Asian Journal of Biotechnology and Bioresource Technology

1(2): 1-6, 2017; Article no.AJB2T.34314

Enzymatic Activity of Bromelain from Crude Extracts of Crown, Peels and Stem of Pineapples from Different Agro-ecological Zones of Thika Region, Kenya

S. K. Kahiro[1], J. M. Kagira[2*], N. Maina[1], S. M. Karanja[3] and F. N. Njonge[2]

[1]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200, Nairobi, Kenya.
[2]Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200, Nairobi, Kenya.
[3]Department of Public and Community Health, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SKK and JMK designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors SMK and NM managed the analyses of the study. Author FNN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2017/34314
Editor(s):
(1) Matheus Poletto, Cellulosic and lignocellulosic Materials, Universidade de Caxias do Sul, Brazil.
Reviewers:
(1) Nazura Usmani, Aligarh Muslim University, India.
(2) Fábio Tonissi Moroni, Universidade Federal do Amazonas, Brazil.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/20112

Original Research Article

Received 23rd May 2017
Accepted 17th June 2017
Published 19th July 2017

ABSTRACT

Bromelain is an enzyme that has great commercial value and is of wide interest in the pharmaceutical, food, and cosmetics industries. In Kenya, large quantities of pineapple by-products are not well utilized although they can be a potential source for bromelain. The objectives of this study were to determine the levels of bromelain in crude extract of different parts of pineapples from different agro-ecological zones (AEZ) of Thika Region, Kenya. Following extraction, protein concentration and bromelain activity was estimated using standard

**Corresponding author: E-mail: jkagira@gmail.com;*

methods. The activity of bromelain for the crown from the upper (UAEZ), mid (MAEZ) and lower (LAEZ) was 89.57, 101.34, 100.78 U/ml and the activity was significantly lower ($p<0.05$) in pineapples from UAEZ compared to that the other two zones. For peels, the bromelain activity (U/ml) of pineapple from UAEZ (88.79 U/ml) was significantly lower ($p<0.05$) than that from MAEZ (98.21 U/ml) and LAEZ (97.65 U/ml). The bromelain activity in the stems of pineapple from LAEZ (89.71 U/ml) was significantly higher ($p<0.05$) compared to those from MAEZ (85.73 U/ml) and UAEZ (82.27 U/ml). In conclusion, the study shows that pineapple by-products in Kenya can be a good source of bromelain, with higher levels of activity being observed in pineapples from the lower and mid-AEZ.

Keywords: Bromelain; pineapple; crown; peels; stem; agro-ecological zones; Kenya.

1. INTRODUCTION

Bromelain is a proteolytic enzyme found in the tissues of plants of the Bromeliaceae family and pineapple (*Ananas comosus*) is its main source. Pineapple is a tropical plant with edible multiple fruit consisting of coalesced berries, and the most economically significant plant in the Bromeliaceae family [1]. Proteolytic enzymes are a class of hydrolytic enzymes capable of cleaving the peptide bonds of protein chains and are important in physiological processes. Indeed, proteolytic enzymes are among the most relevant enzymes from an industrial standpoint because of their involvement in several technological applications [2]. Proteolytic enzymes constitute the major components of pineapple bromelain extract and include stem bromelain (80%), fruit bromelain (10%) and ananain (5%). In addition, the preparation contains a composite mixture of different thiol-endopeptidases and other partially characterized constituents such as phosphates, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates [3]. Bromelain has a number of applications, especially in the food, cosmetics and pharmaceutical industries. It also has several clinical applications, such as an antitumor agent, immune response modulator, sinusitis, bronchitis, angina pectoris, thrombophlebitis, surgical traumas, pyelonephritis, improved antibiotic absorption, mucolytic activity and gastrointestinal action [3-5]. Indeed, it has obtained universal acceptability as therapeutic drug, due to its history of effectiveness and safety [6].

