

# **Characterization and Utilization of *Syzygium cumini* (Jamun) Seeds**

**A**

**Dissertation Report**

**Submitted in Partial Fulfilment of the Requirements**

**For the Award of Degree of**

**Master of Technology**

**In**

**Biotechnology**

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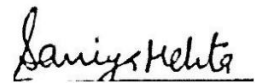
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**July 2022**

## DECLARATION

I hereby declare that the work which is being presented in this dissertation entitled “Characterization and Utilization of *Syzygium cumini* (Jamun) Seeds” in partial fulfillment of the award of the degree of Master of Technology in Biotechnology, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala. This is an authentic record of my work under the guidance of Dr. Ovais Shafiq Qadri (Assistant Professor), Thapar Institute of Engineering and Technology, Patiala. I have not submitted the matter embodied in the thesis for the award of any other degree or diploma.



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Certified that the above statement made by the student is correct to the best of our knowledge and the matter embodied in this dissertation has not been submitted to award of any Degree or certificate in any other university/institute.



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## **ACKNOWLEDGEMENT**

First of all, I am highly grateful to the almighty, the one and only who watches over us from the above, who pours his blessings on me every time and that helped me in completing this work even though I lost my patience sometimes, when I was not able to understand some things or when I was afraid that I won't be able to complete it. Moreover, I would really like to appreciate some of the individuals who were with me on this whole journey and helped me whenever I needed it.

I would really like to express my gratitude, respect, and honour to my supervisor Dr. Ovais Shafiq Qadri, Assistant Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, for his guidance, precious time, and also being patient in dealing with my problems as well as my mistakes and for the continuous encouragement, constant supervision and giving me all the small tips so that I can do my best in completing this project. I would never have done this without his help.

In addition, I would like to extend my sincere thanks to the non-teaching staff who were always available to assist me with the materials that I needed to do my study and also for guiding me with practical suggestions too.

My special thanks go to Mr. Nirmalya Halder and Ms. Darshanjot Kaur, Research Scholars, Thapar Institute of Engineering and Technology, Patiala, who were always available and helped me with the laboratory work. Without their constant cooperation and advice, I would never have been able to succeed in doing my experiments.

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## LIST OF ABBREVIATIONS

JSP	Jamun Seed Powder
MMT	Million Metric Tons
GAU	Gujrat Ayurved University
CIAH	Central Institute for Arid Horticulture
GP	Growth Performance
EAA	Essential Amino Acids
NEAA	Non-Essential Amino Acids
HCN	Horse chestnut
GAE	Gallic acid equivalents
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total phenolic content
AOAC	Association of Analytical Communities
FAO	Food and Agriculture Organization

## ABSTRACT

Jamun (*Syzygium cumini*) seed is a priceless food source because it is a superior source of dietary fiber. Therefore, the proximate, functional and other characteristics of Jamun seeds were investigated in this study. Proximate analysis included the determination of moisture content (10.2 %), crude fat (2.1%), crude fibre (53.7%), crude protein (22.25 %), and ash content (2.74 %), whereas the functional properties included the determination of water holding capacity (3.02 g/g), swelling capacity 2.837 g/g), oil holding capacity (3.65 g/g), and emulsifying activity (1.05 mL/100 mL), and other properties included pH (3.89). Total phenolic content (6.21551, 6.21515, and 6.2135 mg GAE/ g DW at 25, 50, and 100 µg/ml respectively) and antioxidant activity (53.13, 74.23, and 79.91 % 25, 50, and 100 µg/ml respectively) was also determined. It was also examined how the functional and nutritional qualities of cookies would change if Jamun seed powder, were substituted for cookie flour at a percentage of 0–10%. The 7.5% JSP cookie (F4), followed by 5 % i.e., (F3), 2.5% % i.e., (F2), and 10 % i.e., (F5), received the highest ratings from panelists across the five formulations of cookies. Control cookie i.e., (F1) was the formulation that was least favoured of all. The other properties such as proximate composition (which included moisture content, crude fat, crude protein), total phenolic content, and antioxidants activity of five different formulation of cookies were also determined. Additionally, cookies that contained Jamun seed powder showed enhanced antioxidant qualities. As a result, Jamun seed powder can be produced as a useful ingredient in food items and used as a source of nutritional fiber.

## **RESEARCH GAP**

Unused or unconsumed portions of fruits, vegetables, and other food items are being wasted a lot more in recent years due to improper handling procedures or simple disposal for a variety of reasons. In addition, by-products that have been converted into edible products are just being thrown away. Jamun is a tropical fruit that is only grown in the Indian subcontinent and the surrounding areas of Southeast Asia. It is a good source of iron, minerals, and protein. Although Jamun's fresh fruit is used directly in jams, juices, and jellies, other components of the plant, primarily the seeds, bark, and leaves, can also be used as a potent medication to cure a variety of diseases, particularly due to its anti-diabetic effects. The Jamun fruit's characteristics and uses have been the subject of some research, although the seeds have not yet been fully utilized. Therefore, the purpose of this study is to evaluate the antioxidant activities of Jamun seeds and maximize their many distinctive characteristics. Additionally, the quality impact of adding the extracted seed powder to bakery goods was also researched.

## **OBJECTIVES**

1. To investigate the proximate composition, functional properties and other properties of Jamun Seeds.
2. To investigate the phenolic content and antioxidant potential of Jamun Seeds.
3. To utilize the Jamun Seed Powder in bakery products

## CHAPTER 1

### Introduction

*Syzygium cumini* (Jamun) also known as “Jambul”, “Kaala Jamun” and “Jambolan” in different parts of India belong to the family Myrtaceae (Gajera et al., 2017). It is the most important food crop throughout the tropic and subtropic areas of the world. Jamun is a good source of iron, minerals, protein, and carbohydrates. It is native to the Indian Subcontinent and is conventionally used for various medicinal values and industrial applications (Agarwal et al., 2019).



**Figure 1.1: Kaala Jamun**

The different parts of the plant mainly seed, bark, leaves, and pulp are used as an effective medicine for the treatment of various diseases, especially for its anti-diabetic properties (Dagadkhair et al., 2017). Jamun can survive and grow better under low salinity conditions, especially in alkali soils with pH ranging up to 10.5. The physical properties of the leaves of the tree are mostly firm, pinnately veined, and close together; whereas the Jamun fruit is dark purple, and the seed was recorded to be white-pink. The Jamun fruit is often consumed fresh but can also be processed into jelly, wine, jam, squash, and vinegar. The functional constituents present in Jamun impart from different therapeutic activities including antioxidant, antidiabetic, antibacterial, and antidiarrheal activity consisting of myricetin, anthocyanins, tannins, and glucosides (Kalse et al., 2016); whereas seeds of Jamun have shown anti-diabetic, anti-inflammatory and antioxidant properties (Agarwal et al., 2019). The anti-diabetic properties present in Jamun fruit help to convert starch into energy, keeping the blood sugar

levels in check (Joshi et al., 2019). Therefore, it is recommended to consume the fruit regularly as it has hypoglycaemic properties.

Jamun seed extract is a detoxifying herb that helps in the treatment of diabetes and helps maintain healthy blood sugar levels, further reducing the symptoms such as thirst and urination (Kalse et al., 2016). The polyphenolic compounds present in it also help to cure heart disease, asthma, stomach pain, and diarrhea (Singh and Sharma, 2020). It can be used as a good replacement in place of wheat flour for the production of cookies, cakes, and other bakery products as it contains enough protein, fat, and carbohydrates, and has a high amount of energy and minerals.

As a part of the fruit processing industry, it is important to strategically manage the huge amount of fruit waste that is being generated periodically. About 20-30% of Jamun by-products (such as seed, pulp, and flesh) are lost annually due to their processing into jelly, wine, jam, and squash (Shelke et al., 2020).

But these by-products are not only rich in phytochemicals but can also be used for the extraction of dietary fibers, phenolic compounds, sugar derivatives, and other bioactive compounds.

## CHAPTER 2

# Review of Literature

### 2.1 Jamun: History, Scientific Classification, Nutritional Value, and Other Properties

Jamun is one of the most underutilized fruit but is gaining appeal, producing 13.5 million tonnes annually. Although this plant is native to Southeast Asia and India, it has been documented to be grown in Hawaii, Australia, the Philippines, Kenya, and Florida, among other places. Jamun is grown in nearly all of the districts including Gujrat, Bihar, Tamil Nadu, Maharashtra (Shah et al., 2016). The United States Department of Agriculture brought the fruit to Florida in 1911, and it is now widely grown in tropical and subtropical places around the world (Hameed et al., 2020).

