A COMPARATIVE STUDY OF THERMAL, MICROWAVE & ULTRASOUND PROCESS ON BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF PINEAPPLE JUICE

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Roll No.: 0121/04

Registration No.: 986

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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PLAGIARISM VERIFICATION

Thesis Title: A comparative study of Thermal, Microwave and Ultrasonication process on bioactive compounds and antioxidant capacity of pineapple juice.

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The Author

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Abbreviation

g	Gram
mg/dl	mili gram per deciliter
mmol/L	mili mol per liter
ANOVA	Analysis of variance
AOAC	Association of Official AnalyticalChemists
◦B	Degree Brix
TSS	Total Soluble Solids
◦C	Degree Celsius
TAO	Total Antioxidant
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TA	Titratable Acidity
et al	And his/her associates
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic Acid Equivalent
etc.	Et cetera
TE	Trolox equivalent

Abstract

Thermal processing is considered one of the oldest techniques for preservation. Hence it possesses many limitations and causes degradation of bioactive compounds which inspire the researchers to develop other techniques to overcome the flaws and defect of thermal processing. These issues have served as a driver for the development of novel preservation technologies like microwave and ultrasonication. The actual aim of this study is to portray a comparative discussion on different preservative techniques of pineapple fruit juices which has been carried out based on empirical knowledge. To carry out this discussion, three preservative techniques; Thermal, Microwave, Ultrasonication have been applied on pineapple fruit juice and their impact on physicochemical properties, antioxidant capacity, bioactive compounds have been investigated for 21 days maintaining 7 days interval pattern. Though bioactive compounds are non-nutrient plant compounds, they possess many attributes which has substantial positive effect on health and disease prevention. So their stability after processing is crucial and need proper preservative method. Among the three techniques, it has been revealed that microwave and ultrasonication processes are more beneficial than thermal treatment in terms of retention of bioactive compounds and stability of antioxidant capacity. Better retention of total antioxidant 1.997 mg TE/100g, 1.594 mg TE/100g has been found for microwave and ultrasonication respectively. Similar effect has been recorded for flavonoid content. Drastic degradation has been found in terms of phenolic content 0.307mg GAE/100 for thermal sample which is 2 times less than rest of the treatments. In light of this, sonication and microwave pasteurisation may be utilized as a valuable option in place of thermal pasteurisation depending on the needs of the sample.

Keywords: Ultrasonication, Microwave, Bioactive compound, Pasteurization, Antioxidant.

Chapter 1: Introduction

The demand of fruit consumption is ever increasing due to abundance of essential minerals, vitamins, fibers, hence making fruits exceptional nutrient source. Fruits are abundant in phenolic substances, carotenoids, and vitamin C, also known as ascorbic acid, which has significant antioxidant potential and extremely critical for proper human diet according to many peer-reviewed studies. A reduction in the prevalence of cancer, coronary heart disease, cataracts, and inflammation is correlated with a high fruit diet (Bajpai et al., 2017). That is why intake of fruits along with main meal is encouraged. As fruits are perishable in nature, dependent on climate, season and several environmental factors, fruits are processed into many other forms like juice, jam, jelly etc. to keep the positive support of fruits on health available throughout the year.

Bangladesh also focuses on fruit processing methods. Many fruit varieties are processed commercially to reduce loss and store surplus. A major commercial fruit that is consumed either fresh or processed in a variety of ways in Bangladesh is the pineapple (*Ananas cosmosus*). It was originally grown in Sylhet at the beginning of the 20th century after being brought over from India's Assam and Manipur. Apart from Sylhet, Tangail, Dhaka, and Rangamati, it is currently grown in practically all of Bangladesh's districts. Due to agricultural development and feasible weather pattern, at present pineapples are grown abundantly which is another reason of processing it into a variety of products, including juice, jam, jelly, purees, etc. Among them pineapple juice is the most popular and appreciated one due to its very pleasant aroma and flavor (Difonzo et al., 2019). However stability of raw pineapple juices is very low and requires proper strategies to extend its shelf life commercially. Failure of using proper techniques can make this fruit commodity unsafe to consume and can be proved detrimental to health. Hence it claims proper preservative techniques and ensuring food safety is one of the most crucial duties in modern food processing because if there is a problem with food safety, no other quality features will matter (Paniwnyk, 2017). For this reason, the technique that is followed in terms of processing juice in our country is traditional thermal processing.

There are different types of thermal techniques like blanching, hot water immersion etc. Unfortunately, the high thermal processing involved in the heating procedure results in juices losing some of their nutritional value. This is a significant issue when treating liquid foods that have been commercially preserved (Patras et al., 2009). Though arresting microorganisms through these processes are beneficial, yet the deterioration of nutritional and organoleptic quality for example, loss of vitamins, desirable flavor, and bioactive compounds is a critical issue and demands proper examination of quality check due to thermal processing (De Vasconcelos Facundo et al., 2010).Thus, alternative processing techniques have been investigated and developed to create a better preserved juice product. In place of traditional thermal pasteurisation, two alternative processing techniques are being used for juice processing; microwave and ultrasound treatments.

In addition to being a potential replacement or addition to the conventional thermal pasteurisation process, ultrasound processing is a promising non-thermal processing technology (Tiwari et al., 2009). The effect of sonication treatments on some important quality parameters of juice suggested that sonication technique may successfully be implemented an industrial scale for the processing of juice (Ertugay & Başlar, 2014). Compared to traditional procedures, microwave pasteurisation has also some advantages such as enhanced product quality and shorter energy exposure times (Cañumir et al., 2002).Many researches on the microwave pasteurisation of fruit juices have been successful because it maintains the juice's inherent organoleptic qualities and shortens the time it is exposed to energy, which lowers the danger of losing vital thermo labile nutrients (Igual et al., 2010).

1.1 Justification and importance of the study

Fruits are being consumed as a side dish from ancient time with main meal. Nowadays many studies have also revealed the crucial role of consuming fruits on human health through empirical knowledge. Since the availability of fruits varies from place to place and season to season, consumption of their preserved version has been practiced. One of the best ways to preserve fruit is to store it in juice form which is served as a side dish in many food cultures.

