THE EFFECT OF VARIOUS STORAGE CONDITIONS ON BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF POMELO AND ORANGE JUICE

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Roll No.: 0118/02

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June, 2020

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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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DEDICATED TO MY

RESPECTED AND BELOVED

PARENTS AND TEACHERS

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The Author
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Abbreviations

BC	Before Christ
DF	Dilution Factor
DPPH	2,2-diphenyl-hydrazyl-hydrate
°C	Degree Celsius
E	Extinction Coefficient
GAE	Gallic Acid Equivalent
G	Gram
MG	Milligram
ML	Milliliter
Mmol	Millimole
TAC	Total Anthocyanin Content
TPC	Total phenolic content
TFC	Total Flavonoid Content
PC	Phenolic content
TE	Trolox Equivalent
QE	Quercetin
UV	Ultra Violet

Abstract

Storage conditions are important factors for fruits quality and antioxidant potential. This study was assessed the effect of various storage condition on bioactive compounds (Total anthocyanin content, Total flavonoid content, Total phenolic content) and antioxidant activity of pomelo and orange juice. The pomelo and orange juice samples were stored for a period of 30 days at room, chilling and frozen temperature. Total anthocyanin content, Total flavonoid content, Total phenolic content and antioxidant activity of pomelo and orange juice in 0 days, 7 days, 15 days and 30 days were determined by calorimetrically, aluminum chloride colorimetric, Folin-Ciocalteu and DPPH assay method respectively by using UV-visible spectrophotometer. The results of Total anthocyanin content, Total flavonoid content, Total phenolic content and Antioxidant activity of pomelo juice at room temperature, chilling temperature and frozen temperature were varied from 1.34-0.45 mg/100 g, 27.34-8.11 mg QE / 100 g, 88.91-35.16 mg GAE / 100 g and 300.23-160.14 µmol TE / 100 g for different days of stored pomelo juice respectively. The results of Total anthocyanin content, Total flavonoid content, Total phenolic content and Antioxidant activity of orange juice at room temperature, chilling temperature and frozen temperature were varied from 1.53-0.80 mg/100 g, 21.34-6.65 mg QE / 100 g, 106.65-52.88 mg GAE / 100 g and 232.50-101.76 µmol TE / 100 g for different days of stored pomelo juice respectively. The bioactive compounds and antioxidant activity for different days of storage period of pomelo juice at room, chilling and frozen temperature was significantly different ($p<0.05$). The storage time and temperature have an influence on bioactive compounds and antioxidant activity of the pomelo and orange juice. In spite of the reduction in these valuable compounds, the condition of frozen temperature retained good amounts of them than others storage temperature. Regardless of the phenolic compound loss after storage, pomelo and orange juice are good sources of substances with antioxidant potential.

Keywords: Storage conditions, Juice stability, Bioactive compounds, Antioxidant capacity.

Chapter I: Introduction

Citrus fruits are the major group of fruits produced in the world. About 34% of citrus fruits are used to make juices. Citrus fruits and juices are recognized to be healthful components of the human diet because there are many bioactive compounds, such as phenolic acid, limonoid, carotenoid and fiber (Gorinstein et al., 2001; Schieber et al., 2001) in citrus juices and peels.

Orange is the major citrus species in Asia countries such as China, Japan and Korea (Hong et al., 2007). This fruit is very appreciated by people due to its good flavor as well as the property of easy peeling in comparison with other citrus cultivars. Pomelo is one of the tropical fruits which native to Southeast Asia (Toh et al., 2013). In Bangladesh, the fruit is called as *Jambura.* It is a large citrus fruit with a common name of pomelo or shaddock that belongs to the family of Rutaceae (Scora, 1975). The fruit is widely grown in the all districts of Bangladesh. Pomelo is the most popular due to its juiciness, sweetness and delicious taste. The juice from fruits of Citrus is characterized by substantial accumulation (apart from ascorbic acid) of flavonoids and phenylpropanoids (Rousseff et al., 1987). Particularly, the presence of anthocyanins is typical of blood orange varieties; in fact, the red color of blood orange peels and pulp is due to these pigments.

Global fruits and fruits juice consumption has been growing, day by day. Citrus fruits are a natural source of phenolic compounds. Furthermore, juice-processing methods can affect bioactive compounds in the final product (Patras et al., 2010). In recent years, consumers have become more concerned about having a healthy diet, food quality, food safety and sustainable production (Weaver et al., 2014). Furthermore, there is a need to ensure stability during storage of citrus juices, as well as the stability of factors responsible for bioactive properties such as phenolic content, antioxidant capacity and anthocyanins. The distribution and composition of phenolic phytochemicals are affected by maturity, cultivar, horticultural practices, geographic origin, growing season, postharvest storage conditions and duration, and processing procedures (Burda et al., 1990).

The total phenolics, antioxidant activity and anthocyanins of these fruits could be affected during storage (Rababah et al., 2011).

However, there are few papers worldwide, and none in the Asia, concerning the effect of storage on these compounds. The effect of fruit on antioxidant activity, total phenolics, and anthocyanins components were evaluated by few researchers. Fruit juice is a popular way to consume fruit worldwide, and changes during storage to phenolics and other bioactive compounds have been studied (Dawes & Keene, 1999; de Lima et al., 2014; Mendes Lopes et al., 2016). Furthermore, there is a need to increase our understanding of the effect of its storage on nutraceutical contents. For these reasons, the effects of storage upon bioactive compounds and antioxidant activity need to be investigated.