The by-products of pineapple following processing are mainly used as feed for cattle or as fertilizer. However, it is still very low in value when compared to the value of the bioactive compounds that could be obtained from those waste materials, especially in terms of bromelain content [7]. Since pineapple crown and peels make up such a large amount (30–40%) of post processing waste material [8], they can be a potential source for bromelain extraction. Kenya is a major producer of pineapple in the world [9] and thus produces huge quantities of wastes which are not well utilized. Thus, country has unique opportunity for the production of large quantities of bromelain from crown, peels and stem. There have been no concerted efforts to determine the bromelain levels and activity in pineapples grown in Kenya. Further, it is known that the amount of secondary metabolites content in plants such as plant cysteine protease, bromelain [10] is influenced by environmental conditions which are exhibited by different agro - ecological zones where the plants are grown [11]. This study aimed at determining the level of bromelain present in crown, peels and stem of crude extract of pineapples obtained from upper, mid and lower agro-ecological zones of Thika Region, Kenya.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Thika Region which is located in central Kenya and is made up of six administrative sub-counties namely: Kamwangi, Gatundu, Kakuzi, Gatanga, Thika municipality and Ruiru. Kamwangi is located in upper, Gatundu and Gatanga in mid while Kakuzi, Thika municipality and Ruiru are in lower agro-ecological zone. The region has an estimated human population of 828,531 as at 2009. Rainfall is bimodal and ranges from 500 mm in the lower zone to 1,300 mm in the higher zone. The long rains occur from mid-march to June while the short rains occur from mid-October to December. Temperatures in this region range from a minimum of 12.8°C to a maximum of 24.6°C with an average of 18.7°C.Ecologically the region transcends across various ecological zones namely: Upper highlandsUH1, Lower highlands-LH1, Upper Midlands UM2, UM3, UM4, UM5, UM6, and LM4. Gatanga lies in the UH1and Kakuzi in lower midlands-LM4. Majority of farmers in the region are small holders, practicing mixed agriculture including; livestock production, food and cash crops.

2.2 Pineapples Collection

Twenty four mature pineapples were obtained from the upper, mid and lower agro-ecological zones. The pineapples were randomly obtained at farm level where they were harvested, labeled, packed into cool box and transported to the laboratory at Jomo Kenyatta University of Agriculture and Technology. The pineapples were immediately processed as described below.

2.3 Crude Extraction

The fresh pineapples were washed with 0.1% hydrogen peroxide solution. Crown, peels and stem were weighed and cut into small pieces. Juice was extracted from the parts by blending, in the presence of sodium acetate buffer. The amount of juice obtained was measured. Sodium benzoate, preservative was added at a concentration of 1 mg. per gram of the sample used and then filtered. The filtrate obtained was labeled as crown, peels or stem crude extract.

2.4 Bromelain Enzymatic Assay

Bromelain enzymatic assay was undertaken as previously described [12]. Briefly, 1 ml protease sample was added in a test tube and 5 ml of casein. The solution was then allowed to stand for 10 minutes at 37°C and thereafter, 5 ml of TCA was added and allowed to stand for 30 minutes at room temperature. The sample was filtered twice using filter paper and 2 ml of the solution was obtained. 5 ml of sodium carbonate and 1 ml of Folin Ciocalteau reagent was then added and allowed to stand for 30 minutes and absorbance was measured at 660 nm. One unit of bromelain activity was defined as 1 g of tyrosine released in 1 min per mL of sample when casein was hydrolysed under the standard conditions of 37°C and pH 7.0 for 10 min [2].

The formula for determination of specific activity was calculated as:

Specific activity = bromelain activity (U/mL) /protein content (mg/mL) [2].

2.5 Total Protein Quantification

Total protein quantification was done as previously described [13]. Five dilutions of bovine serum albumin (BSA) standard with a range of 5 to 100 µg protein were prepared. The bromelain samples was diluted to obtain 5-100 µg protein/30 µl. 30 µl each of standard solution or bromelain sample was added to an appropriately labeled test tube and protein solutions was assayed in duplicate. Absorbance was then measured at 595 nm.