Pineapple is also preserved in its juice form in large lot commercially in our country. In Bangladesh it is a popular fruit as well as found in abundance during production season. Due to success in agricultural field, it has become easy to cultivate it throughout the country, though it used to be found only in Sylhet region. Besides our country is blessed with fertile land, favorable weather, sufficient rainfall, which leads to abundance production of pineapple throughout the year. That's why it is necessary to store and preserve them for preventing wastage. In our country it is done so by processing it into various forms like juice, jam, jelly, purees etc.

Thermal treatment is the easiest and inexpensive way of preservation for all types of fruit juice. Pineapple juice is also preserved in this manner as it is easy to go process in the perspective of our country. In this study thermally processed pineapple juices are monitored to check stability and retention of bioactive compounds for 28 days maintaining 7 days interval. However, thermal processing might be potential to inactivate the spoilage microorganisms in pineapple fruit juice and extend the shelf life of pineapple fruit juice but it affects severely the amount of bioactive compounds and antioxidant properties. However, according to many studies it has been proved that ultrasonication and microwave treatment are far better than thermal process .That is why, Intervention of appropriate processing and preservation methods will make this fruits available for consumers and manufacturers of the country. Thus processing of pine apple juice has a great significance in economy as well as management of post-harvest losses of Bangladesh.

1.2 Aims and objectives

- To investigate effects of treatment on bioactive compounds and antioxidant capacity of pineapple fruit juice
- To make comparison among the three techniques regarding the retention of bioactive compounds and antioxidant properties during storage.
- To evaluate physicochemical properties of pineapple juice.

Chapter 2: Review of literature

2.1 Overview of pineapple (*Ananas cosmosus)*

The only member of the Bromeliaceae family that is commercially farmed for its delicious and nutritious fruit is the pineapple, *Ananas comosus*. Both the tropics and the subtropics are productive regions. It is the third most significant fruit in terms of global output, behind citrus and banana. The primary producers are in South Central America (Costa Rica and Brazil), Africa (Nigeria and South Africa), and Asia (Thailand, Philippines, Indonesia, India, and China). Thailand was the world's top pineapple producer in 2012, followed by Brazil, Costa Rica, and the Philippines.

Figure 2.1: Pineapple plant with fruit

It was brought to Bangladesh via Assam and Manipur, India, and was initially grown in Sylhet at the start of the 20th century. Although Sylhet, Tangail, Dhaka, and Rangamati have more acres under cultivation, it is now grown in practically all of Bangladesh's districts. The three cultivars are Giant Kew (known as kalendar in the area), Honey Queen (also known as jaldubi), and Red Spanish (ghorashal). Beginning in March, flowering lasts until May, while fruiting lasts until June. Pineapples as non-climacteric fruits should be harvested when ready to eat. Consumers will be guaranteed a base flavour that is agreeable if the fruit's skin colour change from green to yellow at the fruit's base, the soluble solids content is at least 12%, and the acidity is no more than 1%. This delightful tropical fruit contains fibre, bromelain, manganese, copper, vitamin C, vitamin B complex, calcium, zinc, and beta-carotene and is eaten fresh, dried, canned, in juice, and in jams. The flesh is minimal in sodium and calories, free of fat and cholesterol.

2.2 Pineapple physiology and characteristics

The tropical fruit pineapple is prized for its aroma, flavor, and juiciness. There are numerous pineapple kinds with different colors, forms, sizes, and flavors available at this time. When compared to other tropical fruits, pineapples are relatively medium in size. They are made up of several fruitlets and have a characteristic maturation pattern from the top, near the crown, to the bottom of the fruit (Montero-Calderón et al., 2010). Since pineapples are non-climacteric fruits, their quality varies and isn't constant with respect to their level of maturity. Indicators of pineapple maturity are often assessed based on the physical, physicochemical, and chemical features of fruit with appropriate flavor and morphological qualities (Nadzirah et al., 2013).The quality and shelf life of pineapple are significantly impacted by postharvest handling and management in addition to varietal variances and maturation stages. The pineapple tree should typically begin producing fruit within 15 months or up to two years following planting(M. F. Hossain, 2016). One of the crucial factors to consider when evaluating pineapple quality that can affect the chemical make-up of the fruit is the time of harvest. When producing pineapples, it is crucial to look into the plants' resistance to various pests and diseases as well as their various phases of development (Ali et al., 2019). Because overripe pineapple is highly perishable, the best time to collect it is when the color of the peel changes from green to yellow, either manually or somewhat mechanically (Reinhardt et al., 2018).

2.3 Ecology and regional distribution

Tropical plants like pineapples can thrive in most warm areas with temperatures ranging from 20°C to 36°C. Although mild chilly weather during the winter increases fruit quality and encourages flowering, frost is not well-tolerated. Pineapples may reach an altitude of 1800 meters above sea level, although the fruit becomes more acidic at greater altitudes (Lobo & Siddiq, 2016).Regular watering of pineapples is necessary to guarantee the development of high-quality fruit, but they might suffer damage if too much water collects in the soil due to inadequate drainage. For a tropical plant, pineapples can tolerate droughts remarkably well because of their stomata and utilization of the CAM pathway for photosynthesis, even when fruit mass and yields are decreased. For optimum growth, there should be a well-distributed yearly rainfall of 839-1742mm and high relative humidity.

Partial shade is ideal for pineapple growth. An acidic soil with a pH between 4.5 and 5.5 and efficient internal drainage is ideal because it reduces the risk of soil-borne illnesses and makes iron in the soil more accessible to plant roots. FeS2O4 must be applied topically in places where the soil pH is almost neutral in order to sustain proper growth. One characteristic of the pineapple that distinguishes it from other plants is its limited ability to extract iron from an iron-rich soil under specific circumstances. The pineapple tree is typically anticipated to produce fruit within 15 months or as late as two years following planting. Since the plant becomes sensitive to environmental elements like temperature and cloud cover, the first three months following planting are crucial for fruit blossoming and ripening. For pineapple growth, the crop needs ideal conditions with temperatures between 20 and 30 °C and enough sunlight in areas with greater and better-circulated precipitation. Due to its sweet, appealing fruit, pineapple is one of the most widely planted tropical fruits imported to warm climes.

