NUTRITIONAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF *PASSIFLORA EDULIS* AND FATTY ACID ANALYSIS OF ITS EXTRACTED SEED OIL

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Department of Applied Food Science and Nutrition

Faculty of Food Science & Technology

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

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PLAGIARISM VERIFICATION

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List of Abbreviations

SSC	Soluble solids content
TA	Titratable acidity
TSS	Total soluble solid
mg/g	Milligram per Gram
IC50	Half-maximal inhibitory concentration
mg/ml	Milligrams per milliliter
mg/kg	Milligrams per kilogram
$AlCl_3$	Aluminium chloride
NF-kB	Nuclear factor kappa B
µl/dl	microliter per deciliter
ABTS	(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
MUFA	Monounsaturated Fatty Acids
$Na_2S_2O_3$	Sodium Thiosulfate
mL	milliliter
NaOH	Sodium hydroxide
H_2SO_4	Sulfuric acid
$CuSO_4$	Copper sulfate
K_2SO_4	Potassium sulfate
w/v	weight in volume
HCI	Hydrogen chloride
mg/dl	Milligrams per decilitre
mmol/L	millimoles per litre
ISO	International Organization for Standardization

AOAC	Association of Official Analytical Chemists
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
TFC	Total flavonoids content
TPC	Total polyphenol content
C_2H_5OH	Ethanol (ethyl alcohol)
$NaHCO_3$	sodium bicarbonate
USDA	United States Department of Agriculture

Abstract

The study was accomplished to evaluate the nutritional, physicochemical and phytochemical properties analysis of the different parts of Passion fruit and to identify the important nutritional significance as extracted seed oil. In this study, the peel, pulp, and seed were analyzed to compare the physicochemical properties like pH, total soluble solids, titrable acidity, proximate and mineral composition, and bioactive and antioxidant constituents. After comparing the composition, it was found that the three parts had a great source of nutrients, including minerals, ash, protein, fiber, fat, and vitamin C. They also had a notable amount of polyphenol, flavonoid, and antioxidant content. But among the three parts, the seed of passion fruit had 17.623±0.287% fat and a rich source of oil. After that, the oil was extracted from the seed by Soxhlet apparatus using diethyl ether following the analysis of the physicochemical properties of the oil. Then it was found that the iodine value (17.247±0.362), acid value (1.117±0.016), peroxide value (9.267±0.252), and specific gravity (0.971±0.005). These values helped to ensure the oil quality of passion fruit seed. After that, the fatty acid composition was done through GC-FID, and it was found that the presence of Gamma Linolenic acid (10.37%), Heptadecanoic acid (0.43%), Hexanoic acid (3.33%), Behenic acid (0.26%) and so on. This all-fatty acid led to making passion fruit seed oil an excellent source of plant-sourced oil. In conclusion, it is possible to state that passion fruit is a source of excellent nutrition, and its byproducts can be of great use in the future if implemented properly. However, more research is needed in this field to meet the consumer demand for healthier and more sustainable agricultural products.

Keywords: nutritional properties, fatty acid composition, physicochemical properties, bioactive constituents, seed oil.

Chapter 1: Introduction

1.1 Background

There are 520 species of plants in the Passiflora L. family, which is very diverse and found worldwide. The species are primarily found in America, Asia, and Africa, and their values range from 80 to 90 percent. Nonetheless, they can be found in Australia, China, the Pacific Islands, India, Southeast Asia, and surrounding areas (Devi et al.,2020). Only a few of the species that are found all over the world are significant economically. It is found in Hilly areas of Bangladesh like Cox's Bazar, Bandarban, Rangamati etc (M. Das et al., 2013)

The majority of species are edible. Yellow (P. edulis f. edulis) and purple (P. edulis f. flavicarpa) passion fruits are the most famous varieties. They have been examined to understand the underlying processes of having diverse colors. In Bangladesh, mainly yellow colored passion fruit is available. In purple passion fruit, bioactive substances, including flavonols and anthocyanins, are significantly concentrated. They generally differ based on molecular characterization and analysis (Campos et al., 2019).

The Passifloraceae family's plants are grown in tropical and subtropical areas worldwide. It has primarily been farmed in South America. Brazil, Peru, Ecuador, and Colombia are more nations where passion fruit is produced. This is typically ingested as juice or whole fruit. Juice is consumed because pulp makes up 30–40% of the fruit. It is more palatable to consumers because it has nutritional features, color, taste, and aroma. Passifloras come in various kinds, most of which differ phenotypically (Kishore et al., 2006). There are differences between the two varieties in terms of size, flavor, and color. There are wild species that are distinct from domestic species.

Passiflora edulis f. flavicarpa, is yellow-colored and the most widely cultivated species. The growth plates are in Ecuador, Brazil, and Peru. Purple passion fruit is grown primarily in the highlands, whereas yellow passion fruit is planted in the coastal plains. Ecuador is emphasized explicitly because there is the highest amount of passion fruit growth there. Colombian farmers raise P. edulis f. edulis, another common type (Kishore et al., 2006).

The fruit differs from the other P. edulis f. flavicarpa types in size, weight, length, thickness, and diameter. The length is 6-12 cm, while the diameter is 6-7 cm on average.

Peel thickness ranges from 7.2mm to 7.5mm, and weight is from 174 and 175g. Purple passion fruit is more spherical than yellow, with a 5 cm diameter, and has a thinner peel (Kishore et al., 2006).

Some regional variations with the name Criollo need to be accurately characterized botanically. The purple passion fruit, P. edulis f. flavicarpa, is more significant than P. edulis f. edulis.

The interior part of the fruit, which is a sour fruit, is consumed uncooked. The seeds inside the pulp can be consumed with juice or discarded. Passion fruit is consumed in Brazil either as juice or as a concentrate (Mandal and Goutam, 2017). Moreover, it is used to make jam, jelly, and ice cream. The fruit is more agreeable due to its versatility of uses. The fruit can be used industrially to create medications and healthcare items. It will gain appeal in the market shortly. Although the fruit's famous as a juice, its byproducts, like seeds and peel, can also be helpful. These leftovers may play a significant role in the economy in the future.

This tropical fruit is grown for its edible fruits, which have decorative blooms and medicinal applications. Each component of it has an efficient use. Yet, the industry pays close attention to the contribution of passion fruit juice (Gaydou EM et al., 1983). Some byproducts are thrown away. A significant portion is still left as trash, so if the byproducts can be used to create new products, it can significantly lower the cost and can have a great impact on the entire economy (Mandal and Goutam, 2017). There are a lot of oils in the passion fruit seeds, according to studies that have been done. If the seeds are used to make oil instead of being wasted, their usefulness improves four to five times. These seeds are tasty and have crunchiness. It can be helpful using as a topping on salad or ice cream because of its crunchiness. They are nutrient-rich enough and offer advantages for digestive and cardiovascular health (Faleiro et al., 2019). The ability of seeds and fibers to fight free radicals keeps them healthy (Chau et al., 2004). The seeds can provide the agro-industrial waste more value. Cancer risk and obesity can be decreased by insoluble fiber.

Unsaturated fatty acids, including linoleic acid, tocopherols, carotenoids, and phenolic compounds, all of which are known to have antioxidant action, are abundant in the oil contained in the seeds of the passion fruit (Ferreira et al., 2011).

It is crucial that the seeds have been dried beforehand to lower the samples' moisture content in order for the oil extraction to proceed successfully (Rodrgues-Rojo et al.,

2012). It is highlighted that the seeds must be moved, divided, cleansed, and, if necessary, kept before the extraction procedure (Derosya et al., 2020).

Having high-quality seeds is essential for getting high-quality goods. The physical properties of seeds directly influence their quality. Consequently, the physical characteristics and quality of seeds can be directly impacted by the decrease in seed moisture content during drying (Mirzabe et al., 2016). Understanding these qualities is essential for post-harvest operations of agricultural products, including equipment sizing and operation, cost reduction, and proper conservation (Ramashia et al., 2019).

1.2 Significance of the study

The fruit has a significant nutritional and medicinal potential, according to study (Joy et al., 2010). Because citric and malic acids predominate, passion fruit has a high acid content (pH 3.2). The fruit is a good source of vitamins A, B_2, and C as well as phytochemicals like carotenoids and polyphenols that are not nutrients. It is also abundant in protein and minerals including K, P, Ca, Fe, Na, Mg, S, and Cl (Joy et al., 2010). Based on the quantity of nutrients it contains, passion fruit is sometimes referred to as a fruit that is nutritionally dense. The main factor influencing such nutritional rankings is the high concentration of vitamins A, C, and B_2 in passion fruit. It is possible to produce passion fruit for consumption or for its juice, which is frequently added to other fruit juices to boost scent. Fruit is consumed on its own or in fruit salads, sherbets, ice cream, jams, cold beverages, and as concentrates. While the purple type is sold at fresh fruit markets, the yellow version is utilized to make juice according to Zas et al., 2016.

The Passiflora edulis plant has anti-inflammatory, anticonvulsant, antimicrobial, anticancer, anti-diabetic, antihypertensive, anti-sedative, antioxidant properties as well as a variety of restorative measures for treating conditions like osteoarthritis, asthma, and act as a colon cleanser (He et al., 2020). Moreover, many plant parts have been utilized to treat ulcer, hemorrhoids.

Since ancient times, the species of Passiflora have been used as traditional medicine due to their antidepressant and herbal characteristics (Debideen et al., 1978). Passiflora species have proved successful in preventing alcoholic liver damage. Moreover, it possesses anti-platelet, anti-inflammatory, and antidepressant properties (Dos Reis LCR et al., 2018).

The functional and physicochemical properties of passion fruit make the byproducts of it more nutritious. If the byproducts of the fruit convert to useful products, then the agro-industrial cost will reduce and impact on environment also reduces.

Hence, the ability to recycle passion fruit seeds to create functional ingredients may have a variety of uses in the food, pharmaceutical, and cosmetic industries, allowing for the transformation of an agro-industrial waste into goods with added value (Malacrida et al.,, 2012).

1.3 Objectives:

1. To evaluate the physicochemical properties of passion fruit (*Passiflora edulis*) peel, pulp and seed including proximate, bioactive compounds, vitamin and mineral contents.
2. To compare the all nutritional and physicochemical properties of different parts of fruits
3. To extract the oil from fruit seed and determine the physicochemical properties including fatty acid composition.

Chapter 2: Literature Review

2.1 Outline of the passion fruit description

The Passifloraceae family includes the tropical fruit, the passion fruit (Passiflora edulis). Since it has its origins in South America, traditional South American medicine has made extensive use of this. It is used as medicine against asthma, bronchitis, insomnia, anxiety, and infections in the urinary tract because of its therapeutic characteristics (Nesterenko et al., 2014). This fruit is now produced worldwide and used in the food sector as an edible fruit. There are several cultivars of this fruit, including gulupa (P. edulis Sims. f. edulis), purple passion fruit (P. edulis Sims), P. edulis var. flavicarpa Degenerer, granadilla (Passiflora ligularis), and yellow passion fruit (Passiflora angustifolia).

There are two colors of Passiflora edulis, the sour passion fruit: purple and yellow. Both exhibit similar behavior during the fruit's growth, maturity, and ripening stages, but their key differences are in their color, SSC, and TA levels, as well as the elements that make up their aroma (Pertuzatti et al., 2015). Both of them are climacteric fruits with significant ethylene and respiration output. Reduced acidity, which results in a higher perceived sweetness, and weight loss, which causes shriveling, are the critical quality changes that occur during storage and ripening (Silva et al., 2021). Those who prefer a smooth fruit prefer something other than the latter. The need for a specific temperature during storage is another significant distinction. Yellow passion fruit prefers a higher temperature of 10°C, whereas purple passion fruit acts best around 4-5°C. The processing sector is the primary market for both forms, and juice makes up the majority of exports (Biswas et al., 2021).

