Optimal Conditions for Bromelain Extraction from Pineapple Fruit (*Ananas comosus*)

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ABSTRACT

Bromelain is a cysteine protease which is found in the tissues of Bromeliacea plant family of which pineapple *Ananas cosmossus* is best known. The investigated parameters for optimal Bromelain extraction are optimum buffers, pH, Molarity, time, and amounts of husk free pineapple fruit to volume (ml) of buffer ratio. Sodium phosphate was best buffer for bromelain extraction from pineapple fruit because its showed high activity, with casein as a substrate. Subsequent experiments, using sodium phosphate as an optimal buffer for extraction and casein as a substrate, reviled that the optimal bromelain extraction conditions were achieved at pH 7.0, 0.1 M of sodium phosphate, 2.5 min of extraction time, 1:0.5 (gm of pineapple fruit/ v of sodium phosphate buffer) extraction percentage, and 30 min of incubation time. Additionally, Bromelain extracted from pineapple fruit showed a maximum enzyme activity at pH 7and at 30 min of incubation with casein as substrate.

Key words: Bromelain, pineapple, enzyme activity, Bromelain extraction, fruit Bromelain.

INTRODUCTION

The pineapple plant is rich with a mixture of proteolytic enzymes that have the ability to breakdown the proteins into amino acids by hydrolyzing their peptide bonds (1). All the different parts of pineapple plant roots (2), leaves (3), stem (4), crown (5), peel, and fruit have various amount of proteolytic enzymes, with a high level of protease enzyme in stem and fruit and a low level of these enzymes in core, leaves, and peel (6). The group of protease enzymes, along with other non-proteolytic enzymes like peroxidases, phosphatases, and cellulases, in an aqueous crude extracted from pineapple plant is known as bromelain (7,8). The major proteases enzymes within pineapple plant is the cysteine protease (9,10). Since the fruit and stem of pineapple plant contain the highest level of protease enzymes, and depending on the source of extraction, bromelain has been classified into stem bromelain and fruit bromelain which are the two major cysteine proteases within pineapple plant additional to two other minor cysteine protease Ananain and Comosain (11, 12). The proteolytic activity of stem and fruit bromelain is based on sulfhydryl group due to their reduction action, and accordingly they are a sulfhydrylic enzymes (13). Although the cysteine proteases within pineapple plant are similar in their amino acid sequence, the chemical and immunological assay showed difference among these enzymes in substrate specificity, specific activity, and molecular masses (14) despite the unknown function of bromelain within pineapple plant, it has been widely used in so many applications like food, industry, and therapy (15). In medicine, bromelain have been used in the treatment of some diseases like cardiovascular and arthritis. It has been

found that bromelain can act as inhibition factor that reduces the aggregation of blood platelet and prevents their adhesion to epithelial cells (16). Attributed to its painkiller and anti-inflammatory characteristic, bromelain have been used to treat arthritis (17).

A high percentage, about 60%, of commercial enzymes is protease enzymes due to their important role in industry (18). The reason that proteases draw a good attention in industry is due to their features, they are very specific, eco- friendly, and effective. All these properties that proteases have qualifies them to play a major role in industry, starting from detergent industry and ending with therapeutic industry (19). This study was determine to the optimum condition for bromelain extraction from pineapple fruit.

Chemicals and Methods

Materials

The pineapple fruit were obtained from local Iraqi fruitier, while all the chemicals and reagents that have been used in this study were supplied by Hi Media and sigma.

Determining the optimal buffer and pH for Bromelain extraction:

A weight of 25 gm of fresh pineapple fruit free of husk was blended in a blender with 50 ml of 0.1 M of four different buffers include potassium phosphate (pH 7.0), sodium acetate at pH values ranking from 3 to 6, sodium phosphate (pH 7.0), and Tris-Hcl (pH 8 and 9) for 1 min. Also, the bromelain was extracted from 25 gm of pineapple fruit in 50 ml tap and distilled water for 1 min. the blending process were carried out at a high speed and at room temperature. The homogenate was filtered by using filter paper (whatman filter paper No.). Then the filtrate (crude enzyme) was used to determine the protein concentration and bromelain activity against casein.

