Journal of Environmental and Development Studies
Journal homepage: https://talenta.usu.ac.id/jeds

The Effect of Centrifugation Speed on the Characteristics of Bromelin Enzyme Crude Extract from Flesh, Core and Peel Sipahutar Pineapple

Hendri FP Purba[1,4], Hotnida Sinaga[2*], & Amir Husin[3]
[1*]*Natural Resources and Environment Management Study Program, Postgraduate School, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[2]*Department of Food Science, Faculty of Agriculture, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[3]*Department of Chemical Engineering, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[4]*Research Center for Agroindustry, National Research and Innovation Agency (BRIN), Indonesia*

[*]Corresponding Author: hotnida.sinaga@usu.ac.id

ARTICLE INFO

Article history:
Received 22-07-2024
Revised 26-08-2024
Accepted 09-09-2024
Available online 11-09-2024

E-ISSN: 2745-4592

How to cite:
Hendri FP Purba, Hotnida Sinaga & Amir Husin. The Effect of Centrifugation Speed on the Characteristics of Bromelin Enzyme Crude Extract from Flesh, Core and Peel Sipahutar Pineapple. International Journal of Envrionmental and Development Studies 2024. 5(2):54-60.

http://doi.org/10.32734/jeds.v5i01.12510

ABSTRACT

Bromelain enzymes can be utilized as a food supplement and pharmacy manufacturer. Pineapple is a natural source of bromelain enzymes. The centrifugation process is an extraction process to produce a crude extract of enzyme. Wasted parts of the Sipahutar pineapple plant have never been processed into bromelain enzyme. This research was aimed to determine the crude extract characteristics of the Sipahutar pineapple bromelain enzyme extracted with three centrifugation speeds. The research was conducted at the Food Analysis and Biochemistry Laboratory, University of North Sumatra from January to March 2023. The research was arranged in a completely randomized factorial design. The first factor is the part of the fruit used (B), namely the skin, flesh, and core, while the second factor is the centrifugation speed (K) which consists of 3000, 4000 and 5000 rpm. The study results showed that only the part of the fruit used (B) factor that affected the test parameters, especially pH and bromelain enzyme activity. The skin produced the highest pH (4.55) and enzyme activity (0.0035 U/ml) while the flesh produced the lowest pH (4,25), enzyme activity (0.0019 U/ml), and the highest soluble protein content (0.69 µg/ml).

Keywords: Bromelain Enzyme, Crude Extract, Pineapple Plant Waste, Centrifugation Process.

1. Introduction

Sipahutar pineapple is a fruit plant commodity that was included in one of the North Sumatra government's agricultural improvement programs in 1982, increasing Sipahutar pineapple cultivation starting in 1995 until it developed into a center for pineapple production in North Tapanuli Regency, which is in great demand both at domestic and export market (Hardinata et al., 2022). The attractiveness of sipahutar pineapple includes the bigger fruit size and sweet taste with a less juicy texture (Sinulingga & Harahap, 2014). Based on the BPS Statistics of North Tapanuli Regency (2020), Sipahutar pineapple productivity for the last five years (2016-2020) ranged from 17-18 tons per hectare. Pineapple farming in the Sipahutar area is generally managed and developed by the community with private land ownership with an average of 1-3 ha per family (Lubis et al., 2022). The processing or utilization pattern of pineapple differs between developing and developed countries in terms of the percentage of consumption of fresh and processed products, which is 1:9 (Wijeratnam, 2016).

Pineapple categorized as perishable fruit for its short shelf life, so it is necessary to develop postharvest processing technology besides serving it as fresh fruit to increase pineapple economic value(Basumatary et al., 2022). In Sipahutar, the private sector has developed pineapple canning processing practices at the household level, such as dodol, jam, crackers, and other preparations (Lubis et al., 2022). Besides being consumed as

food, pineapple has long been categorized as an herbal plant because of the content of vitamins and the enzyme bromelain, which can cure the disease (Nur et al., 2017). Bromelain enzymes are found in almost all parts of the pineapple plant, including the stems, leaves, peel, tubers, flesh, and crown (Alfiyanti et al., 2019). Technological developments facilitate the use of Bromelain in the health, food, animal feed, beauty products, textile, and beverage industries (Arshad et al., 2014). The U.S. Food and Drugs Administration (FDA) categorizes bromelain products as food supplement that is safe for consumption (Orsini, 2006).

