COMPARISON OF BIOACTIVE COMPOUNDS IN THERMALLY PROCESSED JUICES FROM VARIOUS PINEAPPLE CULTIVARS

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Roll No. : 0121/03

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Food Processing and Engineering

Department of Food Processing and Engineering

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

February 2023

Authorization

I hereby declare that I am the sole author of the thesis. I, Bintu Banik, declare that this thesis is submitted in fulfillment of the requirements for the Degree of Master of Science (MS) in Food Processing and Engineering, Department of Food Processing and Engineering, Faculty of Food Science & Technology, Chattogram Veterinary and Animal Sciences University. It is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

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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

Title of Thesis: **COMPARISON OF BIOACTIVE COMPOUNDS IN THERMALLY PROCESSED JUICES FROM VARIOUS PINEAPPLE CULTIVARS**

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Abbreviation

%	: Percentage
0.1N	: 0.1 Normal
°C	: Degree celcius
TA	: Titratable acidity
et al	: And his / her associates
etc	: Et cetera
TSS	: Total soluble sugar
g	: Gram
sec	: Second
TPC	: Total polyphenol content
TFC	: Total flavonoid content
AOC	: Antioxidant capacity

Abstract

During the peak harvest time, a significant number of pineapples are wasted due to insufficient and inefficient transportation of items from local produce marketplaces to wholesale markets. Pineapple can be processed in many ways to reduce the post-harvest loss of the pineapple. Pineapple juice is one of them and various thermal and non-thermal treatment are used to process the pineapple juice. On the other hand, non-thermal processing requires a significant financial investment as compared to thermal processing. The easiest way of increasing self-life of any product is heat treatment. Therefore, it is critical to investigate the ability of heat treatment to produce high-quality juices from pineapple. In this study, three pineapple cultivars (kalenga, honey queen and ghorashal) were used to determine the effect of heat treatment on their physicochemical properties, bioactive components and antioxidant capacity during storage of 21 days at 4°C. Two temperature margins (50°C and 90°C) were chosen for the heat treatment since conventional heat pasteurization was performed at 90°C. pH values and antioxidant capacity of pineapple juices obtained from three cultivars showed no noticeable changes after the heat treatment. In contrast to kalenga and ghorashal species, the honey queen species showed the highest TSS value and retention of the TSS for 21 days of storage following the heat treatment both at 90°C and 50°C. In case of acidity, high acidity was observed in honey queen and ghorashal species at 90°C. Total polyphenol content showed significant decrease in the three pineapple cultivars. Honey queen and ghorashal species showed greater retention of total flavonoid at 50°C for 14 days of storage. Therefore, heat treatment at 50°C could be an effective method to produce high quality pineapple juices from the honey queen species.

Keywords: pineapple, cultivars, thermal processing, antioxidant, total polyphenol, total flavonoid

Chapter 1: Introduction

Pineapple is one of the popular tropical and subtropical fruits in Bangladesh. A variety of products can be made from pineapple, including canned pineapple slices, dried pineapple, pasteurized pineapple juice, and concentrate (Khalid et al., 2016). However, among all these products, fresh pineapple juice is more popular because of its pleasant aroma, taste and numerous functional properties (Rattanathanalerk et al., 2005). Pineapple juice is high in minerals, particularly manganese, as well as amino acids, carbohydrates, vitamins, and polyphenols (Elkins et al., 1997). Additionally, it has high concentrations of other antioxidants such vitamin C and phenolic compounds (Hossain & Rahman, 2011). Phenolic compounds which give fruits and vegetables their flavor, color, and oxidative stability have also been linked to anti-inflammatory and hydrolytic enzyme inhibition in human cells, as well as antioxidant action by scavenging free radicals (Naczk & Shahidi, 2004). Thus, pineapple is becoming popular among the Bangladeshi due to its health promoting properties.

The huge consumer demand for pineapples in Bangladesh is causing production to increase day by day. (Hossain & Islam, 2017). It is widely produced in Bangladesh as the climate and soils are good fit for the production of pineapple. It is widely cultivated in the districts of Tangail, Mymensingh, Gazipur, Sylhet, Moulovibazar, Chattogram, Bandarban, Khagrachari and Rangamati (Shah, et al., 2015). The world's pineapple production includes at least 90 different varieties (Mondol & Mrittunjoy, 1988). The majority of pineapple farmed in Bangladesh, however, is of three varieties. The cultivated varieties include red spanish (ghorashal), giant kew (kalenga), and honey queen. In the Tangail area, mainly Giant Kew variety of pineapple has intensively been cultivated by the farmers for the last few years. Majority of honey queen was cultivated in the hilly zone of Chattogram (Hossain & Islam, 2017). Palash upazila in the Narsingdi district is known for the production of ghorashal variety. Pineapple comes in fourth place among the major fruits in terms of the overall number of fruits produced in Bangladesh (Shah, et al., 2015). Therefore, there is a huge market in the food business for pineapple juice due to high production of pineapple.

The post-harvest loss of the pineapple hinders the great commercial potential for pineapple juice. Prior research by Hassan (2010) on post-harvest losses of fruits and vegetables in Bangladesh indicates that 43% of pineapples waste during the peak harvest period as a result of insufficient and inefficient transit of goods from the local produce marketplaces to the wholesale markets. In addition, it was found that poor postharvest technical knowledge, severe insect and pest attacks, attacks by bats, squirrels, and monkeys, storage facilities, transportation facilities, and low prices were the main causes of pineapple post-harvest loss in hilly regions of Bangladesh (Hossain & Islam, 2017). In order to reduce the post-harvest loss, different processing and preservation techniques are used.

Fruit juices go through a variety of processes in order to reduce loss and to be preserved for a long time. Fruits must be preserved within four to forty-eight hours of being harvested since fruit deterioration increases as time goes on (Joy et al., 2013). Fresh-cut pineapple and pineapple juices are particularly susceptible to bacterial spoilage, high respiration rates, and ethylene production, which can result in surface deterioration, rapid microbial growth, enzymatic browning, weight loss, and unpleasant volatile production. These effects typically result in a significant reduction in the shelf life of the product (Corbo et al., 2009). Since pineapples are highly perishable, different processing techniques are needed to extend their shelf life. The shelf life of pineapple juice is relatively low if it is not stored properly in a refrigerator (Nwachukwa et al., 2014). Though long-time preservation of fruit juices becomes increasingly challenging due to the presence of bacteria, enzymes that cause spoiling, and unfavorable chemical interactions that can degrade the juice's quality and sensory qualities (Aneja at al., 2014). Several thermal and non-thermal process are used to preserve the fruit juices.

