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The Potential of Pineapple Waste and Proteolytic Content to Enhance Added Value

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Abstract - This review explores sustainable bromelain extraction from pineapple processing waste (PPW), covering distribution, extraction methods, properties, and applications to valorize waste. Pineapple canning industries generate large quantities of solid from pineapple processing waste which can account for up to 50% of the total weight of processed pineapples. Other processing methods produce 25-35% waste on average. Bromelain is distributed across all parts of the pineapple, with significant activity found in the peel, core, and crown. Studies indicate that bromelain can be effectively extracted from these parts using various methods, including ethanol precipitation and membrane filtration techniques. The extraction process not only recovers the enzyme but also contributes to sustainable waste management by valorizing pineapple by-products. The protease activity of bromelain varies by part of the pineapple. Peel exhibits the highest protease activity, with values reported around 3.417 U/µg. Crown shows a protease activity approximately 46.78 units, with an optimum temperature of 35°C and pH of 7. Core has a slightly lower activity than the peel, around 36.93 units, with an optimum temperature of 55°C. Bromelain extracted from pineapple waste demonstrates significant proteolytic activity and can be effectively utilized in various applications, highlighting the importance of utilizing all parts of the pineapple for both economic and environmental benefits. Its proteolytic activity makes it suitable for use in meat tenderization, digestive aids, and as an anti-inflammatory agent, and it also presents a promising opportunity for enhancing poultry nutrition while addressing waste management challenges. Its incorporation into poultry diets can lead to improved growth performance and sustainability in poultry production systems to provides a cost-effective alternative to conventional feed ingredients, particularly during periods of high prices for traditional feed sources. Proper management and utilization of this abundant pineapple waste is crucial to minimize environmental pollution and add value to the byproducts.

Keywords: bromelain, pineapple by products, proteolytic activity, poultry feed

1 Introduction

Concerns about sustainable waste management for agriculture and food industry waste have lately increased [1]. Pineapple peel waste (PPW) is a significant waste management challenge that needs to be solved immediately, considering the effects of pineapple food industries. PPW has since been an incredibly useful substance because it provides considerable antioxidant, starch, phenolic, high fiber and protein content [2].

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The majority of pineapple peels and pulp generated at production sites is discarded indiscriminately, resulting to a range of environmental and health issues [3]. Thus, bioconversion of pineapple waste into bioethanol might aid with trash disposal while also providing value to the bioeconomy. It will also assist in reducing the environmental and socioeconomic difficulties coming from high reliance on fossil-based fuel resources [4].

Pineapple waste can be exploited as a raw material for producing valuable compounds, such as fermentable substrates [5]. Pineapples' high sugar content and low dry matter provide preservation challenges for their by-products [6]. Pineapple byproducts performed similarly to Napier grass and whole corn in terms of growth, ruminal fermentation, and carcass and meat quality. However, using pineapple stem by-product as a feed source lowered prices [7]. Growing native cattle in Myanmar experienced better nutrient intake, energy balance, and body weight increase when fed PWS as a roughage source at 25% of diet [8].

According to Bromelain Enzyme, the waste created during the production of bromelain enzymes amounts to 2 tons per day [9]. Bromelain, a kind of cysteine protease identified in pineapple tissue, belongs to the Bromeliaceae plant family. The proteolytic enzymes produced from pineapple that are commercially accessible are stem bromelain and fruit bromelain [10]. The crude bromelain obtained from the pH 7 preboiled tap water extract had the greatest levels of both proteolytic activity. The weight ratio of fruit waste to water of 1:0.5, as well as a ripeness stage of more than 50% yellow peel, were ideal for obtaining the maximum results in both activities [11].

Pineapple waste, comprising byproducts such as peels, cores, and leaves, has gained popularity as a sustainable poultry feed source. Pineapple waste meal can be used as a replacement to wheat offal in poultry feed. The study intended to analyze the growth performance, nutritional digestibility, carcass, and organ characteristics of chickens given diets with varying amounts of by products [12]. According to Nguyen et al. [13], lyophilization, along with 10% skim milk as a cryoprotectant yielded the maximum activity of 3279.02 Gelatin Digesting Units per gram. The product was identified and measured by Liquid Chromatography with Refractive Index Detector (LC-RID).