The Guarani Indians are thought to have been the original cultivators of pineapples in Brazil and Paraguay and to have brought them to the Caribbean islands. Christopher Columbus brought the pineapple to Europe, where it was grown in greenhouses and became a popular fruit among kings and the affluent. In 1677, Charles II of England had a pineapple painted in his hand.

2.4 Consumption trends

In the past, processed pineapple products (juice, canned, dried) were marketed and consumed primarily alongside fresh fruit in affluent countries whereas pineapples farmed in developing countries were primarily consumed fresh. Pineapple was originally a preferred fruit for processing (FAO 2009), but since the introduction of particularly sweet varieties in the mid-1990s, it has been promoted more and more as a fresh fruit. The growth of ready-to-eat fresh-cut (minimally processed) fruits is directly related to this trend in the fresh pineapple market.

2.5 Emerging benefits of pineapple juice

It is common knowledge that a healthy diet is crucial for maintaining human health. The medical value of pineapple's bioactive components has been established. The fruit is useful for removing intestinal worms, diuretics, and as a contraceptive. (M. F. Hossain, 2016).Moreover, pineapple is frequently used to stimulate the excretion of fat for topical debridement and to promote hunger for dietary nutriment. In place of proteolytic enzyme, pineapple is used as a source of bromelain to treat soft tissue inflammation. According to the findings of Kargutkar & Brijesh,(2018) emphasize has been given on phytochemical, in order to pinpoint the phytochemical components that caused acute inflammatory disorders, the anti-inflammatory activity of pineapple leaf extract was successfully examined. In that investigation, proteins, flavonoids, tannins, carbohydrates, glycosides, and phenols were among the substances. Furthermore abundant in vitamins and micronutrients, pineapple is advised for everyday consumption. In addition, pineapple has few calories and is frequently included in weight watchers' diets. According to the researchers, one ripe pineapple provided around 16% of the recommended daily intake of vitamin C, or 28 mg of vitamin C for every half-glass of pineapple juice (Mohd Ali et al., 2020). In particular, vitamin C is recognized to be an effective antioxidant that protects cells from free radicals and delays osteoblast aging while also observing the growth of diabetes. The thiamine content of pineapple is thought to be crucial for regulating nervous system activity. Thiamine is essential for the synthesis of red blood cells and for minimizing the metabolic alterations brought on by diabetes and high glucose levels, especially in people with nervous system disorders(Cannon & Ho, 2018).Pineapple is a good remedy for constipation, bowel movements, and digestive health as a potential dietary fibre source (Dittakan et al., 2018). Similarly, M. A. Hossain & Rahman, (2011) demonstrated that the pineapple's functional dietary fibre is crucial for lowering the risk of diabetes, colon cancer, and cerebro-vascular illnesses as well as for easing diarrheal symptoms. Also, it is known that pineapple's malic acid promotes immunity, prevents tooth plaque from forming, and helps to maintain oral health (Chaudhary et al., 2019). Manganese, which makes up 72% of the daily recommended intake and is essential for generating working power, is one of the most significant delicate elements found in pineapple. Manganese has been linked to improvements in Type 2 diabetes, insulin resistance, blood glucose regulation, and skeletal abnormalities (Dittakan et al., 2018). The trace element enables the action of oxidizing organic catalysts like ligases and transferases, which is important against free radicals for the breakdown of cholesterol. Chaudhary et al., (2019) demonstrated these beneficial effects were feasible for maintaining congenital adaptability as well as strengthening skeletal increment in growing period. Bromelain, one of the most intricate bioactive chemicals for antioxidant, digestive improvement, and as a cardio protecting agent, is found in pineapple as one of the nutritional components (Asim et al., 2015).

2.6 Processing techniques of pineapple juice

In general, fresh fruits and fruit juices are ingested because they are thought to be very nutrient-dense in nature. Several processing procedures have been developed as a result of technological innovation in the food processing sector in order to extend the shelf life of fruits and fruit products and maintain their availability throughout the year, especially for seasonal ones. Fruit juices are often processed using the traditional thermal pasteurisation technique, which involves subjecting the juice to high temperatures for a set amount of time. Due to having some adverse effect, researchers are trying to find out a substitute of conventional process and interest on microwave and ultrasonication is increasing as they have some extra benefits over thermal process .

2.6.1 Thermal processing

The food industry's most popular process technology for ensuring the products' microbiological safety is thermal processing. These techniques primarily rely on the production of heat outside the object to be heated, whether it be through the combustion of fuels or the use of an electric resistive heater, and the transmission of that heat inside the object via conduction and convection mechanisms (Pereira & Vicente, 2010).Fruits are particularly perishable goods; hence heat processing is done to keep them away from spoiling. In addition to being dehydrated and canned (either whole or in pieces), they are extensively processed into juices, smoothies, purees, nectar, etc.

However thermal practice can lead to the change in bioactive contents of food. The weight of the data points to pasteurisation in the case of exotic fruits causing a decrease in quantities of bioactive chemicals, presumably as a result of how severely these heat treatments are applied (Elez-Martínez et al., 2006).

2.6.2 Microwave processing

Infrared, radio, and television waves all have frequencies higher than that of microwaves, which are electromagnetic radiation waves. As food components contain water, which behaves as an electric dipole because it has both positively and negatively charged molecules, microwave radiation is used to pasteurize juice under this theory. The movement of electrical charges caused by forces of attraction and repulsion causes intermolecular frictions that cause heat energy to be produced when an electromagnetic radiation passes through food (Datta & Rakesh, 2013).Ionic conduction is a different heating method. Ions move toward regions that are oppositely charged when an electromagnetic field is applied. Due to numerous collisions that resemble billiard balls and the breaking of the H-bonds in the water, heat is released as a result (Venkatesh & Raghavan, 2004).