Fruit juices have been processed using a variety of techniques, including traditional pasteurization, ohmic heating, microwave heating, thermosonication, pulsed electric fields (PEF), light therapy, supercritical carbon dioxide, and high hydrostatic pressure (HHP). For instance, PEF treatment lengthens pineapple juice's shelf life without sacrificing its nutritional or antioxidant content (Abu et al., 2020). These cutting-edge technologies have the ability to keep pineapple juice fresh. However, the equipment needed to provide physical treatments still requires a significant cost investment, which must be matched with cutting-edge technological advantages (Fryer et al., 2008).

Among all approaches, conventional pasteurization or moderate heat treatments are unquestionably cheaper for small-scale processing facilities, and their capacity to generate high-quality juices requires further study.

So, this research is trying to figure out the optimal temperature for the pineapple juice preservation and characterize the physicochemical properties and bioactive compounds of three pineapple varieties. This research was designed to compare the bioactive compounds of pineapple juice of three different varieties which were processed thermally in two different temperatures. Hence, the objectives of this study are:

1. To determine the physicochemical properties of the pineapple juice
2. To determine the bioactive compounds and antioxidant capacity of the pineapple juice
3. To assess the changes in the physicochemical properties, bioactive compounds and antioxidant capacity

Chapter 2: Review of literature

2.1 Pineapple

One of the most significant commercial fruit crops in the world is the pineapple (*Ananas comosus* (L.) Merr., Family: Bromeliaceae). As a result of its superior flavor and taste, it is referred to as the queen of fruits (Baruwa, 2013). After citrus and bananas, pineapple ranks third among all tropical fruits in the world (Bartholomew et al., 2003). Thailand is the world's top pineapple producer, accounting for 13% of worldwide production, followed by Brazil and Costa Rica (Baruwa, 2013). The pineapple market has expanded significantly as a result of the appealing aroma compounds and nutritious values, as well as high demand and competitive retail pricing. Pineapple is primarily grown in tropical and subtropical countries because of the temperate climate and rainfall distribution. The crop can bear fruits soon after flowering, allowing for year-round yield production which makes it commercially significant fruits (Shamsudin et al., 2020).

Pineapple fruits have high levels of moisture, sugars, ascorbic acid in soluble solids, and low levels of crude fiber. Thus, pineapple can be employed as an additional fruit source of nutrients for maintaining personal wellness (Hemalatha & Anbuselvi, 2013). Typically, fresh pineapple fruit or fresh pineapple juice are eaten. The crown, rind, eyes, and core only need to be removed from field-ripe fruits before eating them raw for the optimum flavor. Fresh, canned, and juiced pineapple can be found in a variety of foods, including desserts, fruit salads, jam, yogurt, ice cream, candies, and dishes with meat (Debnath et al., 2012).

Different country uses pineapple in various way. Small pineapples are harvested from the vine in Panama and given a handle made of a few inches of the stem. The flesh of larger fruits is prepared in a variety of ways, including cooking it into pies, cakes, puddings, garnishes for ham, sauces, and preserves. It can also be eaten fresh, as a dessert, in salads, compotes, and other dishes. The pineapple is used in Malayan curries and other meat preparations. The fermented pulp is turned into a well-liked sweetmeat in the Philippines. The pineapple tends to develop odd flavors when frozen, thus freezing it is not recommended. Worldwide, people consume canned pineapple. Young, tender shoots are consumed in salads in Africa. Raw or cooked, the terminal bud or "cabbage" and the inflorescences are consumed (Joy, 2010).

2.2 Nutritional value of pineapples

The pineapple is a great tropical fruit that offers a good amount of health advantages along with exceptional juiciness and bright tropical flavor (Hossain et al., 2015). The significant amounts of calcium, potassium, vitamin C, carbohydrates, dietary fiber, water, and other minerals found in pineapple are helpful for the digestive system, aid in maintaining a healthy weight, and promote balanced nutrition. In Bangladesh, pineapple is a popular fruit with low fat and sodium levels (Sabahelkhier et al., 2010). 10–25 mg of vitamin is present in the pineapple (Rasid et al., 1987). The edible part of the pineapple has been the focus of most studies on its composition. Pineapple has a moisture content of 81.2 to 86.2% and a total solids content of 13 to 19%, the main sugars being sucrose, glucose, and fructose. Up to 85% of all solids are made up of carbohydrates, while just 2% to 3% are made up of fiber. Citric acid is the most common organic acid available. Very little ash, nitrogenous chemicals, and lipids (0.1%) are present in the pulp. True proteins make up 25–30% of nitrogenous substances. Out of this percentage, a protease known as Bromelain is responsible for about 80% of the proteolytic activity. Minerals including Calcium, Chlorine, Potassium, Phosphorus, and Sodium are found in fresh pineapple which plays a vital role in our health (Dull, 1971).

According to Debnath et al., (2012), Ascorbic acid is present in pineapple juice, which is also a strong source of vitamin C. As a powerful antioxidant and aid in the body's iron absorption, ascorbic acid, or vitamin C, fights bacterial and viral illnesses. One-half of a cup of pineapple juice contains 50% of the daily recommended intake of vitamin C for adults. Pineapples include a number of critical minerals, including manganese, a trace mineral important for the development of bone as well as the production and activation of specific enzymes. Copper is another trace mineral that is present in pineapples. It helps with iron absorption and controls blood pressure and heart rate.

Table 1: Nutrients in 100g of pineapple juice (Joy, 2010)

Nutrients	Amount
Energy	52 calories
Dietary fibre	1.40g
Carbohydrate	13.7 g
protein	0.54 g
Iron	0.28 mg
Magnesium	12 mg
Calcium	16 mg
Potassium	150 mg
Phosphorus	11 mg
Zinc	0.10 mg
Vitamin A	130 I.U
Vitamin B 1	0.079 mg
Vitamin B 2	0.031 mg
Vitamin B 3	0.489 mg
Vitamin B 6	0.110 mg
Vitamin C	24 mg

2.3 Pineapple production in Bangladesh

Pineapple ranks fourth among all fruits grown in Bangladesh in terms of overall area and production, (BBS, 2009). The months of June, July, and August are the best for pineapple harvesting in Bangladesh. During September and October, only a few pineapples are produced. The Bangladeshi people generally enjoy ripe pineapple. Green pineapple is also utilized in the production of pickles. After the juice is extracted, the leftover is used as cattle feed, and the soft leaves are used for the same reason. Pineapple is used to make a variety of foods such as squash, syrup, and jelly. Pineapple is also used to make vinegar, alcohol, citric acid, calcium citrate, and other products (Moniruzzaman, 1988).