Passion fruit is made up of an exotic climbing vine called Passiflora edulis. Passion fruit, which has its roots in South America, is today cultivated all over the world as a food-grade fruit (Surlehan et al., 2019). In South America and other nations, it has long been a staple of traditional medicine for treating conditions like sleeplessness, asthma, bronchitis, and infections in the urinary tract. The primary phytochemical components of several P. edulis extracts are alkaloids, flavonoids, glycosides, vitamins, minerals, and terpenoid compounds (Fatima A et al., 2013). Unfortunately, there still needs to be more unbiased scientific confirmation of the purported health advantages of P. edulis in people. There has yet to be a long-term study, even though short-term adverse effects

of crude leaf extract in people have been reported. The precise health advantages or hazards of P. edulis have been the subject of shaky findings due to this circumstance (Ngakou Takam P et al., 2019). More research is required to investigate and determine the P. edulis extracts' possible medical benefits.

2.2 Classification within science

Table 2.2: Passiflora edulis's classification in science

Kingdom	Plantae
Family	Passifloraceae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
(Unranked)	Eurosids
Order	Malpighiales
Genus	Passiflora L
Species	Passiflora foetida L.
Synonyms	Passiflora edulis var. verrucifera (Lindl.), Passiflora edulis forma edulis), Passiflora verrucifera (Lindl.)

2.3 Composition of chemical components of passion fruit:

Dietary fiber, carbohydrates, lipids, carboxylic acids, polyphenols, volatile compounds, protein and amino acids, vitamins, minerals, and other nutrients comprise most of P. edulis' nutritional content. Over 110 chemical constituent types have been isolated and identified from P. edulis. The three main categories are flavonoids, triterpenoids, and carotenoids (Adeyeye et al., 2017).

2.4 Passion fruit's macronutrient profile:

2.4.1 Amino Acids and Proteins

Passion fruit pulp contains 0.80 mg/g of total protein. Some proteins in passion fruit have important antifungal activities. For instance, the filamentous fungus Trichoderma harzianum, Fusarium oxysporum, and Aspergillus fumigatus are shown to be inhibited by specific amino acid sequence, and it is obtained after purifying from the seeds of

passion fruit, with IC50values of 32, 34, and 40 mg/ml, respectively (Baindara P and Mandal SM, 2022). Leucine, valine, tyrosine, proline, threonine, glycine, aspartic acid, arginine, and lysine are the primary free amino acids extracted from purple passion fruit. Lysine, threonine, leucine, and valine are essential amino acids for growth among them.

2.4.2 Lipids

20% drying oil, 11.5% solid fat acid (palmitic and stearic acids), and 88.5% liquid acid are present in P. edulis seeds (Baindara P et al., 2020). Unsaturated fatty acids are abundant in seed oil, with linoleic acid accounting for the majority (69.3%), followed by oleic acid (14.4%), palmitic acid (10.1%), and stearic acid (2.9%) (Lucarini, 2019). After generating juice, the leftover passion fruit has a crude oil level of 24 percent.

2.5 Minerals make up micronutrients.

Minerals greatly influence the nutritional content of fruit. In comparison to other commercial fruits like oranges, bananas, and papaya, the level of ash of Passiflora fruit juices was relatively high (0.51–1.37%), which further supports its high mineral content. It was discovered that the Passiflora fruit juices contained a sizable amount of K, Ca, Mg, Na, P, and Fe (Ramaiya et al., 2018). The trend of the micronutrient concentration was K > P > Mg > Ca > Na in all of the Passiflora fruit juices examined (Ramaiya et al., 2018). Even in closely related species, the amount of macronutrient content can differ; for instance, P. edulis was shown to have a lower Ca concentration than Na. The factor could be related to environmental issues linked to the impact on the nutritional characteristics of plants. Compared to other fruits, the mineral K is the most prevalent in Passiflora fruit. For P. quadrangularis mesocarp, K levels vary from 283.338.33 mg per 100g to 453.3332.87 mg 100g for P. edulis. Potassium levels in P. edulis (Purple) were somewhat higher than the value (348 mg 100g) provided by the USDA. This crucial mineral, K, maintains proper blood pressure and acid-base balance. 1119.73 mg of potassium, or around the daily estimated recommendation of 4700 mg (23%), are present in Passiflora fruit juice (Ramaiya et al., 2019).

Phosphorus contributes to the body's basic metabolic process and gives bones and teeth their strength and rigidity.

Passiflora fruit juices vary in Mg content from 75.8330.05 to 170.0076.38 mg 100g. It is excellent to have a balanced diet for people. With the aid of Ca, it works as a co-

factor in several enzyme systems and is involved in neurochemical transmission and muscle excitability (Viera W. et al., 2022). The calcium concentration in Passiflora fruit juices ranged from 14.5090.14 mg 100g in P. quadrangularis to 24.9179.49 mg 100g in P. edulis, and calcium is crucial for bone growth and strength.

The range of Na content in juices from Passiflora fruits was 20.8344.09 to 41.6780.47 mg 100g. Passiflora fruits have a higher range of Na concentration than other fruits, such as papaya (8.00 mg 100g) and guava. 3-6% of the daily requirement for Na is provided by a cup of juice from the Passiflora plant. An adequate amount of micronutrients, particularly Fe content, is provided by one serving of 274 g of Passiflora fruit juice nutritionally. Fe, Zn, and Cu were the trending micronutrients in Passiflora fruit juices.

According to Ramaiya et al., 2018 study, it had been found a trace level of Fe in P. edulis (Purple) and other commercial fruits, such as oranges (0.03 mg 100g), bananas (0.27 mg 100g), and pineapple. The fruit of the Passiflora plant is a fantastic source of plant-based or non-heme iron. Red blood cell production heavily depends on iron. It is essential for pregnant women's and young children's diets. The daily requirement of iron for men (19–30 years old) is 8 mg, for women (18 mg), and pregnant women (27 mg).

Even among closely related species that were produced in various geographic locations, the nutrient content differed, and this could be a result of the impact of various environmental factors.

2.6 Phytochemical substances:

2.6.1 Volatile Substances

The aromatic components of passion fruit should be considered volatile; they also have antioxidant properties. It has been discovered that the esters (59.24%), aldehydes (15.27%), ketones (11.70%), and terpenes and other random substances are still present in passion fruit. And GC-MS analysis showed that 2-tridecanone (62.1%), octadecenoic acid (16.6%), 2-pentadecanone (6.2%), hexadecanoic acid (3.2%), 2-tridecanol (2.1%), octadecanoic acid (2.0%), and caryophyllene oxide (2.0%) are the main volatile components of the fruit shell of P. edulis Sims (Oliveira AC et al., 2009). It is significant that during maturation, the volatile components changed (Haiyan Z et al., 2007).

2.6.2 Flavonoids

The fruit's pulp is a well-known source of flavonoids, with 158.0 mg/ml of total flavonoids, 16.2 mg/ml of isoorientin, and 0.42 mg/g of quercetin (Zeraik & Yariwake, 2010). 0.90 percent of apigenin is present in the aerial portions of P. edulis refluxed with 40 percent ethanol. Thirty-three flavonoids have been found in P. edulis's various sections thus far. The main flavonoids from P. edulis that have been discovered are vitexin, isovitexin, isoorientin, apigenin, quercetin, and luteolin, as well as their derivatives. Due to their numerous biological and medicinal properties, these compounds are significant classes of active substances in P. edulis.

2.6.3 Triterpenoids

P. edulis fruits, leaves, stems, and roots have yielded 29 triterpenoids with various chemical structures. For the treatment of neurodegenerative disease, cycloartane triterpenoids have demonstrated significant protective benefits against cell damage brought on by glutamate. At 50 mg/kg, the cyclobutane triterpenoids cyclopassiflosides IX and XI showed antidepressant-like effects (He et al., 2020).

2.6.4 Alkaloids

P. edulis fruits and leaves include alkaloids such as harmidine, harmine, harmane, harmol, N-trans-feruloyltyramine, and cis-N-feruloyltyramine. A fluorescent harmala alkaloid called harmine can antagonize tumor growth by reversibly inhibiting angiogenesis and monoamine oxidase. It also demonstrated the anti-inflammatory effect by significantly blocking the NF-kB signaling pathway (Liu et al., 2009).

2.6.5 Antioxidant Function

In tests using the antioxidants 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid), the ferric reducing ability of plasma, oxygen radical absorbance capacity, and the bleaching of -carotene, P. edulis seed extract has been shown to have high antioxidant activity (Kawakami S et al., 2022). One study (Krishnaiah et al., 2013) isolated several components from P. edulis seeds and examined how the component level and antioxidant activity varied depending on the extraction method. The findings indicated a favorable association between the extracts' polyphenol concentration and antioxidant activity, indicating that polyphenols play a significant role in antioxidant activity. When the antioxidant activity of P. edulis seed extract was compared to that of other Passiflora species, the IC50 values for P. edulis,

P. tripartita, P. ligularis, and P. pinnatistipula were found to be 2.7-132.6, 3.2, 73.9, and 372.2, respectively, as determined by DPPH assay; however, the IC50 values, as revealed by ABTS assays, were 9 (Krishnaiah et al., 2013).

Piceatannol, a significant portion of polyphenols found in P. edulis seeds, has been shown to have antioxidant action. As a result, it is thought to be in charge of the antioxidant properties of extracts obtained from seeds. Ingestion of ethanol extracts from P. edulis peel and seeds had a protective effect. It worked against the heart, liver, and kidneys against stress, mainly oxidation in a rat model of streptozotocin-induced oxidative stress by increasing superoxide dismutase levels and lowering 2-thiobarbituric acid reactive substance levels. Moreover, efforts have been made to microencapsulate the peel and seed extracts of P. edulis to preserve and improve their antioxidant activity in vivo (Krishnaiah et al., 2013). The encapsulated extracts demonstrated that their antioxidant activity persisted at 60% of the pre-digestion level after digestion. According to a different study, antioxidant activity was similarly retained when P. edulis seed extract was capsuled using rice starch. Hence, using microcapsule technology may be a helpful way to deliver extracts to specific body parts where they can exert their effects while preserving their activity.

Processed foods have also benefited from P. edulis seed extract's high antioxidant activity (Fornari Laurindo et al., 2022). In sesame seed oil, which is high in omega-3 fatty acids, lipid oxidation has been studied to be prevented by the addition of an ethanol extract of P. edulis seeds; the inclusion of the extract enhanced the stability against oxidation of the lipids. A high level of antioxidant activity has been observed for the oil derived from P. edulis, which also includes polyphenols and tocopherol.

2.7 Oil extraction from Passion fruit seed:

2.7.1 Extraction procedure

Examples of commercially significant species are passion fruit plantations, whose oils and fats have a variety of uses in the culinary, pharmaceutical, and cosmetic industries. The traditional methods for extracting edible vegetable oils involve mechanical pressing and/or extraction methods based on liquid solvents. Mechanical pressing has some drawbacks, but the primary ones have to do with potential bioactive component breakdown or shortened shelf life (due to exposure to oxygen and light, and low

extraction rates). It should be remembered that several factors, including the extraction method, the raw material utilized, the storage conditions, and pretreatments, which may set off chemical reactions that may degrade some of these bioactive compounds, might affect bioactive compounds throughout recovery methods. Solvent use typically entails high temperatures, extended periods of time, significant energy expenditure, as well as high toxicity, environmental risk, little selectivity, and potential loss of volatile molecules. N-hexane, which has outstanding solvent qualities in terms of oil recovery and solubility, is the most popular solvent utilized for oil extraction. N-hexane, however, has been identified as an air contaminant that can interact with fruits of the Passiflora plant.

