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Characterization of Bromelain from Parts of Three Different Pineapple Varieties in Nigeria

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Abstract: Bromelain was extracted from crown, flesh, core, and peel of some variant of Nigerian pineapple fruits in order to evaluate its amount and characteristics. Each part of pineapple after separation was weighed, blended, filtered and then filtrate was precipitated with ethanol and centrifuged. All extracts from each stage were collected and assayed for bromelain activity. The concentration of protein was also measured by using BSA as standard while proteolytic activity was determined using Azocasein 1% (w/v) as substrate at standard conditions. The optimum temperature and pH were also evaluated. The result showed that bromelain could be obtained from all parts of pineapple. *Ananas fitzmulleri* (Agric) had the highest weight (1486.42 g) and flesh content (53.5%) compared to other varieties while bromelain obtained from all parts and in the different varieties had optimum activity at 40°C and pH of 7. Hence ethanol precipitation method is viable for bromelain recovery.

Keywords: Bromelain, Azocasein, Pineapple Fruits

1. Introduction

Bromelain is a collective name for all proteases, sulfhydryl proteolytic enzymes belonging to the *Bromeliaceae* family [1]; it is one of the protease enzymes found in the pineapple plant (*Ananas comosus*) [2]. Bromelain is present in all parts of the pineapple plant, but its characteristics depend on the source [3]. The major component, stem bromelain (EC 3.4.22.2), has since been sequenced and shown to be a member of the *papain* superfamily. The enzyme present in the juice of the pineapple stem is of basic character called stem-bromelain and is the major protease present in extracts of pineapple stem while fruit bromelain (EC 3.4.22.3), the enzyme present in the fruit is an acidic enzyme called fruit-bromelain is the major enzyme fraction present in the juice of the pineapple fruit [4]. Muntari *et al.* [5], reported that bromelain concentration was very high in pineapple stems and hence leading to its extraction and utilization as phytomedicinal compound. Unlike the pineapple fruit which is normally used as food, the stems were waste by-product and thus, very cheap source of bromelain [6]. Also, besides the stem and fruit of pineapple as the source of bromelain it was also discovered that some other part of the pineapple, the core peel and crown has been another source as bromelain was extracted from the peel, core, stem and crown of wastes from two pineapple cultivars [7].

Bromelain has a wide range of therapeutic benefit as property of facilitating digestion of proteins, meat softening, ability to facilitate blood clotting [8], other economic importance are related to the food industry and textiles and production of drugs resulting in an increase of its value [9]. It is proved that Bromelain is well absorbed in body after oral administration and has little or no important side effects even after prolonged use [10]. This study aims at extracting crude bromelain from four different pineapple parts (crown, fruit, flesh and peel of three different local species *Ananas fitzmuelleri, Ananas comosus, and Ananas* erectifolius, partially purify the bromelain from the four different parts of each species and characterise the bromelain enzyme.

2. Methodology

Pineapple fruits of the three different species, locally called the Agric (*Ananas fitzmueller)*, Local (*Ananas comosus)* and Erec (*Ananas erectifolius*) were bought from a local market at

Ilara town on Akure - Ilesha express way, Ondo state. The pineapple fruits were identified by Mr Hassan at the Department of Crop Science Production of the Federal University of Technology Akure. The pineapple fruits were washed thoroughly with water and then weighed before been peeled and sliced to separate different parts; crown, fruit, flesh and peel and weighed to ascertain percentage part weights.

2.1. Bromelain Extraction

Each part was blended with sodium phosphate buffer 0.01 M pH 7.0: flesh and core at ratio of 1:1 (w/v) for the peel, at ratio 2:1 (w/v) while for the crown at ratio of 3:1 (w/v) and filtered using cheese cloth, to obtain the juice. The filtered juice was centrifuged at 6000g for 20 minutes at 4˚C. The supernatant obtained (crude bromelain) was collected and refrigerated at -4°C.