It is cultivated generally all-around Bangladesh, especially in hilly and high land areas. It is commonly grown in Tangail, Mymensingh, Gazipur, Sylhet, Moulvibazar, Chittagong, Bnandarban, Khagrachari, and Rangamati are among the cities. Pineapple is widely grown across Tangail District's Madhupur Upazila (Hasan et al., 2010). In Bangladesh, different pineapple cultivars are grown in large quantities each year. However, these three pineapple cultivars are mainly cultivated in Bangladesh. The cultivated cultivars include red spanish (gorashal), giant kew (kalanga), and honey queen(jaldubi). A few farmers in Madhupur, Tangail district, also cultivate a local variety called "Asshini" (late variety), in additional to these three varieties (Shah et al., 2015). In terms of land and productivity, Giant Kew (Kalanga) variety ranks first among cultivated varieties in Bangladesh. This type is usually cultivated in the districts of Sylhet, Moulovibazar, and Tangail in Bangladesh. Giant Kew pineapple has an oval form with a slight taper toward the crown and weighs between 2 and 3 kg. Fruits are better suited for canning since their eyes are shallow and wide. When fully mature, the fruit is yellow. The flesh is light yellow, nearly fibreless, and extremely juicy with 0.6-1.2% acid. The TSS content ranges from 12 to 16 Brix. A typical honey queen ripe pineapple weighs 1 kg, has yellow-colored flesh, eyes that are formed and pointy, and thorns on the leaf. This type is primarily grown in Hill Tract, Chittagong, and Sylhet. Ripe Gorashal pineapple is reddish and off white, with a large eye and an average weight of 1.25 kg. The leaf has a thorn and is wide and wave-like. This variety's cultivation is confined in Bangladesh's Dhaka and Norshindi districts (Ali et al., 2015).

a. b. c.

Figure 1 : Pineapple cultivars (a) Honey Queen, (b) Ghorashal and (c) Kalenga

Compared to the Giant Kew variety of pineapple, the Honey Queen variety is sweeter and higher in nutrients. The Giant Kew cultivar had 1.75 percent non-reducing sugar, 3.88% total sugar, and 6% total soluble solids (TSS). The Honey Queen type, on the other hand, has 10% TSS, 4.84% total sugar, and 1.59% non-reducing sugar. Giant Kew contains more Vitamin C than Honey Queen, but Honey Queen has a higher concentration of all the minerals. Comparing the two species, the Honey Queen has more calcium than the Giant Kew (Kader et al., 2010). In Nigeria, pineapple has an ascorbic acid content of 22.5 - 33.5 mg/100g-fw (Achinewhu & Hart., 1994).

Comparing pineapple pulp to pineapple waste, pineapple pulp had more reducing sugar. Pineapple pulp contains the most reducing sugar (10.5%), according to research. Indian variety trash had a greater overall sugar content (9.75%) than pulp (8.66%). Brazil's pearl pineapple contained 14.5% of the country's total sugar content. TSS ranges in brix from 10% to 14% depending on the season and maturity stage (Hemalatha et al., 2013). Indian varieties of pineapple had a lower level of reducing sugar (10-12.5) (Achinewhu et al., 1994). However, pineapples from Ghana have a higher concentration of reducing sugar (16.5%). In Indian pineapple, pulp had a higher TSS value (13.3%) than waste (10.2%). Josapine pineapple from Malaysia had a TSS value of 13.5%.The ascorbic acid level of pineapple fruits marginally decreased as they ripened. High moisture content (91.35%) and moderate titratable acidity were present in the pineapple waste.

2.4 Pineapple juice

Fresh pineapple juice is a well-known product. On the market, various types of pineapple juice are available. Some single-strength juice is extracted from pineapple chunks squeezed with mills and screw presses. Other types include concentrate juice, mixed juice with other fruits, transparent juice, and many more. Because of the unexpected increase in demand for tropical fruits and fruit products on international markets over the last two decades, many countries have begun processing tropical fruits. Fruit juices, nectars, and drinks are the most popular items manufactured from tropical fruits. Because of the high acidity, approximately 10% to 25% of pineapple juices obtained from canning companies are not suited for or concentrate juice processing (Adhikary et al., 1987). Thus, Fruit juice with natural fruit flavors and no artificial ingredients is becoming increasingly popular.

Because of its high acidity, pineapple juice obtained from the canning sector accounts for around 10%-25% of total pineapple juice production (Sairi et al., 2004). Pineapple juice composition varies according to area, culture, harvest season, and processing time (Khalid et al., 2016). Canned pineapple juice (100 mL) typically contains 0.36 g protein, 0.12 g fat, 12.87 g carbohydrate, and 0.1 g dietary fiber (Gebhardt et al., 1982).

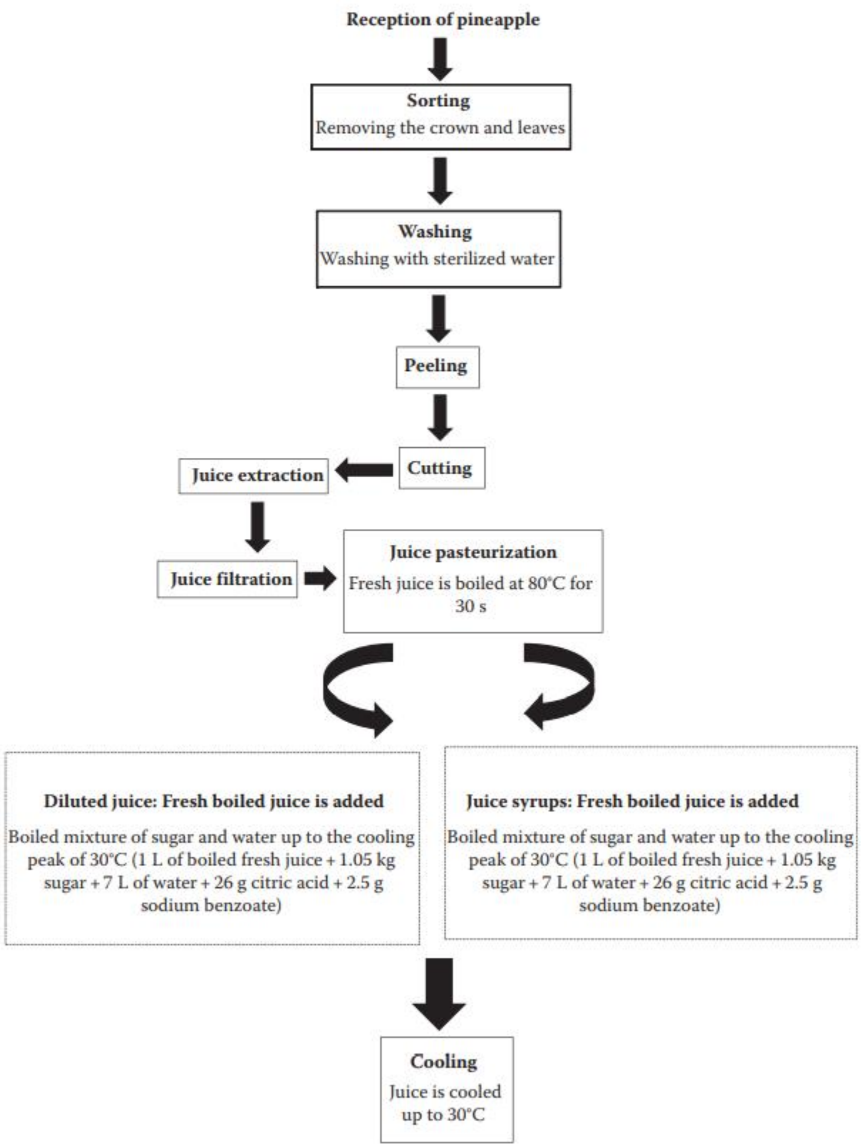

Figure 2: Flow chart of pineapple juice production in a small scale (Khalid et al., 2016)

