CHARACTERIZATION OF PECTIN EXTRACTED FROM POMEGRANATE PEEL (*PUNICA GRANATUM L.*) AND IT'S APPLICATION IN JELLY PREPARATION

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Roll no.: 0219/12

Registration no.: 773

Session: July-December, 2019

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

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh.

August 2022

Authorization

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

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PLAGIARISM VERIFICATION

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Department of Applied Food Science and Nutrition,
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Khulsi-4225

Dedication

This simple effort is dedicated

to

my beloved family members and

teachers.

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The Author

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List of Abbreviation

FAO	: Food and Agricultural Organization
LM	: Low Methoxyl
HM	: High Methoxyl
LMC	: Low Methoxyl Conventional
LMA	: Low Methoxyl Amidated
Kcal	: Kilo calorie
DE	: Degree of esterification
CCl_4	: Carbon tetrachloride
Ca	: Calcium
Mg	: Magnesium
EDTA	: Ethyl di amine tetra acetic acid
CDTA	: Cyclo hexane di amine tetra acetic acid
%	: Percentage
&	: And
ANOVA	: Analysis of variance
AOAC	: Association of Official Analytical Chemists
TSS	: Total Soluble Solids
^{0}B	: Degree Brix
CHO	: Carbohydrate
Dl	: Deciliter
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
^{0} C	:Degree Celsius

et al	: Et alii/ et aliae/ et alia
Etc	: Et cetera
G	: Gram
GAE	: Gallic acid equivalent
Kg	: Kilogramme
Mg	: Miligram
TE	: Trolox equivalent
Cfu	: Colony forming unit
QE	: Quercetin equivalents
GAE	: Gallic acid equivalents
SPSS	: Statistical Package for Social Science
PPM	: Parts per Million
M	: Meter
DNA	: Deoxyribonucleic acid
spp.	: Species
Mg	: Microgram

ABSTRACT

Pectin is a heteropolysaccharide found in plants used for preparation of jelly due to it gelling and texturing capacities under various circumstances. The study was carried out to determine the potential of pomegranate (*Punica granatum* L.) peel as a source of pectin and its application in making jelly. Pectin was extracted from pomegranate (dalim) peel powder using citric acid at 70℃ for 25mins at pH around 3. Pectin yield was 8.2%. The extracted pectin contains high content of methoxyl pectin, its ash content was 1.15% and water holding capacity was 235.25. This study showed that the pomegranate peel has the potential to be utilized in producing pectin commercially due to its moderate content of pectin, high water holding capacity, degree of esterification and availability of raw materials. The proximate analysis of pomegranate juice showed moisture 85.4%, 10.6% total sugars, 1.4% pectin, 0.15g/100ml total acidity (as citric acid), 0.9mg/100ml ascorbic acid and 0.03g/100ml ash %. Pomegranate jelly was made using the extracted pectin, pomegranate juice, sugar and honey. To determine consumer preferences sugar was replaced with honey in two samples. The jelly that was made with 600 ml of fruit juice, 400 g of sugar, and 10 g of pectin was found to have the highest organoleptic quality. Proximate analysis of jelly with honey showed that it has 30.70±0.01% Moisture, 1.30±0.01% crude fiber, 1.61±0.01%, ash, 1.20±0.01%, fat, 1.50±0.01% protein and 63.67±0.01% carbohydrate. Proximate analysis and sensory evaluation showed no significant difference between the jelly containing commercial pectin and prepared pectin. The resulting jelly was bottled in pasteurized Polyethylene Terephthalate (PET) bottles (500 ml capacity) and kept in a refrigerator (5±2℃) up to 90 days. The microbiological development of Jelly remained below the limit until the end of the storage period. Therefore, the prepared jelly was safe for eating for up to three months.

Keywords: Fruit peel, Pectin, Pomegranate Jelly, Proximate analysis, Sensory evaluation.

CHAPTER 1

INTRODUCTION

1.1 General Feature

People from around the world are becoming increasingly concerned about healthy lifestyles. Therefore, it might affect how people eat and what they choose to eat. Sensory qualities of food, such as taste, texture, flavour, and appearance are highly attractive to the consumers today. In addition to sensory qualities, nutritional assessment of consumed food might affect consumers' food selection. Additionally, consumers now place a higher value on wholesome, nutritious food. Fresh fruits and processed fruit products are the finest option to satisfy their requirement for consumption. Fruits in general are quite important for maintaining a balanced diet. They offer an abundance of macro and micronutrients in addition to bioactive substances that support health. An important component of the food supply is made up of both fresh and processed foods. Processed food helps with nutrition security as well as food security (the availability of enough food ensuring that food quality meets human nutrient needs). There have been numerous studies over the past few decades showing the value of fruit eating in reducing health risks, as well as campaigns to include fruit in children's diets (Kandylis et al., 2020). To supply essential nutrients, many food firms have developed fruit supplements such juice, fruit drinks, jam, jelly, fruit-based desserts with milk, fruit powder, etc. These processed foods have taken over the public's choice in snacking.

Pomegranate (*Punica granatum* L.) has been increasingly popular in recent years as people have discovered its delicious flavor, versatile use, and health benefits. The fruit is abundant in phenolics, which have been shown to lower the risk of disease, as well as other biochemicals, including tannins (Viuda Martos et al., 2010). New research shows that all parts of the pomegranate plant—the fruit, blossoms, bark, and leaves contain bioactive phytochemicals with antibacterial, blood-pressure-lowering, and disease-fighting properties. These results support pomegranates' long-held reputation as a healthful treatment (Holland et al., 2006). Punicalagin is regarded to be the main component responsible for the fruit's health-promoting qualities, with

flavonols and anthocyanins playing a less significant role (Syed et al., 2007). Numerous studies suggest that pomegranates may help treat diabetes, heart disease, and cancer. Selected bioactive components, particularly anthocyanins, is responsible for the rich color of the whole pomegranate and its juice (Kandylis et al., 2020). Therefore, the differences in these chemicals concentration between cultivars account for the majority of the color variation. Pomegranate fruit has recently experienced a worldwide surge in popularity. Despite being consumed right away, they are also used to prepare fresh juice, canned drinks, alcoholic drinks, jellies, and coloured and flavoured beverages.

Jelly is described as a semisolid food produced with at least 45 parts by weight of fruit juice and 55 parts by weight of sugar. This mixture has been condensed to at least a 65 percent soluble solid content. A fruit's shortcomings can be made up for by adding gelling agents and acid. Jellies contain a lot of acids and solids (such sugar), which makes them stable. A medium amount of water is used to make jelly, which is an intermediate moisture food. Jellies must also have specific amounts of sugar, acids, and gelling agent in order to effectively gel (Arjun Ringwal, 2019). Since excessive sugar consumption is linked to harmful health issues like obesity, metabolic syndrome, and inflammatory illnesses, substituting honey for sugar can be a healthy choice (Freeman et al., 2018). Additionally, honey has been a staple meal for *Homo sapiens* since the dawn of time. Nearly all religious texts praise this marvelously rich, golden liquid as the nectar of the gods, a healing food (National Honey Board, 2010). Honey contains a variety of phenolic chemicals that show promise in the treatment of cardiovascular disorders (Yaghoobi et al., 2008).

The key element in making jelly is pectin. Pectin can be employed for a variety of purposes because its structural variations produce a range of gelling and texturizing capacities under various circumstances. Pectin can combine with acid and sugar to generate gels. It has been used in confectionary jellies and high-sugar jams since at least the 18th century because of its tendency to gel formation (May, 2014). Pectin is being examined for a variety of uses outside of the conventional jams and jellies because it is a natural food ingredient. These days, pectin is employed as stabilizers, water binders, and thickeners. It serves as a stabilizer in drinkable yogurts and mixtures of milk and orange juice in addition to being used in pastry glazes and yogurts (Wargovich, 2009). Most commercial pectin comes from citrus pulp and peel or apple pomace. Sugar-beet pulp may also be used in the production of certain items

(Arslan & Kar, 1998). Peels from pomegranates can also be used to extract pectin (Melih G uzel et al., 2018). Many pieces of pomegranate peel go untouched when edible components of the fruit are devoured or used. Some farmers consider this peel as waste, although it can be fed to livestock or even used as fertilizer.

Food waste is the term used to describe the loss or disposal of any uneaten food along the whole food supply chain. The highest rates of food waste are found in fruits and vegetables, with an estimated 1.6 billion tonnes wasted annually in the world (FAO, 2016). Pomegranates are an import-focused fruit that are being consumed more frequently in Bangladesh as a wholesome fruit or juice. One ton of fresh fruit produces 669 kg of by-product in the pomegranate juice industry, which is made up of 78% peel and 22% seeds (Qu et al., 2009). This pomegranate peel is a good source of pectin. The pomegranate peel's pectin can be removed using a variety of tools, and this pectin can then be utilized to make jelly. The pomegranate peel can be used more effectively in this way.

1.2 Aims and Objectives

General Objective:

The aim of this study was to formulate pomegranate jelly using pectin extracted from pomegranate peel.

Specific Objectives:

1. To examine the physicochemical characteristics of pectin extracted from pomegranate peel.
2. To prepare pomegranate jelly with extracted pectin and white sugar and honey.
3. To analyze the nutritional comparison of sugar jelly and honey jelly.
4. To analyze production cost.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Pomegranate

Pomegranate (*Punica granatum* L.) belongs to the punicaceae family that is native to Persia, and is grown mostly in west Asia and the Mediterranean region, as well as other parts of the world, including America, where the climate is favourable for its cultivation (Pagliarulo et al., 2016). The words "pomegranate" and "granatus" are Latin for "apple" and "seeded," respectively. Locally, it is known as "Dalim" in Bangladesh. It is one of the earliest edible fruits that is known to have been a significant source of food and herbal medicine. It is mostly cultivated in homesteads in Bangladesh as an aesthetic tree and is usually regarded as an important minor fruit species (Sumaiya et al., 2018).