Modern extraction, processing, and preservation methodologies designed to recover nutraceuticals and reduce environmental impact, as well as the use of relatively green solvents (that fall under the green chemistry category) like acetone and ethanol, have attracted a lot of attention (Liu S et al., 2008). According to one study (Liu S et al., 2008) solvent processing, microwave, ultrasonication, supercritical fluid, enzymatic processing, alkaline processing, subcritical fluid, ionic liquid, accelerated solvent, microemulsion, and others are the main emerging green and sustainable separation approaches towards bio-economy and circular economy contexts. Recent applications of green approaches to passion fruit oil are reported here. Examples of methods that have been utilized to extract this oil include cold pressing (Ferreira et al., 2011) and Soxhlet with various solvents (Oliveira et al., 2013), both of which had comparable yields. According to Oliveira et al., 2013, it had been specifically investigated the extraction of passion fruit oil with green solvents—acetone, ethanol, and isopropanol—using ultrasound, shaker, and Soxhlet techniques, in comparison with n-hexane; the authors came to the conclusion that the ultrasound technique can replace the traditional ones. Moreover, Nyanzi S et al., 2005 assessed the viability of employing green solvents for the ultrasound-assisted extraction of passion fruit oil. Fatty acids, total phenols, and antioxidant capabilities were all observed. In the end, they demonstrated that due to acetone's higher oil recovery, identical fatty acid profile, and oil physicochemical qualities with higher antioxidant activity, it could be a good n-hexane replacement overall. Omega-9 MUFA concentrations were greater in oil extracted with ethanol as well.

In this regard, Perrier A et al., 2017's recent research into the evaluation of subcritical propane, ultrasound-assisted, and Soxhlet extraction of oil from seeds of sweet passion fruit revealed that subcritical propane produced a higher extraction yield (23.68%) at 60°C with an extraction efficiency of 84% in comparison to Soxhlet extraction using n-hexane. Furthermore, the scientists observed that increased tocopherol levels by Soxhlet extraction utilizing n-hexane and subcritical propane at 60 °C as well as substantial quantities of unsaturated fatty acids were achieved (Perrier A et al., 2017). Consequently, the scientists came to the conclusion that a promising method for generating free-solvent products is the extraction of plant source materials using compressed propane as solvent. The use of unconventional extraction techniques, such as compressed propane, a green recovery technology that is still underdeveloped for seed oil extraction, represents an opportunity to add value for this agroindustrial waste, according to subsequent research by the same authors (Perrier A et al., 2017), which focused on yellow passion fruits.

2.7.2 Fatty acid composition analysis of seed oil

Passion fruit seeds have garnered interest in recent years for the manufacture of oil, mostly because of the presence of beneficial chemicals (Giuffré et al., 2007). The chemical makeup of oils extracted from the seeds of various types of passion fruit has been described in several research (Ferrari et al., 2004). According to research, passion fruit seeds contain about 30% oil, with the main fatty acids being linoleic, oleic, and palmitic acids (Malacrida et al., 2012). Linoleic acid makes up roughly 72–73% of the oil, followed by oleic acid (13–16%) and palmitic acid (8%–9%). (Liu et al., 2009). 85% of the fat in passion fruit seed oil is unsaturated, with 69% of it being linoleic acid and 18% being oleic acid (Ferrari et al., 2004). According to Giuffré et al., 2007, linoleic acid C18:3 (73.4%) had the highest ratio, followed by oleic acid C18:1 (13.7%), palmitic acid C16:0 (8.8%), and stearic acid C18:0 (2.4%).

Malacrida et al., 2012 compared the total ratio of saturated to unsaturated fatty acids to the corresponding figures published for popular oils including soybean, corn, and peanut by Borges et al., (2007). They discovered that the profile of the passion fruit seed oil (1/7.06) was similar to that of corn oil (1/6.70). According to Ferreira et al., (2011), Passiflora edulis Sims var. edulis seed oil may serve as a source of essential fatty acids (EFA) and aid in the prevention of cardiovascular ailments such coronary

heart disease, atherosclerosis, and high blood pressure. For instance, Nyanzi et al., (2005) investigated the fatty acid makeup of seed oils from many species of passion fruit, including the yellow fruit, Passiflora edulis Sims var. flavicarpa, and the purple fruit, Passiflora edulis Sims var. edulis.

2.7.3 Passion fruit seed oil's health advantages:

Examples of commercially viable species are the crops of passion fruit, whose lipids and oils are helpful in the food, cosmetics, and pharmaceutical industries (Massa et al., 2021). The seed oil is important for several industrial uses and has characteristics in common with food oils like soybean oil. This product can be used to increase the supply of vegetable oils, which are highly abundant in bioactive ingredients that can be employed in meals and cosmetics (Cesar et al., 2022). The oil which is extracted from the seeds of the passion fruit has been shown by Deus et al., 2015 to contain volatile chemicals that could be exploited as fragrances of industrial interest and produce natural essences with high added value. Moreover, oil may be a source of bio-based substances like biodiesel. Additional researchers demonstrated that the biodiesel mixture made from castor oil and passion fruit boosted thermal stability (Iha et al., 2018). Piceatannol and resveratrol were shown to be significantly present in extracts made from the passion fruit seeds from Madeira Island using the ultrasonic method, according to (Krambeck et al., 2019). Compared to commercial oil, the extracts demonstrated antioxidant and antiaging characteristics, indicating significant promise in the pharmaceutical and cosmetic industries (Jain et al., 2021). In a different study, Krambeck et al., 2020 confirmed that passion fruit seed oil might be used as a liquid lipid to create nanoparticles, with remarkable acceptance for skin application without producing irritation. In order to alter their application, these nanoparticles must be put into semi-solid formulations, as this study also showed their instability. The depigmenting properties of passion fruit seed oil have been demonstrated by Krambeck et al., 2020 for usage in the cosmetics sector.

Chapter 3: Materials and 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 Applied Food Science and Nutrition, Department of Physiology, Biochemistry and Pharmacology, Department of Animal Science and Nutrition, and also in Bangladesh Reference Institute for Chemical Measurements (BRiCM) laboratory.

3.2 Samples Accumulation:

Passion fruit (Passiflora edulis) samples were gathered in the Chattogram, Cox's Bazar, and Kolatoli areas. In order to receive the fruits in the best condition possible, special care was taken during collection. Further necessary components for the experiment were acquired from the laboratory's inventory.

3.3 Study design:

The study had been designed by comparing the nutritional, physicochemical and phytochemical components of passion fruit seed, pulp and peel. After comparing, the seeds was considered the great source of oil and further physicochemical properties of oil was analyzed following the fatty acid composition.

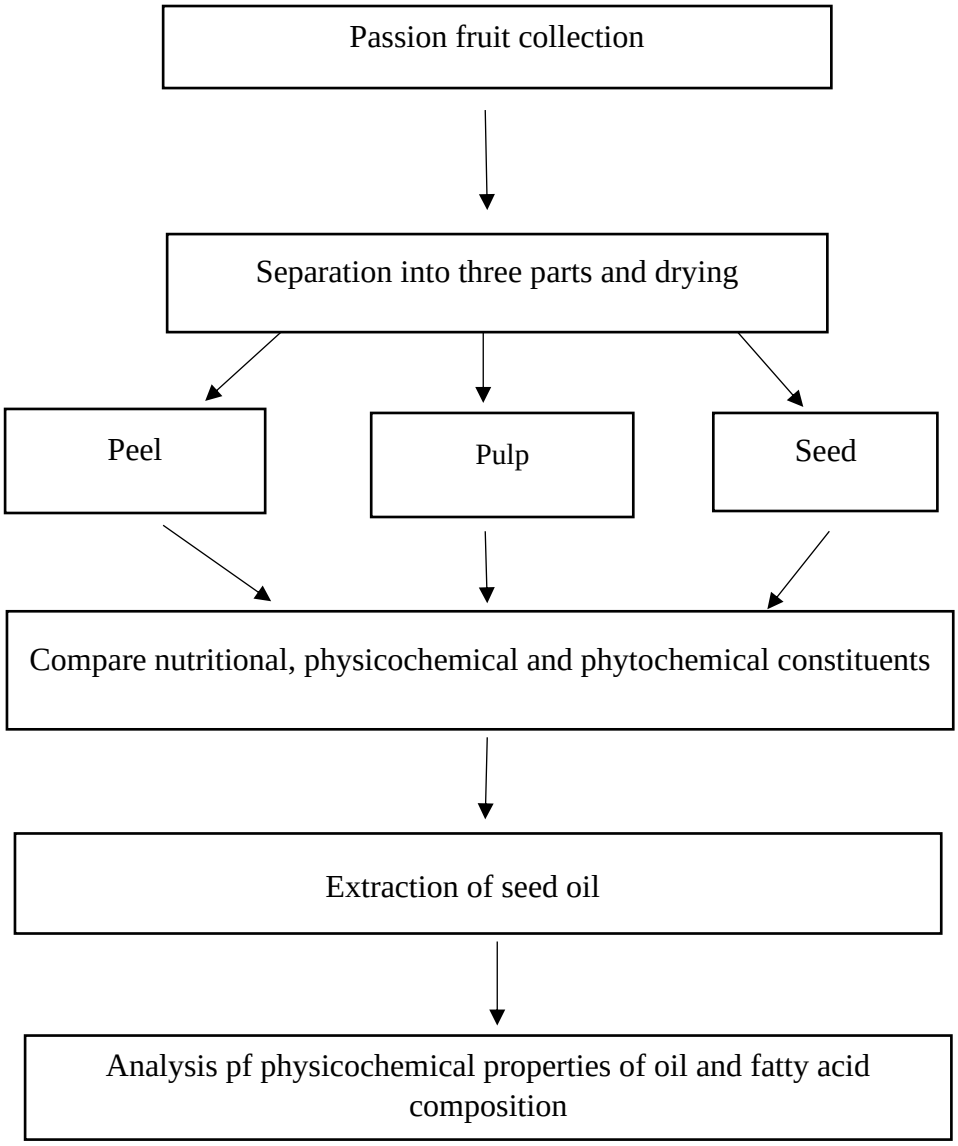


Figure 3.3: Study design of the study

3.4 Analysis of the physicochemical composition of passion fruit

To determine certain qualities, including pH, Total Soluble Solid (TSS), and Total Titratable Acidity (TTA), fresh samples of passion fruit were gathered. Furthermore, proximate analysis, bioactive compound analysis, and antioxidant analysis proceeded against reagents on these samples. Here, the physicochemical characteristics of the peel, pulp, and seeds were compared.

3.4.1. Calculation of pH

The pH of the individual samples was evaluated using a pH meter that had already been calibrated. The pH meter was calibrated before using buffers which have pH values of 4, 7, and 10. Five grams of material were extracted using 50 mL of distilled water and then filtered using Whatman No. 2 filter paper. The pH of the sample aqueous filtrate was then measured using a pH meter. The instrument was immersed in the solution in suspension, and the readings were gathered.