Jamun is a member of the Myrtaceae family, which also includes the Eucalyptus tree and numerous tropical species such as cloves, nutmeg, allspice, and cinnamon (Kour and Arora, 2019). The Jamun fruits are only available once a year, in June and July, and are described as berries with a sweetish sour flavor (Shahnawaz et al., 2009).

#### 2.1.1 Botanical Aspects

Jamun is a genus of flowering plants, which includes myrtles. The genus contains over 1200 species, with a natural range extending from Africa and Madagascar through southern Asia and the Pacific. The root system is only visible on the surface (T. and Mathew, 2007). After the trunk has grown to about 20cm in diameter, it is woody and rigid, with a distinctively smooth, pale speckled bark that peels off in thin flakes (Schwag and Das, 2014).



**Figure 2.1: Jamun Tree**

Flowers are hermaphrodite, Purplish-black oval, and fragrant white blossoms in branching clusters at stem terminals. Jamun fruits come in a variety of shapes. They are big-sized, ranging in length from 1 to 5 cm, with oblong to ovoid form and curving, blunt edges (Shelke et al., 2020).

At maturity, the fruit skin can be smooth, rough, or bumpy. A layer of granular flesh of varying hues lies next to the skin. In mature fruit, the pulp can be deep purple or bluish-black with different degrees of juiciness, acidity, and sweetness. This outer flesh has a thickness of 3–20.3 mm and the juicy core pulp is usually loaded with very hard seeds (Ghosh et al., 2017).

**Table 2.1: Scientific Classification (Ahmad et al., 2019)**

Kingdom	<i>Plantae</i>
	<i>Angiosperms</i>
	<i>Endicots</i>
Order	<i>Myrtales</i>
Family	<i>Myrtaceae</i>
Genus	<i>Syzygium</i>
Species	<i>Syzygium cumini</i>

### 2.1.2 Several Important Cultivars

Some important varieties of Jamun, namely Badama, Bhado, Jethi, Ra-Jamun, Ashada, Katha, and hybrid varieties like Konkan Bahadoli, Rajendra Jamun 1, Goma Priyanka, Narendra Jamun 6 are present in different states of India.

**Goma Priyanka (GJ - 2):** It has a spreading growth habit, lush foliage, drooping branches, and a precocious early bearer. It is semi-dwarf and starts flowering in March (Singh and Singh, 2012).

**Katha Jamun:** It gets ripened in the middle of June, and for processing purposes, these types are preferable (Mishra et al., 2018).

**Narendra Jamun 6:** The fruit is of the oblong kind and has a larger fruit weight and pulp-to-seed ratio (Shakya et al., 2010).

**Krian Duat:** Larger, thicker leaves and red inner bark are present with fresh pulp.

**Ra Jamun:** Fruits are oblong with deep purple or bluish-black color at the fully ripe stage and ripen in the month of June-July (Singh et al., 2007).

**Konkan Bahdoli:** It possesses processing capabilities, small seeds, and a high pulp-to-seed ratio.

**Table 2.2: Jamun varieties in different states of India (Mishra et al., 2018)**

Varieties grown	States
Paras	GAU, Gujrat
Ra-Jamun	North Indian States
Katha	CIAH, Bihar
Konkan Bahadoli	Regional Fruit Research State, Maharashtra
Rajendra Jamun 1	Bihar Agricultural College of Bhagalpur, Bihar
Goma Priyanka	Central Horticultural Experiment Station (CHES), Godhra (Gujrat)
Narendra Jamun 6	Narendra Dev University of Agriculture and Technology, Faizabad (U.P)
CISHJ - 45	Lucknow (U.P.) & Gujrat
CISHJ- 37	Central Institute for Subtropical Horticulture, Lucknow (U.P)
CISHJ-42	Central Institute for Subtropical Horticulture, Lucknow (U.P)



**Figure 2.2: Different Jamun Varieties**

### **2.1.3 Jamun Processing**

Jamun processing is often done with Jamun pulp, which results in the manufacturing of a variety of value-added goods such as juice, jelly, cookies, ice cream, jam, chutney, and many more products. However, all of these procedures result in Jamun peel and seed core waste. However, these waste products/by-products have some value in that they can be used to make new products or as a component in the processing of other products. Jamun processing waste consists primarily of Jamun peel (Ghosh et al., 2017). Because Jamun contains a significant amount of pectin, it can be removed and used for other applications. The powdered form of peel is used in the extraction process, and involves certain chemicals such as HCl and citric acid. The extraction process is greatly aided by the employment of these chemicals for a specific amount of time, temperature, and pH. When compared to citric acid, the use of HCl for extraction results in a significant increase in pectin gain. Furthermore, the extracted pectin can be utilized to manufacture Jamun jelly.

Similarly, Maran et al., (2014) obtained Jamun seed oil utilizing a home-made supercritical fluid extraction process that uses pure carbon dioxide as well as a few other solvents. Thermal dilatation contraction is used to build the entire system. In this instance, ethanol was also utilized as a solvent, and specific conditions, such as various pressures at particular times, aid in the extraction of oil from seeds. Other methods for producing oil include soxhlet extraction, which uses a solvent like hexane. Chromatographic analysis is used to analyze the results. The findings indicated that Jamun seeds are a significant source of phenolic and anthocyanin compounds.

The processing of the Jamun plant necessitates a one-crore investment in apparatus and equipment. The cost of processing 1000 kg of Jamun to make Jamun powder is Rs.30200, which includes packaging (Muhammad and Saghir, 2011). The lack of infrastructure and sophisticated equipment in India is the primary shortcoming of these businesses.

### **2.1.4 Nutritional Importance**

Kaempferol, quercetin, myricetin, isoquercetin, myricetin-3-L-arabinoside, quercetin-3-D-galactoside, dihydromyricetin, oleanolic acid, and acetyl oleanolic acid, eugenol-triterpenoid A and B are among the phytochemicals found in Jamun. It is abundant in antioxidants, protein, fiber, antioxidants, calcium, iron, phosphorous, potassium, manganese, vitamin C and B6 (Baslingappa. et al., 2012)

Tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber, and fatty acids are all abundant in Jamun. Jamun is high in dietary fiber, vitamins A and C, folic acid, and dietary minerals, potassium, copper, and manganese, making them a popular superfruit (Ravi et al., 2003). A single common Jamun (*S.cumini*) fruit provides nearly four times the amount of vitamin C as an orange and has a generally broad, low-calorie profile of vital elements.

Different varieties of Jamun, namely Badama, Bhado, Jethi, Ra-Jamun, Ashada, Katha, and hybrid varieties like Konkan Bahadoli, Rajendra Jamun 1, Goma Priyanka, Narendra Jamun 6 were even studied (Mishra et al., 2018). The nutritional makeup of Jamun was studied, and five preserved goods, jam, jelly, chutney, RTS, and candy, were created from these kinds. The efficiency with which these items are made from various species of Jamun varies. Furthermore, the amount of nutrients available in different Jamun cultivars varies. The composition is also unique. Vitamin C, carbohydrate content, protein content, and mineral content were shown to have distinct chemical compositions in different Jamun.

The processing potential of different Jamun types, including Badama, Bhado, Jethi, Ra-Jamun, Ashada, and Katha. These 5 types were used to make processed items such as squash, RTS, and jelly, which were then tested for quality throughout storage.

**Table 2.3: Nutritional Facts of Jamun (Baraiya et al., 2015)**

<b>Nutrient</b>	<b>Food value</b>	<b>Nutrient</b>	<b>Food value</b>
Moisture	83.7-85.8 gm	Potassium	55 mg
Protein	0.7-0.129 gm	Copper	0.23 mg
Fat	0.15-0.3 gm	Sulphur	13 mg
Crude Fibre	0.3-0.9 gm	Chlorine	8 mg
Carbohydrates	14.0 gm	Vitamin A	80 I.U.
Ash	0.32-0.42 gm	Thiamine	0.008-0.003 mg
Calcium	8.3-15 mg	Riboflavin	0.009-0.01 mg
Magnesium	35 mg	Niacin	0.2-0.29 mg

Phosphorous	15-16.2 mg	Ascorbic Acid	5.7-18 mg
Iron	1.2-1.62 mg	Choline	7 mg

### 2.1.5 Health-Related Attributes

Bioactive substances with favorable physiological and metabolic qualities are abundant in the leaves, seeds, and peels of Jamun fruits. Its antioxidants can help to regulate body weight as well as biochemical factors like glycemia, dyslipidemia, hypertension, and other cardiovascular disease risks. Therefore, it is recommended to consume the fruit regularly as it has hypoglycaemic properties (Arya et al., 2019).

