



COMPARATIVE STUDY ON PHYTOCHEMICAL PROPERTIES OF BEETROOT AND DEVELOPED BEETROOT JELLY

Al Mahamud Ibne Jamal

Roll no: 0121/01

Registration no: 998

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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
Chattogram-4225, Bangladesh**

July 2023

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Al Mahamud Ibne Jamal

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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

Supervisor

Dr. Shireen Akther

Professor

Department of Food Processing and
Engineering

Co-supervisor

Md. Kauser-Ul-Alam

Associate Professor

Department of Food Processing and
Engineering

Chairman of the Examination Committee

(Md. Kauser-Ul-Alam)

**Department of Food Processing & Engineering
Faculty of Food Science and Technology
Chattogram Veterinary & Animal Sciences University
Khulshi, Chattogram-4225, Bangladesh**

July 2023

PLAGIARISM VERIFICATION

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Name of the Student: Al Mahamud Ibne Jamal

Roll number: 0121/01

Reg. no: 998

Department of Food Processing and Engineering

Faculty of Food Science and Technology

Supervisor: Dr. Shireen Akther

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Dr. Shireen Akther

Professor

Department of Food Processing and Engineering

Faculty of Food Science and Technology

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List of Abbreviations

AlCl ₃	Aluminium Chloride
AOAC	Association of Official Analytical Chemists
BJ	Beetroot Juice
BJJ	Jelly Prepared with BJ
BPCJ	Jelly Prepared with Reconstituted Juice of BPC
BPPC	Beetroot Pomace Powder (Cabinet Dried)
BPPS	Beetroot Pomace Powder (Sun Dried)
BPPCJ	Jelly Prepared with Reconstituted Juice of BPPC
BPS	Beetroot Powder (Sun Dried)
BPSJ	Jelly Prepared with Reconstituted Juice of BPS
Bx	Betaxanthin
CF	Crude Fiber
CP	Crude Protein
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
Fe	Iron
HDPE	High Density Polyethylene
H ₂ SO ₄	Sulfuric Acid
IMF	Intermediate Moisture Food
K	Potassium
Mg	Magnesium
mg/g	Milligram per Gram
mmol/L	Millimoles per Litre
Na	Sodium
NaOH	Sodium Hydroxide
P	Phosphorus
TA	Titrateable Acidity
TAC	Total Anthocyanin Content
TFC	Total Flavonoids Content
TPC	Total Polyphenol Content
TSS	Total Soluble Solids
v/v	Volume in Volume
µg/gm	Microgram per Gram

Abstract

Beetroot (*Beta vulgaris* L.) is enriched with phytochemicals such as flavonoids, anthocyanin, betalains etc. and widely known for its numerous health benefits. This study assessed the physicochemical and bioactive compounds along with vitamins-minerals composition, and sensory attributes of different forms of beetroot and developed product (Jelly). Beetroot powder and pomace powder were prepared by cabinet drying and sun drying process and jelly were developed from fresh juice and powder. Bioactive compounds (Total Flavonoids Content-TFC, Total Polyphenol Contents-TPC, Total Anthocyanin Contents-TAC, Betalains; Betacyanins, Betaxanthin, Betalamic acid) and antioxidant capacity were analyzed. Cabinet dried beetroot powder contains a higher quantity of bioactive compounds (TFC: 49.78 mg/100gm, TPC: 5.81 mg/100gm, TAC: 889.55 mg/100gm, Antioxidant Capacity: 31.11%) compared to other beetroot samples. Betalain contents were also found highest in cabinet dried powder and were 332.91, 142.91, 103.94 mg/gm for betacyanins, betaxanthin and betalamic acid respectively. Jelly developed from fresh juice has the best sensory attributes among the jellies. Overall, beetroot contains a significant quantity of nutrients & phytochemicals (Anthocyanin, betalains etc.) and have a bright prospect for developing values added products.

Keywords: Beetroot Juice, Powder, Pomace Powder, Jelly, Bioactive compounds, Betalains.

Chapter 1: Introduction

1.1 Background of The Study

Beetroot (*Beta vulgaris* L.) belongs to the family Chenopodiaceae and usually recognized as red beet, table beet, garden beet, or just beet, also commonly known as beet vegetable in Bangladesh. It's a popular root vegetable known for its distinctive deep purple-red color and earthy flavor. Beetroots are cultivated for their edible taproots and their green leafy tops, which are also edible and referred to as "beet greens" or "beet leaves."

Beetroot originated in Mediterranean Region and has been widely cultivated in the European continent, America, and Asia recently due to its growing popularity (Chawla et al., 2016). It's a winter crops mainly cultivated in Rangpur, Khulna, Dhaka and Rajshahi region of Bangladesh. In Bangladesh, mostly red beets are cultivated for their enormous health benefits and commercial purpose (Rashid et. al., 2020).

Each variety of beetroot has its own unique flavor profile and can be used in various culinary applications, including salads, soups, juices, and even desserts. Red Beetroot (*Beta vulgaris rubra*) is the most common and widely recognized variety, featuring a deep purple-red skin and a vibrant red interior flesh. Golden Beetroot (*Beta vulgaris var. lutea*) have bright golden-orange skin and a yellow or golden flesh. They are sweeter and milder than red beetroots. Chioggia Beetroot (*Beta vulgaris subsp. vulgaris Conditiva group*) Also known as "candy-striped" or "candy-cane" beets have a unique appearance with pink and white concentric rings on the inside, giving them a distinctive striped look when sliced. White Beetroot (*Beta vulgaris subsp. vulgaris Conditiva group*) have a white or pale cream skin and white interior flesh. They have a slightly milder taste compared to the red variety (Park et. al.,2020).

The beetroot has a round or oval shape and is typically 2 to 3 inches in diameter, although some varieties may be larger. The skin of the beetroot is rough and can range in color from deep purple red to shades of orange, yellow, or even white, depending on the variety. The interior flesh of the beetroot is usually a vibrant red or deep purple, though there are also golden and white varieties that have a

different-colored interior. Most species are edible. About 91.03% of the beetroot are edible (Kale et. al.,2018).

Beetroots are not only delicious but also rich in vitamins, minerals, and antioxidants, making them a nutritious addition to a well-balanced diet. Beetroot, a plant originated raw material which has numerous important effects on the human body. It can be consumed raw, steamed, boiled or roasted. Red beetroot is an excellent source of minerals (manganese, iron, sodium, potassium, magnesium, copper). Beetroot contains a lot of antioxidants, vitamins (A, B, C), fiber and natural coloring components. It contains a great amount of Folate (vitamin B₉) which plays a crucial role in human blood formation, specifically in the production of red blood cells (erythrocytes) and white blood cells (leukocytes). Red beetroot is also rich in phenolic compounds, which have antioxidant properties (Mudgal et. al.,2022).

Betanin Also called beetroot red, is the most common pigment in beetroots, responsible for their strong red color. The common name for the coloring agent E162 is beetroot red, also referred to as betanin. E162 is a red / purple coloring, found in beetroots. Although widely used, E162 is quite unstable because of its degrading nature in the presence of light, oxygen and high temperatures (Kumar & Singh, 2018).

Beetroot is one amongst the well-known edible root vegetables and has substantial health beneficiary properties because of the presence of unique natural edible substances. It contains antioxidants, vitamins and minerals varied vital antidepressant, antimicrobial and anti-carcinogenic. Beetroot pigment is also used as a food coloring agent. Beetroot juice is a beautiful crimson red color, nutritionally enriched drink which helps to purify the blood (Kumar et al., 2015).

Beetroot is recognized for its amazing health benefits to almost all body parts. It exhibits significant anti-carcinogenic properties, and also stabilizes blood pressure. The effects that beetroot juice has on inflammation demonstrate a strong role in the development and progression of several clinical conditions including heart disease and cancer; a beneficial effect of beetroot exhibits, may be due to this anti-inflammatory capacity. Beet and their naturally occurring nitrates have been found to be effective to lower blood pressure and boost stamina (Kumar et al., 2015).

There are various value-added products such as juice, powder, jelly, cookies, bread etc. can be prepared by utilizing red beet. Beetroot juice often used as sports beverage for its dietary nitrate supplementation which boost the exercise capabilities of human during sports (Mudgal et al.,2022). Beetroot powder has been found effective as red food coloring agent in meat products to significantly prevent occurrence of lipid oxidation in sausages as it exhibits antioxidant properties with the presence of betalains and phenolic compounds (Dinçer et al., 2020). Beetroot jelly, an intermediate moisture food (IMF) can be prepared using juice, sugar, pectin and citric acid. The prepared jelly had prolonged shelf life with significant phenolic contents, ascorbic acid and nutritive value (Ali et al., 2021).

1.2 Rationale and Significance of The Study

The root vegetable has a significant nutritional and medicinal potential according to established study (Akan et al., 2021). Beetroot is a nutrient-dense vegetable, containing essential vitamins, minerals, antioxidants, and dietary fiber. Understanding its nutrient profile can help identify its role in maintaining good health and preventing nutritional deficiencies. Research suggests that beetroot consumption may be associated with various health benefits, including improved cardiovascular health, lowered blood pressure, enhanced exercise performance, and potential anti-inflammatory effects. Investigating these potential health-promoting properties can provide valuable insights for improving overall well-being.

Beetroot is rich in components like nitrates, which can be converted into nitric oxide in the body. Nitric oxide is a vasodilator that helps widen blood vessels, improving blood flow and potentially benefiting heart health (McMahon et al., 2017).

Beetroot's deep red color is due to betalain pigments, which have antioxidant and anti-inflammatory properties. Understanding the role of these compounds can contribute to the development of natural antioxidant therapies and anti-inflammatory treatments (Clifford et al., 2015).

The functional and physicochemical properties of beetroot make its byproducts more nutritious. If the byproducts of the vegetable convert to useful products, then the agro-industrial cost will be minimized and may have good impact on environment. Hence, the ability to recycle beetroot pomace to create functional ingredients may have a variety of uses in the food industries, allowing for the transformation of an agro-industrial waste into goods with added value (Sahni et

al., 2018). Hence, to analyze the bioactive compounds of different forms of beetroot (juice, powder, pomace powder, jelly) and to utilize its by-product (pomace) and to develop a value-added product, comparative study is required for introducing its vast health beneficiary aspects to human diet.

1.3 Aim and Objectives

1. To evaluate the physicochemical properties of beetroot juice, powder, and pomace powder.
2. To develop beetroot jelly using beetroot juice, powder, and pomace powder.
3. To analyze the physicochemical properties, vitamin C and mineral (Na, K, Mg, Fe, P) contents of beetroot juice and jelly.
4. To compare the phytochemicals (TFC, TPC, TAC, Betalains content) and antioxidant capacity of beetroot juice, powder, pomace powder and developed jelly.

Chapter 2: Literature Review

2.1 Outline of The Beetroot Vegetable Description

Beetroot, scientifically known as *Beta vulgaris* subsp. *vulgaris*, is a root vegetable belonging to the *Chenopodiaceae* family. It is also commonly referred to simply as "beet." The vegetable is characterized by its round, bulbous root, which has a dark red to purple color, although there are also varieties with white, yellow, and striped roots. Beetroot is well-known for its sweet and earthy flavor and is often used in culinary applications, both cooked and raw.

Beetroot cultivation is believed to have started in the Mediterranean region, particularly in ancient Egypt. Historical records suggest that the ancient Egyptians cultivated beetroots for their leaves as early as 4,000 years ago. It is uncertain when the use of the root as a vegetable began, but it is believed to have been popular in ancient civilizations like Greece and Rome.

Beetroot was valued not only for its culinary uses but also for its medicinal properties. Ancient civilizations believed that it had various health benefits, such as aiding digestion and promoting better blood circulation.

Today, beetroot continues to be a popular vegetable worldwide. Its culinary applications have expanded, and it is recognized for its nutritional value and health benefits, making it a staple in many modern diets. Additionally, the natural pigments in beetroot, responsible for its vibrant color, have found use as food dyes and natural colorants in various industries.



Figure 2.1: Beetroot

Beetroot typically has a round to slightly oval shape, resembling a bulbous root. The size of a beetroot can vary, but it is generally around 2 to 3 inches (5 to 7.5 centimeters) in diameter. The length of the beetroot root can range from 2 to 4 inches (5 to 10 centimeters), depending on the variety and growing conditions (Kale et al., 2018).

The most common color of beetroot is a deep, rich red to purple hue, which is the result of pigments called betalains. However, there are various beetroot cultivars, and their color can vary significantly. Some beetroot varieties have a golden or yellow color, which is due to the presence of different types of betalains. There are also striped or multicolored varieties where the flesh of the root may have rings or stripes of different colors.

Beetroot has a smooth, firm, and glossy skin, which protects the root during growth and storage. The skin of the beetroot is usually rough and may have small root hairs, especially in fresh, unpeeled beetroots. When cut or sliced, the inside of the beetroot reveals a dense and solid texture. The flesh of beetroot is crisp and tender, providing a crunchy texture when raw or slightly cooked. Depending on the variety and age, the flesh may range from firm to more tender. When cooked, beetroot becomes soft and takes on a smooth texture while retaining its signature earthy and sweet flavor.