2 Materials and methods

The methodology utilized in this article is a systematic and complete way to examining previous research on a certain issue. Its goal is to integrate data from many studies to offer a clear overview of the present state of knowledge, identify gaps, and influence future research. The primary goal of a Sistematic Literature Review (SLR) to provide a comprehensive overview of the existing literature on a specific topic, allowing researchers to identify trends and gaps in research, assess the strength of evidence for specific interventions or phenomena, and inform clinical practice or policy decisions based on synthesized evidence. Scopus, Sinta, PubMed, Google Scholar, and ScienceDirect were used to conduct a comprehensive search for studies related to this systematic literature review. Systematic Literature Reviews eliminate bias and improve the dependability of findings reached from the body of literature under evaluation by employing a systematic approach. To select references effectively for a systematic review, begin with a focused research question. Develop a search strategy using relevant keywords across appropriate databases. Apply clear inclusion/exclusion criteria to screen results from titles and abstracts. Assess the quality of studies and extract relevant data, synthesize findings to address the research question.

3 Results and discussion

3.1 Bromelain purification from pineapple waste

The variations in relative enzyme activity (REA) of purified bromelain (PB) were studied at 25°C away or under common light sources (15 W) for 5 hours. The REA (Relative Enzyme Activity) for PB solution and PB powder reduced to 12.60% and 25.10%, respectively ($P < 0.01$). The stability of PB at various temperatures was examined. The REA declines essentially linearly from 25 to 75 °C, with substantial differences across groups. Banerje et al. [15] stated that the enzyme's thermal denaturation increased as the temperature above 55°C. This look is caused by bromelain overactivation above 60 °C, rather than by improved enzyme conformation alone The relative absorbance of PB (REA) diminishes as storage duration increases when the water content surpasses 20%, but remains rather steady when the water content is less than 10% (10 to 120 min). It demonstrated that there was a relationship between the water content and PB's stability, with the higher the water concentration, the less stable PB was. We spoke on the variations in PB's REA between pH values of 3.0 and 9.0. According to the findings, the ideal pH range was 4.0 to 5.0 ($P>0.05$). Because pH can have an impact on both the ionic state and the spatial structure of PB, the REA started to drop at pH values higher than 6.0 or lower than 3.0 [14]