Wistar rats given Jamun seed had considerably decreased glycemia as compared to diabetic control (Srikanta et al., 2016). Furthermore, the number of steroids, glycosides, carbohydrates, alkaloids, flavonoids, saponins, tannins and amino acids in these rats increased considerably



**Figure 2.3: Jamun Health Benefits**

### 2.1.6 Physical Properties of Seed and Grains

Physical qualities can help designers create equipment for postharvest handling and processing procedures. It's also useful in farming, harvesting, and storage, as well as in procedures like drying, freezing, and others. This knowledge is critical in the design of harvesting machinery and the preparation of the grain-to-food processing chain. To build accurate machinery and processes in the food chain from harvest to table, it is necessary to first understand the physical qualities of the raw material, without which no further operations can be carried out. When considering bulk or individual units of agricultural material, it's critical to have a precise estimate of shape, size, volume, density, specific gravity, surface area, and other mechanical qualities, which could be used in the design (Dobrzański and Stępniewski, 2013).

These characteristics can then be computed using measuring techniques, which can provide information on the impacts of processing. Color, mechanical parameters, rheological qualities, thermal and electrical resistance, water content, and other physical features are some of the characteristics that help to describe the product quality (Barbosa-Cánovas et al., 2004)

An object's physical properties can contain a wide range of characteristics, which Barbosa-Cánovas et al., (2004) categorized into the following categories:

- Specific heat, boiling point, conductivity, temperature, thermal transfer, diffusivity, and boiling point rise and freezing point depression are examples of thermal properties.
- Color, but also gloss and translucency, are optical qualities.
- Electrical qualities, such as conductivity, resistance, dielectric, conductivity, impedance, inductance, magnetic field, magnetic flux, and permittivity, are all examples of electrical properties.
- Mass, density, particle size, shape, volume, length, location, porosity, surface roughness, and cellularity are structural and geometrical qualities.
- Mass transfer qualities (diffusivity, permeability), surface tension, cloud stability, gelling ability, and radiation absorption are among the others.

Much research has been conducted on various types of waste created by the fruit processing industry, including seeds, peels, and grains, to determine their physical qualities. In the following section, we will look at some of the studies that have been done on the physical properties of various seeds.

The physical properties of any seed play a vital function in the development of any kind of food processing machine, according to Hamdani et al., (2014), which is why it needs to be analyzed. Therefore, the qualities of barley and oats were identified in this study, which included size, shape, density, and gravimetric properties, as well as the properties of flours, which included angle of repose, flowability, and other aspects of different types of grains that have been compared and evaluated with others.

### **2.1.7 Proximate Analysis**

The term "proximate analysis" refers to a method for determining the levels of macronutrients in food samples. Those values are generally disclosed as nutritional information on the labels of finished (end) food products, but they are also determined throughout the manufacturing process.

"Proximate analysis" is a major commercial concern since food manufacturers must verify that their products comply with applicable laws and legal disclosure requirements, as well as the end product's safety when it is distributed to the public. Food manufacturing organizations must develop dependable analytics tools to assess production alongside the existing manufacturing process to achieve industry-required standards and remain competitive in the market.

Proximate Analysis is a classification system that divides molecules in a feed into six groups based on their chemical features (Raza et al., 2015).

Many studies have been conducted to determine the proximate analysis of a variety of foods, including the following:

- Moisture
- Ash
- Crude protein
- Crude fat
- Crude fiber
- Nitrogen-free extracts

In 2013, proximate analysis was performed on *Citrus sinensis* waste, which included dried and ground wastes, as well as phytochemical screening on ethanolic and hydroethanolic extracts. The flavedo and seeds, according to a proximate study, are high in oil. The albedo had an ash

content of 0.85- 0.03 % and the seeds had an ash content of 2.00- 0.06 %. The albedo was found to have the highest moisture and nitrogen-free extract content, as well as the lowest protein, ash, and fat content. The flavedo had the most fiber level, as well as the highest protein content, while the seeds had the lowest. All of the extracts included alkaloids and steroids, according to phytochemical analysis (Oikeh et al., 2013).

Similarly, in 2011, a study of *Datura innoxia* leaf, seed, stem, pod, and rod was conducted. The results of the phytochemical screening revealed the presence of atropine, scopolamine, essential oils, saponins, flavonoids, phenols, and glycosides, but no tannins or carboxylic acids in any of the plant components studied. The proximate study revealed that crude protein content varied from 2.09 in the root to 17.21 percent moisture content (3.5 in the root to 15% in the stem), and crude lipid content varied from 6% in the root to 15.52 percent in the seed. The roots had the largest ash level as compared to the seeds, while the nitrogen-free extract in the pod and stem was 47.97 percent and 20.88 percent, respectively.

### **2.1.8 Utilization of JSP in bakery products**

Many researchers incorporated JSP into wheat flour for the production of different bakery products and to upgrade the quality and quantity of carbohydrates, protein, and fiber content. Even though wheat flour is a good source of nutrients, mixing it with JSP could increase its shelf life and other nutritional properties. Conventionally, Jamun flesh is utilized for the preparation but due to a large amount of by-product wastage, this, distinctive method to prepare value-added products using JSP and other ingredients for the preparation of bakery products such as cookies, cakes, and biscuits and nutrition-enriched bakery products can be developed (Marufa et al., 2019).

According to a study by Nyam et al. (2012), substituting roselle seed powder for cookie flour in varying amounts (0–30%) altered the functional and nutritional characteristics of cookies. In comparison to control cookies (0.90 g/100 g), cookies that had 20 % roselle seed powder added had a higher quantity of total dietary fiber (5.6 g/100 g). Additionally, these cookies showed enhanced antioxidant qualities. Thus, research demonstrated that roselle seed powder may be used as a source of dietary fiber and developed as a useful ingredient in foods.

Another study by Katte et al., (2021), treated cookie samples with jamun powder, which statistically outperformed all other treatments for the overall acceptance measure. Increased

quantities of jamun powder in cookies have been shown to have an impact on the chemical made up of cookies.

## CHAPTER 3

### Materials and Methods

Ripe and fresh Jamun were purchased from the local market, Patiala. Based on the various fruit sizes and shapes, handpicking was done. The Jamun fruit's core, which contains the seeds, was first removed as part of this procedure.

#### 3.1 Extraction of Jamun Seeds from core

There are a few preliminary procedures that must be carried out to prepare the Jamun seeds to analyze their physical characteristics. To get rid of superfluous material, the fruits were rinsed with tap water. In hot water, the fruits were blanched. Blanching is used to remove the enzyme activity in vegetables and some fruits. To avoid hot water damage, the fruits were blanched by immersing them in boiling water for 2-4 minutes, depending on the size of the fruit (the period of blanching increases with increase in size) which is followed by immediately washing it with cold running water to minimize the damage.

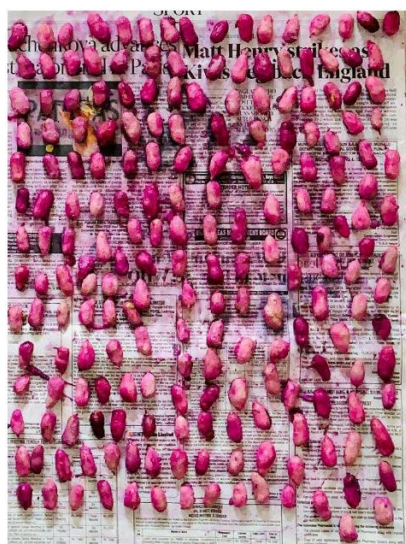


Figure 3.1: Extracted seeds from Jamun

#### 3.2 Drying

Before being examined for various characteristics, the seeds retrieved from the Jamun were dried. To lower the moisture content, hot air drying at 100° C was used. To preserve the viability and vigor of the seed during storage—which would otherwise quickly degrade due to

the formation of mold or increased microbial activity—seed drying lowers the moisture content.



**Figure 3.2: Dried Jamun seeds**

### **3.3 Grinding**

After drying, the seeds were cooled down to room temperature and then grinded into flour and reduced to a very fine powder using sieving and packed in aluminum packs and stored in the cold storage room until further use.



**Figure 3.3: Jamun Seed Powder**

### **3.4 Characterization of Jamun seed powder (JSP)**

#### **3.4.1 Proximate Analysis**

According to the AOAC procedure, the moisture, crude protein, crude fat, crude fiber, ash content, and nitrogen-free extract (NFE) of Jamun seed were measured.