Aims & Objectives

- The effect of various storage condition on bioactive compounds of pomelo and orange juice.
- The effect of various storage condition on antioxidant activity of pomelo and orange juice.

Chapter II: Review of Literature

2.1 Citrus Fruits

Citrus is a genus of flowering trees and shrubs in the rue family, Rutaceae. Plants in the genus produce citrus fruits, including important crops such as oranges, lemons, grapefruits, pomelos, and limes. Citrus fruits are one of the largest fruit crops in the world. About 30% of citrus fruits is processed to obtain various products, mainly juice. Although consumption of fresh citrus fruits is popular in all producing countries, processed products must still be considered almost as luxury products. Breakfast with orange juice is only common in developed countries (Tangpricha et al., 2003). Thus, citrus industries process value-added products whose quality, nutritional characteristics, and purity are appreciated. Since these three aspects are closely related to composition, the analysis of citrus constituents is a frequent subject of research work, supported by governments and industries (González-Molina et al., 2008).

2.2 Main Types citrus fruits on the Market

Citrus fruits are principally marketed according to how they are consumed:

As fresh or dessert fruits – sweet oranges, mandarins (eaten out of hand), grapefruit, or pummelo (spooned). Juice, slices, segments, rind, and leaves are also used to garnish food.

As processed products – juice (fresh, chilled, frozen, canned, blended, or concentrated), syrups and cordials, segments and rind oil, or essence. After the juice is extracted, there remain residues that can be a source material from which over 300 valuable byproducts can be produced. Some specialized types are also candied, dried, or used for marmalade manufacture (Das and Singh, 2004).

2.3 Citrus Fruits-Pomelo

Pomelo [*Citrus grandis (L.) Osbeck*] is one of the tropical fruits which native to Southeast Asia. In Bangladesh, the fruit is called as jambura. It is a large citrus fruit with a common name of pomelo or shaddock that belongs to the family of Rutaceae (Scora, 1975). There are few popular varieties of pomelo in Bangladesh which known as White pomelo and Pink Pomelo. Both type of pomelo is the most popular due to its juiciness, sweetness and delicious taste.

Figure 2.1: Pomelo

2.4 Citrus Fruits-Orange

The orange is the most widely grown and popular citrus species worldwide (Liu et al., 2012). Its origin is believed to be the tropical and subtropical regions of Asia. The mention of oranges in Chinese literature dates to 2200 BC, long before they were introduced to Europe– about 1400 ad Continental America probably received its first oranges in the early-tomid-1500s with the arrival of the explorers Bernal Diaz in Mexico and Ponce de Leon in Florida.

Figure 2.2: Orange

2.5 Bioactive compounds in Pomelo and orange fruits

Citrus fruits are well-accepted by consumers all over the world because of their attractive colors, pleasant flavors and aroma, as well as for their well-known nutrition and health-promoting values (Ballistreri et al., 2019). Citrus fruits are a good source of dietary antioxidants, which are vital in both health promotion and in the prevention and treatment of various human chronic and degenerative diseases. Flavonoids are the most abundant phenolic compounds found in Citrus fruits (Tripoli et al., 2007). Flavonoids identified in Citrus fruits cover over 60 types, according to the five classes mentioned (Horowitz and Gentili, 1977): flavones, flavanones, flavonols, flavans and anthocyanins (the last only in blood oranges). Moreover, these pigments are expressed in the young shoots and some floral tissues (new growth) of lemon, lime, and citron as well as in the peel (flavedo) of 'ISA Red lemon' (Ballistreri et al., 2019). Other phenolic compounds often found in Citrus are hydroxycinnamic and hydroxybenzoic acids. These compounds, and mostly anthocyanins, contribute to protect against certain cancers and cardiovascular diseases, reduce oxidative stress in diabetic patients, and protect DNA against oxidative damage (Spormann et al., 2008).

2.5.1 Total anthocyanin content (TAC)

Anthocyanins are phenolic compounds belonging to the family of flavonoids that constitute the largest group of water-soluble pigments in the Plant Kingdom (He and Giusti, 2010). They are responsible for the colors of many flowers and fruits and are widely distributed in the human diet. Most anthocyanins are heterosides consisting of an aglycone (or anthocyanidin) derived from the 2-phenylbenzopyrylium (flavylium) skeleton diversely hydroxylated/methoxylated, which is linked to one or various sugar moieties that can be further acylated by aromatic or aliphatic organic acids. Most naturally occurring anthocyanins are derived from six aglycones, i.e., pelargonidin, cyanidin, delphinidin, pelargonidin, petunidin, peonidin, and malvidin. Of the different aglycones, cyanidin, delphinidin, and malvidin were estimated to contribute 45, 21, and 15%, respectively, of the total anthocyanin's intake. (Wu et al., 2006).

2.5.2 Total flavonoid content (TFC)

Flavonoids are secondary metabolites corresponding to polyphenols, which have a varied structure, found in the form of aglycones or glycosides in many fruits and vegetables (Ghasemzadeh and Ghasemzadeh, 2011). Flavonoids have a chemical structure of 15 carbons constituted by a common skeleton of phenyl-benzo-γ-pyran (C6–C3–C6), also known as nucleus flava, composed of two phenyl rings (A and B) and a ring heterocyclic (pyran) C. Flavonoids include flavanols, flavones, flavonoids, flavanones, anthocyanidins, and isoflavones (Burda and Oleszek, 2001).