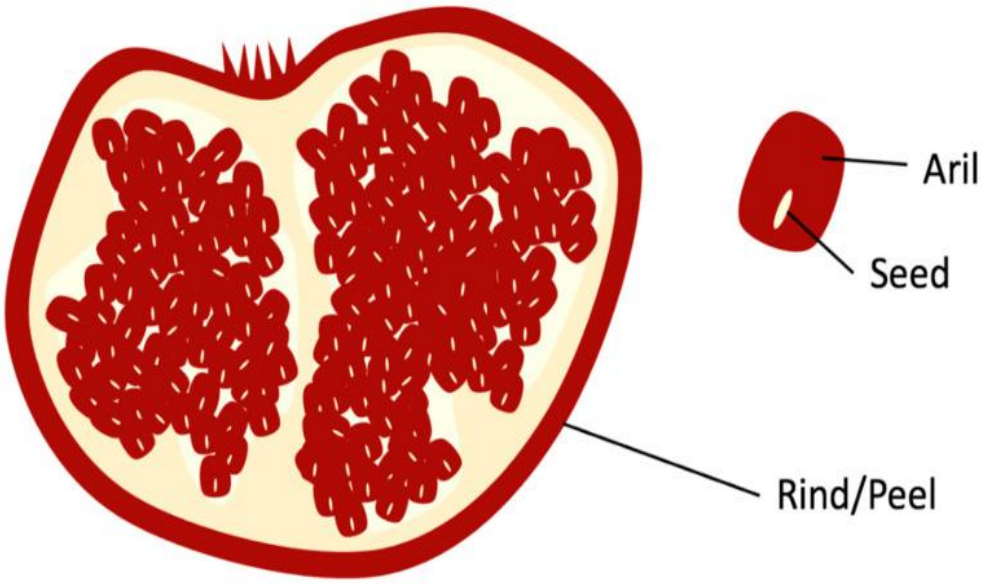

Figure 2.1: Dissection of Pomegranate

Taxonomical classification

Kingdom: Plantae

Divison: Magnoliophyta

Class: Magnoliopsida

Sub-Class: Rosidae

Order: Myrtales

Family: Lythraceae

Genus*: Punica*

Species**: *P. granatum***

The pomegranate is a fleshy berry having a crown like calyx, and it has a nearly spherical shape with a diameter of up to 10 cm. A fleshy mesocarp is housed inside the leathery exocarp and is divided into chambers by membranes. The fruit's edible part is found in the arils. Pomegranate exocarp, or the peel, makes up about 50% of the entire fruit, while the edible portion is made up of 10% seeds and 40% arils (Sreekumar et al., 2014). Pomegranate peel is only utilized in a modest amount of traditional herbal medicines. Pomegranate peel is a prospective and unique pectin resource because it has been estimated to contain 10% pectin (Abid, et al., 2016). The extraction and structural characterization of pomegranate peel pectin has been the subject of some papers recently (Abid et al.,2017), however further research is needed to fully understand its functional qualities.

2.2 Chief Constituents of Pomegranate

Pomegranate fruits have a variety of elements in their seeds, skins, and arils, among other parts. These substances play a therapeutic role in the management of health through the control of numerous biological processes. The pomegranate's peels make up around 60% of the fruit and include a variety of nutrients, including minerals like calcium, magnesium, phosphorus, potassium, and salt as well as flavonoids, ellagitannins, and pro anthocyanidin compounds (Mirdehghan et al., 2007). Massive concentrations of organic acids, carbohydrates, minerals, vitamins, and polyphenols with antioxidant properties can be found in pomegranate fruit arils (Jaiswal et al., 2010). Flavonoids are also the primary fruit polyphenols, along with condensed tannins and

hydrolysable tannins (MI et al., 2000). The common components of pomegranates include hydrolyzable tannins including ellagitannins and gallotannins, with punicalagin being the most abundant hydrolyzable tannin (Fischer et al., 2011). Gallagic acid, ellagic acid, and EA glycosides are among the hydroxybenzoic acids found in peels. Additionally, seeds contain components like protein, crude fibers, vitamins, minerals, carbohydrates, polyphenols, and the phytoestrogen coumestrol estrone (Singh et al., 1990). An experiment was conducted to investigate the antioxidant activity of pomegranate peels and seeds, and the results revealed that both peel and seed extracts have antioxidant activity. Additionally, the maximum antioxidant activity of all of the studied extracts was found in the methanol extract of peel (Singh et al., 2002). According to a different study, enzymes like catalase, peroxidase, and SOD were preserved after pre-treatment of peel extract with CCL_4. In addition, lipid peroxidation was reduced by 54% against control (Kotamballi et al., 2002). The evaluation of the antioxidant activity of pomegranate peel powder and whey powder produced the finding that both powders have antioxidant activity. Another study showed that extracts from seeds had varying levels of antioxidant activity, with sour white peel having the strongest antioxidant activity. Leaf and peel both showed very strong antioxidant activity (Sadeghi et al., 2010).

2.3 Pectin

All plant tissue contains pectin, a carbohydrate that is present naturally. Henry Braconnot initially defined and isolated the word pectin in 1825 (Braconnot, 1825). Fruit cell walls contain pectin in variable levels, and it has significant nutritional and technical qualities (Drake at el., 2002). They may be bonded covalently to other polymers and act as one of the primary factors cementing the cellulose fibrils in the cell walls. The routes for the movement of water and nutrients are provided by intracellular pectins (Mohnen et al., 2008). Pectins are a class of structurally heteropolysaccharides found in the middle lamella and primary cell walls of higher plants. They are mostly made up of covalently bonded D-galacturonic acid (GalA) units (Chan et al., 2017). For a long time, the food and beverage sectors have used these polysaccharides. Pectin is primarily used as a thickening, stabilizing, emulsifying, and gelling agent (Min et al., 2010; Nakamura et al., 2002). This conventional use in the food industry is being complimented by the rising use of pectin as a fat substitute and functional component that promotes health (Min et al., 2010).

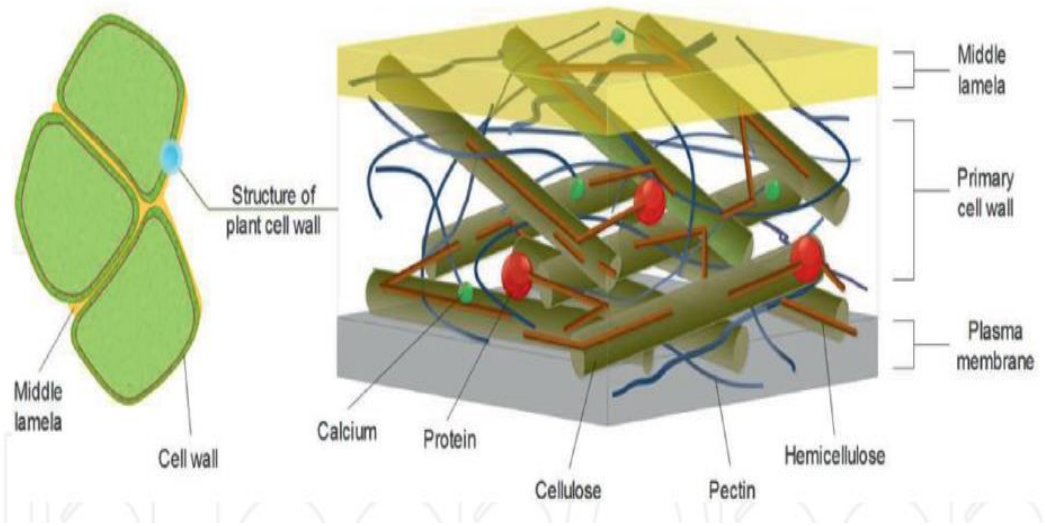

Figure 2.2: Pectin as a component of fruit cell wall

Pectin is a significant dietary fiber source that has been recognized as a new prebiotic with enhanced therapeutic benefits for gut microbiota management (Babbar et al., 2016). Pectin can also be utilized as a stabilizer in acidic protein beverages and to enhance the mouthfeel and pulp stability in drinks with juice as the foundation. Pectin also boosts the gel strength of low-calorie jams and decreases syneresis in jams and marmalades. In confectionary jellies, pectin is employed to provide a solid gel structure and a clean bite. Pectin is typically used in food applications at a dosage of 0.5% to 1% (Buren and J.P, 2012).

2.4 Classification of Pectin:

Typically, pectin is categorized based on its methoxylation level (DM). Methoxylation is measured as a percentage of esterified galacturonic acid units to total galacturonic acid units in a pectin molecule. It affects pectin's features, particularly its solubility and gel-forming abilities.

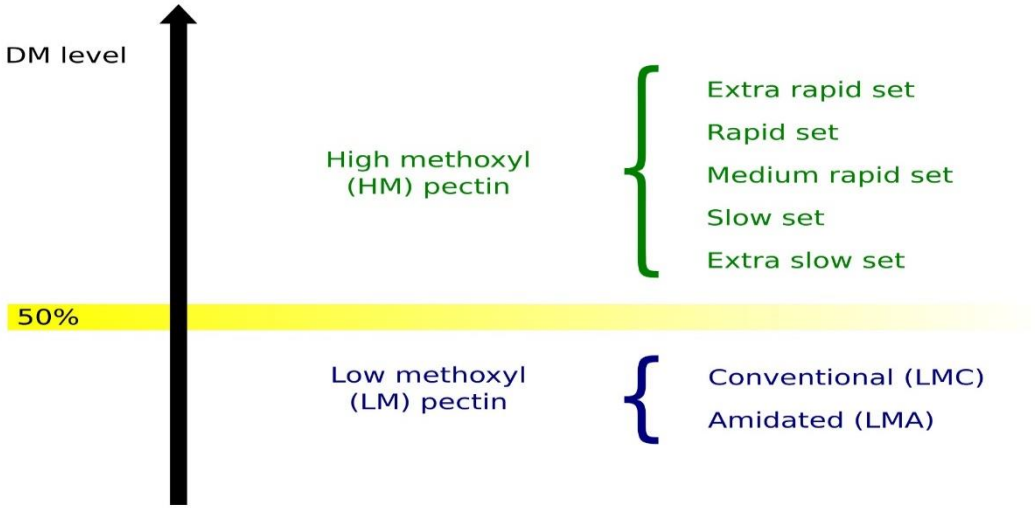

Figure 2.3 : Pectin classification

HM Pectin

High methoxyl (HM) pectin, produced using the standard extraction method, is defined as having more than 50% methoxyl groups. In aqueous systems with large concentrations of soluble solids and low pH values, they can form gels (Sharma et al., 2006).