### 3.4.1.1 Moisture Content

A.O.A.C. technique (2011) was used to determine the moisture content of samples. 1.2111 gm of the sample was weighed and transferred to pre-weighed Aluminium foil. The weighed sample was then dried for two hours at 100 degrees in a hot air oven. The aluminium foil containing the dried sample was weighed when it reached room temperature. The following equation was used to determine the moisture content (Raza et al., 2015);

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

### 3.4.1.2 Crude Protein

Using two to four repetitions for each sample, the micro-Kjeldahl method (AOAC, 1984) was used to assess the total nitrogen content of the seeds. Johann Kjeldahl, a brewer, created the Kjeldahl technique in 1883. A suitable titration approach can be used to determine the nitrogen that is released when food is digested with a strong acid. The protein content is not directly assessed by the Kjeldahl method; rather, a conversion factor (F) is required to convert the nitrogen concentration that was measured to a protein concentration. However, this is simply an average figure, as each protein has a distinct conversion factor depending on its amino-acid makeup. Many applications utilize a conversion factor of 6.25, which is equivalent to 0.16 g nitrogen per gram of protein whereas, 5.18-5.46 is for nuts and seeds. The method can be divided into three steps: Digestion, Neutralization, and Titration (Raza et al., 2015).

Digestion: The digestion tube containing the flour sample (1 g) is filled with 25 ml of sulfuric acid, 10 g of potassium sulfate, and 1 g of copper sulfate. The digestion tube is then heated to 350°C for three hours.



**Figure 3.4: Sample Preparation for Kjeldahl Digestion Process**

Distillation: After three hours, the machine is turned off, and 180ml of 40 percent NaOH and 50ml of distilled water are poured into the tube. Then, 4% boric acid is added to a flask containing 100 ml of ethyl alcohol, 0.099 gm of bromocresol green, and 0.066 gm of methyl red. In the end, green color is seen which contained ammonia in it and that is our Nitrogen content extracted from the sample.



(a.)



(b.)

**Figure 3.5: (a.) Sample Preparation for Distillation process and (b) Result after the distillation process of Protein Estimation**

Titration: Treatment of solution with 0.1N HCl, and endpoint came out to be a light pink color. The following equation was used to determine crude Protein;

$$\text{Nitrogen (\%)} = \frac{(\text{Sample Titre} - \text{Blank Titre}) \times 14 \times N \times 100}{W \times 1000}$$

where, N = Normality of the standard HCl solution

W = Weight of the sample (g).

The amount of protein is calculated by multiplying with a conversion factor of 5.46

Protein (%) = Nitrogen (%)  $\times$  5.5

### 3.4.1.3 Crude Fat:

The crude fat content was measured using petroleum ether (solvent) with the Soxhlet system. In the Soxhlet method, 4 gm of seed flour was placed inside a filter paper thimble that had a diameter large enough to fit inside the main chamber. A round bottom flask, a condenser, and an extracting tube make up the Soxhlet extractor. The entire extraction procedure took up to 10 to 12 cycles of solvent, with the solvent continuously refluxing over the sample to produce the final result of the glass bulb's fat content. The Round bottom flask was placed in a hot air oven set at 50°C for 17 hours after the extraction to eliminate the solvent. The following equation was used to determine crude fat (Raza et al., 2015);

$$\text{Crude fat (\%)} = \frac{(W2 - W1) \times 100}{S}$$

where W2 = Weight of sample after drying

W1 = Weight of Round bottom flask

S = Weight of Sample



(a.)



(b.)

**Figure 3.6: (a.) Soxhlet Apparatus Setup and (b.) Fat Content extracted from JSP**

#### **3.4.1.4 Crude Fibre:**

As indicated in the AOAC Method (2011), the crude fiber in fat-free samples was measured by first digesting it with 1.25 % H<sub>2</sub>SO<sub>4</sub> for 30 minutes and then with 1.25 percent NaOH solution, Following filtering, the sample was cleaned with distilled water. The remainder was weighed and put in a muffle furnace set to a temperature of 550–650°C till grey or white ash was produced. The following equation was used to determine crude fiber (Raza et al., 2015);

$$\text{Crude Fibre (\%)} = \frac{\text{Weight of digested sample} - \text{Weight of ash}}{\text{Weight of sample (g)}} \times 100$$

#### **3.4.1.5 Total Ash:**

As indicated in the AOAC Method (2011) total Ash was determined by incineration of 3.04 gm of dry sample in a Muffle furnace at 550-600°C for 2-3 hours, till greyish-white residue was formed. The following equation was used to determine Total Ash (Raza et al., 2015);

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

#### **3.4.1.6 Nitrogen Free Extract (NFE):**

NFE was calculated using the following equation (Raza et al., 2015);

$$\text{NFE (\%)} = 100 - (\text{Moisture \%} + \text{CP \%} + \text{CF1 \%} + \text{CF2 \%} + \text{Ash \%})$$

where, CP = Crude protein

CF1= Crude fat

CF2= Crude fibre

### **3.4.2 Functional Characteristics**

#### **3.4.2.1 Water holding capacity (WHC)**

30 mL of distilled water was added to 2.019 g of the dried sample, and it was then put for 24 hours at room temperature to hydrate. After the mixture was filtered, the weighted wet sample was dried in an oven at 100 °C until it reached a consistent weight. The dried sample was brought to room temperature for 15 minutes in a desiccator that included silica gel. The sample mass was measured and weighed. The following equation was used to determine the water holding capacity (Nyam et al., 2012);

$$\text{Water holding capacity (g/g)} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Dry weight (g)}}$$

#### **3.4.2.2 Swelling Capacity**

30 mL of distilled water was used to hydrate 2 g of dried material for 24 hours at room temperature. It was measured how much the swelled sample weighed. The volume of the sample was recorded. The following equation was used to determine the swelling capacity (Nyam et al., 2012);

$$\text{Swelling capacity (ml/g)} = \frac{\text{volume occupied by the sample}}{\text{Original Sample weight}}$$

#### **3.4.2.3 Oil holding capacity (OHC)**

20 mL of soyabean oil and 2 g of the dried sample were combined in a centrifuge tube. The combination was kept at room temperature for 24 hours. For 30 minutes, the sample was centrifuged at 2,200 g. Supernatant volume was measured and noted. Oil holding capacity was expressed as grams of oil held per gram of sample (Nyam et al., 2012).

#### **3.4.2.4 Emulsifying Activity**

2.0597 g of dried material was homogenized for 2 minutes at 250 rpm in 100 mL of distilled water. 100 mL of soyabean oil was then added to the mixture and homogenized for 1 minute at 250 rpm. A 40 mL sample of the created emulsion was centrifuged at 1,200 g for 5 minutes. The volume of the emulsion layer per 100 mL of the mixture was used to measure emulsion activity (Nyam et al., 2012).

### **3.4.3 Other Properties**

#### **3.4.3.1 Determination of pH**

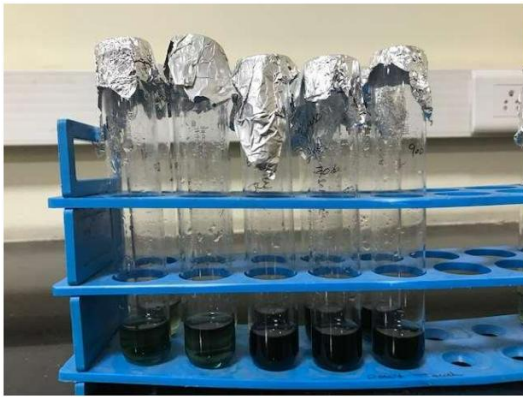
A.O.A.C. technique (2000) was used to determine the pH of the sample. The pH was measured using a pH meter. The electrode was placed right into the sample solution that had been taken in the beaker. After the initial reading was finished, the electrode was cleaned with distilled water and then dried with tissue paper (Muhammad and Saghir, 2011).

#### **3.4.4 Sample preparation for total phenolic and nutraceutical potential**

The flour was first extracted with petroleum ether to remove lipophilic components before being used as an extract for testing the total phenolic and antioxidant activity. After that, 2.011 g of defatted flour was combined with 80 % methanol (1:10 w/v) and sonicated for 30 minutes at room temperature using a water bath at 40 kHz. After obtaining the sample extracts, the clear supernatant was retrieved and evaporated to dryness in a hot air oven at 50° C overnight. For further analysis, the dried extract was re-dissolved in methanol at concentrations of 25, 50, and 100 g/ml (Manzoor et al., 2021)

##### **3.4.4.1 Total Phenolic content**

Gallic acid was used as a standard in the Folin-Ciocalteu assay, which was used to determine the total phenolic content. 100 mL of the phenolic extract made as described above was mixed with 250 ml of the Folin-Ciocalteu reagent and 4 ml of distilled water. After vortexing, the mixture was let stand for three minutes. The solution mixes were incubated for five minutes at room temperature. Following that, 20% of a 1 mL sodium carbonate solution was added to the water, and 10 mL of distilled water was used to make the volume. A UV-Vis spectrophotometer was used to measure the sample absorbance at 685 nm after the solution mixtures had been incubated in the dark for 20 minutes. The amount of total phenol per gm of material was represented as mg gallic acid equivalents (Sharma et al., 2020).