The potential utilization of pineapple as a source of bromelain enzyme is remarkably high because about 75% of pineapple plant parts will be wasted, such as stems, peel, cores, and crowns, except for the flesh, which can be consume as processed and fresh fruit (Vasiljevic, 2020). Commercial bromelain enzymes can be found in powder and liquid form (Sukendar et al., 2020) and have a unique odor with a clear white or yellowish color (Ujiani, 2021). The bromelain enzyme found in pineapple plant fiber is a thermostable endopeptidase enzyme, which functions to break down protein into smaller parts so that it is easier to digest (Arti et al., 2019). Enzymes work to unravel the combination of substrates with enzymes to produce products that are not bound together (Xuanyuan, 2022).

Obtaining the pineapple bromelain enzyme is conducted by taking the extract and then centrifuging, ultrafiltration, and lyophilization with using of gelatin, casein, or tripeptide chromogenic substrates (Silaban & Rahmanisa, 2016). The bromelain products on the market are derived from the stem and fruit of the pineapple plant.

The aims of this study were to obtain the activity of bromelain enzyme value in the fruit flesh, core, and peel of Sipahutar pineapple fruit. Testing of the bromelain enzyme activity contained in the Sipahutar pineapple needs to be carried out with the consideration that no previous research has been conducted on the handling of waste, as well as the benefits and economic value of the bromelain enzyme. The treatment was carried out by taking the bromelain enzyme extract from flesh, core (tube), and peel of pineapple at several levels of centrifugation speed (rpm). The parameters observed on this study are the characteristics of the crude bromelain enzyme produced, including enzyme activity, pH, and dissolved protein.

2. Method

The study activity began with collecting ripe and ready to consume Sipahutar pineapples directly from the Sipahutar District with the condition of the fruit being ready to be consumed or ripe. The research was conducted at the AKBP Laboratory, Food Technology Study Program, Faculty of Agriculture, University of Sumatera Utara. The materials used in the study were Sipahutar pineapple, 60% Ammonium Sulfate, 0.1 M Sodium Acetate, Bradford Reagent, 1 M Phosphoric Acid pH 6.5, and 1000 ppm Gelatin. Bromelain enzyme extraction from fruit flesh, core (hump) and pineapple peel. The research procedures were carried out following Masri's research (Masri, 2014). Centrifuge is used in this study for the extraction or separation method. The centrifugation method can perform finer cell breakdown with accelerated separation of substances. The centrifugal force of the centrifugation method produces a clearer supernatant because the sediment has stuck firmly to the bottom of the tube.

2.1. Preparation of pineapple crude extract

Peel, chop, and puree the pineapple flesh, core, and peel by adding water in a 1:1 ratio. Homogenize, 250 g of pineapple peel, core, and flesh, using 33.33 ml of Sodium Acetate Buffer (pH 6,5). Filter using cheesecloth to produce pineapple extract. Centrifuged at 3000 rpm, 4000 rpm, and 5000 rpm, then filtered using Whatman filter paper.

2.2. Precipitation of Bromelain Crude Extract

Pineapple extract, 3 ml each of pineapple peel, core, and flesh extracts were added with 7 ml of 60% Ammonium Sulphate. Stir using a magnetic stirrer for 45 minutes. Incubate overnight in the refrigerator at 4-8 ºC. Next, centrifuged for 25 minutes at 3000 rpm, 4000 rpm, and 5000 rpm. In this study, the crude bromelain extract observed was the supernatant of the centrifuged extract.