2.2. Determination of Enzyme Activity

The enzyme activity assayed by the azocasein method as described by Oliveira *et al*. [11], where azocasein 1.0% (w/v) (Sigma) was solubilized in 4% ethanol (v/v) and 0.1 M phosphate buffer, pH 7.0, and used as substrate. The assay mixture, containing 125 µL of substrate and 125 µL of extract enzymatic was incubated for 10 min at 37°C and the reaction stopped by addition of 750 µL of 5% trichloroacetic acid (w/v). The samples were centrifuged at 6000g for 10 minutes and at a temperature 4°C. One unit of activity was defined as the amount of enzyme required to produce the increase in optical density by one unit within 1 hour.

2.3. Protein Concentration Assay

Protein content in each bromelain extract was measured by Bradford method [12] using bovine serum albumin (BSA) as standard.

2.4. Precipitation by Ethanol

Bromelain precipitation was performed according to methodology described Englard and Seifter [13]. Ethanol 98% (w/w) cooled to 0°C was added dropwise until concentrations of 30 and 70% (w/w) was reached. The solution was then centrifuged at 10,000 for 20 min at 4°C and the resulting pellet was suspended in 20 mM buffer phosphate pH 7.0.

2.5. Enzyme Partial Characterization

Optimum pH and temperature was evaluated before and after ethanol precipitation. Effect of pH assays were performed as described during bromelain assay with exchanged buffers so the desired pH could be reached. Likewise, the effect of temperature was accessed by changing the incubation temperature.

The enzyme preparation used for the assay for the effect of pH on bromelain activity was stabilized in different buffer of different pH range, from; Glycine/HCL buffer with pH 4, sodium acetate/acetic acid buffer with pH 4, 5, and 6, Sodium phosphate buffer with pH 7 and pH 8. These different buffers were used to prepare the substrate (casein) for the enzyme, while effect of temperature on bromelain activity was evaluated by azo-casein as substrate. Bromelain activity was assayed at temperature between 30 and 70°C.

3. Results and Discussion

From table 1, the weight in gram and their percentage value of the different parts was *Ananas fitzmulleri* variety had the highest weight in grams followed by *Ananas comosus* and *Ananas erectifolius* respectively, with their flesh accounting for the largest percentage for all varieties.

Table 1. *Weight of pineapple parts from the three different species.*

	***Ananas fitzmulleri* (Agric)**		***Ananas comosus* (Local)**		***Ananas erectifolius* (Erec)**	
Parts	**Weight (g)**	**(%)**	**Weight (g)**	**(%)**	**Weight (g)**	**(%)**
CROWN	227.95	15.30	239.42	23.30	193.24	23.50
CORE	256.08	17.20	172.15	16.80	138.57	16.80
FLESH	794.15	53.50	474.80	46.30	395.6	48.00
PEEL	208.24	14.00	139.56	13.60	96.82	11.70
TOTAL	1486.42	100.0	1025.93	100.0	824.23	100.0

Similar report to that obtained in this study was seen by Sobir and Duri [14], who noted that for all the weight in gram and percentage of the different part of the pineapple varieties, the flesh of all the pineapple varieties constitutes the largest weight of the pineapple in gram and percentage.

Table 2. *Purification table for Agric pineapple (Ananas fitzmuelleri).*

PARTS	**PURIFICATION STEP**	**Vol. (ml)**	**Enz. Activity (U/ml)**	**Protein Conc. (µg/ml)**	**Specific activity (U/µg)**	**yield**	**Fold**
AGRIC CROWN	FIRST CENTRIFUGATN	200	0.0155	1.44	0.01	100	1
	70% ETHANOL PRECIPITATION	5	0.011	1.07	0.01	100	1
AGRIC CORE	FIRST CENTRIFUGATN	200	0.011	1.63	0.005	100	1
	70% ETHANOL PRECIPITATION	5	0.06	0.67	1	200	0.5
AGRIC FLESH	FIRST CENTRIFUGATN	200	0.15	1.33	0.113	100	1
	70% ETHANOL PRECIPITATION	5	0.007	0.11	0.064	56.64	1.76
AGRIC PEEL	FIRST CENTRIFUGATN	200	0.014	1.48	0.0095	100	1
	70% ETHANOL PRECIPITATION	5	0.01	0.74	0.0135	142.1	0.7