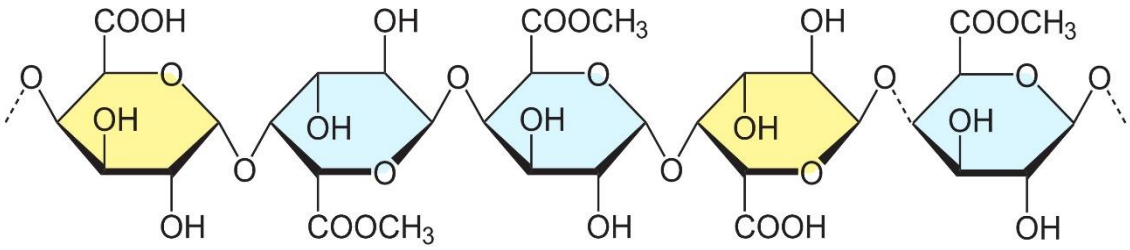

Figure 2.4: HM Pectin

LMC Pectin:

Changing the extraction method or continuing acid treatment will result in LMC pectin with less than 50% methoxyl groups.

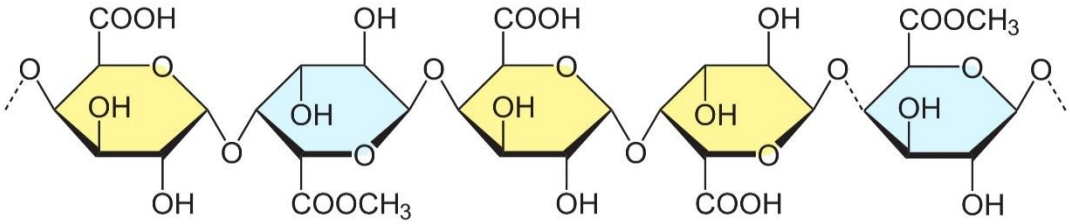

Figure 2.5: LMC Pectin

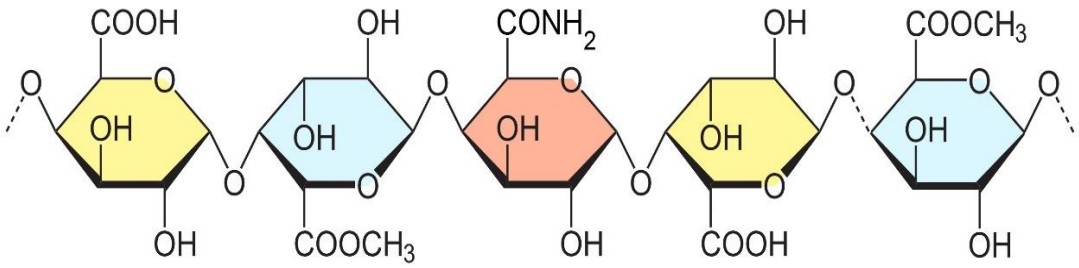

Figure 2.6: LMA Pectin

Pectin gel formation depends on methylation. Low methoxyl (LM) pectins are utilized in low-sugar goods because they can form gels without or with very little sugar and Ca2+. LM pectin forms gel at high sucrose concentrations (Wang at el., 2012).

2.5 Characteristics of Pectin

If pectin is adequately dissolved in water and stored in a cool, dry environment, it has gel-forming, thickening, and stabilizing qualities. The following are the general features of pectin:

Solubility

To enable effective use and prevent heterogeneous gel formation, pectin must be fully dissolved. Any lumps that develop during the solution's preparation cause the gel's strength to decrease since the lumps of pectin are inactive. Before dissolving the pectin in hot water (85-90°C) with a soluble solid's concentration below 20%, it is recommended that the pectin be pre-blended with sugar in a minimum 1:3 ratio using an appropriate high-speed stirrer. In the presence of gelling conditions, pectin will not dissolve in the medium (Logfgren et al., 2017).

Storage stability

Pectin must be kept in a cold, dry area for storage. Pectin will degrade at higher temperatures than ambient because of a decrease in molecular weight. Pectin's optimum pH range is 2.8-4.7.

Viscosity

Pectin solutions have a lower viscosity than other thickening agents. The viscosity of solutions containing LM pectin is increased by polyvalent salts (such Ca++ and Mg++). When the acidity is raised in calcium-free solutions, the viscosity decreases.

2.6 Sources of pectin

Most of the cell wall's pectin is located in its middle lamella, and it decreases as it moves through the primary wall and into the plasma membrane (Kertesz, 1951). Although pectin is found in abundance in the majority of plant tissues, there are just a few sources that can be utilized in the production of pectin for commercial application. This is due to the fact that pectin's capacity to gel depends on its molecular size and esterification level (DE). Variations in many parameters affect pectin's gelling ability. So, finding a lot of pectin in a fruit isn't necessarily enough to label it as a commercial pectin source (Thakur, 1997). Currently, apple pomace or citrus peel, both of which are leftovers from the production of juice, are the primary sources of commercial pectin. Pectin makes up 15–20% of the dry matter in apple pomace. Compared to apple peel, citrus peel has a considerably greater pectin content, between 20 and 30 percent and 9.5-10.5% wet matter. Citrus and apple pectins are nearly identical in terms of applications (May, 1990). Alternative sources for pectin extraction include mango waste, pomegranate peel, sunflower heads (seeds used for edible oil), and sugar beet waste from the production of sugar (Rolin, 1993). Cherries, grapes, and strawberries have very little pectin compared to hard fruits like apples, quince, plums, gooseberries, oranges, and other citrus fruits. Pectin concentrations in plants are typically between: Carrots, around 1.4; Apricots, 1; Cherries, 0.4; Oranges, 0.5–3.5; and Citrus Peels, 30% (Srivastava et a.l, 2011).

2.7 Extraction procedures of pectin

Direct boiling and microwave heating are the most widely used method for pectin extraction. A good yield of pectin can be obtained using the traditional method of direct boiling, which takes about two hours. The extracted pectin experiences thermal deterioration as a result of a prolonged time of direct heating. Microwave heating extracts enough pectin in 15 minutes. In terms of pectin output, microwave heating methods tend to be more efficient, and the resulting products tend to be of higher quality. The extraction solvents and the use of chelating chemicals, such as EDTA and CDTA, can affect pectin production (JP Maran et al.,2013).

Water based extraction

Traditional water-based pectin extraction uses acidified water (pH 2–2) and a maximum temperature of 70°C (Joye and Luzio, 2000). Pectin compounds are precipitated using ethanol or isopropyl alcohol after the assembly runs for 2-4 hours.

Pectin extraction from plant tissues has been reported using a wide range of different agents. The simplest and most traditional approach for eliminating the pectic materials is extraction with hot water. Meyer's and Rouse reported using several zeolites in order to extract pectin. Zeocarb was used as the extractant, and the production of pectin from dried orange peel was reported to be very low at 85-90°C (Joseph and Huang, 1973). Pectin is commercially generated by processing the source material with hot, diluted mineral acid that has a pH of around two. The specific extraction time varies depending on the source material, the desired type of pectin, and the manufacturer.

Microwave heating extraction

A Milestone Ethos Microwave Lab Station has been used for microwave extraction. The pH was maintained at or below 2 by the addition of 10% ethanol, 0.05M ethylene di-amine tetra acetic acid, and 1M sodium hydroxide as solvents during the microwave extraction procedure. Microwave heating extraction significantly shortens the extraction time as compared to water-based extraction. The quantity of pectin that can be obtained from a water-based extraction with a three-hour extraction duration can practically be achieved with a fifteen-minute microwave heating session [http/lsbu.ac.uk]. During microwave heating considerable pressure builds up inside a material. The high pressure then alters the material tissues' physical characteristics by destroying the cell structure and enhancing the capillary-porous structure of the tissues. This characteristic enhances the subsequent extraction of pectin by allowing better extraction solvent penetration into the tissues. In comparison to straightforward water-based approaches, increased extraction rates and yields were also achieved with microwave technology (United States Patent, 2007).

2.8 Honey and Its application to processed fruit items:

Honey is a sweet and natural product made by bees, particularly those of the species Apis mellifera (Havsteen, 2002), which collects nectar from flowers to produce blossom honey, which unlike other sweeteners, has no refining processes like white cane sugars and sugar beets. It does not require preservatives, which are frequently utilized in industrial manufacture. The primary ingredients in honey are sugars, primarily fructose and glucose (Steeg et al., 1988). Other components, such as water and free amino acids, are present from the floral source where the bee collects the nectar, although in smaller amounts and vary according to the type of honey (White et

al., 2007). Proline is the one that is most prevalent. Invertase, amylase, and glucose oxidase are the main enzymes in honey. Catalase and phosphatase are also present (Bogdanov et al., 2008). Additionally, honey contains organic acids including formic, acetic, butyric, oxalic, lactic, succinic, folic, malic, citric, and glycolic that give honey its distinct flavor and ensure its remarkable stability against microorganisms (Sato and miyata, 2000). In comparison to inorganic nutrients and vitamins, honey does not include fat or fiber, and their impact is minimal. Because the conversion of levulose into sugar is a much slower process than is necessary, the body has adequate time to move on to the process of eliminating sugars when honey is consumed in moderation. Because honey causes less harm than regular sugar, stable diabetic patients can take it (medical diet: consuming minimal amounts of sugar) (Ulloa, 2010). One of honey's many attributes is its potent energizing ability; 100 grams of honey contain 330 kcal. It is ideal for sportsmen since it enables speedy recovery from demanding exertion and hides signs of exhaustion. Elderly and tired people are also advised to use it (Mendizábal, 2005). The population's food requirements are diverse; many of them call for novel flavors, colors, and scents. Given this innovative products such as fruit jams, jelly are not required, such as cranberry, replacing sugar with honey with a very wide commercial potential.