2.3. Determination of Soluble Protein Content of Bromelain Crude Extract

The stages used in determining the soluble content of bromelain crude extract up to the measurement of bromelain enzyme activity begin with the determination of protein standard solution, measurement of soluble

protein content, measuring of bromelain enzyme activity, and continuing with measuring the pH of the bromelain enzyme, with the following description:

2.3.1 Determination of Protein Standard Solution

Measurements for the soluble protein content of bromelain crude extract in this study used is the Bradford Test. The Bradford test is a colorimetric method involving Coomassie Brilliant Blue (CBB) dye to measure total protein concentration in solution. CBB dye will indicate a bluish color after binding to proteins from acidic solutions—comparison of absorbance of bromelin enzyme extract after measuring using spectrometry at a wavelength of 595 nm. Determination of protein standard solution using gelatin with concentrations of 10, 20, 30, 40, 50, and 60 ppm by adding 2,5 ml of Bradford reagent.

2.3.2 Measurement of Soluble Protein Content

Mix the 0.5 ml of bromelain enzyme extract with 2.5 ml of Bradford reagent. Vortex for 30 minutes. Then incubated for 60 minutes at room temperature. A bluish color will appear after mixing with CBB dye as an indication that it has bound to proteins from an acidic solution. Observations were repeated 3 times. Next observations using a 595 nm spectrophotometer wavelength.

2.3.3 Measurement of Bromelain Enzyme Activity

Determination of protein content was done by comparing the absorbance of the bromelain enzyme extract with the linear equation of the gelatin standard curve. During the absorbance determination process, observations were made of the hydrolyzed substrate. Because in the measurement of the enzyme effectiveness test, the hydrolyzed substrate becomes a multiplying factor for the ratio of solution volume and enzyme weight.
Bromelain enzyme activity is determined based on the formula (Masri, 2014):

$$\text{Enzyme activity} = \text{Hydrolyzed substrate x } \frac{1}{\text{MW of enzyme}} \text{ x } \frac{\text{Solution volume}}{\text{Weight of enzyme}}$$

Note: Molecule weight (MW) of Enzyme = 181.19 g/mol

2.3.4. pH measurement

Measurement of pH using a pH meter with the principle of combining standard Hydrogen glass electrodes from polymers and reference calomel electrodes. The working step is to calibrate the pH meter with a pH buffer solution, which is conducted every time before taking a measurement. The pH meter electrode was inserted into the solution; the scale number indicated on the pH meter was recorded as the measured pH extract value.

3. Discussion

3.1. Bromelain's Activity

According to the results of ANOVA, the single factor of material (B) had a significant effect on bromelain enzyme activity; in contrast, the single factor of centrifugation speed (K) and the interaction of the two factors (BxK) had no significant impact. The results of further DMRT analysis, as presented in Table 1, showed that the enzyme activity of the Sipahutar pineapple flesh (D) and peel (K) were not significantly different, with values of 0.0033 and 0.0035 U/ml, respectively. The core (B) of Sipahutar pineapple in this study produced an average value of enzyme activity of 0.0019 U/ml, significantly different from the value of enzyme activity of the flesh and peel parts.

Although not significantly different, the enzyme activity value of the Sipahutar pineapple in this study has a tendency as reported by Misran et al., (2019), for the Morris pineapple without crown, specifically that the peel has a slightly higher enzyme activity. This tendency differs from the results of other studies, which found that the enzyme activity in the pineapple flesh was higher than the peel, as reported by Mohan et al., (2016) for the Morris pineapple with a crown. This variation is not surprising considering that the value of bromelain enzyme activity can be influenced by various factors such as purification /extraction method, pineapple plant parts, species/variety, growing environment, level of maturity, and drying process.