2.2 Nomenclature of Beetroot

Table 2.2: *Beta vulgaris* classification in science

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Super division:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Caryophyllidae
Order:	Caryophyllales
Family:	Chenopodiaceae
Genus:	Beta
Species:	B. vulgaris

Source: Deshmukh et al., 2018

2.3 Composition of Chemical Components of Beetroot

Beetroot (*Beta vulgaris* subsp. *vulgaris*) is a nutritionally rich vegetable with a diverse range of chemical components that contribute to its color, flavor, and health benefits. The primary chemical components of beetroot include:

Betalains: Betalains are the pigments responsible for the vibrant red to purple color of beetroot. They are water-soluble antioxidants that belong to two main types: betacyanins (responsible for red color) and betaxanthins (responsible for yellow color). Betalains are known for their significant health benefits, including antioxidant and anti-inflammatory properties.

Dietary Fiber: Beetroot is a good source of dietary fiber, which aids in digestion, promotes a feeling of fullness, and helps in the regulation of blood sugar levels.

Dietary Nitrate: Beetroot is notably high in nitrate, a naturally occurring compound. When consumed, nitrate converts into nitric oxide in the body, which may help lower blood pressure and improve blood vessel function, leading to potential cardiovascular benefits.

Vitamins: Beetroot is rich in various vitamins, including vitamin C, which is a potent antioxidant that supports the immune system and helps in collagen formation. It also contains vitamin B9 (folate), which is essential for DNA synthesis and cell growth.

Minerals: Beetroot is a good source of essential minerals such as potassium, which is crucial for maintaining a healthy balance of fluids and electrolytes in the body, as well as for proper muscle and nerve function. It also contains manganese, which is involved in various enzymatic reactions in the body, and iron, important for red blood cell production.

Carbohydrates: Beetroot contains carbohydrates, mainly in the form of sugars like sucrose, glucose, and fructose, which contribute to its naturally sweet flavor.

Antioxidants: Besides betalains, beetroot contains other antioxidants such as carotenoids, flavonoids, and phenolic compounds. These antioxidant components help to neutralize free radicals and protect the body from oxidative stress and cellular damage.

Phytochemicals: Beetroot contains various phytochemicals, including betaine, a natural compound that may support liver health and provide cardiovascular benefits.

2.4 Nutritional Profile of Beetroot

Table 2.4: Nutritional profile of 100 gm beetroot

Constituents	Amounts	Constituents	Amounts
Carbohydrates	9.96 gm	Vitamin B₆	0.067 mg
Sugars	7.96 gm	Folate	80 µg
Dietary Fiber	2.0 gm	Vitamin C	3.6 mg
Fat	0.18 gm	Calcium	16 mg
Protein	1.68 gm	Iron	0.79 mg
Vitamin A equiv.	2 µg	Magnesium	23 mg
Thiamine	0.031 mg	Phosphorus	38 mg
Riboflavin	0.027 mg	Potassium	305 mg
Niacin	0.331 mg	Zinc	0.354 mg
Pantothenic Acid	0.145 mg	Sodium	77 mg

Source: Kumar et al., 2015

2.5 Bioactive Compounds in Beetroot

Beetroot consists of a wide range of bioactive components including dietary nitrate, phenolics, flavonoids, carotenoids, betalains etc. Among them betalain is the most abundant and responsible for the vibrant red color of the vegetable. Betalains comprises two pigments mainly named betacyanins(red) and betaxanthins(yellow).

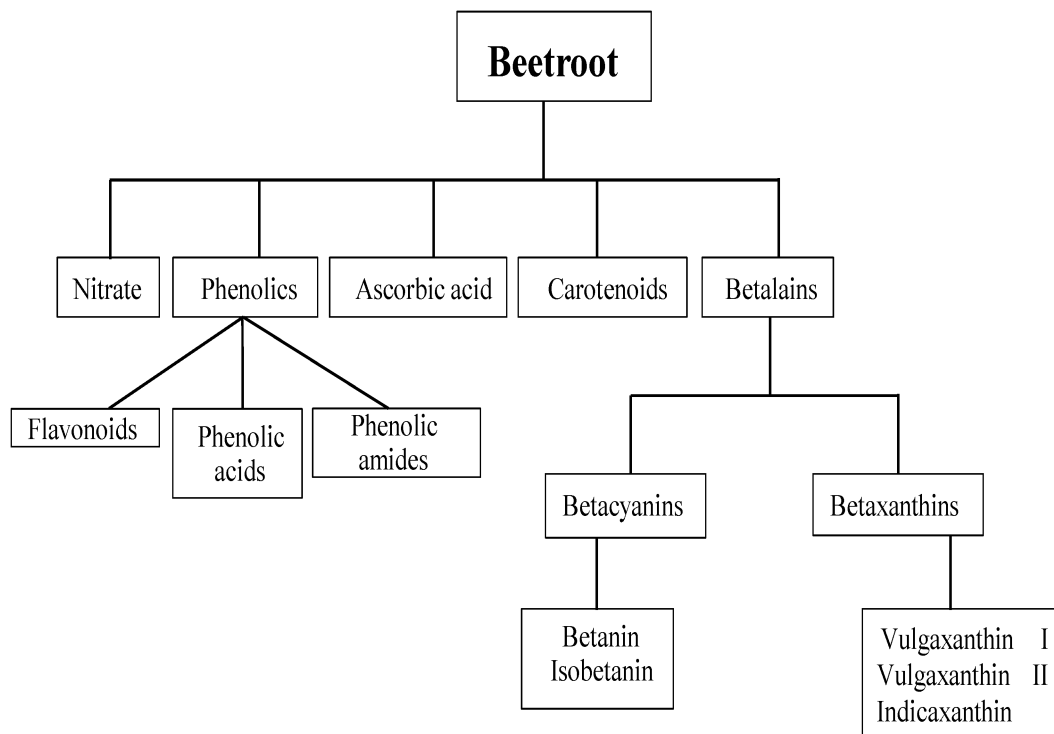


Figure 2.5.1: Overview of potentially bioactive compounds in beetroot (Clifford et al., 2015)

The high antioxidant capacity of beetroot is related to the presence of high betalain contents, and also other phenolic compounds such as flavonoids and polyphenolic acids. Significant quantity of catechins and polyphenolic acid (ferulic, protocatechuic, vanillic, p-coumaric, p-HBA, syringic, and caffeic) have been found in beetroot (Kavalcová et al., 2015).

2.6 Betalains

Betalains are a group of water-soluble pigments naturally found in beetroots (*Beta vulgaris subsp. vulgaris*). They are primarily responsible for the distinct red, purple, and yellow colors in beetroots and other vegetables such as Swiss chard and some cacti fruits. Betalains are considered a unique class of pigments as they are not as commonly found in other plant families.

There are two main types of betalains:

Betacyanins: These are responsible for the red to purple colors in plants. The most common betacyanin found in beetroots is called betanin. It is responsible for the deep red color of the vegetable.

Betaxanthins: These are responsible for the yellow to orange colors in plants. One well-known betaxanthin found in beetroots is vulgaxanthin.

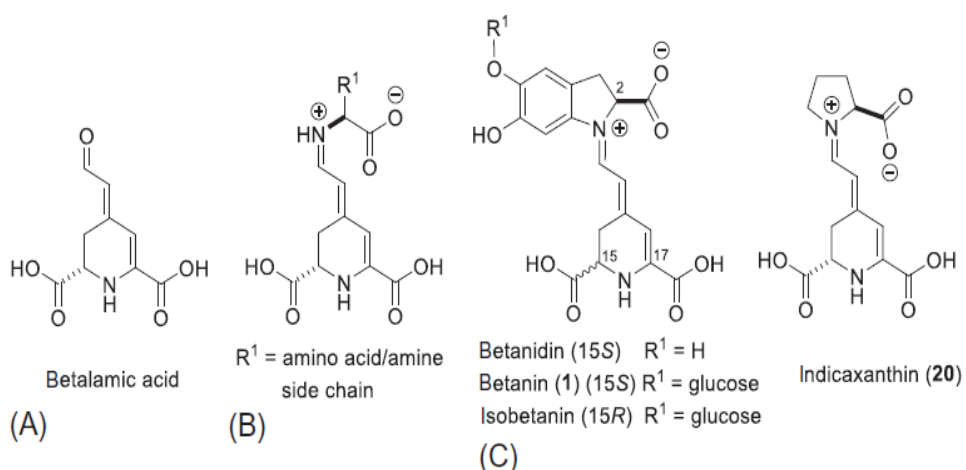


Figure 2.5.2: General structures of betalains. (A) Betalamic acid; (B) betaxanthin-like structure; (C) betacyanin-like structure. (Source: Coy-Barrera, 2020)

Betalains are considered bioactive compounds because of their numerous health benefits. They have been proven in scientific study for their antioxidant and anti-inflammatory properties. As antioxidants, betalains help to neutralize harmful free radicals in the human body, reduce oxidative stress and cellular damage. Their anti-inflammatory properties may contribute to reducing the inflammation and support overall health.

Apart from their potential health benefits, betalains are also used as natural food colorants in the food industry. They can provide vibrant and stable colors to a wide range of food products, including beverages, candies, and cosmetics.

It's important to note that betalains are sensitive to heat and light, and their color can fade when exposed to high temperatures or prolonged cooking. To preserve their vibrant color and potential health benefits, it is recommended to consume beetroots and other betalain-rich foods in their raw or minimally processed forms.

2.7 Health Benefits

Beetroot offers several health benefits due to its rich nutrient and bioactive compound content. Some of the notable health benefits associated with consuming beetroot include:

Cardiovascular Health: The high nitrate content found in beetroot is converted into nitric oxide in the body, which helps in relaxing and dilating blood vessels, leading to an improved blood flow and reduced blood pressure. Regular consumption of beetroot may improve heart health and reduce the risk of cardiovascular diseases.

Antioxidant Properties: Betalains, the pigments responsible for the vibrant color of beetroot, act as antioxidants that neutralize harmful free radicals in the body. Antioxidants help to protect cells from oxidative damage and may decrease the risk of chronic diseases and aging-related conditions (Sawicki et al., 2018).

Anti-Inflammatory Effects: Beetroot's betalains have been studied for their anti-inflammatory properties. Reducing inflammation in the body can have positive effects on overall health, as chronic inflammation is linked to various diseases.

Improved Athletic Performance: The nitrates in beetroot have been shown to enhance exercise performance by improving oxygen utilization and increasing stamina during physical activities. Consuming beetroot or beetroot juice before exercise may help athletes and active individuals boost their performance.

Digestive Health: Beetroot is a good source of dietary fiber, which aids in digestion and supports a healthy digestive system. Fiber promotes regular bowel movements and can help prevent constipation.

Brain Health: Some research suggests that the nitrate content in beetroot may improve blood flow to the brain, which could have potential cognitive benefits. Additionally, beetroot's antioxidant properties may help protect brain cells from oxidative stress.

Nutrient Dense: Beetroot is rich in essential vitamins and minerals such as vitamin C, folate, potassium, and iron. These nutrients found in beetroot may carry out vital roles in supporting various bodily functions, including immune function, red blood cell production, and electrolyte balance.

Weight Management: Beetroot is relatively low in calories and contains dietary fiber, which can contribute to a feeling of fullness and satiety. Including beetroot in a balanced diet may help with weight management and weight loss goals.

Chapter 3: Materials & Methods

3.1 Study Location

The study was conducted in the laboratory of Chattogram Veterinary and Animal Sciences University (CVASU), in Department of Food Processing and Engineering, Department of Physiology, Biochemistry and Pharmacology and Department of Animal Science and Nutrition.

3.2 Samples Accumulation

Beetroot (*Beta vulgaris* L.) samples were bought from the local market of Reazuddin Bazar, Chattogram. To receive the vegetable in the best condition possible, special care was taken during collection. Beetroots were kept in the refrigerator during the study period. Further necessary components for the experiment were acquired from the laboratory's inventory.

3.3 Study Design

The study was focused on developing a value-added beetroot product along with utilizing the by-products. It had been designed to determine the physicochemical properties of beetroot juice, powders, and jelly. The key focus of this study was to determine and compare the presence of bioactive compounds (TFC, TPC, TAC, Betalains) in beetroot juice, powder, and jelly.