Potassium (124-130 mg/100 mL), magnesium (12-15.4 mg/100 mL), phosphorus (3.1-8.0 mg/100 mL), iron (0.2-0.31 mg/100 mL), and manganese (0.3-0.99 mg/100 mL) are the major minerals in fresh pineapple juice (Elkins et al., 1997). Vitamin C (ascorbic acid), an effective antioxidant, is abundant in pineapple juice (Luximon-Ramma et al., 2013). Fresh pineapple juice included 84.2 mg of vitamin C per 100 ml(Cárnara et al., 1995). Pineapple juice and concentrate each include 5 and 50 IU of vitamin A per 100 grams, respectively (USDA, 2014). Asparagine, proline, aspartic acid, serine, glutamic acid, tyrosine, valine, and isoleucine are some of the significant free amino acids that have been found in pineapple juice (Gawler et al., 1962). The juice is abundant in asparagine, serine, threonine, and glycine(Elkins et al.,1997). Sucrose (4.1 g/100 mL), fructose (2.5 g/100 mL), and glucose (2.3 g/100 mL) are also present in pineapple juice(Cámara et al., 1996). The amino acids and sugars have a significant impact on the Maillard process, which yields hydroxymethylfurfural at pH less than 7 and concentrations ranging from 0.1-22.0 mg/100 mL of fresh pineapple juice (Ooghe et al., 1995). In pineapple juice processing, the Millard reaction is regarded as a crucial quality parameter (Khalid et al., 2016).

2.5 Thermal processing

One of the main objectives of the food industry is the preservation of the organoleptic qualities of food. In order to maintain a balance between raw material safety and nutritional quality, heat treatment optimization is a crucial technique (Traffano-Schiffo et al., 2014). Thermal processes are classified based on the degree of heat treatment (Miller & Silva, 2012). There are two types of thermal processing. One is conventional heat treatment, and the other is unconventional heat treatment. Conventional heat treatments categorized in 4 groups, and these are high temperatures long time (HTLT), high temperature short time (HTST), mild temperature-long time (MTLT) and mild temperature-short time (MTST). In addition to the conventional thermal processing, other unconventional thermal methods are ohmic heating and microwave heating (MHW) (Petruzzi et al., 2017).

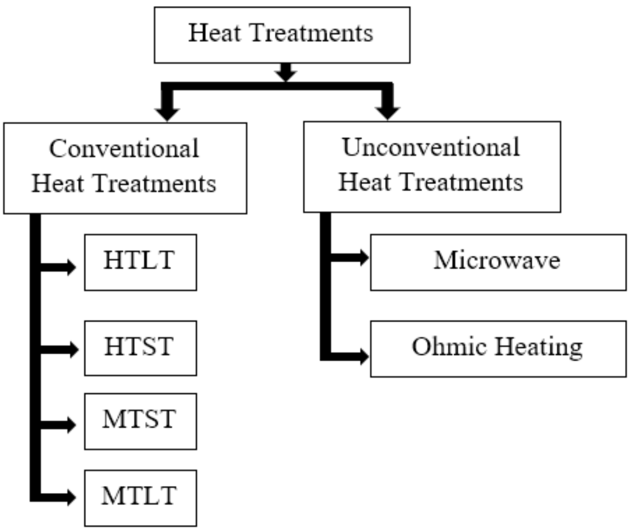

Figure 5: Types of the heat treatments (Petruzzi et al., 2017)

The most popular method for processing juices and beverages is known as high temperature low time (HTLT); it can be categorised as pasteurization (temperature <100 °C), canning (temperature 100 °C), or sterilizing (temperature >100 °C) (Miller and Silva 2012). This technique uses mechanisms of conduction and convection to transfer heat from the outside to the food (Chen et al., 2013). HTLT treatments may decrease or inactivate several enzymes, such as polyphenoloxidase (PPO), peroxidase (POD), pectin esterase (PE), and polygalacturonase (PG), whose activities result in unfavorable changes in sensory quality attributes and nutritional value of the goods (Miller and Silva 2012).However, many antioxidant compounds may be affected by HTLT, diminishing their favorable health benefits. The decrease in antioxidant capability was caused by a loss of total anthocyanins and vitamin C (Miller and Silva 2012). Despite its disadvantages, HTLT remains the most common method among food processors due to its capacity to eliminate microorganisms.

HTST thermal pasteurization (temperature ≥ 80 °C and holding durations ≤ 30 s) has been utilized to overcome the drawbacks of the HTLT which ensures product safety and keep the desired bioactive components (Petruzzi et al., 2017). It has been shown that HTST heat treatment increases the concentration of total phenolics, ()-epicatechin, ferulic acid, and p-coumaric acid in apricot nectar (Huang et al., 2013), lemon/pomegranate juice's color hue (Mena et al., 2013), nutritional value in fermented orange juice (Cerrillo et al., 2015); and and viscosity in carrot juice (Chen et al., 2012).

For minimally processed items, MTLT heat treatments (temperature 80 °C and holding periods >30 sec) are employed to extend shelf life (Petruzzi et al., 2017). It has been reported that MTLT can increase the amount of total phenolics in black jamun juice (Saikia et al., 2015), preserve color in cucumber juice (Wang et al., 2013), increase the stability and viscosity of color in pineapple juice (Saeeduddin et al., 2015), increase the amount of ascorbic acid and other phenolic compounds in prickly pear juice (Cruz-Cansino et al., 2015), and increase the amount of beta-carotene in reduced- (Wang et al., 2015). There are some disadvantages of MTLT, and these are a decrease in total antioxidant capacity, total phenols, anthocyanin content, and instrumental color variables in smoothie (Keenan et al., 2012), total phenolic content and ferric reducing antioxidant property in litchi juice (Saikia et al., 2015), total flavonoid content in pineapple juice (Saikia et al., 2015).

