NUTRITIONAL COMPOSITION, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF AMLOKI (*PHYLLANTHUS EMBLICA*) - PAPAYA (*CARICA PAPAYA*) JAM

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

Department of Applied Food Science and Nutrition

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Chattogram Veterinary and Animal Sciences University

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MARCH 2023

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

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PLAGIARISM VERIFICATION

Title of Thesis: **Nutritional composition, phytochemical and antioxidant activity of amloki (*Phyllanthus emblica*) - papaya (*Carica papaya*) jam**

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DEDICATED TO MY BELOVED FAMILY &RESPECTED TEACHERS

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Abbreviation

%	**:**Percentage
&	**:**And
°B	**:**Degree brix
°C	**:**Degree Celsius
µg	**:**Microgram
ABTS	**:**2,2-Azinobis-3-Ethylbenzthiazolin-6-Sultonic Acid
ANOVA	**:**Analysis of variance
AOAC	**:**Association of Official Analytical Chemists
cfu	**:**Colony forming unit
CHO	**:**Carbohydrate
dl	**:**Deciliter
DPPH	**:**2,2-diphenyl-1-picrylhydrazyl
et al	**:**Et alii/ et aliae/et alia
etc.	**:**Et cetera
g	**:**Gram
GAE	**:**Gallic Acid Equivalent
Ibs.	**:**Pounds
kcal	**:**kilocalorie
Kg	**:**Kilogram
L.	**:**Linn
m	**:**Meter
mg	**:**Milligram
ml	**:**Milliliter
N	**:**Normality
nm	**:**Nanometer
PPM	**:**Parts per million
QE	**:**Quercetin equivalents
SD	**:** Standard deviation
Spp.	**:**Species
SPSS	**:**Statistical Package For Social Science
TE	**:**Trolox equivalent
TSS	**:**Total soluble solids

Abstract

Amloki and papaya are rich in nutrients but making jam with them is not common in Bangladesh due to the bitter taste of amloki. Because of this, efforts have been made in the present research to create amloki-papaya jam using various amloki and papaya ratios in order to determine the sensory acceptance and then to examine the nutritional content, phytochemicals, and antioxidants capacity. The open kettle technique was used to make the amloki-papaya jam. One-way analysis of variance (ANOVA) was used to estimate the significance level at 5% ($P<0.05$). Amloki-papaya jam was made for the experiment, along with sugar, honey, commercial pectin, and citric acid. The ranges for the amounts of carbohydrate, fat, protein, ash, and fiber were 66.51% to 70.34%, 0.98% to 1.68%, 1.05% to 1.40%, 0.31% to 0.59% and 0.47 to 2.11% respectively. Energy content was found ranging from (285.86-301.83) kcal/100 g. The calculated vitamin C content and antioxidant capacity of amloki-papaya jams ranged from (98.28- 184.25) mg/100 g and (30.19-30.31) mg TE/100 g. As compared to control sample A, sample B (jam prepared from 80% amloki and 20% papaya) had greater total phenolic contents and flavonoids contents with values ranging from (20.48±0.01) mg QE/100 g and (21.29±0.03) mg GAE/100 ml respectively. Among four formulations of jam, amloki-papaya jam made with 80% amloki and 20% papaya (sample B) had the highest (8.43±0.79) sensory acceptability rating. After 15 days of storage at a cool temperature (8±2°C), total viable count was determined to be within an acceptable range and fungal activity was not observed. So, it may be recognized as a functional food because of the high concentration of outstanding phytochemicals, such as antioxidants and bioactive compounds.

Keywords: Amloki, Papaya, Jam, Antioxidants capacity, Phytochemicals, Sensory properties.

Chapter 1: Introduction

The 2,500-year-old maxim of Hippocrates, "Let food be the medicine and medicine be the food," is currently the focus of many studies. Natural meals, especially those that are filling and comprised of vegetables, have seen a noticeable increase in popularity over the last ten years. This demand has been made by advertisements and academic studies that have shown the potential health advantages of certain foods. As a result of their high concentrations of physiologically active compounds, notably vitamins, polyphenolic acids, flavonoids, and anthocyanins, these foods are now referred to as functional foods (Hasler, 2002).

Amloki (*Phyllanthus emblica*), a member of the Phyllanthaceae family, is indigenous to India and also thrives in tropical and subtropical areas of Pakistan, Uzbekistan, Sri Lanka, South East Asia, China, and Malaysia (Khan, 2009). It may be cultivated effectively in areas with both tropical and dry climates. According to Indian mythology, Amloki is said to be the first ever tree to be produced in the cosmos. It is a significant horticultural crop in India and is also known as Indian Gooseberry. It is known as Amloki in Bangladesh, but is also known as Amla, Aonla, Nelli, Amlika, Dhotri, Emblica, and Usuri in India (Nayaka, 2006).

Amloki is one of the finest sources of vitamin C, amino acids, and minerals and is very nutrient-dense (Varier et al., 1997). It has several different chemical components, including tannins, alkaloids, and phenols. Emblicanin A and B, gallic acid, and ellagic acid are the only hydrolysable tannins known to have biological action. Almost every part of the plant has therapeutic qualities, but the fruit stands out because it is a potent rasayana in Ayurveda and is used in conventional medicine to treat inflammation, jaundice, diarrhea, and a number of other condition (Mirunalini and Krishnaveni, 2010). The fruit has virtually little table value because of its sour and astringent flavor. Due to their extreme astringency, raw fruits are often not eaten, although processed versions of them have immense potential. However, Amloki fruits are not generally available. They are processed into a range of culinary items such as preserves, jams, jellies, sweets, toffee, pickles, sauces, squash, juice, beverage, cider, shreds, dry powder, and so on (Singh et al., 2005).

Papaya (*Carica papaya*) belongs to the Caricaceae family. Its low cost and great nutritional content have earned it the nickname "common man's fruit." Papayas are a popular fruit that are often found in tropical areas. It is prevalent in Mexico, Southern Asia, Central America, and certain parts of Africa (Macalood et al., 2013). The fruit is also known as pawpaw or papaw in Africa and the United Kingdom. In the past, it was thought to be a fruit that should not be eaten. However, it is now commonly grown. Papayas often have green skin while they are young, which becomes entirely reddish-orange when they are fully matured. Fruit's changing exterior color is a sign of maturity, and this shift is thought to be mostly caused by an increase in carotene concentration and a reduction in chlorophyll. The presence of carotenoid pigments has a significant role in determining the color of papaya fruit flesh. Lycopene is present in papaya fruit with red flesh, but not fruit with yellow flesh (Devitt et al., 2010). Lycopene, which is missing in yellow-fleshed fruit, makes up 63.5% of the total carotenoids in the red-fleshed papaya (Yamamoto, 1964).

Besides being a good source of vitamins A (retinol) and vitamin B (Thiamin, riboflavin, niacin etc.), papaya is also high in calcium, iron, and vitamin C (ascorbic acid). A wide variety of phytochemicals are found in papaya, including enzymes (found in the latex), carotenoids (found in the fruits and seeds), alkaloids (found in the leaves), phenolics (found in the fruits, leaves, and shoots), and glucosinolates (in seeds and fruits) (Shinde et al., 2020).

People are increasingly interested in consuming fruits and vegetables to preserve excellent health as they become more health-conscious. Whereas they often like eating both fresh and cooked foods. As consumers' interest in eating nutrient-dense food has increased, research has been conducted to identify the phytonutrient levels and particular health advantages in a variety of fruits and vegetables. To our knowledge, neither nutritional analyses nor the making of jam from Indian Gooseberries were done in the setting of Bangladesh.

Jam is a fruit preservation technique that works well because of its high concentration of sugar, which prevents bacteria, yeast, and molds growing as well as other types of deterioration. This indicates that it is possible to produce pleasant items while maintaining the nutritional value of fruits (Ashaye and Adeleke, 2009). This particular

fruit preserver is produced from the pulp and juice of only one fruit (the complete fruit), which is often used as a bread spread. Pectin is often used in the manufacture of fruit jam as a gelling agent, however sugar, honey, and citric acid may also be used (Ihediohanma et al., 2014).

In order to create or alter the consistency of jams, jellies, confections, pectin, and low-fat dairy products are crucial as well as lowers the glycemic response of goods and is a component in the pharmaceutical sector. To evaluate the type and quantity of pectin, pectin is separated based on their solubility by stepwise extraction in buffer solutions, chelating agent solutions, dilute acids, or dilute sodium hydroxide or sodium carbonate solutions. There are no restrictions on allowable daily consumption since it is also regarded as a safe addition (Da Silva and Rao, 2006).

Fruits and vegetables are essential for both business and human nutrition. Yet, since they are seasonal and very perishable, they must be processed into more stable forms like jams, jellies, and juice to reap their full benefits (Sinha et al., 2012). Although one enticing and useful method to utilize fruit is to make jam (Jalgaonkar et al., 2022). Only a few investigations on Amloki-papaya jam have been done, given existing research. This research was conducted to ascertain the nutritional content, phytochemicals, sensory assessment, microbiological analysis, antioxidant and bioactive components of jam prepared from Amloki and Papaya in order to get more insightful information about this topic.

Aims and objectives:

1. To prepare amloki-papaya jam by using different ratio of amloki and papaya.
2. To analyze and compare nutritional composition, phytochemicals and antioxidant activity among the prepared jam.
3. To compare the overall acceptability of the developed product.

Chapter 2: Review of literature

2.1 Overview of amloki

Amloki (*Phyllanthus emblica*) tree is a small to medium-sized deciduous tree that typically grows to a height of 8 to 18 meters. Its greenish-grey bark and greenish - yellow flowers are formed in axillary clusters. The main stem is often split into two to seven scaffolds very close to the base (Yahia, 2011). The pinnate faishon leaves are tightly packed, giving the branches an overall fluffy look. Leaves form after the fruits have set. Unisexual, light green flowers appear in groups of 6 to 10 on the leaf axils. Fruits have a fleshy, almost globose form, a diameter of 2.1–2.4 cm, a weight of 5.3–5.7 g, and a volume of 4.5–5.0 ml. The fruit's stone has six ribs and divides into three segments, each of which normally has two seeds. When ripe, the green, meaty, globose, and sparkling fragile fruits become pale yellow or brick red (Kulkarni and Ghurghure, 2018).

Figure 2.1: Amloki tree, amloki leaves and amloki

Amloki (*Pyllanthus emblica*) is referred to by a variety of distinct common names all across the globe including Amla, Aonla, Nelli, Amlika, Dhotri, Emblica and Usuri. It is referred to as Indian gooseberry in English-speaking nations. Mirabolano emblico is the name in Italian, Amba in Nepalese, and Popok Melaka in Malaysian. Amloki is sometimes referred to as *Phyllanthe emblica* in French. Amloki is referred to as An Mole in Chinese. Aaamlaka, Dhaatri, Kaayastha, Amogha, Hattha, Nellikkani, Dhatriphala, and Vayastha are some of the common names for amloki (Khan, 2009).

2.1.1 Taxonomy of amloki

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Phyllanthaceae

Genus: *Phyllanthus*

Species: *P. emblica*

Binominal Name: *Phyllanthus emblica*

2.1.2 Origin and distribution of amloki

Phyllanthus emblica, which is indigenous to India, is also grown in a number of other tropical and subtropical nations, including Bangladesh, Malaysia, Mascarene Islands, Myanmar, Pakistan, SriLanka, and Uzbekistan (Thilaga et al., 2013).

2.2 Utilization and economic importance of amloki

2.2.1 Amloki fruit

Amloki is one of the best sources of vitamin C and low molecular weight hydrolysable tannins, making it a superior antioxidant. The processes behind antioxidant activity include the recycling of the sugar moiety and the transformation of polyphenol into medium and high molecular weight tannins. Amloki contains elagic acid, a powerful antioxidant may repair chromosomal defects and prevent gene mutations (Govind, 2011). Breast, uterine, pancreatic, stomach, and liver cancers are only a few of the malignancies that amloki stops from growing and spreading. It may lessen or completely eliminate the negative effects of radiation and chemotherapy (Bhattacharya et al., 2002).