Table 2.1 Average Composition of honey (Murty et al.,2001, Alveraz et al.,2010)

Component	Average (%)
Water	~ 17
Fructose	32.56-38.2
Glucose	28.54-31.3
Other sugars	~ 9.8
Proteins	~ 0.3
Dietary fiber	~ 0.2
Free acid as gluconic	~ 0.4
Lactone as gluconolactone	~ 0.2
Total acid as gluconic	o.6
Ash	~ 0.2
Nitrogen	~ 0.04
Minerals	~ 0.2
pH	3.2-4.5

2.9 Sugar

Sucrose, commonly referred to as sugar, is a crystalline carbohydrate with a sweet flavor and a caloric content of 4K per gram (Cseke et al., 2016). Beet sugar and cane sugar are the primary sources of sugar, although sugar can also be obtained from a variety of other foods, including honey, corn syrup, fruits, and vegetables (Erdat et al., 2007). Sugar's principal role in food is as a flavor enhancer, but it also contributes significantly to the perishability of the dish, as well as its color, texture, and fermentation. Rising rates of obesity, cardiovascular disease, and type 2 diabetes have all been related to increased sugar consumption; as a result, in recent years, food organizations have published stringent standards for measuring sugar intake in meals (Rosa et al., 2009).

2.10 Citric Acid

Citrus fruits contain naturally occurring citric acid, also known as 2-hydroxy-propane-1, 2-tricarboxylic acid (C6H8O7.H2O). Citrus acid, from the Latin citrus, is an organic acid produced in living cells via the Krebs cycle (Swain et al., 2012). According to a review by Angumeenal et al., (2013), pure citric acid is odorless, tasteless, and colorless, solid at room temperature.

One of the most significant commercially value-added products is citric acid (CA), a Krebs cycle intermediary that is used in the food processing sector (70%), pharmaceutical industry (12%), and other industries (18%) (El-Hussein et al., 2009; Yalcin et al., 2010). It is also regarded as a chemical that is generally recognized as safe (GRAS), non-toxic, and pleasing to the taste. Citrus fruits including grapes, oranges, limes, lemons, and tangerines naturally contain citric acid. Citric acid is a widely used component in the food business for its preservative, acidifying, antioxidant, emulsifying, and buffering properties. As a result, citric acid is constantly employed as a common ingredient in a wide range of food products. It is a useful compound, and its daily consumption is highly desired throughout the world (Soccol et al., 2006).

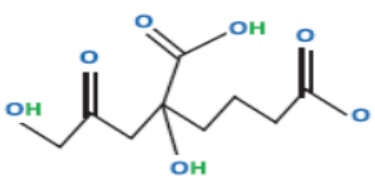

Fig 2.7: Citric Acid

2.11 Jelly

Fruit juice-based products with a semi-solid or gelatinous consistency are known as jelly (Madrid et al., 1994). Fruit juices and pulp, sugar, sulphurous anhydride, benzoic acid, sodium, potassium, or calcic benzoate, antifoaming agents, thickeners, natural jellifiers (agar-agar, pectins, carob gum, alginates, and carrageenates), and flavors are all used to make jellies.

Fruit jellies are preserved fruit juice and sugar mixes with a semisolid consistency. Crystallized sugar or a gummy texture are not desirable in jelly. The jelly shouldn't taste bland or have any synergy, and it shouldn't be tough or rubbery either. Making jelly is a smart technique to save fruit flavor for year-round use. Fruit jelly may be made at home with minimal specialized equipment and is ideal for the novice canner. Jelly is heavy in sugar and, as a result, is primarily a calorie source, so it should be consumed in moderation by those trying to lose weight. Most jellies have 50 calories in a spoonful.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

The experiment was conducted in the laboratory of the department of Applied Food Science and Nutrition, Department of Food Processing and Engineering and Animal Science and Nutrition Department of Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chattogram-4225.

Fig 3.1: Sample Collection and Study

3.2 Study Duration

The study was conducted over a three-month period, from May to July 2022.

3.3 Collection of Sample

Fresh pomegranate was collected from the local market of Chattogram City Corporation. Collected fruits were washed and peeled.

3.4 Preparation of Pomegranate Peel Powder

The peels of pomegranate were submerged for two minutes in a hot water bath (90 °C) to inactivate the pectinase enzyme. The peels were then dried for 48 hours at 60°C to a constant weight in an air cabinet dryer. The peels were then processed into a powder and kept at 4°C (Bosch MKM 600, Germany).

3.5 Extraction of Pectin

Pectin extraction was carried out using a modified version of the Kliemann et al., (2009). 10gm of sample was mixed with 150ml distilled water. Then the pH was adjusted to around 3 with citric acid. Filtration was done to remove the peel after boiling the sample for 25 minutes. After the filtration, equal volume of ethanol was added to extract and allowed to stand for 1hour. The extracted pectins were then separated by filtration, precipitated with 96% ethanol, and cleaned with 1ml of acetone. Then the separated pectin was stored at 4°C after being dried at 50°C for 24 hours.

3.6 Physiochemical Characterization of Pectin Sample

The dried pectin sample obtained from pomegranate peel waste was subjected to the following qualitative and quantitative tests in order to characterize them.

3.6.1 Qualitative test

3.6.1.1 Color

Visual observation was done to do this. Normally pectin appears brown or light brown color.

3.6.1.2 Solubility of dry pectin in hot and cold water

0.25g of pectin sample was taken in two conical flasks separately, then 10ml of 95% ethanol and 50ml of distilled water were added in these two flasks. The second flask's mixture was vigorously heated to create a suspension, which was then heated at 85–95°C for 15 minutes (Fishman et al., 2003).

3.6.1.3 Solubility of Pectin solution in hot and cold alkali

5ml of the pectin solution was added to two separate conical flasks containing 1ml of 0.1N NaOH, and the second flask was heated at 85–95°C for 15 minutes

(Joslyn,1980)

3.6.2 Quantitative test

3.6.2.1 Moisture Content

The weight of an empty crucible was measured after it had been dried in an oven and chilled in a desiccator. 5gm pectin sample was placed in a hot air oven at 100°C for 1 hour. After this procedure was carried out three times (AOAC 2016). The following formula was used to compute the moisture content:

Moisture content % $= weight\ of\ the\ residue * \frac{100}{weight\ of\ sample}$

3.6.2.2: Esterification, amidation and galacturonic acid contents of pectins

FAO JECFA, 2009 method was used to determine the esterification, amidation and galacturonic acid contents of pectin. Pectins (5 g) were combined with 100 mL of 60% ethyl alcohol and 5 mL of 2.7 M HCl solutions, filtered, and then washed six times with 15 mL each of the same alcohol-acid solution and 96% ethanol before being dried at 105°C. 1/10 of the dried sample was mixed with 100 mL of water and 2 mL of ethanol before being titrated against 0.1 M of NaOH. V1 represents the titration's spent volume. The solution was titrated against 0.1 M NaOH after being combined with 0.5 M NaOH and 0.5 M HCl. The spent volume of the titration is denoted by V2. The mixture was then distilled, titrated against 0.1 M of NaOH, and the spent volume of the titration was noted as S1. NaOH (10%, 20 mL) was added to the solution. For the blank determination, HCl (0.1 M, 20 mL) was titrated against 0.1 M of NaOH. The spent volume was recorded as B1 and the B1-S1 difference (in mL) was recorded as V3. The dried samples, after mixing with ethanol, were dissolved in 0.125 M NaOH (25 mL) for saponification. Finally, the volume was brought up to fifty milliliters with distilled water. This solution was distilled after being combined with 20 mL of Clark's solution. The distillate's volume was measured as S2 after being titrated against 0.05 M NaOH. Water (20 mL) was used as the blank solution, titrated, and the volume of NaOH (mL) and S2-B2 difference (mL) were recorded as B2 and V4, respectively. According to the equations below, the ratio of esterification, amidation, and total galacturonic acid was computed (FAO JECFA, 2009). The outcomes were determined by averaging at least three replications, and they were presented as mean± standard deviation.

DE % (w/w) = $\frac{v2}{v1+v2} * 100$…… (2)

DA % (w/w) =$\frac{v3}{v1+v2+v3-v4} * 100$ …..……(3)

GA (mg) = $(v1 + v2 + v3 - v4) * 19.41$…….(4)

3.6.2.3 Ash content

The ash content was measured using AOAC procedures (2016). The inorganic residue left over following the breakdown of organic matter is referred to as ash content. In a pre-dried weighted crucible, 10 gram dried jelly was placed. It was then charcoalized. The charcoal was then placed in a muffle furnace and heated for 4 hours at roughly 600°C until it was totally removed. The crucible was then removed from the furnace. It was carefully cooled in a desiccator before being weighed. The ash content was determined using the following formula:

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

3.6.2.4 Liquid Holding Capacity

Liquid holding capacity (LHC) was determined by mixing 1g pectin with water/acetone/dimethyl sulphoxide/acetic acid (40 g), left to stand for 2 hours. The mixture was then centrifuged at 3500 rpm for 30 minutes, weighed, and the liquid holding capacity (LHC) was calculated using the equation below (Tappi, 1991).

$$\text{LHC (\%)} = \frac{(\text{B} - \text{A})}{A} * 100$$

Here, LHC stands for liquid holding capacity. A is the dry weight of pectin (g), and B is the weight of wet pectin (g).

3.6.2.5 FT-IR analysis

On a Jasco FT/IR430 spectrophotometer, FTIR analysis of pectins (made as KBr pellets) was carried out (Japan). To figure out the samples' esterification degree (DE), the peak area values of the free carboxyl groups (1630 cm-1) and the esterified groups (1740 cm-1) were added up (Pappas et al., 2004).

Esterification Degree $= 124.7 * R + 2.2013$

$$\text{R} = \frac{A1740}{(\text{A1740} + \text{A1630})} * 100$$

A1740 and A1630 are the the absorbance densities at 1740 cm-1 and 1630 cm-1 respectively.

3.6.2.6 pH

In chemistry, pH is a measure of the acidity or basicity of an aqueous solution. In technical terms, pH is the negative logarithm of the activity of the (solvated) hydronium ion, more often expressed as the measure of the hydronium ion concentrations. The pH scale is traceable to a set of standard solutions whose pH is established by international agreement. Primary pH standard values are determined using a concentration cell with transference, by measuring the potential difference between a hydrogen electrode and a standard electrode such as the silver chloride electrode. Measurement of pH for aqueous solutions can be done with a glass electrode and a pH meter, or using indicators, pH is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution (McClements and Decker, 2010).