Table 1. Bromelain enzyme activity from different parts of pineapple at several centrifuge speeds

Material (B)	Speed (rpm)			Means
	3000	4000	5000	
	(V1)	(V2)	(V3)	
		(U/ml)		
Flesh (D)	0.0034	0.0029	0.0035	**0.0033b**
Core (B)	0.0023	0.0018	0.0017	**0.0019a**
Peel (K)	0.0036	0.0030	0.0040	**0.0035b**
Means	**0.0029**	**0.0025**	**0.0033**	

Note: Numbers in columns and rows followed by the same notation show no significant difference real according to the DMRT test with α=5%

3.2. pH

The ANOVA results showed that the plant material, (B) significantly affected the pH of the produced bromelain crude extract. In otherwise, the centrifugation speed (K) and its interaction (BxK) had no effect. According to the results of the DMRT further test, it is known that the pineapple peel has the highest pH (4.55) while the lowest was found in the flesh (4.25). The average pH values of the three tested parts of the pineapple plant, that is the flesh (D), the core (B), and the peel (K), were significantly different from each other.

Overall, the pH of the crude bromelain extract of each plant part tested classifies as acidic, with a tendency that the pH of the peel was higher than the flesh and core from the part of Sipahutar pineapple. The same result was found in the studies of Nordin et al., (2023), who stated that the high citric acid and malic acid in the fruit flesh and cores contributed to the low pH of the extract. Weak citric and malic acids are the primary organic acids in the flesh; in contrary, secondary metabolites from the flavonoid group, specifically catechins and epicatechins, and organic acids such as gallate and ferulic acid (Paull et al., 2020), are found in the pineapple peel.

Table 2. The pH of bromelain crude extract from different pineapple parts at several centrifuge speed

Material (B)	Speed (rpm)			Means
	3000	4000	5000	
	(V1)	(V2)	(V3)	
Flesh (D)	4.25	4.24	4.25	**4,25a**
Core (B)	4.33	4.34	4.31	**4,33b**
Peel (K)	4.52	4.61	4.54	**4.55c**
Means	**4.38**	**4.38**	**4.37**	

Note: Numbers in columns and rows followed by the same notation show no significant difference real according to the DMRT test with α=5%

3.3. Dissolved Protein

The ANOVA results show that material (B) and speed (K) each have an effect natural to dissolved proteins, whereas interaction both (BxK) do not have significant effect. Further DMRT analysis results, as listed in Table 3 showed that pineapple fruit meal (B1) produced the highest protein content with an average value of 686.75 µg/ml. In contrast, the peel has the lowest dissolved protein (233.69 µg/ml). Although the average value of the dissolved protein is most deficient in the treatment peel fruit, no difference is accurate with the average value in the treatment pineapple crown. The average of dissolved protein value for varieties Sipahutar in this research is similar to Huang et al, (2021) research result at Cayenne varieties, i.e., the value of dissolved protein from flesh is relatively higher more than the peel's value dissolved protein. Meanwhile, if the result of this research was compared to the dissolved protein value pineapple peel from Kenya reported by Kahiro et al, (2018), the dissolved protein Sipahutar pineapple peel is lower by 35-40%. Differences in dissolved protein values can be influenced by various factors like variety, level maturity, environment, the section of the plant, kind of enzymes, amount of samples, and stuff related method extraction are used (Kader et al., 2010). Furthermore, for speed, the highest protein content (0.52 mg/ml) was shown in the treatment speed centrifugation at 5000 rpm (K3); its value was no different from the speed of 4000 rpm of 0.44 mg/ml (K2). Speed 3000 rpm (K1) in the study produces the lowest protein content with a value of 0.28 mg/ml and is different in accuracy from speed 4000 (K2) and 5000 rpm (K3). Based on the formula style centrifugal relative

(RCF) with the value of r is 10 cm, it is known that the speed of 3000, 4000, and 5000 rpm of speed centrifugation in this study is equivalent to 1006.2 g, 1788.8 g, and 2.795 g.

