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Optimization of Pasteurization Conditions for Pineapple Juice Quality Preservation

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Abstract: The food industry and consumers are interested in the health advantages of bromelain and vitamin C. Since thermal treatment has been shown to degrade the nutritional value of fruit juices, it was necessary to optimize the pasteurization conditions in order to retain as much bromelain and ascorbic acid as possible in pineapple juice. This study investigates the effect of pasteurization on pineapple juice using N36 variety by optimizing pasteurization conditions, which are temperature and time. The optimization study was done using central composite design. The pasteurization was done accordingly within the temperature range of 75°C to 95°C and time range of 10 seconds to 1800 seconds. Total soluble solids, ascorbic acid content and bromelain activity were determined as the response for the optimization of pasteurization conditions. Fresh pineapple juice which is an unpasteurized sample was assessed for its physicochemical properties, nutritive qualities, and microbiological count to compare these attributes with the optimized pasteurized sample. This study found that optimal pasteurization settings of pineapple juice are at temperature of 81.5°C for 784.6 seconds, with desirability near to 1. The response of total soluble solids, ascorbic acid content and bromelain activity are all maximized and yielded values of 9.695°Brix, 0.063 mg/L, and 0.026 CDU/mL, respectively. The optimized pasteurization conditions discovered in this study may be used as a reference by the pineapple juice manufacturers.

Keywords: Ascorbic acid, Bromelain, Pasteurization, Pineapple juice, Optimization

1. Introduction

Pineapple (*Ananas comosus*) is a significant subtropical fruit that can be consumed fresh or processed in a variety of ways [1]. Further processing is carried out to produce some products that are more shelf stable such as pineapple pulp, concentrated pineapple juice, and dried pineapple and most crucially, pasteurized pineapple juice [2]. Pineapple on its own has minerals and antioxidants, such as ascorbic acid, carotenoid, flavonoids, and bromelain which are abundant [3]. Bromelain is a naturally occurring anti-inflammatory enzyme that is also a good source of dietary fiber for the immune and

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digestive systems [4]. Due to the presence of bioactive compounds that are beneficial for human health, people are becoming more aware of the importance of pineapple juice. People take much more interest in the product as it is easier and convenient to consume as liquid rather than in fruit form.

Pineapple juice is pasteurized with a mild heat treatment, which is also known as partial sterilization, to make it safe for human consumption as well as to improve shelf life and storage quality by killing pathogens with heat. Due to high temperature of pasteurization, vitamin C and some major elements such as potassium can be easily lost [5]. Other than that, freshly produced pineapple juice has a short shelf life and must be stored in the refrigerator [6]. After opening pasteurized pineapple juice from can or bottle, it must be continuously refrigerated for it to last for about 7 to 10 days without being contaminated [6]. It is a challenge for the industry to process the fresh pineapple juice into pasteurized juice that retains the maximum amount of nutrients and extended shelf life without addition of additives and preservatives [7].

As a result, research into pasteurization condition optimization is essential for high-quality pineapple juice preservation. Optimization of pineapple juice can be done by using an experimental design which is response surface methodology (RSM). It is vital to find out what had already been done in this regard as part of pasteurized pineapple juice quality research. This study focused on optimizing the pasteurization conditions for pineapple juice quality preservation by minimizing the loss of nutrients such as vitamin C, and especially bromelain while obtaining pineapple juice with the highest amount of nutrients possible after pasteurization of pineapple juice. The physicochemical properties, nutritional qualities and microbiological quality between the optimized pasteurized pineapple juice and unpasteurized pineapple juice were also compared.

2. Materials and Methods

2.1 Materials

The fresh pineapple (N36 cultivar) was bought from a local market in Pagoh Jaya, Johor, Malaysia. Pineapple fruit N36 cultivar was used because it has particularly higher sugar and less acidity than other cultivar which is suitable for juice production [8]. Pineapple was obtained by buying the harvested fruits that are free from bruises or damage. The fruit was selected at the maturity stages of fully ripe (index 7) which is determined by observing its color (100% bright yellow).