3.7 Preparation of Juice

Uniformly ripe pomegranate fruits were selected for extraction of juice for making of jelly. The fruits were cut into 2-4 pieces and crushed in a grinder. Then the juice was collected after filtering the crushed mass through muslin cloth and weighed as per the flow chart shown in Fig 1 (Chopra et al., 2003).

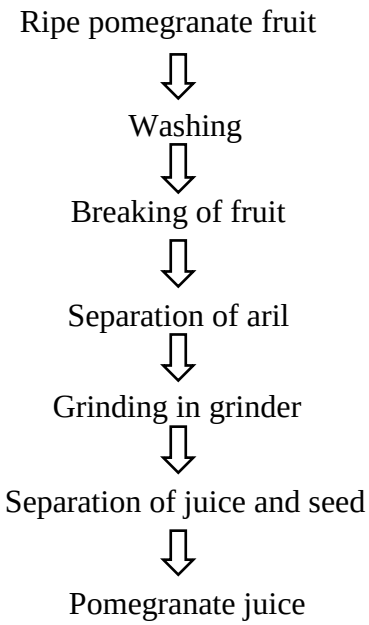

Fig 3.2 : Flow chart for extraction of juice

3.8 Physiochemical analysis of juice

3.8.1 pH determination

pH determination was done by the pH meter (McClements and Decker, 2010).

3.8.2 Chemical composition

3.8.2.1 Moisture content

The standard method of the Association of Official Analytical Chemists was used to figure out the amount of moisture (AOAC, 2016).

Calculation: The moisture percentage was calculated as follows:

$$\text{Moisture \%} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Sample weight}} * 100$$

3.8.2.2 Ash content

Ash content was determined by methods of AOAC (2016).

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

3.8.2.3 Determination of Vitamin C

Chemically assay of the Vitamin C depends on the market reducing properties of the Vitamin C. Generally, Vitamin C is determined in plant or animal extract by its reducing action on the dyes stuff 2,6-dichloride phenol indophenols. In this matter, Vitamin C oxidized by the color dye to the dehydroascorbic acid. Concurrently, the dye is converted into the colorless compound. So that the endpoint of the reaction may be easily determined. Rapid excretion and filtration are desirable. Oxidation is presented by the use of metaphosphoric acid during extraction. Strongly acidic solution will provide most accurate result. The titration should be finished in one minute. In aqueous solution, the dye is blue, pink in an acidic solution, and colorless when totally reduced (AOAC, 2016).

Reagent requirement

Dye Solution

1. 260 mg of dye (2,6-dichlorophenol indophenols)
2. 210 mg of NaHCO3 dissolved in 100 ml of distilled water.

Metaphosphoric acid solution (3%)

1. 15/7.5 mg of Metaphosphoric acid.
2. 40/20 ml of glacial acetic acid is diluted with distilled water to get 500/250 ml solution.

Standard ascorbic acid solution

500 ml/250 ml of a solution of metaphosphoric acid and 50/25 mg of crystalline ascorbic acid were combined.

Procedure

In the burette, the dye solution was taken. Then, in a conical flask, 5 mL of Vitamin C solution was then added. The dye was added dropwise to the conical flask by using a burette. After the pink color appeared and lasted for 20 seconds before fading, the titration was complete. At least three separate readings were taken.
A similar procedure was used with a solution of ascorbic acid, the concentration of which was unknown. Mg %, or milligram percentage, was used to represent the outcome.

3.8.2.4 Titratable Acidity

10 ml of juice was placed into a 100 ml volumetric flask and diluted to 100 ml with distilled water. 10 ml of the diluted juice was titrated against N/10 NaOH with phenolphthalein as an indicator. The titration's endpoint is indicated by the appearance of pink color. The titration was reported three times, with each report recording the average value (AOAC, 2016).

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

Where, TV = 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

3.9 Preparation of jelly

At first, pomegranate juice, sugar, pectin were weighed. At medium heat, pomegranate juice and sugar/honey were simmered for 5 minutes with continuous stirring. After that, pectin was added, followed by citric acid to adjust the pH level. The heating was maintained while stirring. After 15 minutes the endpoint was indicated at 65-67 degree Brix TSS in the mixture as measured by a refractometer. The jelly was then placed in a glass jar. After chilling, the cans or jars are labeled and saved for future research.

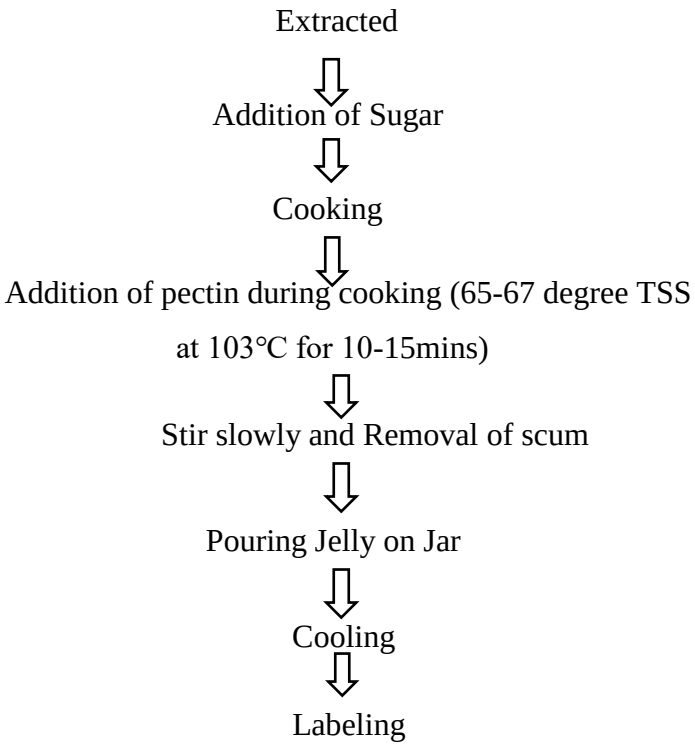

Fig 3.3 : Flow chart for preparation of jelly from juice

3.10 Design of experiment:

The jelly was prepared with pomegranate juice. The different percentage of sugar/ Honey and pomegranate juice were used for preparation of jelly. The treatment combinations are presented in Table 3.1.

Number of Treatment : 4

Table 3.1 Number of Treatment

Treatment	Treatment Combination
T1 (Sample B)	Sugar 40% + Pomegranate Juice 60% + Extracted Pectin (1gm)
T2 (Sample C)	Honey 40% + Pomegranate Juice 60% + Extracted Pectin (1gm)
T3 (Sample D)	Honey 50% + Pomegranate Juice 50% + Extracted Pectin (1gm)
T4 (Sample E)	Sugar 50% + Pomegranate Juice 50% + Extracted Pectin(1gm)
Control (Sample A)	Pomegranate jelly made with commercial Pectin (1gm)

3.11 Physiochemical analysis

3.11.1 Determination of pH

pH is usually stated as the negative logarithm of hydronium ion concentrations. In a concentration cell with transference, the potential difference between a hydrogen electrode and a standard electrode, such as the silver chloride electrode, is measured to generate primary pH standard values. A glass electrode and a pH meter can be used to measure the pH of aqueous solutions, alternatively indicators can be used (McClements and Decker, 2010).

3.11.2 Total Soluble Solids

Digital refractometer was used to measure total soluble solids (Atago RX 1000), and the result were expressed as percent soluble solids (Brix) in accordance with AOAC standards.

3.11.3 Moisture content

Utilizing the established procedure of the Association of Official Analytical Chemists, the moisture content was calculated (AOAC, 2016).

3.11.4 Total solids

AOAC procedures were used to calculate total solid (2016). Using the data received during moisture estimation, the following formula was used to calculate the percent total solid content:

% Total solids = 100 – % moisture content.

3.11.5 Ash content

AOAC (2016) method was used to determine the ash content.

3.11.6 Estimation of Crude Fat

Principle: Food samples are dissolved in organic solvents (chloroform: methanol) and the filtrate is filtered to determine fat content. Placing the filtrate into separating funnels and then separated mixture is then dried to measure the extract and finally, the percentage of fat is estimated. The samples' crude fat content was measured using AOAC (2016) techniques and a soxhlet apparatus.

Calculation: The percentage of crude fat was represented as follows:

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

3.11.7 Estimation of Crude Protein

Principle:

The Kjeldahl method is used to determine the nitrogen concentration of organic and inorganic substances. It is a recognized procedure that is described in various normative sources, including (AOAC, 2016).

Calculations: The kind of receiving solution and any dilution variables used during the distillation process must be taken into consideration when calculating the percentages of nitrogen or protein.

In the equations below, "N" denotes normality , "ml blank" refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When boric acid is used as the receiving solution the equation is :

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

3.11.8 Determination of Crude Fiber

Principle: Cellulose, hemicellulose, and lignin make up the majority of crude fiber, the portion of carbohydrates that are insoluble in water. It is calculated by first boiling a fat-free known amount of food sample for 30 minutes in a weak acid solution (1.25% H2SO4), followed by 30 minutes in a weak alkali solution(1.25% NaOH) and then subtracting ash from the residue obtained. The crude fiber was measured using the AOAC technique (2016). The residue was then ignited in the muffle furnace until the produced white ash (550-600°C, 4-6 hours).

Calculation: Calculation of the crude fiber percentage as follows:

$$\text{\% Crude fiber} = \frac{W - W1}{W2} * 100$$

Where,

W= Weight of crucible, crude fiber and ash

W1=Weight of crucible and ash

W2= Weight of sample

3.11.9 Determination of total carbohydrate

The carbohydrate content was estimated by calculating the difference between the Nitrogen Free Extractive (NFE). It was specified as the difference between 100 and the sum of the other proximate components.

Calculation: As a result, the following formula was used to calculate it:

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

3.12 Determination of Antioxidant capacity by DPPH scavenging method

Extract preparation

The Felcon tube was filled with a 1 gram sample. After that, 10 mL of absolute methanol was added and the mixture was allowed for 72 hours. After every 4 hours, continuous straining was performed. After 72 hours, the filtrate was collected, and methanoic extract obtained.