2.5.3 Total phenolic content (TPC)

Citrus fruits and juices are rich sources of bioactive compounds, like phenolic content, carotenoids, limonoids, coumarin-related compounds, folates, essential oils, pectin's and vitamin C (Marti et al. 2009). Phenolic compounds (PCs) are ubiquitously distributed phytochemicals found in most plant tissues, including fruits and vegetables (Laura et al., 2019). They are secondary metabolites synthesized through the shikimic acid and phenylpropanoid pathways. PCs possess numerous bioactive properties and, although they are not nutrients, dietary intake provides health-protective effects, therefore postharvest treatments have been used to enhance or preserve the contents of PCs in fruits and vegetables (Ignat et al., 2011). TPC (Total phenol content) ranges from 7.84–13.57 mg GAE L^{-1} were found for Citrus sp. Pomelo (Kumar et al 2019).

2.6. Antioxidant capacity in Pomelo and orange fruits

There were many attempts to find the antioxidant content, capacity, and specific antioxidant compounds in citrus fruits (Al-Sayyed, et al., 2019). Antioxidants are substances that known to delay or inhibit oxidation (Halliwell, 1995). Plant component especially fruit has antioxidant components that are able to reduce oxidative stress (Agudo et al., 2007). Fruits are also important sources of vitamins, minerals and other kinds of phytochemicals. Bioactive compounds in fruit that having antioxidant properties are carotenoids, polyphenols, anthocyanins and vitamins (Dillard and German, 2000; Ness and Powles, 1997). The peel of citrus fruit contained higher amount of antioxidant as compared to its pulp as the peel is to protect the antioxidants in the fruit from oxidation (Toh et al., 2013). Moreover, previous studies reported that the peel of pomelo fruit contained a higher amount of antioxidant content and antioxidant capacity as compared to its pulp. (Abudayeh et al., 2019).

2.7 Various storage condition on bioactive compounds and antioxidant activity

A food facility operation needs to have clearly defined storage areas and procedures for several reasons.

2.7.1 Room Temperature

It means "room temperature" or normal storage conditions, which means storage in a dry, clean, well ventilated area at room temperatures between 15° to 25°C (59°-77°F) or up to 30°C, depending on climatic conditions (Appleman and Arthur, 1919).

2.7.2 Chilling Temperature

The legal requirement is that chilled food is kept at 8°C or below. The recommendation is to keep the fridge or display unit at 5°C or below to ensure the food is maintained at 5°C or below (James et al., 2008).

2.7.3 Frozen temperature

-4 to 2 temperature storage conditions are known as the fridge (Arthur, 1942).

2.8 The effect of various storage condition on bioactive compounds and antioxidant activity

There is a considerable demand for fresh fruits as well as their products. Since many types of fruit are seasonal and their shelf life is limited, they must be processed to keep the quality (Scibisz and Mitek, 2007).The food qualities of juice such as color, acidity, soluble solid, texture, total phenolics, antioxidant activity and anthocyanins can be affected during storage (Wicklund et al., 2005).The antioxidant activity, total phenolics, and anthocyanins fruit contents were investigated by Wicklund et al. (2005) in berry fruits (i.e. strawberry and cherry), in fig by Veberic et al. (2008) and in apricot by Bureau et al. (2009).The total phenolics, antioxidant activity and anthocyanins of these fruits and their products could be affected during storage. However, there are few papers worldwide, concerning the effect of juice and products processing on these compounds. The effect of fruit jam and similar processing on antioxidant activity, total phenolics, and anthocyanins components were evaluated by few researchers. Kim and Padilla-Zakour (2004) evaluated the Antioxidant capacity of fruits ranged from 354.8 to 692.3 mg/100 g, expressed as vitamin C equivalent antioxidant capacity. Toh et al, 2013 showed that The AC value was found in Tambun Pink pomelo juice (0.3 mmol TE/100 g). Rababah et al. (2011) showed that The Antioxidant activity of orange fruits, 30.22% inhibition. Rababah et al. (2011) also showed that The Antioxidant activity of orange fruits jam (13.51% inhibition) during storage zero to five months at 25 ◦C temperature. Studies that focus on the behavior of total phenolics, antioxidant activity and anthocyanins of these fruit jams during long-term storage are rather limited. Furthermore, there is a need to increase our understanding of the effect of juice processing and its storage on nutraceutical contents. For these reasons, the effects of storage upon bioactive compounds and antioxidant activity need to be investigated. The aim of the present study was to evaluate bioactive compounds and antioxidant activity of pomelo and orange fruits and their juice during 30days of storage.

Chapter III: Materials and Methods

The research work was done at the Department of Food Processing and Engineering and Department of Applied Food Science and Nutrition of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh, 1st Jan 2020 to 30th Dec 2020 for evaluation the effect of various storage condition on bioactive compounds and antioxidant activity of pomelo and orange juice.

3.1 Study design

Firstly, pomelo and orange fruits were collected from local market. After collection of samples, it was used for juice extraction. Then this juice was stored at different types of storage condition for evaluation of bioactive compounds and antioxidant activity.

3.2. Sample collection and juice extraction

The pomelo and orange fruits were bought from local market (Zawtala Bazar, Pahartali) in Chattogram, Bangladesh. The pomelo and orange fruits were peeled by hand, extracted pulp and blended in a household blender (Philips HR 7761, China) for obtaining the pomelo and orange fruits juice. Before the storage process, the juice was sieved, in order to retain large particles.