Determining the optimal buffer molarity for Bromelain extraction:

An optimum buffer concentration for Bromelain extraction was determined by blending 25 gm of pineapple fruit free of husk in a blender with 0.05, 0.1, 0.2, 1, and 2 M of sodium phosphate at pH 7 and at room temperature. The homogenate was filtered throughout filter paper. The protein concentration and Bromelain activity were estimated.

Determining the optimal time for Bromelain extraction:

An optimal time for Bromelain extraction was investigated by blending 25 gm of pineapple fruit free of husk with 0.1 M of sodium phosphate at pH 7 and at various period of time (30 sec, 45 sec, 1 min, 1.5 min, 2 min, and 2.5 min). The homogenate was filtered and the filtrate was used to determine the protein concentration and Bromelain activity.

Determining the optimal extraction percentage:

To estimate the optimal extraction percentage of Bromelain enzyme from pineapple fruit, various amounts of pineapple fruit to volumes (ml) of 0.1 M of sodium phosphate were applied. The investigated percentage were 1:0.5, 1:1, 1:1.5, 1:2, and 1:3 (w:v). This experiment were done at pH 7 and at 2 min extraction time. The protein concentration and Bromelain activity were estimated.

Determining the optimal pH for Bromelain activity:

The enzyme activity was estimated under different pH values ranging from 4-9, with maintaining other parameter at their optimal level. The protein concentration and Bromelain activity were estimated.

Determining the optimal incubation time for Bromelain activity:

The extracted Bromelain was incubated with casein for various period of time. The investigated periods of time were ranking from 10-50 min. with an interval of about 5 min. The protein concentration and Bromelain activity were estimated.

Assay of proteolytic activity of Bromelain:

The proteolytic activity of Bromelain against casein was investigated by Lower's method (20). A mixture of 200 µl of extracted Bromelain and 1.8 ml of 1% (w/v) casein were incubated in water bath at 37 C°. After 30 min of incubation the reaction was stopped by the addition of 3 ml of 15 % trichloroacetic acid (TCA). Centrifuge at 6000 rpm for 15 min was applied to the mixture. A blank was prepared by mixing 3 ml of TCA with 1.8 ml of 1% casein, then 200 µl extracted enzyme was added. The blank was subjected to the same steps as the investigated samples by using distilled water instead of plant extract. 3 ml of supernatant were applied to spectrophotometer cuvette. The absorbance were measured at 280 nm to estimate the proteolytic activity of Bromelain.

Assay for Protein concentration:

Bradford method was used to estimate and determine the protein concentration within samples (21).

Results:

Buffers and pH for Bromelain extraction:

One of the most substantial factors that might effect on enzymes extraction is buffer. The different extraction buffers used in this study are shown in figure (1). The highest specific activity for Bromelain (131.25 U/mg) was obtained when sodium phosphate buffer was used at pH 7.0 while the lowest specific activity for Bromelain (41 U/mg) was obtained when sodium acetate pH 3.0 was used for enzyme extraction. According to the obtained data in figure (1), sodium phosphate (pH 7.0) was estimated to be the optimal buffer for Bromelain extraction and it has been used for further investigation.

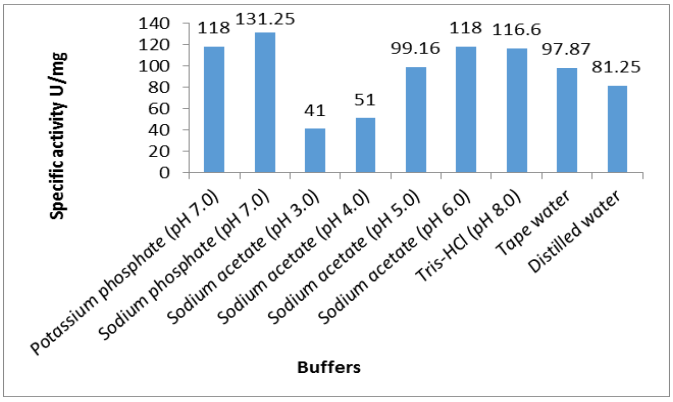


Figure (1): Effect of buffer and pH on Bromelain extraction from pineapple fruit.