Food produces heat in direct proportion to its dielectric characteristics. In contrast to traditional thermal pasteurization, microwave heating uniformly heats the entire volume of the meal; as a result, items like fruit juices with a high water content heat up quickly (Venkatesh & Raghavan, 2004). Since that microwaves can distribute energy throughout a material's bulk, there is the potential to speed up processing and improve quality overall (Thostenson & Chou, 1999).The rate of degradation of heat-labile nutrients and bioactive substances is reduced by the quick heating and significantly shorter heat exposure times to the product (Cañumir et al., 2002).

2.6.3 Ultrasonication

A non-thermal processing technique used in the production of fruit juice is ultrasound processing, often known as sonication. When ultrasound is applied at low frequencies (20–100 KHz), it causes cavitation, which causes the development and collapse of bubbles in a liquid media (Aadil et al., 2013).It has a substantial antimicrobial effect, improves functionality, and little affects quality metrics(Gabriel, 2012).Sonication is simple and less time consuming with improved efficiency (Gogate, 2011).

Vegetables, meat, fish, chocolates, cheese, fermented goods, emulsions, carbonated beverages, and fruit juices have all been explored for their potential to benefit from ultrasonic processing (Chemat et al., 2011).Prior to traditional food processing and preservation methods like freezing, frying, brining, cutting, extruding, drying, defrosting, degassing, filtration, homogenization, and accelerated fermentation, the technology exerts a number of effects, such as heat transfer, mass transfer, and vibrations or cavitation (Chemat et al., 2011).

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Chapter 3: Materials and Methods

3.1 Study area

The whole study was conducted in the laboratory of Applied Food Science and Nutrition, Food Processing and Engineering, Applied Chemistery and Chemical Technology at Chattogram Veterinary and Animal Sciences University (CVASU).

3.2 Sample collection

To conduct this study ripe and fresh pineapple the first priority as a sample. Keeping these criteria under consideration, pineapple was purchased from the local market of Chawkbajar, Chattogram, as they are famous for selling fresh fruit commodities.

3.3 Preparation of pineapple juice

To prepare the pineapple juice for running the experiment, some specific ways have been followed. Those steps were initiated by washing the pineapple with tape water to remove all the impurities and dirt from the surface. Then the whole fruit was peeled and core was thrown out using a sharp stainless-still knife. Afterwards, the whole fruit was cut to make small pieces. It had made the blending and extraction of juice easier. After that, the pineapples pieces were finely blended using household blender. A muslin cloth was used to strain the blended portion so that only fruit juice could be obtained. Then the juice is homogenized at 10,000 rpm (revolution per minute) for 5 minute. Homogenization causes uniform mixing and reduces particle size which leads to increase juice stability. Fresh juice samples were ready to carry out the rest of the process after making juice following aforementioned steps.

3.4 Methodological framework of study

3.4.1 Sample type

1. Control (with no treatment)
2. Thermally processed juice sample
3. Ultrasonicated juice sample
4. Microwave juice sample

3.4.2 Thermal processing

1. Juice is run in hot water bath
2. Temperature: 95degree Celsius for 3 min

3.4.3 Ultrasonication

1. Juice sample is kept in ultrasonication chamber at room temperature maintaining medium vibration for 15 minute.

3.4.4 Microwave treatment

1. Juice sample is run in a microwave machine and microwave treatment is applied for 5 minute.

3.4.3 Storage condition

1. Stored at 4 degree Celsius in the dark for 28 days
2. Quality determination of thermally, microwave and sonicated sample maintaining 7 days interval e.g.: 0, 7, 14, 21 days.

3.5 Physicochemical properties of fruit juice

3.5.1 Measurement of pH

In the study of chemistry, an aqueous solution's acidity or basicity is determined using the pH scale. pH is defined as the concentration of hydronium ions or, more generally, the negative logarithm of the activity of the (solvated) hydronium ion. There have been international agreements to create a collection of standard solutions whose pH can serve as a benchmark for the pH scale. Primary pH standards are calculated using a concentration cell with transference by contrasting the strong difference between a hydrogen electrode and a standard electrode, such as a silver chloride electrode. Glass electrodes and pH meters, or indicators, can be used to assess the acidity or basicity of aqueous solutions. The reciprocal of the hydrogen ion activity in a solution, expressed as a decimal logarithm, is known as pH (Igual et al., 2010).

3.5.2 Total soluble solids

Using a refractometer, the total soluble solids of the jelly sample were measured. Total soluble solids (TSS) were measured using the (AOAC, 2016) suggested method, which required a digital refractometer (Atago RX 1000). The outcomes were represented as a percentage of dissolved solids (Brix)

3.5.3 Titratable acidity

The percentage of acidity was measured by titrating 0.1N NaOH with phenolphthalein indicator to determine the level of anhydrous citric acid. A 100 mL volumetric flask containing 5 mL of juice was supplemented with 10 mL of diluted juice to titrate against 0.1N NaOH using phenolphthalein. The titration's end point is specified when a pink color appears. The average titration value was noted on three different times (AOAC, 2016).

Titratable acidity can be assessed using following formula:

$$\% \text{ of T.A} = \frac{(T.V \times 0.1N\ NaOH \times Factor \times 4)}{Sample\ volume \times 1000}$$

Where,

TV = Titer value of the sample in ml

Factor =Molecular weight of Citric acid: 64 (Citrus Fruit);

3.6 The DPPH radical scavenging assay for measuring antioxidant activity

3.6.1 Preparation of extract

1 gram of the sample was taken into a Felcon tube. The sample was mixed with10 ml of 100%ethanol, left it in dark condition for 3days for maturation. Then the ethanoic extract was poured through whattman filter paper. That specific extract was used for evaluating the antioxidant and bioactive compounds of pineapple juice.

3.6.2 Procedure

The scavenging activity of the extracts was measured by the DPPH test in a manner similar to that described by (Chemat et al., 2011).

