National Pingtung University Science and Technology, Taiwan
*Corresponding Author-- Voice: +886-8-7740283, Email: pomin@mail.npust.edu.tw

Abstract: This study investigated the possibility of an agricultural waste, paddy oats (*Gnetum gnemon* L.) fruit peel, as a source of protease extract, particularly used as a meat tenderizer. The effect of paddy oats protease extract on meat tenderness was evaluated and compared to those of commercial papain, commercial bromelin, and control. Paddy oats protease extract showed the high meat tenderizing activity since it resulted in shear force value of meat sample 46% lower than that of control, and similar ($p<0.05$) with those of commercial papain and commercial bromelin. Panelists scored the meat sample treated with paddy oats protease extract much more tender (7.4) than that of control (5.8), using a 10 point hedonic scale and no different ($p<0.05$) from those of commercial papain and commercial bromelin with score 7.3 and 7.7, respectively. Paddy oats protease extract also consistently ranked high for all evaluated parameters, therefore paddy oats protease extract could have a high potential as a meat tenderizer for improving tenderness quality of meat.

Key Words: Paddy oats, protease extract, new source, meat tenderizer

INTRODUCTION

Meat tenderness has been identified as the most important quality attribute in consumer demand for beef product (Miller et al. 1995). The importance of meat tenderness to consumer can be seen from the relationship between the tenderness and the meat prices. Boleman et al. (1997) reported that there was a strong relationship between the increase in the price of meat and meat tenderness, as well as the general willingness of consumers to pay a premium for more tender steaks. The consumer demanded tender meat products and they often rejected tough meat.

Treatment by a proteolytic enzyme (protease) is one of the popular methods to enhance meat tenderness. Study on the exogenous protease as meat tenderizer has been conducted for over tents years and has investigated protease from bacteria, fungal and plant sources. (Calkins & Sullivan 2007). Plant protease, especially papain and bromelin are the most popular meat tenderizer enzyme in the market. For instance, the major area of consumption of meat tenderizers in United State is in consumer households. This consumer use probably accounts for 90% of meat tenderizing enzyme sales, typically two products are papain and bromelin (Enzyme Development Corporation, 2016).

Paddy oats (*Gnetum gnemon*) are grown commercially in Indonesia, India, Malaysia, Thailand, and Fiji. It is a medium size tree, growing to 15-20 m tall. The leaves are evergreen, opposite, 8-20 cm long and 3-10 cm wide, entire, emerging bronze-colored, maturing glossy dark green. The fruit-like strobilus consists of a small amount of skin or peel and a large nut-like seed 2-4 cm long inside (Lim 2012), with both the fruits and leaves being very popular in Indonesian cuisines. Approximately 38% of the whole fruit is in paddy oats fruit peel, which is only discarded during seed processing, as waste or used as organic fertilizers. These discarded peels may cause environmental problems, and the treatment of such waste has been very costly for industries. The utilization of agricultural waste as a source of protease is an alternative means.

MATERIALS AND METHODS

MATERIAL

Fresh paddy oats fruit peel was obtained from a local market. Paddy oats fruits peel was selected based on size uniformity at the same stage of maturity (red colored ripe peel) and lack of visual defect. The fruit peel was chopped into small pieces, and then it was quickly blended (HR-2011 Philips Blender) with cold 0.1 M sodium phosphate buffer at pH 7.2 containing EDTA 5mm for 4 min. The resulting blend was filtered through a cheese cloth and then centrifuged at 10,000 x g at 4 ° C for 10 min. The pellet was discarded and the supernatant (crude protease extract) was collected and subjected to 40% ammonium sulfate precipitation and then protein content was measured. Protease solution of commercial papain and commercial bromelain was prepared by dissolving them with distillate water, then filtered through a Whatman paper No. 1. The protein content of proteolytic extracts was determined according to the Bradford method, using BSA as a protein standard (Bradford, 1976). The final protein concentration of protease solutions was adjusted to be 75mg/100mL.