Procedure

Using a slightly modified version of the method described by (Azlim et al.,2010), the DPPH test was used to measure the antioxidant mobility of the extracts. 6 mg of DPPH was dissolved in 100 mL of 100% methanol to develop a methanoic DPPH solution. To make a methanoic DPPH solution, 6 mg of DPPH was dissolved in 100 mL of 100% methanol. The methanoic extract was then diluted with 2 mL of DPPH solution. After lightly shaking the mixture, it was left in the dark for 30 minutes at room temperature. A UV-VIS spectrophotometer was used to detect absorbance at 517 nm (UV-2600, Shimadzu Corporation, USA). Control was made by combining 1 mL of methanol with 2 mL of DPPH solution, with methanol serving as a blank. The scavenging mobility was determined by comparing the absorbance of the samples to that of the DPPH reference solution. The antioxidant capability of extracts is determined using the following equation based on their DPPH free radical scavenging mobility.:

% of inhibition = Blank absorbance - Sample absorbance× 2 / Blank absorbance

Here, 2 = Dilution Factor

Trolox was used as the standard, while TEAC composite was used as the calibration standard curve (Trolox equivalent antioxidant mobility). The results have been reported in milligrams (mg) per 100 grams of powder on a dry weight (DW) basis.

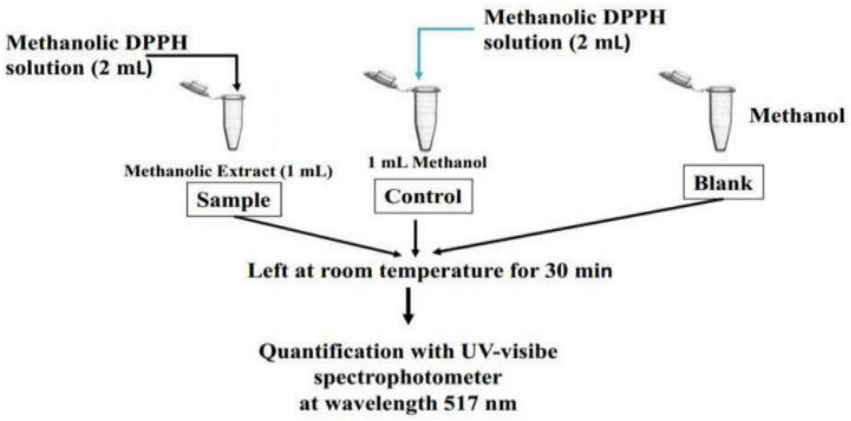

Figure 3.2: Determination of antioxidant capacity

3.13 Microbiological analysis

3.13.1 Fungal analysis

Media Preparation

Sabouraud Dextrose Agar (SDA) is a selective medium that can support the growth of filamentous bacteria such as Nocardia, as well as dermatophytes, various fungi, and yeasts. This medium's acidic pH (about 5.0) inhibits bacterial growth but promotes the growth of yeasts and most filamentous fungi. Antibacterial agents can be used to increase the effectiveness of antibacterial agents. SDA medium is an enzymatic digest of casein and animal tissues that provides a rich source of amino acids and nitrogenous compounds for the growth of fungi and yeasts. 10 g Mycological peptone (enzymatic digest of casein and animal tissues), 40 g Dextrose, and 15 g Agar with pH 5.6 at 25 0C are used for 1 liter SDA media. All media were sterilized in an autoclave at 121°C for 15 minutes after being prepared according to the manufacturer's instructions. Although there are many selective agars for the development and detection of mold and yeast cultures, the bulk of them do not have rigorous nutritional requirements for growth. Sabouraud Dextrose Agar supports the growth of a wide range of fungal strains. The methods and techniques described by Chen and Gu (2000), FSSAI (2012), and APHA are used here (1996).

Procedure for media preparation

In the beginning, 65 g of the medium were suspended in one liter of cleaned water. The medium was then thoroughly dissolved by heating it with frequent agitation for one minute. Autoclaved at 121°C for 15 minutes. The mixture was then cooled to 45°C to 50°C before being put into petridishes. In order to obtain isolated colonies, the material was streaked onto the medium using a sterile inoculating loop. The plates were then incubated at 25-30°C inverted (agar side up) with high humidity. Cultures were checked for fungal development on a weekly basis and stored for 4-6 weeks before being declared as negative (Aryal, 2015).

Interpretation

The plates should show isolated colonies in streaked areas and confluent development in areas of strong inoculation after appropriate incubation. Inspect plates for fungi with normal coloration and morphology. To confirm results, additional processes should be carried out. Yeast colonies can range in color from creamy to white. Molds will develop as multicolored filamentous colonies (Aryal, 2015).

3.13.2 Fecal Choliform Test Procedure

Eschericia coli was inoculated in the proper selective media for the quantitative detection of fecal choliform, as per the FDA's 2001 recommendations (Codi et al.,1999)

1. To make 0.2N NaOH solution, 8.0g of sodium hydroxide were dissolved in 1L of deionized or distilled water.
2. Rosolic acid solution was made by mixing 10ml of 0.2N NaOH with 0.1g of rosolic acid crystals.
3. A 250 ml flask was filled with 5.210 grams of dehydrated mFC medium and 100 ml of deionized or distilled water.
4. For several minutes, the mixture was vigorously stirred to break up clumps and keep the medium from clinging to the flask.
5. Flask was placed in a heated water bath or on a hot plate and heated slowly to reach temperature at 90℃. Stirring was done frequently to keep the mixture from burning.
6. For every 100 ml of medium, 1 ml of rosolic acid solution was added, using a clean pipette.
7. The medium's temperature was lowered to between 45 and 50 °C. Then medium was put into 90mm petridis bottoms to a depth of 20–25 ml.
8. Following the medium's 10-minute solidification, petridishes were sealed by firmly pressing the tops.After the medium had solidified, those plates were utilized.
9. On a selective petridish the samples were spread out. Alcohol-dipped sterilized forceps were held in a Bunsen burner to be speared out. Forceps was chilled before use.
10. Petridis was incubated 24 hours at 44.5℃.

3.13.3 Confirmation of E.Coli in MacConkey Agar Media

1. In a 250ml flask, 5.15gm of dehydrated MacConkey agar medium was mixed with 100ml of deionized or distilled water.
2. The mixture was vigorously agitated for several minutes to break up clumps and keep the medium from sticking to the flask.
3. The flask was placed in a warm water bath or on a hot plate and gradually heated to 90°C. To avoid burning, the mixture was regularly stirred. It had not been autoclaved.

4. A clean pipette was used to add 1 ml of rosolic acid solution per 100ml of medium. Heating was continued until the medium was completely disintegrated.
5. The medium was cooled to approximately 45-50°C. Then put into 90mm Petridis bottoms to fill 20-25ml.
6. After the medium had solidified (approximately 10 minutes), the petridishes were closed by firmly pushing the tops together. After the medium had solidified, those plates were used.
7. The sample was applied to a selective petridish. Sterilized forceps were dipped in alcohol and held over a Bunsen burner to be speared. Before using the forceps, they were chilled.
8. Petridish was incubated at 370.5°C for 24 hours.
9. The colonies were examined using a long wave UV laser. The presence of E.Coli is indicated by fluorescence. Some UV lamps may not use the proper power and can produce inaccurate readings. UV lamp bulbs were tested to ensure they met specifications.

3.14 Sensory evaluation

Sensory evaluation was performed in order to obtain the highest level of consumer approval for the finished product. A taste-testing panel determined whether the developed product was appropriate or not. The panel test took place on the CVASU campus, and the panelists were both CVASU teachers and students. The product developed from pomegranate juice using extracted pectin was given to a panel of 15 people. The panelists tasted five formulations which were encoded with sample A, sample B, sample C, sample D and Sample E. The panelists were asked to give suitable ratings for the sensory qualities of jelly's appearance, color, flavor, texture, taste, and overall acceptability. While this method may not be indicative of how consumers feel about a product, it does highlight features that should be present in a top-tier product (Sing et al., 2008). Five samples were sampled, and ratings were given based on the feedback. Sensory evaluation of the five samples' qualitative parameters (taste, color, flavor, consistency, and overall acceptability) was performed using nine-point Hedonic scales (Larmond, 1977).

The scale was set up in such a way 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.

3.15 Statistical Analysis

Data were collected and stored in a Microsoft Excel 2013 spreadsheet for statistical analysis. All samples were repeated three times. For the proximate composition and sensory evaluation of Pomegranate jelly, descriptive statistics (mean and standard deviation) were calculated. Data is organized, coded, and recorded with the help of IBM SPSS Statistics 25. After that, statistical analyses were performed. Data on proximate composition, phytochemicals, antioxidant capacity, and sensory evaluation were analyzed using the One-way ANOVA method to investigate significant levels of variance at the 95 % confidence interval. The degree of variation between samples was calculated using a postdoc Fisher test . The significance level used in the analysis was 5% ($p \leq 0.05$).

CHAPTER 04

RESULTS

4.1 Pectin Recovery

The extraction procedure of pectin from fruit peels was trialed three times. The quantity of pectin was found 22gm from 268gm of pomegranate peel. That means, the average pectin yield percentage was 8.2% which contains 78.48% GA (monomer of pectin).

4.2 Characterization of Pectin

The characterization of powdered pectin obtained from pomegranate peel waste was carried out for various parameters to determine its suitability in food system. The moisture content, ash content, and degree of esterification are given in Table 4.1

Table 4.1 : Characteristics of pectin

Sample	Moisture (%)	Ash(%)	DE(%)	Galacturonic Acid (GA%)	Amidation
Pomegranate Peel Pectin	10.5	1.15	56.74	78.48	3.75

4.2.1 Color

The color of the pomegranate peel pectin was grayish to light brown color.

4.2.2 Liquid holding capacity

A polymer with a high liquid-holding capacity can increase the volume of food while decreasing its caloric content.

Table 4.2 : Liquid Holding Capacity (LHC)

LHC				
Peel	**Water (% w/w)**	235.25	**DMSO (% w/w)**	276.72
	Acetone (% w/w)	82.04	**Acetic Acid (%,w/w)**	192.06

4.2.3 Solubility

In Table 4.3, the solubilities of pectins in various solvents are reported. The pectins were soluble in NaOH (1M), hot water but not in cold water, acetone and methanol.