Temperatures of <80 °C and holding periods of ≤30 sec are used in MTST heat processing. *Listeria innocua* (NCTC 11288) population in apple, mango, orange, and pineapple smoothie was found to be reduced by 6 to 7 logs after MTST heat treatments (Palgan et al., 2012), and the native microorganisms were reduced by 3.5 to 3.7 logs (Walkling-Ribeiro et al., 2010). MTST preserves sensory quality (appearance, sweetness, and acidity) in apple/cranberry juice blend (Caminiti et al., 2011), as well as biological properties related to peroxidation inhibition (Azofeifa et al., 2015) and enhances the anthocyanin content in pomegranate juice (Mena et al., 2013b), and total phenolic content in sweet cherry juice (Queiros et al., 2015).

2.6 Bioactive compounds

Laorko et al. (2010) investigated how membrane filtration affected the total phenolic content and antioxidant capacity of pineapple juice. The 0.2 m membrane had the highest total phenolic content (69.34 mg gallic acid equivalents [GAE]/100 mL) and free radical scavenging capacity (25.76 mg ascorbic acid equivalents (AAE)/100 mL), and antioxidant activity declined with pore size. In compared to other fruit juices, pineapple juice has intermediate antioxidant activity (pineapple juice > orange juice > cherry juice) (Ramadan-Hassanien, 2010). After 120 minutes of incubation, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of pineapple juice fell from 78.1% to 71.9% (Ramadan-Hassanien , 2010). Mahdavi et al.(2010) measured and compared the total polyphenols in fresh and packaged fruit juice. The total polyphenol concentration of natural fresh pineapple juice was 36.2 mg GAE/100 mL, compared to 35.7 mg GAE/100 mL in commercial pineapple juice, indicating no significant difference ($P > 0.05$).

Gardner et al. (2000) studied the composition and antioxidant activity of several juices, including orange, apple, pineapple, grapefruit, and vegetables. They discovered that ascorbic acid contributed more than 66% of the antioxidant activity of citrus juices, while it contributed less than 5% to the other products and 0.8% to pineapple juice. The phenolic chemicals in pineapple juice are thought to be responsible for the majority of the antioxidant activity. Apart from phenolic components, pineapple juice also contains l-ascorbic acid and dehydroascorbic acid.

Chapter 3: Materials & method

3.1 Site and period of study

The study was conducted in the laboratory of the department of Food Processing and Engineering at Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram-4225. The study was conducted for a period of six months from the 1^{st} of July to 30^{th} December 2022

3.2 Equipment

Digital analytical balance was used to measure all the liquid samples, juicer, beakers, watch glass, glass rod, burette, pipette, conical flask, test tubes, volumetric flasks were used in the assessment and preparation of sample and standard solutions. Hot air bath was used for the heat treatment of the samples. UV-visible spectrophotometer was used for the determination of concentration and absorbance of the targeted compounds.

3.3 Chemical and reagents

Sodium hydroxide (NaOH), potassium acetate (CH_3CO_2K), sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), ethanol, methanol, folin ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), distilled water.

3.4 Sample collection and preparation

Pineapples from three cultivars (Honey Queen, Giant Kew and Ghorashal) are collected from the local fruit market situated in Bohoddarhat and Newmarket area. All visible dirt and the crown were removed from the pineapples. The pineapples were then washed with tap water. They were then peeled with a knife and the core was removed. The flesh was sliced into small pieces, and the juice was extracted using a regular juicer. The pineapple pulp was then filtered through a filter cloth (100 mesh) to obtain a sample of pineapple fruit juice. Before processing, all fresh juice samples were kept at 4 degrees Celsius for less than an hour.

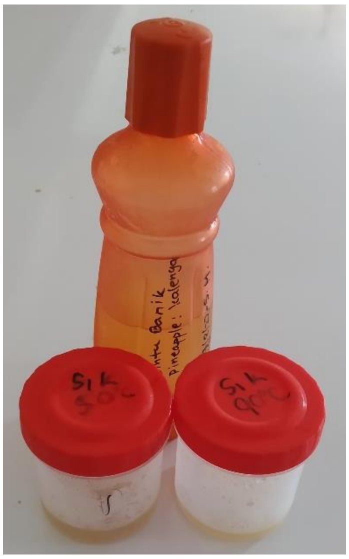

Figure 4(a) : Kalenga juice samples

Figure 4(b) : Honey queen juice samples

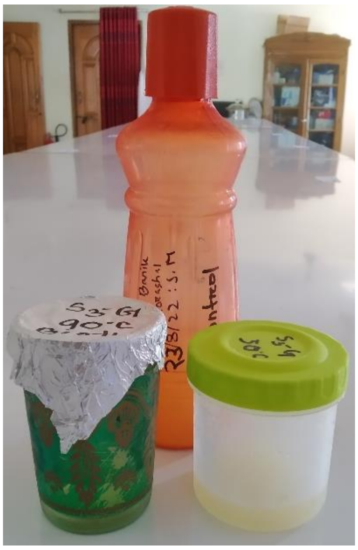

Figure 4(c) : Ghorashal juice samples

3.5 Sample extraction

For the sample extraction, absolute ethanol was used. The 1g juice sample was mixed with 10 ml ethanol and stored in the dark at room temperature for three days. After three days of maturation, the mixture was filtered and the supernatant was collected, which was then used for bioactive compound analysis.

3.6 Thermal processing

The freshly squeezed pineapple juices were then thermally processed. The juices were heated in a rotary evaporator at 50°C and 90°C. The juice samples were treated for 10 minutes. The samples were immediately cooled to room temperature with an ice water bath after thermal processing. Every treatment was done in triplicate.

Juice without any heat treatment was used as a control group. After treatment, all samples were stored at 4±1°C in the dark for 14 days. Samples were carried out at 0, 7, 14 and 21 days to determine the physicochemical properties and bioactive compounds changes during storage.

3.7 Determinations of pH and total soluble solids (TSS)

The pH values of pineapple fruit juice samples were measured at room temperature by a pH meter (pHep, H198107). The TSS of pineapple fruit juice was measured using a digital refractometer (RP-101, Atago, Tokyo, Japan) at an ambient temperature (20°C) and expressed as ˚Brix.

3.8 Determinations of acidity percentage

The acidity percentage of pineapple fruit juice was titrated using a standardized 0.1 mol/L NaOH to the end point with phenolphthalein (pH = 8.2). First, 5 mL of juice sample and 20 mL of deionized water were mixed in a beaker with a pipette. A few drops of phenolphthalein were added to it. Following the addition of 0.1 N NaOH to the burette, the initial reading was taken. Titration was then carefully performed until a bright pink color produced. It determines the end point of the titration. The final reading of the burette was recorded. The same procedure was repeated three times using juice samples, and the mean value was recorded.