(a.)



(b.)

**Figure 3.7: (a.) Standard preparation and (b.) Sample preparation for TPC**

### 3.4.4.2 Nutraceutical potential

#### 3.4.4.2.1 DPPH radical scavenging activity

DPPH solution (0.1 mmol/L in methanol) was combined with 2 mL of sample extracts at different concentrations (25, 50, and 100 g/mL) and incubated for 30 minutes in the dark. Using a UV-vis spectrophotometer, the decrease in absorbance of the sample extracts was measured against a blank (methanol) at 517 nm. Ascorbic acid (VC) was used as a positive control. The following equation was used to determine the scavenging effect (Manzoor et al., 2021):

$$\% \text{ DPPH * scavenging activity} = \frac{A_0 - A}{A} \times 100$$

A

where, A= Absorbance of sample extract after 30 mins

A<sub>0</sub>= Absorbance of DPPH



(a.)



(b.)

**Figure 3.8: (a.) Standard preparation and (b.) Sample preparation for DPPH**

### **3.5 Utilisation of Jamun Seed Powder (JSP)**

Wheat Flour (WF) and other ingredients used in cookies were purchased from the local market, Patiala.

#### **3.5.1 Preparation of cookies**

The recipe provided by Tyagi et al. (2007) was used to make the cookies. Based on the flour weight the recipe for the control cookies was 100 g of fine wheat flour, 23 g of sugar, 14 ml of oil, 14 g of margarine, 0.3 g of salt, and 0.2 g of sodium bicarbonate, and 16 ml of water. In place of flour, Jamun seed powder was added to cookies in percentages of 2.5, 5, 7.5, and 10% without affecting the other ingredients. Dough sheeters were used to manually prepare the batter in a lab and sheet it out to a thickness of 14 mm. The cookies were moved to a baking tray that had been lightly oiled after being cut with a cookie die to the correct diameter. The cookies were baked in an electric oven that had been preheated to 180 °C for 12-15 min.



(a.)



(b.)



(c.)



(d.)



(e.)

**Figure 3.9: Sample preparation of cookies at (a.) ctrl (b.) 2.5% (c.) 5% (d.) 7.5% (e.) 10% conc.**

### **3.5.2 Quantitative Analysis of cookies**

For this, Proximate Analysis (Moisture content, Crude Fat, and Crude Protein), Total Phenolic Content, and Nutraceutical potential (DPPH radical scavenging activity) tests were carried out. According to the procedures given above, whereas, 7 untrained panelists used the Hedonic test to assess the cookies' appearance, scent, texture, flavor, and overall acceptability (Hussain et al., 2006).

## CHAPTER 4

# RESULTS AND DISCUSSION

### 4.1 Jamun Seed Powder Analysis

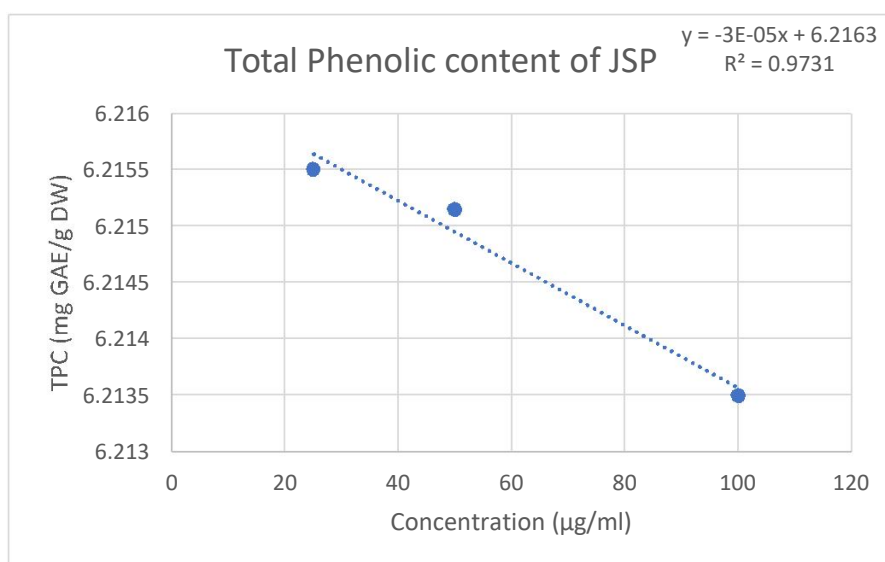
The results of the proximate analysis, functional properties and other properties produced from JSP are displayed in Table 4.1.

The moisture content of JSP was observed to be 10.2 %. Jamun seed has substantially less crude lipid i.e., 2.1% as compared to Nyam et al. (2014) (i.e., 17.4 %). This was most likely caused by variations in the organic solvents utilized in the analysis of crude fat as opposed to the study, which employed hexane, we used petroleum ether in the process. In addition, environmental and genetic factors may have an impact on the lipid content (Izquierdo et al., 2012). The Jamun seed's high crude fiber content i.e., 53.7 % suggested that it might be a reliable source of dietary fiber. Nyam et al. (2014) showed that the amount of soluble and insoluble fractions in JSP was balanced. Therefore, the physiological effects of Jamun seed are efficient in reducing the risk of obesity, glycaemic response, colon cancer, gastrointestinal disease, and cardiovascular disease. The effects of processing, such as boiling, and heat treatment, led to a significantly higher protein content i.e., 22.25 %, than that reported by Nyam et al. (2014) (i.e., 13%). The ash content was higher i.e., 2.74% than that reported by Nyam et al. (2014) (i.e., 1.1%).

High Water holding capacity (WHC) (3.02 g/g) than that reported by Nyam et al. (2014) (i.e., 2.1 g/g) was found in JSP due to the high quantity of soluble fiber content, along with pectin and mucilage (Betancur et al., 2004). A high Oil holding capacity (OHC) (3.65 g/g) than that reported by Betancur et al. (2004) (i.e., 2.7 g/g) was also observed, which could be attributed to the sample's high cellulose content, as a result of which oil is being trapped on the surface of the fiber, with sources rich in lignin and hemicellulose having a higher capacity to store oil and greater particle size (Betancur et al., 2004). Low Emulsifying Activity (EA) (1.05 ml/100 ml) in JSP than that reported by Betancur et al. (2004) (i.e., 49.3 and 8.6 ml/100 ml, for *P. lunatus* and *C. ensiformis* respectively) is probably associated with high fiber as dietary fiber's ability to bind bile acids reduces absorption in the small intestine, enables excretion in the faeces and lowers blood cholesterol levels, all of which contribute to emulsification. High

Swelling capacity (SC) (2.837 g/g) than that reported by Nyam et al. (2014) (i.e., 1 g/g) in the JSP was also observed which could be due to a higher number of free hydroxyl groups.

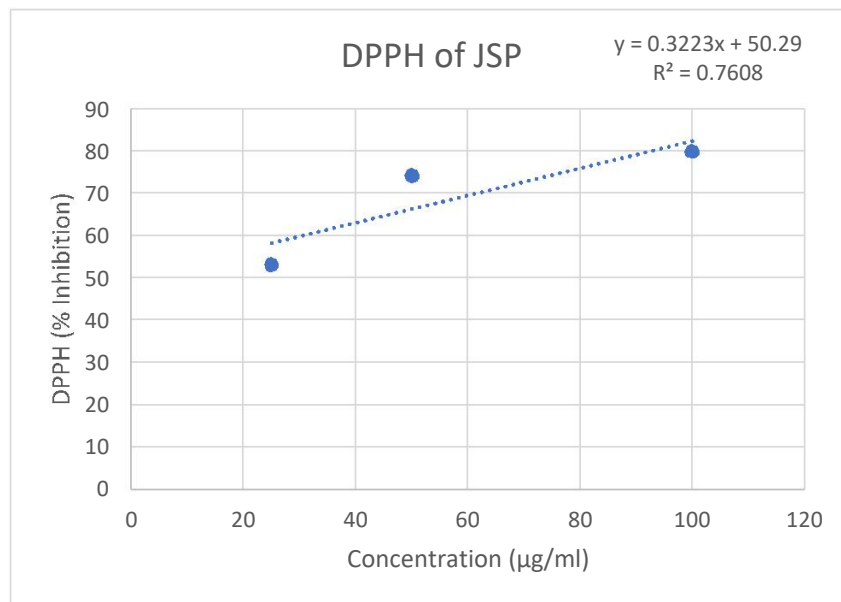
High pH (3.89) than that reported by Muhammad and Saghir, 2011 (3.87) was observed in JSP due to the presence of different phytochemicals such as gallic acid and ellagic acid (EA).