Chemicals used in this experiment were potassium permanganate ($KMnO_4$) for ascorbic acid determination, as well as 0.65% casein solution, 110 mM trichloroacetic acid, Folin-Ciocalteau reagent and 500 mM sodium carbonate for bromelain activity determination. Sodium hydroxide and phenolphthalein were used for total titratable acidity (TTA) determination. Buffer solutions of pH 4 and pH 7 were used during pH determination. For microbial analysis, nutrient agar and saline solution were used.

2.2 Method

2.2.1 Pineapple juice preparation

Pineapple juice preparation method was conducted according to Oforiwaah [7]. The crown of pineapple was removed before washing the fruit to eliminate dirt. The skin of pineapple was peeled by hand using sharp knife and sliced into small parts. Then, pineapple juice was extracted using multifunction blender (MX800S, Panasonic, Malaysia). After extraction, pineapple juice was filtered using a sieve or cheesecloth to remove any foreign particles.

2.2.2 Pasteurization of pineapple juice

Condition of pasteurization was optimized by Response Surface Methodology (RSM) according to Chin & Lee [1]. Central composite design (CCD) was used as the experimental design to optimize the pasteurization conditions of pineapple juice. Design Expert software (Version 13, Stat-Ease Inc., Minneapolis) was used for the optimization. It was done by generating a set of experimental trials with 11 runs as shown in Table 1. This experiment was conducted using two factors (temperature and time) and three responses (total soluble solids, ascorbic acid content and bromelain concentration).

Pasteurization method was done according to Hounhouigan et al. [2]. 15ml of filtered pineapple juice was put into sterilized bottles and then was pasteurized using water bath (SWB Series, Stuart, China) at varying temperature (75℃ – 95℃) and time (10 – 1800 s). After treatment is done, the bottle was immediately put in the ice-water bath for cooling down.

Table 1: Physicochemical properties for all samples (unpasteurized and pasteurized)

Run No.	Factor		Response				
	Temp. (℃)	Time (s)	TSS (°Brix)	AA (mg/L)	Bromelain (CDU/mL)	TTA (% citric acid)	pH
0	0	0	13.63±0.0577	0.062±0.0012	0.101±0.0005	1.321±0.0005	3.46±0.0702
1	95	905	5.4±0.0577	0.060±0.0015	0.020±0.0025	0.726±0.0017	3.26±0.0152
2	95	1800	5.4±0.0577	0.049±0.0021	0.022±0.0020	1.089±0.0005	3.21±0.0208
3	95	10	5.6±0.0577	0.059±0.0021	0.027±0.0015	0.961±0.0010	3.32±0.0152
4	85	10	8.2±0.100	0.061±0.0011	0.026±0.0005	0.960±0.0005	2.96±0.1205
5	85	905	9.8±0.0577	0.061±0.0005	0.027±0.0010	1.046±0.0020	4.28±0.0754
6	75	10	8.4±0.0601	0.059±0.0010	0.026±0.0012	0.865±0.0015	3.03±0.0550
7	85	905	9.8±0.0577	0.061±0.0015	0.026±0.0006	1.046±0.0005	4.27±0.0585
8	85	905	9.9±0.100	0.061±0.0005	0.027±0.0010	1.046±0.0017	4.28±0.0754
9	85	1800	8.7±0.1154	0.060±0.0015	0.027±0.0005	1.063±0.0010	4.07±0.0808
10	75	1800	9.0±0.2081	0.060±0.0006	0.026±0.0010	0.845±0.0011	2.90±0.0556
11	75	905	8.1±0.100	0.063±0.0015	0.026±0.0006	0.820±0.0005	3.12±0.0057

The data was expressed in mean ± standard deviation

2.2.3 Determination of total soluble solid (TSS) content

Determination of TSS was done according to Leneveu-Jenvrin et al. [6]. TSS was expressed in °Brix by measuring juice using pocket refractometer (PAL-1, Atago CO. LTD, Japan). Before it is used for measuring TSS, it was calibrated with distilled water. Then, two or three drops of samples were put on the refractometer prism, and the reading was observed after a few seconds. The procedure was done for all the pasteurized samples after ensuring that the prism was rinsed with distilled water and dried with a soft, lint-free tissue.

