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IMPORTANCE OF PINEAPPLE (*Ananas comosus* L.) WASTE AS A POSSIBLE SOURCE OF INDUSTRIAL PROTEASES

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ABSTRACT

Pineapple (*Ananas comosus* L.) is a tropical fruit highly valued for its flavor, sweetness, aroma, and nutraceutical properties. The 'Cayena Lisa' cultivar is the most widely used for both fresh consumption and industrial purposes in Mexico. The municipalities of Tuxtepec and Loma Bonita, in the state of Oaxaca, produce around 582 thousand Mg of solid waste per year from fruit processing, to which the waste from plantations attacked by pests and diseases must be added. Consequently, these wastes must be processed in order to generate added value. Isolation of cysteine proteases from industrial residues is one possibility. In the present study, the parameters of the Mexican Official Standard for measuring pH (NOM-F-317-S-1978), total soluble solids (SST) (NMX-F-103-1982), and cysteine-protease activity in pineapple fruit processing residues at two stages of ripening over four months of harvest were used to determine whether there is a factor of quality attributes that correlates with the activity of this protein. In both ripening stages, cysteine-protease activity is higher in August. Regardless of the degree of ripening, peel and core are the best sources of cysteine-proteases. SST and pH values have no correlation with cysteine-protease activity, so they cannot be used as indicators of such activity. The findings show that 'Cayena Lisa' pineapple residues grown in Loma Bonita, Oaxaca, could be a good source of cysteine-proteases.

Keywords: agro-industrial wastes, peptidases, revalorization of agricultural wastes.

INTRODUCTION

Pineapple (*Ananas comosus* L.) is a tropical plant highly valued for the flavor, sweetness, aroma, and nutraceutical properties of its fruit (Mohd *et al.*, 2020). In Mexico, the 'Cayena Lisa' variety accounts for nearly 80 % of pineapple production, both for fresh consumption and for industrialization of pulp, juices, and peptidases, due to its size, weight, pale yellow color, pulp softness, and reducing sugar content (Uriza-Ávila *et*

al., 2018). Pineapple harvesting generates two types of waste: harvesting and canning waste, both of which produce a large amount of solid waste. Some authors mention that waste production from pineapple processing can constitute 25 to 35 %, or even up to 50 %, of the total weight of the fruit (Seleni *et al*., 2014; Conesa *et al*., 2015), depending on the commercial form of the product. It is worth mentioning that waste derived by food processing generates solid waste management and pollution problems (Hikal *et al*., 2021).

Banerjee *et al*. (2018) pointed out that the low valorization of pineapple residues is due to the lack of knowledge of their potential economic value. Pineapple residues represent raw materials that can be converted into high-value products, such as silage, paper, fabrics, biopolymers, cellulose and hemicellulose, fertilizers, methane, ethanol, citric acid, antioxidant compounds, and proteolytic enzymes. Pineapple proteases are proteins that have applications in the food industry as a meat tenderizer, in the hydrolysis of soluble proteins, beer and wine production, cosmetic, and pharmaceutical industries. In the clinical area, they have been successfully employed as phytotherapeutics (Arshad *et al*., 2014; Banerjee *et al*., 2018; Santos *et al*., 2021). Bromelain is the most well-known pineapple cysteine endopeptidase, with a global market worth $2 956 120 000 USD and a compound annual growth rate (CAGR) of 4.72 % from 2022 to 2027 (Market Reports World, 2022). The 'Cayena Lisa' variety's potential utility for this purpose needs to be confirmed.

The Mexican state of Oaxaca contributes 13 % of national pineapple production, and the municipalities of Loma Bonita and San Juan Bautista Tuxtepec generate 80 % of state production (SIAP, 2022). Pineapple pulp and juice are the most commonly used in industrial processing; the rest of the plant material (crown, peel, core, stem, and trimmings) is discarded. In the Papaloapan Basin region, 582 thousand Mg of solid waste are generated annually, to which the waste from plantations whose fruits are attacked by pests and diseases must be added. There is a clear need to seek a biotechnological solution for processing and generating added value from these agroindustrial wastes (Hikal *et al*., 2021).

The objectives of this study were to determine: the cysteine-protease activity in the pulp and in the different parts that make up the pineapple fruit residues (peel, core, and crown); the changes in cysteine-protease activity during the harvest period (from May to August); and the relationship between this activity and quality attributes (total soluble solids (SST) and pH), in order to decide on the destination of the harvest and its residues.

MATERIALS AND METHODS

Sigma-Aldrich reagents were used. Plant material was provided by the Unión Estatal de Productores de Piña (State Union of Pineapple Producers) in Loma Bonita, Oaxaca, during the period May-August 2019, in a commercial plantation (18° 4' 0.4'' N, 95° 53' 32.4'' W). Each month, fruits were selected from *Ananas comosus* (L). Merr, cultivar 'Cayena Lisa', without disease or pest symptoms, at two stages of physiological

maturity, based on color criteria corresponding to Code 2 (C2) and Code 5 (C5) according to NMX-FF-028-SCFI-2008 (Garcia-Tain *et al*., 2011).