**Figure 4.1: Total phenolic content at different conc.**

According to reports, Jamun seeds are a good source of polyphenols. These compounds mostly include different combinations of flavonols, dihydrochalcones, and flavan-3-ols. The result was expressed as the mg gallic acid equivalents per gram of sample. The total phenolic content of JSP was observed to be 6.21551, 6.21515, and 6.2135 mg GAE/ mg DW at 25, 50. and 100 µg/mL.

Given the foregoing, the phenolic content of Jamun fibrous residues may be responsible for its antioxidant effects. The result of total phenolic content is displayed in Table 4.2.



**Figure 4.2: DPPH at different conc.**

For a reasonably quick assessment of free radical scavenging ability, DPPH is a reliable method. Even at a low concentration, the assay is very sensitive to active components. The stable DPPH radical is reduced to the equivalent hydrazine in the presence of a proton-donating material, and the photochemical evaluation is done by measuring the absorbance at 517 nm. The DPPH radical scavenging activity of JSP was observed to be 53.13%, 74.23%, and 79.91% at 25, 50, and 100 µg/mL respectively, which was found to be high as that reported by Manzoor et al. (2021) in apple seed flour (i.e., 42.7, 55.3, 82.3% at 25, 50, and 100 µg/mL respectively). A high DPPH radical scavenging activity may be attributed to the presence of a greater concentration of intrinsic phenolic compounds that can act as hydrogen or electron donors to free radical species for the prevention of chain initiation. The results of the antioxidant produced from JSP properties are displayed in Table 4.2.

**Table 4.1: Proximate composition, functional properties and other properties of JSP**

Analysis	Results
<b>Proximate properties</b>	
Moisture (%)	10.2
Crude fat (%)	2.1
Crude fiber (%)	53.7

Crude protein (%)	22.25
Ash content (%)	2.74
Nitrogen-free extract (%)	9.01
<b>Functional Properties</b>	
Water holding capacity (g/g)	3.02
Swelling capacity (g/g)	2.837
Oil holding capacity (g/g)	3.65
Emulsifying activity (mL/100mL)	1.05
<b>Other Properties</b>	
pH	3.89

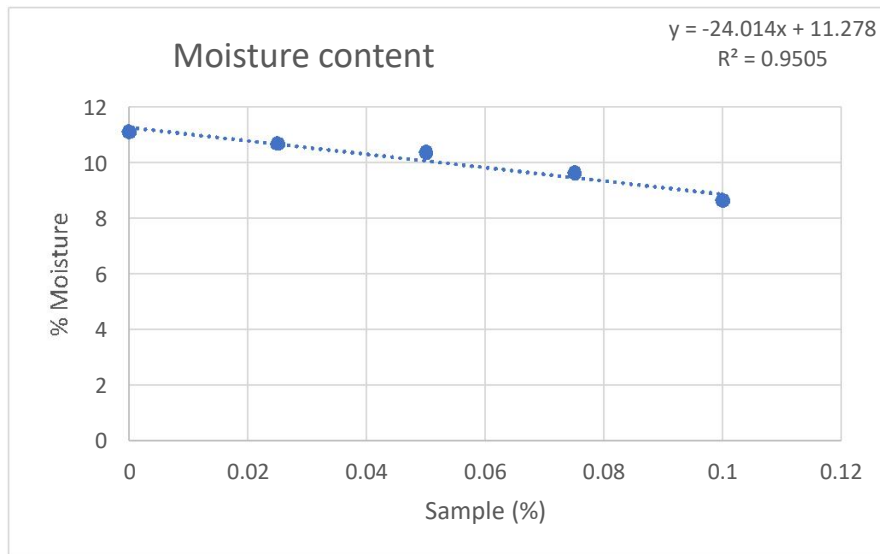
**Table 4.2: Total Phenolic content and Antioxidant activity of JSP**

<b>Analysis</b>	<b>25 µg/ml</b>	<b>50 µg/ml</b>	<b>100 µg/ml</b>
Total phenolic content (mg GAE/g DW)	6.21551	6.21515	6.2135
DPPH radical scavenging capacity (%)	53.13	74.23	79.91

## 4.2 Cookie analysis

### 4.2.1 Physical Analysis of cookies

Five formulations of cookies were prepared. 0.0 % (control, F1), 2.5 % (F2), 5.0 % (F3), 7.5 % (F4), and 10% (F5) of JSP was incorporated into cookie flour, respectively and their physicochemical properties are shown in Table 4.4. The moisture content of all cookie formulas was in the range of 7.0- 12.0 %. The formulations of the cookie with the higher amount of JSP had lower moisture content. The results showed a significant difference between the control cookies with the other four formulations of cookies that were added with JSP.



**Figure 4.3: Moisture content of cookies**

#### 4.2.2 Sensory evaluation

The five samples were subjected to a hedonic test to measure customer approval for the sensory qualities of appearance, aroma, texture, flavor, and overall acceptance. The results were summarized in Table 4.3. The appearance of F4 was the most preferred by panelists, followed by F3, F2, and F5. The appearance of F1 was the least preferred because the color of F1 was pale as compared to F4. F5 looked burnt after baking due to the high-level incorporation of JSP added into cookie making. The aroma for F4 was the most preferred by panelists, followed by F3, F2, F5, and lastly F1. However, F2 and F5 did not show significant differences because their aroma was similar even though different levels of JSP were incorporated into the cookie. In terms of flavor, most panelists preferred both F2 and F3, followed by F4, F5, and F1. The flavor of the cookies was improved with the incorporation of JSP because these cookies had a typically pleasant Jamun seed flavor. The control cookie (F1) was significantly different from the other three formulations (F2, F3, F4, and F5). The texture of cookies F4 was the most preferred by panelists, followed by F3, F5, and F2, while the least preferred cookie was F1. F4 was the crunchiest and crispiest cookie of all. On the other hand, F1 was the hardest and the least crisp cookie. Cookie with a harder texture and the least crispness was not favoured by the panelists. In overall acceptance, the F4 cookie scored the highest among all formula cookies. This indicated that a 7.5% level of incorporation of JSP into flour was the optimum.

### 4.2.3 Proximate analysis and antioxidant activities of cookies

The proximate analysis including crude fat and crude protein was carried out and antioxidant activities, as well as total phenolic content, were also carried out. It was seen that the formulations of the cookie with the 10% JSP (F5) had lower lipid content (36.82%) when compared with the control cookies (F1) (44.92%) (as seen in Figure 4.4), than that reported by Nyam et al., (2014) where with the increase of roselle seed powder formulation (i.e., 20%) there was an increase in lipid content (26.6%), which could be attributed to the sample's high cellulose content as a result of which oil being trapped on the surface of the fiber. Whereas, the cookies with 10% JSP (F5) showed high protein content (7.62%) when compared with control cookies (F1) (6.71 %) due to the effects of processing, such as boiling, and heat treatment, which led to a significantly higher protein content (as seen in Figure 4.5), according to Nyam et al., (2014) there was also a rise in crude protein with the increase of roselle seed powder (i.e., 5.9-9.4 %). Murafa et al., 2019 found that the protein content for cakes formulation were 6.34- 8.56% i.e., the protein content of control cookies was lower than the other samples.

Other researchers found that the protein composition of composite cakes under development was generally comparable, proteins found in developed foods and cakes changed because the protein content of the flours. Temperature increases cause the degradation of Foods contain sugar and protein, which leads to denaturing of the protein (Pascual et al., 2002). This Protein denaturation could possibly be a factor in the varying levels of protein (Akter and Alim, 2018)

So, it might be concluded that JSP incorporated cookies promote the nutritional composition as well as prolong the shelf life of products due to which it could be utilized for the preparation of bakery products and other food products.