Table 3. *Purification table for local pineapple (Ananas comosus).*

PARTS	PURIFICATION STEP	Vol. (ml)	Enzyme activity. (U/ml)	Protein conc. (µg/ml)	Specific activity (U/µg)	Yield	Fold
LOCAL CROWN	FIRST CENTRIFUGATN	200	0.0068	1.29	0.0053	100	1
	70% ETHANOL PRECIPITATION	5	0.014	0.85	0.0165	311.32	0.32
LOCAL CORE	FIRST CENTRIFUGATN	200	0.011	0.96	0.0115	100	1
	70% ETHANOL PRECIPITATION	5	0.013	0.62	0.02	173.91	0.58
LOCAL FLESH	FIRST CENTRIFUGATN	200	0.0068	1.11	0.006	100	1
	70% ETHANOL PRECIPITATION	5	0.006	0.41	0.015	250	0.4
LOCAL PEEL	FIRST CENTRIFUGATN	200	0.016	1.07	0.015	100	1
	70% ETHANOL PRECIPITATION	5	0.01	0.67	0.015	100	1

Table 4. *Purification table for Erec (Ananas erectifolius).*

PARTS	PURIFICATION STEP	Vol. (ml)	Enzyme activity. (U/ml)	Protein conc. (µg/ml)	Specific activity (U/µg)	Yield	Fold
EREC CROWN	FIRST CENTRIFUGATN	190	0.0038	1	0.0038	100	1
	70% ETHANOL PRECIPITATION	5	0.0087	0.85	0.01	263.2	0.38
EREC CORE	FIRST CENTRIFUGATN	190	0.007	1.18	0.0011	100	1
	70% ETHANOL PRECIPITATION	5	0.0085	1.37	0.0063	572.73	0.18
EREC FLESH	FIRST PRECIPITATION	190	0.0014	1.18	0.012	100	1
	70% ETHANOL PRECIPITATION	5	0.009	1.15	0.008	66.67	1.45
EREC PEEL	FIRST PRECIPITATN	190	0.0094	0.96	0.0098	100	1
	70% ETHANOL PRECIPITATION	5	0.006	0.59	0.01	102.04	0.98

Tables 2, 3 and 4 shows the level of purification fold obtained with results of the enzyme activity, the protein concentration and the specific activity of the bromelain extracted after first centrifugation and 30 – 70% ethanol precipitation, for the three variety of pineapple considered in this study. The purification factor for the first centrifugation and the 70% ethanol precipitation showed a decrease in the total enzyme activity and the total protein concentration after 70% ethanol precipitation of bromelain for all part of the *Ananas fitzmulleri,* except for its core, whose total enzyme activity for 70% ethanol precipitation (3.35 U/ml), was higher than its first centrifugation (2.20 U/ml). Also the specific activity, yield and fold of the *Ananas fitzmulleri* parts increases after 70% ethanol concentration. Rabelo *et al*. [15], reported similar increase for the pineapple variety he studied. except for its flesh where the specific activity and yield decreases while the fold increases.

Similarly findings was observed for *Ananas comosus*, with the total enzyme activity and total protein concentration decreasing after 70% ethanol precipitation, while specific activity and yield increases at this level of purification for all parts of *Ananas comosus*. However, bromelain recovery fold at the precipitation level decreases for all parts considered except for the peel where the bromelain fold at the first centrifugation level and 70% ethanol precipitation level were the same. The total enzyme activity and total protein concentration decreases at precipitation level for all parts of *Ananas erectifolius,* its specific activity and yield increases and fold decreases for all its parts at precipitation level except for the fleshy part whose specific activity (0.008) and yield (66.67) decreases and the fold (1.45) increases at the 70% ethanol precipitation level of purification.

Figure 1, 2 and 3 shows the effect of temperature on the activity of bromelain for all varieties of the pineapple. A gradual increase was observed within the range of 30 – 35°C while a peak was obtained at temperature of 40°C.