The experimental unit consisted of five randomly selected fruits from each ripening stage, in each sampling month. The fruits were transported to the Food Workshop of the Universidad del Papaloapan (UNPA) in Tuxtepec, Oaxaca, where they were washed with a water jet to remove soil and air-dried. The fruits were cut, and the pulp and residues (peel, crown, and core) were separated. The pulp was used to evaluate enzyme activity. Each type of residue from the five fruits was mixed to have a composite sample.

To prepare the homogeneous fresh extract, 100 g of each composite mixture were taken; they were ground in a centrifugal juice extractor (Oster) to obtain the crude extracts. The resulting mixture was filtered using gauze, and 2 mL aliquots of each crude extract were taken in triplicate and centrifuged at 10 000 xg for 20 min at 4 °C. The supernatant was kept at 4 °C until enzymatic evaluation, which was performed the same day upon completion of sample extraction of each residue and pulp.

Subsequently, the solid portion of each extract was removed by centrifugation at 5000 xg for 8 min at room temperature. The pH was measured according to Mexican Official Standard NOM-F-317-S-1978 in a HI 2211 ORP Meter (Hanna® Instruments, Mexico). SST was quantified as indicated in NMX-F-103-1982. Zero calibration of the optical refractometer (Atago Master-M, Bellevue, WA, USA) was performed by adding 40 μL of distilled water at room temperature through the prism; for the experimental samples, 40 μL of the supernatant of the centrifuged crude extract were used, waiting one minute to obtain the sample result. The results were expressed in °Brix. Readings were taken in triplicate for each crude extract.

For the calculation of the specific activity, total proteins were quantified by the Bradford method, using bovine serum albumin (BSA) as a standard (Bradford, 1976). From the crude extracts of each residue, 2 mL were taken in triplicate, centrifuged at 10 000 xg for 20 min at 4 °C; 25 μL of the supernatant of each sample were used and mixed with 1 mL of Bradford's reagent; absorbance was recorded at 595 nm in a UV-Vis spectrophotometer (Thermo Scientific™ Waltham, MA, USA).

The proteolytic activity of cysteine endopeptidases in crude enzyme extracts was determined by the method modified by Ketnawa *et al*. (2012): the assay is based on the proteolytic hydrolysis of casein by cysteine endopeptidases to release L-tyrosine. The peptidase activity calibration curve was performed with six concentrations of L-tyrosine (0, 100, 200, 300, 400, and 500 μg mL^{-1}) in reaction buffer (casein 1 % w/v, cysteine 0.03 M, EDTA 0.006 M, potassium phosphate 0.05 M, pH 7.0).

For the samples, 1 mL of the supernatant of the crude extracts and 1 mL of the reaction buffer were mixed. The reaction was carried out at 37 °C for 10 min and stopped by adding 3 mL of 5 % w/v trichloroacetic acid (TCA) to precipitate the casein. The reaction mixture was centrifuged at 8000 xg for 10 min and the supernatant with L-tyrosine was measured at 275 nm. The reactions were carried out in triplicate. A unit of enzyme activity was defined as the amount of enzyme releasing a product

equivalent to 1 µg tyrosine min^{-1} mL^{-1} under standard assay conditions and was expressed as casein digesting units (CDU mL^{-1} $mg\ protein^{-1}$).

Statistical analysis

Statistical analyses were performed in Minitab Statistical Software (19) and Microsoft Excel (2019). Differences between samples for pH, SST, protein concentration, and enzyme activity were determined by analysis of variance; comparison of means was performed by Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Representative images of the samples used in the study are shown below (Figure 1). The average weight of the peel, core, and pulp of the four monthly samplings was higher in the fruit at the most advanced ripening stage (C5, 88 % w/w) than in the fruit at the C2 stage (80.2 % w/w). For the crowns, the highest weight corresponded to C2 (19.8 % w/w) (Table 1). The higher crown weight in C2 may be because the fruit filling process had not yet been completed, so this structure was not yet entering senescence (Uriza-Ávila *et al*., 2018).