Characteristics of centrifugation, like speed and time as part of method extraction, have varying influences on protein characteristics. For example, Cvjetkovic et al., (2017), reported that protein correlated positively with centrifugation time but not speed, while Baskoro et al., (2017), suggested that increasing speed affected decreasing the frequency of processed proteins. In the case of crude bromelain extraction, centrifugation speed was reported to affect the stem and fruit of pineapple bromelain protein differently, negatively and positively, respectively Gautam et al., (2010), whereas in this study, increasing the centrifugation speed positively increased the protein content of all three crude parts of the pineapple plant.

Table 3. Extract protein content of rough Bromelain from Pineapple parts differ on a few centrifuge speeds

Material (B)	Speed (rpm)			Means
	3000	4000	5000	
	(V1)	(V2)	(V3)	
Flesh (D)	0.49	0.76	0.81	0.69 b
Core (B)	0.21	0.33	0.42	0.32a
Peel (K)	0.13	0.24	0.33	0.23a
Means	0.28a	0.44 b	0.52 b	

Note: Numbers in columns and rows followed by the same notation show no significant difference real according to the DMRT test with α=5%

The results of this study inform the characteristics of crude bromelain enzyme from three parts of the Sipahutar pineapple fruit, namely peel, flesh, and core. The pH value, total soluble protein, and crude bromelain enzyme activity of Sipahutar pineapple peel, flesh, and core extracts ranged from 4.24-4.61; 0.13-0.81 µg/ml; and 0.0017-0.0040 u/ml. The results showed that the highest enzyme activity value was obtained in pineapple peel with an average of 0.0035 U/ml, so the handling of Sipahutar pineapple waste in the form of bromelain enzyme products is potential.

4. Conclusion

The extraction method in this study produced a clearer supernatant. The centrifugation method produces finer cells with sediment stuck to the bottom or on the tube wall. The results showed that the highest bromelain enzyme activity was found in the enzyme produced from the Sipahutar pineapple peel. This shows that the potential of the peel which is the highest residue of pineapple fruit can be reprocessed into a more useful bromelain enzyme. Pineapple peel waste processed into bromelain enzyme can be used as a base for environmentally friendly soaps, medicines, and cosmetics. Pineapple waste into bromelain enzyme reduces the amount of waste and can increase farmers' income and government solutions in pineapple producing centers in managing pineapple waste into more economic value. The activity value of the Sipahutar pineapple enzyme in this study uses crude extract without any further purification treatment, so further research is needed to add treatment after the extraction method.

Acknowledgement

I wish to express my deep thanks to all the laboratory assistant at the AKBP Laboratory, Food Technology Study Program, Faculty of Agriculture, Universitas Sumatera Utara for help and cooperation during this study.

References

Alfiyanti, R. D., Prihatiningrum, B., & Budirahardjo, R. (2019). Efek Enzim Bromelin Buah Nanas (Ananas comosus (L.) Merr) Berbasis Sediaan Gel terhadap Lebar Intertubulus Dentin. *E-Journal Pustaka Kesehatan*, *7*(3), 195–200. https://doi.org/10.19184/pk.v7i3.11705

Arshad, Z. I. M., Amid, A., Yusof, F., Jaswir, I., Ahmad, K., & Loke, S. P. (2014). Bromelain: An overview of industrial application and purification strategies. *Applied Microbiology and Biotechnology*, *98*(17), 7283–7297. https://doi.org/10.1007/s00253-014-5889-y

Arti, I. M., Huda, A. N., & Pratama, E. Y. (2019). Karakteristik Fisik Daging Sapi Has Dalam Pada Perendaman Berbagai Bagian Buah Nanas. *Jurnal Teknologi Pangan*, *2*(2), 11–23. https://doi.org/http://dx.doi.org/10.26418/jft.v2i2.40798

Badan Pusat Statistik Tapanuli Utara. (2020). *Kecamatan Sipahutar dalam Angka 2020*. Badan Pusat Statistik Kabupaten Tapanuli Utara. https://tapanuliutarakab.bps.go.id/publication/2020/09/28/ad80d74fe1cce23b00578595/kecamatan-sipahutar-dalam-angka-2020.html