3.4.2. Total Soluble Solids Calculation

Using the same extract, the sample's Total Soluble Solids (TSS) content was determined using a hand refractometer. Total soluble solids (TSS) were measured directly using an Atego RX 1000 digital refractometer, and the findings were reported as percent soluble solids ("Brix") in accordance with ISO.

3.4.3 Determine total titratable acidity

According to AOAC guidelines, the percentage of acidity was determined using anhydrous citric acid by titrating against the basic solution of 0.1N NaOH and utilizing the 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. Then, 0.1N NaOH was used to titrate 10ml of diluted filtrate using phenolphthalein as the indicator. The titration's endpoint was signaled by the appearance of a pink tint. After the titration was reported three times, the average result was recorded.

3.5 Proximate analysis conclusion

The proximate components of the sample (passion fruit seed, peel, and pulp) were evaluated in accordance with AOAC standard technique. 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.

3.5.1 Calculation of moisture

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

Principle: Food staffs usually contain 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:

Moisture %= Initial weight-final weight /Sample weight × 100

3.5.2 Determining the amount of ash

The total ash content was ascertained using the AOAC method 14.006 (2005).

The mineral components are all mixed together 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:

Ash % of Sample=The amount of ash in the supplied sample/ Sample weight× 100

3.5.3 Determination of proteins

The protein content of whole passion fruit 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 H_2SO_4 in the presence of the digestion mixture ($CuSO_4$ and K_2SO_4 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 (.1N) HCI 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 necessary:

- Sulfuric acid in concentrated form

- Digestion blend
- Solution of boric acid
- Solution of alkali.
- mixture of indicators
- HCI standard: 0.1 N

Calculation: Calculated nitrogen and protein percentages are as follows:

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

3.5.4 Calculation of crude 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 (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 Soxhlet device

Calculation: The crude fat percentage was given as follows:

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

3.5.5 Determination of Crude Fiber

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

Basic principle: Cellulose, hemicellulose, and lignin make up the majority of the water-insoluble portion of carbohydrates known as crude fiber. By boiling a known amount of fat-free food sample in weak acid solution (1.25% H_2SO_4) for 30 minutes, followed by weak alkali solution (1.25% NaOH) for 30 minutes at constant volume, and then subtracting ash from the residue obtained, it can be estimated through digestion.

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.

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

3.5.6 Calculation of the total amount of Dry matter

Moisture content 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:

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

3.6 Analysis of minerals:

Biochemical analysis was used to determine the mineral content (Humalyzer 3000). For the biochemical assay, a commercially available biochemical kit (Randox®) was employed.

3.6.1 Sample preparation

Five grams of passion fruit (peel, pulp, and seed) were heated to 550 °C in a muffle furnace for 1 hour, allowed to cool, moistened with a little de-mineralized water, and then heated again for a short time before cooling. It was filtered using filter paper, transferred to a volumetric flask (100 ml), and built up to the required level for the analysis of the mineral contents. The peel, pulp, and seeds of a passion fruit sample were prepared according to the prescribed protocols.

3.6.2 Potassium (K+) measurement

Principle: A fine turbidity of potassium tetraphenylboron is created when potassium and sodium tetraphenylboron combine. The amount of turbidity is inversely related to the amount of potassium present in the sample.

Analysis/Assay:

- Wavelength/Filter: 630 nm (Hg 623)/Green for the test
- Room-temperature
- 1 cm light path

Calculations:

$$\text{Potassium (mg/dl)} = \frac{(A)\text{sample}}{(A)\text{standard}} \text{ x Standard conc. (mg/dl)}$$

3.6.3 Calcium (Ca) determination:

Colorimetry technique: (Without deproteinization) O-cresolphthalein complexone

Analysis/Assay:

- Hg 578 nm wavelength/filter (550-590) 570nm
- 20-25°C/37°C is the temperature.
- 1 cm light path

Calculations: Concentration in mg/dl= $\frac{(A)\text{sample}}{(A)\text{standard}}$ × Standard conc. (mg/dl)

3.6.4 Magnesium Determination

Principle: The approach is based on the specific binding of magnesium and calmagite, a metallochromic indicator, at alkaline pH, which causes a shift in the complex's absorption wavelength. The intensity of the cromophore generated is related to the concentration of magnesium in the sample.

Analysis/Assay:

- Wavelength/filter: 520 nm, Hg 546 nm, 500-550 nm, for the assay (Increase of absorbance) Hg 623 nm, 628 nm, and 570–650 nm (Decrease of absorbance)
- 20 to 25°C or 37°C
- 1 cm light path Testing against a reagent blank

Calculation:

$$\text{Magnesium (mg/dl)-=} \frac{(A)\text{sample}}{(A)\text{standard}} \text{ x Standard conc. (mg/dl)}$$

3.6.5 Phosphorous determination

Analysis/Assay:

- Wavelength/filter: 340 nm, Hg 334 nm, and Hg 365 nm for the test
- Temperature:20-25°C/37°C
- 1 cm light path testing against a reagent blank

Calculation:

$$\text{Phosphorus concentration (mg/dl)} = \frac{(A)\text{sample}}{(A)\ \text{Standard}} * \text{Standard conc. (mg/dl)}$$

3.6.6 Iodine determination

Principle: Mercuric thiocyanate is converted into thiocyanate when chloride ions mix with free mercuric ions. A reddish-brown ferric thiocyanate complex is created when the released thiocyanate mixes with the ferric ions. The amount of chloride in the sample directly correlates with how intense the color is.

Analysis/Assay:

- Wavelength/filter: 505 nm (Hg 546) /Green
- Room-temperature
- Lightpath: 1 cm

Calculaion:

$$\text{Iodine in mmol/L} = \frac{(A)\text{sample}}{(A)\text{standard}} \times \text{Standard conc. (mg/dl)}$$

3.7 Vitamin Identification

The water-soluble substance ascorbic acid is essential for life. Vitamin C is required for the cellular chemistry that generates energy, aids in sperm formation, and forms the collagen protein, which is necessary for the growth and health of blood vessels, cartilage, joints, and other connective tissues. As a powerful antioxidant, vitamin C helps guard against stress, cancer, and heart disease. The vitamin content of passion fruits, plays a role in human. Since vitamin C is highly heat-sensitive, it can be said that heating the samples for long time had an impact on their vitamin C concentration.

3.7.1 Calculation of vitamin C levels

Health experts agree that vitamin C is essential, yet heat and air during food preparation, packaging, and storage swiftly diminish or destroy it. The 2, 6-dichloroindophenol titrimetric technique is advised for use in determining 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 material. Consequently, the termination point of the reaction may be determined easily. Rapid excretion and filtering are preferable because excess 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.

Reagent necessary

Metaphosphoric acid solution (3%), dye solution (260 mg of dye (2,6-dichlorophenol indophenols) and 210 mg of $NaHCO_3$ 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. There were at least three readings taken. The ascorbic acid solution was treated using the same process but with an unknown concentration. Mg%, or milligram percentage, was used to represent the outcome.

3.8 Determination of phytochemical content

Making of an Extract by adding 10 ml of 100% ethanol to 1 g of material in a Felcon 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.

3.8.1 Measurement of polyphenols (TPC)

Procedure

With a few modest modifications, the Folin-Ciocalteu (FC) reagent procedure described for detecting TPC was applied to determine the TPC of the extracts (Al-Owaisi et al., 2010). One milliliter of ethanoic extract was combined with 1.5 milliliters of FC reagent in a falconer tube, which was then left at room temperature for three minutes. After adding 1.5 ccs of 7.5% Na_2CO_3, the mixture was permitted 60 minutes to settle. The absorbance was determined at a wavelength of 765 nm using a UV-VIS Spectrophotometer with C_2H_5OH as the blank. Calculations show that for every gram of extracts, TPC is equivalent to mg of gallic acid equivalents (GAE).

3.8.2 Measurement of flavonoids (TFC)

The total flavonoid content of the samples was determined by adopting the aluminum chloride colorimetric method reported by Chang et al. (2002). Using 15 ml of 95 percent C_2H_5OH in a cuvette, prepared extract stock solution (1 mg/ml) was diluted in aliquots of 0.5 ml. Thereafter, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 mol/L potassium acetate, and 2.8 ml of distilled water were added to the liquid in the cuvette. The combination was left at room temperature for 30 minutes. The blank was made out of an equivalent volume of distilled water with 10% aluminum chloride. In order to quantify the absorbance, a UV-visible spectrophotometer was used at a wavelength of 415 mm (UV-2600, Shimadzu Corporation, USA). The total amount of flavonoid present in the sample was calculated by comparing the absorbance of the sample extracts to a standard quercetin curve. A measure of the anticipated total flavonoids is the number of quercetin equivalents (QE) per gram of extract (mg QE/g) (TFC).

3.8.3 Antioxidant capacity measurement using the DPPH scavenging technique

Procedure

The antioxidant mobility of the extracts was evaluated using the DPPH test, with a few minor modifications. Around 6 mg of DPPH was dissolved in 100 ml of 100% methanol to create a methanoic DPPH solution. Afterwards, 1 ml of methanoic extract was combined with 2 ml of DPPH solution. The mixture was then given a gentle shake and left to stand at room temperature in the dark for 30 minutes. A UV-VIS spectrophotometer was used to measure the absorbance at 517 mm wavelength (UV-2600, Shimadnu Corporation, USA). In the control, which was created by mixing 1 mL of methanol with 2 mL of DPPH solution, methanol served as a blank. In comparison to the DPPH standard solution, the samples' decreased absorbance served as a surrogate for the scavenging mobility. The antioxidant capacity of the extracts was assessed based on their potential to scavenge DPPH free radicals. TEAC composite (Trolox equivalent antioxidant mobility), which was also used as the standard, was used to create the standard calibration curve. The results were represented as mg/100 g of Trolox equivalents per gram of powder on a dry weight (DW) basis.

3.9 Essential Oil Extraction

The soxhlet extraction method recommended by AOAC was used (AOAC, 2005). A cotton-coated, porous thimble that had been weighted with 10g of solids was placed in the center chamber of the Soxhlet apparatus. A 250 mL clean, oven-dried, round-bottomed flask was fitted with the Soxhlet siphon and condenser, and 80 mL of diethyl ether (40-60°C) was put into it. Following that, the flask was permitted to reflux for three hours. The heating flow rate was kept low enough during refluxing to prevent the solvent from escaping from the condenser's top. Once the solvent had vanished, the remaining oil was retrieved and weighed (Nazir et al., 2017).

3.10 Making a decision of oil Physicochemical Properties

Three replications were used to assess the physical properties of the extracted essential oil, such as its solubility in alcohol and refractive index, density, and optical rotation (de Araujo et al., 2020). Once more, three replications were utilized to determine the chemical composition of the extracted oil using the prescribed standard protocols,

including the acid value, free acid value, saponification value, peroxide value, iodine value after acetylation, and phenol content.

3.10.1 Calculation of Acid Value

The amount of potassium hydroxide (KOH) needed to neutralize the free fatty acids in one gram of oil is known as the acid value. The acid value serves as a proxy for the amount of FFA present in the oil. Free fatty acids are created when the oil is hydrolyzed. Using the standard procedure for oils and fats described in AOAC, the acid value of oil samples was assessed (2016).

Reagents: Phenolphthalein indicator: 1% solution in 95% (v/v) ethanol, 0.1 N aqueous NaOH solution, and ethanol (95%).

A 250 mL conical flask was filled with 25 mL of ethyl alcohol before being filled with 0.5 mL of phenolphthalein indicator solution. The mixture boiled for around five minutes. The mixture was then boiled once more after 1.5 mL of the sample was added to a flask. A total of 0.5 ml of the phenolphthalein indicator was combined. After that, titration was done using 0.1 N alkali solutions. Wait 30 seconds while titrating with 0.1 N KOH for a faint pink hue to occur.