2.2.4 Determination of ascorbic acid (AA) content

The ascorbic acid was determined using spectrophotometric method according to Elgailani et al. [8]. UV-Visible spectrophotometer (T60U, PG Instruments, UK) was used for the analysis. The samples of pasteurized juice were accurately taken 10 mL and was transferred into test tubes. Each test tube was added with 1.0 mL of potassium permanganate (100 μg/mL). Each test tube contents were thoroughly mixed and then before stood for five minutes. The prepared solutions were then read at 530 nm against blank using a suitable concentration by a spectrophotometer for the analysis.

2.2.5 Determination of Bromelain concentration

The bromelain concentration analysis was done using spectrophotometric method according to Dolino et al [15]. One casein digestion unit (CDU) was defined as 1 g of L-tyrosine liberated per minute per mL of a sample when casein is digested at 37°C and pH 7.0 for 10 min. Bromelain activity was quantified in CDU/ml. By using a UV-Visible spectrophotometer (T60U, PG Instruments, UK) set at 660 nm, the samples were read in comparison to an L-tyrosine calibration curve.

2.2.6 Optimization and validation

The optimization of pasteurization conditions and validation of data were done using ANOVA with temperature and time desirability close to 1. The response of TSS, ascorbic acid and bromelain are all expected to be maximized.

2.2.7 Determination of total titratable acidity (TTA)

Simple titration with 0.1 N sodium hydroxide according to Chin & Lee [1] was used to determine the total acidity of pasteurized pineapple juice. In a conical flask containing 45 mL of distilled water and 2 drops of phenolphthalein as an indicator, 5 mL of juice was added. Drop by drop, while whirling, the sodium hydroxide was added to the solution until the color changes from colorless to bright pink at the end point. The volume of sodium hydroxide used to reach the end point was recorded and the total acidity can be calculated as shown in Eq 1.

$$TTA = \frac{N * V * \frac{M}{\#\ Hydrogen\ ions}}{S * 10} g/100mL \qquad Eq\ 1$$

where
N = normality of sodium hydroxide,
V = volume of sodium hydroxide,
M = molecular weight of predominant acid (acetic acid),
S = sample in g/mL

2.2.8 pH

The pH of samples was determined using method by Oforiwaah [7]. At room temperature, pH of the juice was determined by using digital pH meter (pH 700, Eutech Instruments, Singapore) which was calibrated first using buffer solutions of pH 4 and pH 7. The juice sample was put in a 100-ml beaker and properly mixed. Then, the pH electrode was submerged in the beaker and the reading of pH probe was taken after the reading is stabilized.

2.2.9 Microbial analysis

Total viable count of pasteurized pineapple juice was investigated according to Chadaré et al. [3] to examine if the pasteurization method had any effect on microbial count. Selective media, nutrient agar was prepared and agar plates was incubated at 37°C for 24 hours. For total plate count, 1.0 mL of juice was diluted with 10 mL of saline solution. Juice samples was diluted in saline serially and spread plate method was used. The plates were incubated at 37°C for 24 hours after inoculum was distributed evenly. In the proper selective media, this experiment was done in triplicates. To count bacteria, colony forming units (CFU) was used. The average number of bacteria per plate was divided by the sample volume and represented as CFU/100mL (AOAC, 2005).

2.2.10 Statistical analysis

Statistical analysis method was done according to Oforiwaah [7]. The findings of the study were collected to determine the mean and standard deviation. Then, it was analyzed through two-way analysis of variance (ANOVA). At a 5% probability, ANOVA was used to determine the statistical significance of the model terms. Then, multiple comparison test, Tukey test was used as to determine that the means for comparison of parameters between optimized pasteurized samples and unpasteurized samples are significantly different.