2.6 Data Analysis

Analyses were performed with Microsoft excel 2013 and Statistical Package for the Social Sciences (SPSS) version 20. For the statistical analysis, multiple comparison was performed using Tukey's test employing 95% confidence interval where $p<0.05$ was considered significant.

3. RESULTS

3.1 Activity of Bromelain in Crown from Different Agro-ecological Zones

In descending order, the bromelain proteolytic activity was highest in the crown of pineapples from lower-agro-ecological zone (LAEZ) and lowest in the upper agro-ecological zone (UAEZ). The activity was significantly ($p<0.05$) lower in the crowns of pineapples from UAEZ compared to that from the other two zones. There was no significant ($p>0.05$) difference in bromelain proteolytic activity in extracts of crowns of pineapples from mid agro-ecological and lower agro- ecological zone (Table 1).

3.2 Activity of Bromelain in Peels from Different Agro-ecological Zones

The bromelain proteolytic activity in peels was significantly ($p<0.05$) higher in the peels obtained in pineapple samples from MAEZ and LAEZ compared to those from UAEZ. There was no significant ($p>0.05$) difference in bromelain proteolytic activity in samples from MAEZ and LAEZ (Table 2).

3.3 Activity of Bromelain in Stems of Pineapple from Different Agro-ecological Zones

The bromelain activity of LAEZ stem bromelain was significantly ($p<0.05$) higher than that from UAEZ and MAEZ. But there were no significant ($p>0.05$) differences in bromelain activity of stems of pineapples from UAEZ and MAEZ (Table 3).

Table 1. Average protein content, proteolytic activity, specific activity of crown bromelain from different agro-ecological zones in Thika region, Kenya

Crown origin	Mean protein content (mg/ml) (95% CI)	Bromelain activity (U/ml) (95% CI)	Specific activity (U/mg) (95% CI)
Upper AEZ	1.98 (1.93-2.01)[a]	89.57 (87.25-91.89)[a]	45.52 (43.78-47.26)[a]
Mid AEZ	1.99 (1.95-2.04)[a]	101.34 (99.02-103.66)[b]	50.91 (49.17-52.65)[b]
Lower AEZ	2.00 (1.96-2.05)[a]	100.78 (98.46-03.10)[b]	50.50 (48.76-52.24)[b]

AEZ = Agro-ecological zone, CI-Confidence Interval
Mean with same superscript within the same column are not significantly different (p>0.05), mean with different superscript within the same column are significantly different (p<0.05)

Table 2. Average protein content, proteolytic activity, specific activity of peels bromelain from different agro-ecological zones in Thika region, Kenya

Peels origin	Protein content (mg/ml) (95% CI)	Bromelain activity (U/ml) (95% CI)	Specific activity (U/mg) (95% CI)
Upper AEZ	1.90(1.86-1.93)[a]	88.79(86.21-91.38)[a]	47.01(45.27-48.76)[a]
Mid AEZ	1.94(1.90-1.97)[a]	98.21(95.62-100.79)[b]	50.76(49.01-52.50)[b]
Lower AEZ	1.95(1.91-1.98)[a]	97.63(95.04-100.22)[b]	50.22(48.48-51.97)[b]

AEZ = Agro-ecological zone, CI-Confidence Interval
Mean with same superscript within the same column are not significantly different (p>0.05), mean with different superscript within the same column are significantly different (p<0.05)

Table 3. Average protein content, proteolytic activity, specific activity of stem bromelain from upper, mid and lower agro-ecological zones

Stem origin	Protein content (mg/ml) (95% CI)	Bromelain activity (U/ml) (95% CI)	Specific activity (U/mg) (95% CI)
Upper AEZ	2.15(2.12-2.18)[a]	82.27(79.46-85.08)[a]	38.37(36.90-39.84)[a]
Mid AEZ	2.18(2.16-2.21)[a]	85.73(82.92-88.54)[a]	39.30(37.84-40.78)[a]
Lower AEZ	1.90(1.87-1.92)[b]	89.71(86.90-92.52)[b]	47.37(45.90-48.84)[b]

AEZ = Agro-ecological zone, CI-Confidence Interval
Mean with same superscript within the same column are not significantly different (p>0.05), mean with different superscript within the same column are significantly different (p<0.05)

3.5 Comparison of Bromelain Activity among Crown, Peel and Stems Pineapple

The bromelain activity of stem bromelain was significantly lower (p<0.05) than that from crown and peels. However, there were no significant (p>0.05) differences in bromelain activity from crown and peels (Table 4).