The following equation was used to determine the acid value:

$$\text{Acid Value}=\frac{\text{N of alkali (0.1) x ml of alkali x 56.1}}{\text{wt of sample}}$$

3.10.2 Peroxide Value Calculation

According to the peroxide value, each kilogram of fat or oil included a certain amount of peroxide oxygen. Calculating the peroxide value was done using the AOAC Official Procedure (PV).

Reactions in peroxide value:

$$H\text{-}O\text{-}O\text{-}H+H_2O+I\,(KI)=H\text{-}O\text{-}H+20H+I_2$$

$$I_2+S_2O_3^{2-}=S_4O_6^{2}+2I^{-}$$

Reagents: Acetic acid (glacial), chloroform, 15% potassium iodide solution, 0.1 N sodium thiosulphate solution, 1% starch solution, and potassium dichromate are the chemicals used as reagents (0.01 N)

Procedure: Peroxide value, or PV, is a very sensitive biomarker of early-stage fat and oil oxidative deterioration.

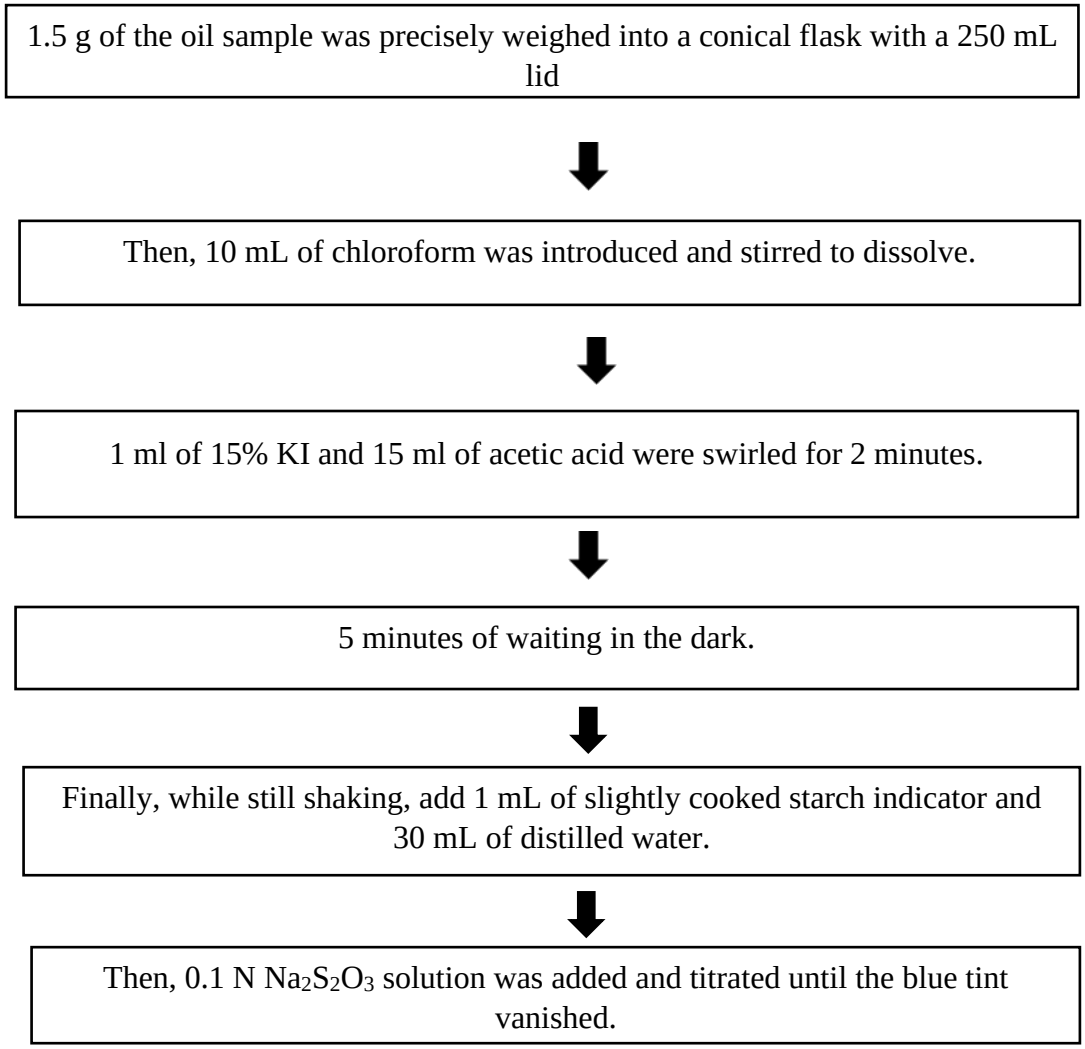


Figure 3.10.2: Peroxide value determination

The equation below was used to compute the peroxide value:

$$\text{Peroxide Value(meq/kg)} = \frac{\text{Nx(a-b)x1000}}{\text{Wt of sample}}$$

Here,

N = the $Na_2S_2O_3$ normality,

B = the amount of $Na_2S_2O_3$ eaten by the sample (ml), and

a = the amount consumed by the blank (ml).

3.10.3 Calculation of Iodine value

It is determined in grams of iodine how much iodine 100 grams of oil is absorbed. The iodine value, which is measured in centigrams of absorbed iodine per gram of sample, represents how unsaturated the oil is. The iodine value was determined using the AOAC's Standard Method (2016).

Reagents: 1% Starch solution, glacial acetic acid, pure bromine, chloroform, 15% KI solution, and 0.1N $Na_2S_2O_3$ solutions.

Procedure:

1.5g of passion fruit seed oil was introduced to a conical well-stopper flux that holds 250 ml.

↓

The oil was dissolved using 25 mL of the Hanus solution and 10 mL of chloroform.

↓

The mixture was then allowed to settle for 30 minutes in a sealed area while intermittently shaking.

↓

Following this period of time, the mixture produced by 10mL of KI (15%) solution and 100mL of distilled water.

↓

1 ml of a little hot starch ($C_6H_{10}O_5$-H_2O) indicator was added.

↓

Standard 0.1N $Na_2S_2O_3$ solutions were used to tritrate the solution until its dark color practically vanished.

↓

The blank procedures used for the test sample were used for the blank determination, but no oil was used.

Figure 3.10.3: Process flow for determining the iodine value.

Using the following formula, the value of iodine was determined:

$$\text{Iodine value: } \frac{(a-b)xN \text{ x}1.267 \text{ x}100}{\text{Wt of sample (g)}}$$

In this case, 'N' is the $Na_2S_2O_3$'s normality, 'b' is the amount of $Na_2S_2O_3$ that the sample consumed (in milliliters), and 'a' is the amount of $Na_2S_2O_3$ that the blank consumed (ml).

3.10.4 Specific gravity determination:

Pycnometer standardization: A cleaning solution containing chromic acid was poured into the pycnometer and allowed to sit for a number of hours.

It was recently boiled, allowed to cool to around 20°C, and then placed in a water bath with a steady 30°C temperature. The item was removed, cleaned off using a towel, and weighed after 30 minutes of soaking.

Procedure: Before using the prepared oil sample in the pycnometer, the side arm's cap was removed. The instrument was then filled without any air bubbles being trapped. The container was shut, and it was left to sit for 30 minutes in a water bath that had been heated to 30°C.

Properly cleaned the capillary hole to get rid of any possible oil leaks.

Removed the bottle from the water, gave it a thorough rinse, and dried it entirely. Remove the side arm's cap as soon as the temperature drops below 30 °C and weigh it right away.

Specific Gravity, C = $\frac{A-B}{C-B}$

Where,

A = wt. in g of specific gravity bottle at 30°C (filled with oil)

B = wt. in g of specific gravity bottle at 30°C

C = wt. in g of specific gravity bottle at 30°C (filled with water)

3.11 GC-FID analysis of the fatty acid composition

Conditions for the Instruments and the Method:

For this application, a Shimadzu NexisTM GC-2030 with a split/splitless injector port, a flame ionization detector (FID), and an autosampler were used. Because hydrogen is combustible, a hydrogen sensor was mounted on the GC as a precaution. The machine also has Shimadzu's innovative automatic Gas Selector, which enables method development without the requirement for human gas replumbing. In-line filters were used to pass through all gases. The 37-component FAME mixture was initially examined using the helium carrier gas-required AOAC method 996.06. The accompanying table includes the GC parameters for this analysis as well as the modified fast technique using a hydrogen carrier gas.

Table 3.11: Methods and conditions for the AOAC method 996.06 and Shimadzu's faster method using hydrogen carrier gas, at an increased linear velocity, with a modified oven temperature program.

	Original AOAC Method 996.06	Shimadzu Nexis GC-2030 Fast Hydrogen Method
Inlet	1 µL Split Injection; 225 °C; Split Ratio 200:1	
Column	Rt-2560 100 m × 0.25 mm ID × 0.20 µm film thickness	
Carrier	Helium; Constant Linear Velocity 18 cm/s	Hydrogen; Constant Linear Velocity 35 cm/s
Oven	100°C (4 min hold); 3°C/min to 240 °C (15 min hold)	150°C (2 min hold); 4°C/min to 220 °C; 2°C/min to 240°C (8 min hold)
FID	285°C; H_2 32 mL/min; Air 200 mL/min; Make-up (N_2) 24 mL/min	

3.12Statistical analysis

Statistical analyses were carried out using Minitab statistical package (version 21). The results of the experiment are presented as mean ± SD of measurements. One way analysis of variance (ANOVA) was done and their statistical significance ($p<0.05$) was carried out by Tukey's pairwise comparison analysis.

Chapter 4: Results

The results of physicochemical and nutritional analysis of the Passion fruit of Bangladesh, achieved by the different tests are represented below through graphs and tables:

4.1 Physicochemical properties analysis:

Different samples of passion fruit peel, pulp and seed were subjected to analyses the physicochemical properties. Table showing the properties according to p^h, titrable acidity and TSS. In the table, overall mean differences of values for various parameters of the samples, one way ANOVA (Analysis of variance) test was conducted. The findings indicated that there was a significant mean difference in the values of peel, pulp and seed.

Table 4.1: Physicochemical properties analysis test results for passion fruit different parts.

Parameters	**Passion fruit peel**	**Passion fruit pulp**	**Passion fruit seed**
p^H	5.20 ± 0.100^a	3.433 ± 0.1528^b	3.000 ± 0.100^c
TSS (^{0}Brix)	1.290 ± 0.216^c	2.500 ± 0.100^a	2.210 ± 0.105^b
Titrable acidity (% citric acid)	5.293 ± 0.178^a	3.667 ± 0.152^c	4.133 ± 0.090^b

Legends: Statistics are significant ($P<0.05$) when the mean, standard deviation, and values are in the same row and have the same superscript.

From the table, it had been cleared that there were significant differences among the values of physicochemical properties.

4.2 Proximate composition:

Here, the three samples including passion fruit peel, pulp and seeds were analyzed to know the proximate composition keeping them respectively sample 1, 2 and 3. Among the three samples, dry matter is highest in sample 2 and it was 88.863±0.229 and lowest

was 14.407±0.467. For moisture content, the high value was 85.557±0.309 (sample 1) and the low value was 18.503±0.349 (sample-3). For crude fiber content, highest value was 53.200±0.195 in sample-3 and lowest was 33.453±0.268. For ash content, highest value was 8.397±0.266 in sample-1 and lowest value was 3.770±0.225 in sample-2. For fat content, the highest value was 17.623±0.287 in sample-3 and lowest value was 0.770±0.154 in sample-1. For protein content, highest value was 9.940±0.078 in sample-3 and lowest value was 8.547±0.234 in sample-1. The results had been obtained form one way ANOVA analysis and there is significant difference in the dry matter, moisture, crude fiber, ash, fata and protein content.