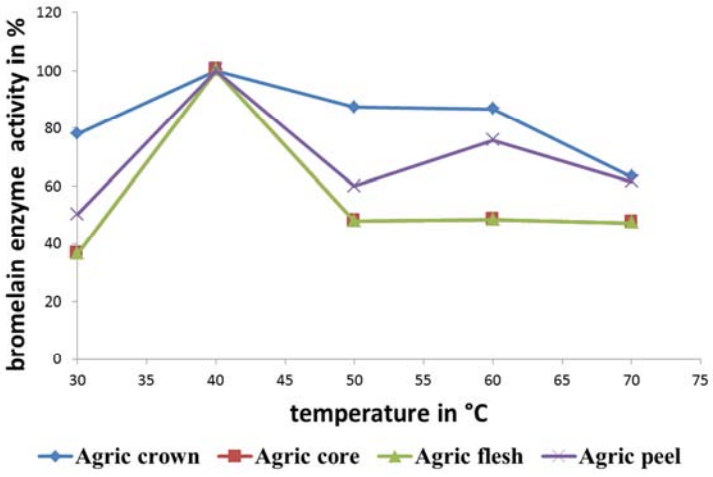

Figure 1. *Effect of temperature on bromelain activity obtained from Agric pineapple (Ananas fitzmuelleri).*

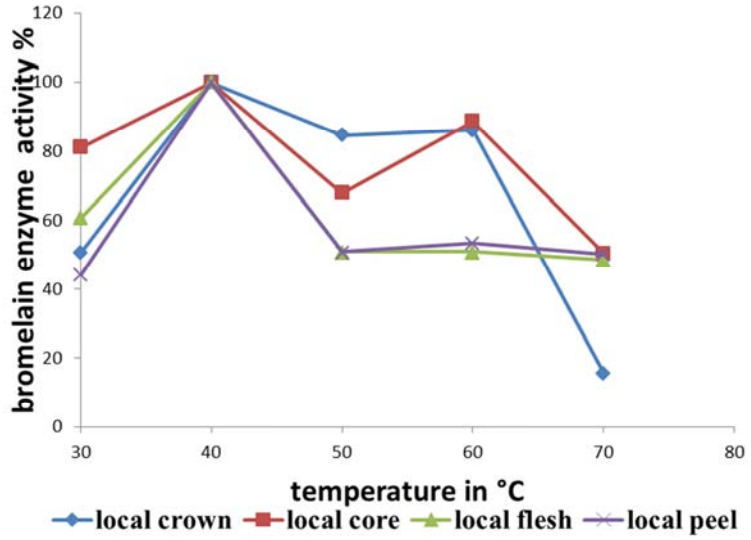

Figure 2. *Effect of temperature on bromelain activity obtained from Local pineapple (Ananas comosus).*

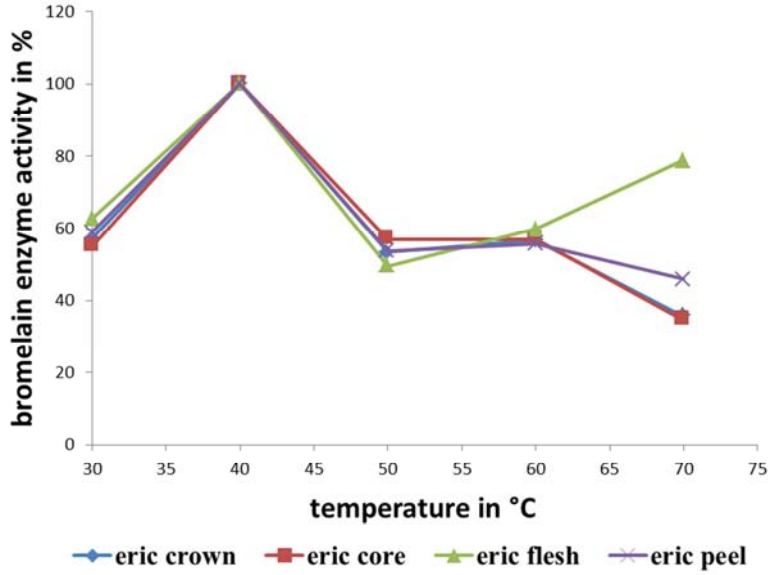

Figure 3. *Effect of temperature on bromelain activity obtained from Erec pineapple (Ananas erectifolius).*