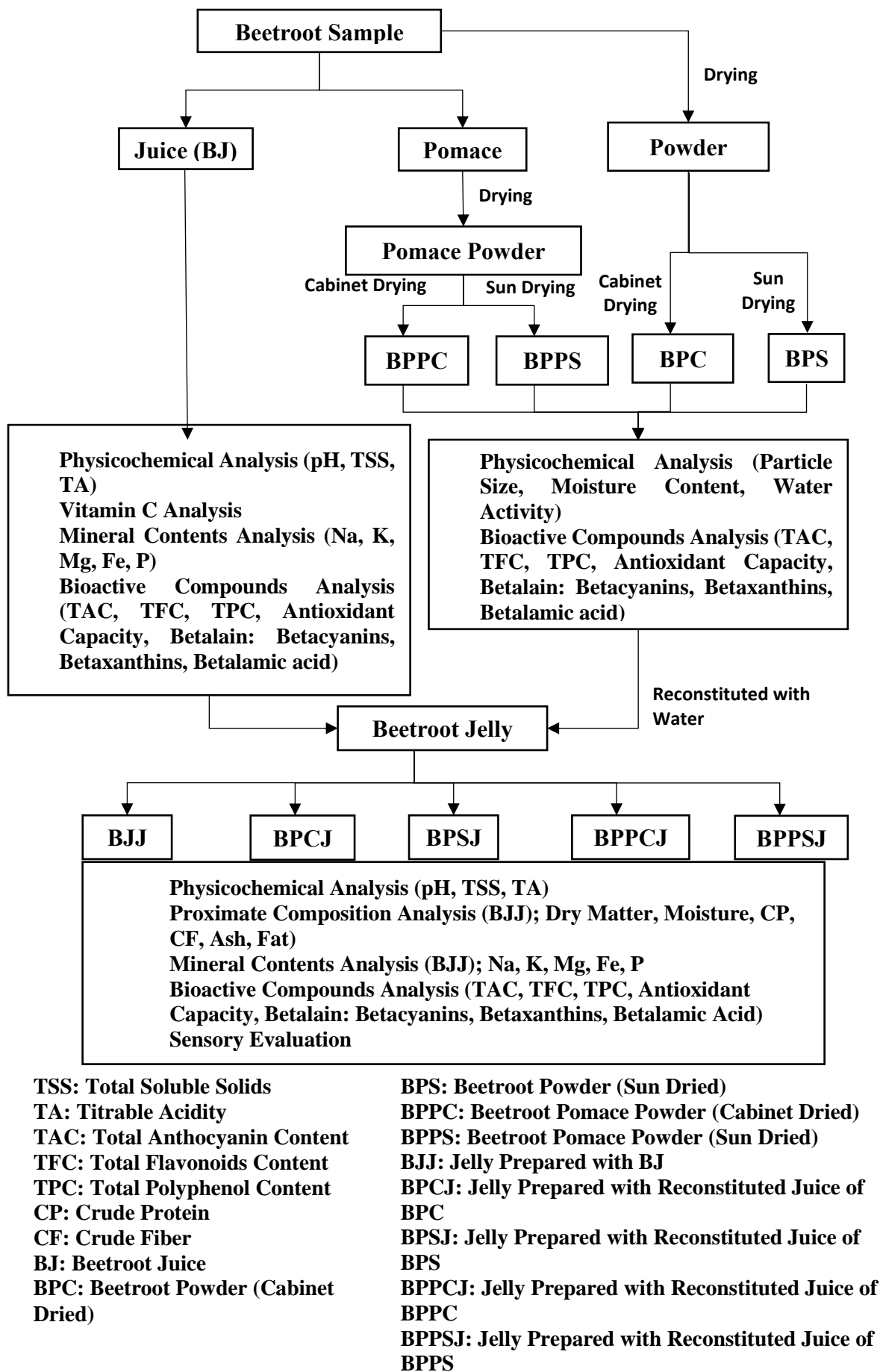


Figure 3.3: Study design of the thesis

3.4 Preparation of Beetroot Juice

Beetroots were first subjected to washing with clean water to remove dust, dirt and outer material. The weight of beetroot samples was taken in an electrical weighing balance (AND GLILF). Sorting and grading were done manually. The beetroot was then peeled and cut into slices of about of 2-3 mm thickness using Panasonic MK-5086M slicer and subjected to extraction using PanasonicMX-AC300 Mixer Grinder (Vidhya et. al., 2018). Then the ground pulp was filtered through muslin cloth and the beetroot juice (**BJ**) was finally filled in 500 ml hot filling pet bottle.

3.5 Preparation of Beetroot Powder

Beetroot powder was obtained through mechanical drying (cabinet drying) and traditional sun drying process. Fresh beetroots were subjected to washing, blanching, peeling and finally reduced to a size of 1-3 mm thickness using slicer (Panasonic MK-5086M). Sliced beetroot was dried in a cabinet dryer (E3 Drying Cabinet, Genlab, UK) at 60°C for 24 hours. Sun drying was carried out during a sunny day for 24 hours. The temperature of the day was recorder 35°C. The dried beetroot slices were then ground using PanasonicMX-AC300 Mixer Grinder to obtain a fine beetroot powder of 63-125 µm particle size and packed into airtight HDPE zipper bags, sealed, and stored in a cool and dry place for further use (Sahni et al., 2018). Two types of beetroot powder were obtained: **BPC**; Cabinet dried beetroot powder and **BPS**; Sun dried beetroot powder.

3.6 Preparation of Beetroot Pomace Powder

Beetroot powder was also obtained through mechanical drying (cabinet drying) and traditional sun drying process. Beetroots were washed, peeled, and subjected to juice extraction. After the extraction of juice, remaining pomace was spread on aluminum trays (16"×12") and kept in a drying bed thickness of 0.5 cm. Drying was carried out at 60°C for 8 hours in a cabinet dryer (E3 Drying Cabinet, Genlab, UK). Sun drying was carried out similarly during a sunny day for 8 hours. The temperature of the day was recorded 35 °C. The dry pomace was ground by using domestic grinder (PanasonicMX-AC300 Mixer Grinder) and sifted through sieve of 63-125 µm particle size and packed into airtight HDPE zipper bags, sealed, and stored in a cool and dry place for further use (Sahni et al., 2018).

Two types of beetroot pomace powder were obtained: **BPPC**; Cabinet dried beetroot pomace powder and **BPPS**; Sun dried beetroot pomace powder.

3.7 Preparation of Beetroot Jelly

Jelly was prepared according to Srivastava et al., 2002. Five formulated treatments of red beetroot juice (BJ) and reconstituted beetroot juice from beetroot powder and beetroot pomace powder were heated, 70 % sugar with 1.6% pectin were added to the juice during heating, then 0.8% citric acid was added. Heating was continued with constant stirring till reaching desired consistency and TSS (65°Brix). The samples were formulated as follows:

Table 3.7: Ingredients of Jelly Preparation

Sample	Ingredients	Juice	Sugar	Pectin	Citric Acid
BJJ (Beetroot Jelly)		500 ml beetroot juice	350 gm	8 gm	4 gm
BPCJ (Beetroot Jelly with Reconstituted Juice of BPC)		50 gm BPC reconstituted with 500 ml water	350 gm	8 gm	4 gm
BPSJ (Beetroot Jelly with Reconstituted Juice of BPS)		50 gm BPS reconstituted with 500 ml water	350 gm	8 gm	4 gm
BPPCJ (Beetroot Jelly with Reconstituted Juice of BPPC)		50 gm BPPC reconstituted with 500 ml water	350 gm	8 gm	4 gm
BPPSJ (Beetroot Jelly with Reconstituted Juice of BPPS)		50 gm BPPS reconstituted with 500 ml water	350 gm	8 gm	4 gm

3.8 Physicochemical Analysis of Juice and Jelly

3.8.1 Measurement of pH

A pH meter that had already been calibrated was used to determine the pH of the juice and jelly compositions. Before using, the pH meter was calibrated using buffer solutions with pH values of 4, 7, and 10. A sample of approximately 1 gm was suspended in 9 mL of deionized water (Mamede et al., 2013) and then filtered using Whatman No. 2 filter paper. The pH reading was obtained when the electrode of the pH meter was submerged in the suspended solution.

3.8.2 Total Soluble Solids

To determine the total soluble solids of the juice and jelly sample, a hand refractometer was used. The (AOAC, 2016) recommended method was followed for measuring total soluble solids (TSS) using a portable hand refractometer and the findings were reported as the percentage of soluble solids (°Brix).

3.8.3 Titratable acidity (TA)

The percentage of acidity level was calculated in terms of anhydrous citric acid via titration with regard to 0.1N NaOH with phenolphthalein indicator. Each time, five grams of sample was placed in a 100ml volumetric flask, and 100ml of distilled water was added so that it was up to the volume of 100ml. When a pink color develops, it indicates the end point of titration. On three separate occasions, the average titration value was recorded (AOAC, 2016).

Titrate acidity was determined using the equation given below:

$$(\%) \text{ of TA} = \frac{(TV \times \text{Factor})}{W}$$

TV = Titer value of the sample in ml

W = Quantity of the sample taken for the test in ml

Factor (Citric acid): 0.0064

3.9 Physicochemical Analysis of Beetroot and Beetroot Pomace Powder

3.9.1 Particle Size

Particle size of beetroot powder and beetroot pomace powder obtained through cabinet drying and sun drying process were analyzed by using a vibratory sieve shaker (ANALYSETTE 3 SPARTAN, FRITSCH) in which an electromagnetic drive causes the sieves to oscillate in a vertical direction.

3.9.2 Moisture Contents

The moisture content of the dried powder sample was analyzed using OHAUS Moisture Analyzer (MB25). A certain amount of sample was taken in the pan of the analyzer and the initial weight and final weight of the sample were observed.

$$\text{Moisture percentage (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

3.9.3 Water Activity

The water activity (a_w) of the dried powder sample was analyzed using Labstart a_w , Novasina. An amount of sample of powder was taken into the dispenser and placed into the measuring cell of the analyzer. After completion of the analysis the results were observed.

3.10 Vitamin C Analysis

Health experts agree that vitamin C is essential, yet the presence of heat and air during food preparation, packaging, and storage swiftly diminishes or destroy it. The 2, 6-dichloroindophenol titrimetric technique was used to determine the amount of vitamin C in drinks (AOAC, 2010). In this instance, the color pigment induced the oxidation of vitamin C into dehydroascorbic acid. At the same time, the dye is transformed into a colorless component. Consequently, the end point of this reaction may be observed easily. Rapid extraction and filtering are preferable for vitamin C analysis because excess amount may be added into plant products by oxidized vitamin C that is partially removed during sampling and grinding. Metaphosphoric acid is used during extraction to stop oxidation. The most accurate result will be obtained with a very acidic solution. It should take one minute to finish the titration. The dye has three different states of dissolution: blue in water, pink in acid, and colorless when entirely reduced.

Reagents

Metaphosphoric acid solution (3%), dye solution (260 mg of dye (2,6-dichlorophenol indophenols) and 210 mg of NaHCO₃ dissolved in 100 ml of distilled water) [500/250 ml of distilled water diluted with 15/7.5 mg of metaphosphoric acid and 20/40 ml of glacial acetic acid].

Standard ascorbic acid solution: 500 ml/250 ml of metaphosphoric acid solution with 50/25 mg of crystalline ascorbic acid dissolved in it.

Procedure

Up to 0 marks of dye solution were applied to the burette. Later, a conical flask with 5 ml of a solution of vitamin C was in use. The conical flask was placed beneath the burette, and the dye was added drop by drop. When a pink color appeared and remained for 20 seconds before dissipating, the titration was complete. The ascorbic acid solution was measured using the same process with an unknown concentration. mg %, or milligram percentage was used to represent the outcome.

3.11 Mineral Contents Analysis

According to AOAC (2010), this method uses digestion to extract minerals from the food source. Juice or jelly was dissolved in a 2:1 HNO₃/HClO₄ acid solution and then digested. One gram of sample was weighed and placed in a conical flask. After adding 7 ml of HNO₃ and 3 ml of HClO₄, the conical flask was set on a hot plate set to 200W for 3 minutes to ensure thorough digestion. After being cooled, the prepared solution was filtered into a 100 ml standard flask, via filter paper, and then it was diluted to volume with distilled water. The AAS method of mineral content analysis was applied to this solution.

3.11.1 Determination of Sodium (Na)

In this procedure, sodium is precipitated as a triple salt in the presence of magnesium and uranyl acetate. Subsequently, excess uranyl ions, in an acidic environment, react with ferrocyanide, leading to the development of a brownish color. The intensity of this color is inversely related to the amount of sodium present in the sample. To carry out the experiment, small volumes of sodium standard and precipitating reagent (0.02 ml and 1 ml, respectively) are added to a cuvette during the precipitation process. Similarly, a sample solution consisting of 0.02 ml of the

test sample and 1 ml of precipitating reagent is prepared in a separate cuvette. After letting the mixtures sit for 5 minutes, they are vigorously shaken to ensure complete mixing. The next step involves centrifuging the solutions at 2500 to 3000 RPM to separate the clear supernatant from the precipitate. During the color development phase, a blank solution is prepared using 1 ml of acid reagent. Then, the cuvettes containing the standards and samples are injected with 0.02 ml of the precipitating reagent and 0.1 ml of the coloring reagent using a pipette. The mixtures are then incubated at room temperature for 5 minutes. After incubation, the absorbance of the blank, standard, and sample solutions is measured against distilled water within 15 minutes. By multiplying the sample's absorbance by the standard's absorbance at a specific concentration (mmol/L), the sodium concentration in the sample can be determined in mmol/L.