Subsequently, 1 ml of methanoic acid extract was added with 2 ml of DPPH solution. The mixture was given a light shake before usage after 30 minutes of resting in a shadowy condition at room temperature. The absorbance was determined at 517 nm using a UV-VIS spectrophotometer (UV-2600, Shimadzu Company, USA). 1 mL of methanol and 2 mL of DPPH solution were mixed to create the control, which functioned as a blank. By comparing the samples' absorbance with that of a DPPH standard solution, it was possible to determine the samples' scavenging activity. According to the mobility of extracts' ability to scavenge free radicals, antioxidant capacity was calculated.

The calibration standard curve was based on the TEAC composite (Trolox equivalent antioxidant mobility), which was used as the standard. The results were given as milligrams per 100 grams of trolox equivalents (TE) for each gram of powder on a dry weight (DW) basis.

3.7 Determination of bioactive component

Preparation of extract For TPC and TFC, a Felcon tube containing 1 gram of material was utilized. The combination was then given 10 ml of 100% ethanol, and it was let to settle for 72 hours. Every four hours, the straining process was repeated. Ethanoic extract was found in the collected filtrate after 72 hours.

3.7.1 Total phenolic content (TPC)

The total phenolic content of the extracts was calculated using a modified version of the Folin-Ciocalteu reagent technique (Al-Owaisi et al., 2014).A modified Folin Ciocalteu technique was used to determine the jelly's total polyphenol content (TPC). 1 ml of ethanoic extract and 1.5 ml of FC reagent were combined in a falcon tube and let to sit at room temperature for 3 minutes. A further 60 minutes passed before 1.5 ml of 7.5% $Na2CO3$ was added to the mixture. The absorbance was measured at 760 nm using a UV VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA) with $C2H5OH$ serving as the blank. Per gram of extracts, the total phenolic content (TPC) was calculated and expressed as milligrams of Gallic acid equivalents (mg GAE/g).

3.7.2 Total flavonoid content (TFC)

The total flavonoid content (TFC) of the samples were calculated by following the aluminum chloride colorimetric method (Willett, 2002). To do so sample were prepared by taking aliquots of 0.5 mL of diluted extract and then mixed up with some other chemicals like 2.8 mL of pure water, 0.1 mL of 10% $AlCl3$, and 0.1 mL of 1 mol/L potassium acetate. The combination was kept for 30 minutes at room temperature. Using a blank solution containing the same volume of distilled water and 10% aluminum chloride, the absorbance was measured at 415 nm using a UV-visible spectrophotometer (UV2600, Shimadzu Company, USA). The amount of flavonoids in each milligram of extract were determined and displayed as milligrams of quercetin equivalents (mg QE/g).

3.8 Statistical analysis

In a Microsoft Excel 2019 Spreadsheet, data was gathered and saved for statistical analysis. For samples of jelly, descriptive statistics, including mean and standard deviation, were computed. Using MINITAB 19, the data was arranged, coded, and recorded. After that, a statistical analysis was done on the outcomes of these experiments. Using one-way ANOVA, it was possible to calculate the amount of significant variance at a 95% confidence level for the data on proximate and physiochemical composition, mineral content, phytochemical content, and sensory evaluation. A level of 5% significance (p 0.05) was used for the statistical analysis.

Chapter 4: Result

4.1 Physicochemical properties of pineapple Juice

4.1.1 pH

One of the most crucial aspects of fruit juice is its pH and pineapple juice is not an exception of it. Generally processed fruits are undergone through different treatments to make the juice stable in form which may or may not the alteration of pH. From the following table (4.1) it is prominent that various processing techniques have not shown any remarkable change in the pineapple juice. Highest ph has been recorded is 4.8 for control, thermally treated and microwave processed juice sample. However, lowest ph 3.7 is found in sonicated juice sample during 28 days storage time.

Table 4.1.1 Effects of different treatment on pH of pineapple juice

Treatment	Storage time (Days)	pH
Control	0	4.8 ± 0.05^a
Thermal	0	4.7 ± 0.05^a
	7	4.8 ± 0.05^a
	14	4.7 ± 0.01^a
	21	4.7 ± 0.05^a
Microwave	0	4.7 ± 0.05^a
	7	4.7 ± 0.05^a
	14	4.8 ± 0.01^a
	21	3.9 ± 0.01^b
Ultrasonication	0	4.6 ± 0.05^a
	7	4.7 ± 0.05^a
	14	4.7 ± 0.01^a
	21	3.7 ± 0.01^b

Data are expressed as Means ± SD, different letter (a, b, c, d) indicates statistically different ($p<0.05$).

4.1.2 Total soluble solids (TSS)

The total soluble sugar content (expressed as °Brix) of the pineapple juices analyzed in this study are comparable to those reported in the literature (Wu et al., 2021). The soluble solid content of pineapple juices has not been impacted by heat treatment, microwave cooking, or sonication, as shown by statistical analysis, which found no significant changes for the parameters taken into account and produced a range of 15.10–15.11°Brix. After 28 days of storage, the sonicated juice sample's lowest TSS was 14.71.

Table 4.1.2 Effects of different treatment on TSS of pineapple juice

Treatment	Storage time	TSS (°Brix)
Control	0	15.10±0.005[a]
Thermal	0	15.10±0.005[a]
	7	15.11±0.005[a]
	14	15.11±0.010[a]
	21	14.93±0.057[b]
Microwave	0	15.10±0.005[a]
	7	15.11±0.005[a]
	14	15.10±0.005[a]
	21	14.94±0.002[b]
Ultrasonication	0	15.10±0.005[a]
	7	15.11±0.005[a]
	14	14.91±0.005[b]
	21	14.71±0.002[c]

Data are expressed as Means ± SD, different letter (a, b, c, d) indicates statistically different ($p<0.05$).

4.1.3 Titratable acidity

Juice taste and consumer acceptance are significantly influenced by titratable acid (TA). The results of this study's analysis of pineapple juices' TA content are shown in Table 4.3. The juice that received thermal treatment for 28 days had the lowest TA concentration (0.311-0.254 g/L), whereas the juice that received ultrasound treatment for 28 days had the greatest TA content (0.386-0.258 g/L) (Table-3). Microwave also had similar effect on TA of juice.