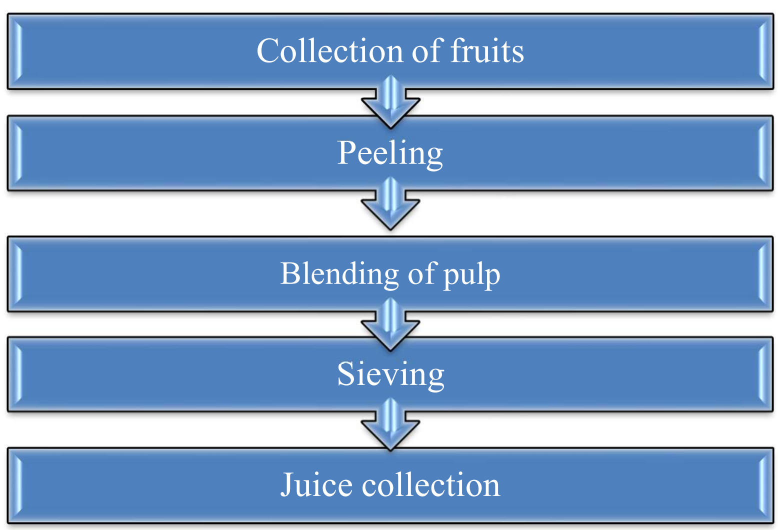

Figure 3.1 Flow Chart of Juice Extraction

3.3 Juice storage and stability

In order to investigate the effects of storage condition these juices were evaluated for, bioactive compounds and antioxidant capacity during a storage period of 0 days to 30 days. Immediately after juice processing, samples were collected in sterile tube and stored at room temperature in the absence of light, chiller, fridge temperature. Samples were analyzed at 0, 7, 15 and 30 days at room (25°C) temperature, chilling (5°C) temperature and frozen (-4 °C) temperature.

3.4. Bioactive compounds of Juice samples

3.4.1. Preparation of extracts

Preparation of extracts were determined according to a modified method described by Unal et al. (2014). Pomelo and orange juice samples were transferred into respective beakers added with absolute ethanol, and left to shake on a shaker for 72 hr at room temperature. The solvent was then separated from residue by straining. The filtrate was collected and stored at room temperature while the residue was re-extracted twice, each time with fresh solvent.

Finally, all the filtrates were combined and evaporated under reduced pressure at 60 °C using a rotary evaporator to obtain the crude extracts. The crude extracts were weighed and stored at 4 °C until further analysis.

3.4.2 Total anthocyanin content (TAC)

TAC of the Pomelo and orange juice extracts was determined calorimetrically following the method described by Unal et al. (2014). Stock solutions of 15 mg/mL of extracts were prepared. Extract solution (3 mL) was pipetted into a cuvette. The intensity of the extract color was measured at wavelength 520 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu, Japan). Ethanol will be used as a blank. TAC was calculated and expressed as milligrams per 100 g (mg/100 g) using the following equation:

$$TAC = \text{Absorbance of sample} \times DF \times 100 / m \times E$$

Where, DF stands for dilution factor; m means weight of sample used to make stock solution; E refers to extinction coefficient (55.9).

3.4.3 Total flavonoid content (TFC)

TFC of the Pomelo and orange juice extracts was determined using the aluminum chloride colorimetric method described by Chang et al.,(2002). Stock solution (1 mg/mL) of extracts was prepared. Quercetin will be dissolved in 80% ethanol to make standard solutions (1.0, 3.0, 5.0, and 7.0 mg/mL) to plot a standard curve. Aliquots of 0.5 mL of diluted extract or standard solution will be mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in the cuvette. The mixture was left at room temperature for 30 min. The absorbance was read at wavelength 415 nm in UV-visible spectrophotometer (UV-2600, Shimadzu, Japan). For the blank, 10% aluminum chloride was substituted with distilled water of the same amount. TFC was calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

3.4.4 Total phenolic content (TPC)

TPC of the Pomelo and orange juice extracts was determined according to the method described by Unal et al., (2014). Stock solutions (1 mg/mL) of extracts and standard solutions of gallic acid (1.0, 2.0, 4.0, 6.0, 8.0 mg/mL) was prepared. Extracts or gallic acid standard solution (0.3 mL) was pipetted into a cuvette. Diluted FC reagent (1.5 mL) was then added and mixed. The mixture was left for 3 min before adding 1.5 mL of sodium carbonate (75 g/L) solution and left for 60 min. The absorbance was read at wavelength 765 nm using a UV spectrophotometer (UV-2600, Shimadzu, Japan). and ethanol was used as the blank. TPC was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

3.5. Antioxidant's properties of Juice samples

3.5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Antioxidant capacity of the extracts was determined using DPPH assay as described byAzlim Almey et al.,(2010). Stock solution (1 mg/mL) of extract was diluted to concentrations of (0.50, 1.00, 1.50, 2.00, 2.50) mg/mL in methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL methanol. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution. Methanol was used as a blank while Trolox was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated and expressed as milligrams of Trolox equivalents (TE) per gram of extracts (µmol TE/g).