The cookies incorporated with JSP showed enhanced antioxidant capacity. The addition of JSP during cookie production significantly improved the antioxidant activity in different concentrations (i.e., 50 and 100  $\mu\text{g/ml}$ ). The total phenolic content of cookies enriched with JSP was higher (0.818 and 1.348 mg GAE/ g DW at 50 and 100  $\mu\text{g/ml}$ ) due to the presence of different polyphenols such as flavonols, dihydrochalcones, and flavan-3-ols (as displayed in figure 4.10 a.), as that of Nyam et al., (2014) where phenolic content of cookies enriched with roselle seed powder was higher than that of control samples (i.e., 60.2 and 28.4 mg GAE/ g DW for roselle seed powder and control sample respectively) and their scavenging activity was higher (98.3 and 98.4 percent at 50 and 100  $\mu\text{g/ml}$ ) than those of control cookies (0.001 and 0.153 mg GAE/ g DW, 91.3 and 85.5 at 50 and 100  $\mu\text{g/ml}$ , respectively) (as displayed in Table

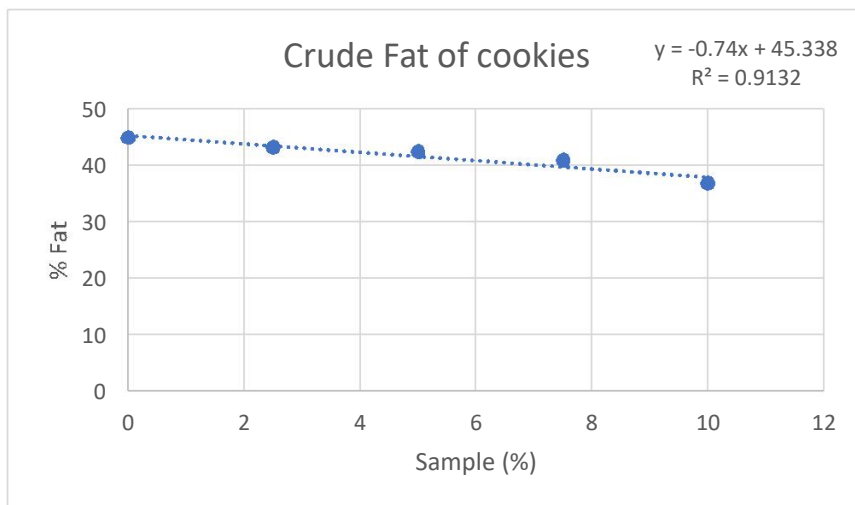
4.4 and figure 4.10 b.). It can be said that adding 10% roselle seed powder to cookies improved their antioxidant activity. A high DPPH radical scavenging activity may be attributed to the presence of a greater concentration of intrinsic phenolic compounds that can act as hydrogen or electron donors to free radical species for the prevention of chain initiation, which was found to be same as that of Nyam et al., (2014) i.e., with the increase of sample formulation, antioxidant activity increased (98.0 and 82.2 % for sample and control cookies respectively).

**Table 4.3: Sensory quality (Hedonic test) properties and proximate composition of different formulations of cookies**

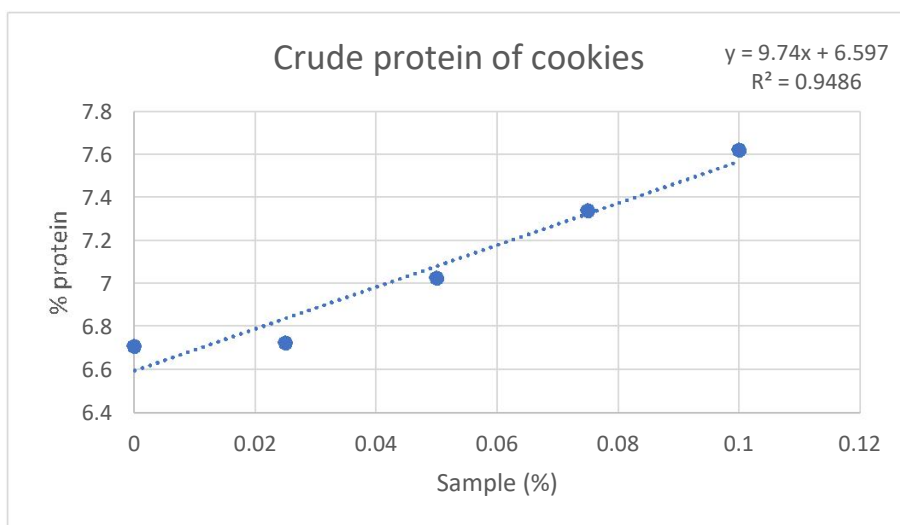
Components	F1	F2	F3	F4	F5
Moisture content (%)	11.101	10.683	10.354	9.6175	8.632
Crude fat (%)	44.92	43.2	42.35	40.9	36.82
Crude fibre (%)	6.71	6.725	7.025	7.34	7.62
Appearance	6	7	7	8	7
Aroma	4	7	8	9	7
Texture	5	6	7	8	7
Flavour	5.5	9	9	8	8
Overall Appearance	6.5	8	8	9	7

**Table 4.4: TPC and DPPH of different formulations of cookies**

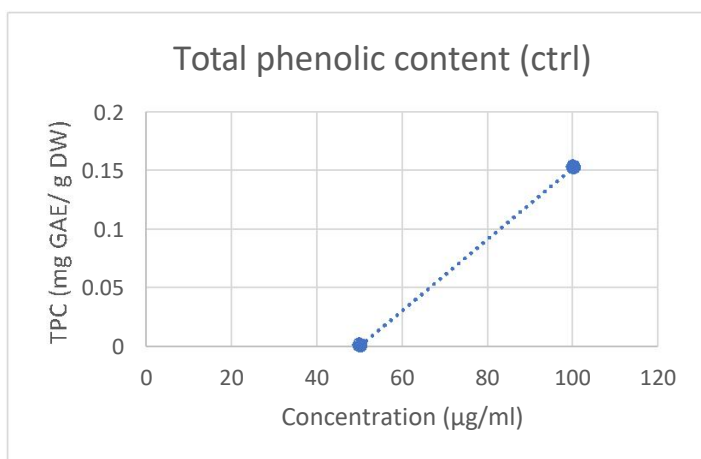
Concentration (µg/ml)	F1		F2		F3		F4		F5	
	TPC	DPPH	TPC	DPPH	TPC	DPPH	TPC	DPPH	TPC	DPPH
50	0.001	91.3	0.0013	93.2	0.356	95.3	0.519	97.6	0.818	98.3
100	0.153	85.5	0.29	88.3	0.475	95.17	0.74	97.6	1.348	98.4



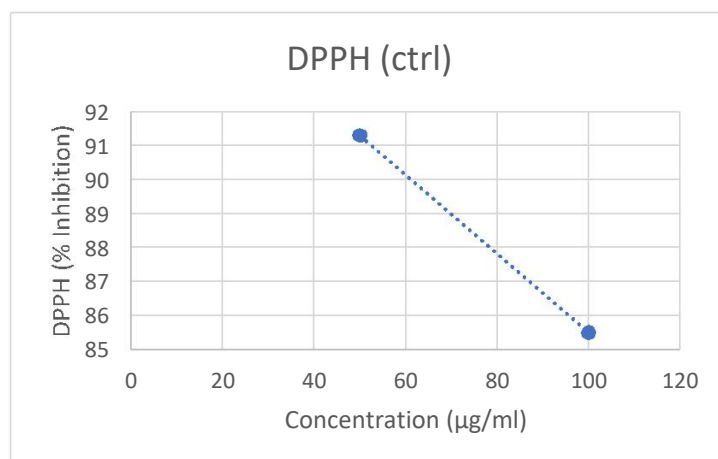
**Figure 4.4: Crude Fat of different formulations of cookies**



**Figure 4.5: Protein content of different formulations of cookies**

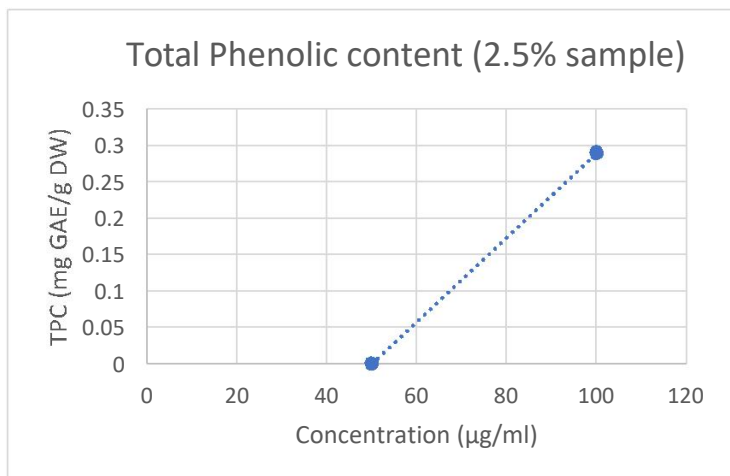


(a.)