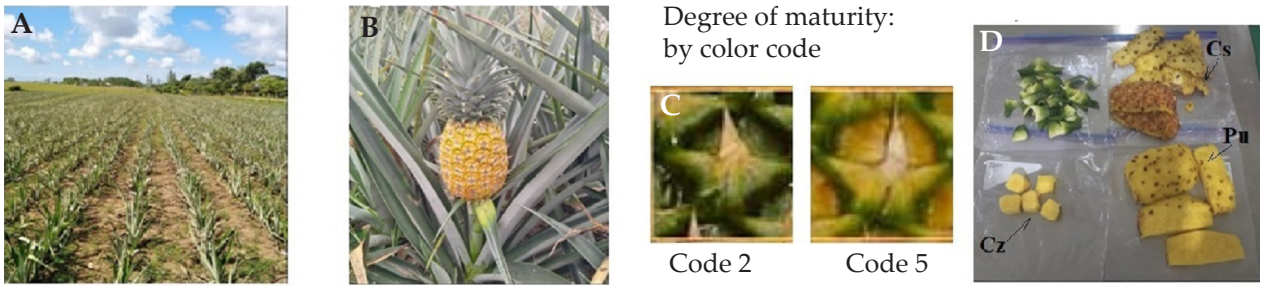

Figure 1. Representative images of the *Ananas comosus* L. plantation and fruits used in the study. A: plantation in Loma Bonita, Oaxaca; B: ripe fruit (C5); C: maturity degree classification; D: material used to obtain the fresh pulp extract (Pu) and the peel (Cs), crown (Co), and core (Cz) residues.

Table 1. Average weight of pulp and residue of pineapple (*Ananas comosus* L.) fruits at two ripening stages. Averages of four monthly samplings, May to August 2019.

Part of the fruit	Maturity stage			
	C2		C5	
	kg	% w/w*	kg	% w/w*
Peel	0.843	30.7	0.978	32.1
Crown	0.543	19.8	0.358	11.8
Core	0.462	16.8	0.586	19.2
Pulp	0.899	32.7	1.124	36.9
Total:	2.747	100	3.046	100

*Weight proportion of residues compared to the whole fruit.

At both ripening stages, pulp accounted for 33 and 37 % (w/w) of the average total fruit weight, while the total sum of residue weight (peel, core, and crown) accounted for 67 and 63 % (w/w) in C2 and C5 pineapples, respectively. These data were similar to those obtained by Ketnawa *et al*. (2012) and Conesa *et al*. (2015) for 'Nang Lea' and 'Phu Lea' varieties, where the residues (peel, core, stem, and crown) produced during processing represented approximately 50 % (w/w) of the total fruit weight. These authors indicate that peel and core constituted the main residues, which was confirmed in the present study as peel and core represented 47.5 and 51.3 % w/w of the residues in pineapples at C2 and C5 stages, respectively. The municipality of Loma Bonita produced approximately 123 038 Mg of pineapple in 2021, of which 74 901 were of the 'Cayena Lisa' variety (SIAP, 2022). The usable industrial waste in the process of extracting by-products, including cysteine-proteases, is approximately 37 450 Mg per season; therefore, the potential for useful raw material is enormous.

Regarding the physicochemical characterization (Table 2), at the C2 ripening stage, the pH values of the peel were the lowest compared to the other residues, and did not show significant variations in any of the four months of harvest (Figure 2A).

Table 2. pH values of various parts of the fruit of pineapple (*Ananas comosus* L.) varieties.

Variety	Part of the fruit			
	Peel	Core	Other	Reference
Morris	4.04 ± 0.02	3.89 ± 0.01	3.92 ± 0.01 (pulp)	Misran *et al.* (2019)
N36	3.94 ± 0.01	--	--	Nadzirah *et al.* (2012)
Phu Lea	4.01± 0.13	4.09± 0.22	4.64± 0.22 (stem)	Ketnawa *et al.* (2012)
Nang Lea	4.02 ± 0.30	4.27 ± 0.24	4.76 ± 0.16 (stem)	
Cayena Lisa C2	**3.91 ± 0.17**	**4.98 ± 0.22**	**4.61± 0.97 (pulp)**	**This study**
Cayena Lisa C5	**4.20 ± 0.49**	**4.48± 0.47**	**5.26 ± 0.84 (pulp)**	**This study**