3. Results and Discussion

3.1 Effect of pasteurization on total soluble solid (TSS) of pineapple juice

Table 1 and Figure 1 show that compared to other pasteurized samples, unpasteurized samples have considerably higher TSS (13.63°Brix). While samples pasteurized at 85°C/905s are the highest among pasteurized samples with a total Brix value of 9.8–9.9, samples pasteurized at 95°C/905s and 95°C/1800s both had the lowest total Brix values (5.4°Brix). This indicates that the samples pasteurized at temperature of 85°C is sweeter than pasteurized at temperature of 95°C. The decline may have been caused by microbial metabolic processes that converted the samples carbohydrates to organic acids, causing pH to drop, deterioration, and a shorter shelf life [9].

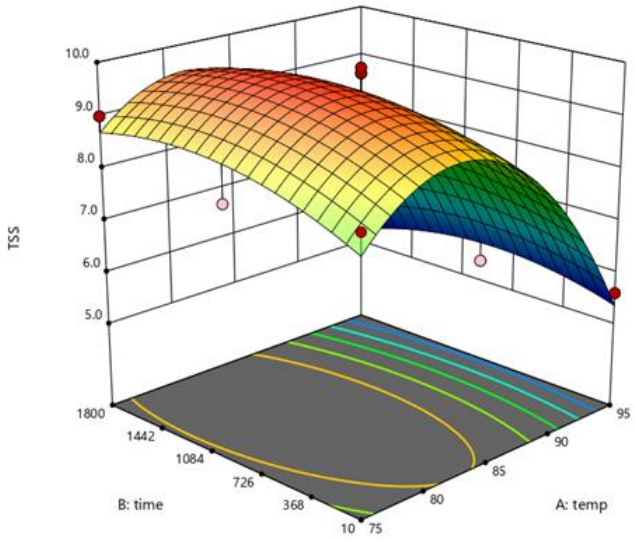

Figure 1: Surface plot for the effect of temperature (°C) and time (s) on the TSS of pineapple juice

3.2 Effect of pasteurization on ascorbic acid (AA) content of pineapple juice

Table 1 and Figure 2 show the information on ascorbic acid concentrations in unpasteurized and pasteurized samples. As displayed in Table 1, the sample that was pasteurized at 75°C/905s has the maximum content of ascorbic acid (0.06265 mg/L). Meanwhile, the sample that has been pasteurized at 95°C for 1800s has the lowest content of ascorbic acid (0.049 mg/L). Ascorbic acid is a polar molecule that is thermally sensitive. Therefore, once a raw material has been pasteurized, it is vulnerable to deterioration [17].

Heat treatment, especially at high temperature and lengthy time, certainly affected the ascorbic acid content in pineapple juice. This study demonstrates that pasteurization at 95°C for 1800 s is not suitable for keeping the maximum quantity of ascorbic acid. Therefore, low-temperature long-time (LTLT)

pasteurization might be more suitable for retaining the content of ascorbic acid in pineapple juice such as in this study at 75°C for 905 s and 75°C for 1800 s which yield 0.063 mg/L and 0.06077 mg/L.

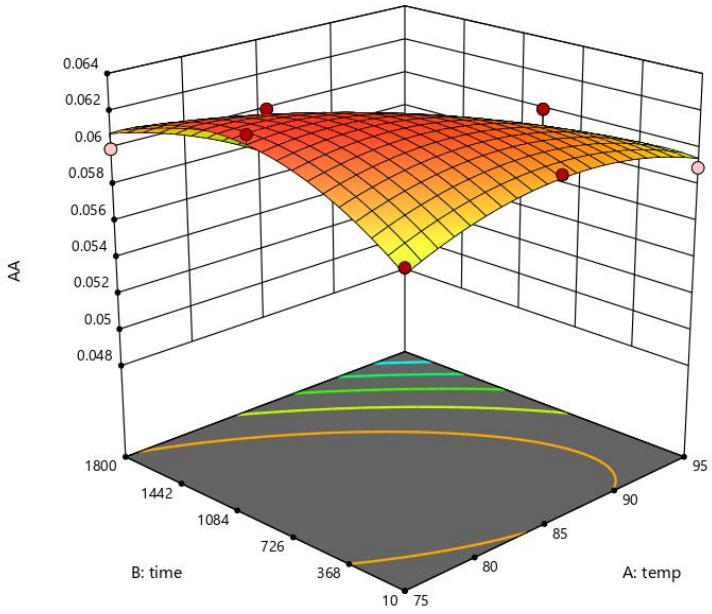

Figure 2: Surface plot for the effect of temperature (°C) and time (s) on the ascorbic acid content of pineapple juice