The given result is in accordance with the result obtained from (22) which revealed that sodium phosphate was an optimum buffer for bromelain extraction from pineapple peel. Also this result is

similar to the results obtained from (23, 24, 25), which showed that the best pH for bromelain activity was at pH 7.

Optimal buffer molarity for Bromelain extraction:

The specific activity of extracted Bromelain was enhanced (132 U/mg) when the molarity of sodium phosphate at pH 7 was 0.1 M while it decreased (68.2 U/mg) when the molarity was 2 M (see figure 2). As shown in figure 2, 0.1 M of sodium phosphate at pH 7.0 is the optimal molarity for Bromelain extraction.

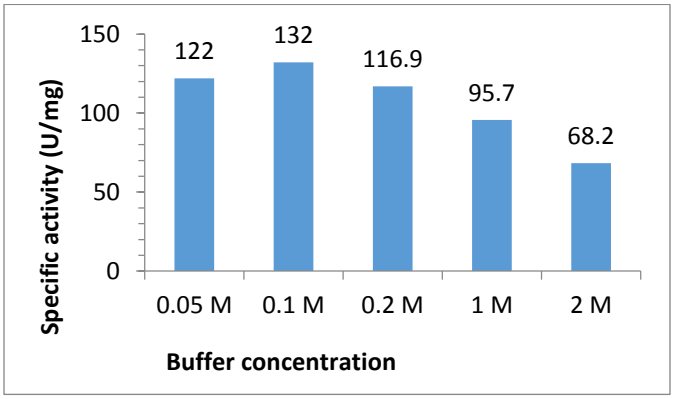


Figure (2): Effect of buffer concentration on Bromelain extraction from pineapple fruit.

This result is similar to that obtained from a study conducted by Ketnawa *et al.* in 2012, (6) they have found that optimal Bromelain extraction from pineapple stem obtained at 0.1 M of phosphate Buffer.

Extraction time:

Different times for Bromelain extraction were determined (figure 3). The best time was after 2 minutes with specific activity 147 U/mg, while in 30 sec. the specific activity of enzyme was decreased and reduced to 116 U/mg, and the reason is the low release of enzyme from pineapple fruit. While 0.45 sec., 1, 1.5, 2.5 and 3 minutes showed low specific activity 123, 130, 137, 141 and 134 U/mg respectively, increasing temperature for blender, causes effect on total activity for Bromelain enzyme. The decrease in activity at elevated temperature degrees may change the structure of the enzyme that blocks the active sites, with denaturation of enzyme.

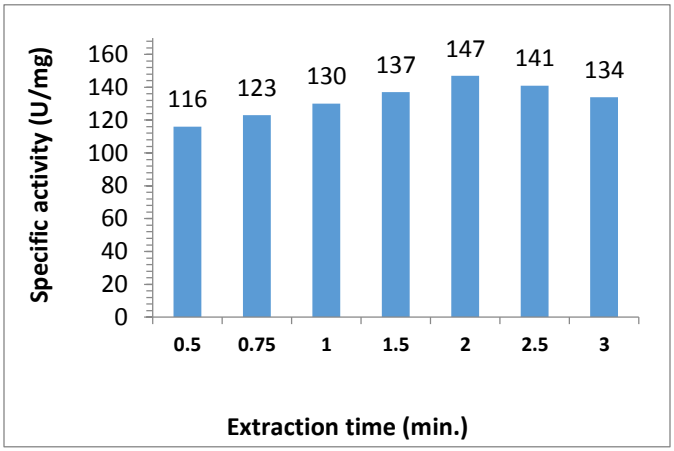
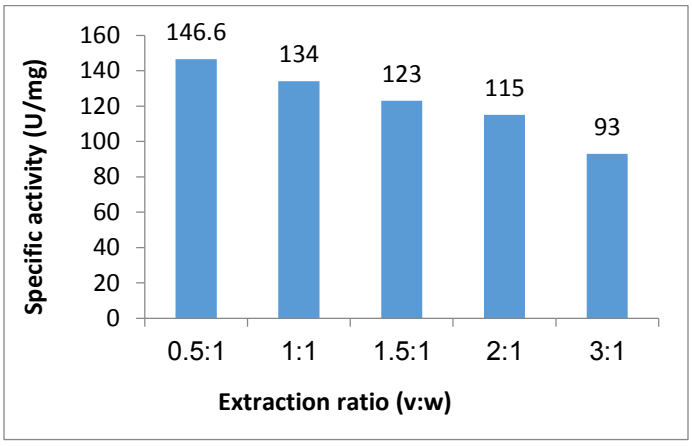


Figure 3: Effect of extraction time on Bromelain extraction from pineapple fruit.