Baskoro, B., Nugraha, R., Puspitawati, R., & Redjeki, S. (2017). Effect of centrifugation at 7, 000 g, 8, 000 g, and 9, 000 g on the salivary protein profile ≥ 30 kDa. The 1st Physics and Technologies in Medicine and Dentistry Symposium. IOP Conf. Series: Journal of Physics: Conf. Series, 884(1), 1–4. https://doi.org/10.1088/1742-6596/884/1/012013

Basumatary, I. B., Mukherjee, A., Katiyar, V., Dutta, J., & Kumar, S. (2022). Chitosan-based active coating for pineapple preservation: Evaluation of antimicrobial efficacy and shelf-life extension. Lwt-Food Science and Technology, 168(2022), 113940. https://doi.org/10.1016/j.lwt.2022.113940

Cvjetkovic, A., Lötvall, J., & Lässer, C. (2017). The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. Journal of Extracellular Vesicles ISSN: 3078 (March). https://doi.org/10.3402/jev.v3.23111

Gautam, S. S., Dash, V., Goyal, A. K., & Rath, G. (2010). Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant Abstract: The Thai Journal of Pharmaceutical Sciences, 34(2), 67–76. https://doi.org/10.1016/j.ijbiomac.2020.04.220

Hardinata, A., Harahap, F., Rahmatsyah, Rosmiati, R., Adnin, V., & Flower, M. (2022). Pengembangan Sentra Produksi Selai Nanas Di Desa Sipahutar Ii Kecamatan Sipahutar Kabupaten Tapanuli Utara Prosiding Seminar Nasional Hasil Pengabdian Kepada Masyarakat Lppm Unimed 2022, 13–17. http://digilib.unimed.ac.id/50376/

Huang, C. W., Lin, I. J., Liu, Y. M., & Mau, J. L. (2021). Composition, enzyme and antioxidant activities of pineapple. *International Journal of Food Properties*, *24*(1), 1244–1251. https://doi.org/10.1080/10942912.2021.1958840

Kader, A., Hossain, F. M. J., Islam, M. M., Kabir, G., Sarkar, S. K., & Absar, N. (2010). A comparative analysis on the nutritional contents of two varieties of pineapple of Chittagong region. Chittagong University Journal of Biological Sciences, 5(1&2), 105–112. https://doi.org/10.3329/cujbs.v5i1.13375

Kahiro, S. K., Kagira, J. M., Maina, N., & Karanja, S. M. (2018). Specific Activity of Pineapples Bromelain Extracts at Different Purification Stages Obtained from Different Agro-Ecological Zones of Thika Region, Kenya. 17(2), 1–8. https://doi.org/10.9734/JALSI/2018/39678

Lubis, R. H., Purba, J., Sutiani, A., Juliani, R., Adnin, V., & Sulandari. (2022). Peningkatan Ekonomi Masyarakat Desa Sipahutar I Melalui Pengolahan Kreatif Buah Nanas (Ananas comosus). Prosiding Seminar Nasional Hasil Pengabdian Kepada Masyarakat Lppm Unimed 2022, 181–187. http://digilib.unimed.ac.id/50489/

Lubis, R. H., Purba, J., Sutiani, A., Juliani, R., Adnin, V., & Sulandari. (2022). Peningkatan Ekonomi Masyarakat Desa Sipahutar I Melalui Pengolahan Kreatif Buah Nanas (Ananas comosus). Prosiding Seminar Nasional Hasil Pengabdian Kepada Masyarakat Lppm Unimed 2022, 181–187. http://digilib.unimed.ac.id/50489/

Masri, M. (2014). Isolasi Dan Pengukuran Aktivitas Enzim Bromelin Dari Ekstrak Kasar Bonggol Nanas (*Ananas comosus*) pada Variasi Suhu dan pH. *Biogenesis*, *2*(2), 119–125. https://doi.org/https://doi.org/10.24252/bio.v2i2.478