3.3 Effect of pasteurization on bromelain activity of pineapple juice

Data in Table 1 and Figure 3 show that 0.101 CDU/mL is the highest amount of bromelain activity which is contained in the unpasteurized sample. All the pasteurized samples mostly have nearly the same amount of bromelain activity. The heat treatment that the pasteurized samples underwent has greatly affected the bromelain activity in pineapple juice. The bromelain activity completely inactivated at higher temperature (80°C) than lower temperature (40°C - 50°C) [11]. Due to processing or other treatments, the bromelain concentration in commercially available pineapple juice is typically low or totally lost, which is why the bromelain from pineapple was isolated to be utilized for other purpose, mainly as medicine due to its health benefits [12].

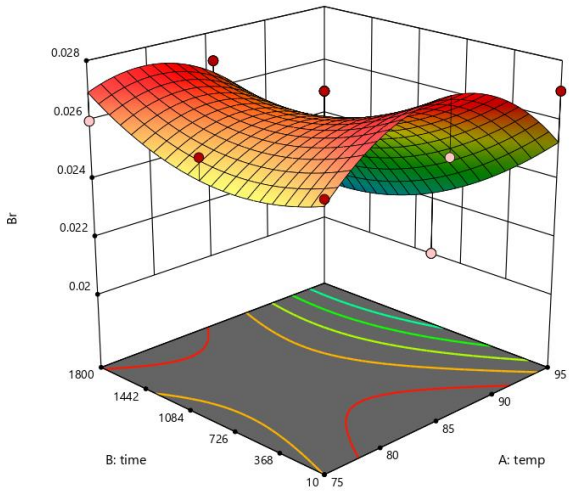

Figure 3: Surface plot for the effect of temperature (°C) and time (s) on the bromelain activity of pineapple juice

3.4 Optimization and validation of pasteurization conditions

The optimal pasteurization conditions for pineapple juice are at temperature 81.5°C for 784.6 s, with desirability near to 1, which is 0.947. The response of TSS, AA content and bromelain activity are all maximized and yielded predicted and experimental values as shown in Table 2 with error less than 10%.

Table 2: Predicted and experimental values at optimum conditions (81.5°C/ 784.6s)

Responses	Predicted	Experimental
TSS (°Brix)	9.695	9.800
AA content (mg/L)	0.063	0.067
Bromelain activity (CDU/mL)	0.026	0.025

3.5 Total titratable acidity (TTA) of pineapple juice

As pineapple juice is regarded as a fruit juice with a high acidity level, TTA is a crucial characteristic. It is clear from Table 1 that the unpasteurized sample has the highest TTA (1.321%). The unpasteurized sample had the maximum titratable acidity since it was promptly analyzed after extraction. It is freshly made and is not processed in any way; citric acid can therefore be maintained in fresh pineapple juice. The lowest TTA (0.726%) can be seen in pasteurized samples at 95°C/905s. This is because pasteurization takes a long time and at a high temperature.

Since the pineapple fruit from the N36 cultivar is inherently lower in acidity and higher in sugar than other cultivars, the pineapple variety N36 employed in this experiment may also have an impact on the proportion of acidity present in the juice [10]. Othman [13] revealed that the decline in the titratable acidity of pineapple juice is caused by the loss of the predominant acid which is citric acid during pineapple ripening.