2.2.2 Amloki fruit powder

Amloki fruit powder improves blood pressure management. Amloki, harada, and bihara are the three plants that make up triphala. The liver contains an enzyme called alanine transaminase, which may cause the blood sugar level to rise. To normalize this enzyme, take one or two teaspoonful’s of this mixture, which is made from powdered bitter gourd and amloki jamun in equal proportions. Constipation is characterized by irregular

and infrequent bowel movements. To cure this condition, use 1 teaspoon of amloki powder every morning with milk or water (Devalaraja et al., 2011).

2.2.3 Amloki extract and juice

Although lipoperoxidase and catalase levels were only reversed by 50 mg/kg of amloki fruit extract, 100 mg/kg of amloki extract was shown to be much more efficient in reducing oxidative stress (Lee, 2004). However, a mixture of 4 teaspoons of fresh Amloki juice, 3 teaspoons of honey, and water may help with constipation. If parasites are the cause of constipation, taking 20 grams of fresh Amloki juice each day might kill the worms (Thakur et al., 1989).

2.3 Nutritional properties of amloki

According to many research, the nutritional makeup of fresh amloki varies, most likely as a result of the plant's genetic diversity, environmental factors, ecological setting, and growing and harvesting circumstances. The average composition of amloki fruits is as follows: moisture.81.2%, protein.0.5%, fat.0.1%, carbohydrates.14.1%, mineral matter 0.7%, fiber 3.4%, calcium 0.05%, potassium 0.02%, iron 1.2 mg/100 g, vitamin C 600 mg/100 g (Ghosal, 1996).

The seed contains phosphatides, a fixed oil, and a minor amount of essential oil. The following physical and chemical characteristics apply to the fixed oil production (16%): Acid value 12.7, saponification value 185, acetyl value 2.03, iodine value 139.5, unsaponifiable matter 3.81%, sterol 2.70%, saturated fatty acids 7%, linolenic acid 8.78%, linoleic acid 44.0%, oleic acid 28.40%, stearic acid 2.15%, palmitic acid 2 (Arora et al., 2011).

2.4 Overview of papaya

Papaya tree has a single stem and reaches a height of 20 to 30 feet. The leaves are likewise long and broad (up to 212 feet wide), palmate lobed or deeply incised, with entire margins. They also have petioles that are 1-3 feet long. The huge, round fruits are sometimes referred to as "pepo-like berries" because of their center chamber holding the seeds. Fruits are produced in an axillary position on the main stem, usually singly but sometimes in little clusters. Fruits vary in weight from 0.5 to 20 lbs. and are green when unripe and becoming yellow or red-orange when they are mature. Individual fruits

mature about 5 to 9 months, depending on the grower and environment. After six to twelve months, plants begin to produce fruit. Papaya plants that are trioecious or hermaphrodite can only produce male, female, or hermaphrodite (hermaphrodite) flowers. Different papaya trees may produce blossoms that are either male, female, or bisexual, which is referred to as "trioecious" in botanical terminology. Since bisexual plants self-pollinate and produce the most delectable fruit, they are preferred over female or male plants (Aravind et al., 2013).

Figure 2.2: Papaya tree with papaya, unripe papaya, and ripe papaya

Papaya (*Carica papaya*) is known by a number of distinct familiar names all across the globe including Papita, Tree melon, Paw paw, Mamao. Papita is the name in Indian and Paw paw in Australian. Papaya is sometimes referred to as Mamao in Brazilian. Pepe, Omakai, Papai, Pappali, Pharagi, and Eerankari are some of the common names for papaya (Milind and Gurditta, 2011).

2.4.1 Taxonomy of papaya

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Brassicales

Family: Caricaceae

Genus: *Carica*

Species: *C. papaya*

Binominal Name: *Carica papaya L.*

2.4.2 Origin and distribution of papaya

Tropical America is the native home of the genus *Carica* and the papaya must have developed by a process of natural hybridization. In the sixteenth century, the Spaniards carried it from tropical America to the Caribbean and South-East Asia. Then, it quickly expanded to India, Oceania, and Africa. Today, it is extensively endemic to tropical and warmer subtropical regions of the globe (Villegas, 1997).

2.5 Utilization and economic importance of papaya

2.5.1 Leaves

Papaya leaf provides a plethora of advantages. In certain parts of Asia, immature papaya leaves are prepared and consumed much like spinach.

2.5.2 Papaya fruit

A few of the elements present in great quantities in papaya fruit are pro-vitamin A, vitamin B (thiamin, riboflavin, niacin etc), vitamin C (ascorbic acid), carotenoids lycopene, dietary minerals, and dietary fiber. The papaya fruit contains a phytoalexin called danielone. When tested against the papaya pathogen *Colletotrichum gloesporioides*, this substance shown strong antifungal properties.

2.5.3 Seeds

Papaya's edible black seeds taste spicy and acrid. They may sometimes be pulverized and used in lieu of black pepper. *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* infections may be treated using papaya seeds antibacterial capabilities. Papaya seeds may prevent renal failure brought on by toxins. Papaya seeds are used to treat skin irritation and reduce fever. Besides this, seeds are anthelmintic, antiamoebic, and a cure for piles and typhoid (Aravind et al., 2013).

2.5.4 Peel

In cosmetics papaya peel is often used. In numerous home treatments papaya peels are used which contain vitamin A (retinol, retinol esters) that helps in skin regeneration and restoration when used in sunscreen and soothing lotion. Applying papaya peel to the skin may brighten it. When applied to the skin with honey, peel may sooth and moisturize it and treat dandruff (rub papaya vinegar and lemon juice over the scalp for 20 minutes before taking a shower to treat dandruff) (Aravind et al., 2013).

2.5.5 Root

In several Asian nations, papaya root juice is used to treat urinary issues (Aravind et al., 2013).

2.6 Nutritional properties of papaya

Papaya's nutritional value may vary depending on a variety of variables, including the exact cultivar, the growing environment, and the processing techniques. According to early research, 100 g of ripe papaya contains 0.6 g of protein, 0.1 g of fat, 7.2 g of carbohydrate, and 0.8 g of fiber. They include 14 mg of vitamin C, 8.94mg of b-carotene, 1.72 mg of calcium, and 57 mg of iron per 100 g.

Table 2.1: Nutritive value of 100 g of *Carica papaya* fruit (Villegas, 1997).

Constituents	Green (Unripe) Papaya	Ripe Papaya
Carbohydrates	5.7 g	7.2 g
Protein	0.7 g	0.6 g
Fat	0.2 g	0.1 g
Fiber	0.9 g	0.8 g
Minerals	0.5 g	0.5 g
Carotene	274 μg	8.94 mg
Energy	27 kcal	32 kcal

2.7 Functional properties and phytochemicals

2.7.1 Functional foods

Japan Explores the Boundary between Food and Medicine was the first publication to use the phrase "Functional Food" (Swinbanks and O'Brien, 1993). A functional food is any meal or food component that may provide health advantages in addition to the regular nutrients it contains. It can be said that these whole, enriched, fortified or enhanced foods offered health benefits beyond the provision of essential nutrients when consumed at effective levels as a regular part of a varied diet (Rama, 2019). The ability of functional foods to improve health, prevent disease, and reduce medical costs (Nicoletti, 2012).

2.7.2 Functional foods from plant sources

According to epidemiological, in vivo, in vitro, and clinical trial findings, a plant-based diet may lower the prevalence of chronic diseases, including cancer. According to the Global Cancer Research Fund, eating a lot of fruits and vegetables has been shown to be preventative against a variety of digestive and respiratory cancers (Boffetta et al., 2010). Many epidemiological studies have shown a negative correlation between the consumption of fruits and vegetables and chronic diseases such as different types of cardiovascular disease and cancer. Phytochemicals have been connected to the protective outcome evident observed by Schreiner and Huyskens-Keil (2006). Health professionals are increasingly becoming comprehend about phytochemicals' edge for enhancing health (Sharma, 2011). In the USA, the Nutrition Labeling and Education Act (NLEA) of 1990 was established that, most desired goods have nutrition labels and allowing the inclusion of information on diseases or health conditions on food labels (Marietta et al., 1999).

The leading causes of cardiovascular morbidity and mortality nowadays in the majority of industrialized and developing countries are atherosclerosis and hyperlipidemia. According to Felix-Redondo et al. (2013) elevated plasma cholesterol levels significantly rise the chance of developing cardiovascular diseases. In order to maintain the body's normal functions, it is essential to bring the elevated serum to the proper concentrations. A growing variety of functional meals manufactured from plants are being developed as adjuvant therapy for certain illnesses since the development of functional food technology (Demigne et al., 1998).

Various phenolic compounds, which may be associated to health benefits such as a reduction in heart disease and cancer owing in part to their antioxidant activity, have lately gained increasing interest in studies (Seeram et al., 2002). As it is anticipated that the global market for functional foods and drinks would reach $109 billion by 2010, several sources of phytochemicals are being researched (Watkins, 2008). Polyphenols are often included in beverages due to their positive physiological effects on health (Croft, 1998). .

2.7.3 Phytochemicals

Phytochemicals, which are bioactive, non-nutritive plant compounds, are found in fruits, vegetables, grains, and other plant foods and have been demonstrated to lower

the risk of significant chronic disorders (Zhang et al., 2015). Foods with plant origins are said to include a variety of phytochemicals and bioactive substances, which have drawn attention from researchers interested in functional foods. Shahidi (2004) claims that while developing functional foods and making the choice to adopt a healthy diet, it is crucial to take into account the complementary nature of phytochemicals from diverse sources and their synergistic effects.

According to estimation, around 5000 phytochemicals have reportedly been found, but a significant portion of them are still unidentified and must be found before their health advantages can be completely appreciated (Shahidi et al., 1996). The benefits of phytochemicals in fruits and vegetables may be far larger than currently acknowledged since oxidative stress brought on by free radicals is connected to the genesis of a wide range of incurable diseases (Ames and Gold, 1991).

Many oxidizing substances are continuously present in human and other organismal cells. These compounds could be produced by cellular metabolism or they might be present in food, water, or the air. Maintaining a balance in opposition to antioxidants is essential for maintaining optimal physiologic conditions in the body. Particularly in persistent bacterial, viral, and parasite infections, an excess of oxidants may result in an imbalance and oxidative stress (Liu and Hotchkiss, 1995). A higher risk of cancer and cardiovascular disease may come from oxidative stress because it can damage major macromolecules including proteins, deoxyribonucleic acid, and lipids (Ames and Gold, 1991). In order to prevent or lessen the oxidative stress resulting from free radicals, a suitable amount of antioxidants must be consumed. Numerous phytochemicals (antioxidant substances) found in fruits and vegetables, including phenolics and carotenoids, may assist to protect cellular systems from oxidative harm and lower the likelihood of developing chronic disease (Van Breda and De Kok, 2018).

Phenolic compounds

Dietary components known as phenolic compounds are present throughout the plant world. Numerous molecules with different chemical structures make up phenols. Examining these ingredients in foods and beverages has evolved over the last several decades due to their influence on sensory aspects (color and astringency) (Monagas et al., 2005). According to Yasoubi et al. (2007) the pomegranate contained 40.3 mg

GAE/100 g of total phenol. According to reports, amloki's total phenolic content ranges from 44.9 to 55.5 mg GAE/100 g (Kannaujia et al., 2019) and the total phenolic content of Papaya has been observed to 27.2 mg GAE/100g.

Flavonoids

There has been a great deal of interest in the flavonoid content of foods and plants since the early 1980s, when studies by Steinmetz and Potter (1991) revealed a link between a diet rich in fruits and vegetables and a lower risk for chronic diseases. Because decreasing risk did not correlate with typical nutrients, researchers have focused on a category of non-nutrient, potentially bioactive molecules called flavonoids.

Natural polyphenolic compounds having a C_6-C_3H_6 spinal column are called flavonoids. The six structural groups of this set of plant pigments such as flavones, flavonols, flavonones, flavanonols, flavan-3-ols (catechins) and anthocyanidins which may be found in fruits, grains, vegetables, bark, stems, roots, stems, flowers, and tea can be chemically classified. These substances (aglycones) may also be alkoxylated or esterified and are typically glycosylated (at one or more sites with a variety of sugars). As a consequence, researchers have identified almost 5000 different flavonoids present in plant materials (Harborne and Williams, 2000). The techniques that have been published for determining flavonoids are based on the creation of an aluminum chloride complex, which is often utilized in analytical procedures to figure out the amount of flavonoids in different plants (Grubesic et al., 2007). A study of the literature revealed that Mallow sabdariffa extracts include two different kinds of flavonoids such as flavonols (gossypetin) and anthocyanins (Wichtl, 2004).