3.6. Statistical tools to be used for data analyses

Data for statistical analysis were determined and recorded in Microsoft Excel 2013 spread sheet. All samples were in three replicates. Data were then sorted, coded and recorded in IBM SPSS Statistics 16. These data were further analyzed using one-way ANOVA tests to assess significant levels of variation at 95% confidence interval. The statistical analysis was conducted at 5% level of significance ($P < 0.05$).

Chapter IV: Results

4.1 Total anthocyanin content (TAC) of pomelo juice at various storage condition

Total anthocyanin content (TAC) of pomelo juice at various storage condition are presented in Table 4.1. The results of total anthocyanin content (TAC) at room temperature, chilling condition and frozen condition were ranged from 0.45-1.34 mg/100 g, 1.13-1.34 mg/100 g and 1.20-1.34 mg/100 g for different days of stored pomelo juice respectively.

Table:4.1 Total anthocyanin content (TAC) of pomelo juice at various storage condition

Storage condition (Temperature)	Storage time (Days)			
	0	7	15	30
Room	$1.34{\pm}0.03^{a}$	$1.01{\pm}0.01^{b}$	$0.75{\pm}0.02^{c}$	$0.45{\pm}0.01^{d}$
Chilling	$1.34{\pm}0.02^{a}$	$1.25{\pm}0.04^{ab}$	$1.19{\pm}0.06^{bc}$	$1.13{\pm}0.01^{c}$
Frozen	$1.34{\pm}0.01^{a}$	$1.28{\pm}0.02^{ab}$	$1.25{\pm}0.05^{ab}$	$1.20{\pm}0.03^{b}$

** Significant at $P < 0.05$; Values followed by different superscript letters denote a significant difference; comparison done across storage condition.

4.2 Total flavonoid content (TFC) of pomelo juice at various storage condition

Total flavonoid content (TFC) of pomelo juice at various storage condition are presented in Table 4.2. The results of Total flavonoid content (TFC) at room temperature, chilling condition and frozen condition were ranged from 5.11-27.34 mg QE / 100 g, 20.70-27.34 mg QE / 100 g and 21.76-27.34 mg QE / 100 g for different days of stored pomelo juice respectively.

Table: 4.2 Total flavonoid content (TFC) of pomelo juice at various storage condition

Storage condition (Temperature)	Storage time (Days)			
	0	**7**	**15**	**30**
Room	27.34±0.06^{a}	19.18±0.02^{b}	10.32±0.10^{c}	8.11±0.02^{d}
Chilling	27.34±0.02^{a}	25.49±0.04^{b}	23.18±0.03^{c}	20.70±0.14^{d}
Frozen	27.34±0.03^{a}	25.45±0.05^{b}	23.89±0.01^{c}	21.76±0.04^{d}

** Significant at P <0.05; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

4.3 Total phenolic content (TPC) of pomelo juice at various storage condition

Total phenolic content (TPC) of pomelo juice at various storage condition are presented in Table 4.3. The results of Total phenolic content (TPC) at room temperature, chilling condition and frozen condition were ranged from 35.16-88.91 mg GAE / 100 g, 75.61-88.91 mg GAE / 100 g and 76.14-88.91 mg GAE / 100 g for different days of stored pomelo juice respectively.

Table:4.3 Total phenolic content (TPC) of pomelo juice at various storage condition

Storage condition (Temperature)	**Storage time (Days)**			
	0	**7**	**15**	**30**
Room	88.91±0.05^{a}	76.13±0.01^{b}	55.43±0.20^{c}	35.16±0.04^{d}
Chilling	88.91±0.08^{a}	85.45±0.10^{b}	80.98±0.01^{c}	75.61±0.05^{d}
Frozen	88.91±0.06^{a}	86.43±0.01^{b}	82.98±0.02^{c}	76.14±0.02^{d}

** Significant at P <0.05; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

4.4 Antioxidant activity pomelo juice at various storage condition

Antioxidant activity of pomelo juice at various storage condition are presented in Table 4.4. The results of Antioxidant activity at room temperature, chilling condition and frozen condition were ranged from 160.14-300.23 µmol TE / 100 g, 266.22-300.23 µmol TE / 100 g and 270.55-300.23 µmol TE / 100 g for different days of stored pomelo juice respectively.

Table:4.4 Antioxidant activity pomelo juice at various storage condition

Storage condition (Temperature)	Storage time (Days)			
	0	7	15	30
Room	300.23±0.20^{a}	270.67±0.02^{b}	210.34±0.10^{c}	160.14±0.04^{d}
Chilling	300.23±0.03^{a}	294.15±0.05^{b}	282.54±0.02^{c}	266.22±0.08^{d}
Frozen	300.23±0.05^{a}	295.87±0.03^{b}	285.65±0.05^{c}	270.55±0.01^{d}

** Significant at P <0.05; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

4.5 Total anthocyanin content (TAC) of orange juice at various storage condition

Total Anthocyanin Content (TAC) of orange juice at various storage condition are presented in Table 4.5. The results of total anthocyanin content (TAC) at room temperature, chilling condition and frozen condition were ranged from 0.80-1.53 mg/100 g, 1.23-1.53 mg/100 g and 1.25-1.53 mg/100 g for different days of stored orange juice respectively.