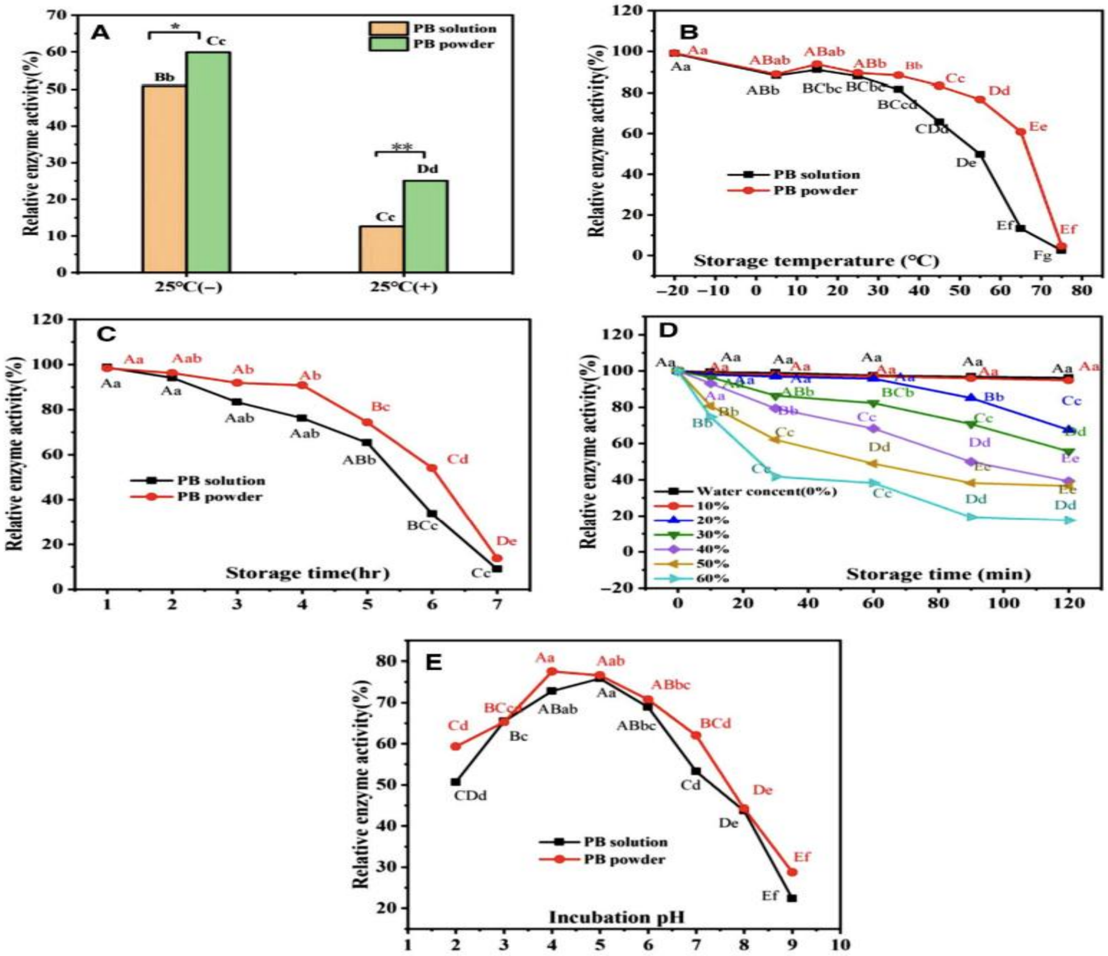


Fig 1. The stability of purified bromelain (PB) concerning storage conditions and chemical additives. Relative changes in PB's enzyme activity were observed at 25 °C in the absence of light (A), across temperatures ranging from -20 to 75 °C (B), over time (C), in relation to water content (D), and at different pH levels (E) [14].

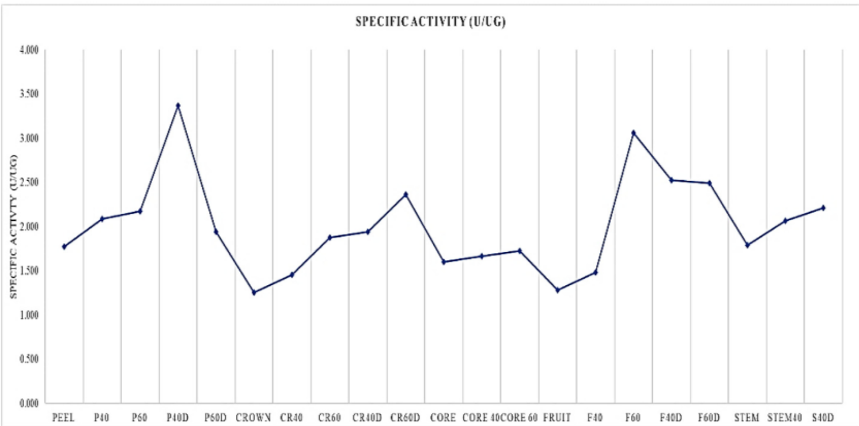
It suggested that PB was more unstable the more water it had. The discussion focused on the variations in PB's REA between pH values of 3.0 and 9.0. According to the findings, the ideal pH range was 4.0 to 5.0 (P>0.05). Since pH may have an impact on both the ionic state and the spatial structure of PB, the REA started to decline at pH values higher than 6.0 or lower than 3.0 [14]. The outcome deviates from the findings of [15] who reported that pH 7.0 is the point at which free bromelain's enzymatic activity recovers to its maximum. This might be because the bromelain isolated from the two research samples differed in purity (Fig 1.) The pH range of pineapple crude extracts is generally between 4.0 and 5.0, while the TSS (Total Soluble Solids) range is between 2.6 and 6.3, according to Ketnawa et al. [21]. Expanding on this, Kanjanarong et al. [22] discovered that the greatest amounts of total protein (215.5 ± 0.5 mg), enzyme activity (182.5 ± 1.0 Units/ml), and specific activity (16.9 ± 0.5 Units/mg) were obtained from stem residue trash when it was extracted using a 0.1M sodium phosphate buffer at pH 7.0.