3.11.2 Determination of Potassium (K)

The combination of potassium and sodium tetraphenyl boron leads to the formation of a finely dispersed turbidity comprising potassium tetraphenyl boron. The turbidity level is inversely proportional to the concentration of potassium in the sample being tested. For this experiment, a blank solution is prepared by pipetting 1 ml of potassium reagent and 0.02 ml of deionized water into a cuvette. For the sample solution, 1 ml of potassium reagent, 0.02 ml of potassium standard, and 1 ml of the sample extract are mixed in another cuvette. These mixtures are then incubated for 5 minutes to allow for the development of turbidity. The absorbance of both the blank and the standard solution is measured within 15 minutes. Subsequently, the sample absorbance-to-standard absorbance ratio is calculated, and this ratio is multiplied by the known concentration of the standard (expressed in mmol/L) to determine the potassium concentration in the sample (also in mmol/L).

3.11.3 Determination of Magnesium (Mg)

An alkaline pH is utilized to facilitate the specific binding of the metallochromic indicator calmagite to magnesium. This binding induces a shift in the absorption wavelength, forming the foundation of the technique. The strength of the resulting chromophores is directly proportional to the concentration of magnesium in the sample.

To establish the reagent blank solution, 1 ml of the reagent is placed in a cuvette. The sample solution, requiring 10 L to prepare, is mixed with 1 ml of the reagent in a separate cuvette. For the standard solution, 1 ml of reagent is added to 10 ml of magnesium standard in a cuvette. After thorough mixing, the cuvettes are allowed to incubate at room temperature for two minutes. Subsequently, the absorbance at 520 nm is measured for both the sample and standard solutions, and these values are compared to the absorbance of the reagent blank. By multiplying the sample's absorbance value with the known standard concentration, the magnesium concentration is expressed in milligrams per gram (mg/gm).

3.11.4 Determination of Iron (Fe)

Iron is released from the transferring complex upon dissolution in a mildly acidic solution. Ascorbic acid aids in converting the free iron back to its bivalent form. When ferrozine reacts with iron ions, it forms a vividly colored compound. The intensity of the resulting color is directly related to the amount of iron present in the sample. To create the reagent blank solution, 1 ml of the reagent is transferred into a cuvette using a pipette. For the standard solution, 200 μ L of the standard and 1 ml of the reagent are combined. Similarly, for the sample solution, 200 μ L of the sample extract and 1 ml of the reagent are mixed. After thorough mixing, the cuvettes are incubated at room temperature for ten minutes. The absorbance of both the standard and the sample is measured by comparing them to the reagent blank. By applying the appropriate calculations, the iron concentration is expressed in micrograms per gram (μ g/gm).

3.11.5 Determination of Phosphorus (P)

To create the reagent blank solution, 1 ml of phosphorus reagent was utilized. For the sample solution, 1 ml of phosphorus reagent and 10 μ l of phosphorus standard, as well as 1 ml of phosphorus reagent and 10 μ l of the sample extract, were pipetted into a cuvette. These components were mixed and left to incubate for 5 minutes. The reagent blank served as a reference point for comparing the absorbance of the sample and standard solutions. The phosphorus concentration was calculated in milligrams per deciliter (mg/dl) by multiplying the sample's absorbance by the standard concentration (mg/gm).

3.12 Proximate Composition Analysis

The proximate components of the sample (beetroot jelly) were evaluated in accordance with AOAC standard technique (DM Basis). The moisture, ash, crude protein, crude fiber, and crude fat contents were determined using the dry ash method, oven drying method, Kjeldahl's method, gravimetric method, and soxhlet method, respectively.

3.12.1 Calculation of Moisture

The Association of Official Analytical Chemists' (AOAC, 2005) standard technique was used to calculate the moisture content.

Principle: Food stuff usually contains moisture. Simple heating at 104-105°C for 3–4 hours in the oven and cooling in a desiccator to absorb moisture is used to estimate moisture. The procedure is performed numerous times until the sample exhibits a stable weight.

Apparatus: Desiccator, hot air oven, the crucible, and weighing scale

Calculation: This is how the percentage of moisture was determined:

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{final weight}}{\text{Sample weight}} \times 100$$

3.12.2 Determining the Amount of Ash

The total ash content was ascertained using the AOAC method 14.006 (2005). The mineral components are all mixed in the ash fraction. Using this technique, all organic material is burned to oxidize it, and the amount of ash that remains is calculated.

Apparatus: Porcelain, a gas burner, and a muffle furnace

Calculation: The following phrase was used to determine the ash content:

$$\text{Ash \% of Sample} = \frac{\text{The amount of ash in the supplied sample}}{\text{Sample weight}} \times 100$$

3.12.3 Determination of Crude Proteins

The protein content of beetroot was calculated using AOAC method (2005).

Principle: The Kjeldhal method is employed to calculate nitrogen. By measuring the material's nitrogen content and multiplying the nitrogen factor by 6.25, the

protein content of food items can really be determined. Plant protein is thought to contain 16% nitrogen on average. As a result, the plant protein factor is 100/16-6.25. A known amount of the sample is almost always digested with H₂SO₄ in the presence of the digestion mixture (CuSO₄ and K₂SO₄ in the ratio of 1:20). Following diluting the digested material and trapping the released ammonia in a 2% boric acid solution; surplus acid is neutralized with alkali (40% NaOH, w/v). A standard (0.1N) HCl solution is used to titrate the recovered distillate. By multiplying by 6.25, one can calculate crude protein and calculate the percent nitrogen.

Apparatus: Kjeldahl digesting unit, condenser, and flask are examples of equipment.

Reagent:

- Sulfuric acid in concentrated form
- Digestion blend
- Solution of boric acid
- Solution of alkali.
- Mixture of indicators
- HCl standard: 0.1 N

Calculation: Calculated nitrogen and protein percentages are as follows:

$$\text{Protein \%} = \frac{\text{Titration value} \times \text{Normality of HCl (0.1)} \times 0.014}{\text{Sample weight}} \times 6.25 \times 100$$

3.12.4 Calculation of Fat

To ascertain the samples' crude fat content, a soxhlet device was utilized in accordance with the AOAC (2005) technique.

Principle: Food samples are dissolved in organic solvents such as chloroform, methanol and the filtrate is then separated to determine the amount of fat present. Putting the filtrate into two funnels, separating the mixture, drying it to measure the extract, and then estimating the fat content.

Apparatus: Thimble, the solvent extractor

Calculation: The crude fat percentage was given as follows:

$$\text{Fat \% of sample} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.12.5 Determination of Crude Fiber

The AOAC method was used to calculate crude fiber (2005).

Basic principle: Crude fiber, the water-insoluble fraction of carbohydrates, mainly consists of cellulose, hemicellulose, and lignin. The estimation of crude fiber in a fat-free food sample involves a sequential process. First, a known quantity of the sample is boiled in a weak acid solution (1.25% H₂SO₄) for 30 minutes. Then, it undergoes boiling in a weak alkali solution (1.25% NaOH) for another 30 minutes while maintaining a constant volume. Finally, the ash content is subtracted from the obtained residue to calculate the crude fiber content through this digestion process.

Apparatus: Leibig condenser, Reflux condenser, and Gooch crucible are the instruments.

Reagent necessary:

- Sulfuric acid solution, 0.255N
- Potassium sulfate solution, grade Asbestos-Gooch, 10%.

Calculation: The weight loss reflects crude fiber.

$$\text{Crude fiber \%} = \frac{\text{Weight of residue with crucible} - \text{weight of ash with crucible}}{\text{Weight of sample (moisture and fat free)}} \times 100$$

3.12.6 Calculation of the Total Amount of Dry Matter

Moisture contents are used to estimate the dry matter content. In it, protein, fat, fiber and ash are present. Hence, it was determined by using the following formula:

$$\% \text{ Dry matter} = 100(\%) - \% \text{ Moisture content}$$

3.14 Determination of Bioactive Compounds

Extract Preparation

Making of an Extract by adding 10 ml of 100% ethanol to 1 g of material in a Falcon tube and then letting it sit for 72 hours. After 72 hours, filter the solvent and collect the filtrates. Then, the ethanoic extract was discovered.

3.13.1 Measurement of Flavonoids (TFC)

The total flavonoid content in the samples was determined following the aluminum chloride colorimetric method as described by Chang et al. (2002). To prepare the extract stock solution (1 mg/ml), 1.5 ml of 95 percent C₂H₅OH was used in a test tube, which was then diluted in 0.5 ml aliquots. Subsequently, 0.1 ml of 10% AlCl₃, 0.1 ml of 1 mol/L potassium acetate, and 2.8 ml of distilled water were added to the test tube. The mixture was left at room temperature for 30 minutes. For the blank, an equivalent volume of distilled water with 10% aluminum chloride was used. The absorbance was measured using a UV-visible spectrophotometer at a wavelength of 415 nm (UV-2600, Shimadzu Corporation, USA). The total flavonoid content in the sample extracts was determined by comparing their absorbance to a standard quercetin curve. The result was expressed as the amount of quercetin equivalents (QE) per gram of extract (mg QE/g) or TFC. The measurements were replicated three times to calculate means and standard deviation for improved accuracy and reliability.

3.13.2 Measurement of Polyphenols (TPC)

The Folin-Ciocalteu (FC) reagent method used to quantify total phenolic content (TPC) was adapted with some minor adjustments to determine the TPC of the extracts, as described by Al-Owaisi et al. (2014). In this process, 1 milliliter of ethanoic extract was mixed with 1.5 milliliters of FC reagent in a falcon tube and left at room temperature for three minutes. Following this, 1.5 ml of 7.5% Na₂CO₃ was added, and the mixture was allowed to settle for 60 minutes. Absorbance was measured at a wavelength of 765 nm using a UV-VIS Spectrophotometer (UV-2600, Shimadzu Corporation, USA), with C₂H₅OH as the blank reference. The TPC of the extracts was determined by making calculations that show the equivalence of TPC to mg of gallic acid equivalents (GAE) for every gram of extracts. To enhance accuracy and reliability, measurements were replicated three times to calculate means and standard deviation.

3.13.2 Measurement of Anthocyanins (TAC)

The total anthocyanin content is determined using the pH difference method, where the content is expressed as cyanidin 3-glucoside. The anthocyanin extract is first dissolved in a potassium chloride buffer (0.025 M, pH 1) and a sodium acetate buffer (0.4 M, pH 4.5) with a ratio of extract to buffer set at 1:5 (v/v). After

incubation for 15 minutes at room temperature, the absorbance of each solution is measured at both the maximum wavelength and 700 nm. The obtained results are then utilized in a specific formula to calculate the total anthocyanin content.

$$A = [(A_{\lambda_{maks}} - A_{700})_{pH = 1}] - [(A_{\lambda_{mak}} - A_{700})_{pH = 4.5}]$$

$$\text{Total Anthocyanin Content} = \frac{A \times MW \times DF \times 1000}{\epsilon \times b}$$

Where, DF is the dilution factor, b is the thick solution, ϵ is molar absorptivity and MW is the molecular weight (cyanidin 3glucoside 449.2 g / mole) (Wrolstad, 2005). Measurement was replicated 3 times for calculating means and standard deviation.

3.14 Antioxidant Capacity Measurement by the DPPH Scavenging Technique

The antioxidant activity of the extracts was evaluated using the DPPH test, with slight modifications from the procedure described by Azlim et al., 2010. Approximately 6 mg of DPPH was dissolved in 100 ml of 100% methanol to prepare a methanoic DPPH solution. Subsequently, 1 ml of the methanoic extract was mixed with 2 ml of the DPPH solution. The mixture was gently shaken and allowed to stand at room temperature in the dark for 30 minutes. Using a UV-VIS spectrophotometer, the absorbance was measured at a wavelength of 517 nm. For the control, 1 ml of methanol was mixed with 2 ml of the DPPH solution, and methanol served as the blank. To assess the scavenging activity of the samples, the decrease in absorbance relative to the DPPH standard solution was used as an indicator. The antioxidant capacity of the extracts was evaluated based on their ability to scavenge DPPH free radicals. A standard calibration curve was created using TEAC composite (Trolox equivalent antioxidant capacity), which was also used as the standard. The results were expressed as milligrams of Trolox equivalents per gram of powder on a dry weight (DW) basis. For enhanced accuracy, the measurements were replicated three times to calculate means and standard deviation.

3.15 Betalain Analysis

For the extraction of betalains, approximately 0.1 g of the sample was mixed with 10 mL of deionized water. The mixing was done using a Standard Mini Vortexer (XH-D Vortex, Hinotech) until a homogeneous mixture was achieved. Subsequently, the mixture was centrifuged at 3300g for 10 minutes using a Centrifuge Model 228 centrifuge (Fisher Scientific, Hampton, NH). The resulting supernatant was collected, and the insoluble pellet was subjected to two more extractions with

10 mL of water each time. The supernatants from all three extractions were combined and then brought up to a final volume of 50 mL with water (Kujala et al., 2000).