Anthocyanins

A further class of plant pigments is called anthocyanins. These anthocyanins have various acylation groups, sugar molecules, and anthocyanidin kinds in their structures. Anthocyanins are seen as a possible natural pigment to replace synthetic food colorings because of their vivid color and great water solubility (Mazza and Miniati, 2018). Foods containing anthocyanins have additional health-promoting qualities in addition to their coloring capabilities. For example, according to Barbosa-Canovas et al. (2009) anthocyanins may be able to reduce the risk of cardiovascular diseases in those who consume wine, berries, and grapes. According to the proposed mechanism,

anthocyanins function as antioxidants by supplying highly reactive free radicals with hydrogen atoms, stopping the series of reaction started by free radicals. Functional food claims must be supported by reliable scientific evidence (Clydesdale, 1997).

Antioxidants

Antioxidants are the compounds in charge of scavenging free radicals and protecting our bodies from a wide range of diseases associated with free radicals. The method includes an oxidative process that is initiated, propagated, and finished by the use of free radicals. Production of antioxidants is possible both naturally in numerous foods and within the body (Alam et al., 2020).

An aqueous extract of the amloki fruit demonstrated an 85.7% to 87.3% efficiency in scavenging DPPH radicals at a concentration of 250 g/ml to 500 g/ml (Middha et al., 2015) and papaya extract demonstrated a 44.6% DPPH radical scavenging action at a dosage of 50 g/ml (Ang et al., 2012). In an acidic environment, the anthocyanins demonstrated some heat endurance as well as a favorable shade balance.

According to a research in the journal Food and Chemical Toxicology, amloki extract may guard against oxidative stress brought on by the chemical compound t-BHP in rat primary hepatocytes (tert-butyl hydro peroxide) and treatment with amloki extract significantly decreased oxidative stress indicators including protein carbonylation and lipid peroxidation while significantly increasing the activity of antioxidant enzymes (Hiraganahalli et al., 2012). Additionally, papaya extract proved effective in preventing the oxidative stress that the chemical compound CCl_4 (carbon tetrachloride) caused in rat primary hepatocytes and treatment with papaya extract significantly reduced oxidative stress indicators including protein carbonylation and lipid peroxidation while also significantly increasing the activity of antioxidant enzymes (Ang et al., 2012).

2.8 Antimicrobial activity

Plants, especially those that have long been utilized in traditional and non-orthodox medicine from many countries to treat microbial diseases, might be interesting sources for novel antimicrobials (Abdallah, 2011). Because of the existence of substances including chymopapain, papain and caricain, which are proteolytic enzymes with antibacterial, antifungal, and antiviral activity, papaya may have antimicrobial effects (Kaur and Arora, 2014).

2.9 Medicinal and health benefits of amloki

i. Ample amounts of low molecular weight hydrolysable tannins and vitamin C may be found in amloki fruit. Amloki becomes an excellent source of antioxidants as a result of these contents (Ghosal, 1996).

ii. The fruit of Amloki has a high concentration of vitamin C, which lowers blood sugar levels. It activates the isolated group of cells known as the islets of Langerhans, which generate the hormone insulin (Bhattacharya et al., 2002).

iii. Amloki may aid in boosting the immune system and improving general health. For instance, a research discovered that amloki extract was able to stimulate the production of antibodies and immune cells in mice, indicating that it may have immune-boosting qualities (Suja et al., 2009).

iv. Fruit juice from *Phyllanthus emblica* is a good choice. Plaques in the aorta are reduced. It is efficient in reducing cholesterol amounts and oxidizing low-density lipoprotein (LDL), which prevents atherosclerotic alterations (Kim et al., 2005)

v. Constipation is characterized by irregular and infrequent bowel movements. Take 1 teaspoon of amloki powder each morning with milk or water to treat this condition. Take 20 grams of fresh Amloki juice each day to kill any worms that may be causing your constipation (Thakur et al., 1989)

vi. The major component of amloki fruit is tannin, which has a strong potential for treating intestinal illnesses including diarrhea and dysentery (Srivasuki, 2012).

vii. In Ayurveda, amloki is referred to as chakshyushya. It is efficient in treating chronic illnesses including pterygium or pinguecula, conjunctivitis, glaucoma, diabetic eye diseases such Retinopathy, mucosa xerosis (dry eye), and surgical cataract (Head, 2001).

viii. Amloki fruit extracts have the ability to protect cells from oxidative damage brought on by chromium (Sai Ram et al., 2003).

ix. Triphala powder that helps the digestive system and keeps the eyes bright and dazzling is created by combining behde, hirda and amloki powder with honey (Biswas et al., 2001).

2.10 Medicinal and health benefits of papaya

Since papaya contains a lot of nutrients and antioxidants, there are a variety of possible health advantages. The following are a few possible advantages of papaya:

i. By using the agar cup plate technique, the bacteriostatic properties of papaya seed and pulp against a variety of enteropathogens have been identified, including *Bacillus subtilis, Enterobacter cloacae*, and *Salmonella typhi*. Purified fruit extracts, both from ripe and unripe fruits, have extremely strong antibacterial effects against microorganisms like *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* (Osato et al., 1993).

ii. In tropical populations, the air dried papaya seeds ingestion provides an affordable, natural, risk-free, easily accessible immunotherapy and preventative approach against intestinal parasitosis (Okeniyi et al., 2007).

iii. Significant antimalarial activity is shown in the petroleum ether extract of the raw papaya fruit's rind (Bhat and Surolia, 2001).

iv. When rats were given the medication orally at a dose of 10 mg/kg, papaya aqueous root extract significantly increases urine production and displays urinary electrolyte excretion patterns that are comparable to those of hydrochlorothiazide (Sripanidkulchai et al., 2001).

v. Beta-carotene and vitamin A in papaya may support good skin maintenance and may even assist to minimize the look of wrinkles. It lessens the odor of persistent skin sores. It is thought to be more efficient than other topical treatments for treating persistent ulcers and is more affordable (Hewitt et al., 2000).

vi. Papaya fruit extracts in ethanol and water have impressive hepatoprotective properties against CCl_4 prompted liver injury (Rajkapoor et al., 2003).

vii. It has shown promise to mitigate the oxidative and inflammatory damage brought on by the hepatitis C virus in cirrhosis (Marotta et al., 2007).

2.11 Conclusion

The interest in incorporating active ingredients like dietary fiber and phenolic antioxidants into common foods like Amloki-papaya jam has significantly increased as a result of growing consumer health awareness. Eating this sort of food may help individual’s live healthier lives and avoid sickness. It's critical to produce jam and other

functional foods that will be aesthetically pleasing to customers as well as physiologically valuable in terms of look, taste, and texture. Besides this, Amloki-papaya jam is a healthy and nutritious option for those who want to enjoy the benefits of these two super foods. Papaya is known for its high level of vitamin C, fiber and antioxidants, while amloki is a potent source of vitamin C and other important nutrients. Consequently, employing natural ingredients in jam while retaining crucial jam quality traits such surface color, texture, and taste may provide a number of advantages in the advancement of human health.

Chapter 3: Materials and Methods

3.1 Study area

The experiment was carried out in the laboratory of the Department of Applied Food Science and Nutrition, Food Processing and Engineering, Poultry Research and Training Center, Animal Science and Nutrition, and Physiology, Biochemistry and Pharmacology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram.

3.2 Study duration

The experiment was carried out from 15th September 2022 to 30th January 2023, for a total of four and a half months.

3.3 Collection of sample

Fresh samples of ripe papaya and amloki were collected from Chattogram district's local market. Fruits such as amloki and papaya were picked with care for their range in color. The scientific and super shop were visited to obtain sugar, honey, pectin, and citric acid. The laboratory stocks provided additional pertinent supplies that were needed for the experiment.

3.4 Jam preparation

Sample A

Fresh amloki, sugar, honey, pectin, and citric acid were used to make jam. Amloki jam was made using 550 g boiling amloki paste, 400 g sugar, 40 g honey, 5.5 g pectin, and 0.2% citric acid as the control sample A.

Sample B

Fresh amloki, papaya, sugar, honey, pectin, and citric acid were used to make jam. Where, to prepare the amloki-papaya jam designated as sample B, 440 g boiled amloki paste, 110 g papaya paste, 400 g sugar, 40 g honey, 5.5 g pectin, and 0.2% citric acid were used.

Sample C

Fresh amloki, papaya, sugar, honey, pectin, and citric acid were used to make jam. Where, to prepare the amloki-papaya jam designated as sample C, 300 g boiled amloki paste 400 g sugar, 40 g honey, 5.5 g pectin, and 0.2% citric acid were used.

Sample D

Fresh amloki, papaya, sugar, honey, pectin, and citric acid were used to make jam. Where, to manufacture amloki-papaya jam designated as sample D, 220 g amloki paste, 330 g papaya paste, 400 g sugar, 40 g honey, 5.5 g pectin, and 0.2% citric acid were used.

Table 3.1: Formulations of amloki-papaya jam (Kumar et al., 2019)

Ingredients	Sample A (amloki:papaya =100:0)	Sample B (amloki:papaya = 80:20)	Sample C (amloki:papaya =60:40)	Sample D (amloki:papaya =40:60)
Amloki	550	440	300	220
Papaya	0	110	220	330
Sugar	400	400	400	400
Honey	40	40	40	40
Pectin	5.5	5.5	5.5	5.5
Citric acid(%)	0.2	0.2	0.2	0.2

The open kettle techniques were used to make jams using amloki and papaya. To separate the clean from the damaged fresh amloki and papayas were separated. Fruits that were uniform in size, shape, and color as well as firm, ripe fruits were chosen and cleaned. After cleaning the amloki and papaya fruits were washed separately in excess of potable water so as to remove the impurities. Amloki fruit seeds were taken out before pulping. Papaya fruits were also cut in half and their peels removed. After the seeds and white portion were removed, the halves were cut into thin slices. And then the fruit samples were independently blended in a high-speed blender for 5-10 minutes. After that, sugar is added and the entire mixture is then brought to a boil while being constantly stirred to produce the desired soluble solid. Commercial pectin is also used along with citric acid as a preservative. After the TSS of around 68% was reached, the jam was judged. The jam was then hot-filled into sterilized bottles with the barest amount of headspace. It was covered and left to cool (Kumar et al., 2019). The whole process is shown in figure 3.1.