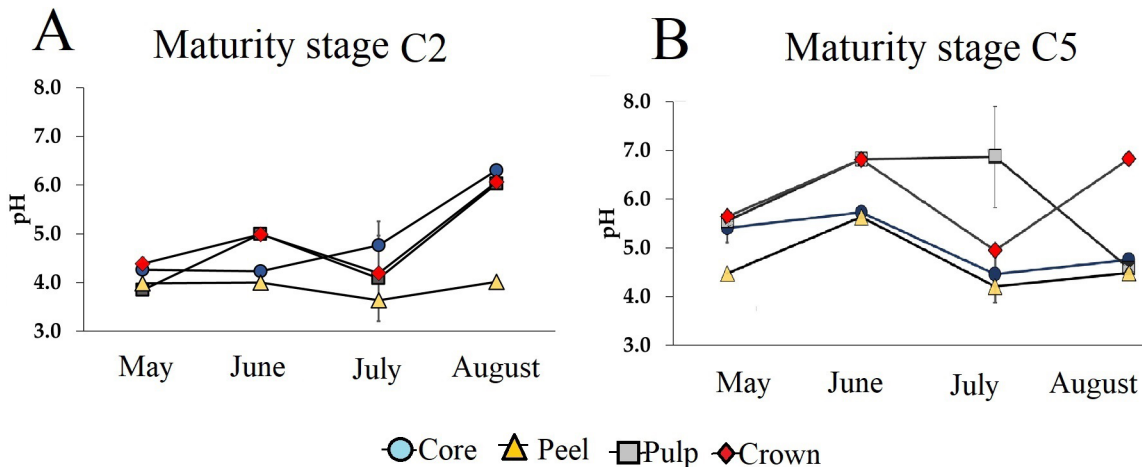

Figure 2. pH values in pineapple (*Ananas comosus* L.) from different parts of the fruit at two maturity stages, during the months of harvest (n = 3).

The pH values of the core, pulp, and crown remained at around 4 to 5 for the first three months and increased significantly ($p \geq 0.05$) in August with comparison to the peel values, showing no differences between them. The final pH value of the fruit components, except the peel at this stage of ripening, was close to 6 (Figure 2A).

In the case of samples at the C5 ripening stage, the pH values of each fruit component varied throughout the months (Figure 2B). In the month of May, the pH of the peel was 4.01±0.01, which was significantly lower than that of the other residues (around 5, on average). In June, the pH of the pulp, peel, and crown increased; the core showed no significant changes. In July, except for pulp, pH decreased in all residues. In August, the pH values of the crown (6±0.01) were significantly higher than those of the peel, core, and pulp (around 4).

The average pH of the residues during the period analyzed was 4.64±0.69 and 4.82±0.45 for C2 and C5, respectively, which are higher than the values described for 'Cayena Lisa' grown in China, which ranged from 3.58 to 3.86 (Lu *et al*., 2014), or for 'Cayena Sarawak' at maturity stage 5 (maximum maturity). Soloman *et al*. (2016) observed that the mean pH value was around 3.88±0.18, and suggested that it is an internal indicator of maturity that can be used to determine the best harvesting period. The pH of a fruit is the result of the mixture of various compounds; Truc *et al*. (2008) observed that the acid composition of unripe pineapple includes mainly citric acid, malic acid, and succinic acid, which decrease during fruit ripening following a conversion of organic acids to reducing sugars (Siti Rashima *et al*., 2021). These physicochemical changes give pineapple its characteristic flavor and odor.

Another important parameter in the physicochemical characterization of fruit is SST, which is usually expressed as a percentage or °Brix. At ripening stage C2 (Figure 3A),

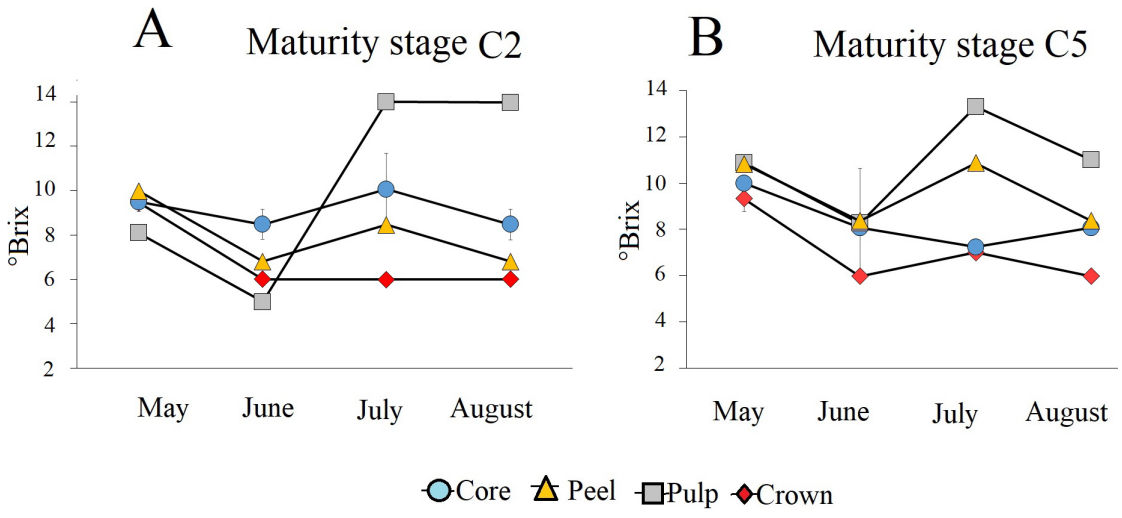

Figure 3. Average total soluble solids content (°Brix) of pineapple (*Ananas comosus* L.) pulp and residues at two maturity stages, during the harvest months (n = 3).