4. DISCUSSION

The current study was geared towards evaluating the bromelain activity of crude extracts of different parts of pineapples from different AEZ in Thika region of Kenya. In many cases, the commercial use of bromelain in the food, cosmetics, and nutritional, medicinal and pharmacological supplement industries does not require a high purity bromelain enzyme preparation [14]. Thus, crude extracts of bromelain can be used in various processes. In the case of industrial enzymes, yield of the enzyme recovery rather than purity is the major concern [15]. The use of crude bromelain in the crude extract can enable lowering of the costs of the production process since downstream processing may account up to 60–90% of the total enzyme production cost [16]. Crude extract bromelain activity is higher than that observed by [2].

Results of the current study showed that the highest proteolytic activities were obtained from the extracts of the pineapples from lower agro-ecological zone while lowest values were recorded from the upper agro-ecological zone. Sucrose content affects bromelain accumulation in pineapple cells as described previously [17]. Sucrose content in pineapples is influenced by environmental conditions which are exhibited by different agro-ecological zones and cultural practices [18].

Table 4. Table of average protein content, proteolytic activity, specific activity of bromelain from different pineapple parts

Origin	Protein content (mg/ml) (95% CI)	Bromelain activity (U/ml) (95% CI)	Specific activity (U/mg) (95% CI)
Crown	1.99(1.97-2.02)[a]	97.23(95.46-99.00)[a]	48.98(47.83-50.13)[a]
Peel	1.93(1.90-1.95)[b]	94.88(93.10-96.65)[a]	49.33(48.18-50.48)[a]
Stem	2.08(2.05-2.10)[c]	85.90(84.13-87.68)[b]	41.68(40.53-42.83)[b]

Mean with same superscript within the same column are not significantly different (p>0.05), mean with different superscript within the same column are significantly different (p<0.05)

More so, it has been shown that cysteine proteases such as bromelain are differentially expressed in plants depending on different environmental conditions, such as amount of rainfall, temperature and salt concentration as previously described [19] which characterizes different agro-ecological zones. Bromelain was extracted from the crown, peel and stem and the highest proteolytic activity were obtained from the extracts of the crowns while lowest values were recorded from the stem and this is similar to previous findings [20].

The commercial production process involves obtaining bromelain from cooled pineapple juice using filtration and lyophilisation yielding a yellowish powder which has 30 to 40% protein content, and is of uncertain biological activity [21-22]. Thus there is need for the determination of bromelain activity in pineapple extracts. The addition of protease such as bromelain to animal feed can increase protein inversion and availability, decrease the cost of animal feed, and increase the source of protein and increase animal production [23]. Thus, the crude extract from pineapple processing and by-products can provide ample source for bromelain which could be used as a feed additive in livestock production.