Calculation:

$$\% \text{ of citric acid} = \frac{volume\ of\ alkali\ used \times (N) of\ NaOH \times 64 \times 4}{volume\ of\ juice\ sample \times 1000} \times 100$$

3.9 Determination of total polyphenol content (TPC)

TPC of pineapple juice was measured using a slightly modified Folin Ciocalteu method reported by Alothman et al. (2009). 1 mL of extracts were pipetted into a 1.5 mL FC reagent-containing test tube. The solutions were properly mixed and left at room temperature for 3 minutes. After that, 1.5 mL of sodium carbonate (7.5%) solution was added and incubated at room temperature for another 60 minutes. A UV-VIS Spectrophotometer (UV-2600, Shimadzu Corporation, USA) was used to measure absorbance at 765 nm, with ethanol serving as the blank. A gallic acid standard curve was drawn with several standard solutions of gallic acid, i.e., 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL, to quantify TPC concentration. The pineapple juice absorbance was compared to the gallic acid standard curve. TPC was determined and represented per gram of extracts (mg GAE/g) as milligrams of Gallic Acid Equivalents (GAE).

3.10 Determination of total flavonoid content (TFC)

TFC of pineapple juice extracts was assessed using a slightly modified version of the aluminum chloride colorimetric method reported by Chang et al. (2002). 0.5ml of the extract was transferred to a test tube containing 5ml of ethanol. The mixture in the cuvette was then treated with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water. The mixture was left at room temperature for 30 minutes. The absorbance was measured at 415 nm using a UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA) and 10% aluminum chloride substituted with the same amount of distilled water as the blank. Standard solutions of quercetin (0.025, 0.050, 0.075, and 0.100 mg/mL) were prepared by dissolving it in 80% ethanol. Total flavonoid concentration was calculated by comparing sample extract absorbance to a quercetin standard curve. TFC is determined as milligrams Quercetin Equivalents (QE) per 100ml (mg QE/100 ml).

3.11 Determination of antioxidant capacity (AOC)

AOC of the extracts was measured using the DPPH test, which was slightly modified from the methods published by Chan et al., (2007). Methanolic DPPH solution was made by dissolving 6 milligram of DPPH in 100 mL of methanol. An aliquot (0.5 mL) of extract methanolic solution with concentrations of 0.10, 0.20, 0.30, 0.40, 0.60, and 0.80 mg/mL was added to 2.5 mL of DPPH methanolic solution. The mixture was given a light shake before being left at room temperature for 30 minutes in the dark. Using a UV-VIS spectrophotometer, the absorbance was measured at 517 nm (UV-2600, Shimadzu Corporation, USA). Methanol was used as a blank and as the control, which was made by combining 1 mL of methanol with 2 mL of DPPH solution. The reduction in absorbance of the samples when compared to the DPPH standard solution served as a measure for the scavenging activity. Trolox was employed as the reference material, and the TEAC compound (Trolox equivalent antioxidant capacity) was used to create the calibration standard curve. The results were represented as µmol of Trolox equivalents per 100 ml (µmol TE/100 ml).

3.12 Statistical Analysis

The data were recorded and sorted in Microsoft Excel 2013. Statistical analysis was performed) using Minitab Statistical Software (Version 18.1). Obtained data were presented as Mean Value±Standard Deviation (SD).

Chapter 4: Results and discussion

The findings of the study have been given and analyzed in this chapter with the aim of comparing bioactive components in thermally processed juices from three pineapple cultivars. The data is presented in various tables and figures. The findings have been discussed and possible interpretations are provided under the headings listed below.

4.1 pH

Table 2: pH of pineapple cultivars(treated and untreated juice sample)

Cultivars	Days	Control	50°C	90°C
Kalenga	0	4.1	4	4
Honey Queen	0	4.2	4.2	4
Ghorashal	0	4.2	4.1	4
Kalenga	7	4.3	4.3	4
Honey Queen	7	4.2	4.2	4.1
Ghorashal	7	4.3	4.3	4.2
Kalenga	14	4.2	4.2	4.1
Honey Queen	14	4.2	4.1	4
Ghorashal	14	4.1	4.3	4.2
Kalenga	21	4.1	4.2	4
Honey Queen	21	4.1	4	4.2
Ghorashal	21	4.1	4.2	4.2

pH is one of the parameters that influence microbe growth and survival during processing and storage. Most fruit juice has a pH between 6 and 7, indicating a low level of acidity. The exception is pineapple juice, which has a pH between 3 and 4 since pineapples are strongly acidic. In our study, the pH ranges from 4.1 to 4.2 in control and 4 to 4.3 in treated juice samples. This correlates to the pH range of 4 to 4.5 in pineapple fruits, as measured by Kim et al., (2001).

Furthermore, there was no noticeable change in the pH of thermally processed pineapple juice during storage. These findings are comparable with those of Rivas et al. (2006), who found no pH changes in thermally treated juice (mixed orange and carrot juice) during refrigerated storage at 2°C and 12°C. A previous study that examined the pH of thermally treated Valencia and Navel orange juice discovered no significant changes during storage at 4°C and 10°C (Bull et al., 2004).

As can be seen from the Table 2, The pH of the pineapple juice did not change significantly as a result of the heat treatments, which is consistent with prior investigations (Yeom et al., 2000). These quality characteristics are significant because they are linked to the stability of bioactive chemicals in fruit products (Kaddumukasa et al., 2017).

4.2 Total soluble sugar (TSS)

Table 3: TSS of pineapple cultivars(treated and untreated juice sample)

Cultivars	Days	Control (°Brix)	50°C (°Brix)	90°C (°Brix)
Kalenga	0	10	11	11
Honey Queen	0	9	12	16
Ghorashal	0	8	6	7
Kalenga	7	9	10	11
Honey Queen	7	10	9	17
Ghorashal	7	10	8	9
Kalenga	14	9	10	11
Honey Queen	14	10	10	17
Ghorashal	14	14	15	15
Kalenga	21	11	10	10
Honey Queen	21	10	10	15
Ghorashal	21	14	15	13

TSS is an important characteristic in fruit juice and is frequently used to assess its quality (Bartholomew et al., 2003). According to Shah, et al., (2015), pineapples have a total sugar content that ranges from 9.9 to 14.7 with only slight changes between varieties. The TSS of the kalenga, honey queen, and gorashal cultivars ranges from 8 to 10°brix, as shown in the table 3. The data for pineapple cultivars included in the study fall within these parameters. The Kalenga pineapple cultivar had the highest TSS (10°brix) of all the experimental units used in pineapple juice processing. The Ghorashal pineapple variety had the lowest TSS concentration (8°brix) in pineapple juice. The highest TSS (16°brix) was recorded after thermal processing for honey queen varieties at 90˚C. For honey queen species, the retention of TSS at 90˚C was high compared to the giant kew and ghorashal species. As per Tandon et al. (2003), the higher soluble solids in pasteurized juice are caused by water evaporation during thermal processing.