MEAT TENDERIZING TREATMENT

Samples prepared by cutting frozen steak into a size of 3x3x3 cm^3 chunks, then samples were thawed at room temperature. Steak chunks were marinated in different protease solution for 2 hours, placed in a sterile box at room temperature. After marinating, samples were cooked in the oven to an internal temperature of 75^{o}C.

PSYCHOCHEMICAL ATTRIBUTES

a. Shear force

The Warner-Bratzler shear force (WBSF) was employed to analyze meat tenderization. Samples were tested as per the manufacturer's instruction, according to the following conditions: test speed of 60.00mm / min; inch speed of 20.00mm / min; width 9.00mm; 6:00 depth mm; 20:00 mm gauge length. Samples were pulled in the direction of meat fiber, and tenderization expressed as the maximum force (Newton) is required.

b. Water-holding Capacity

The method to determine water holding capacity by Wardlaw et al. (1973) was adopted to analysis water holding capacity of meat samples. Briefly, minced meat mixed with 0.6 M NaCl solution and stirred well, kept at 4^{o}C for 15 min and then centrifuged at 3000g for 25 min.

c. pH and moisture

pH and water content of meat samples were measured according to standard procedure (AOAC, 1995).

d. Sensory Attributes

The sensory evaluation of the meat samples was assessed by a trained panel of 7 members. Panelist evaluated the samples using a 10 point hedonic scale (Thompson et al. 2005). Tenderness (1= least tender and 10= most tender); Juiciness (1= least juicy and 10= most juicy); Flavor (1= least preferred and 10= most preferred); Color (1= least preferred and 10= most preferred).

RESULTS & DISCUSSION

PSYCHOCHEMICAL ATTRIBUTES

Tabel 1. Physicochemical properties of beef samples treated with different protease solution

Protease solution	Shear force (N)	WHC (%)	Cooking loss (%)	pH	Moisture (%)
Control	38.59 [a]	23.85 [a]	29.84 [d]	6.15	74.51
Bromelin	22.14 [b]	21.19 [b]	32.96 [c]	5.97	75.22
Papain	19.71 [c]	22.38 [b]	35.14 [a]	6.08	74.14
Paddy oats	20.77 [bc]	22.64 [b]	33.37 [b]	5.92	74.19

Differences between means were evaluated by Least Significance Different (LSD) test. Different letter superscripts in the same column indicate significant difference ($p < 0.05$).

a. Shear force

The effect of different proteolytic extracts on shear force value of meat sample was studied and reported as shown in Table 1. The table shows the efficacy of various proteolytic extracts in decreasing shear force value of meat sample. It can be clearly seen that all of the treatments have a significant effect on meat sample tenderization compared to control. Protease extract of paddy oats showed the high meat tenderizing activity since it resulted in shear force value of meat sample 46% lower than that of control (20.77 N and 38.59 N), respectively. The meat tenderness differed significantly ($p<0.05$) between control and treated samples. The most tenderness meat, represented as the lowest value of shear force (19.71 N), was obtained from meat sample treated with commercial papain. There was a marked reduction ($p<0.05$) in tenderness of bromelin-treated samples (22.14 N) compared to papain-treated sample, while the tenderness of paddy oats-treated sample did not differ ($p<0.05$) from those of samples that were treated with papain and bromelin.

Tenderness of meat increased during marination the meat sample in a protease solution. During immersion, proteases hydrolyzed myofibril protein as well as connective tissue protein of meat, as the result tenderness of meat increase. Previous research conducted by Lee et al. (1986), using ginger as a meat tenderizer extract concluded that the protease from ginger rhizome could be used to improve the tenderness of meat. Tenderness of meat occurred because of the hydrolysis in the connective tissue and myofibril fragmentation. Improvement the tenderness of treated meat samples may be also due to collagen solubility. Study on the effect of protease on beef collagen revealed that the quality of meat tenderness improved because of collagen solubilization (Sugiyama et al. 2005).