The optimum temperature observed for bromelain activity in all parts of pineapple varieties considered (*Ananas fitzmulleri*, *Ananas comosus* and *Ananas erectifolius*), was 40°C. This result corresponds and was also supported by the work of Harrach *et al*., [16] who reported the optimum temperature of the bromelain activity at 40 – 65°C for stem bromelain, Amid et al. [17] used a purified recombinant bromelain and this exhibited the highest hydrolytic activity at 45ºC under routine assay conditions, however, the enzyme was devoid of detectable activity at 65ºC. Ferreira *et al.*, [18] noted that the ideal temperature range for the characterization of bromelain from pineapple stem bark is between 30°C and 40°C, which supports the similar behaviour observed in this study between bromelain from the different pineapple varieties. These results are also comparable to the characteristics of pineaple bromelain, which is active between 40ºC and 60ºC [19]. Furthermore bromelain activity was observed to be stable with approximately 50% enzyme activity at temperature range of 30 - 60ºC until it finally began to reduce in activity at 70ºC. This was observed for all parts of *Ananas comosus and Ananas fitzmulleri* except for the fleshy part of *Ananas erectifolius* where bromelain activity was seen to increase in activity at 70ºC. Similar result was reported for fruit bromelain which had its maximum activity at 70ºC [20], also, bromelain from other pineapple, such as Perola variety from Brazil, had their maximum activity also at 70ºC [21]. Temperature is a critical agent on the enzyme activity. When the temperature rises, the activity initially increases, however the process thereafter declines due to the denaturing action of heat [22].

The effect of pH on the activity of bromelain is shown in Figure 4, 5 and 6. The optimum activity was observed at pH 7.0. The enzyme was more active in the pH of 4, 6, and 7. There was a gradual decline in the activity of the enzyme after attaining the optimum pH of 7.0.

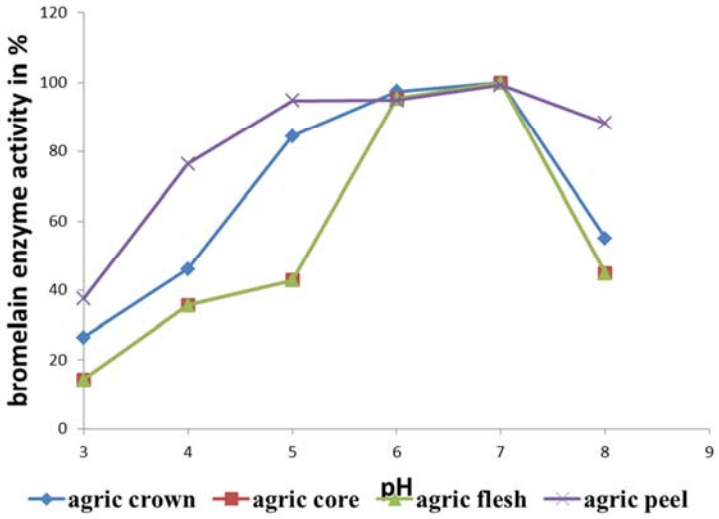

Figure 4. *Effect of bromelain on bromelain activity obtained from Agric pineapple (Ananas fitzmuelleri).*