Abdulrahman *et. al*. (26) found that 1.30 min. was best extraction time for Bromelain enzyme from *Ananas comosus*. While Glider and Hargrove (27) found that best time for Bromelain extraction from pineapple was 2 min.

Extraction ratio:

Five extraction ratios were examined to determine the best proportion for Bromelain extraction. Figure (4) shows that 1:0.5 (v:w) was the best ratio, with specific activity 146.6 U/mg, while 1:1 (v:w) ratio was 134 U/mg. Other ratios were 1:1.5, 1:2 and 1:3, (v:w) with specific activity 123, 115 and 93 U/mg respectively, because the protein concentration increased.



Figure(4): Effect of extraction ratio on Bromelain extraction from pineapple fruit.

Abdulrahman *et. al*. (26) found that best extraction ratio for Bromelain enzyme from *Ananas comosus* was 1:2 (v:w). While Ketnawa *et. al.* (28) found that 1:1 (v:w) was better extraction ratio for Bromelain extraction from pineapple peel.

Effect of substrate pH on Bromelain activity:

Maximum Bromelain specific activity 147.3 U/mg was obtained at pH 7.0 (see figure 5). While other pH's of casein which was as substrate for Bromelain enzyme decreased above and below pH 7.0.

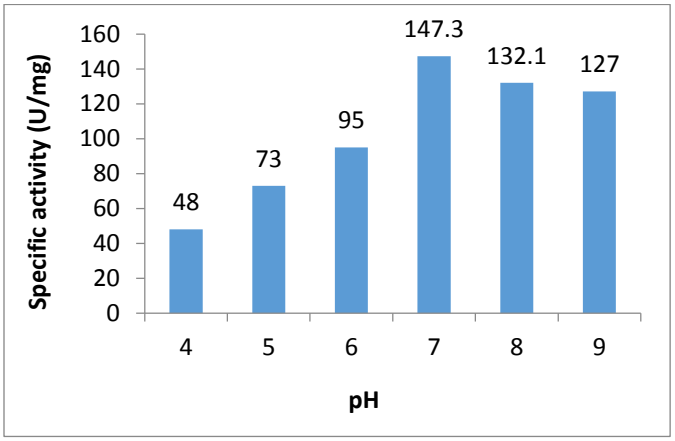


Figure (5): effect of substrate pH on Bromelain activity.

Ferreira *et.al*. (29), locate that pH 7.0 was best pH for Bromelain activity, also Abdulrahman *et. al*. (26) found the same result. The pH of enzyme environment affects the activity of the enzyme in several ways. First each enzyme has its own optimum pH, at which the maximum enzyme activity, but the enzyme is stable within certain limits under and above the optimum. Secondly, enzyme stability is influenced by environmental pH, at extremes acidity or alkalinity the enzyme may be denatured. Thirdly, the reaction mixture pH may effects on association of the substrate with the enzyme (30).

Effect of incubation period on Bromelain activity:

The results showed that the best specific activity (146.9 U/mg) for Bromelain enzyme was 30 min. after incubation with casein as substrate (see figure 6). While other specific activity, more and less than 30 min incubation time, were decreased.