3.6 pH of pineapple juice

As can be seen in Table 1, the pH for each sample is able to maintain within the range suggested by United States of Food and Drug Administration (USFDA) 2004 which is in range of 3.50 until 4.00. Higher pH that goes beyond 4.6 is not desirable in pasteurized pineapple juice because the spore of *Clostridium botulinum* can grow in food products that have pH above 4.6 with the absence of oxygen [17]. The spore is deadly as it produced extremely potent neurotoxin [17]. It is also hard to kill and can survive for longer time in food and beverage products, therefore it is critical for pH to be maintained under 4.6 [16]. Foods with pH above 4.6 is considered as low-acid foods and pineapple juice is categorized as high-acid food. However, low pH can favor the growth of acid-resistant microorganisms such as *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* to survive in the juice [18].

3.7 Microbiological content of pineapple juice

Table 2 indicates that because the temperature and time do not exceed the highest permissible level ($3\log_{10}$ CFU/mL) of bacteria in food products suggested by the International Commission on Microbiological Specification of Foods [19], they are sufficient to kill microorganisms that are unsafe for consumption [19]. In addition, it also follows the United States of Food and Drug Administration (USFDA) 2004, where the requirement of microbiological count for fruit juice must be low (< 25 CFU/ml) for it to be marketable and safe for human consumption. As shown in Table 2, all the microbial

count for optimized pasteurized samples is under 25 CFU/ml with range from 6.6 x 10^{-5} to 7.1 x 10^{0} CFU/mL.

Table 2: Microbiological count for unpasteurized and optimized pasteurized pineapple juice

Sample / Dilution	Microbial count (CFU/mL)			
	Unpasteurized	Optimized pasteurized	Percent reduction	Log reduction
10^{-1}	1.68 x 10^{1}	7.1 x 10^{0}	60.71	0.406
10^{-2}	1.37 x 10^{0}	6.7 x 10^{-1}	73.72	0.580
10^{-3}	2.62 x 10^{-1}	0.3 x 10^{-2}	79.77	0.694
10^{-4}	1.73 x 10^{-2}	5.3 x 10^{-3}	82.66	0.761
10^{-5}	3.13 x 10^{-3}	1.36 x 10^{-3}	78.59	0.669
10^{-6}	3.02x 10^{-4}	6.6 x 10^{-5}	76.49	0.629

4. Conclusion

The pasteurization temperature of 81.5°C and 784.6 s of pasteurization time were discovered to be the best in this study with total soluble solids of 9.695°Brix, ascorbic acid content of 0.063 mg/L and bromelain activity of 0.026 CDU/mL. The number of microorganisms present in the optimized pasteurized juice is lower than that of the unpasteurized juice and adhering to the standards for marketable and safe consumption of fruit juice.

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References

[1] Y.L. Chin & S.C., Lee, "Optimization of Concentrating Process Using Rotary Vacuum Evaporation for Pineapple Juice," The Italian Association of Chemical Engineering, vol. 78, 2020, doi:103303/CET2078002

[2] M.H. Hounhouigan, A.R. Linnemann, M.M. Soumanou & M.A.J.S. Van Boekel, "Effect of heat treatment on yeast inactivation, vitamin C and physicochemical quality of fresh pineapple juice," African Journal of Food Science, vol. 14, no. 8, pp 256-264, 2020, https://doi.org/10.5897/AJFS2020.1997

[3] F.J. Chadaré, M.H. Hounhouigan, A.K. Carole Sanya, A. Gbaguidi, J.M., Dekpemadoha, A.R. Linnemann & D.J. Hounhouigan, "Microbial and Nutritional Stability of Pineapple Juice during Storage: Effect of Harmonized Thermal Pasteurization Technologies," American Journal of Food Science and Technology, vol. 9, no. 3, pp 82–89, 2021, https://doi.org/10.12691/ajfst-9-3-3

[4] M.H. Hanhouigan, Quality of pasteurised pineapple juice in the context of the Beninese marketing system. Wageningen University & Research, 2014. https://edepot.wur.nl/312562

[5] K.M. Phillips, M. Council-Troche, R.C. McGinty, A.S. Rasor, M.T. Tarrago-Trani, "Stability of vitamin C in fruit and vegetable homogenates stored at different temperatures. Journal of Food Composition and Analysis," 2015. S0889157515001982–. doi:10.1016/j.jfca.2015.09.008