core SST values did not show significant differences in any of the months and remained between 8.3 and 10 °Brix, while those of the pulp and peel decreased significantly in July and August compared to May (Figure 3A). This indicates that from May and June onwards, the concentration of soluble solids, probably sugars, increases. The residue with the lowest SST was the peel, while the pulp showed the highest SST values (around 13) and these remained stable over time. On the other hand, at the C5 ripening stage, the pulp had the highest SST values, followed by the peel, core, and crown; this trend repeated itself in all harvesting months. Pineapple 'Cayena' is described as one of the cultivars with high SST values compared to other varieties. The results of this study for *A. comosus* cv. 'Cayena Lisa' (8 to 14 °Brix) are higher than the cultivars 'Nang Lae', 'Phu Lae', 'N36', and the 'Morris' variety, where the °Brix range from 2.55 for peel to 6.27 for crown (Ketnawa *et al*., 2012; Misran *et al*., 2019).

Finally, the results indicated the presence of cysteine protease activity in the pulp and in all pineapple residues (Figure 4). The CDU activity was very similar, regardless of residue type or ripening stage. Proteolytic activity was minimal in the period from May to July in all parts analyzed, except the crown in May. In August, activity increased significantly in the pulp and in all residues.

Regardless of maturity stage, the maximum activity for all tissues occurred in August, though the parts showed differential proteolytic activity in this month. This could be due to the functions of proteases within the plant, as explained below. At stage C5, the peel had the highest activity and the crown had the lowest (Figure 5). The activity of cysteine proteases at the C2 ripening stage in the pulp and peel (20.48 and 17.39 mL^{-1} mg^{-1}) was significantly higher than that in the core and crown (16 and 2.84 CDU mL^{-1} mg^{-1}, respectively). In the case of C5 samples core and peel (15.73 and 21.85 CDU mL^{-1} mg^{-1}, respectively) had significantly higher activity compared to pulp and crown.

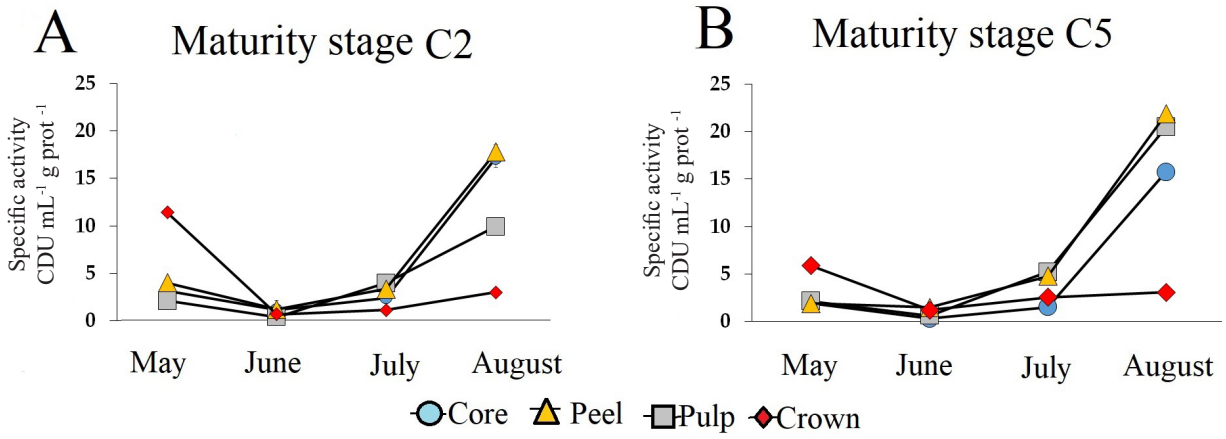

Figure 4. Cysteine protease activity in pulp and residues of pineapple (*Ananas comosus* L.) of two ripening stages, during the months of harvest (n = 3).