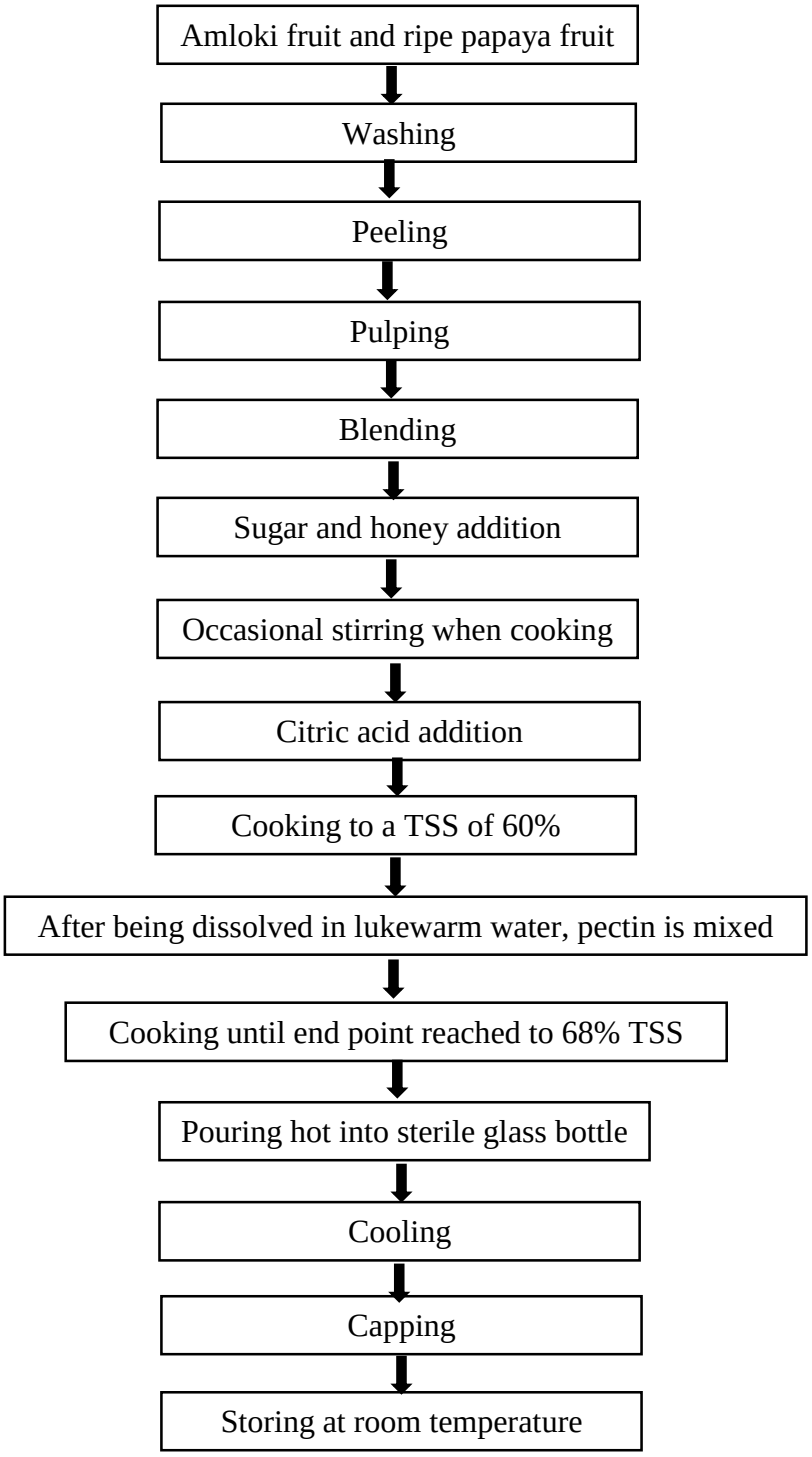

Figure 3.1: Processing steps of amloki-papaya jam

3.5 Physicochemical analysis of amloki-papaya jam

According to procedures of AOAC (2016) fresh jam samples were examined for moisture, total solids, ash, total soluble solids, pH, and titratable acidity. Moreover, for these samples, proximate, bioactive component, and antioxidant studies were performed.

3.5.1 Determination of pH

In chemistry, pH scale is used to measure an aqueous solution's acidity or basicity. While pH is technically the negative logarithm of the activity of the (solvated) hydronium ion, it is most often described as a measurement of the concentration of the hydronium ions. A set of standard solutions whose pH has been defined by international agreement can be used to trace the pH scale. The potential difference between a hydrogen electrode and a standard electrode, such as the silver chloride electrode, is measured using a concentration cell with transference to get the main pH standard values. The pH of aqueous solutions may be determined using a glass electrode and a pH meter. The decimal logarithm of the reciprocal of the hydrogen ion activity in a solution is used to determine pH (De Medeiros et al., 2017).

3.5.2 Total soluble solids

Total soluble solids in the fruits were calculated using a hand refractometer. Using a digital refractometer (Atago RX 1000), total soluble solids (TSS) were measured directly, and the results were presented as percent soluble solids (Brix) in accordance with AOAC recommendations.

3.5.3 Titratable acidity

The percentage of acidity was determined using phenolphthalein indicator and titrating against N/10 NaOH to compute anhydrous citric acid. Each time, 10 ml of juice was placed in a 100ml volumetric flask and the volume was increased to 100 ml by adding distilled water. After this, 10 ml of the diluted juice was titrated against N/10 NaOH using phenolphthalein as the indicator. The appearance of a pink color marks the titration's endpoint (AOAC, 2016). The stated result was three times the titration's average value. Titratable acidity can be determined as below:

$$\text{Titratable acidity (\%)} = \frac{\text{T.V} \times \text{Factor}}{\text{W}}$$

Where,

T.V = Titer value of the sample in ml

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

Factor - Citric acid: 0.0064 (Citrus fruit); Malic Acid: 0.0067

3.5.4 Determination of Vitamin C

The market-reducing effects of vitamin C play a role in its chemical assay. Typically, the amount of vitamin C in a plant or animal extract is evaluated by how much 2, 6-dichloride phenol indophenols it can reduce. Vitamin C in this instance was oxidized into dehydroascorbic acid by the color pigment. At the same time, the dye is transformed into a colorless material. The moment at which the reaction ends may be easily determined. Rapid excretion and filtration are desirable since too much might be released by oxidized Vitamin C during sample and grinding. By using metaphosphoric acid during extraction, oxidation is shown. The results will be most accurate when the solution is quite acidic. It should take one minute to finish the titration. In an aqueous solution, the dye is blue. Acidic solutions become pink, and when entirely decreased, they become colorless (AOAC, 2016).

The Chemicals required for making dye solutions include 260mg of dye (2, 6-dichlorophenol indophenols), 210 mg of $NaHCO_3$, 100 ml distilled water and metaphosphoric acid solution (3%). 500/250 ml Metaphosphoric acid solution (3%) is prepared by 15/7.5 mg of metaphosphoric acid and 40/20 ml of glacial acetic acid dilutes. And in a solution of 500 ml/250 ml metaphosphoric acid, 50/25 mg of crystalline ascorbic acid were dissolved to make standard ascorbic acid solution.

Procedure

A dye solution was put within the burette. The next step was to add 5 ml of vitamin C solution to a conical flask. The conical flask was placed under the burette, and the dye was added drop by drop. The titration was finished when a pink tint first appeared, persisted for 20 seconds, and then disappeared. There were at least three distinct readings done. The same procedure was used to treat the ascorbic acid solution, but at an unknown concentration. Milligram percentage(mg %), was used to represent the outcome.

3.5.5 Moisture content

One of the most significant and often utilized metrics in the production and testing of food products is moisture measurement. The moisture content is directly important economically for both the processor and the consumer since the amount of dry matter in a serving of food is inversely related to the amount of moisture it contains. Yet, the effect of moisture on the stability and quality of food is far more important. The moisture content was determined using a method that is standardized by the Association of Official Analytical Chemists (AOAC, 2016). The percent of moisture was calculated as follow

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

3.5.6 Total solids

Total solid was determined using methods of AOAC (2016). The percentage of total solid content was determined using the data amassed during the measurement of moisture.

$$\text{\% Total solids} = 100 - \text{\% moisture content}$$

3.5.7 Ash content

Ash content was calculated using methods of AOAC (2016). When organic matter is destroyed, an inorganic residue known as ash is left behind. A pre-dried, weighted crucible contained 10 g of dried jam. Then charcoal was made out of it. After that, the charcoal was put into a muffle furnace and burned for four hours at a temperature of around 600°C until the charcoal was completely consumed. The crucible was then taken out of the furnace. Before weighing it, carefully chill it in a desiccator. Following is the formula used to determine the ash content.

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

3.5.8 Estimation of crude fat

To determine the amount of fat in food samples, the samples may be dissolved in organic solvents like chloroform or methanol. The filtrate can then be filtered to separate the different components. To calculate the amount of the extract, the filtrate is separated into several funnels, the mixture is dried, and the estimated fat percentage is

then calculated. According to AOAC (2016) guidelines, a Soxhlet apparatus was used to measure the samples' crude fat content. The following formulation was used to represent the crude fat percentage.

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

3.5.9 Estimation of crude protein

The nitrogen content of both organic and inorganic materials is calculated using the Kjeldahl technique. To determine the protein content, Kjeldahl nitrogen is tested in foods and drinks, meat, feeds, cereals, and forages. Additionally, nitrogen levels in wastewater, soils, and other materials are determined using the Kjeldahl technique. It is a legitimate technique that is specified in several normative, including AOAC (2016). Calculating the percentages of nitrogen or protein requires taking into account the kind of receiving solution that was used as well as any dilution variables that were utilized throughout the distillation process. In the equations below, the letter "N" stands for normality. "ml blank" stands for the milliliters of base needed to back titrate a reagent blank when the receiving solution is standard acid; it stands for the milliliters of standard acid needed to titrate a reagent blank when the receiving solution is boric acid. Equation for getting solution using boric acid is:

$$\text{Nitrogen \%} = \frac{(\text{ml Standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007}{\text{wt of sample in gram}}$$

3.5.10 Estimation of crude fiber

The "crude fiber," or water-insoluble part of carbohydrates, is mostly made up of cellulose, hemicellulose, and lignin. It may be estimated by digestion by first cooking a known amount of fat-free food in a weak acid solution (1.25% H_2SO_4) for 30 minutes. Next, cook it in a weak alkali solution (1.25% NaOH) for 30 minutes at a consistent volume. Finally, deduct the ash from the residue that results. The crude fiber was identified using the method of AOAC (2016). The residual material was then burned in a muffle furnace for 4-6 hours to 550–600°C (white ash). The crude fiber percentage is calculated as follows:

$$\text{\% Crude fiber} = \left(\frac{W - W_1}{W_2}\right) \times 100$$

Where,

W = Weight of crucible, crude fiber and ash

W_1=Weight of crucible and ash

W_2= Weight of sample

3.5.11 Determination of total carbohydrate

Using the distinction approach as stated by Edeogu et al. (2007) the total percentage of carbohydrates was calculated. The amount of readily accessible carbohydrates is calculated by deducting the total of moisture, ash, protein, and fat values (per 100 g) from 100. Therefore, it was estimated using the following formula:

$$\% \text{ CHO} = 100 - (\text{Protein} + \text{Fat} + \text{Ash} + \text{Moisture content})$$

3.6 Energy estimation

By determining the amounts of protein fat, and carbohydrates in each kind of food and using the following equation, the energy content of amloki jam was ascertained (Baer et al., 1997).

$$\text{Energy} = (\text{Protein} \times 4.1) + (\text{Fat} \times 9.2) + (\text{Carbohydrate} \times 4.1)$$

3.7 Determination of antioxidant capacity by DPPH scavenging method

Extract preparation

1 g sample was put into the Felcon tube. The combination was then given 10 ml of 100% methanol, and it was allowed to sit for 72 hours. Continuous straining was done after a 4-hour interval. After 72 hours, the filtrate was collected, and methanoic extract was found.

Procedure

The DPPH assay was used to evaluate the extracts' antioxidant mobility. It was significantly modified from the procedure described by Azlim et al. (2010). 100 ml of 100% methanol were used to dissolve around 6 mg of DPPH in order to create a methanoic DPPH solution. Subsequently, 1 ml of methanoic extract was combined with 2 ml of DPPH solution. After giving the mixture a moderate shake, it was left to stand for 30 minutes at room temperature in the dark. A UV-visible spectrophotometer was

used to detect the absorbance at 517 nm (UV-2600, Shimadzu Corporation, USA). Control, which was created by mixing 1 mL of methanol with 2 ml of DPPH solution, employed methanol as a blank. The scavenging mobility was approximated by the decrease in absorbance of the samples compared to the DPPH reference solution. The antioxidant capacity of extracts was calculated using the following equation based on their capability to scavenge DPPH free radicals.

$$\% \text{ of inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100$$

The calibration standard curve was constructed using the TEAC composite (Trolox equivalent antioxidant mobility), which was also utilized as the standard. The results were represented as mg/100 g of Trolox equivalents (TE) per gram of powder on a dry weight (DW) basis.