Table 4.2: Test results for proximate obtained from the passion fruit peel, pulp and seed samples

Sample name	**Peel (Sample-1)**	**Pulp (Sample-2)**	**Seed (Sample-3)**
Dry matter (%)	14.407 ± 0.467^{c}	88.863 ± 0.229^{a}	81.427 ± 0.476^{b}
Moisture (%)	85.557 ± 0.309^{a}	11.013 ± 0.090^{c}	18.503 ± 0.349^{b}
Crude fiber (CF) (%)	33.453 ± 0.268^{a}	34.583 ± 0.402^{b}	53.200 ± 0.195^{a}
Ash (%)	8.397 ± 0.266^{a}	3.770 ± 0.225^{b}	1.580 ± 0.310^{c}
Ether Extract/Fat (%)	0.770 ± 0.154^{c}	6.073 ± 0.125^{b}	17.623 ± 0.287^{a}
Crude Protein (CP) (%)	8.547 ± 0.234^{c}	9.20 ± 0.100^{b}	9.940 ± 0.078^{a}

Legends: Statistics are significant ($P<0.05$) when the mean, standard deviation, and values are in the same row and have the same superscript.

4.3 Determination of minerals:

The minerals content of the passion fruit had been analyzed by AAS in the laboratory. Results of the passion fruit peel, pulp and seed samples are shown in the below table.

Table 4.3: Test results for mineral obtained from the passion fruit peel, pulp and seed samples.

Minerals	**Peel (Sample-1)**	**Pulp (Sample-2)**	**Seed (Sample-3)**
Calcium (mg/dl)	2.833 ± 0.0160^{a}	3.193 ± 0.060^{a}	1.833 ± 0.208^{b}
Magnesium (mg/dl)	3.633 ± 0.125^{a}	2.646 ± 0.100^{c}	3.203 ± 0.090^{b}
Phosphorus (mg/dl)	2.770 ± 0.226^{a}	2.300 ± 0.100^{b}	2.500 ± 0.100^{b}
Iodine (µl/dl)	94.33 ± 6.03^{b}	150.47 ± 9.85^{a}	100.00 ± 10^{b}
Potassium (mmol/dl)	10.200 ± 0.300^{a}	3.900±0.100c	8.800 ± 0.100^{b}

Legends: Statistics are significant (P<0.05) when the mean, standard deviation, and values are in the same row and have the same superscript.

Among the analyzed micronutrients, Iodine was the most abundant mineral with 150.47 µl/dl in sample 2. Other minerals like Calcium, magnesium, phosphorus, potassium were also present and there was present significant differences among them (p<0.05)

4.4 Vitamin C determination:

The below table shows the results obtained from the passion fruit juice, peel and pulp.

Table 4.4: Test results for vitamin C determination

Parameter	**Peel (sample-1)**	**Pulp (sample-2)**	**Seed (sample-3)**
Vitamin C	1.300 ± 0.105^{c}	3.33 ± 0.152^{b}	3.70 ± 0.100^{a}

Legends: Statistics are significant (P<0.05) when the mean, standard deviation, and values are in the same row and have the same superscript.

The value of passion fruit peel, pulp and seed are significantly different as the p value is less than 0.05.

4.5 Bioactive compound determination:

Bioactive components and antioxidant compounds were analyzed by applying UV-visible spectrophotometer. Results are shown in the below table:

Table 4.5: Bioactive and antioxidant compounds analysis test results

Parameter	Peel (Sample-1)	Pulp (Sample-2)	Seed (Sample-3)
Total flavonoids content (TFC) (mg QE/100g)	48.640 ± 0.212^{c}	150.39 ± 0.607^{a}	89.264 ± 0.955^{b}
Total polyphenol content (TPC) (mg QE/100g)	2.059 ± 0.022^{b}	4.589 ± 0.014^{a}	4.650 ± 0.149^{a}
Antioxidant capacity (DPPH) (%inhibition)	2.748 ± 0.004^{c}	2.449 ± 0.005^{a}	3.432 ± 0.102^{b}

Legends: Statistics are significant ($P<0.05$) when the mean, standard deviation, and values are in the same row and have the same superscript.

There is significant differences among the samples. For flavonoid content, the highest value is 150.39 in sample 2 and the lowest value is 48.640 in sample 1. The polyphenol content is ranged from 2.059 to 4.650. It's the range from lower to higher. The antioxidant content is higher in sample 3 and it is 3.432 and in sample 2, the value is lower and it is 2.748. All the value shares a significant variance among them.

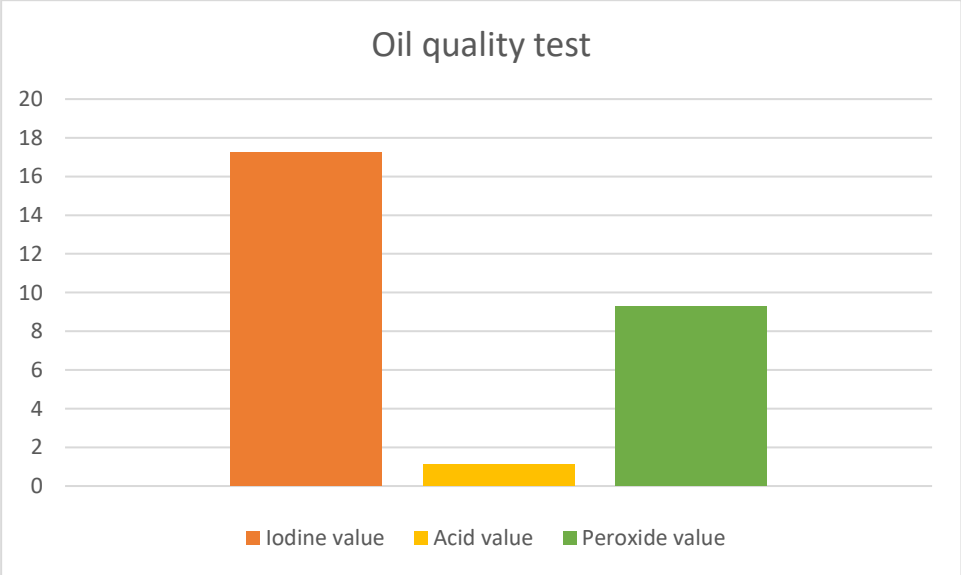
4.6 Physicochemical properties of oil:

After the analysis of proximate composition, vitamin, mineral and bioactive compounds, the seed was found to have the most significant oil and the quality was good. The oil was extracted from the seed and then the quality was checked by applying iodine value, acid value and peroxide value. As the quality was so good of the oil, then fatty acid composition was analyzed to check the edible properties of the oil. The iodine value, acid value and peroxide value are shown in the below table:

Table 4.6: Test results of Passion fruit seed oil iodine value, acid value and peroxide value including specific gravity

Chemical properties	Passion fruit seed oil
Iodine value	17.247±0.362
Acid value	1.117±0.016
Peroxide value	9.267±0.252
Specific gravity	0.971±0.005

This table provides information of passion fruit seed oil. The value of this quality test indicates the oil functional properties. The related graph of iodine value, acid value and peroxide value are given below in graphs:



Here the mean is shown for iodine value, which was done in three days. Same procedure followed for acid value and peroxide value.

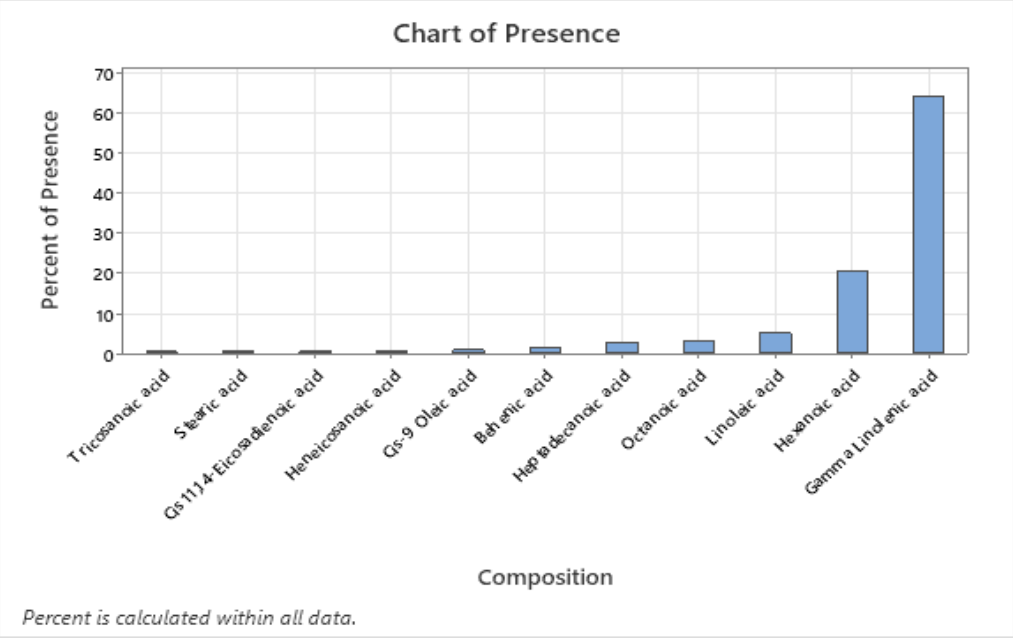
4.7 Fatty acid composition analysis:

There are so many fatty acid presents in passion fruit seed oil which was analyzed by using GC-FID. After the anlysis, the composition of fatty acid were shown in the below table:

Table 4.7: Test results of fatty acid composition of passion fruit seed oil

Composition	Presence
1) Hexanoic acid	3.33%
2) Octanoic acid	0.47%
3) Heptadecanoic acid	0.43%
4) Stearic acid	0.09%
5) Cis-9 Oleic acid	0.15%
6) Linoleic acid	0.83%
7) Gamma Linolenic acid	10.37%
8) Heneicosanoic acid	0.10%
9) Cis 11,14-Eicosadienoic acid	0.09%
10) Behenic acid	0.26%
11) Tricosanoic acid	0.07%

The table shows the fatty acid constituents. These fatty acids make the passion fruit seed oil edible and make the oil quality so good. The content is shown in the below graph:



Chapter 5: Discussion

The goal of the study was to evaluate the nutritional content and bioactive substances present in different parts of passion fruit.

5.1 Physicochemical Characteristics of different parts of passion fruit

The results from table 4.1 represent the pH, total soluble solids (TSS), and titrable acidity of passion fruit peel, pulp, and seed. The pH of the passion fruit peel was found to be 5.20 ± 0.100^{a}, while the pH of the pulp and seed were 3.433 ± 0.1528^{b} and 3.000 ± 0.100^{c}, respectively. The letters (a, b, and c) represent statistically significant differences between the samples. Based on this data, the passion fruit peel has the highest pH, while the passion fruit pulp and seed have lower pH values.