b. Water holding capacity (WHC) and cooking loss

Water holding capacity is the ability of meat to retain water content or moisture when force is applied. It is one of the important psychochemical attributes because it will affect the sensory quality, especially the juiciness parameter of meat. Tabel 1 presents the value of water holding capacity of meat sample due to the effect of various protease solution. Among the treated samples with different proteolytic extract, the sample soaked in bromelin solution achieved the lowest WHC (21.19%). It was not significantly different ($p<0.05$) from those of samples treated with papain and paddy oats protease of 22.38% and 22.64%, respectively. However, the water holding capacity of all treated samples was significantly lower than that of control (23.85%). Several studies showed that protease improved the meat tenderness by fragmenting meat protein (Lewis & Luh 1988; Kumar & Berwal 1998). Degradation of meat protein such as sarcoplasmic proteins and myosin could be the reason for its ability to bind water resulting in decreasing water holding capacity of meat (Marino et al. 2014).

Cooking loss, measured as the difference in weight of the meat before and after cooking, it expresses the amount of water released from the meat after cooking process using thermal. As we can see in Table 1, an increase in cooking loss due to submersion meat samples in various proteolytic extract solution. Cooking loss value of meat samples ranged from 29.84% (control) to 35.14% (papain solution) while cooking loss value of meat samples treated with bromelin and paddy oats protease were recorded of 32.96% and 33.37%, respectively. Those cooking loss values slightly increased comparing to that of control, and differ significantly ($p<0.05$) among them. There is a strong relationship between water holding capacity and cooking loss of muscle. Wagner & Añón (1985) reported the decrease of water holding capacity would be followed by the increase of cooking loss of the muscle due to protein fragmentation.

c. pH and moisture

Table 1 displays the pH and moisture of the meat samples were treated with various proteolytic extracts compared with control. There was no significant difference ($p<0.05$) both pH and moisture of all meat samples. This finding was not in agreement with the previous study. A study on the application of bromelin extract for improving muscle tenderness concluded that a significant decreased in pH and moisture were found in all of the treated samples when compared to the control (Ketnawa & Rawdkuen 2011). Another study reported that pH of duck breast muscle increased significantly when marinated in protease extract of ginger (Tsai, et al. 2012). pH and moisture of meat are as a function of many factors such as rigor mortis, meat treatment, and buffering capacity of muscle. In this study, the various protease solution had no effect on pH and moisture of meat samples. This could be due to some compound dilute into solution when meat samples were dipped in protease solution, and vice versa the water from solution come inside meat samples so that pH and moisture of samples were disrupted.

SENSORY ATTRIBUTES

Means for sensory attributes are presented in Table 2. The highest value of tenderness was obtained for meat samples treated with bromelin (7.7); however, it was not statistically different ($p<0.05$) from tenderness value for samples treated with papain (7.3) and paddy oats protease (7.4). Meat sample without extract treatment (control) was scored lowest for tenderness (5.8) and it was statistically different from others ($p<0.05$). The sensory evaluation score for tenderness is in good agreement with the results of texture measurement of the meat sample, which were more tender for meat samples treated with all protease extract since they showed the lower shear force value than control (Table 1).

Tabel 2. Sensory attributes of beef samples treated with different protease solution

Protease solution	Tenderness	Juiciness	Flavor	Color
Control	5.8 [b]	6.3 [b]	6.8 [a]	6.7
Bromelin	7.7 [a]	6.8 [a]	6.4 [ab]	7.0
Papain	7.3 [a]	6.8 [a]	6.2 [b]	6.9
Paddy oats	7.4 [a]	6.6 [ab]	6.5 [ab]	7.2

Differences between means were evaluated by Least Significance Different (LSD) test. Different letter superscripts in the same column indicate significant difference ($p < 0.05$).

Meat samples treated with a different type of protease solution were also received significantly higher scores ($p<0.05$) for all parameter describing juiciness, flavor, except color (Tabel 2). All meat samples were ranked juicer ($p<0.05$) than untreated meat sample (control), except the sample treated with paddy oats protease, where its juiciness score was not significantly different from those of other treated samples and control. The lowest flavor score (6.2) addressed to meat sample treated with papain, while other samples had higher flavor value and not statistically different from control (6.8). Meat samples treated with paddy oats proteolytic extract scored highest for color but it was not different ($p<0.05$) from other samples, including control. These scores indicated that the types of protease extract had no effect on meat samples color.