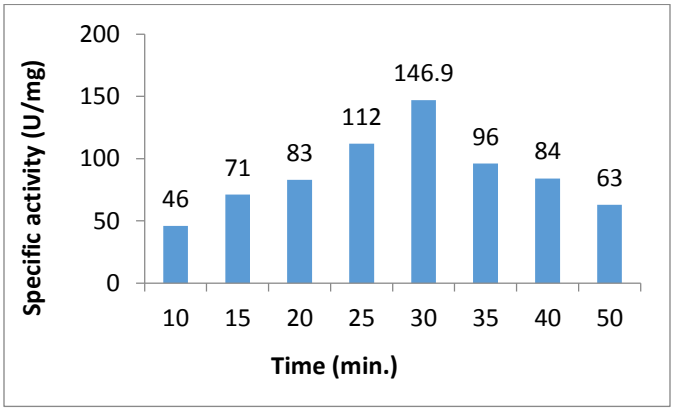


Figure (6): Effect of incubation period on Bromelain activity.

Abdulrahman *et. al*. (26) found that the best incubation period for Bromelain activity after incubated with casein was 50 min. Priya *et.al* (31) found that 30 min. incubation time was best time for Bromelain activity after incubated with gelatin as substrate.

CONCLUSION

In this study, Bromelain have been extracted from husk free pineapple fruit obtained from local Iraqi store. Extraction process subjected to different parameters and conditions in purpose to seek an optimal conditions for extraction and proteolytic activity. Optimal Bromelain extraction was obtained with sodium phosphate at pH 7 and 0.1 M at 1:0.5 extraction ratio for 2 min mixing time. Optimal Bromelain specific activity was at pH 7 and for 30 min incubation time.

REFERENCES:

[1]. Anshu, G. and Khare. S.K. (2007). Enhanced production and characterization of a solvent stable Protease from solvent tolerant Pseudomonas aeruginosa. *Enzyme and Microbial Technology*. 42; 11-16.

[2]. Kelly, G.S. (1996). Bromelain: A literature review and discussion of its therapeutic applications. *Alternative Medicine Review*, 1, 243-257.

[3]. Hale, L.P. ; Greer, P.K. ; Trinh, C.T. and James. C.L. (2005). Proteinase activity and stability of natural bromelain preparations. *International Immunopharmacology* 5: 783-793.

[4]. Hebbar, H.U. ; Sumana, B. and Raghavarao, K. (2008). Use of revere micellar system for the extraction and purification of bromelain from pineapple waste. *Bioresourse technology* 99:4896-4902.

[5]. Leite, N.S. ; De lima, A.A.B. ; Santana, J.C. ; Lopes, F.L.G. ; Ledo, A.D. ; Tambourgi, E.B. ; and De souza, R.R. (2012). Determination of optimal condition to obtain the bromelain from pineapple plant produced by Micropropagation. *Brazilian archives of biology and technology* 55:647-652.

[6].Ketnawa, S. ; Chaiwut, P. and Rawdkuen, S. (2012). Pineapple wastes: A potential source for bromelain extraction. *Food Bioprod. Process*., 90, 385-391.

[7]. Heinicke, R. M. (1953). Complementary Enzyme Actions in the Clotting of Milk. *Science* 118, 753-754.

[8]. Chobotova, K. ; Vernallis, A.B. and Abdul Majid, F.A. (2009). Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives. *Cancer Letters* 20: 1-9.

[9]. Maurer, H.R. (2001). Bromelain: Biochemistry, pharmacology and medical use. *Cell Mol. Life Sci*. 58, 1234–1245.

[10]. Lopez –Carcia, P. ; Hernandez, M. and Segundo, B.S. (2012). Bromelain, a cysteine protease from pineapple (*Ananas comosus)* stem, is an inhibitor of fungal plant pathogen. *Lett Appl Microbiol*.55 (1):62-7.

[11]. Rowan, A.D. ; Buttle, D.J. and Barrett, A.J. (1990).The cysteine proteases of the pineapple plant. *Biochem. J.*, 266, 869–875.

[12]. Saffle, R.L. and Galbreath, J.W. (1964). Quantitative determination of salt soluble protein in various types of meats *food technol*., 18:1943-1944.

[13]. Bartholomew, D.P ; Paull, R.E. and Rohrbach. K.G. (2003). The pineapple: botany, production and uses. Bartholomew, D.P., Paull, R.E., Rohrbach, K.G.(eds). CABI puplishing, Wallingford, UK. Pp1-301.

[14]. Gautam, S.S. ; Mishra, S.K. ; Dash, V. ; Goyal, A.K. and Rath, G. (2010). Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant. *Thai J. Pharm. Sci*. 34, 67–76.