In the first two weeks of storage, the total soluble solids of the heat-treated Kalenga pineapple juice (at 50 ˚C and 90 ˚C) remained essentially constant. However it started to decline after 2 weeks of storage. In contrast, the TSS of honey queen variety pineapple juice started to decline after first week of storage. According to Rivas et al.

(2006), the change in total soluble solids is caused by the presence of microorganisms that cause the fruit juice to deteriorate due to sugar fermentation. Microorganism-caused fermentation is the process of breaking down glucose through the biochemical pathway (Rosen and Gothard, 2010). The juice of Kalanga and honey queen pineapple varieties may have developed this condition around the third week and first week of storage respectively, as the studies found that the juice began to smell fermented the moment the bottles were uncapped. On the other hand, the TSS of Gorashal cultivars decreased in the first week but increased after the second week. According to Saad (2017), higher temperatures can promote cell wall breakdown, making the cell wall less stable and more permeable for leaching.

4.3 Titratable acidity (TA)

Table 4: TA of pineapple cultivars(treated and untreated juice sample)

Cultivars	Days	Control (%)	50°C (%)	90°C (%)
Kalenga	0	1.89±0.01	2.02±0.11	2.15±0.03
Honey Queen	0	1.46±0.01	1.32±0.06	1.95±0.01
Ghorashal	0	0.76±0.01	1.55±0.02	1.88±0.20
Kalenga	7	1.01±0.01	1.13±0.01	1.13±0.01
Honey Queen	7	1.39±0.01	1.37±0.05	1.62±0.07
Ghorashal	7	0.61±0.21	1.43±0.13	1.56±0.10
Kalenga	14	1.29±0.06	1.41±0.40	1.63±0.03
Honey Queen	14	1.41±0.03	1.54±0.01	2.14±0.11
Ghorashal	14	1.12±0.14	1.40±0.10	1.23±0.01
Kalenga	21	1.33±0.01	1.46±0.09	1.57±0.08
Honey Queen	21	1.28±0.01	1.70±0.02	1.96±0.04
Ghorashal	21	1.07±0.01	1.67±0.41	1.29±0.04

TA is the measurement of the overall acid concentration in a food. Titratable acidity in this experiment ranges between 0.76 – 1.89% in the control and 1.13 – 2.02% at 50°C and 1.23 – 2.15% at 90°C in treated juice sample from week 1 to week 3. TA decreased following three weeks of storage in pineapple juice prepared from the Kalenga cultivar, both in the control and treatment samples. According to Sodeko et al. (1987), microbes decrease acidity and result in the fermentation of organic acid, which causes spoilage.

However, juices from Honey Queen and Ghorashal cultivars showed opposite trend. Over the course of the three weeks of storage, the titratable acidity marginally increased both in the control sample and the treated samples. The oxidation of reducing sugar may be the cause of the increase in titratable acidity, and this process may also be responsible for the increase in fruit juice's acidity (Kumar et al., 2017). Pineapples have a variety of organic acids, including malic and citric acid. Titratable Acidity approximates total acidity that indicates the fruit's keeping quality or storability. The high acidity of Honey Queen and Ghorashal cultivars make them appropriate for processing products that require high acidity and low pH, such as jam.

4.4 Total polyphenol content (TPC)

Table 5: TPC of pineapple cultivars(treated and untreated juice sample)

Cultivars	Days	Control (mg GAE/100 ml)	50°C (mg GAE/100 ml)	90°C (mg GAE/100 ml)
Kalenga	0	1.531±0.01	1.507±0.01	1.261±0.02
Honey Queen	0	0.916±0.01	1.441±0.02	2.388±0.03
Ghorashal	0	0.649±0.03	-0.115±0.01	-0.574±0.01
Kalenga	7	1.146±0.04	-1.028±0.01	-0.965±0.03
Honey Queen	7	-1.188±0.02	-1.456±0.01	-0.758±0.03
Ghorashal	7	-1.086±0.02	-0.895±0.01	-0.979±0.01
Kalenga	14	-0.995±0.06	-0.926±0.04	-1.024±0.03
Honey Queen	14	-1.358±0.06	-1.855±0.01	-0.846±0.01
Ghorashal	14	-1.158±0.01	-1.855±0.01	-0.846±0.01
Kalenga	21	-0.103±0.01	-0.456±0.03	-0.536±0.01
Honey Queen	21	-0.192±0.01	-0.231±0.03	-0.108±0.01
Ghorashal	21	0.084±0.03	0.002±0.03	-0.363±0.01

TPC of juice obtained from the Kalenga pineapple cultivar is in the range of -0.456±0.03 mg GAE/100 ml to 1.531±0.01 mg GAE/100 ml. TPC was higher in the untreated juice sample, which was 1.531±0.01 mg GAE/100 ml. When the juice sample was thermally processed at 50°C and 90°C, the TPC content decreased at 1.507±0.01 mg GAE/100 ml and 1.261±0.02 mg GAE/100 ml respectively. Moreover, the TPC decreased gradually throughout the three weeks of storage period. TPC Content of juice obtained from the Ghorashal Species showed the similar trend. The TPC content was in the range of -0.979±0.01 mg GAE/100 ml to 0.649±0.03 mg GAE/100 ml. TPC was higher in the untreated juice sample, which was 0.649±0.03 mg GAE/100 ml. Then it reduced when the juice was thermally processed and stored for the three weeks. However, Honey Queen Species showed an opposite trend. The concentration of the total polyphenol was higher in the thermally treated juice. The TPC of untreated juice was 0.916±0.01 mg GAE/100 ml. Then it gradually increased at 1.441±0.02 mg

GAE/100 ml and 2.388±0.03 mg GAE/100 ml when thermally processed at 50°C and 90°C respectively. However at the third week of storage, a slight increase in the TPC was observed in three of the juice sample.

According to the Mahdavi et al., (2010), the polyphenol content is affected by many factors such as processing techniques, clarification, and heat treatment of the juices. The polyphenolic content of fruit juices may be reduced by clarifying, according to Ritter et al. (1992) findings. Klopotek et al., (2005) also found that pasteurization had an impact on the polyphenol levels (-27% reduction). Thermal processing may reduce total phenolics due to oxidative destruction of thermally unstable phenolic compounds. These finding explains the decrease of TPC of juice samples after thermal processing of juices (Nayak et al., 2013). However, Tavarini et al. (2008) reported that during storage, certain chemicals may develop and react with the Folin-Ciocalteau reagent to increase the phenolic content. Among the three varieties, the stability of the TPC was relatively high in the Kalenga Species. Ghorashal Species showed lower stability than the Honey Queen Species. Therefore, Kalenga cultivars may use for the further processing of different food items from it.