3.2 Polysaccharide extraction of bromelain from pineapple byproducts

The proteolytic activity of each juice could be determined following the application of the model and the optimization of the complex formation between the enzyme and the polysaccharide. Consequently, the peel crude juice showed 3.1 U mg-1 of protein at pH 9 and the stem crude juice showed 2.7 U mg-1 of protein, for comparison with normal enzymatic formulations. These results match those reported for conventional bromelain (Sigma–Aldrich, St. Louis, Missouri, USA), which has a protein content of >3 U mg-l. However, it should be noted that in this instance, a low-cost approach was used to get the same level of proteolytic activity but a greater purity level without the need of organic solvents or inorganic salts [16].

3.3 Proteolytic activity estimation

The Azocasein technique was utilized by Abbas et al. [17] to measure the proteolytic activity. As shown in Graph 2, the amount of azocasein was adjusted using bromelain to ensure optimal digestion. Proteolytic activities of both crude and purified extracts were calculated using this. Proteolytic activity increased upon purification. The maximum proteolytic activity was found in the peel and fruit, with 3.417 and 2.556 U/lg, respectively (Fig 2.)



KEY
P – Peel
Cr – Crown
C – Core
F – Fruit
S – Stem

40 – 40% amm. Sulp saturation

60 – 60% amm. Sulp. Saturation

40D – 40% saturation – subjected to dialysis

60D – 60% saturation – subjected to dialysis

Fig 2. Azocasein test determines the specific activity of bromelain fractions [17].

3.4 Impact of Fermented Pineapple Peel Waste on Laying Hen Performance and Cholesterol Content in Egg Yolks

Adrizal et al. [18] performed a study to assess the effects of fermented pineapple peel byproduct (FPPB) combined with an Local bacteria (LB) solution derived from bamboo shoots on the performance of laying hens, as well as cholesterol content in yolks. This experiment employed 200 ISA Brown laying chickens who were 32 weeks old, produced 700% eggs, and had an average egg weight of 58.58 g/egg The experiment included 200 ISA Brown laying hens, each 32 weeks old, which produced 700% of eggs with an average weight of 58.58 g per egg. A completely randomized design was implemented, utilizing five levels of FPPW supplementation (0%, 5%, 10%, 15%, and 20%) in the feed, with four hens assigned to each treatment group. The parameters measured were daily dietary intake, Daily egg output, weight of egg, mass of egg, feed conversion ratio (FCR), and yolk cholesterol content (as detailed in Tables 1 and 2). The findings revealed that FPPW can be incorporated into laying hen diets at levels up to 20% without adversely affecting performance indicators such as daily feed dietary, Egg production per hen per day, weight of egg, mass of egg, and FCR. Fermenting pineapple peel can enhance its nutritional profile by reducing crude fiber content and increasing the bioavailability of its beneficial compounds. Fermented pineapple peel has been shown to lower serum cholesterol levels significantly, which may translate to lower cholesterol levels in egg yolks when included in poultry diets [8][22]. The high fiber content in pineapple peel waste can bind to cholesterol in the digestive tract, preventing its absorption. This process encourages excretion of bile acids, which are synthesized from cholesterol, thereby reducing overall cholesterol levels and influencing the cholesterol content of egg yolks [23][24]. Additionally, it was observed to lower egg yolk cholesterol by up to 16.86%. At the 20% supplementation level, daily feed intake was measured at 117.89 g per bird, hen day egg production at 66.28%, egg weight at 63.68 g, egg mass at 42.21 g, FCR at 2.83, and egg yolk cholesterol 430.70 mg/100 g.