Table 4.1.3 Effects of different treatment on TA of pineapple juice

Treatment	Storage time (Days)	TA (g/L)
Control	0	0.304±0.003[a]
Thermal	0	0.311±0.002[a]
	7	0.256±0.002[b]
	14	0.256±0.002[b]
	21	0.254±0.002[b]
Microwave	0	0.389±0.001[a]
	7	0.374±0.002[b]
	14	0.363±0.002[c]
	21	0.259±0.001[c]
Ultrasonication	0	0.386±0.001[a]
	7	0.379±0.003[b]
	14	0.376±0.003[c]
	21	0.258±0.001[c]

Data are expressed as Means ± SD, different letter (a, b, c, d) indicates statistically different ($p<0.05$).

4.2 Antioxidant activity of pineapple juice

Pineapple has high bioactive ingredients content, resulting in a high ability of antioxidant (Ayón-Reyna et al., 2019). The application of various preservative techniques and their impact on retention of antioxidant capacity has been shown in table 4.2. From the statistical data, it can be said that antioxidant capacity was the highest in the untreated juice 4.793 mg TE/100 g. However, severe degradation had been occurred during storage time of untreated sample. Among the three techniques, high antioxidant capacity had been recorded in US sample (4.123 mg TE/100 g). Slight changes had been found between the MW and TP juice throughout the 28 days storage time.

Table 4.2 Effect of different treatment on TAO of pineapple juice

Treatment	Storage time (Days)	TAO (mg TE/100g)
Control	0	4.793±0.002[a]
Thermal	0	3.266±0.004[a]
	7	2.950±0.002[b]
	14	2.286±0.003[c]
	21	1.605±0.002[d]
Microwave	0	3.835±0.001[a]
	7	3.575±0.001[a]
	14	2.353±0.003[b]
	21	1.997±0.001[c]
Ultrasonication	0	4.123±0.001[a]
	7	3.934±0.005[b]
	14	2.566±0.001[c]
	21	1.594±0.001[d]

Data are expressed as Means ± SD, different letter (a, b, c, d) indicates statistically different ($p<0.05$).

4.3 Phytochemical components of pineapple juice

4.3.1 TPC

Pineapple fruit juice is stuffed with lots of goodness due to presence of some phytochemical components. Two of them are flavonoid and polyphenol. They display many beneficial effects on health and it has already been proved through empirical knowledge. As a result, their retention and degradation after several processing techniques is crucial to find out. Statistical analysis shows and proves that heating process causes substantial destruction. In terms of polyphenol, it is evident that amount of total polyphenol content (TPC) was higher in control pineapple juice (1.552mg GAE/100g FW) sample and no significant change occurred in thermally processed juice (1.265 mg GAE/100g FW) in 0 day. The TPC started to degrade in high amount after 7days both in control and thermally treated pineapple juice. During 28 days storage time, there was a little polyphenol remaining in the both sample. Hence data were recorded 0.302mg GAE/100g FW and -2.307mg for thermally treated juice sample. It is an evidence of major negative impact of thermal treatment. It was one kind of indication which implies phenolic contents gets destroyed when stored for long time and the rate became high when sample undergo thermal treatment. But in terms of microwave and ultrasonication treated sample, retention was far better than the thermal treatment and no negative value has been recorded.

Table 4.3.1: Effect of different treatment on TPC of pineapple juice

Treatment	Storage time (Days)	TPC (mg GAE/100)
Control	0	1.552±0.003[a]
Thermal	0	1.265±0.004[a]
	7	0.724±0.001[a]
	14	0.096±0.001[c]
	21	0.302±0.001[c]
Microwave	0	3.264±0.005[a]
	7	2.123±0.005[b]
	14	1.103±0.004[c]
	21	0.697±0.002[d]
Ultrasonication	0	3.446±0.001[a]
	7	2.122±0.005[b]
	14	1.008±0.005[c]
	21	0.606±0.001[d]

Data are expressed as Means ± SD, different letter (a, b, c, d) indicates statistically different ($p<0.05$).

4.3.2TFC

Flavonoids, which are mostly found in plants as colored pigments, can serve as strong antioxidants at different doses. According to certain research, flavonoids can shield membrane lipids from oxidation (Terao et al., 1994). Positive result has been exerted by different process in terms of TFC retention. From the statistical data, a better retention has been recorded than control. Findings of this study for thermal, microwave and sonicated samples are 4.562mg QE/g, 4.488mg QE/g, and 4.184mg QE/g respectively. As the day has been passed, every sample has shown remarkable minimization and weak retention.

Table 4.3.2 Effects of different treatment on TFC of pineapple juice

Treatment	Storage time (Days)	TFC (mg QE/g)
Control	0	3.924±0.005^{a}
Thermal	0	4.562±0.001^{a}
	7	3.056±0.001^{b}
	14	2.125±0.002^{c}
	21	1.055±0.003^{d}
Microwave	0	4.488±0.005^{a}
	7	3.810±0.001^{b}
	14	2.948±0.001^{c}
	21	1.634±0.001^{d}
Ultrasonication	0	4.184±0.005^{a}
	7	3.109±0.001^{a}
	14	2.542±0.001^{c}
	21	0.606±0.001^{d}

Data are expressed as Means ± SD, different letter (a, b, c, d) indicates statistically different ($p<0.05$).