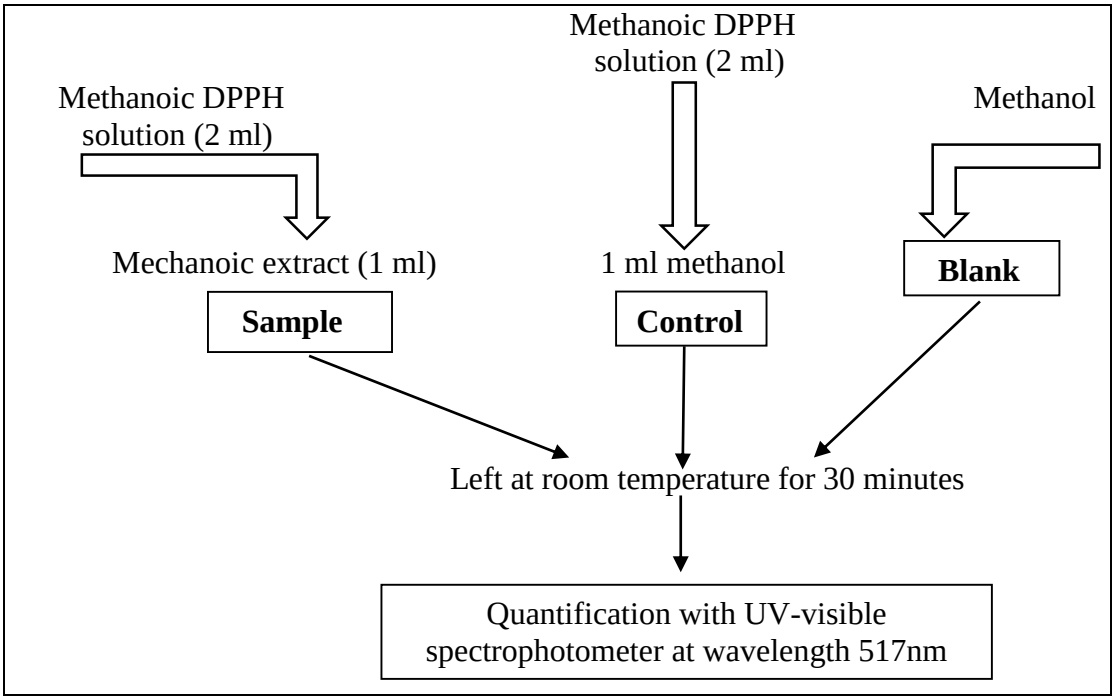

Figure 3.2: Determination of antioxidant capacity

3.8 Determination of bioactive compounds

Extract preparation

5 g of material were gathered for the TAC in a Felcon tube, whereas 1 g of sample were taken for the other TPC and TFC. The combination was then given 10 ml of 100% ethanol, and it was allowed to sit for 72 hours. Continuous straining was done after a 4-hour interval. After 72 hours, the filtrate was collected, and an ethanol extract was found.

3.8.1 Total phenolic content (TPC)

With a few modest adjustments, the Folin-Ciocalteu (FC) reagent technique was employed to determine the TPC of the extracts. (Al-Owaisi et al., 2014). The Folin-Ciocalteu method, as described by Vergani et al. (2016) was significantly adjusted in order to determine the total polyphenol content (TPC) of the amloki jam. 1 ml of ethanoic extract was combined with 1.5 ml of FC reagent in a falconer tube, which was then left at room temperature for three minutes. After adding 1.5 ml 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-visible spectrophotometer (UV2600, Shimadzu Corporation, USA) with C_2H_5OH as the blank. TPC was calculated to be mg of gallic acid equivalents (mg GAE/g) per gram of extracts.

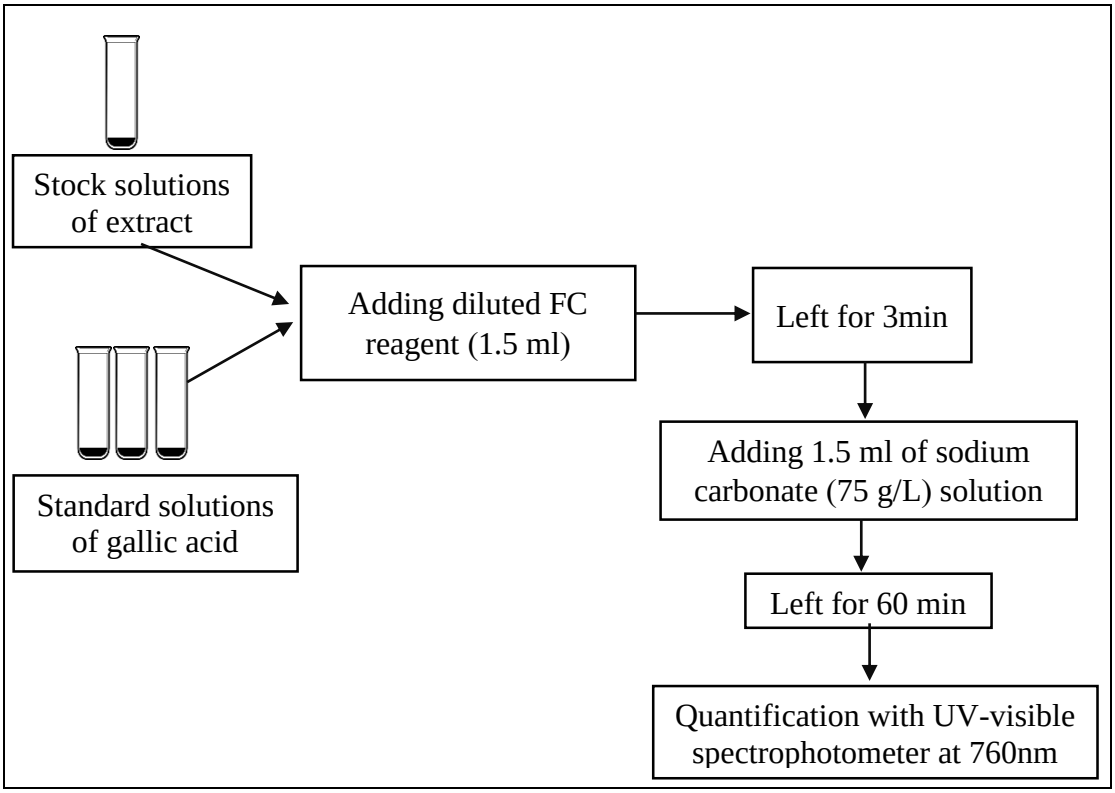

Figure 3.3: Determination of total phenolic content (TPC)

3.8.2 Total flavonoid content (TFC)

With a few minor adjustments, the aluminum chloride colorimetric method described by Chang et al. (2002) was used to assess the samples' total flavonoid content (TFC). A stock solution of the extracts (1 mg/ml) was prepared, and aliquots of 0.5 ml of the diluted extract were diluted with 1.5 ml of 95% C_2H_5OH in a cuvette. After that, 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 immixture in the cuvette. 30 minutes were spent letting the mixture sit at

room temperature. The absorbance was assessed using a UV-visible spectrometer, model UV 2600, made by Shimadzu Company in the United States. 10% aluminum chloride was substituted with the equivalent quantity of Distilled H_2O in the blank. The total quantity of flavonoids in the sample was determined by calculating the extract's absorbance in relation to a standard curve for quercetin. TFC was determined and reported as mg of quercetin equivalents (mg QE/g).

3.8.3 Total anthocyanin content (TAC)

10 mg/ml extract stock solutions were prepared. A cuvette was filled with the 3 ml of extract solution. Using a UV-visible spectrophotometer, the extract color's intensity was determined at 520 nm. TAC will be computed using ethanol as a reference and represented as milligrams per 100 ml (mg/100 ml) using the following formula:

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

Where,

M represents the sample's weight, which was utilized to create the stock solution and DF denotes the dilution factor. E stands for the extinction coefficient (55.9) (Giusti and Wrolstad, 2001).

3.9 Microbiological analysis

3.9.1 Aerobic plate count (bacterial plate count)

A sample's bacterial population is estimated by counting the number of aerobic plates on the sample. Aerobic Colony Count (ACC), Mesophilic Count, Standard Plate Count (SPC), and Total Plate Count (TPC) are other names for Aerobic Plate Count (APC). The total number of live bacteria was determined using the Standard Plate Count (SPC) technique. The test is based on the idea that when coupled with nutrient-rich agar, every cell would appear as a visible colony. It is a generic test for organisms that live aerobically at mesophilic temperatures (25 to 40°C), not a count of all bacteria. The different microorganisms used to evaluate safety, sanitary quality, adherence to good manufacturing practices, and organoleptic acceptability cannot be distinguished by APC. APC may provide information on the shelf life or probable organoleptic change of a food (Banwart, 2012).

Sample preparation and dilution

The accuracy with which the sample was collected has a significant bearing on the validity of the analysis and interpretation of the findings. The sample must accurately reflect the whole bulk. In order for the sample to accurately reflect the whole mass of the items, the product was fully blended for this purpose. In a 250 ml flask, 25 g of this well-blended amloki jam were added. Phosphate buffer saline (0.6 KH_2PO_4 which has a pH of 7.2) was used to dilute the sample. Around 100 ml of buffer saline was added, and it was well mixed in the beaker by rocking it back and forth. The volume was filled with the same buffer water. Sterilization calls for heating each piece of gear, solution, or other instrument to 121°C for 15 minutes. The produced sample was then utilized as a stock solution after being diluted 10 times, or 1×10^{-1} times (Andrews, 1992). Using 9 ml blanks, the following dilutions were created. First, 1/10 dilution (1 ml in 9 ml) was made (a). A vortex mixer (b) was used to blend this. From (a), 1 ml was taken, added to the next tube, and well combined. It was diluted by a factor of 10^{-2}. In this way the dilution was raised to 10^{-6} times.

Standard plate count, counting and recording

An SPC (standard plate count) was used to estimate the number of microorganisms present in the collected and preserved samples. This data might be used as a predictor of product shelf life or a food quality indicator. Thereafter, 1 ml of the diluted material was pipetted into each of the sterile empty petri dishes containing the nutrition agar (Plate count agar) medium. On a flat surface, the plates were combined by swirling. When the medium had solidified, the plates were turned over and incubated in an incubator at 37°C for 24 hours. After that, based on the quantity and simplicity of the bacterial colony count, the incubated plates were chosen for counting after incubation. It was best to stay away from the plate with the dispersed, overlapping, and confusing colonies. We chose the plates with 30 to 250 colonies that were clear, bright, and countable. Number of colony forming units (cfu)/g or ml = average cfu plate multiplied by the dilution factor Sample preparation, sample dilution, standard plate counts, counting, and recording procedures were used to determine the viable bacterial count. The incubation was place for 24 hours at 37°C (AOAC, 1990; Sharf, 1966).

3.9.2 Fungal analysis in jam

Procedure for preparation of media

Sabouraud Dextrose Agar (SDA) is a selective medium that can grow filamentous bacteria like Nocardia and is largely used for the isolation of dermatophytes, various fungi, and yeasts. This medium's acidic pH (around 5.0) stops bacteria from growing, but encourages the growth of yeasts and the majority of filamentous fungus. To strengthen the antibacterial action, antibacterial agents may also be included. Enzymatically digested casein and animal tissues make up the SDA medium, which serves as a nourishing supply of amino acids and nitrogenous chemicals for the development of fungi and yeasts. For 1 liter of SDA medium, 10 g of Mycological Peptone (an enzyme digest of casein and animal tissues), 40 g of Dextrose, and 15 g of Agar with a pH of 5.6 at 25°C are employed. All of the media were sterilized for 15 minutes at 121 °C in the autoclave after being prepared according to the manufacturer's guidelines. For the development and identification of mold and yeast cultures, there are various selective agars available, however most of them do not have rigorous nutritional requirements for growth. Sabouraud Dextrose Agar supports the growth of several fungi strains. We adhere to the procedures and techniques outlined in Chen and Gu (2000). In the beginning, 65 g of the medium were dissolved in 1 liter of distilled water. It was then heated while being stirred frequently and cooked for one minute to thoroughly dissolve the medium. 15 minutes at 121 °C in an autoclave. The mixture was then placed onto petri dishes after cooling to 45° to 50°C. To process the sample, isolated colonies were obtained by streaking the sample onto the medium using a sterile inoculating loop. The plates were then incubated at 25–30°C with high humidity while they were upside down (agar side up). Weekly fungal growth checks were performed on the cultures, which were retained for four to six weeks before being deemed negative (Aryal, 2015).

Interpretation

After sample incubation and confluent proliferation in regions of vigorous injection, the plates should show single colonies in streaked regions. Look for fungus colonies on plates that have the expected color and form. The results should be confirmed by other procedures. Yeast colonies will develop in shades of cream to white. Molds will develop into filamentous colonies of different colors (Aryal, 2015).

3.10 Cost analysis

The entire cost of the ingredients needed to manufacture the jam, which included amloki and papaya, was utilized to calculate the price of the jam. The amount, which was given in taka, was calculated based on the price per kg of jam.