Table 4.3 : Solubility of pectin in different solvent

Pomegranate Peel Pectin		
Solubility	**NaOH**	+
	Methanol	_
	Acetone	_
	Hot Water	Soluble
	Cold Water	Insoluble

4.2.4 pH

The pH of extracted pectin was 4.2

4.2.5 FTIR Analysis

In Figure 4.1 the FTIR spectra of the isolated pectins were presented. The x axis represennted wavenumber (cm^{-1}) and Y axis represented the transmittance (%).

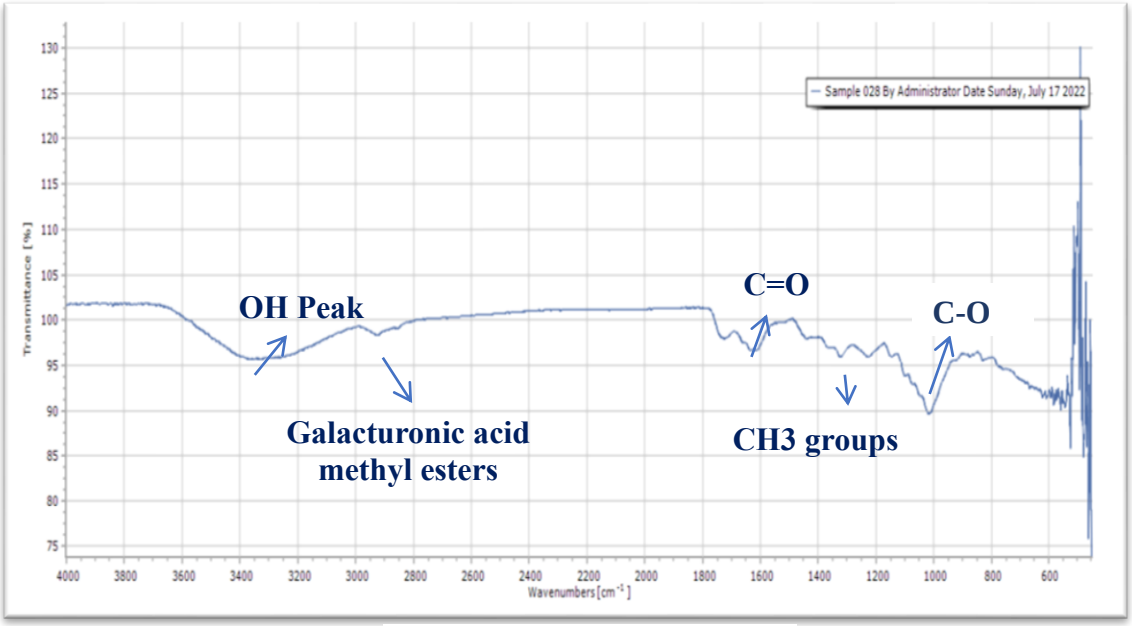

Fig 4.1 : FTIR Result

4.3 Physiochemical properties of Pomegranate juice

4.3.1 pH

The pH of pomegranate juice was 3.2.

4.3.2 Chemical Composition

Before preparing products fresh juice was tested and analyzed. The result of the chemical composition of pomegranate juice is tabulated in table 4.4.

Table 4.4 Chemical Composition of pomegranate juice

Parameters	Pomegranate Juice
Moisture (%)	85.4
Total sugar (%)	10.6
Total acidity(g/100ml)	0.15
Ascorbic acid (mg/100ml)	0.9
Ash(g/100ml)	0.03

4.4 Physicochemical properties of jelly

4.4.1 pH , Acidity and TSS

pH of Jelly is an important factor for optimum gel condition. In table 4.5, lowest (2.81±0.01) pH found in sample D and highest (3.02±0.01) in sample B. TSS (total soluble solids) was highest (0.67^{o}B) in sample B and lowest in (0.61^{o}B) in sample E. The highest value (1.03±0.01%) of acidity was observed in sample B while the lowest value (0.60±0.01%) was obtained in sample D.

Table 4.5 Physicochemical analysis test result of Pomegranate jelly

Sample	pH	Acidity	TSS
Sample A	2.93±0.02^{b}	0.64±0.01^{b}	0.62±0.01^{c}
Sample B	3.02±0.01^{a}	1.03±0.01^{a}	0.67±0.01^{a}
Sample C	3.00±0.02^{a}	1.00±0.02^{a}	0.66±0.01^{a}
Sample D	2.81±0.01^{c}	0.60±0.01^{bc}	0.65±0.01^{b}
Sample E	2.9±0.01^{b}	0.63±0.01^{b}	0.61±0.01^{c}
P value	0.01	0.02	0.01

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

4.5 Proximate analysis of jelly

One way ANOVA (Analysis of Variance) test was conducted to see the overall mean differences of values for different parameter of pomegranate jelly treated with sugar and honey and comparison with control.

Table 4.6 Proximate analysis of Pomegranate jelly

Parameters (%)	Sample A	Sample B	Sample C	Sample D	Sample E	P Value
Dry matter	63.24 ± 0.01[b]	67.23 ± 0.01[a]	64.28± 0.01[b]	65.25± 0.02[b]	68.93± 0.01[a]	0.01
Moisture	36.76 ± 0.01[a]	30.70± 0.01[c]	33.82± 0.01[b]	34.75± 0.01[b]	31.07± 0.01[c]	0.01
Crude Fibre	1.59±0.01[a]	1.30±0.01[a]	1.80±0.01[a]	1.92±0.01[a]	1.37± 0.01[a]	0.01
Ash	0.81±0.01[a]	1.61±0.01[a]	1.30±0.01[a]	1.37±0.01[a]	0.71±0.01[a]	0.01
Fat	0.92±0.01[a]	1.20±0.01[a]	1.58±0.01[a]	1.79±0.01[a]	1.35±0.01[a]	0.01
Protein	1.58±0.01[a]	1.50±0.01[a]	1.98±0.01[a]	1.87±0.01[a]	1.40±0.01[a]	0.01
Carbohydrate	58.34 ±0.01[b]	63.67± 0.01[a]	59.52± 0.01[b]	58.3± 0.01[b]	64.1±0.01[a]	0.01

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

4.6 Anti-oxidant compound of jelly

From the table 4.7, that it was found that there was no significant differences among all the samples in terms of antioxidant capacity .

Table 4.7 Antioxidant activity analysis of Pomegranate jelly

Sample	Antioxidant capacity (mg/100g sample) TE
Sample A	6.63 ± 0.01^{a}
Sample B	6.52 ± 0.01^{a}
Sample C	6.56 ± 0.01^{a}
Sample D	6.51 ± 0.01^{a}
Sample E	6.61 ± 0.01^{a}

4.7 Microbial analysis

To determine total viable count and the fungal count jelly was stored at 5°C upto 90 days. The microbiological development of Jelly remained below the limit until the end of the storage period.

Table 4.8 Microbial analysis of jelly

Storage Days		Sample A	Sample B	Sample C	Sample D	Sample E
0	*E. coli*	Not found	Not found	Not found	Not found	Not found
	Yeast and mold (10^3 cfu/ml)	Not found	Not found	Not found	Not found	Not found
30	*E. coli*	Not found	Not found	Not found	Not found	Not found
	Yeast and mold (10^3 cfu/ml)	Not found	Not found	Not found	Not found	Not found
60	*E. coli*	Not found	Not found	Not found	Not found	Not found
	Yeast and mold (10^3 cfu/ml)	1.00	0.9	0.4	0.2	1.00
90	*E. coli*	Not found	Not found	Not found	Not found	Not found
	Yeast and mold (10^3 cfu/ml)	2	2	1	1	1.9

4.8 Sensory Quality Evaluation

After doing the ANOVA test it was interpreted that, there were no significant differences between sample B and E in regard to appearance, color, sweetness, taste and texture. But sample E ranks higher than sample B in case of overall acceptance. In contrast to the other samples, sample C and D had the lowest levels of acceptance.

Table 4.9 Hedonic scale scoring test results

Sample / parameters	Pomegranate Jelly				
	Sample A	**Sample B**	**Sample C**	**Sample D**	**Sample E**
Appearance	5.62 ± 0.01^{a}	4.9 ± 0.01^{b}	4.4 ± 0.02^{c}	4.1 ± 0.01^{c}	5.2 ± 0.01^{b}
Color	6.4 ± 0.015^{a}	6.0 ± 0.01^{a}	5.75 ± 0.015^{b}	5.45 ± 0.01^{b}	6.1 ± 0.01^{a}
Smell	5.13 ± 0.01^{a}	5.11 ± 0.01^{a}	5.20 ± 0.01^{a}	5.15 ± 0.01^{a}	5.25 ± 0.01^{a}
Taste	5.75 ± 0.01^{a}	5.45 ± 0.01^{a}	4.9 ± 0.02^{b}	4.7 ± 0.02^{b}	5.6 ± 0.01^{a}
Sweetness	5.0 ± 0.01^{b}	5.3 ± 0.01^{a}	$4.55.5\pm0.01^{b}$	4.25 ± 0.01^{b}	5.5 ± 0.01^{a}
Texture	5.3 ± 0.01^{a}	5.1 ± 0.01^{a}	4.9 ± 0.01^{a}	5.0 ± 0.01^{a}	5.2 ± 0.01^{a}
Overall Acceptibility	5.65 ± 0.01^{a}	5.3 ± 0.01^{b}	4.8 ± 0.01^{c}	4.5 ± 0.01^{c}	5.5 ± 0.01^{b}

Legends: Values represent mean± SD and the presence of different superscript along a column indicates a significant differences at $P < 0.05$.

Sample A: Pomegranate jelly made with commercial pectin (Control)

Sample B: 40% Sugar + 60% Pomegranate Juice + Extracted Pectin

Sample C 40% Honey + 60% Pomegranate Juice + Extracted Pectin

Sample D: 50% Honey + 50% Pomegranate Juice + Extracted Pectin

Sample E: 50% Sugar + 50% Pomegranate Juice + Extracted Pectin

4.9 Cost analysis

Table 4.10: Production cost of Pectin (4.2g pectin from 10gm peel powder) (BDT)

Used chemicals/materials	Unit Price(Tk)	Used quantity	Cost(Tk)
Ethanol	1115 per liter	100ml	111.5
Acetone	90 per liter	1ml	0.9
Filter paper	30 per 25gm	2gm	2.4
Total cost			114.8

According to table 4.10, total cost for producing 4.2gm pectin was 114.8 Tk. That means, average pectin production cost in laboratory was about 1350Tk/50gm. If commercialized then production cost will be further reduced. On the other hand, the price range of 50gm commercial pectin in the market is 1200-1700 Tk. So it proves that production of pectin from pomegranate peel will be profitable.