4.5 Total flavonoid content (TFC)

Table 6: TFC of pineapple cultivars(treated and untreated juice sample)

Cultivars	Days	Control (mg QE/100ml)	50°C (mg QE/100ml)	90°C (mg QE/100ml)
Kalenga	0	8.172±0.05	6.503±0.04	9.237±0.03
Honey Queen	0	7.038±0.06	7.794±0.05	14.980±0.01
Ghorashal	0	10.867±0.04	11.631±0.05	9.897±0.16
Kalenga	7	4.388±0.06	7.464±0.05	5.161±0.09
Honey Queen	7	5.385±0.08	10.769±0.07	4.603±0.01
Ghorashal	7	4.548±0.02	8.768±0.09	16.940±0.07
Kalenga	14	6.961±0.19	5.160±0.05	6.870±0.04
Honey Queen	14	5.459±0.01	6.452±0.01	10.204±0.03
Ghorashal	14	5.647±0.02	6.139±0.02	7.973±0.03
Kalenga	21	1.470±0.01	1.291±0.03	1.042±0.01
Honey Queen	21	1.319±0.01	1.067±0.03	1.883±0.01
Ghorashal	21	0.197±0.03	0.011±0.03	1.188±0.01

The TFC of Kalenga species was in the range of 1.042±0.01 mg QE/100ml to 9.237±0.03 mg QE/100ml. The flavonoid concentration of untreated juice sample was 8.172±0.05 mg QE/100ml. However the concentration increased at 6.503±0.04 mg

QE/100ml and 9.237±0.03 mg QE/100ml at 50°C and 90°C respectively. Overall, a decreasing trend was observed in the concentration of juice sample during the three weeks of storage period. At third weeks, the concentration decreased to 1.291±0.03 mg QE/100ml. For honey queen cultivars, the total flavonoid concentration was in the range of 1.067±0.03 mg QE/100ml to 14.980±0.01 mg QE/100ml. The concentration of juice sample fluctuates between 4.603±0.01 mg QE/100ml and 10.769±0.07 mg QE/100ml for the first two weeks. However, a sudden drop in the concentration was observed in the third week and the range was 1.067±0.03 mg QE/100ml to 1.883±0.01 mg QE/100ml.On the other hand, the TPC of Ghorashal species was between 0.011±0.03 mg QE/100ml and 16.940±0.07mg QE/100ml. Ghorashal species also shown similar trend as the kalenga and honey queen varieties.

The findings are consistent with the findings of Saikia et al (2015). They discovered that the flavonoid concentration of pineapple juice decreased after processing.

4.6 Antioxidant capacity (AOC)

Table 7: AOC of pineapple cultivars(treated and untreated juice sample)

Cultivars	Days	Control (µmol TE/100 ml)	50°C (µmol TE/100 ml)	90°C (µmol TE/100 ml)
Kalenga	0	2.796±0.05	2.896±0.04	2.597±0.03
Honey Queen	0	3.016±0.06	2.954±0.05	2.994±0.01
Ghorashal	0	2.941±0.01	3.036±0.01	3.141±0.03
Kalenga	7	2.200±0.06	2.796±0.05	2.265±0.09
Honey Queen	7	1.963±0.08	2.127±0.07	2.274±0.01
Ghorashal	7	2.486±0.05	3.226±0.02	2.508±0.03
Kalenga	14	2.534±0.19	2.163±0.05	2.414±0.04
Honey Queen	14	2.100±0.01	2.157±0.01	2.275±0.03
Ghorashal	14	2.533±0.01	2.538±0.02	2.485±0.02
Kalenga	21	2.469±0.01	2.161±0.03	2.269±0.01
Honey Queen	21	2.488±0.01	1.479±0.03	2.011±0.01
Ghorashal	21	2.524±0.06	2.217±0.07	2.807±0.02

AOC of Kalenga species was in the range of 2.161±0.02 µmol TE/100 ml to 2.896±0.04 µmol TE/ml. The highest antioxidant was observed in thermally treated (50°C) juice sample, which was 2.896±0.04 µmol TE/ml. The AOC remain stable throughout the three weeks of storage. In case of honey queen species, the highest AOC was 3.016±0.09 µmol TE/ml, which was found in untreated fresh juice sample. The AOC of thermally processed honey queen species fluctuated between the 1.479±0.03 µmol

TE/ml and 2.994±0.02 03 μmol TE/ml. The AOC of Ghorashal Species was in the range of 3.226±0.02 μmol TE/ml to 2.217±0.01 μmol TE/ml. The highest AOC was observed during the first week of storage. However among the three cultivars, the total antioxidant value was more stable in the juice sample of kalenga cultivars, followed by Ghorashal cultivar.

The breakdown of heat labile phenolic compounds found in the juices could be the cause of the decrease in phytochemicals and antioxidant activity (Cortes et al., 2008). When heated, phenolic compounds tend to undergo structural rearrangement, which can result in either increased or decreased antioxidant activity (Saikia et al., 2015)

Chapter 6: Conclusion

There was no significant change in pH among the three pineapple cultivars after the heat treatments and storage of three weeks. TSS was greatly affected by the thermal processing. TSS increased after heat treatment at 50°C and 90°C. The highest TSS was observed in honey queen species at 90°C followed by kalenga species. Ghorashal species had the lowest TSS in both untreated and treated juice samples. Furthermore, high acidity was found in honey queen and ghorashal species. Kalenga species has the maximum retention of total phenolic components and antioxidant capability when compared to honey queen and ghorashal cultivars. However, TPC decreased significantly after the three weeks of storage. Therefore, heat treatment at 90°C resulted in favorable outcomes for physicochemical properties but poor retention of bioactive components. Mild heat treatment at 50°C resulted in moderate alterations in physicochemical attributes as well as bioactive components. Thus, heat processing at 50°C would be ideal for pineapple juice processing.

Chapter 7: Future prospects and recommendation

Limitations:

1. Research fund was inadequate.
2. Lab facility should be more improved.
3. Specific bioactive compounds present in the pineapple juice were not determined for insufficient fund and facility.
4. Sample size of this investigation was not representative to the population due to short period of the study.

Suggestions for future research work:

From the present study, the following suggestions can be made for future work.

1. Future research should include a larger number of samples and a more detailed examination of the pineapple juice.
2. More research is needed to determine the microbiological quality of pineapple juice after heat treatments.
3. Sensory analysis needed to do to determine the consumer acceptability of the pineapple juice.

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