Overall, the result of this study showed that meat samples treated with paddy oats protease extract had a similar characterization to samples treated with bromelin and papain. Paddy oat protease extract could be used as a meat tenderizer because it consistently ranked high for all evaluated parameters. These results suggested that, by utilizing this information, the tenderness quality of meat could be improved by using paddy oat seed peel, that is new and cheaper material for producing proteolytic enzyme.

CONCLUSIONS

Paddy oats protease extract enhanced meat sample characteristics were similar to those of meat sample treated with commercial bromelin as well as commercial papain. These results could be an opening possibility for production new commercial protease from paddy oats fruit peel, particularly protease as a meat tenderizer.

REFERENCES

AOAC. 1995. Official methods of analysis (16^{th} ed.) Washington DC: Association of official analytical chemists.

Boleman, S.J., S.L. Boleman, R.K. Miller, J.R. Taylor, H.R. Cross, T.L Wheeler, M. Koohmaraie, S.D. Shackelford, M.F. Miller, R.L. West, D.D. Johnson, and J.W. Savell. 1997. Consumer Evaluation of Beef of Known Categories of Tenderness. Journal of Animal Science 75:1521-1524.

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of dye binding. Analytical Biochemistry 72: 248-254.

Calkins, C.R and G. Sullivan. 2007. Adding Enzymes to Improve Beef Tenderness.

Cattlemen’s Beef Board. University of Nebraska.
Enzyme Development Corporation. Meat Tenderizing Enzymes. Retrieved from http://www.enzymedevelopment.com/applications/tenderizer, accessed Marc 2016.
Ketnawa, S. and S. Rawdkuen. 2011. Application of Bromelain Extract for Muscle Foods Tenderization. Food and Nutrition Sciences 2: 393-401.
Kumar, M. and J.S. Berwal. 1998. Tenderization of spent hen meat with Cucumis trigonus Roxb (kachri). Indian Journal of Poultry Science 33: 67-70.
Lee, Y.B., D.J. Sehnert and C.R. Ashmore. 1986. Tenderization of meat with ginger rhizome protease. J. Food Sci. 51: 1558-1559.
Lewis, D. A. and B.S. Luh. 1988. Application of actinidin from kiwifruit to meat tenderization and characterization of beef muscle protein hydrolysis. Journal of Food Biochemistry 12: 147-158
Lim, T. 2012. Edible Medicinal and Non-Medicinal Plants Vol. 3. London, New York, Springer.
Marino, R., M Albenzio, A. Della-Malva, M. Caroprese, A. Santillo and A. Sevi. 2014. Changes in meat quality traits and sarcoplasmic proteins during aging in three different cattle breeds. Meat Science 98: 178–186.
Miller, M.F., K.L. Huffman, S.Y. Gilbert, L.L. Hammon, and C.B. Ramsey. 1995. Retail Consumer Acceptance of Beef Tenderized with Calcium Chloride. Journal of Animal Science 73:2308.
Sugiyama, S., A. Hirota, C. Okada, T. Yorita, K. Sato and K. Ohtsuki. 2005. Effect of kiwifruit juice on beef collagen. Journal of Nutrition Science and Vitaminology 51: 27-33.
Thompson, J.M., A. Gee, D.L. Hopkins, D.W. Pethick, S.R. Baud and W.J. O'Halloran. 2005. Development of a sensory protocol for testing palatability of sheep meats. Australian Journal of Experimental Agriculture 45: 469–476.
Tsai, L., N. Yen and R.R. Chou. 2012. Changes in Muscovy duck breast muscle marinated with ginger extract. Food Chemistry 130: 316–320
Wagner, J. R. and M.C. Añón. 1985. Effect of freezing rate on the denaturation of myofibrillar proteins. International Journal of Food Science and Technology 20: 735-744.
Wardlaw, F.B., L.H. Maccaskill and J.C. Acton. 1973. Effect of postmortem muscle changes in poultry meat loaf properties. Journal of Food Science 38: 421-424.