The total betalain contents, measured in milligrams per 100 grams of fresh tissue, were determined using a spectrophotometer (UV-2600, Shimadzu Corporation, USA). The betalain content (AC_d) was calculated using the formula reported by Cai et al., 2000 (Eq. 1). This equation considers the absorption value (A) at the absorption maxima of 536 nm for betacyanins, 480 nm for betaxanthins, and 430 nm for betalamic acid. Additionally, it considers the dilution factor (DF), total extract volume (V_a) in milliliters, fresh weight of the extracting material (W_a) in grams, and the path-length (L) of the cuvette (1 cm). For the quantification of betacyanins, the molecular weight (MW=726.6) and molar extinction coefficient ($\epsilon = 5.66 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$) of amaranthin were employed. The major betaxanthins (Bx) were quantified using the molecular weight (MW=309) and the mean molar extinction coefficient ($\epsilon = 48,000 \text{ M}^{-1}\text{cm}^{-1}$). The determination of betalamic acid involved the application of the molecular weight (MW=212) and molar extinction coefficient ($\epsilon = 24,000 \text{ M}^{-1}\text{cm}^{-1}$) (Biswas et al., 2013). To ensure accuracy, measurements were replicated three times, and means and standard deviations were calculated.

$$AC_d = \frac{A (MW) V_a (DF)}{\epsilon L W_a} \text{ (mg/gm of dried extract) [Eq. 1]}$$

3.16 Sensory Evaluation of Jelly

The samples of jelly were assessed for their sensory qualities. Color, flavor, texture, and overall acceptability of the samples were assessed. The degree of acceptance of the samples were determined using a 1–7 point hedonic rating test. 20 panelists were chosen from the academics, students, and staff members of the Chattogram Veterinary and Animal Sciences University's Department of Food Processing and Engineering and instructed on the process before the review. The 20 panelists each received a portion from each sample. On a scale of 1 to 7, where 1 - dislike extremely; 2 - a moderate dislike; 3 - dislike slightly; 4 - neither like nor dislike; 5- like slightly; 6 - like moderately; 7- like extremely; the taste panelists were asked to score the sample for color, appearance, smell, texture, taste, flavor and overall acceptability (Amerine et al., 2013).

3.17 Statistical Analysis

Data was sorted, coded and recorded in Microsoft Excel 2019 spreadsheet. Descriptive statistics (mean and standard deviation) were done for bioactive compounds (TPC, TFC, TAC), antioxidant capacity and betalain (betacyanins, betaxanthins, betalamic acid) analysis. After that statistical analysis (Tukey's pairwise comparison analysis) were conducted with MiniTab software (version 21.0) by using One-way ANOVA (Analysis of Variance) procedures to assess significant level of variation at 95% confidence interval. The statistical analysis was conducted for at 5% level of significant (≤ 0.05).

Chapter 4: Results

4.1 Physicochemical Properties of Beetroot Juice, Powder, and Jelly

Samples of beetroot juice, powder and jelly were subjected to different physicochemical analysis.

Table 4.1: Physicochemical Properties of Beetroot a) Juice, b) Powder, and c) Jelly

a) Juice

Parameters	Beetroot Juice
pH	6.0
Total Soluble Solids (TSS)	10 °Brix
Titration Acidity (TA)	0.014

b) Powder

Sample	Particle Size (μm)	Moisture content (%)	Water activity (a_w)
BPC	63-125	6.01	0.35
BPS		9.86	0.38
BPPC		5.21	0.41
BPPS		7.48	0.52

BPC: Beetroot Powder (Cabinet Dried)

BPS: Beetroot Powder (Sun Dried)

BPPC: Beetroot Pomace Powder (Cabinet Dried)

BPPS: Beetroot Pomace Powder (Sun Dried)

c) Jelly

Parameters	BJJ	BPCJ	BPSJ	BPPCJ	BPPSJ
pH	4.2	4.0	4.1	4.0	4.0
Total Soluble Solids (TSS) (°Brix)	69	68	69	70	68
Titration Acidity (TA)	0.49	0.45	0.486	0.51	0.49

BJJ: Jelly Prepared with BJ

BPCJ: Jelly Prepared with Reconstituted Juice of BPC

BPSJ: Jelly Prepared with Reconstituted Juice of BPS

BPPCJ: Jelly Prepared with Reconstituted Juice of BPPC

BPPSJ: Jelly Prepared with Reconstituted Juice of BPPS

pH, total soluble solids (TSS) and titration acidity (TA) were found as 6.0, 10 °Brix and 0.014 for beetroot juice sample (BJ).

Particle size, moisture content and water activity of beetroot powder and beetroot pomace powder from cabinet drying and sun drying process were measured. Particle size of BPC, BPS, BPPC and BPPS were found 63-125 µm. Moisture content (%) of BPC, BPS, BPPC and BPPS were recorded 6.01, 9.86, 5.21 and 7.48 respectively. Water activity (a_w) of BPC, BPS, BPPC and BPPS were recorded 0.35, 0.38, 0.41 and 0.52 respectively.

The pH, total soluble solids (TSS) and Titration acidity of developed beetroot jelly (BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ) were measured as part of the physicochemical analysis. pH of BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ were found 4.2, 4.0, 4.1, 4.0 and 4.0 respectively. Total soluble solids (TSS) of BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ were measured 69, 68, 69, 70 and 68 °Brix respectively. Titration acidity (TA) of BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ were found 0.49, 0.45, 0.486, 0.51 and 0.49 respectively.

4.2 Vitamin C of Beetroot Juice and Jelly

The vitamin C of the juice and jelly samples were calculated by using AOAC, 2010 method. The vitamin C content of beetroot juice and jelly were found 0.23 and 0.12 mg/gm respectively.

4.3 Mineral Contents of Beetroot Juice and Jelly

The minerals content of the beetroot juice and jelly had been analyzed by AAS in the laboratory. Results of minerals content in beetroot juice (BJ) and jelly (BJJ) samples are shown in the below table.

Table 4.4: Minerals contents data obtained for beetroot juice (BJ) and jelly (BJJ) sample.

Minerals	Juice	Jelly
Sodium (Na)	111.1 mmol/gm	73.9 mmol/gm
Potassium (K)	40.7 mol/gm	0.2 mol/gm
Magnesium (Mg)	15.7 mg/gm	0.1 mg/gm
Iron (Fe)	3648 µg/gm	15 µg/gm
Phosphorus (P)	35.2 mg/gm	0.2 mg/gm

4.4 Proximate Composition of Beetroot Jelly

Jelly prepared with beetroot juice was subjected to proximate composition analysis on dry basis. The dry matter, moisture, crude protein (CP), crude fiber (CF), ash and ether extract (fat) were found 63.70, 36.30, 1.45, 3.80, 0.96, 0.32 in percentage (%) respectively.

4.5 Bioactive Compounds of Beetroot Juice, Powder, and Jelly

Bioactive components and antioxidant capacity were analyzed by using a UV-visible spectrophotometer. Results were subjected to descriptive statistical analysis followed by Tukey.s comparison analysis. Results are shown in the below table:

Table 4.5: Bioactive compounds and antioxidant capacity analysis test results of a) juice, b) powder and c) jelly samples

Sample	Total flavonoids content (TFC) (mg QE/100g)	Total polyphenol content (TPC) (mg GAE/100g)	Total anthocyanin content (TAC) (mg/100g)	Antioxidant Capacity (% Inhibition)
a) Juice				
BJ	31.75 ± 0.16	0.31 ± 0.01	144.03 ± 0.32	30.18 ± 0.01
b) Powder				
BPC	49.78 ± 0.12 ^a	5.81 ± 0.01 ^a	889.55 ± 0.65 ^a	31.11 ± 0.005 ^a
BPS	11.14 ± 0.07 ^c	4.31 ± 0.02 ^b	215.77 ± 0.001 ^c	2.71 ± 0.001 ^c
BPPC	41.30 ± 0.09 ^b	2.25 ± 0.01 ^c	449.06 ± 1.61 ^b	30.82 ± 0.005 ^b
BPPS	3.44 ± 0.033 ^d	1.51 ± 0.01 ^d	35.40 ± 0.32 ^d	2.30 ± 0.004 ^d
c) Jelly				
BJJ	1.63 ± 0.04 ^d	1.50 ± 0.01 ^a	44.16 ± 0.001 ^c	2.04 ± 0.003 ^a
BPCJ	4.53 ± 0.04 ^a	0.54 ± 0.01 ^b	55.15 ± 0.32 ^b	1.56 ± 0.001 ^b
BPSJ	1.91 ± 0.07 ^c	0.36 ± 0.001 ^c	40.24 ± 0.001 ^d	1.41 ± 0.01 ^c
BPPCJ	2.71 ± 0.02 ^b	0.04 ± 0.01 ^d	58.32 ± 0.85 ^a	1.16 ± 0.002 ^d
BPPSJ	0.501 ± 0.033 ^e	0.01 ± 0.001 ^e	15.09 ± 0.001 ^e	0.44 ± 0.006 ^e

Results are presented as mean ± SD. *Different superscripted letters (a-d) in each column of Table 4.5.b and (a-e) in 4.5.c shows statistically significant differences (p value < 0.05) for all the samples.

BJ: Beetroot Juice

BPC: Beetroot Powder (Cabinet Dried)

BPS: Beetroot Powder (Sun Dried)

BPPC: Beetroot Pomace Powder (Cabinet Dried)

BPPC: Beetroot Pomace Powder (Sun Dried)

BJJ: Jelly Prepared with BJ

BPCJ: Jelly Prepared with Reconstituted Juice of BPC

BPSJ: Jelly Prepared with Reconstituted Juice of BPS

BPPCJ: Jelly Prepared with Reconstituted Juice of BPPC

BPPSJ: Jelly Prepared with Reconstituted Juice of BPPS

There are significant differences among the samples for powders and jellies. For flavonoids content, the highest value is 49.78 in BPC sample and the lowest value is 0.501 in BPPSJ sample. The polyphenol content is ranged from 0.01 to 5.81. The highest & lowest amount of polyphenol was found in BPC and BPPSJ sample and they were 5.81 & 0.01 respectively. For anthocyanin content, the highest value is 889.55 in BPC sample and the lowest value is 15.09 in BPPSJ sample. The antioxidant capacity is higher in BPC sample, and it is 31.11 and in BPPSJ sample, the value is lower, and it is 0.44. BPPSJ sample contains minimum value of anthocyanin, polyphenol, flavonoids and have the lowest antioxidant capacity. BPC sample have relatively higher amount of anthocyanin, polyphenol, flavonoids, and antioxidant capacity compared to all other samples. All the value shares a significant variance among them.

Among the analyzed bioactive compounds anthocyanin contents were found highest and beetroot contains comparatively lesser amount of polyphenol contents. BPC sample contains highest amounts of TFC, TAC, TPC and exhibits best antioxidant capacity among other powder samples. Among the jelly samples, TFC was found highest in BPCJ and TAC was found highest in BPPCJ.