[6] C. Leneveu-Jenvrin, B. Quentin, S. Assemat & F. Remize, "Maintaining Physicochemical, Microbiological, and Sensory Quality of Pineapple Juice (Ananas comosus, Var. "Queen Victoria") through Mild Heat Treatment," Processes, vol. 8, no. 9, pp 1186, 2020, doi:10.3390/pr8091186

[7] K.S. Oforiwaah, "Optimization of microwave pasteurization and storage on the physicochemical properties of juice from three pineapple varieties," University of Cape Coast, 2020. http://hdl.handle.net/123456789/6697

[8] I.E.H. Elgailani, M.A.M.G. Elkareem, E.A.A. Noh, O.E.A. Adam & A.M.A. Alghamdi, "Comparison of Two Methods for the Determination of Vitamin C (Ascorbic Acid) in Some Fruits," American Journal of Chemistry, vol. 2, no. 1, pp 1–7, 2017 https://doi.org/10.20448/812.2.1.1.7

[9] P.P. Kaddumukasa, S.M. Imathiu, J.M. Mathara, J.L. Nakavuma, "Influence of physicochemical parameters on storage stability: Microbiological quality of fresh unpasteurized fruit juices," Food Sci Nutr. vol. 5, pp 1098–1105, 2017, https://doi.org/10.1002/fsn3.500

[10] B.A.M.S. Kumara & K.D.T. Hettige, "Ripening Stage Affects the Quality of Fresh and Dehydrated Pineapples (Ananas comosus (L.) Merr.) cv. Mauritius in Sri Lanka," Sustainable Food Production, vol. 8, pp 29–37, 2020, https://doi.org/10.18052/www.scipress.com/sfp.8.29

[11] R. Jutamongkon & S. Charoenrein, "Effect of Temperature on the Stability of Fruit Bromelain from Smooth Cayenne Pineapple," Kasetsart Journal. Natural Sciences, vol. 44, pp 943-948, 2010

[12] A.N.M. Ramli, T.N.T. Aznan, & R.M. Illias, "Bromelain: from production to commercialisation. In Journal of the Science of Food and Agriculture, John Wiley and Sons Ltd, vol. 97, no. 5, pp. 1386–1395, 2017, https://doi.org/10.1002/jsfa.8122

[13] O.C., Othman, "Physicochemical characteristics and levels of inorganic elements in off-vine ripened pineapple (Ananas comosus L.) fruits of Dar es Salaam, Tanzania," KIST Journal of Science and Technology, 1(1), 23-30, 2011

[14] M. Elewa, G. El-Saady, K. Ibrahim, M. Tawfek & H. Elhossieny, "A novel Method for Brix Measuring in raw Sugar Solution," In Egyptian Sugar Journal, vol. 15, 2020, https://doi.org/10.21608/esugj.2020.209517

[15] O.G.L. Dolino, D.B. Montrecastro & A.M. Basilio, "Low Cost Recovery of Bromelain Solids from Industrial Peel, Pulp, Core Wastes Using Ethanolic Cashew Leaf Polyphenol," Philippine Journal of Science, vol. 149, no. 3, pp 581-587, 2020

[16] S. Zhang, & R. Zhang, "Effects of microwave treatment of apple raw material on the nutrients and antioxidants activity of apple juice," Journal of Food Processing, vol. 2014, 2014

[17] G. A. Alonzo, "Fruit Juice Processing: Addressing Consumer Demands for Safety and Quality," In Microbial food safety and preservation techniques. 2009, pp. 33–52.

[18] R. G. Labbé, & S. García, “Guide to Foodborne Pathogens,” Second Edition. Edited, 2013, https://doi.org/10.1002/9781118684856.ch6

[19] International Commission on Microbiological Specifications of Foods (ICMSF), “Microorganisms in Foods”, Microorganisms in Foods, University of Toronto Press, Canada, 1, 110-117, 1978.