Chapter 5: Discussion

Antioxidant compounds found in fruits and vegetables are considered one of the most beneficial things that make commodities conducive to health. These components are known as free radical scavengers that keep our body free from free radicals. Many studies have also discovered the occurrence of diseases that have linkage with free radicals generates in our body (Diplock, 1994). To diminish the negative impact of these, consuming fruits are considered as an easy and inexpensive way. Pineapple juice is a good source of antioxidant. That is why in this study, the retention of TAO after implementation of different techniques has been observed. In this study, all samples have showed decreasing trend of TAO throughout storage compared to control sample. Thermally treated juice has showed massive destruction in TAO due to elevated temperature during 28 days storage than the other two processes and the findings are similar to the study of Gómez et al., (2011). By comparing the rest of the two processes; microwave and ultrasonication; ultrasonication has showed positive impact in the retention of TAO in pineapple juice. The reason could be the inactivation of polyphenol oxidase enzyme (responsible for browning reaction) due to the cavitation produced during ultrasound treatment (Cheng et al., 2007).

Phytochemical compounds are important trace ingredients of every fruit. Pineapple fruit juice is also a rich source of phytochemicals. Among the all phytochemical compounds, phenolic compounds have attracted the attraction of researchers as it provides many beneficial effects on human body. In addition to their antioxidant action in scavenging free radicals, phenolic chemicals that give fruits and vegetables their bitterness, astringency, flavour, colour, and oxidative stability have also been linked to anti-inflammatory and hydrolytic enzyme inhibition in human cells (Naczk & Shahidi, 2004). However, during process of fruits and fruit commodities phenolic compounds are degraded. This study aims to find out the most effective preservative techniques that will be helpful to conserve phenolic contents. During thermal treatment, TPC has been reduced in drastic manner as well as complete destruction has been recorded at the last phase. And the decrease of total phenolic by thermal treatment might be due to the oxidative degradation of thermally unstable phenolic compound (Nayak et al., 2015). On the contrary, sonication and microwave has showed completely an opposite scenario. Both of these treatments have increased the phenolic content in remarkable way. The likely cause of the partial increase in phenolic content could be related to the phenolic acids' molecular rearrangements and degradation during processing (Naczk & Shahidi, 2004). Both the free and bound phenolic chemicals are typically hydrolyzed and leached by sonication (Herrera & Luque De Castro, 2004). Similar to how partial hemicellulose and lignin degradation releases bound phenolic acids, microwave heating enhances tissue rupture and releases some of the phenolic acids into the liquid medium or solvent used for extraction (Jokić et al., 2012). Above all, it can be said that different processing techniques have both positive and negative impact on TPC of pineapple juice.

Flavonoids are also included in phytochemical group; hence need proper investigation on their character and sensitivity towards different preservative actions. Unlike others, effect of different processes on TFC has been investigated. For flavonoids, the total flavonoids content (TFC) of thermally treated juice has no significant differences compared to control sample. However, slight increment in TFC was observed. It has been noted that thermal treatment increases the amount of flavonoids released from the cell matrix; this may be the cause of the increase in TFC of thermally treated juice seen in this study (De Brujin et al., 2008). A significant increment of TFC has also been evidenced in sonicated sample. During ultrasound processing, mechanical waves propagate by rarefactions and compression, resulting in surface peeling, erosion, and particle break down, which might be beneficial in releasing more bioactive compounds and enhancing the total flavonoids content (Wang et al., 2019). At the same way, microwave treated sample has shown enhancement of TFC.

Chapter 6: Conclusion

.The study revealed that the type of processing treatment had an influence on the concentration of phytochemical and bioactive compounds in the processed juice. The phytochemical content responded more favorably to microwave and sonication treatments than to conventional thermal pasteurization. Both provide favorable feedback about the preservation of antioxidant capability. With rare exceptions, both microwave treatment and sonication of samples were found to have a favorable impact on the phenolic content and flavonoid content. Microwave treatment required less exposure time to temperature and sonication required usage of low temperature. Therefore, microwave and sonication treatment could be used in place of thermal pasteurization depending on the sample requirements. Because upholding the phytochemical and antioxidant components is directly linked to their contribution to human health. So aim of preservation should not be limited just to make safe food, emphasize should be given on making nutritious food. As a future scope, the current study can be expanded upon to examine changes in the antioxidant activity and phytochemical composition of additional fruit juice samples.

Chapter 7: Recommendation and Future Perspective

There is a saying that "Let food be the medicine and medicine be the food". This saying is almost true and has been proved logical and relevant through empirical knowledge. Scientists all around the world have already started effortlessly to find the medicinal properties within the fruits to prevent and reduce disease occurrences. These positive effects of fruits are found due to the presence of phytochemicals and bioactive compounds. It has made people attracted towards higher consumption of fruits and fruit commodities. That's why fruits are also conserved for future intake by applying some preservative methods. This study reflects the application of various treatments on pineapple juice and reveals their output on quality attributes. Considering the outcome of this present study, following recommendations and perspectives can be proposed

- For better understanding and confirmation, this study can be performed repetitively.
- This study can be performed on other seasonal and exotic fruits as well as the feedback of them can be investigated and compared with this current study.
- This study can be considered as a comparative experimental model among different processing techniques.
- This study can provide strategies to improve retention during conventional and unconventional process.
- This study can be applied and may prove fruitful during processing of fruit commodities like jam, jelly, canned items etc.
- Considering the findings of this study, the techniques can be followed in terms of large scale production of juices commercially.
- Other preservative techniques can be combined with aforementioned processes and the compilation result can be recorded and compared.

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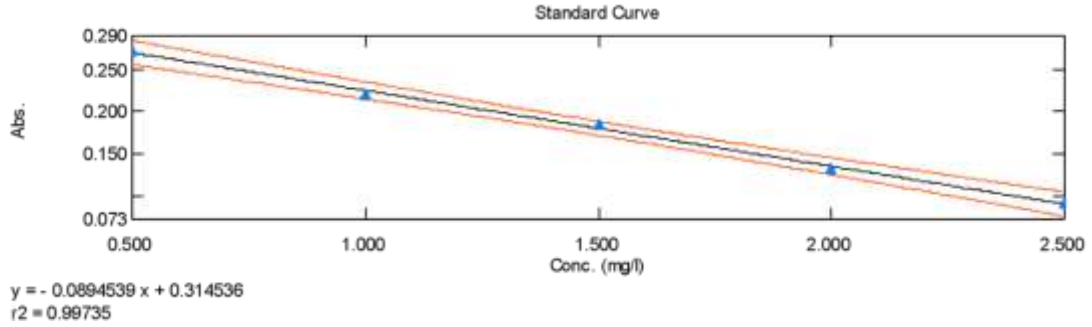
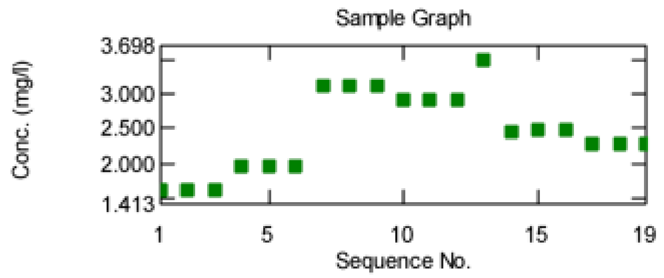
Appendices