The TSS (measured in Brix) of the passion fruit peel was found to be 1.290 ± 0.216^{c}, while the TSS of the pulp and seed were 2.500 ± 0.100^{a} and 2.210 ± 0.105^{b}, respectively. Based on this data, the passion fruit pulp has the highest TSS, while the passion fruit peel has the lowest TSS. The titrable acidity (% ascorbic acid) of the passion fruit peel was found to be 5.293 ± 0.178^{a}, while the titrable acidity of the pulp and seed were 3.667 ± 0.152^{c} and 4.133 ± 0.090^{b}, respectively. Based on this data, the passion fruit peel has the highest titrable acidity, while the passion fruit pulp has the lowest titrable acidity.

Overall, these results suggest that the passion fruit peel, pulp, and seed have different pH, TSS, and titrable acidity levels, which may influence their sensory properties, nutritional content, and potential uses in food and beverage products.

5.2 Nutritional Composition

5.2.1 Dry matter

The dry matter content is very high in passion fruit pulp and then seeds. The contents of dry matter vary from the results obtained from (Marques DJ et al., 2019). It indicates the nutrients presence in the pulp and seed rather than the peel where comparatively lower dry matter was present. The content higher in passion fruit pulp leads to the production of fruit juice in the industry.

5.2.2 Moisture content

Moisture content is the factor that is most frequently examined to determine a food product's shelf life (Niazi et al., 2017). The average percentage of moisture content for the passion fruit different parts varies. The moisture content is highest in peel (85.55g), then in seed (18.503g). The lowest moisture content was in pulp (11.013) of passion fruit. The result of moisture content is similar to the results of Morais et al., (2016) where the moisture content of peel is higher (93.3g) than the seed moisture content. But the pulp has higher moisture content (65.8g) than the investigated result. According to Celestino et al., (2010), it asserts that items with lower moisture content are typically more resistant to microbial and chemical deterioration. The high moisture peel content found implies that the peels should be dried for optimum product preservation.

5.2.3. Ash Volume

The overall mineral composition of food can be gauged by counting how much ash is present. Ash content is a good predictor of the nutrients in food. The ash content of the passion fruit peel is highest (8.397g) than the pulp (3.770g) and seed (1.580g) content in the investigated study. The obtained result is fully similar to the results of Morais et al., (2016) where the peel has the highest ash content (6.4g) than the pulp (3.8g). The seed has the lowest ash content (1.4g). This ash composition is very important for the mineral composition in passion fruit different parts.

5.2.4. Raw Fiber

Eating adequate fiber may help treat or prevent constipation, which may facilitate the body's ability to expel waste more quickly. It also encourages a healthy gut microbiota. According to a 2007 review, dietary fiber increases stool volume, promotes regular bowel movements, and reduces the amount of time waste remains in the intestines (Al-Farsi et al., 2007). The average crude fiber in passion fruit samples, according to Morais et al., (2016), ranged from 5.03 to 38.8, which is lower than the stated value. The amount of crude fiber in passion fruit peel is almost similar to passion fruit pulp; whereas the seed has the highest fiber content. This crude fiber increases the nutritional values of the seed and add values to the overall qualities.

5.2.5. Crude Fat

Fat helps to fuel our bodies, protects our organs, fosters cell growth, decreases blood pressure and cholesterol, and facilitates the body's assimilation of vital nutrients. According to the investigated study from table 4.2, the passion fruit seed has the highest fat content than the fruit peel and pulp. The values of total fat are almost 17.623g and it is also similar to the results from Lopes et al., 2010. This high level of crude fat helps to determine the production of oil from the seed. This is plant-based oil and very good for human health.

5.2.6 Crude protein:

Protein is used by the body in a variety of ways. In addition to supporting tissue growth and repair and synchronizing biological processes, it also encourages metabolic reactions. Protein content of passion fruit peel, pulp and seeds are almost same (Table 4.2). It is due to the absence of any treatment to the passion fruit peel, pulp and seed. The value ranges from 8.854 to 9.940 whereas according to Morais et al., 2016, the value ranges from 7.0 to 13.1. The investigated result is almost same to the findings of the referred value.

5.3 Mineral composition:

Our bodies need minerals to stay healthy and use them for a range of functions, such as preserving the strength of our bones, muscles, hearts, and brains. Minerals are necessary for the creation of hormones and enzymes. Passion fruit have been shown to contain a wide range of minerals, including sodium, potassium, magnesium, calcium, phosphorus, iron, zinc, copper, nickel, cobalt, (Ramaiya et al., 2018). The trend of the macronutrient concentration was K > P > Mg > Ca > I in all of the Passiflora fruit juices examined. Even in closely related species, there might be variations in the micronutrient trend. These may be attributed to environmental conditions which have effects on the plant nutrition attributes (Martin E, 1997).

From the table 4.3, it had been found Potassium is higher in the passion fruit (Pulp, 10.200 ± 0.300^{a} >seed, 8.800 ± 0.100^{b} >peel, 3.900 ± 0.100^{c}). But in case of phosphorus, the amount is almost same in passion fruit pulp, peel and seed. This mineral is not varied in different parts of passion fruit.

Magnesium content is present from 2.646 ± 0.100^{c} to 3.633 ± 0.125^{a} (High in peel and low in pulp). In seed there is average amount of magnesium. A balanced diet for humans must include magnesium. Together with Ca, it plays a role in neuro-chemical transmission and muscle excitability as a co-factor in several enzyme systems (Martin E, 1997).

Calcium content is highest in pulp (3.193 ± 0.060^{a}) compared than peel and seed percentage. This calcium is very important for human body and provides the nutrients for bone formation. This mineral works with magnesium as co factor.

Iodine content is highest in pulp (150.47 ± 9.85^{a}) rather than in seed (100.00 ± 10^{b}) and peel (94.33 ± 6.03^{b}). The findings demonstrate that fruit pulp and seeds had high amounts of the minerals tested, which suggests that pulp and seeds might be thought of as an alternate source of nutrition.

5.4. Vitamin composition:

The table 4.4 shows the vitamin C content in three different parts of a fruit or vegetable (Peel, Pulp, and Seed). The values are presented as the mean ± standard deviation, and each sample is labeled with a letter (a, b, or c) to indicate significant differences between them. Based on the table, it can be observed that sample-3 (Seed) has the highest vitamin C content with a mean of 3.70 ± 0.100, followed by sample-2 (Pulp) with a mean of 3.33 ± 0.152. Sample-1 (Peel) has the lowest vitamin C content with a mean of 1.300 ± 0.105. The letters a, b, and c are used to indicate significant differences between the means of the samples. In this case, sample-3 (Seed) has a significantly higher vitamin C content than sample-1 (Peel), while sample-2 (Pulp) has no significant difference with either of the other two samples. Sample-1 (Peel) is labeled with letter c, sample-2 (Pulp) is labeled with letter b, and sample-3 (Seed) is labeled with letter a.

5.5. Phytochemical composition:

5.5.1. Total flavonoid content

A large family of phenolics known as flavonoids is found in almost all plant sections. They have a significant role in the flowers' and fruits' colors. According to study, polyphenols can help control blood pressure and maintain healthy blood vessels, both of which will increase circulation. They also reduce chronic inflammation, another risk factor for heart disease. Once more, polyphenols can help us control and lower our

blood sugar levels (Al-Farsi et al., 2007). Moreover, they are essential for secondary antioxidant defense against a variety of biotic and abiotic stressors. The flavonoids content higher in passion fruit pulp indicates that it is rich in phytonutrients. The findings from Table 4.5 (150.39 mg QE/100g) and Zeraik M.L. et al., 2010 (158.29 mg QE/100g) have no significant differences which indicates that the products from passion fruit pulp may have great impact. The seeds contain about 59% of flavonoids lower compared to the pulp.

5.5.2. Total polyphenol content

Polyphenol has greater impact on human body. Studies have shown a substantial link between diets high in polyphenols and a reduced risk of developing some malignancies, cardiovascular illnesses, diabetes, and neurological diseases (Cory et al., 2018). The polyphenol content is almost 50% lower in passion fruit peel rather than pulp and seed.

5.5.3. Antioxidant content:

Antioxidant efficacy is influenced by other bioactive chemicals' presence, interactions, and synergistic effects in addition to phenolic content. Passion fruit seed has highest antioxidant content whereas the pulps have 71% less antioxidant compared to seeds and 80% less compared antioxidant present in peel. The value denotes the seeds is much more nutritious than peel and pulp.

5.6. Physicochemical properties of oil:

It is possible to determine an oil's authenticity, quality, and purity by looking at its physicochemical qualities.

The iodine value measures the degree of unsaturation of a fat or oil. A higher iodine value indicates a higher degree of unsaturation, which means that the oil has more double bonds between its carbon atoms. In this case, the iodine value of the passion fruit seed oil after three days of extraction is 17.247±0.362, which is a moderately low value, indicating that the oil has a relatively low degree of unsaturation.

The acid value measures the amount of free fatty acids present in the oil. A higher acid value indicates a higher concentration of free fatty acids, which can indicate the oil is rancid or has undergone hydrolysis. In this case, the acid value of the passion fruit seed oil is 1.117±0.016, which is within the acceptable range for edible oils.

The peroxide value measures the number of peroxides present in the oil, which are indicators of oxidative rancidity. A higher peroxide value indicates a higher degree of oxidative rancidity. In this case, the peroxide value of the passion fruit seed oil is 9.267±0.252, which is slightly higher than the recommended maximum value for edible oils, indicating that the oil may be starting to undergo oxidative rancidity.

The specific gravity of oil becomes normal due to the absence of any other fortified ingredients. The results is almost same to the findings of Liu et al., 2008.

Overall, the passion fruit seed oil has a moderately low degree of unsaturation, an acceptable acid value, and slightly elevated peroxide value, indicating that the oil may be starting to undergo oxidative rancidity.

5.7 Fatty acid composition of passion fruit seed oil:

Table 4.7 shows the percentage of each fatty acid present in the sample. Here's what each of the fatty acids in the sample represents:

- Hexanoic acid: a six-carbon saturated fatty acid, which can be found in some animal fats and plant oils. The percentage is about 3.33%
- Octanoic acid: an eight-carbon saturated fatty acid, which can be found in coconut oil and some dairy products.
- Heptadecanoic acid: a 17-carbon saturated fatty acid, which can be found in some animal fats and dairy products. The amount of it is 0.43%
- Stearic acid: an 18-carbon saturated fatty acid, which is commonly found in animal fats and some plant oils.
- Cis-9 Oleic acid: an 18-carbon monounsaturated fatty acid, which is commonly found in olive oil and other plant oils.
- Linoleic acid: an 18-carbon polyunsaturated fatty acid, which is an essential fatty acid that the body needs but cannot produce on its own. It is commonly found in vegetable oils, nuts, and seeds.
- Gamma Linolenic acid: an omega-6 polyunsaturated fatty acid with 18 carbons and 3 double bonds, which is found in some plant oils and has been associated with various health benefits. The percentage is highest among others and it is about 10.37%

- Heneicosanoic acid: a 21-carbon saturated fatty acid, which is found in some animal fats and plant oils.
- Cis 11,14-Eicosadienoic acid: an omega-6 polyunsaturated fatty acid with 20 carbons and 2 double bonds, which is about 0.09% in passion fruit seed oils.
- Behenic acid: a 22-carbon saturated fatty acid, which is found in some animal fats and plant oils. In passion fruit seed oil, the amount is 0.26%
- Tricosanoic acid: a 23-carbon saturated fatty acid, which is found in some animal fats and plant oils and it is about 0.07%

Overall, this composition indicates that the sample contains a mix of saturated, monounsaturated, and polyunsaturated fatty acids, with gamma linolenic acid being the most abundant at 10.37% and it is significantly different from the findings of Ramaiya et al., (2019). These fatty acids can have different effects on health and nutrition, so the specific fatty acid composition of a food or oil can be an important consideration for dietary planning and health management. The fatty acid composition alone is not sufficient to determine the quality of an oil. Other factors, such as the extraction and processing methods, storage conditions, and potential contaminants, can also affect the quality of an oil. However, the presence of a mix of saturated, monounsaturated, and polyunsaturated fatty acids, as well as the presence of essential fatty acids like linoleic acid, are generally considered to be indicators of a good quality oil. This is because a varied fatty acid profile is associated with a range of health benefits and can provide a balance of different nutrients.