3.11 Sensory evaluation

For the purpose of determining if customers would find the finished product generally acceptable, sensory assessment was carried out. A group of tasters evaluated whether the manufactured product will be accepted by consumers. The panel test was held on the campus of CVASU, and both teachers and students from the institution served on the panel. The product manufactured from amloki and amloki-papaya jam was distributed to the panel's fifteen participants. Sample A, sample B, sample C, and sample D were the four formulas that were encoded. The panelists sampled each of the four samples without being informed of the compositions. The panelists were asked to give the jam the proper score for its sensory qualities, including its appearance, color, flavor, texture, taste, sweetness, and general acceptability. This technique clearly suggests characteristics that a high-quality product should have, while it obviously does not represent real customer perception. They sampled four items and gave each one a score based on their assessment. Hedonic nine point measures were used to assess the four samples' sensory appraisal of their qualitative criteria (taste, mouth feel, appearance, flavor, and acceptability) (Larmond, 1977). The scale was constructed so as to:

Table 3.2: Rating scale for sensory evaluation

Ranks	Scores
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

3.12 Statistical analysis

Data were collected and recorded in a Microsoft Excel 2013 spread sheet in order to evaluate statistical analysis. Three duplicates of each sample were used. For the proximate composition, phytochemicals, antioxidant capacity and sensory analysis of amloki-papaya Jam, descriptive statistics (mean and standard deviation) were performed. Statistics were computed using Minitab 19.0 software. The amount of variation that is statistically significant at a 95% confidence level was calculated using a one-way analysis of variance (ANOVA) on the obtained data. The difference in variance between the sample groups was calculated using a post hoc "Tukey" test. The statistical evaluation was done at a significance level of 5% ($P<0.05$).

Chapter 4: Result

4.1 Physiochemical properties of jam

A key component of ideal gel state is jam's pH. In table 4.1, sample A has the greatest (2.98 ± 0.02^{a}) pH while sample B has the lowest (2.83 ± 0.03^{c}) pH. Sample C had the greatest (69 degree brix) TSS whereas samples A, B, and D had the lowest (68 degree brix) TSS. The highest value (0.83±0.03%) of acidity was identified in sample A, while the lowest value (0.68±0.01%) was discovered in sample D.

Table 4.1: Physicochemical properties of amloki-papaya jam

Components	Formulations of amloki-papaya jam				1−ANOVA (P value)
	Sample A	Sample B	Sample C	Sample D	
pH	2.98 ± 0.02^{a}	2.83 ± 0.03^{c}	2.87 ± 0.03^{bc}	2.90 ± 0.01^{b}	0.001
TSS(°B)	68 ± 0.00^{a}	68 ± 0.00^{b}	69 ± 0.00^{c}	68 ± 0.00^{d}	0.001
Acidity(%)	0.83 ± 0.03^{a}	0.75 ± 0.01^{b}	0.71 ± 0.02^{bc}	0.68 ± 0.01^{c}	0.001

Legends: Means ± SD and values in the same rows with the same superscripts are not statistically significant ($P<0.05$).

In the table,

Sample A- jam made just from amloki without any additional papaya.

Sample B-jam made with 80% amloki and 20% papaya

Sample C-jam made with 60% amloki and 40% papaya

Sample D-jam made with 40% amloki and 60% papaya

4.2 Nutritional composition

Table 4.2 provides information on the nutritional value of amloki-papaya jam, almost all samples varied considerably. The largest percentage of crude fiber (2.11 ± 0.01^{b} %) was found in sample B, whereas the highest percentages of crude protein (1.40 ± 0.03^{c} %) and crude fat (1.68 ± 0.03^{c} %) were found in sample C. The lowest levels of crude fiber (0.47 ± 0.01^{d} %), crude fat (0.91 ± 0.01^{bd} %), and crude protein (1.05 ± 0.01^{ad} %) respectively, were found in samples D, B, and A.

Table 4.2: Nutritional composition of amloki-papaya jam

Components	Formulations of amloki-papaya jam				1−ANOVA
	Sample A	Sample B	Sample C	Sample D	(P value)
Moisture(%)	30.20 ± 0.02^{a}	30.82 ± 0.05^{b}	29.47 ± 0.02^{c}	27.04 ± 0.03^{d}	0.001
Crude fiber(%)	1.07 ± 0.02^{ac}	2.11 ± 0.01^{b}	1.05 ± 0.01^{ac}	0.47 ± 0.01^{d}	0.001
Ash(%)	0.31 ± 0.01^{a}	0.59 ± 0.02^{b}	0.48 ± 0.01^{c}	0.55 ± 0.01^{d}	0.001
Crude fat(%)	1.11 ± 0.06^{a}	0.91 ± 0.01^{bd}	1.68 ± 0.03^{c}	0.97 ± 0.01^{bd}	0.001
Crude protein(%)	1.05 ± 0.01^{ad}	1.17 ± 0.02^{b}	1.40 ± 0.03^{c}	1.10 ± 0.02^{ad}	0.001
CHO(%)	67.33 ± 0.03^{a}	66.51 ± 0.07^{b}	66.97 ± 0.06^{c}	70.34 ± 0.04^{d}	0.001
Vitamin C (mg/100g)	184.25 ± 0.02^{ab}	161.17 ± 0.03^{ab}	148.87 ± 0.03^{c}	98.28 ± 0.02^{d}	0.001

Legends: Means ± SD and values in the same rows with the same superscripts are not statistically significant ($P<0.05$).

4.3 Energy content

According to figure 4.1, sample D had the greatest energy content (301.83 kcal/100 g), Whereas sample B had the lowest (285.86 kcal/100 g).

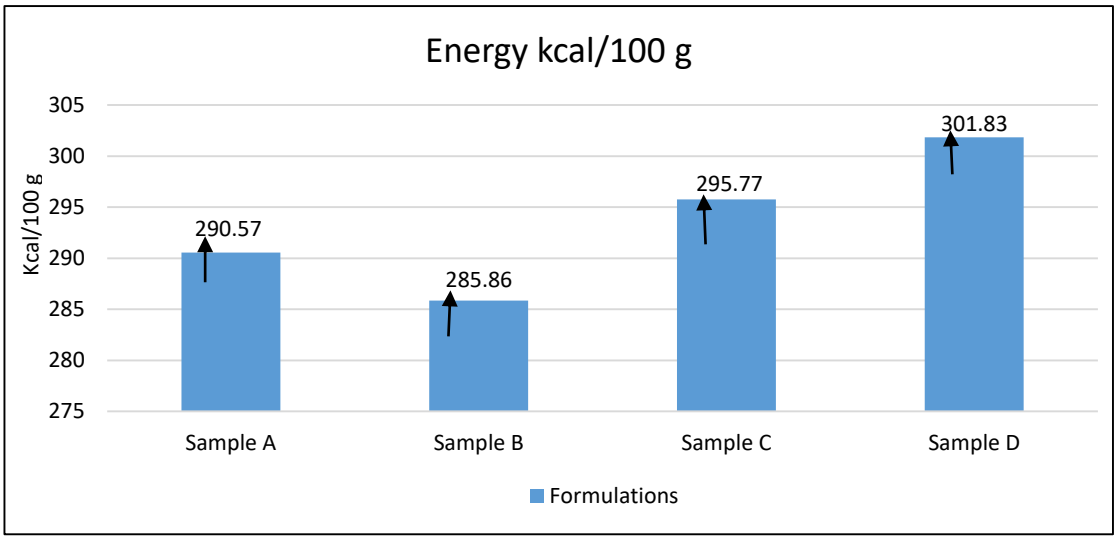

Figure 4.1: Comparison of energy content among four sample of amloki-papaya jam

4.4 Phytochemical composition of amloki-papaya jam

Table 4.3 provides the findings for the bioactive components (TPC, TFC and TAC). Each samples values were determined to be considerably different. The greatest level of total phenolic content (20.48 ± 0.01^{a}) mg GAE/100 ml and total flavonoid content (21.29 ± 0.03^{a}) mg QE/100 g measurements were found in sample B and the greatest level of total anthocyanin content (8.81 ± 0.01^{a}) mg TA/100 ml found in sample D. The lowest values of total phenolic content (10.22 ± 0.02^{d}) mg GAE/100 ml, flavonoid content (6.27 ± 0.05^{c}) mg QE/100 g and total anthocyanin content (7.03 ± 0.04^{ab}) mg TA/100 ml were respectively discovered in sample C, sample D and sample A.

Table 4.3: Phytochemical composition of amloki-papaya jam

Components	Formulations of amloki-papaya jam				1−ANOVA (P value)
	Sample A	Sample B	Sample C	Sample D	
Total Phenolic Content (TPC) (mg GAE/100 ml)	20.07 ± 0.00^{b}	20.48 ± 0.01^{a}	10.22 ± 0.02^{d}	10.56 ± 0.04^{c}	0.001
Total Flavonoid Content (TFC) (mg QE/100 g)	21.23 ± 0.04^{a}	21.29 ± 0.03^{a}	14.37 ± 0.04^{b}	6.27 ± 0.05^{c}	0.001
Total Anthocyanin Content (TAC) (mg TA/100 ml)	7.03 ± 0.04^{ab}	7.66 ± 0.01^{c}	8.23 ± 0.02^{b}	8.81 ± 0.01^{a}	0.001

Legends: Means ± SD and values in the same rows with the same superscripts are not statistically significant ($P<0.05$).

4.5 Antioxidant capacity

From table 4.4, it can be shown that the sample A had the greatest antioxidant capacity (30.31 ± 0.02^{a} mg TE/100 g.) and sample D had the lowest antioxidant capacity (30.19 ± 0.04^{b} mg TE/100 g).

Table 4.4: Antioxidant capacity of amloki-papaya jam

Components	Formulations of amloki-papaya jam				1−ANOVA (P Value)
	Sample A	Sample B	Sample C	Sample D	
Total Antioxidant Capacity(TAC) (mg TE/100 g)	30.31 ± 0.02^{a}	30.27 ± 0.01^{a}	30.23 ± 0.02^{ab}	30.19 ± 0.04^{b}	0.003

Legends: Means ± SD and values in the same rows with the same superscripts are not statistically significant ($P<0.05$).

4.6 Microbial analysis

Table 4.5 showed that both the total viable count and fungal count were calculated from 0 to 15 days after the preparation of jam. For the assessment, samples of jam were kept at a temperature of 4°C for 15 days. When the products were made yeast and mold were not there and after 15 days, there was no evidence of their existence.

Table 4.5: Microbiological evaluation of amloki-papaya jam

Formulations of amloki-papaya jam	TVC (cfu/ml)		Mold and Yeast	
	0 day	15 days	0 day	15 days
Sample A	2.8×10^{1}	1.8×10^{2}	No growth	No growth
Sample B	2.8×10^{1}	1.6×10^{2}	No growth	No growth
Sample C	3.4×10^{1}	1.1×10^{3}	No growth	No growth
Sample D	3.6×10^{1}	1.7×10^{2}	No growth	No growth

4.7 Cost analysis

Table 4.6: Production cost of amloki-papaya jam

Heads	Tk./Kg	Quantity used g/kg products)	Total Tk. For sample A	Total Tk. for sample B	Total Tk. For sample C	Total Tk. for sample D
1)Expenditure raw materials						
Amloki or amloki-papaya mixture	120(for Amloki) 50(for papaya)	550	66	52. 8 5.5	36 11	26.4 16.5
Sugar	85	400	34	34	34	34
Honey	700	40	28	28	28	28
Pectin	12000	5.5	66	66	66	66
Citric Acid	1000	0.2	0.2	0.2	0.2	0.2
Sub total			194.2	186.5	175.2	171.1
2)Processing cost@15% of raw material			29.13	27. 98	26.28	25.67
3)Bottling Cost 2 piece		15 Tk./piece	30	30	30	30
Total production cost of per kg amloki and amloki-papaya jam			253.33	244.48	231.48	226.77

In the table,

Sample A- jam made from amloki without any additional papaya.

Sample B-jam made with 80% amloki and 20% papaya

Sample C-jam made with 60% amloki and 40% papaya

Sample D-jam made with 40% amloki and 60% papaya

Additionally, the amounts of sugar, honey, pectin, and citric acid in each sample are the same.

This recipe may be used to make 1 kg of jam. The cost of jam, therefore, is:

Sample A per kg jam is= 253.33 Taka

Sample B per kg jam is= 244.48 Taka

Sample C per kg jam is=231.48 Taka

Sample D per kg jam is= 226.77 Taka

4.8 Sensory evaluation

The greatest (8.43±0.79) acceptance rate was seen in sample B across all criteria. In contrast to the other samples, sample D had the lowest acceptability rating.