Appendix A: Antioxidant capacity of pineapple juice

Standard curve of Antioxidant Capacity

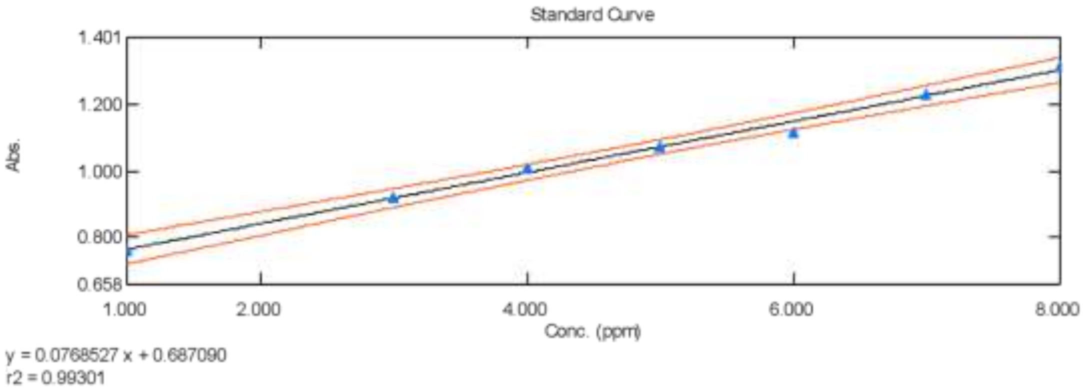


Sample graph

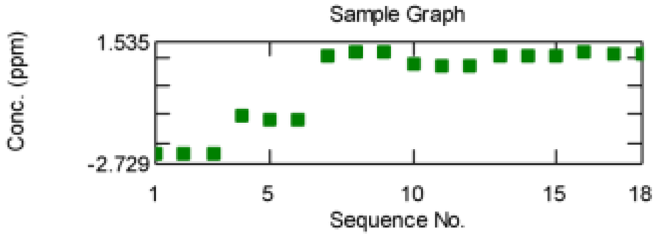


Appendix B: Phytochemical contents of pineapple juice

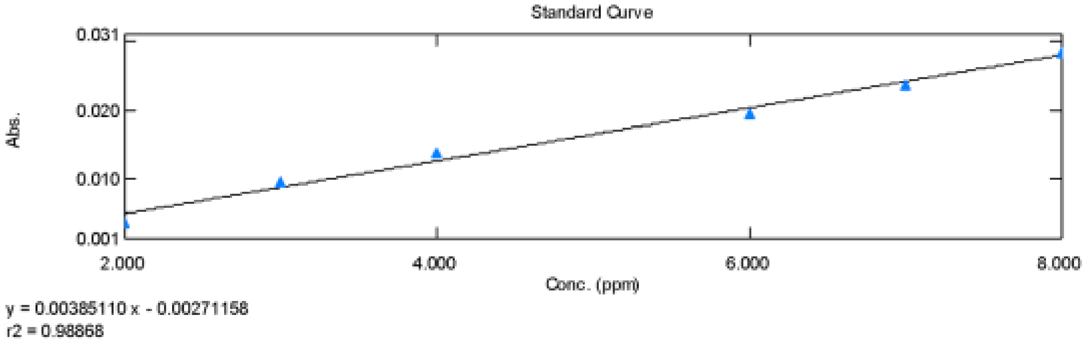
Standard curve of TPC (Total Phenolic Content)



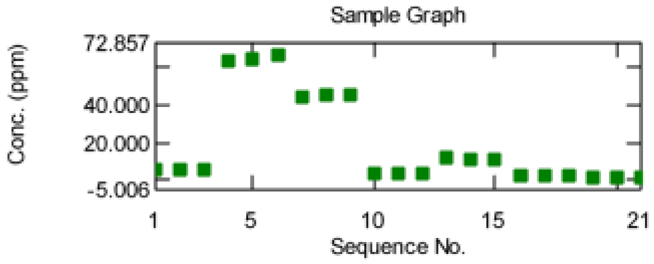
Sample graph



Standard curve of TFC (Total Flavonoid Content)



Sample graph



Appendix C: Photo Gallery

Homogenization of pineapple juice

Thermal treatment of pineapple juice

Microwave treatment of pineapple juice

Ultrasound treatment of pineapple juice

Pineapple juice sample after treatment(thermal, microwave, sonicated respectively)

pH determination process of pineapple juice sample

Titratable acidity determination of pineapple juice

End point of titration

Sample preparation for DPPH test

Sample insertion in UV machine

Sample preparation for TPC test

Brief Biography

At Dr. Khastagir Govt. Girls High School in Chattogram, Toma Das took the Secondary School Certificate Exam in 2012, and at Govt. City College in Chattogram, she passed the Higher Secondary Certificate Exam in 2014. She received her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University in Chattogram, Bangladesh. She is currently a student at Chattogram Veterinary and Animal Sciences University, where she is pursuing a Master of Science in Applied Human Nutrition and Dietetics (CVASU). She is extremely interested in working in the food industry, enhancing people's health through appropriate direction and advice, and raising knowledge of nutrition and food safety among the public.