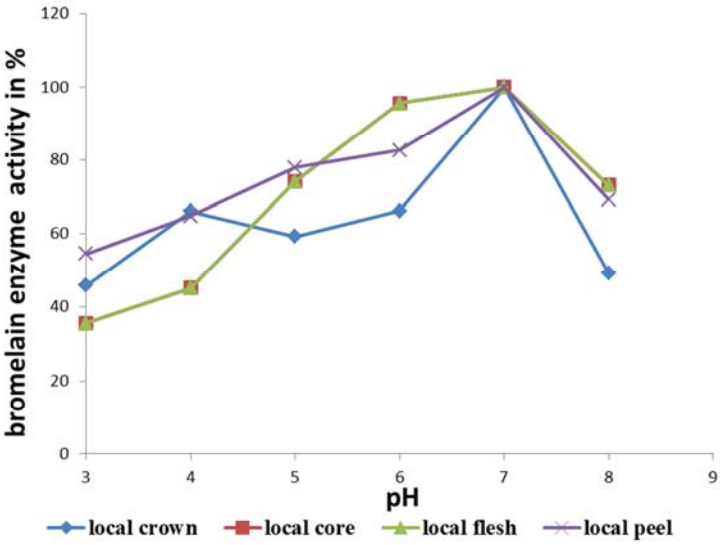

Figure 5. *Effect of bromelain on bromelain activity obtained from Local pineapple (Ananas comosus).*

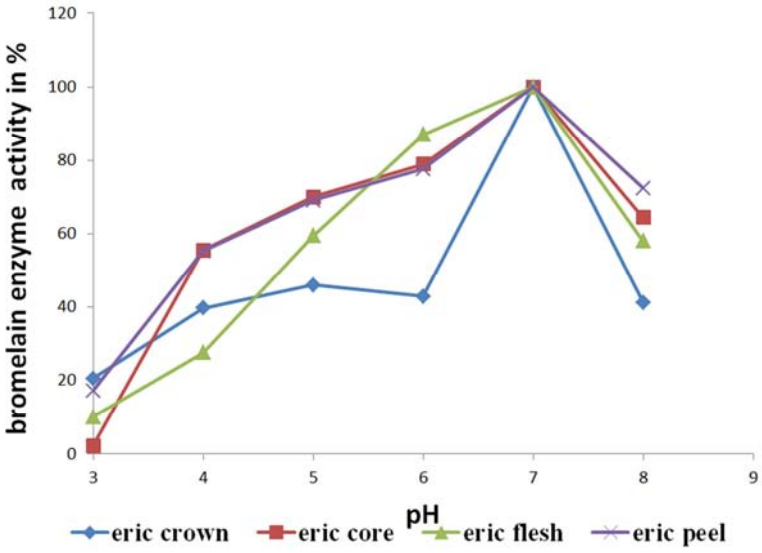

Figure 6. *Effect of bromelain on bromelain activity obtained from Erec pineapple (Ananas erectifolius).*

Optimal pH for bromelain activity was at pH 7.0 for all parts of the pineapple varieties considered in this study. This result corresponds to bromelain activity as reported by Martowibowo *et al*., [23]. Bromelian active at pH 3 through to pH 7 where it activity is at maximal until it finaly falls at pH 8 for all parts of the pineapple. Corzo *et al*., [24] and Manzoor *et al*., [25] support the result as it was reported that bromelain act well within pH range 3 – 8. Beyond this pH value, the activity declined progressively. The effect of pH can be explained considering the surface charge on the adsorbent material. At low pH, due to the high positive charge density, electrostatic repulsion will be high, resulting in lower uptake of positively charged bromelain. The isoelectric point of bromelain is 9.55 [26], hence the adsorption decreases at a higher pH. Some proteins contain acid labile groups and even relatively mild acid treatment may cause irreversible loss of function. The optimum pH of bromelain from pineapple fruits was reported to be 7.0. Enzymes are generally sensitive to pH changes in their environment and have optimum pH 7.0 at which they have their maximum activity, beyond which their activity decreases [27].

4. Conclusion

Crude bromelain extracted and partially purified by ethanol precipitation from pineapple parts of three different varieties (*Ananas fitzmuelleri, Ananas comosus, and Anana erectifolius*) showed considerable physiochemical properties, with activity at temperature range of 30 – 70ºC and pH range of 5 – 8. This precipitation method shows that bromelain recovery process is a viable process in which results in a good quality enzyme which can be considered for industrial purposes.

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