Chapter 6: Conclusion

It can be concluded from this study that there is significant difference among the physicochemical properties of different parts of Passion fruit. Mineral composition and bioactive compounds are significantly different also. The difference between the parts led to analyze the seed separately and extraction of seed oil. The results obtained from physicochemical properties of oil were also significantly notable and can be great source of plant oil in Bangladesh.

The perspective of passion fruit worldwide is positive, with a growing demand for this tropical fruit in different markets. In recent years, the global passion fruit market has experienced steady growth, driven by increasing consumer awareness of its nutritional and health benefits. Moreover, passion fruit is becoming more popular as a natural ingredient in the cosmetics and skincare industry, particularly in Europe and North America. In the food industry, passion fruit is used as a flavoring agent for different products, such as juices, desserts, and ice creams. The demand for natural and organic ingredients is increasing, and passion fruit seed oil is seen as a potential alternative to synthetic ingredients in skincare and haircare products.

While passion fruit is not widely cultivated in many parts of the world, it has the potential to become more popular due to its unique flavor and potential health benefits. Further research is needed to fully understand the benefits and potential uses of this fruit and its byproducts.

Chapter 7: Recommendation and future perspectives

In Bangladesh, passion fruit is cultivated in some areas, but it is not a major crop. However, as the country is experiencing growth in the cosmetics and food industries, passion fruit seed oil has potential for future applications in these sectors.

- In the cosmetic industry, there is a growing demand for natural and organic ingredients, which makes passion fruit seed oil an attractive option. As a result, Bangladeshi companies involved in the production of cosmetics, skincare, and hair care products could potentially benefit from incorporating passion fruit seed oil into their formulations due to its high content of essential fatty acids, including omega-6 and omega-9. These fatty acids help to nourish and moisturize the skin and hair. It also provides antioxidant and anti-inflammatory benefits.In the food industry, passion fruit seed oil can be a healthier alternative to other vegetable oils that are commonly used in Bangladesh, such as soybean and palm oil. This can help to address the growing concern over health issues related to the consumption of unhealthy fats. Moreover, passion fruit is also consumed as a fresh fruit in Bangladesh, and there is potential to increase its consumption and promote its health benefits
- In addition to its use in the cosmetic industry, passion fruit seed oil is also being investigated for its potential health benefits. Studies have suggested that the oil may have antimicrobial and anticancer properties, as well as the ability to lower cholesterol levels and reduce inflammation.
- Furthermore, passion fruit seed oil has potential applications in the food industry as a source of edible oil. It is rich in linoleic acid, which is an essential fatty acid that cannot be produced by the body and must be obtained through the diet. Therefore, it can be a good alternative to other vegetable oils for people who are looking for a healthier option. Overall, the future perspective of passion fruit seed oil looks promising due to its diverse potential applications in different industries, including cosmetics, healthcare, and food. Further research is needed to fully explore its benefits and potential uses.

Overall, the future perspective of passion fruit seed oil in Bangladesh looks promising, and there are opportunities for the country to explore its potential applications in

different industries, especially in the cosmetic and food sectors. However, more research and development are needed to fully understand the benefits and potential uses of this oil in Bangladesh.

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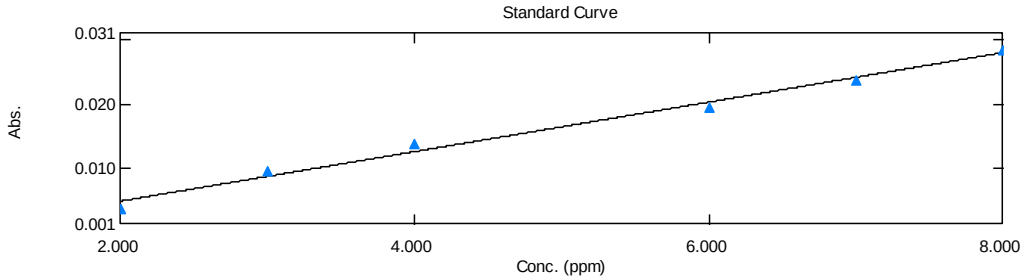
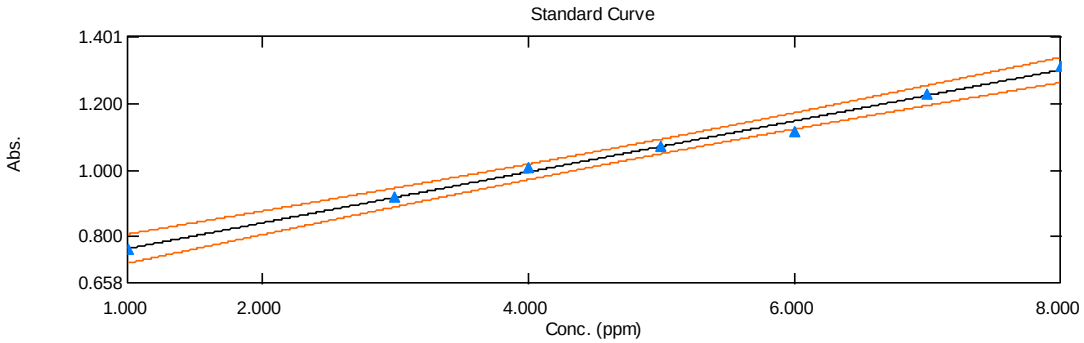
Appendix I: Standard curve for flavonoids, polyphenol and antioxidant

1) Standard curve for flavonoids:



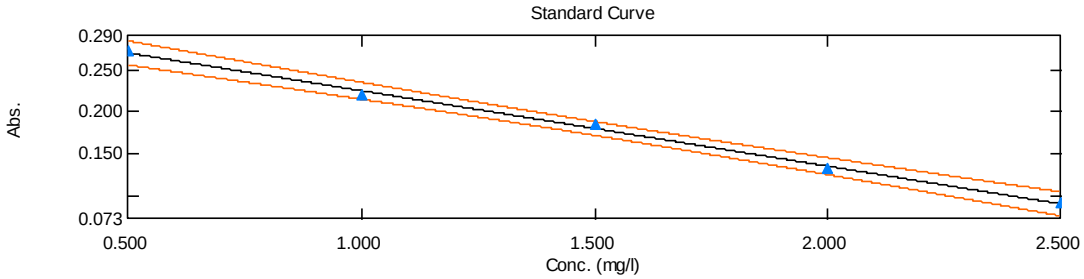
y = 0.00385110 x - 0.00271158
r2 = 0.98868

2) Standard curve for polyphenol:



y = 0.0768527 x + 0.687090
r2 = 0.99301

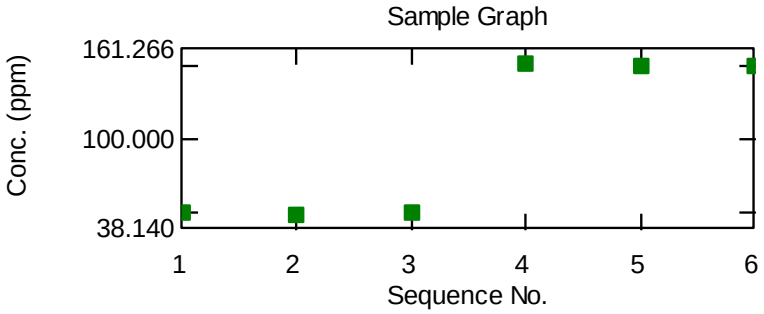
3) Standard curve for antioxidant:



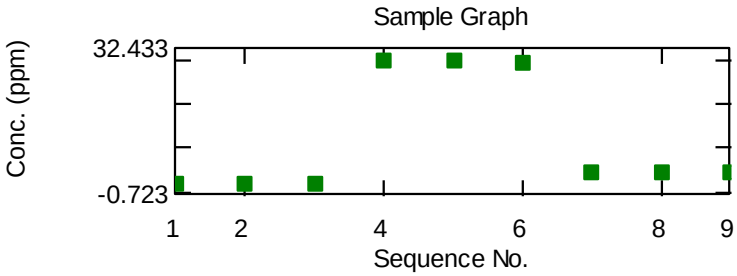
y = - 0.0894539 x + 0.314536
r2 = 0.99735

Appendix II: Sample curve of Flavonoids, polyphenol and antioxidant

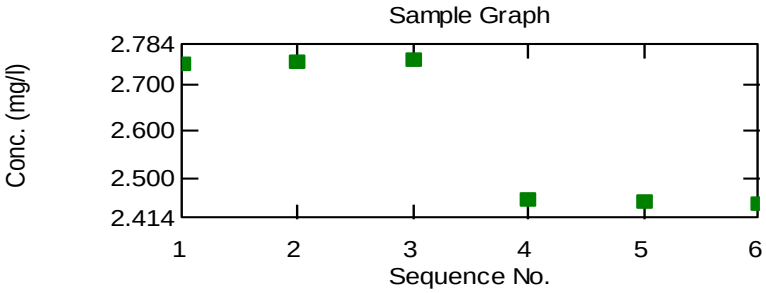
1) Sample curve for flavonoids:



2) Sample curve for polyphenol:



3) Sample curve for antioxidant:



Appendix III: Photo gallery

A) Passion fruit collection and drying

1) Passion fruits after collecting

2) Peel separation from pulp and seed

3) Drying seed

4) Drying the pulp and peel

B) Proximate composition analysis:

1) Dry matter

2) Fiber analsis

3) Protein determination

4) Ash content

5) Fat content

C) Bioactive determination:

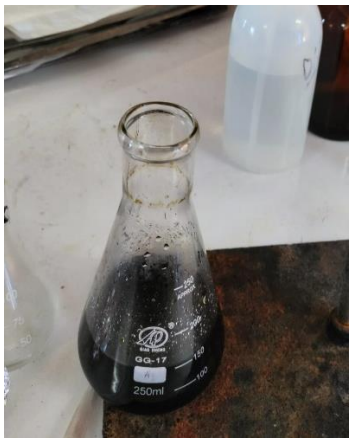
Sample extraction

Polyphenol, flavonoid and antioxidant determination

D) Seed oil preparation:

Seed oil preparation by Soxhlet apparatus

E) Physicochemical properties of oil



Acid value, Iodine value and peroxide value determination of Passion fruit seed oil

Brief biography

Tahiya Tahsin completed a B.Sc. (Hons) in Food Science and Technology from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh, in 2019 (held in 2020). Before that, she passed the Higher Secondary Certificate (HSC) Examination in 2015 with a grade point average (GPA) of 5.00 after passing the Secondary School Certificate (SSC) Exams in 2013 with a grade point average (GPA) of 5.00. She is currently a candidate for the MS in Applied Human Nutrition and Dietetics at the CVASU Faculty of Food Science and Technology's Department of Applied Food Science and Nutrition. His professional goal is to get a demanding job as a food engineer. She has a strong passion for engaging in academic research-based work and has interest to work in the environment where her capacity for innovative problem-solving tasks may be put to good use.