Table 4.7: Hedonic rating test for sensory evaluation of amloki-papaya jam

Parameters	Formulations of sample				1−ANOVA
	Sample A	Sample B	Sample C	Sample D	(P value)
Taste	7.43±0.79	8.29±0.76	8.14±0.38	7.71±0.76	0.453
Sweetness	7.43±0.79	7.86±0.38	8.0±1.16	7.57±0.79	0.826
Mouth feel	7.86±1.46	7.29±1.11	8.29±0.49	7.43±0.98	0.670
Flavor	7.86±0.69	8.29±0.76	8.14±0.69	7.71±1.11	0.824
Appearance	7.71±0.49	8.14±0.69	8.00±1.0	7.86±1.07	0.931
Overall acceptability	7.86±0.38	8.43±0.79	8.14±0.38	7.43±0.79	0.315

Legends: Means ± SD and values in the same rows with the same superscripts are not statistically significant ($P<0.05$).

Chapter 5: Discussions

5.1 Physicochemical properties of amloki-papaya jam

Jam's pH must be taken into account for the optimal gel quality. Low pH in food also inhibits the development of microorganisms. According to this research, Samples A had higher pH value (2.98 ± 0.02^{a}), whereas Sample B had the lowest value (2.83 ± 0.03^{c}). According to the findings in table 4.1, all samples pH levels varied significantly, falling between (2.83 ± 0.02^{c}) to (2.98 ± 0.01^{a}), which was within the range of usual jams. According to Srinu and Suseela (2016) the pH of amloki-papaya jam was 2.98.

Jam failure is often brought on by an inadequate acid content, which is one of the most frequent reasons. When the jam is concentrated enough to pour, the pH value should be measured. Citric acid should be added if the pH is more than 3.3 to bring it down to a range of 3.0 to 3.4. At a pH of 3.2 to 3.4 and in the presence of a lot of sugar, citric acid has the ability to create a viscous semi-solid. By adding the citric acid towards the end of the boiling process, the pH may be better controlled, and the batch's pre-gelling and pectin hydrolysis may be reduced. Depending on the initial acidity of the extract as well as its buffering ability, various extracts will need varying quantities of added acid. To get the best taste, regulate or vary the pace of setting, and alter the degree of sugar inversion, the pH may be changed (Eke-Ejiofor and Owuno, 2013).

Among the different amloki-papaya jam tests, 0.68 and 0.69 were the least and most notable TSS values. It is likely that the hydrolysis of polysaccharides caused the increase in TSS. Shah et al. (2015) observed that the TSS of apple and olive jam was close to 0.69 and that it grew with time. Additionally, they said that the apple-olive jam's rising total soluble solid may be caused by the breakdown of polysaccharides in the presence of acid.

All samples in this investigation revealed slightly discernible change in the overall titratable acidity level of jam. Sample A yielded the highest result ($0.83\pm0.03\%$), while sample D had the lowest value ($0.68\pm0.01\%$). The primary contributing factor to acidity may be the use of citric acid during jam making. According to Shah et al. (2015) five different kinds of apple and olive fruit blended jams were employed in this experiment, and their titratable acidity ranged from (0.68 to 0.83%), which is within the range of amloki-papaya jam.

5.2 Nutritional composition of jam

When making amloki-papaya jam, pectin and sugar are combined with the ripe amloki and papaya to offer the necessary nutrients including protein, fat, fiber, CHO, and vitamins and minerals. In table 4.2, the proximate composition of four different forms of jam was shown. In comparison to control sample A, sample B has more moisture, whereas sample D contains less. It could be because jam is cooked at a higher temperature. According to Siddiqui et al. (2015) pectin is mostly utilized to build the appropriate texture of goods, which has the effect of regulating the moisture or water in the product. Moisture has a considerable effect on the shelf life and freshness of goods. Food items with a high moisture content have a short shelf life. According to Srinu and Suseela (2016) the moisture content of jam produced with various proportions of amloki and papaya varied from 32.08% to 34.02%. Jam's moisture content might change depending on storage circumstances and the temperature used during the cooking process (Broomes and Badrie, 2010).

When compared to the control sample A, sample B had the most abundant value of crude fiber (2.11±0.01%), Ash (0.59±0.02%) and sample C contain highest value of crude protein (1.40±0.03%), crude fat (1.68±0.03%) whereas sample D contained highest value of carbohydrates (70.34±0.04%). It's possible that the addition of commercial pectin or fruit sugar naturally altered the nutritional makeup of jam significantly. Pectin is a kind of polysaccharide that improves a product's CHO profile (Brejnholt, 2009). Ash content reveals the minerals that are present in food products (khan et al., 2012). According to Srinu and Suseela (2016) research, the jam made from amloki and papaya in the current study had a greater nutritious value.

Vitamin C (Ascorbic acid)

Ascorbic acid is fundamental for life. Sample B contained highest amount (161.17±0.03) mg/100 g of vitamin C which is higher than sample C and D. According to Kumar et al. (2019) six different ratios of amloki-papaya jams were employed in this experiment and their ascorbic acid ranged from (84 to 186) mg/100 g. Martinsen et al. (2010) showed that high processing temperature decreased ascorbic acid content. According to Gonzalez-Molina et al. (2010) eating fruit that is high in vitamin C may help avoid obesity and cardiovascular disorders. Since the human body cannot synthesis these elements, daily intake of fruits and vegetables must serve as a supplement.

5.3 Phytochemicals of amloki-papaya jam

Bioactive chemicals are important for maintaining the immune system and avoiding chronic diseases in humans in addition to giving the body the nourishment it needs. (Cencic and Chingwaru, 2010). As a consequence, it is crucial to quantify these compounds. Table 4.3 reports the results of the analysis of the bioactive chemical content found in amloki-papaya jam.

Total polyphenol contents

Total polyphenol contents of sample B (20.48±0.01^{a}) mg GAE/100 ml was higher than those of control sample A, despite sample A being made from 100% amloki and the other three samples (B, C, and D) being made from various ratios of amloki and papaya. Rababah et al. (2011) found that apricot jams ranged in total polyphenol content from (20.14±0.12 mg GAE/100 ml) to (51.48±5.12 mg GAE/100 ml). Our findings for amloki-papaya jam was also within the range. When raw materials are processed into jam, the cell structure is disrupted. As a result, the cells are more vulnerable to non-enzymatic oxidation, which might be one of the causes of the reduction in total polyphenol. Besides this, the type and variety of fruit, the amount of sugar and pectin, as well as other components, all affect how bioactive chemicals alter during processing (Shinwari and Rao, 2018).

Total flavonoids contents

Total flavonoid contents of sample B (21.29±0.03^{d} mg QE/100 g) was higher than those of control sample A, despite sample A being made from 100% amloki and the other two samples (C and D) being made from various ratios of amloki and papaya. According to Diaconeasa et al. (2019) total flavonoid concentrations in jams varied from 2.61 mg QE/100 g in blueberry jam to 11.43 mg QE/100 g in blackcurrant jam. Blackcurrant jam has the greatest concentration of total flavonoids, similar to its total phenolic content. They might have different cultivars utilized to make jam, a different harvesting period, a different production process, or even different storage conditions, all of which could have an impact on their quantitative deviations from our findings (Diaconeasa et al., 2019).

Total anthocyanin contents

Total anthocyanin content of sample D (8.81±0.01[a] mg TA/100 ml) was higher than those of control sample A, despite sample A being made from 100% amloki as papaya is a superior source of anthocyanin contents than amloki. For this reason, anthocyanin content in sample D (made with 40% amloki and 60% papaya) was shown to be greater when compared with sample A (made with 100% amloki). The degree of brix has a significant impact on the percentage of anthocyanin degradation in jams. Normally 64° brix to 76° brix led to 20–30% degradation, whereas 80 degree brix led to 50–60% degradation (Queiroz et al., 2009).

5.4 Antioxidant capacity

All of the samples in table 4.4's antioxidant capacity did not substantially differ from one another. In specifically, the ability of chemical and biological substances to scavenge free radicals was investigated using the commonly used substrate DPPH. According to table 4.4, sample A, a control sample, has the maximum (30.31±0.02[a] mg TE/100 g) antioxidant capacity, although samples B, C, and D have antioxidant capacities that are almost identical(30.27±0.01[a]) mg TE/100 g, (30.23±0.02[ab]) mg TE/100 g, (30.19±0.04[b]) mg TE/100 g respectively to those of sample A (30.31±0.02[a]) mg TE/100 g. Tannins, in particular, which are recognized for their potent antioxidant capabilities, are abundant in amloki. The high content of vitamin C in amloki, a powerful antioxidant that aids in the body's ability to combat dangerous free radicals, is another factor contributing to the fruit's high antioxidant capacity. Amloki really has up to 20 times more vitamin C per serving than an orange (Khopde et al., 2001). There are several research that support the idea that heat that comes during cooking reduces the antioxidant level and that adding components like sugar might diminish the antioxidant content in the final product (Rodgers Dinstel et al., 2013). Despite the fact that after being turned into jams they only preserve 65% of their antioxidant content (Ceron et al., 2014)

5.5 Microbial analysis

For each of the four samples of amloki-papaya jam, microbiological studies (total viable count, yeast, and mold count) were carried out. Amloki-papaya jam shown in Table 4.5 did not contain any yeast or mold. According to Muck (2010) mold is an aerobic

creature and cannot thrive in environments with insufficient oxygen. On the other hand, yeast may grow both aerobically and anaerobically. For yeast and mold to develop in a variety of food products, there are quite a few different acid/alkaline needs, ranging from pH 2 to over pH 9. Jam was preserved in airtight bottles, which prevented the formation of yeast and mold. Extreme heat is used while making jam, and this, together with the product's high pH and high sugar content, may cause a decrease in the number of microorganisms found in the final product (Makanjuola et al., 2019).

Jam made with amloki and papaya had a Total viable count ranging from (2.8×10^1 cfu/ml) to (3.6×10^1 cfu/ml). The bacterial count increased after 15 days of storage at a low temperature (8±2°C) and ranged from (9.2×10^1 cfu/ml) to (1.7×10^2 cfu/ml). With a total number of bacteria, yeasts, and molds fewer than 10 cfu/g, microbiological investigations indicated that the mulberry and roselle mixed fruit jam had the best overall acceptance (Wongchalat and Chatthongpisut, 2017).

5.6 Sensory evaluation

In order to determine which jam had the most organoleptically palatable percentage, sensory analysis of amloki-papaya jam was conducted. Sensory analysis data from Table 4.8 demonstrate that, the variation in taste, flavor, appearances, mouth feel, sweetness and overall acceptability were found to be statistically insignificant at the 5% ($P<0.05$) level of significance. Though sample B (jam prepared with 80% amloki and 20% papaya) scored highest (8.43±0.79) in overall acceptability rating. It may be due to taste, flavor and appearance. Since this jam appears like a blend of papaya and amloki during the sensory assessment of jam, panelists preferred it. Moreover, the taste and texture of the jams might fluctuate depending on the individual. However sample C (jam prepared with 60% amloki and 40% papaya) scored almost similar to sample B.

Sample D (composed of 40% amloki and 60% papaya) had the lowest (7.43±0.79) hedonic score when compared to control sample A. It could be caused by the jam's greater sweetness, diminished taste or outward appearances. This jam loses its acceptability in terms of appearances and sweetness because of the larger proportion of papaya, which causes the amloki's greenish tint to diminish. While sample B received the best score in terms of overall acceptability.