Misran, E., Idris, A., Sarip, S. H. M., & Ya'akob, H. (2019). Properties of bromelain extract from different parts of the pineapple variety Morris. *Biocatalysis and Agricultural Biotechnology*, *18*(2019), 1878–8181. https://doi.org/https://doi.org/10.1016/j.bcab.2019.101095

Mohan, R., Sivakumar, V., & Muralidharan, C. (2016). Optimisation of Bromelain Enzyme Extraction from Pineapple (Ananas comosus) and Application in Process Industry. *American Journal of Biochemistry and Biotechnology*, *12*(3), 188–195. https://doi.org/10.3844/ajbbsp.2016.188.195

Nordin, N. L., Sulaiman, R., Bakar, J., & Noranizan, M. A. (2023). Comparison of Phenolic and Volatile Compounds in MD2 Pineapple Peel and Core. *Foods*, *12*(11). https://doi.org/10.3390/foods12112233

Nur, S., Surati, & Rehalat, R. (2017). Aktifitas Enzim Bromelin Terhadap Peningkatan Protein Tepung Ampas Kelapa. *Jurnal Biology Science & Education 2017*, *6*(1), 84–93. https://doi.org/http://dx.doi.org/10.33477/bs.v6i1.136

Orsini, R. A. (2006). Bromelain. *Plastic and Reconstructive Surgery*, *118*(7), 1640–1644. https://doi.org/10.1097/01.prs.0000242503.50548.ee

Paull, R. E., Uruu, G., & Chen, N. J. (2020). Rapid field assay for pineapple fruit acidity. *HortTechnology*, *30*(5), 593–596. https://doi.org/10.21273/HORTTECH04664-20

Silaban, I., & Rahmanisa, S. (2016). Pengaruh Enzim Bromelin Buah Nanas (Ananas comosus L.) Terhadap Awal Kehamilan. *Majority*, *5*(4), 80–85. https://juke.kedokteran.unila.ac.id/index.php/majority/article/view/889/797

Sinulingga, S., & Harahap, F. (2014). Pengaruh Pemberian Zat Pengatur Tumbuh (Zpt) Indole Acetic Acid (Iaa) dan Benzyl Amino Purin (Bap) Terhadap Pertumbuhan Planlet Nanas (Ananas comosus l.) Sipahutar secara In Vitro. Seminar Nasional Biologi : Optimalisasi Riset Biologi dalam Bidang Pertanian, Peternakan, Perikanan, Kelautan, Kehutanan, Farmasi Dan Kedokteran, 204–209. http://digilib.unimed.ac.id/4904/1/Fulltext.pdf

Sukendar, N. K., Laga, A., & Siade, T. P. (2020). Study Of Preservation Crude Bromelain Enzyme by Filtration And Addition Of Sodium Benzoate. *Canrea Journal: Food Technology, Nutrition, and Culinary Journal*, 63–68. https://doi.org/https://doi.org/10.20956/canrea.v1i1.23

Ujiani, S. (2021). Effectiveness of Pineapple Extract (Ananas comosus (L.)) on Streptococcus Pneumococcus Growth. *American Journal of Health Research*, *9*(4), 107. https://doi.org/https://doi.org/10.20956/canrea.v1i1.23

Vasiljevic, T. (2020). Pineapple. *Valorization of Fruit Processing By-Products*, 203–225. https://doi.org/10.1016/B978-0-12-817106-6.00010-1

Wijeratnam, S. W. (2016). Pineapple. Encyclopedia of Food and Health, Academic Press, 380–384. https://www.sciencedirect.com/science/article/abs/pii/B978012384947200547X

Xuanyuan, T. (2022). How different factors affect the activity of bromelain. *International Journal of Frontiers in Medicine*, *4*(10), 30–42. https://doi.org/10.25236/ijfm.2022.041006