Table:4.5 Total anthocyanin content (TAC) of orange juice at various storage condition

Storage condition (Temperature)	Storage time (Days)			
	0	**7**	**15**	**30**
Room	1.53 ± 0.05^{a}	1.32 ± 0.08^{b}	1.02 ± 0.03^{c}	0.80 ± 0.04^{d}
Chilling	1.53 ± 0.03^{a}	1.45 ± 0.02^{ab}	1.38 ± 0.04^{c}	1.23 ± 0.04^{d}
Frozen	1.53 ± 0.05^{a}	1.48 ± 0.02^{a}	1.40 ± 0.03^{a}	1.25 ± 0.05^{b}

** Significant at P <0.05; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

4.6 Total flavonoid content (TFC) of orange juice at various storage condition

Total flavonoid content (TFC) of orange juice at various storage condition are presented in Table 4.6. The results of Total flavonoid content (TFC) at room temperature, chilling condition and frozen condition were ranged from 6.65-21.34 mg QE / 100 g, 13.88-21.34 mg QE / 100 g and 15.43-21.34 mg QE / 100 g for different days of stored orange juice respectively.

Table:4.6 Total flavonoid content (TFC) of orange juice at various storage condition

Storage condition (Temperature)	Storage time (Days)			
	0	**7**	**15**	**30**
Room	21.34±0.04[a]	17.45±0.06[b]	11.35±0.01[c]	6.65±0.04[d]
Chilling	21.34±0.07[a]	18.56±0.03[b]	16.43±0.01[c]	13.88±0.04[d]
Frozen	21.34±0.04[a]	19.11±0.02[b]	17.20±0.06[c]	15.43±0.03[d]

** Significant at $P < 0.05$; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

4.7 Total phenolic content (TPC) of orange juice at various storage condition

Total phenolic content (TPC) of orange juice at various storage condition are presented in Table 4.7. The results of Total phenolic content (TPC) at room temperature, chilling condition and frozen condition were ranged from 52.88-106.65 mg GAE / 100 g, 89.29-106.65 mg GAE / 100 g and 92.65-106.65 mg GAE / 100 g for different days of stored orange juice respectively.

Table:4.7 Total phenolic content (TPC) of orange juice at various storage condition

Storage condition (Temperature)	Storage time (Days)			
	0	7	15	30
Room	106.65±0.03[a]	86.39±0.05[b]	62.11±0.03[c]	52.88±0.05[d]
Chilling	106.65±0.02[a]	98.35±0.05[b]	94.13±0.01[c]	89.29±0.07[d]
Frozen	106.65±0.05[a]	100.07±0.03[b]	96.56±0.01[c]	92.65±0.06[d]

** Significant at P <0.05; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

4.8 Antioxidant capacity of orange juice at various storage condition

Antioxidant activity of orange juice at various storage condition are presented in Table 4.8. The results of Antioxidant activity at room temperature, chilling condition and frozen condition were ranged from 101.76-232.50 µmol TE / 100 g, 215.18-232.50 µmol TE / 100 g and 218.10-232.50 µmol TE / 100 g for different days of stored orange juice respectively.

Table:4.8 Antioxidant Capacity of orange juice at various storage condition

Storage condition (Temperature)	**Storage time (Days)**			
	0	**7**	**15**	**30**
Room	232.50±0.05^{a}	210.13±0.05^{b}	177.56±0.02^{c}	101.76±0.04^{d}
Chilling	232.50±0.05^{a}	227.80±0.08^{b}	220.13±0.08^{c}	215.18±0.05^{d}
Frozen	232.50±0.06^{a}	227.31±0.02^{b}	224.54±0.02^{c}	218.10±0.05^{d}

** Significant at P <0.05; Values with different superscript letters in a row denote a significant difference; comparison done across storage condition.

Chapter V: Discussions

5.1 The effect of various storage condition on bioactive compounds and antioxidant capacity of pomelo juice.

Bioactive compounds and antioxidant capacity are the most abundant part in the human diet, and are widespread constituents of fruits and vegetables. These compounds are of significant interest due to their antioxidant properties. Total anthocyanin content (TAC) of different days of storage period of pomelo juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, Total anthocyanin content (TAC) gradually decreased as storage time increased. Significant losses in total anthocyanin content were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because total anthocyanin content is usually susceptible to different factors like storage time and temperature. Hamedani et al. (2012) observed Total anthocyanin content of blood orange (Citrus sinensis cv. Tarocco) as 12.39 mg/L after 85 days of storage at 8ºC. Habibi et al. (2020) observed total anthocyanin content of Blood Orange Cultivars "Sanguinello" as (160.8 ± 5.6 mg L−1) and "Tarocco" as (53.6 ± 6.4 mg L−1) after 180 days of storage at 5ºC.

Total Flavonoid Content of different days of storage period of pomelo juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, Total flavonoid Content gradually decreased as storage time increased. Significant losses in total flavonoid Content were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because total flavonoid content is usually susceptible to different factors like storage time and temperature. Hamedani et al. (2012) observed Total flavonoid content of blood orange (Citrus sinensis cv. Tarocco) as 142.56 mg/L after 85 days of storage at 8ºC.

Total phenolic content of different days of storage period of pomelo juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, Total phenolic content gradually decreased as storage time increased. Significant losses in total flavonoid Content were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because total phenolic content is usually susceptible to different factors like storage time and temperature. Hamedani et al. (2012) observed Total phenolic content of blood orange (Citrus sinensis cv. Tarocco) as 537.0 mg/L after 85 days of storage at 8ºC. Habibi et al. (2020) observed Total phenolic content of Blood Orange Cultivars "Sanguinello" as 636 ± 24 mg eq. of gallic acid L^{-1} and "Tarocco" as 368±14 mg eq. of gallic acid L^{-1} after 180 days of storage at 5ºC.