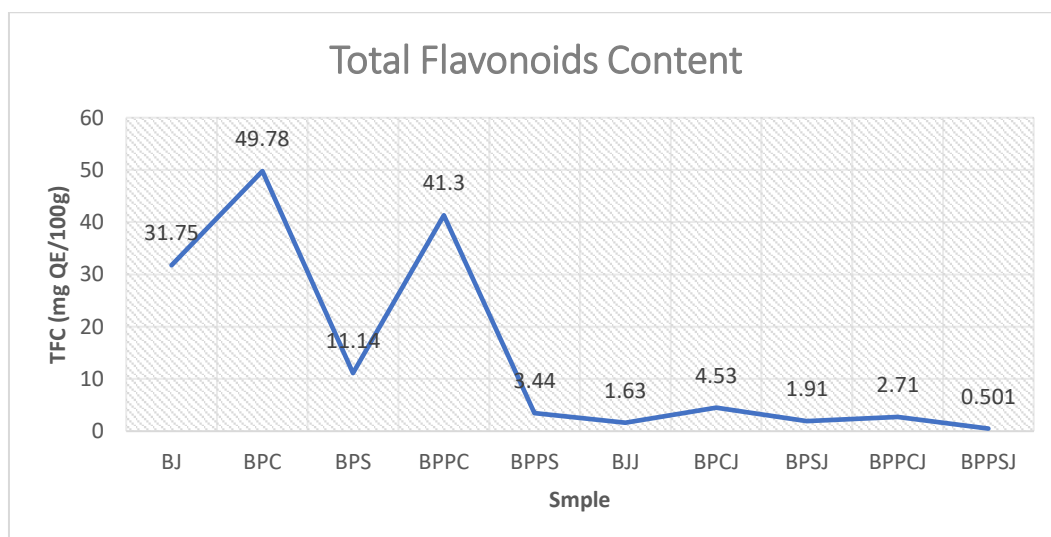


Figure 4.5.1: Total flavonoids content (TFC) (mg QE/100g) of beetroot sample

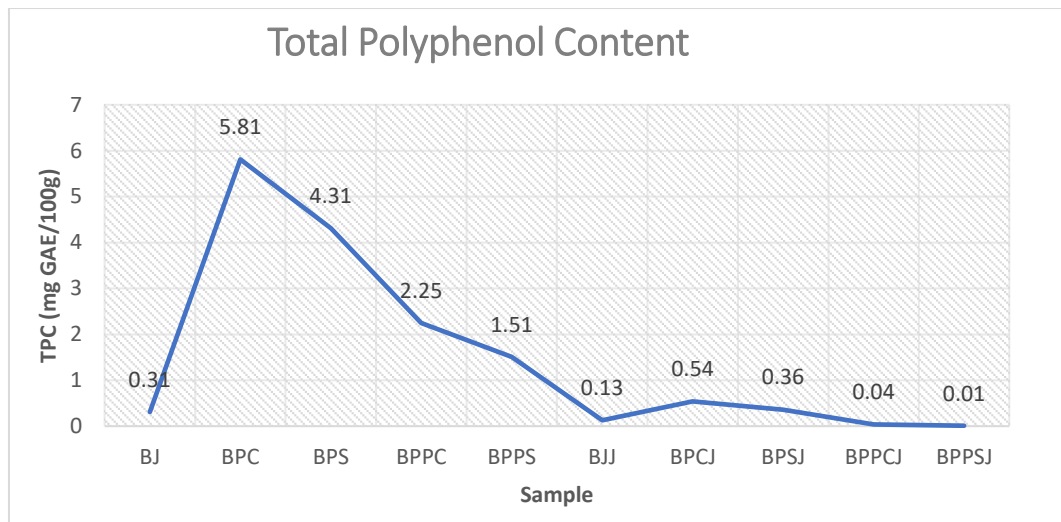


Figure 4.5.2: Total polyphenol content (TPC) (mg GAE/100g) of beetroot sample

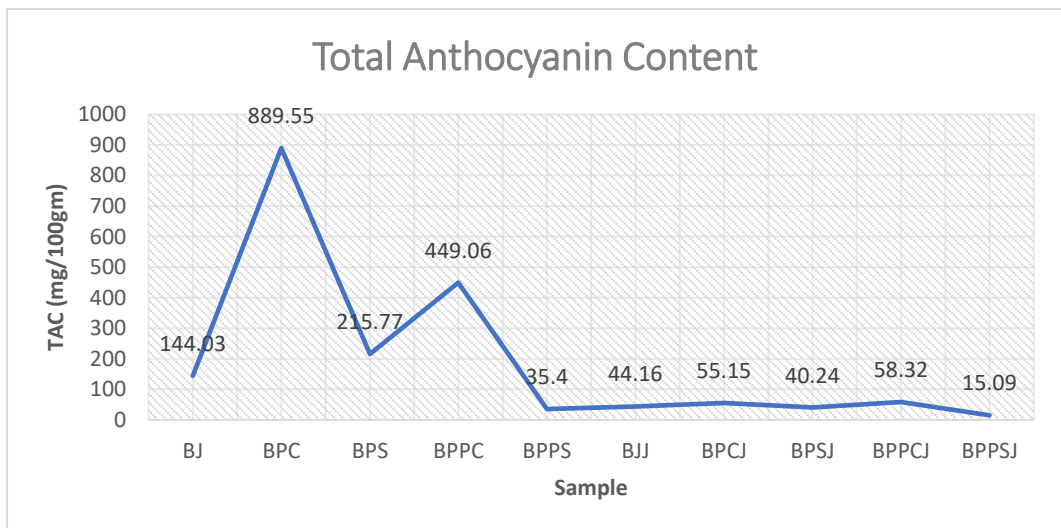


Figure 4.5.3: Total anthocyanin content (TAC) (mg/100g) of beetroot sample

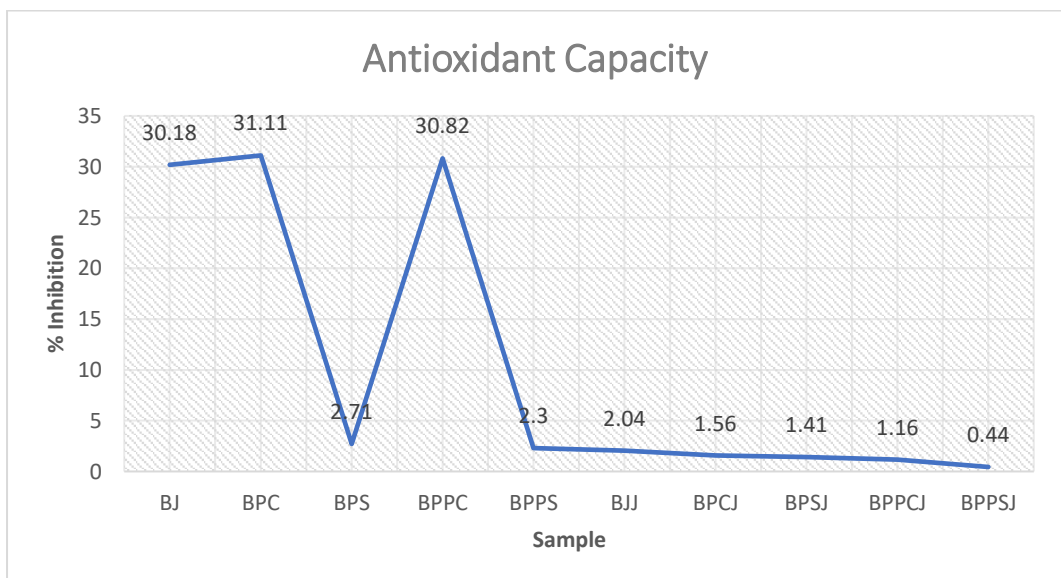


Figure 4.5.4: Antioxidant Capacity (% (Inhibition)) of beetroot sample

4.6 Betalain Content Determination

Betalain contents (betacyanin, betaxanthin, betalamic acid) were quantified by using UV-visible spectrophotometer. Results were subjected to descriptive statistical analysis followed by Tukey's comparison analysis. Results are shown in the below table:

Table 4.6: Betalain contents analysis test results

Sample	Betacyanins (mg/gm of sample)	Betaxanthins (mg/gm of sample)	Betalamic Acid (mg/gm of sample)
a) Juice			
BJ	47.15 ± 0.19	18.93 ± 0.001	19.05 ± 0.05
b) Powder			
BPC	332.91 ± 0.37 ^a	142.91 ± 0.001 ^a	103.94 ± 0.25 ^a
BPS	173.09 ± 0.37 ^b	84.01 ± 0.001 ^b	83.32 ± 0.25 ^b
BPPC	147.63 ± 0.001 ^c	65.67 ± 0.001 ^c	65.66 ± 0.25 ^c
BPPS	58.41 ± 0.001 ^d	31.87 ± 0.001 ^d	45.64 ± 0.25 ^d
c) Jelly			
BJJ	10.53 ± 0.001 ^d	4.83 ± 0.001 ^d	5.01 ± 0.33 ^e
BPCJ	25.67 ± 0.13 ^a	11.27 ± 0.13 ^a	11.66 ± 0.08 ^a
BPSJ	16.90 ± 0.07 ^b	7.75 ± 0.04 ^b	9.89 ± 0.001 ^b
BPPCJ	14.55 ± 0.07 ^c	5.71 ± 0.04 ^c	8.33 ± 0.05 ^c
BPPSJ	7.06 ± 0.001 ^e	4.39 ± 0.04 ^e	6.04 ± 0.13 ^d

Results are presented as mean ± SD. * Different superscripted letters (a-d) in each column of Table 4.6.b and (a-e) in 4.6.c shows statistically significant differences (p value < 0.05) for all the samples.

BJ: Beetroot Juice
 BPC: Beetroot Powder (Cabinet Dried)
 BPS: Beetroot Powder (Sun Dried)
 BPPC: Beetroot Pomace Powder (Cabinet Dried)
 BPPS: Beetroot Pomace Powder (Sun Dried)

BJJ: Jelly Prepared with BJ
 BPCJ: Jelly Prepared with Reconstituted Juice of BPC
 BPSJ: Jelly Prepared with Reconstituted Juice of BPS
 BPPCJ: Jelly Prepared with Reconstituted Juice of BPPC
 BPPSJ: Jelly Prepared with Reconstituted Juice of BPPS

There are significant differences among the samples for powders and jellies. For betacyanins content, the highest value was found in BPC sample (332.91) and the lowest value was found in BPPSJ sample (7.06). The highest & lowest amount of betaxanthins were found in BPC & BPPSJ sample and they were 142.91 & 4.39 respectively. For betalamic acid content, the highest value is 103.94 in BPC sample and the lowest value is 5.01 in BJJ. BPC sample contains maximum value of betacyanins, betaxanthins and betalamic acid. BPPSJ sample has relatively lower amount of betacyanins and betaxanthins compared to all other samples. BJ has relatively lower betalamic acid compared to all other samples. All the value shares a significant variance among them.

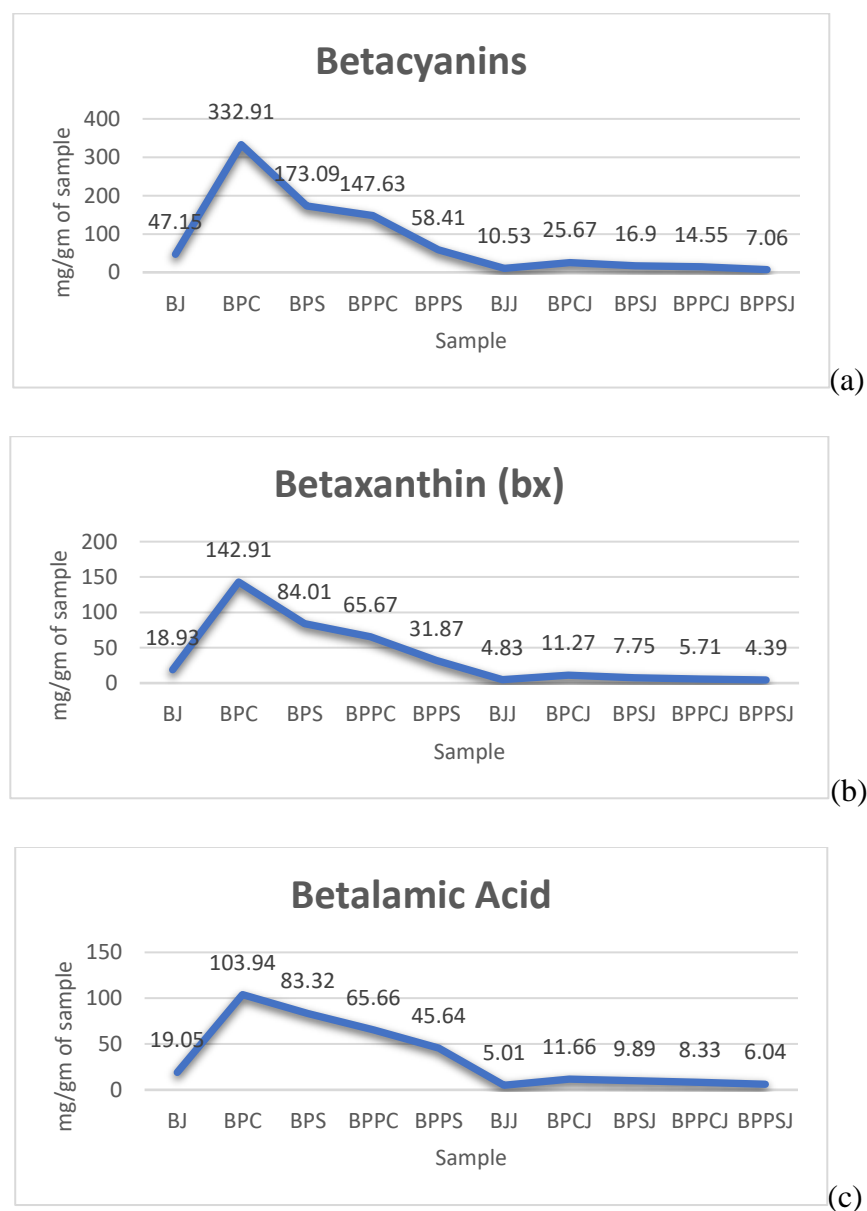


Figure 4.6: a) Betacyanin; b) betaxanthins; c) betalamic acid of beetroot samples

4.7 Sensory Evaluation

Prepared five jellies were presented before 20 panelists consisting of 10 male and 10 female panelists. Each panelist scored their perceived data on samples independently and recorded the scores on the prescribed evaluation sheets provided. These jellies were compared to one another in terms of color, appearance, smell, texture, taste, flavor, and overall acceptability. Results were subjected to descriptive statistical analysis followed by ANOVA (Analysis of Variance) Tukey's comparison analysis.