Chapter 6: Conclusion

In general, amloki-papaya jam will be a tasty and healthful addition to any pantry because of its positive spin on conventional fruit jam. The spread is great because it mixes the acidic, somewhat bitter flavor of amloki with the sweet, tropical taste of papaya. For those seeking to experience something novel and fascinating, its distinct taste and nutritional advantages make it a fantastic option. The amloki-papaya jam was subjected to a proximate analysis, and the results revealed that sample B (made with 80% amloki and 20% papaya) contain highest amount of crude fiber percentage (2.11 ± 0.01^{b}) and vitamin C (161.17±0.03 mg/100 g) compared to other sample. According to phytochemical analysis, sample B of amloki-papaya jam was a reliable source of total phenolic content (20.48 ± 0.01^{a} mg GAE/100 ml) and total flavonoid content (21.29 ± 0.03^{d} mg QE/100 g). Besides this, due to the quantity of superior antioxidants presents in sample B (30.27 ± 0.01^{a} mg TE/100 g) it is acknowledged as a functional food. According to this study, sample B (made with 80% amloki and 20% papaya) had the highest acceptability in terms of sensory perception. According to the findings of the research, the nutritional and phytochemical quality of jam made with 80% amloki and 20% papaya was superior to other samples. The consumer can utilize this method because it is inexpensive and straightforward to make jam. For the benefit of Bangladeshi growers, processors, and consumers, this study indicates a promising option for amloki and papaya to be processed into jam. Additionally, it can also be demonstrated that exporting jam of the highest caliber and meeting international standard may lead to the creation of foreign exchange, which is favorable for Bangladesh's national economy.

Chapter 7: Recommendations and Future perspectives

In our country, malnutrition affects more than half of the population at the moment. In this situation, amloki-papaya jam might be an excellent source of nutrients and energy since they are plentiful in rural parts of Bangladesh. Our study on the manufacturing of amloki-papaya jam reached a pleasing conclusion with fruitful outcomes. Additionally, this has increased its marketability and financial value. Contemporary food businesses may use the strategy utilized on medium and large sizes of production. Considering the outcomes of the present investigation, the following recommendation and perspective are provided for the ongoing study activity:

a) In order to confirm the experiment's findings, the present study may be repeated.
b) The composition may be further altered, and one may attempt to make mixed jam using other recipes and fruit ratios.
c) Mostly because preparation is not difficult it is also suggested for usage off-season since it may be kept for a lengthy period of time. On the other side, it will be advantageous for those who belong to the economically disadvantaged section.
d) Since the findings have therapeutic significance, they will be helpful from a therapeutic perspective.
e) In order to raise the standard of amloki-papaya jam, a more sophisticated packaging and preservation technique would be developed.
f) The enormous sample size made it possible to compare the different sets of data in a manner that was statistically significant. Due to the limited number of samples that were examined, our conclusion should be treated with care, and the results need to be verified in a bigger research.
g) Similar research should also be done on other fruits like mango and jackfruit that are not readily accessible in the market during the off-season.
h) It is necessary to take the necessary steps to boost the nutritional value of jam that is sold commercially.

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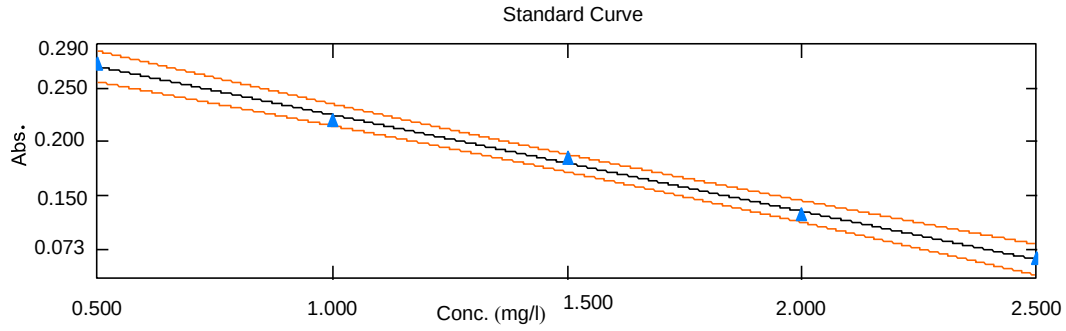
Appendices

Appendix A: Antioxidant capacity of amloki-papaya jam

Standard curve of antioxidant capacity



y = - 0.0894539 x + 0.314536
r2 = 0.99735

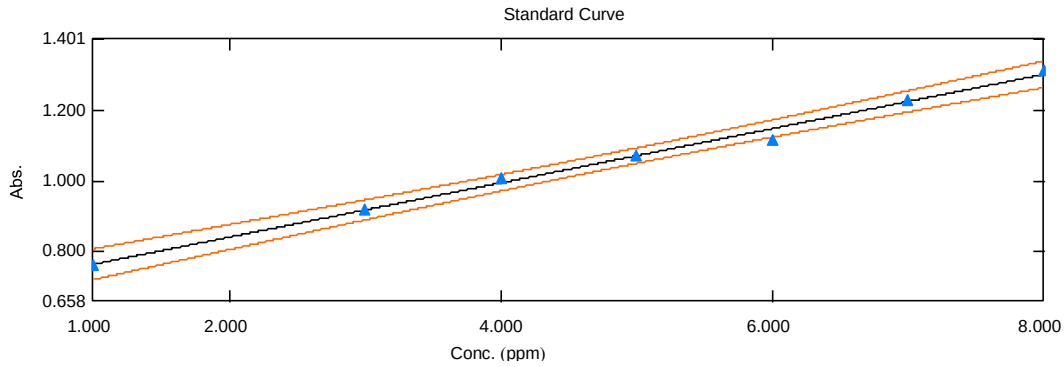
Descriptive statistics

Antioxidant capacity

Sample	N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
Sample A	3	30.3100	0.0200	0.0115	30.2765	30.3435	30.29	30.33
Sample B	3	30.2700	0.0100	0.0058	30.2365	30.3035	30.26	30.28
Sample C	3	30.2470	0.0208	0.0120	30.2132	30.2802	30.23	30.27
Sample D	3	30.1900	0.0400	0.0231	30.1565	30.2235	30.15	30.23
Total	12	30.2540	0.0502	0.0145	30.2206	30.2877	30.15	30.33

Appendix B: Bioactive compounds of amloki-papaya jam

Standard curve of TPC (Total phenolic content)



y = 0.0768527 x + 0.687090

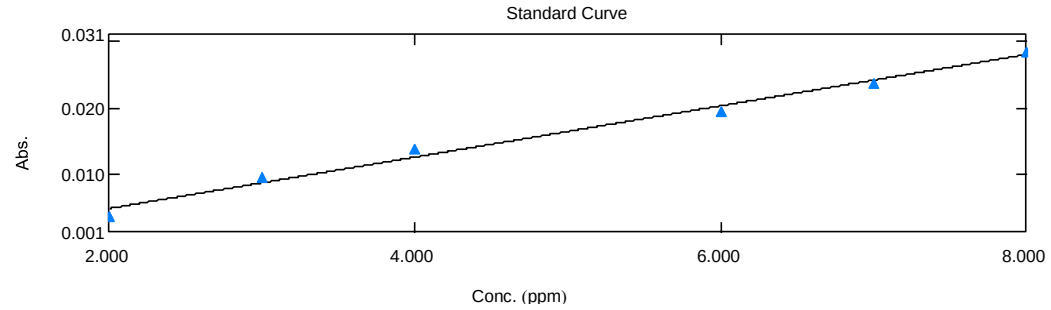
r2 = 0.99301

Descriptive statistics

TPC (Total phenolic content)

Sample	N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Mini-mum	Maxi-mum
					Lower bound	Upper bound		
Sample A	3	20.0700	0.0000	0.0000	20.0400	20.1000	20.07	20.07
Sample B	3	20.4800	0.0100	0.0058	20.4495	20.5105	20.47	20.49
Sample C	3	10.2200	0.0200	0.0115	10.1895	10.2505	10.20	10.24
Sample D	3	10.5600	0.0400	0.0231	10.5295	10.5905	10.52	10.60
Total	12	15.3300	5.1700	1.4900	15.3021	15.3629	10.20	20.49

Standard curve of TFC (Total flavonoid content)



y = 0.00385110 x - 0.00271158
r2 = 0.98868

Descriptive statistics

TFC (Total flavonoid content)

Sample	N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Mini-mum	Maxi-mum
					Lower bound	Upper bound		
Sample A	3	21.2300	0.0400	0.0231	21.1789	21.2841	21.19	21.27
Sample B	3	21.2900	0.0300	0.0173	21.2359	21.3441	21.26	21.32
Sample C	3	14.3700	0.0400	0.0231	14.3159	14.4241	14.33	14.41
Sample D	3	6.2700	0.0500	0.0289	6.2159	6.3241	6.22	6.32
Total	12	15.7900	6.4500	1.8600	15.7365	15.8441	6.22	21.32

Descriptive statistics

TAC (Total anthocyanin content)

Sample	N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Mini-mum	Maxi-mum
					Lower bound	Upper bound		
Sample A	3	7.0333	0.0379	0.0219	7.0014	7.0653	6.99	7.06
Sample B	3	7.6600	0.0100	0.0100	7.6281	7.6919	7.65	7.67
Sample C	3	8.2267	0.0252	0.0145	8.1947	8.2586	8.20	8.25
Sample D	3	8.8133	0.0115	0.0068	8.7814	8.8453	8.80	8.82
Total	12	7.9330	0.6900	0.1990	7.9015	7.9653	6.990	8.820

Appendix C: Questionnaire for Hedonic test of amloki-papaya jam

Name of the Taster: …… **Date: …….**

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as color, flavor, texture and overall acceptability. Use the appropriate scale to show your attitude by checking at the point that best describe your sense and feeling about the sample please give a reason for this attribute. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us. For Taste/Flavor/Mouth feel/Appearance/Overall Acceptability.

The scale is arranged such that; Like extremely =9, Like very much =8, Like moderately =7, Like slightly=6, Neither like nor dislike =5, Dislike slightly =4, Dislike moderately =3, Dislike very much =2, and Dislike extremely =1.

Here,
Sample A- jam made just from amloki without any additional papaya.
Sample B-jam made with 80% amloki and 20% papaya
Sample C-jam made with 60% amloki and 40% papaya
Sample D-jam made with 40% amloki and 60% papaya

Hedonic	Taste				Flavor				Mouth feel				Sweetness				Appearance				Overall acceptability			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Like Extremely																								
Like very much																								
Like moderately																								
Like slightly																								
Neither like or dislike																								
Dislike slightly																								
Dislike moderately																								
Dislike very much																								
Comments																								

Appendix D: Photo Gallery

Separating seeds from amloki fruits

Separating seeds from papaya fruits

Boiling amloki-papaya jam

Amloki-papaya jam

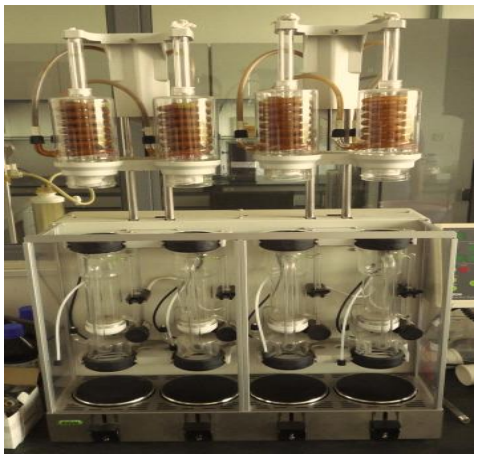
Amloki-papaya jam

pH determination

Acidity determination

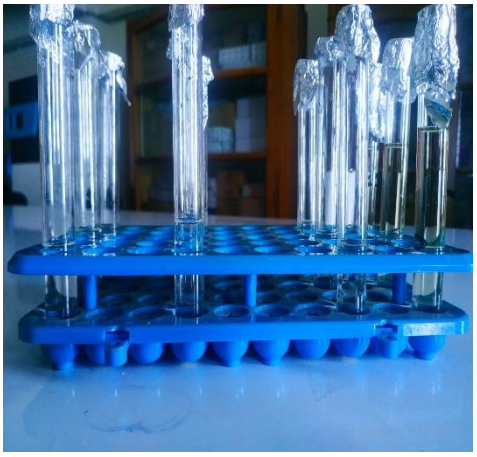
Crude fiber determination

Protein determination



Fat determination

Preparing sample for ethanoic extract preparation



Ethanoic extract preparation

Preparing sample for spectrophotometric analysis

Working in UV-visible spectrophotometer

Microbial analysis

Sensory evaluation

Brief Biography

Israt Yeasmin Rafe passed the Secondary School Certificate Examination in 2012 from Dhurung Khulshi Lions High School, Chattogram and then Higher Secondary Certificate Examination in 2014 from Kapasgola City Corporation Women College, Chattogram. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.