Antioxidant capacity of different days of storage period of pomelo juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, antioxidant capacity gradually decreased as storage time increased. Significant losses in antioxidant capacity were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because antioxidant capacity is usually susceptible to different factors like storage time and temperature. Hamedani et al. (2012) observed antioxidant capacity of blood orange (Citrus sinensis cv. Tarocco) as 36.0 % after 85 days of storage at 8ºC. Habibi et al. (2020) observed antioxidant capacity of Blood Orange Cultivars "Sanguinello" as 78% and "Tarocco" as 74% after 180 days of storage at 5ºC.

5.2 The effect of various storage condition on bioactive compounds and antioxidant capacity of orange juice.

Phenolics are the major health promoting compounds in orange juice and major determinants towards antioxidant capacity. Biologically active compounds are the nutritional constituents such as ascorbic acid, flavonoids, limonoids etc. that have potential health promoting properties and have been widely used as medicine. Antioxidants scavenge the harmful free radical in the body and combat the pathogenesis of major diseases including arthritis, diabetes and cancer. The changes Total Anthocyanin content (TAC) of different days of storage period of orange juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, Total anthocyanin content (TAC) gradually decreased as storage time increased. Significant losses in total anthocyanin content were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because total anthocyanin content are usually susceptible to different factors like storage time and temperature. Touati et al. (2016) prepared grape nectars reported anthocyanins content as 7.58 mg MvE/100 mL after 30 days of storage.

Total flavonoid content of different days of storage period of orange juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, Total flavonoid content gradually decreased as storage time increased. Significant losses in total flavonoid content were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because total flavonoid content is usually susceptible to different factors like storage time and temperature.

Total phenolic content of different days of storage period of orange juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, Total phenolic content gradually decreased as storage time increased. Significant losses in total flavonoid content were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because total phenolic content is usually susceptible to different factors like storage time and temperature. Touati et al. (2016) prepared orange nectars reported Total phenolic content as 45.88 mg GAE/100 mL after 30 days of storage.

Antioxidant capacity of different days of storage period of orange juice at room, chilling and frozen temperature was significantly different ($p<0.05$). However, antioxidant capacity gradually decreased as storage time increased. Significant losses in antioxidant capacity were observed as a result of storage, after 30 days at room, chilling and frozen temperature. This is because antioxidant capacity is usually susceptible to different factors like storage time and temperature. Touati et al. (2016) prepared orange nectars reported antioxidant capacity as 4 µM TE/100 mL after 30 days of storage.

Chapter VI: Conclusion

Pomelo and orange juice are considered as a new source of natural bioactive compounds and antioxidants. The results of the present study confirmed that storage resulted in a decrease of bioactive compounds such as anthocyanins, phenolic compounds and flavonoids, and antioxidant activity of pomelo and orange juice. Since there is a great interest in these compounds because of increasing evidences of beneficial effects to human health, it is important to know to what extent they are affected by storage time and temperature. Bioactive compounds and antioxidant activity of pomelo and orange juice were relatively low at more storage time. Thus, the effects of storage on the phytochemicals and antioxidant capacity of pomelo and orange fruits should be considered prior to selection of storage conditions.

Chapter VII: Recommendations & future perspectives

The worldwide consumption of fruits and fruits juice has importantly increased during recent periods, due to a number of distinct factors. Foremost among these factors is the growing knowledge that pomelo and orange constitutes an important and healthy part of the human diet, mainly owing to the presence of bioactive compounds and antioxidant activity, which play an essential role in human health, also to the presence of vitamins, minerals. Present study is conducted to investigate the effect of various storage condition on bioactive compounds and antioxidant activity of pomelo and orange juice**.** Establishment of proper processing and storage unit at grower's level could utilize the pomelo and orange juice, which will be helpful to get this product during off season, ultimately minimizes postharvest losses of pomelo and orange and generates income to the growers. The following recommendation are:

- More researches are to be conducted at different operating conditions on the extraction of bioactive compounds and antioxidant activity of pomelo and orange juice.
- More researches can be conducted at different heat treatment on the extraction of bioactive compounds and antioxidant activity of pomelo and orange juice.
- More researches are to be conducted at different correlation between bioactive compounds and antioxidant activity of pomelo and orange juice.

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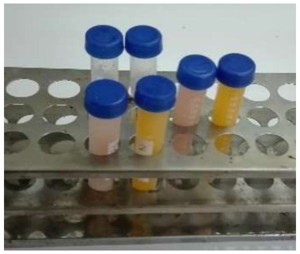
Appendix A: Photo Gallery