[15]. Nadzirah, K.Z. ; Zainal, S. ; Noriham, A. and Normah, I. (2013). Efficacy of selected purification techniques for bromelain. *International food research Journal* 20 (1): 43-46.
[16]. Metzig, C. ; Grabowska, E. ; Eckert, K. and Rehse, K. M. (1999). Bromelain proteases reduces human platelet aggregation in vitro, adhesion to bovine endothelial cells and thrombus formation in rat vessels in vivo. *In vivo.* 13 (1): 7-12.
[17]. Brien, S. ; Lewith, G. ; Walker, A.; Hicks, S.M. and Middleton, D. (2004). Bromelain as a treatment for osteoarthritis: A review of clinical studies. *Evid Based Complement Alternat Med.* 1(3):251-257.
[18]. Feijoo-Siota, L. and Villa, T. G. (2010). Native and Biotechnologically engineered Plant Proteases with Industrial applications. *J Food Bioprocess Technol*. 4, 1066-1088.
[19]. Poldermans, B., (1990). Proteolytic enzymes. In: proteolytic enzyme in industry: production and applications, (ed.) Gerhartz, W., VCH publisher, *Weinheim, Germany*. pp: 108-123.
[20]. McDonald C.E. and Chen L.L.(1965). Lowry modification of the Folin reagent for determination of proteinase activity, *Ann Biochem*. (10): 175-186.
[21]. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72, 248-254.
[22]. Ketnawa, S. ; Chaiwut, P. and Rawdkuen, S. (2011). Extraction of Bromelain from pineapple peel, *food science and technology international.* (4):395-402.
[23]. Minami, Y. ; Doi, E. and Hata, T. (1971). Fractionation, purification and some properties of proteolytic enzymes from stem bromelain. *Agricultural and Biological Chemistry*, 35(9), 1419–1430.
[24]. Valles, D. ; Furtado, S. and Cantera, A. (2007). Characterization of news proteolytic enzymes from ripe fruits of Bromelia antiacantha Bertol. (Bromeliaceae). *Enzyme Microb. Technol*., 40,409-413.
[25]. Larocca, M. ; Rossano, R. ; Santamaria, M. and Riccio, P. (2010). Analysis of pineapple [*Ananas comosus* (L.)Merr.] fruit proteinases by 2-D zymography and direct identification of the major zymographicspots by mass spectrometry.. *Food Chem*. 123, 1334–1342.
[26]. Abdulrahman, A. ; Milala1, A.M.A. and Gulani, I.A., (2015). Antimicrobial effects of crude bromelain extracted from pineapple fruit (*Ananas comosus* (Linn.) Merr.). *Advances in Biochemistry*. 3(1): 1-4.
[27]. Glider, W.V. and Hargrove, M.S., (2002). Using Bromelain in Pineapple Juice to Investigate Enzyme Function. *J. Biochemistry*. 275-295.
[28]. Ketnawa,S. ; Sai-Ut, S. ; Theppakorn, T. ; Chaiwut, P.and Rawdkuen, S., (2009). Partitioning of bromelain from pineapple peel (Nang Lae cultv.) by aqueous two phase system. *As. J. Food Ag-Ind*. 2(04), 457-468.
[29]. Ferreira, J.F. ; Santana, J.C.C. and Tambourgi, E.B., (2011). The Effect of pH on Bromelain Partition from *Ananas comosus* by PEG4000/Phosphate ATPS. *Braz. Arch. Biol. Technol.* 54(1): 125-132.
[30]. AL-Sa'ady, A.J.R., (2014). Optimum conditions for invertase production from *Saccharomyces cerevisiae* using solid state fermentation. *Current Research in Microbiology and Biotechnology*. 2(3): 373-377.
[31]. Priya, S.S.P. ; Jayakumar, K. ; Mathai, V. ; Chintu, S. and Babu, K.S., (2012). Immobilization and Kinetic Studies of Bromelain: A Plant Cysteine Protease From Pineapple (*Ananas Comosus*) Plant Parts. *Int J Med Health Sci*. 1(3): 10-1