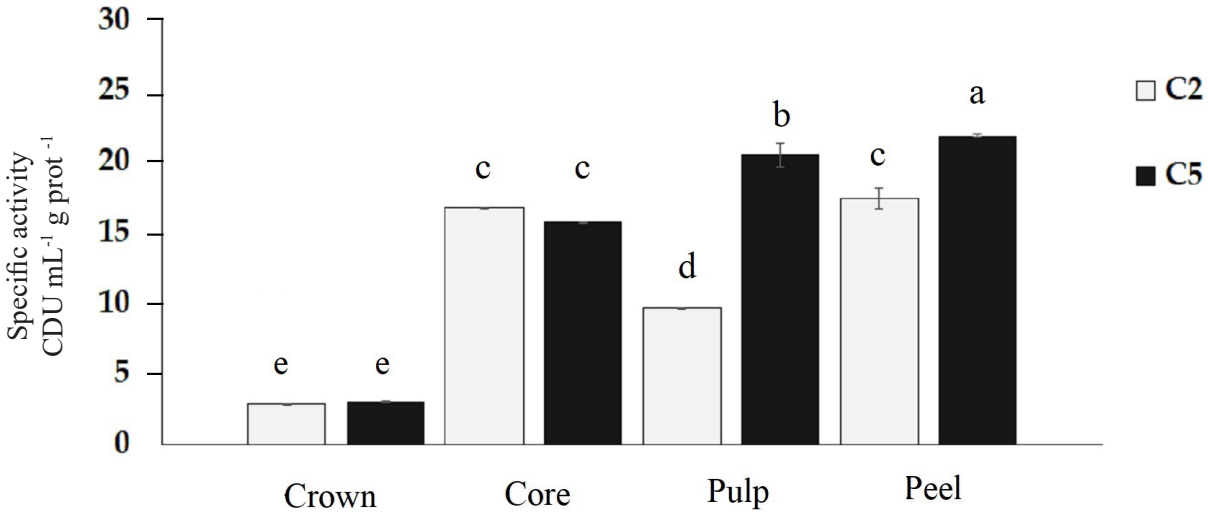

Figure 5. Proteolytic activity of different pineapple parts corresponding to the month of August for two ripening stages (n = 3; different letters indicate significant differences, Tukey $p \leq 0.05$).

This behavior is similar to that described by other authors; for example, in cultivars 'Nang Lae' and 'Phu Lae', it was observed that the extract from each type of residue exhibited different proteolytic activity and protein content: the highest value was from the crown, while the extract from the stem had the lowest value in both cultivars (Vasiljevic, 2019). On the other hand, pineapple core extracts have greater effect on meat tenderizing than peel and pulp extracts; this may indicate that pineapple core contains bromelain with higher enzymatic activity than that of any other part of the fruit (Clavijo *et al*., 2012).

Cysteine-proteases are involved in the control of apoptosis, the hypersensitive response (HR), senescence, embryogenesis, floral development, several types of environmental stress, and pathogen resistance in various plant species (Grudkowska and Zagdańska, 2004; López-García *et al.*, 2012). Hydric stress is one of the most common stresses in plants; although studies of its effect on pineapple are scarce, drought is known to delay flowering and affects fruit morphology and quality (Ortiz, 2022).

One of the mechanisms of stress response includes protein degradation by proteases, which is closely related to the synthesis of new proteins. On the other hand, although the effect of excess moisture on protease activity has been less studied, it is known to affect fruit morphology (water core) and increase susceptibility to pathogen attack, particularly fungi (Shu, 2019). The above suggests that seasonal environmental factors could influence protease activity. From May to August, in the Loma Bonita area, rainfall gradually increases; the month with the most rainfall is August (CONAGUA, 2022) which coincides with the maximum peak of cysteine protease activity (Figure 6). Temperature is another environmental factor to consider.

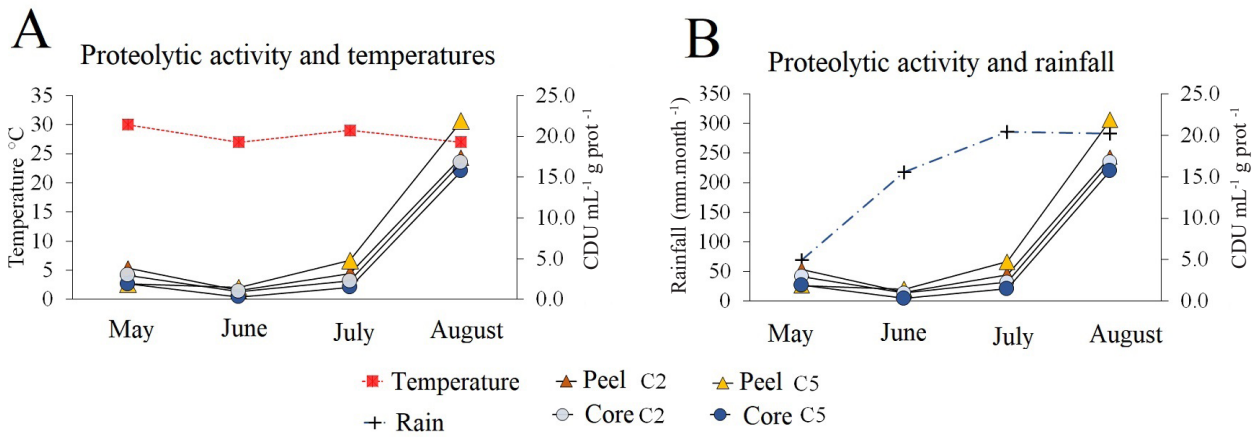

Figure 6. Relationship of proteolytic activity of pineapple fruit parts with environmental conditions of temperature and rainfall during the harvest months.

The record does not show significant differences in temperature during the sampling months; however, such months correspond to the hottest time of the year in Mexico (CONAGUA, 2022). In particular, the period of highest temperature during the year is known as "canícula", which in 2019 lasted 40 days and officially ended on August 20 of that year.