Table 1. The average daily feed consumption, egg production per hen per day, and egg weight of laying hens that received fermented pineapple peel waste

FPPB Treatment (%)	Daily feed intake (g per bird per day)	Hen day egg output (%)	Weight of the egg (g)
0	117.86 ± 0.89	70.92 ± 1.67	62.96 ± 2.75
5	116.01 ± 2.24	69.64 ± 4.26	63.24 ± 0.74
10	118.25 ± 1.07	65.66 ± 7.10	62.88 ± 1.95
15	117.17 ± 0.98	69.08 ± 6.80	62.95 ± 1.14
20	117.89 ± 0.47	66.28± 5.72	63.68 ± 0.30
SE	0.64	2.74	0.82
Level of significance	Insignificant	Insignificant	Insignificant

Notes: FPPB stands for fermented pineapple peel byproduct [18].

Table 2. The average mass of egg, feed conversion ratio, and yolk cholesterol content in laying hens that were fed FPPB

FPPB Treatment (%)	Mass of the eight (g)	Feed conversion ratio (FCR)	Yolk cholesterol content (mg/100 g)
0	44.65 ± 2.35	2.65 ± 0.13	518.07a
5	4.06 ± 2.36	2.66 ± 0.19	519.55a
10	41.49 ± 3.57	2.88 ± 0.25	480.35ab
15	43.24 ± 3.53	2.74 ± 0.27	468.02bc
20	42.21 ± 3.61	2.83 ± 0.24	430.70c
SE	1.57	0.11	4.81
Level of significance	Insignificant	Insignificant	Insignificant (<0.01)

Notes: Level of significance represents range of error, and FPPB stands for fermented pineapple byproduct, accompanied by different letters are significantly different at (<0.01) [18].

3.5 The impact of bromelain enzyme on the performance of growth, biochemistry, the metabolism of antioxidant, flesh quality, and the structure of broilers' intestinal

The impacts of bromelain enzyme on the performance of growth, biochemistry, the metabolism of antioxidant, Flesh quality, and the structure of broilers' intestinal were significant. Bromelain significantly enhanced the number of goblet cells (GCN), crypt depth (CD), villus length (VL), and epithelial height (EH) in the small intestine (< 0.05). Overall, while bromelain had a limited impact on the microbiological quality of meat, it positively influenced intestinal structure and performance indicators. In terms of morphological parameters, the groups treated with bromelain showed notably higher goblet cell counts, increased villus length, deeper crypts, and taller epithelial layers in small intestine tissues in comparison with the control. The increased villus-to-crypt ratios indicate enhanced digestive and absorption capabilities. Former studies have shown that Bromelain treatment reduced intestinal Escherichia coli levels, boosted Lactobacillus populations, and improved intestinal villus height in broiler chickens [19]. Additionally, bromelain supplementation at 30 g/kg of diet led to improvements in final body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR). Bromelain treatments produced varying effects on tissue antioxidant and oxidant states, increasing malondialdehyde (MDA) levels in breast and drumstick tissues. Conversely, bromelain decreased serum levels of high-density lipoprotein (HDL), low-density lipoprotein (LDL), and cholesterol (COL). While bromelain influenced meat color properties differently, it also decreased. The microbial content in breast and drumstick meat during storage. was affected. Additionally, bromelain improved the structure of the small intestine. Thus, bromelain can be regarded as a valuable dietary supplement for broiler [20].

Table 3. The effect of adding dietary bromelain on the growth performance of broiler chickens