Table 4.7: Hedonic scale scoring test results for beetroot jelly samples

Sample	BJJ	BPCJ	BPSJ	BPPCJ	BPPSJ
Sensory Properties					
Color	6.25 ± 0.55	5.3 ± 0.57 ^a	5.55 ± 0.75 ^a	5.8 ± 0.69 ^a	5.5 ± 1.35 ^a
Appearance	6.1 ± 0.78	5.25 ± 0.63 ^a	5.25 ± 1.02 ^a	5.45 ± 1.19 ^a	5.55 ± 1.19 ^a
Smell	5.55 ± 1.05	5.25 ± 0.96 ^a	4.75 ± 0.85 ^a	4.8 ± 0.95 ^a	5.05 ± 0.68 ^a
Texture	6.2 ± 0.69	4.8 ± 0.83 ^{ab}	4.2 ± 1.05 ^{bc}	3.85 ± 1.38 ^c	5.6 ± 1.09 ^a
Taste	6.3 ± 0.57	5.2 ± 0.83 ^a	5.05 ± 1.19 ^a	4.8 ± 1.47 ^a	5 ± 1.07 ^a
Flavor	5.9 ± 0.85	4.9 ± 0.85 ^a	5 ± 0.72 ^a	4.65 ± 1.26 ^a	4.75 ± 0.85 ^a
Overall Acceptability	6.3 ± 0.57	5.05 ± 0.60 ^a	4.65 ± 0.87 ^a	4.9 ± 1.21 ^a	4.95 ± 1.05 ^a

Legends: All values in the table shown (Mean ± Standard Deviation) of data, where superscripts a, b, c denotes significant difference (p value ≤ 0.05) among samples of BPCJ, BPSJ, BPPCJ and BPPSJ in each row.

BJJ: Jelly Prepared with BJ

BPCJ: Jelly Prepared with Reconstituted Juice of BPC

BPSJ: Jelly Prepared with Reconstituted Juice of BPS

BPPCJ: Jelly Prepared with Reconstituted Juice of BPPC

BPPSJ: Jelly Prepared with Reconstituted Juice of BPPS

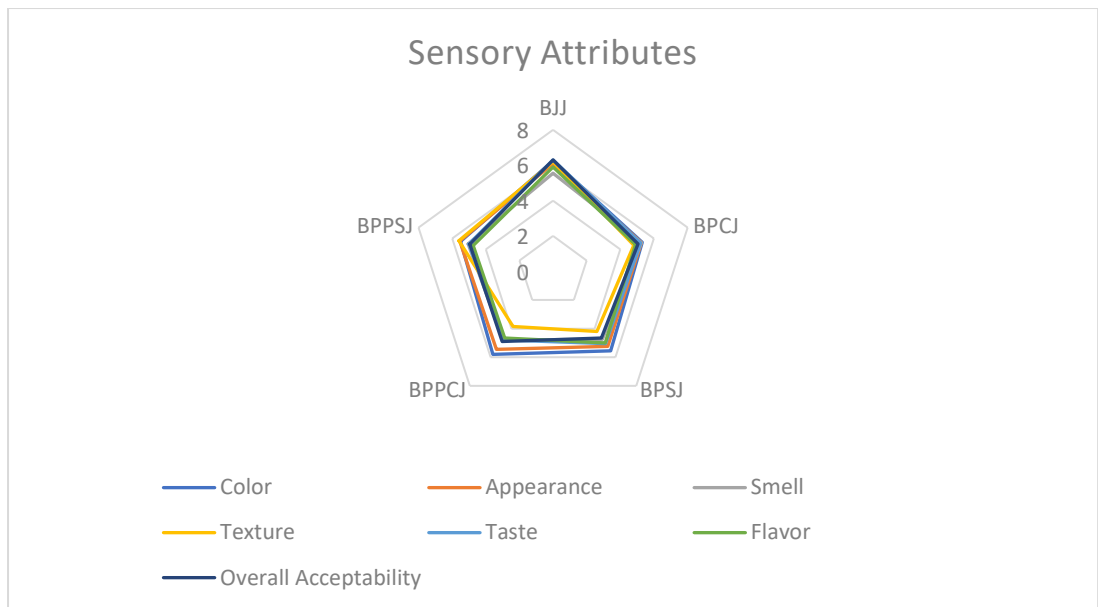


Figure 4.7: Sensory attributes of developed jellies

BJJ sample has better sensory attributes compare to jellies made with reconstituted beetroot powder and pomace powder. There are no significant differences among BPCJ, BPSJ, BPPCJ, and BPPSJ sample in terms of color, appearance, smell, taste, flavor, and overall acceptability. Panelists had the best preference for texture in BPPSJ sample.

Chapter 5: Discussion

5.1 Physicochemical Characteristics of Beetroot Juice, Powder, and Jelly

The physicochemical properties including pH, total soluble solids (TSS) and titrable acidity (TA) were measured for beetroot juice and jelly. Particle size, moisture content and water activity were analyzed for beetroot powder and beetroot pomace powder.

pH, TSS and TA of beetroot juice were found 6.0, 10 °Brix and 0.014 respectively. Similar results were observed for beetroot juice according to established study (Kale et. al., 2018).

Beetroot and beetroot pomace powder obtained through cabinet drying and sun drying (BPC, BPS, BPPC and BPPS) had a particle size ranging from 63-125 µm. Moisture content (%) of BPC, BPS, BPPC and BPPS were found 6.01, 9.86, 5.31 and 7.48 respectively. Water activity (a_w) of BPC, BPS, BPPC and BPPS were found 0.35, 0.38, 0.41 and 0.52 respectively. Cabinet drying process yield powder with lesser moisture content and water activity than sun drying process. Beetroot powders both from cabinet drying and sun drying had lesser moisture content and water activity compared to beetroot pomace powder.

pH, TSS and TA were measured for beetroot jelly samples (BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ). pH of BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ were found 4.2, 4.0, 4.1, 4.0 and 4.0 respectively. TSS (°Brix) of BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ were found 69, 68, 69, 70 and 68 respectively. TA of beetroot jelly samples (BJJ, BPCJ, BPSJ, BPPCJ and BPPSJ) were found 0.49, 0.45, 0.486, 0.51 and 0.49 respectively. pH, TSS and TA value of the jelly samples implicate that the jelly was slightly acidic in nature, high in sugar contents, had similar composition and underwent similar processing techniques.

These results suggest that these physicochemical characteristics of beetroot juice, powder and jelly may influence their sensory attributes, nutritional content, potential uses in food and beverage products as well as utilization of byproducts.

5.2 Proximate Composition of Beetroot Jelly

The dry matter, moisture, crude protein (CP), crude fiber (CF), ash and ether extract (fat) of beetroot jelly (BJJ) were found 63.70, 36.30, 1.45, 3.80, 0.96, 0.32 in percentage (%) respectively. Tiwari and Singh, 2019 described similar proximate composition for beetroot jelly.

High dry matter contents of beetroot jelly implicate the presence of total solids mainly including sugar along with carbohydrates, fiber, protein, ash. The jelly has similar moisture content of commercial mango and pineapple jelly from Bangladesh (Sarower et. al., 2015). The protein content in the beetroot jelly might be derived from sources like proteins naturally present in the beetroot. Crude fiber, ash content of the jelly sample was concentrated compared to fresh beetroot sample reported by Kale et. al. (2018). Beetroot jelly consist very low percentage of fat content mainly derived from fresh beetroot which has similar quantity of fat content reported by Kale et. al. (2018).

5.3 Vitamin C Contents of Beetroot Juice and Jelly

The vitamin C content of beetroot juice and jelly were found 0.23 and 0.12 mg/gm respectively. Vitamin C contents was depressed when the beetroot juice is processed into jelly. The effect of heat treatment is responsible for the degradation of vitamin C contents. Washing, presence of light and oxygen also contributes to the degradation of vitamin and explains the lower vitamin C contents in the jelly samples (El-Ishaq and Obirinakem, 2015). Both beetroot juice and jelly have significant amounts of vitamin C presence compared to other study (Kale et. al., 2018)

5.4 Minerals Contents of Beetroot Juice and Jelly

The minerals content of the beetroot juice (BJ) and jelly (BJJ) had been analyzed by AAS in the laboratory. The quantity of sodium (mmol/gm), potassium (mmol/gm), magnesium (mg/gm), iron ($\mu\text{g/gm}$) and phosphorus (mg/gm) in beetroot juice sample were found 111.1, 40.7, 15.7, 3648 and 35.2 respectively. In jelly the quantity of sodium (mmol/gm), potassium (mmol/gm), magnesium (mg/gm), iron ($\mu\text{g/gm}$) and phosphorus (mg/gm) were 73.9, 0.2, 0.1, 15 and 0.2 respectively.

The amount of sodium, potassium, magnesium, iron, and phosphorus in jelly samples are depressed than that of in juice samples. As reported by Kumar et al., 2015 and Kale et. al., 2018 the amount of Na, K, Mg, Fe and P present in juice and jelly are lesser than that of in raw beetroot. The differences in mineral content between beetroot juice and beetroot jelly can be attributed to the processing methods used to prepare the jelly. Juice extraction and jelly-making processes can lead to the removal or loss of some minerals from the original beetroot source.

Consequently, the nutritional composition of the final products varies.

Mineral stability and availability in foods are often less impacted by processing than other macro and micronutrients. It's prone to leaching into the water used for processing or cooking. Mineral contents losses are usually associated with leaching or the formation of insoluble metal complexes, which reduces absorption. Thermal processing has no effect on the availability of minerals associated with proteins unless there is significant thermal destruction of the protein (Arora et al., 2023).

5.5 Bioactive Compounds of Beetroot Juice, Powder, and Jelly

Bioactive compounds (TFC, TPC, TAC) and antioxidant capacity of beetroot juice (BJ), beetroot powders (BPC, BPS), beetroot pomace powders (BPPC, BPPS) and beetroot jellies (BJJ, BPCJ, BPSJ, BPPCJ, BPPSJ) were measured using UV-Vis spectrophotometer.

TFC, TPC and TAC were found highest in BPC and lowest in BPPSJ. Both beetroot powder and beetroot pomace powder had comparatively higher number of flavonoids, polyphenol, and anthocyanin presence than of juice and jelly sample. TFC, TPC and TAC gets concentrated in the powder form. Powders obtains through cabinet drying process (BPC, BPPC) had comparatively higher number of flavonoids, polyphenol and anthocyanin content compared to the powder obtained by sun drying process (BPS, BPPS). Beetroot powder (BPC, BPS) had more flavonoids, polyphenol, and anthocyanin content presence than that of beetroot pomace powder (BPPC, BPPS). Due to the presence of light and heat, a significant number of TFC, TPC and TAC get lost during sun drying process. Jelly had lesser number of flavonoids, polyphenol and anthocyanin content compared to juice and powder sample. The difference between BPC-BPS and BPPC-BPPS of flavonoids, polyphenol and anthocyanin implicate that cabinet drying process can retain much more of flavonoids, polyphenol, and anthocyanin content than sun drying process. The presence of lower amount of TFC, TPC and TAC in jelly samples implicate the loss of flavonoids, polyphenol, and anthocyanin during the thermal processing for the preparation of jelly. Silva et. al., 2016 reported similar findings for flavonoids in beetroot juice and gel sample.

The highest antioxidant capacity was exhibited by BPC and the lowest was exhibited by BPPSJ. Beetroot juice contains a great presence of antioxidant capacity

compared to jelly and sun-dried powder sample. Both BPC and BPPC had higher amount of antioxidant capacity which implicate the retention of inhibition capability of oxidation process through cabinet drying process. Both BPS and BPPS sample had very little amount of antioxidant capacity implicating the suffer to factor such as light, oxygen and heat during sun drying process. The significant difference between jelly samples and other samples describes the vulnerability of bioactive compounds and antioxidant capacity to heat treatment.

5.6 Betalain Content

Betalain contents including betacyanin, betaxanthin, betalamic acid were quantified by using UV-visible spectrophotometry method for beetroot juice (BJ), beetroot powders (BPC, BPS), beetroot pomace powders (BPPC, BPPS) and beetroot jellies (BJJ, BPCJ, BPSJ, BPPCJ, BPPSJ).

Betalain consists of mainly betacyanin (betanin, isobetanin) and betaxanthins (vulgaxanthin-I, vulgaxanthin-II, vulgaxanthin-III) (Clifford et al., 2015). Kerr et al. (2019) reported betalain contents of beetroot powder obtained through different drying methods. BPC, BPS, BPPC and BPPS exhibits similar results with other drying method from this study. Here, BPC have the highest amount of betalain presence than other samples and BPPSJ have the lowest. Cabinet drying process was found effective to retain the betalain content than that of sun drying process. Jelly had lower number of betalains content compared to juice and powder due to undergoing thermal processing which reduced the amount of betalains presence in the jellies.

5.8 Sensory Evaluation of Beetroot Jelly

Sensory quality of beetroot jelly samples (BJJ, BPCJ, BPSJ, BPPCJ, BPPSJ) based on color, appearance, smell, texture, taste, flavor, and overall acceptability were evaluated. BJJ sample had better characteristics for all the attributes compared to jelly of reconstituted juice presented before the panelists.

BPCJ, BPSJ, BPPCJ and BPPSJ sample have similar acceptance in terms of color, appearance, smell, taste, flavor, and overall acceptability and had no significant differences among them. Both BPPSJ and BPPCJ sample had significant differences in terms of texture. Beetroot juice, pulp, and powder are often used as food additives for cereal, baked and confectionary products, milk and milk products etc. according to Punia et. Al. (2022).