Pictures of laboratory activities

Fruits

Peeling



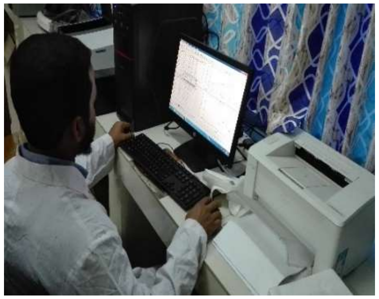
Juice

Storage

Storage

Sample preparation

UV Visible



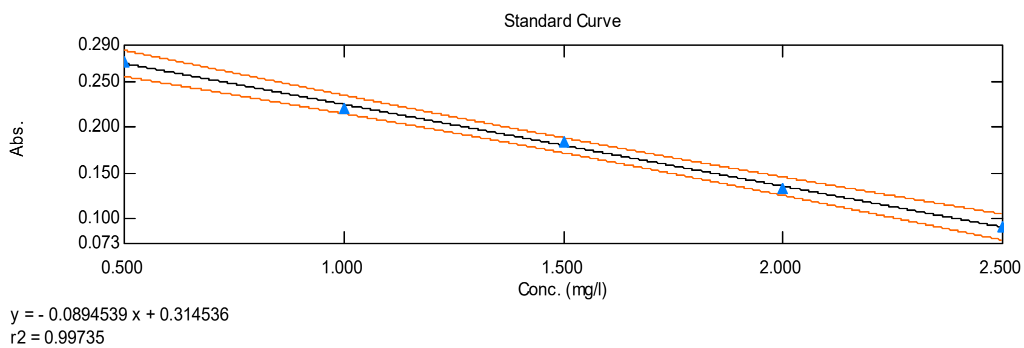
UV Visible

Appendix B: Standard Curve for bioactive compounds

Antioxidant Capacity Standard Table

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt.Factor	Comments
1	Std2	Standard	1.000	0.221	1.000	
2	Std3	Standard	1.500	0.185	1.000	
3	Std4	Standard	2.000	0.133	1.000	
4	Std5	Standard	2.500	0.092	1.000	

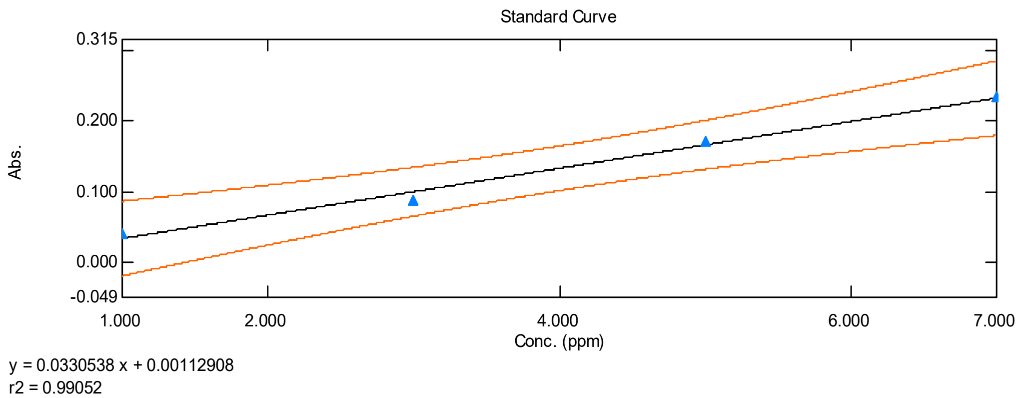
Standard Curve



TFC Standard Table

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt.Factor	Comments
1	Std1	Standard	1.000	0.041	1.000	
2	Std2	Standard	3.000	0.088	1.000	
3	Std3	Standard	5.000	0.171	1.000	
4	Std4	Standard	7.000	0.234	1.000	

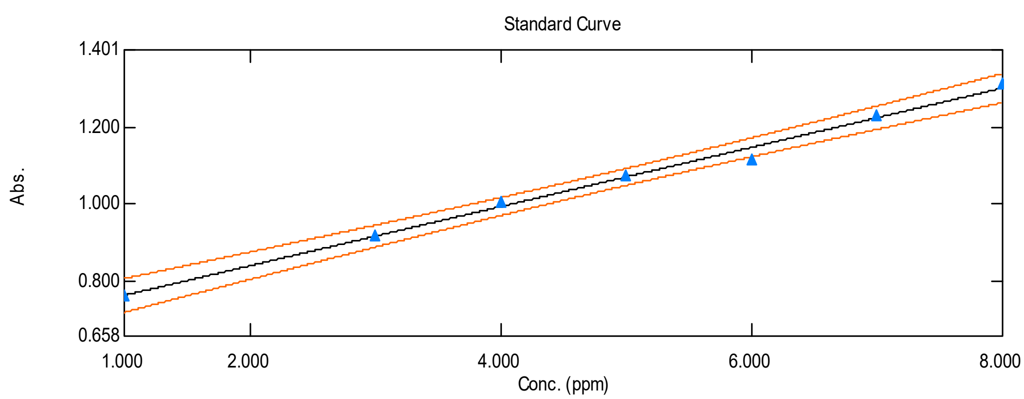
Standard Curve



TPC Standard Table

Sample ID	Type	Ex	Conc	WL 517. 0	Wgt. Factor	Comments
1	Std1	Standard	1.000	0.763	1.000	
2	Std2	Standard	2.000	0.780	1.000	
3	Std3	Standard	3.000	0.920	1.000	
4	Std4	Standard	4.000	1.007	1.000	
5	Std5	Standard	5.000	1.074	1.000	
6	Std6	Standard	6.000	1.115	1.000	
7	Std7	Standard	7.000	1.230	1.000	
8	Std8	Standard	8.000	1.314	1.000	

Standard Curve



y = 0.0768527 x + 0.687090
r2 = 0.99301

Brief Biography

Md. Fakhrul Islam passed the Secondary School Certificate Examination in 2009 and then Higher Secondary Certificate Examination in 2012. He obtained his B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, he is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). His research interests are functional food product development and nutritional value analysis.