As indicated, the edible portion of pineapple fruit is about 35 %. Waste in the canning industry ranges between 45 and 65 %, and includes, among others, peel and trimmings (Difonzo *et al*., 2019), which, due to their large volume, can become a focus of microbiological and environmental infection. Therefore, revaluing waste by obtaining by-products is an attractive alternative; in particular, obtaining enzymes is interesting, as there is a market for them. Extracts obtained from *A. comosus* residues can also be a source of phosphatases, glucosidases, peroxidases, cellulases, glycoproteins, and carbohydrates, in addition to cysteine-endopeptidases.

CONCLUSIONS

Two stages of pineapple fruit ripening (C2 and C5) were evaluated, and both showed different behaviors for pH and SST. The pH of fruit increased during the C2 stage and peaked in August; on the contrary, in the C5 stage, pH increased in June and decreased again in August. On the other hand, SST decreased compared to May and remained stable until August. As for cysteine protease activity, the maximum values occurred in August in all parts of the fruit. In particular, the peel and core sections had a significantly higher activity than the pulp and crown in both ripening stages, which represents an advantage since proteases can be obtained from these residues, remaining as an element to be considered in the value chain of the commercialization of the 'Cayena Lisa' pineapple in the Papaloapan Basin region.

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REFERENCES

Arshad ZIM, Amid A, Yusof F, Jaswir I, Ahmad K, Loke SP. 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

Banerjee S, Ranganathan V, Patti A, Arora A. 2018. Valorisation of pineapple wastes for food and therapeutic applications. Trends in Food Science & Technology 82 (1): 60–70. https://doi.org/10.1016/j.tifs.2018.09.024

Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of Protein-Dye binding. Analytical Biochemistry 72 (1–2): 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Clavijo D, Portilla-Martinez MC, Quijano-Parra A. 2012. Cinética de la bromelina obtenida a partir de la piña perolera (*Ananas comosus*) de Lebrija-Santander. Bistua: Revista de la Facultad de Ciencias Básicas 10(2): 41–49.

CONAGUA (Comisión Nacional de Agua). 2022. Información estadística climatológica. Estación Loma Bonita 20063. Gobierno de México, Comisión Nacional de Agua. Ciudad de México, México. https://smn.conagua.gob.mx/es/climatologia/informacion-climatologica/informacion-estadistica-climatologica (Retrieved: June 2023).

Conesa C, García-Breijo E, Loeff E, Seguí L, Fito P, Laguarda-Miró N. 2015. An electro chemical impedance spectroscopy-based technique to identify and quantify fermentable sugars in pineapple waste valorization for bio-ethanol production. Sensors 15 (9): 22941–22955. https://doi.org/10.3390/s150922941

Difonzo G, Vollmer K, Caponio F, Pasqualone A, Carle R, Steingass CB. 2019. Characterisation and classification of pineapple (*Ananas comosus* [L.] Merr.) juice from pulp and peel. Food Control 96: 260–270. https://doi.org/10.1016/j.foodcont.2018.09.015

García-Tain Y, Pérez-Padrón J, García-Pererira A, Hernández-Gómez A. 2011. Determinación de las propiedades de calidad de la piña (*Ananas Comosus*) variedad Cayena Lisa almacenada a temperatura ambiente. Ciencias Técnicas Agropecuarias 20 (1): 62–65.

Grudkowska M, Zagdańska B. 2004. Multifunctional role of plant cysteine proteinases. Acta Biochimica Polonica 51 (3): 609–624. https://doi.org/10.18388/abp.2004_3547

Hikal WM, Mahmoud AA, Said-Al Ahl HAH, Bratovcic A, Tkachenko KG, Kačániová M, Rodriguez RM. 2021. Pineapple (*Ananas comosus* L. Merr.), waste streams, characterisation and valorisation: an overview. Open Journal of Ecology 11 (9): 610–634. https://doi.org/10.4236/oje.2021.119039

Ketnawa S, Chaiwut P, Rawdkuen S. 2012. Pineapple wastes: A potential source for bromelain extraction. Food and Bioproducts Processing 90 (3): 385–391. https://doi.org/10.1016/j.fbp.2011.12.006

López-García B, Hernández M, Segundo BS. 2012. Bromelain, a cysteine protease from pineapple (*Ananas comosus*) stem, is an inhibitor of fungal plant pathogens. Letters in Applied Microbiology 55 (1): 62–67. https://doi.org/10.1111/j.1472-765x.2012.03258.x

Lu XH, Sun DQ, Wu QS, Liu SH, Sun GM. 2014. Physico-chemical properties, antioxidant activity and mineral contents of pineapple genotypes grown in China. Molecules 19 (6): 8518–8532. https://doi:10.3390/molecules19068518

Market Reports World. 2022. Global bromelain market 2022 by manufacturers, regions, type and application, forecast to 2028. Pune, India. https://www.marketreportsworld.com/global-bromelain-market-19899147 (Retrieved: July 2023).