Chapter 6: Conclusion

The perspective of beetroot is positive due to its vast health benefits, with a growing demand for this vegetable in domestic and international markets. From this study, it can be said that beetroot juice, beetroot powder, beetroot pomace powder and beetroot jelly have significant presence of bioactive compounds (flavonoids, polyphenol, anthocyanin) and antioxidant capacity. Betalains (betacyanin, betaxanthin, betalamic acid), a bioactive compound group primarily responsible for the dark red color of beetroot with its numerous health benefits have also found in significant quantity in this present study. Beetroot also has significant vitamins and minerals presence. Beetroot powder obtained from the cabinet drying process (BPC) exhibits very good retention of bioactive compounds (TFC, TPC, TAC, Betalains) and antioxidant capacity and have better physicochemical properties. Beetroot pomace which is a by-product from juice preparation can be utilized by processing into powder. Beetroot jelly prepared from fresh juice (BJJ) can be processed as a value-added product. While beetroot is not widely cultivated in Bangladesh, it has the potential to become more popular due to its unique color and potential health benefits. Further research is needed to fully understand the benefits and potential uses of this vegetable and its byproducts.

Chapter 7: Recommendation and Future Perspectives

In Bangladesh, beetroot is cultivated in some areas, but it is not a major crop. However, as the country is experiencing growth in the food industries, beetroot has potential for future applications in this sector.

- a) Due to the presence of excellent health benefits beetroot juice can be a potential value-added product for health-conscious people and also have prospects of being a super sports drink.
- b) Beetroot powder prepared by cabinet drying is recommended for higher retention of bioactive compound including flavonoids, polyphenol, anthocyanin, betalains.
- c) Beetroot pomace, which is a by-product of beetroot juice can be utilized with prospective value by transforming into powder.
- d) Beetroot jelly is a novel product and can be marketed as an attractive value-added product in future.
- e) Further product optimization, shelf life and cost-benefit studies are suggested to develop and improve commercial beetroot jelly formulation.

Overall, the future perspective of beetroot in Bangladesh looks promising, and there are opportunities for the country to explore its potential applications in in the food sectors. However, more research and development are needed to fully understand the benefits and potential uses of this vegetable in Bangladesh.

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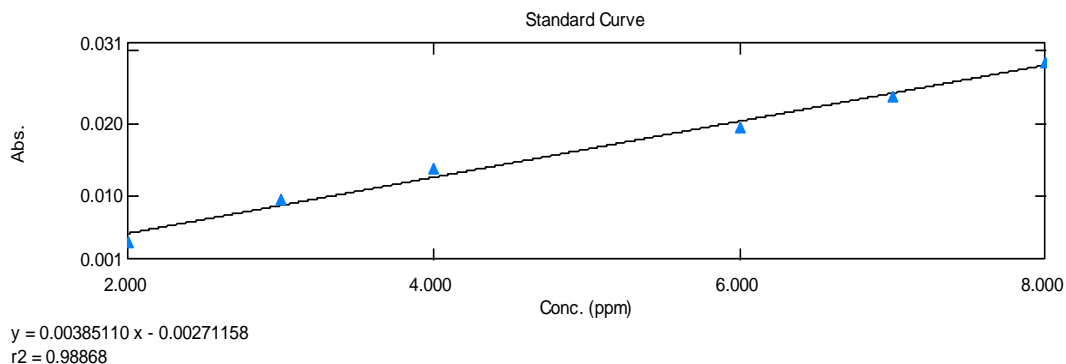
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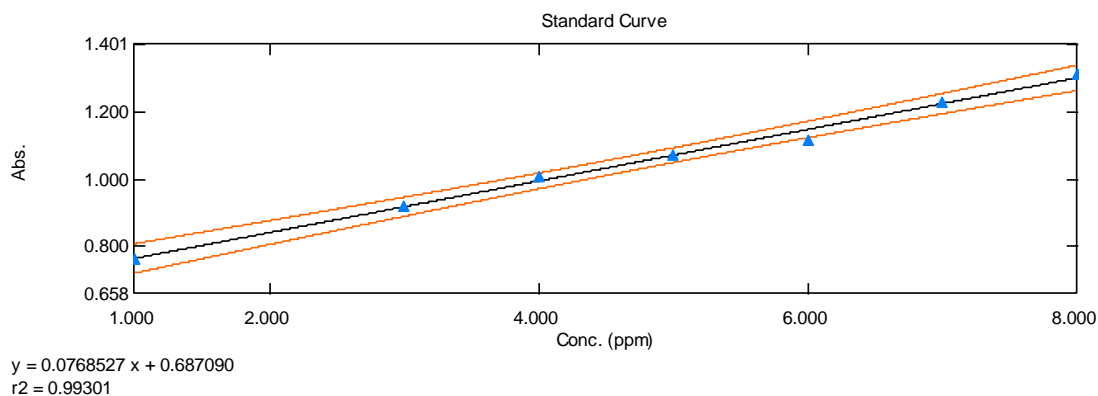
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Appendix I: Standard Curve for Flavonoids, Polyphenol and Antioxidant

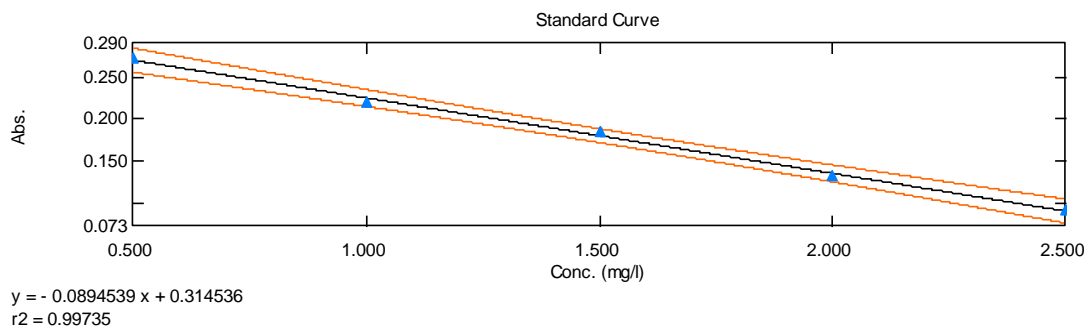
Standard Curve for Flavonoids



Standard Curve for Polyphenol



Standard Curve for Antioxidant



Appendix II: Photo Gallery



(a)



(b)

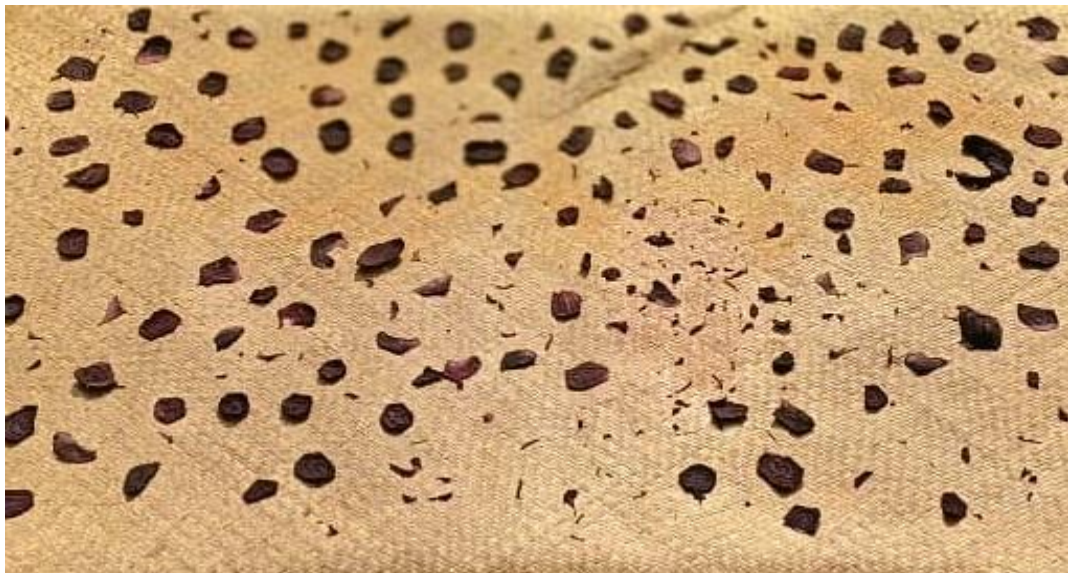
a, b) Beetroot



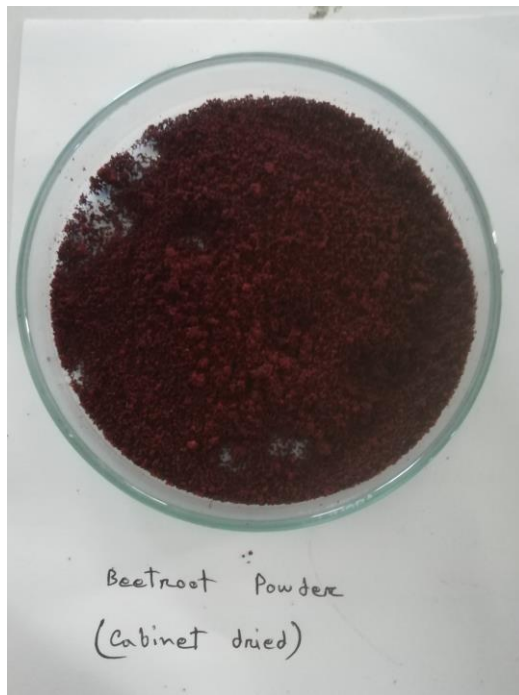
c) Beetroot Juice



d) Cabinet Drying



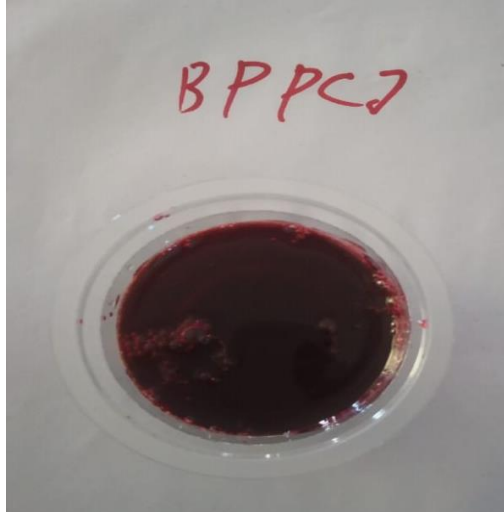
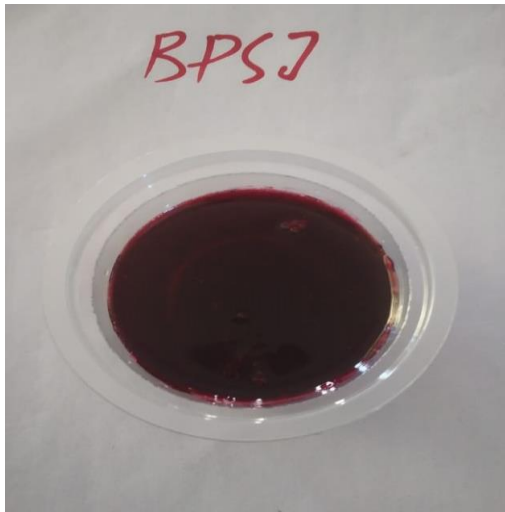
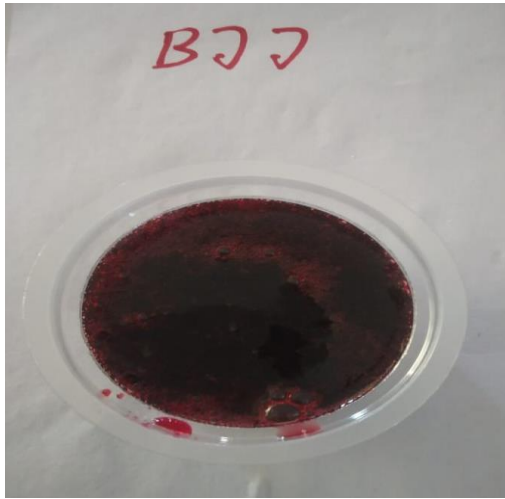
e) Sun Drying



f) Beetroot Powder



g) Beetroot Pomace Powder



h) Beetroot Jelly



i) Preparation of Beetroot Jelly



j) UV-Vis Spectrophotometry Analysis



k) Sensory Evaluation

Brief Biography

Al Mahamud Ibne Jamal passed the Secondary School Certificate (SSC) Examinations in 2013 with Grade Point Average (GPA) 5.00 followed by Higher Secondary Certificate (HSC) Examination in 2015 with GPA 4.58. He received the B.Sc. (Hon's) in Food Science and Technology in 2019 (held in 2020) from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of M.Sc. in Food Processing and Engineering under the Department of Food Processing and Engineering, Faculty of Food Science and Technology, CVASU. His career objective is to obtain and secure a challenging position as a Food scholar. He has profound interest to work in a challenging environment where his skill can be put to good use for problem solving and come up with innovative solutions.