5. CONCLUSION

In conclusion the study has shown that the concentration of bromelain was found to be higher in the lower agro-ecological zone than in the mid agro-ecological zone and was lowest in the upper agro-ecological zone. More studies should be conducted to determine the effects of agro- ecological condition on primary metabolites which in turn affects secondary metabolites such as bromelain activity level in pineapple extracts. To ensure proper production of bromelain from pineapple processing by-products, it is important to develop various production and downstream processes which can be stopped at different stages of purification depending on the destination of bromelain resulting to cost effectiveness.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hebbar HU, Sumana B, Raghavarao KS. Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes. Bioresources Technology. 2008;99(1):4896-4902.
2. Costa HB, Fernandes PMB, Romão W, and Ventura JA. A new procedure based on column chromatography to purify bromelain by ion exchange plus gel filtration chromatographies. Industrial Crops and Products. 2014;59:163–168.
3. Maurer HR. Bromelain biochemistry, pharmacology and medical use. Cell and Molecular biology for Life Sciences. 2001;58(9):1234-1245.
4. Bala M, Ismail NA, Mel M, Jamed MS, Mohd H and Azura AS. Bromelain production. Current trends and perspective. Archives of Sciences. 2012;65(11):369-399.
5. Secor ER, Singh A, Guernsey LA, McNamara JT, Zhan L and Maulik N. Bromelain treatment reduces CD25 expression on activated CD4+ T cells *in vitro*. International Immunopharmacology. 2009;9(3):340-346.
6. Bhattacharya R, Bhattacharyya D. Preservation of natural stability of fruit bromelain from *Ananas comosus* (pineapple). Journal of Food Biochemistry. 2009;33(1):1-19.
7. Umesh HH, Sumana B, Raghavarao K. Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes. Bioresource Technology. 2008;99(11):4896-4902.

8. Ketnawa S, Rawdkuen S, Chaiwut P. Two phase partitioning and collagen hydrolysis of bromelain from pineapple peel Nang Lae cultivar. Biochemical Engineering Journal. 2010;52(2-3):205-211.
9. Koech W, Gicuru KI, Kibet LK, Ngenoh E. Evaluation of allocative efficiency of small-scale pineapple (*Ananas comosus*) production in Bureti district, Kenya. Agriculture Journal. 2014;9(1):92-196.
10. Perez A, Napoles L, Lorenzo JC, Hernandez M. Protease excretion during pineapple micropropagation in temporary immersion bioreactors. *In Vitro* Cellular and Developmental Biology. 2003;39(3): 311–315.
11. Kunert KJ, Wyk SGV, Cullis CA, Vorster BJ, Foyer CH. Potential use of phytocystatins in crop improvement, with a particular focus on legumes. Journal of Experimental Botany. 2015;66(12):3559–3570.
12. Murachi T. Bromelain enzymes. Methods in Enzymology. New York Academic Press. 1976;XLV:475–485.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72(1-2):248-254.
14. Ketnawa S, Chaiwut P, Rawdkuen S. Pineapple Wastes: A potential source for bromelain extraction. Food and Bioproducts Processing. 2012;90(3):385-391.
15. Illanes. Enzyme production. In: Enzyme biocatalysis. Brazil Springer Science and Business Media. 2008;B:57-106.
16. Lightfoot EN. Protein purification from molecular mechanisms to large scale processes. ACS Publications. 1990;427: 35–51.
17. Ngampanya B, Phongtongpasuk S. Effects of sucrose concentration on crude bromelain production of *in vitro* culture of pineapple (*Ananas comosus* var. 'Pattavia'). Kasetsart Journal (National Science). 2006;40:129–134.
18. Bartolome P, Ruperez P, Fuster C. Pineapple fruit-morphological characteristics, chemical composition and sensory analysis of Red Spanish and smooth cayenne cultivars. Food Chemistry. 1995;53(1):75–79.
19. Rabbani MS, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Monitoring expression profiles of rice genes under cold, drought, and high salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiology. 2003;133(4):1755–1767.
20. Ketnawa S, Chaiwut P, Rawdkuen S. Pineapple wastes: A potential source for bromelain extraction. Food and Bioproducts Processing. 2012;90(3):385-391.
21. Coelho DF, Silveira E, Pessoa Junior A, Tambourgi EB. Bromelain purification through unconventional aqueous two-phase system (PEG/ammonium sulphate). Bioprocess Biosystem Engineering. 2013; 36:185-192.
22. Larocca M, Rossano R. Santamaria M. and Riccio P. Analysis of pineapple [*Ananas comosus (L.) Merr.*] Fruit proteinases by 2-D zymography and direct identification of the major zymographic spots by mass spectrometry. Food Chemistry. 2010;123:1334-1342.
23. Tománková O, Kopečný J. Prediction of feed protein degradation in the rumen with bromelain. Animal Feed Science and Technolology. 1995;53:71-80.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/20112