Weight					
Variables	**Control**	**Bromelan-15**	**Bromelain-30**	**Bromelain-45**	**Significance Level**
BW, (g)					
Weight-1	220.38±2.12	216.39±1.71	215.49±1.76	218.60±3.12	I
Weight-2	544.54±1.33	536.59±6.93	534.41±5.08	548.92±5.79	I
Weight-3	1039.08±16.20	1033.10±9.81	1037.51±15.78	1034.29±22.54	I
Weight-4	1556.73±24.87	1640.90±17.75	1592.00±31.17	1616.57±38.06	I
Weight-5	2267.83±42.33	2383.55±49.43	2335.27±53.93	2272.88±82.52	I
Weight-6	2766.99±92.06^{a}	3060.21±101.28^{ab}	3106.93±98.14^{b}	2836.53±106.14^{ab}	*
BWG, (g)					
Weight-1	23.61±0.23	23.18±0.18	23.09±0.19	23.42±0.33	I
Wegiht-2	46.31±0.24	45.74±0.78	45.56±0.49	47.19±0.43	I
Weight-3	70.65±2.40	70.93±0.77	71.87±1.58	69.34±2.44	I
Weight-4	73.95±1.95^{a}	86.83±1.65^{b}	79.21±2.42^{ab}	83.18±3.98^{b}	*
Weight-5	101.59±2.84	106.09±4.98	106.18±5.24	93.76±7.64	I
Wegiht-6	71.17±10.77^{a}	96.67±8.98^{ab}	110.24±7.89^{b}	80.52±4.82^{a}	*
FI, g					
Weight-1	24.58±0.64	23.58±0.58	23.03±0.65	23.79±0.54	I
Weight-2	58.15±1.60	58.75±0.46	57.88±0.91	60.32±1.10	I
Weight-3	105.23±1.81^{b}	98.02±1.26^{a}	94.15±0.76^{a}	98.89±2.02^{a}	*
Weight-4	112.27±1.58^{a}	125.17±1.68^{b}	125.68±1.91^{b}	126.84±1.63^{b}	*
Weight-5	174.03±0.89^{b}	174.51±5.41^{b}	176.16±4.17^{b}	160.60±3.39^{a}	*
Weight-6	132.56±6.20^{a}	169.74±10.77^{bc}	180.59±3.89^{c}	147.99±7.47^{ab}	*
FCR					
Weight-1	1.04±0.02	1.02±0.02	1.00±0.02	1.02±0.01	I
Weight-2	1.26±0.03	1.29±0.02	1.27±0.01	1.28±0.02	I
Weight-3	1.49±0.04^{c}	1.38±0.02^{ab}	1.31±0.02^{a}	1.43±0.05^{bc}	*
Weight-4	1.52±0.03^{ab}	1.44±0.02^{a}	1.59±0.03^{b}	1.54±0.06^{ab}	*
Weight-5	1.72±0.05	1.65±0.03	1.68±0.07	1.79±0.15	I
Weight-6	2.14±0.24^{b}	1.78±0.05^{ab}	1.68±0.09^{a}	1.85±0.07^{ab}	*
Carcass yield (%)	67.08 ± 1.11	68.26 ± 0.80	71.298 ± 3.54	67.46 ± 0.76	I

Table 4. The effect of dietary bromelain supplementation on serum parameters [20].

Variables					
Parameter	**Control**	**Bromelain-15**	**Bromelain-30**	**Bromelain-45**	**Significance level**
ALP (U/L)	2243.33±237.91	2597.67±682.65	2079.83±313.70	1700.67±289.34	I
TG (mg/dL)	31.00±2.10^{b}	40.50±3.52^{ab}	47.17±5.44^{a}	33.17±2.33^{b}	*
COL (mg/dL)	173.50±9.68^{a}	141.67±2.32^{bc}	128.67±6.03^{c}	154.67±3.77^{b}	*
HDL (mg/dL)	112.67±4.77^{a}	96.83±2.15^{bc}	87.83±3.02^{c}	101.33±3.26^{b}	*
Low Density Lipoprotein (mg/dL)	98.67±6.07^{a}	75.67±1.33^{bc}	65.00±4.30^{c}	82.17±1.85^{b}	*
Calsium (mg/dL)	109.83±3.34^{a}	104.67±1.91^{ab}	69.17±18.94^{b}	81.50±14.53^{ab}	*
Phospor(mg/dL)	64.50±1.98	59.50±10.56	49.33±13.66	60.00±2.18	I
Thymidine Phosphorylase (g/dL)	42.50±1.09^{a}	29.67±5.35^{ab}	34.67±1.38^{ab}	23.50±8.92^{b}	*
Blood Albumin (g/dL)	90.17±24.29	98.83±17.70	96.83±19.03	128.50±5.41	I
Blood Sugar Level (mg/dL)	229.83±4.71	258.67±16.23	245.67±5.50	255.67±12.00	I

Note: The information is displayed as mean ± standard error of the mean (SEM). Means in the same row that feature different superscripts are significantly different (< 0.05), while "I" denotes no significant difference (>0.05). ALP stands for alkaline phosphatase, TG refers to triglycerides, COL indicates cholesterol, HDL represents high-density lipoprotein, LDL signifies low-density lipoprotein, and TP denotes total protein.