Misran E, Idris A, Mat Sarip SH, Ya'akob H. 2019. Properties of bromelain extract from different parts of the pineapple variety Morris. Biocatalysis and Agricultural Biotechnology 18: 101095. https://doi.org/10.1016/j.bcab.2019.101095

Mohd Ali M, Hashim N, Abd Aziz S, Lasekan O. 2020. Pineapple (*Ananas comosus*): A comprehensive review of nutritional values, volatile compounds, health benefits, and potential food products. Food Research International 137 (1): 109675. https://doi.org/10.1016/j.foodres.2020.109675

Nadzirah KZ, Zainal S, Noriham A, Normah I, Siti AM. 2012. Physico-chemical properties of pineapple crown extract variety N36 and bromelain activity in different forms. APCBEE Procedia 4: 130–134. https://doi.org/10.1016/j.apcbee.2012.11.022

NMX-F-103-1982 (Norma Mexicana). 1982. Alimentos-determinación de grados Brix en alimentos y bebidas método de ensayo http://diariooficial.gob.mx/nota_detalle.php?codigo=5075420&fecha=23/12/2008#gsc.tab=0 (Retrieved: July 2023).

NMX-FF-028-SCFI-2008 (Norma Mexicana). 2008. Productos alimenticios no industrializados para consumo humano —fruta fresca—Piña (*Ananas comosus* var. *comosus*)–especificaciones. http://www.economia-nmx.gob.mx/normas/nmx/2009/nmx-ff-028-scfi-2008.pdf (Retrieved: July 2023).

NOM-F-137-S-1978 (Norma Oficial Mexicana). 1978. Determinación de pH en alimentos". Secretaría de Patrimonio y Fomento Industrial. https://dof.gob.mx/nota_detalle.php?codigo=4704689&fecha=23/05/1978&print=true (Retrieved: July 2023).

Ortiz RA. 2022. Abiotic stress factors influencing calcium nutrition of export bananas (*Musa* sp.) and pineapples (*Ananas comosus*) in Central America. Acta Horticulturae 1333: 99–106. https://doi.org/10.17660/ActaHortic.2022.1333.14

Santos DI, Martins CF, Amaral RA, Brito L, Saraiva JA, Vicente AA, Moldão-Martins M. 2021. Pineapple (*Ananas comosus* L.) by-products valorization: novel bio ingredients for functional foods. Molecules 26 (11): 3216. https://doi.org/10.3390/molecules26113216

Seleni MM, Canniatti SG, dos Santos CT, Ratnayake WS, Flores RA, Bianchini A. 2014 Characterization and potential application of pineapple pomace in an extruded product for fiber enhancement. Food Chemistry 163: 23–30. https://doi.org/10.1016/j.foodchem.2014.04.076

Shu HY, Sun W, Li KM, Xu GY, Zhan RL, Chang SH. 2019. The cause for water-heart fruit of pineapple and protective measurements. American Journal of Plant Sciences 10 (6): 885–892. https://doi.org/10.4236/ajps.2019.106063

SIAP (Servicio de Información Agroalimentaria y Pesquera). 2022. Anuario Estadístico de la Producción Agrícola. Gobierno de México, Servicio de Información Agroalimentaria y Pesquera. Ciudad de México, México. https://nube.siap.gob.mx/cierreagricola (Retrieved: June 2023).

Siti Rashima R, Azhar ME, Maizura, M. 2021. Influence of post-harvest physiology on sensory perception, physical properties, and chemical compositions of Morris pineapples (*Ananas comosus* L.). Journal of Food Sciences 86 (9): 4159–4171. https://doi.org/10.1111/1750-3841.15877

Soloman DG, Razali Z, Somasundram C. 2016. Physiochemical changes during growth and development of pineapple (*Ananas comosus* L. Merr. cv. Sarawak). Journal Agricultural Sciences and Technology 18: 491–503.

Truc TT, Binh LN, Muoi NV. 2008. Physico-chemical properties of pineapple at different maturity levels. The First International Conference on Food Science and Technology, Can Tho, Vietnam, 130–134.

Uriza-Ávila DE, Torres-Ávila A, Aguilar-Ávila J, Santoyo-Cortés VH, Zetina-Lezama R, Rebolledo-Martínez A. 2018. La piña mexicana frente al reto de la innovación. Avances y retos en la gestión de la innovación. Colección Trópico Húmedo. Universidad Autónoma Chapingo: Texcoco, México. 479 p.

Vasiljevic T. 2019. Chapter 10 – Pineapple. *In* Charis M. Galanakis. (ed.), Valorization of fruit processing by-products. Academic Press: Cambridge, MA, USA. pp: 203–225.