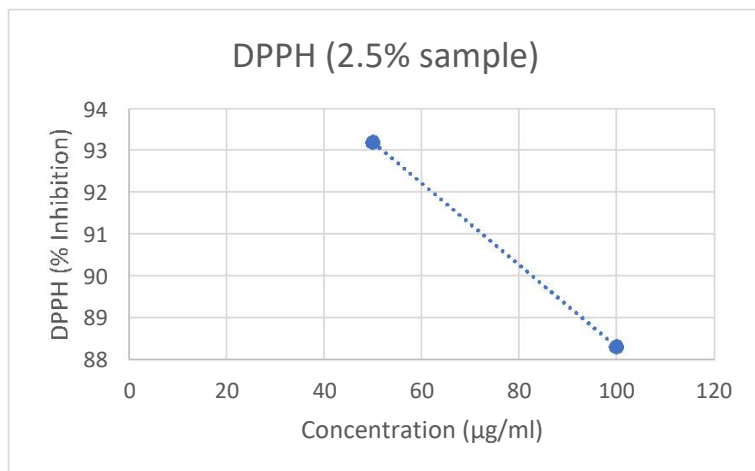


(b.)

**Figure 4.6: (a.) TPC and (b.) DPPH at 0% formulation**

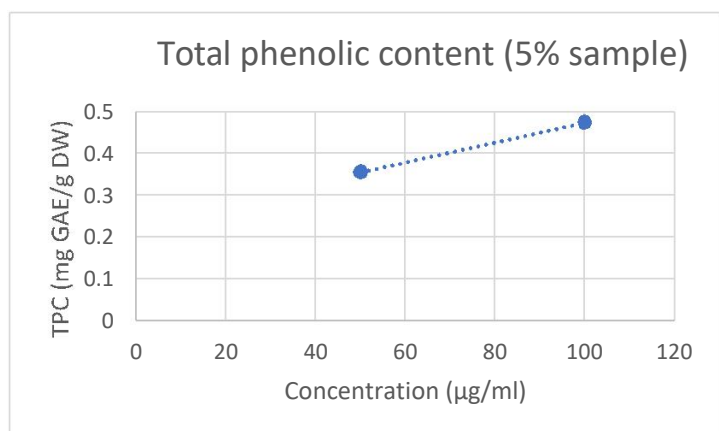


(a.)

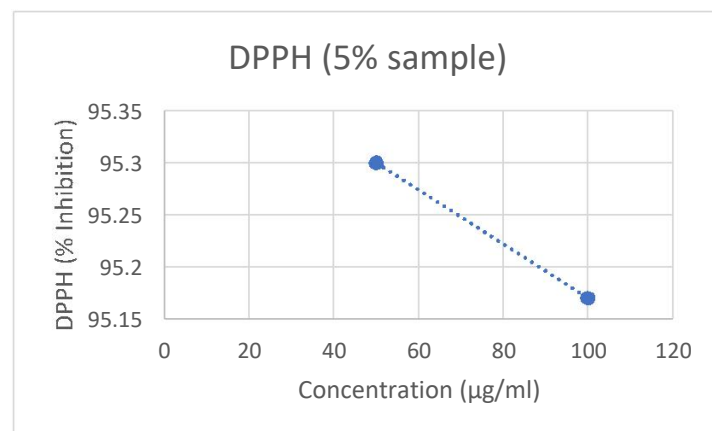


(b.)

**Figure 4.7: (a.) TPC and (b.) DPPH at 2.5% formulation**

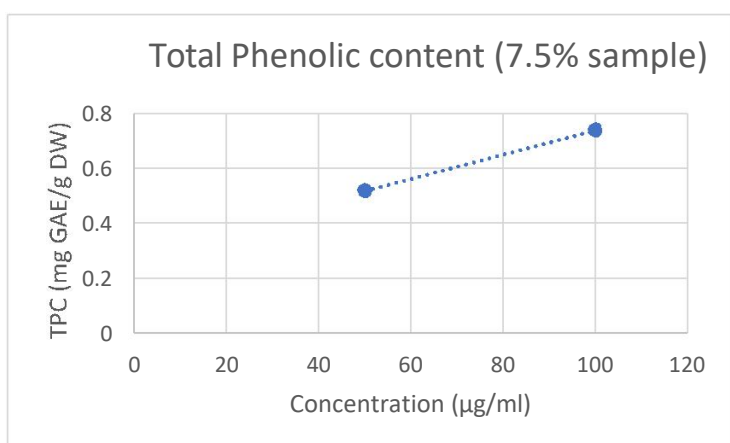


(a.)

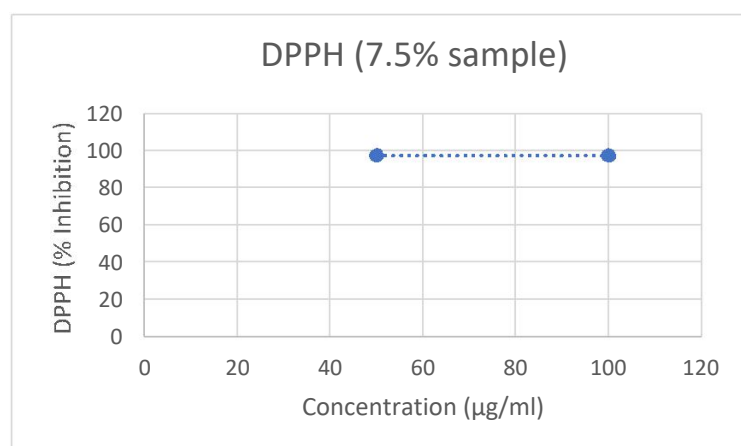


(b.)

**Figure 4.8: (a.) TPC and (b.) DPPH at 5% formulation**

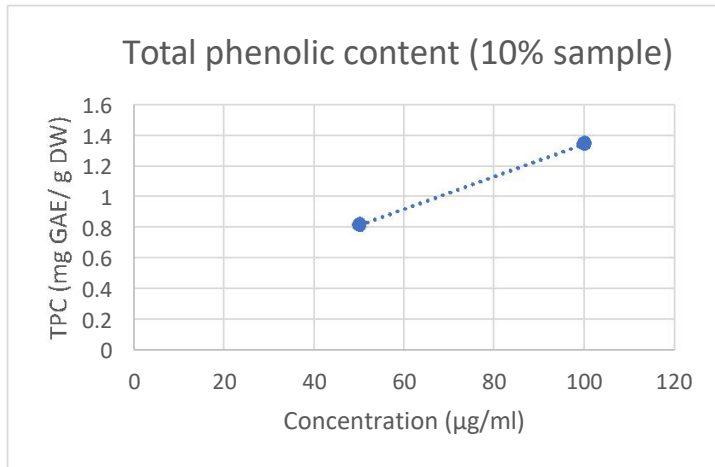


(a.)

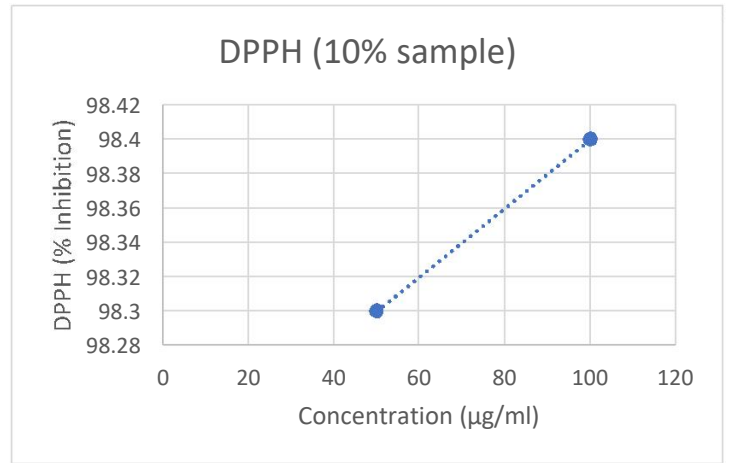


(b.)

**Figure 4.9: (a.) TPC and (b.) DPPH at 7.5% formulation**



(a.)



(b.)

**Figure 4.10: (a.) TPC and (b.) DPPH at 10% formulation**

## CONCLUSION

This study highlighted on the structural, anti-inflammatory, and anti-diabetic potential of Jamun seeds. According to this study, there are significant differences across flour samples in terms of proximate, functional, and other aspects. The flour samples were also discovered to be biologically active and abundant in polyphenols and antioxidants, which have the potential to disrupt lipid and carbohydrate metabolism for the successful control of obesity and hyperglycemia. Additionally, these seed extracts worked just as well as value-added dietary supplements. The information from this study will provide a new strategy for valuing underutilized Jamun seeds as a viable source of natural bioactive chemicals with potential use in the creation of goods that are nutritional and useful.

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