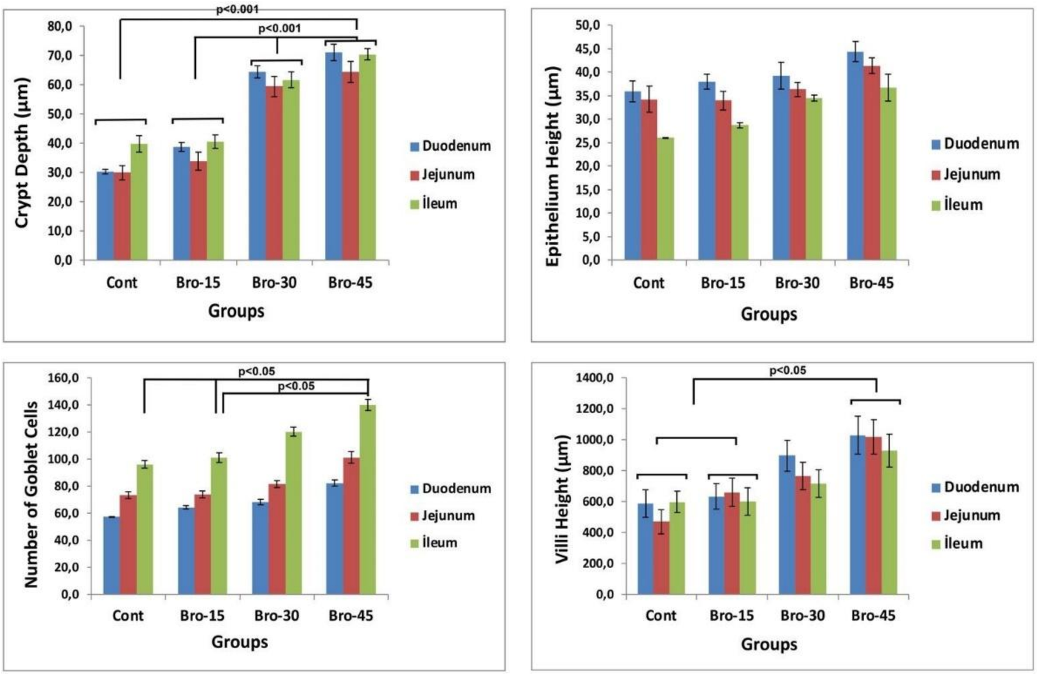


Fig 3. The histological characteristics of digestive organs in control and bromelain-treated groups [20].

Note : Histometric measurements were taken from sections of the superior part of the small intestine, second part of the small intestine, and Last part of the small intestine. Control refers to the basic dietary; B-15 indicates the basic dietary supplemented with 15 gram /kilogram of bromelain enzyme ; B-30 represents the basic dietary with 30 gram/kilogram of bromelain; and B-45 denotes the basic dietary with 45 g/kg of bromelain enzyme The findings are expressed as the average value plus or minus the standard deviation. Significance level of 0.05 or lessened is commonly regarded as empirically relevant.

4 Conclusion

Bromelain is present throughout the pineapple, with the peel, core, and crown exhibiting the highest levels of activity. According to studies, bromelain may be efficiently extracted from these sections utilizing a variety of procedures, including ethanol precipitation and membrane filtering. The extraction procedure not only recovers the enzyme, but it also helps to manage trash in a sustainable way by valorizing pineapple byproducts. The protease activity of bromelain changes depending on the portion of the pineapple. Peel has the greatest protease activity, with recorded levels of 3.417 U/µg. Crown has a protease activity of around 46.78 units, with an optimal temperature of 35°C and pH of 7. Its integration into chicken diets has the potential to increase growth performance and sustainability in poultry production systems by providing a cost-effective alternative to conventional feed components, particularly during periods of high feed costs. Proper management and exploitation of this considerable pineapple waste is critical to reducing environmental damage and increasing the value of byproducts.

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