Table 4.11: Production cost of Pomegranate jelly (BDT)

Line Item	**TK/Kg**	**Quantity used** (kg/1kg products)	**Total Tk (for sample B)**	**Total Tk (for sample E)**	**Total Tk (for sample C)**	**Total Tk (for sample D)**
Pomegranate	200	2.5	500	500	500	500
Sugar	90	0.32	28.8	29.25	-	-
Honey	1250	0.16	-	-	200	196
Citric Acid	1200	0.0028	3.36	3.36	3.36	3.36
Subtotal			532.16	532.61	703.36	699.36
2) Processing cost @ 15% of raw material			79.82	79.89	105.50	104.90
3) Bottling cost	35 tk/piece	4 piece	140	140	140	140
Total production cost			751.98	752.5	948.86	944.26

According to table 4.11, Sample B and E contains sugar and the price per kg jelly is around 750 Tk , hence the price per 250gm jelly is 187 Tk. Similarly, Sample C and D per kg jelly are around 950 Tk as honey was used. The market price for mango or orange jelly is Tk. 440/kg. In this study, jelly prepared from pomegranate costs more than processed jelly since the price of pomegranate is high, but using other fruits instead of pomegranate will reduce the price.

CHAPTER 5
DISCUSSION

5.1 Pectin

5.1.1 Yield

The pectin yields of the pomegranate peel (PP) was determined as 8.2%. According to earlier research, Pectin yields for kiwifruit pomace ranged from 3.62 to 4.48% (Yuliarti et al., 2015), citrus peels (lemon, mandarin, orange, and grapefruit) ranged from 11.46 to 22.09%, banana and mango peels yielded 2.8% and 8.8%, respectively (Panchami and Gunasekaran), and quince yielded 1.99 and 2.86% (Mollea et al., 2008). The peels examined in this study had better pectin yields than those reported in the literature for kiwifruit pomace, banana peels, quince, and cocoa husks, and they might be utilized to make pectin.

5.1.2 Ash

The extracted pectin had an ash level of 1.15 percent. According to reports, the ash levels for kiwifruit pectins ranged from 1.05 to 12.87%, apple pomace pectin was found to have an ash content of 1.84%, mandarin peel pectin had an ash content of 0.50%, and lemon pectin had an ash content of 0.70% (Yuliarti et al., 2015; Dang, 1968). Since a low ash concentration is crucial for pectin purity, these results suggest that the pectins generated in this work are of high quality (Miyamoto and Chang, 1992).

5.1.3 Liquid holding capacity

Liquid holding capacity was presented in table 4.2. By utilizing a polymer with a high liquid-holding capacity, the food's volume can be raised while its caloric content is decreased. Additionally, it has a direct impact on the food's viscosity and texture. Consequently, the material's ability to store liquid is crucial for food both medically and technologically (Rodriguez et al., 2006). The extracted pectin was found to have higher water and dimethyl sulfoxide holding capabilities than acetic acid and acetone holding capacities.

According to research in the literature, commercial citrus pectin has a water holding capacity of 1035% (w/w), commercial apple pectin has a water holding capacity of 200% (w/w), and olive oil byproducts have a holding capacity of 34-187% (w/w) (Senent et al., 2015). It was discovered that the extracted pectin had water-holding

capabilities that were higher than those of apple pomace and olive oil byproduct pectins but lower than those of commercial citrus peel pectin.

5.1.4 Solubility

Table 4.3 displays the solubilities of pectins in various solvents. In comparison to acetone and methanol, pectin was soluble in NaOH (1M). Tyagi (2016) revealed that the pectin from orange peels is insoluble in acetone and methanol, which is also confirmed in our experiment. According to the results of investigations on the solubility of pectins from lemon, grapefruit, and orange peels (Aina et al., 2012), all pectins formed yellow solutions in cold alkaline (20°C, 0.1 N NaOH) and dissolved and formed milky structures in hot alkaline (85-95°C, 0.1 N NaOH).

5.1.5 Esterification

The isolated pectin was high methoxyl pectins (Table 4.1) as the esterification degree ranged in 56.74. Pectin esterification was influenced by the degree of fruit ripeness and extraction techniques (Azad et al., 2014, Yuliarti et al., 2015). Esterification degrees for kiwifruit pectins were found to be 82% and 90% (Yuliarti et al., 2015), 63% and 75% for orange peel pectins (Georgiev et al., 2012; Venzon et al., 2015), and 55.61% for lemon peel pectin ((Mohamed and Mohamed, 2015). The amounts of esterification in the pectin extracted for this study were equivalent to those reported in the scientific literature.

5.1.6 Amidation degree

amidation degrees are essential for flexibility and gelling (Lootens et al., 2003). The amidation degree for Pomegranate pectin was 3.75% and galacturonic acid was 78.48%. When the results were contrasted with earlier studies, it was observed that, pomegranate peel pectin had higher amidation degrees than orange and lemon peel pectins (2.20% and 1.44%, respectively), (Georgiev et al., 2012) although extracted pectins had higher galacturonic acid levels than gold kiwifruit pectin (28.96% to 58.57%) (Yuliarti et al., 2015).

5.1.7 FTIR

Results from analyzing the FT-IR spectrum of the purified pectin in Table 4.1 indicated that spectra at 3500-3300 cm-1 was for non-associate OH peak. The galacturonic acid methyl esters' -CH, -CH2, and -CH3 stretches are responsible for the absorbance at about 2926 cm-1. The C=O stretch, which was seen in the ester and came from the acetyl (COCH3) group, is what causes the peak at 1740 cm-1. The peaks at 1320 and 1445 cm1 are indicative of -CH3 groups, whereas the peak at 1630

cm1 is related to the -OH tensile vibration band. The bands at 1026 cm-1 are C-O bending or stretching bands (Fig. 1) (Ferreira et al., 2001; Nesic et al., 2011; Fajardo et al., 2012). The esterification degrees of the pectin were determined and shown in table 4.1 based on the areas of the peaks at 1740 cm-1 and 1630 cm-1 of the FT-IR spectra of the pectin. Pectin was found to have a high degree of esterification (DE> 50), and these results were consistent with those of the titration technique.

5.1.8 pH

pH affects a jelly batch's setting time, a lower pH causes jellification to occur more quickly. Higher electrostatic repulsion between pectin molecules on account of the charge density of pectin molecules for incereasing pH prevents gel formation (Joseph et al., 1949). The extracted pectin's pH is appropriate for gel formation.

5.2 Pomegranate Juice

5.2.1 pH

The pH of pomegranate juice was 3.2. Gel sets work best when the pH is between 3.1 and 3.3. When the pH is above 3.5, gel formation is frequently poor, and when the pH is below 3, the gel is frequently rigid and prone to synergesis or weeping.

5.2.2 Chemical Composition

The chemical components of fresh pomegranate juice are nearly similar to the report published by BioMed Research International in 2014. According to their findings, fresh juice includes 85 % moisture content, 10% total sugars, primarily fructose and glucose, and 1.5% pectin, 0.1g/100ml total acidity(as citric acid) , 0.7mg/100ml ascorbic acid and 0.05g/100ml ash(Sreeja et al., 2014). It was found 85.4% moisture, 10.6% total sugars, 1.4% pectin, 0.15g/100ml total acidity (as citric acid), 0.9mg/100ml ascorbic acid and 0.03g/100ml ash (Table 4.4). A little differences may be due to instrumental error or inefficient measurement. In this experiments soil nutrients and composition of the growing area and inefficient measurement or instrumental error has influenced in varietal differences. It is noted here that the variety of pomegranate cultivar used in this experiment is unknown.

5.3 physicochemical properties of Pomegranate Jelly:

5.3.1 pH

The pH of the jelly is crucial for maintaining the best gel condition. The appropriate pH level in food also inhibits microbial development. The pH level should be assessed when the jelly has reached a pourable consistency. Citric acid should be used to lower the pH to a range of 3.0 to 3.4 if it is greater than 3.3, where it has the ability to create

a viscous semi-solid when the pH is between 3.2 and 3.4 and there is a lot of sugar present. The pH may be better controlled by adding the citric acid at the end of the boiling process, which also reduces batch pre-gelling and pectin hydrolysis. Depending on the original acidity and buffering capabilities of the extract, different extracts will need varying amounts of added acid. The best flavor can be achieved by adjusting the pH, which can also be done to control or alter the rate of setting and the degree of sugar inversion (Eke Ejiofor and Owuno, 2013). According to table 4.5, there was a statistically significant difference in pH value amongst the samples, with readings ranging from 2.81±0.02 to 3.02±0.01 and these values were within the range of typical jellies. Since pomegranate juice has a pH level of 3.2, it was within the pH level.

5.3.2 Total Soluble Solid (TSS)

Pomegranate jelly tests in this study yielded TSS of 0.62 and 0.67. The hydrolysis of polysaccharides is probably what caused the increase in TSS (Shah et al., 2015) discovered that the TSS of fruit jelly was found to be close to 0.69. They also stated that the polysaccharide breakdown in the presence of acid may be the cause of the apple-olive jam's rising total soluble solid content.

5.3.3 Nutritional composition

Table 4.2 shows the approximate composition of jelly. From the table, it is clear that moisture content of control samples is higher than those of other samples prepared with extracted pectin. The relatively low moisture content observed in sample B and E might be due to sugar as sugar reduces the moisture content which inhibits the growth of food spoiling microbes. Sample C and D has higher moisture content than Sample B and E. It might be due to the honey which act as a hydrophilic substance in low humidity. Therefore ,the lower moisture content observed in sample will promote its longer shelf life than sample with honey when stored under the same condition (Owuno, 2013).

Fiber, ash , protein and fat content do not have any significant differences than control A which means that extracted pectin has similar quality as commercial pectin as pectin does not alter these composition (Mokhtar and T A,1982).

Sample B and E has higher sugar content than sample C and D as these have been prepared with sugar.

5.3.4 antioxidant capacity

From Table 4.7, it was found that there were no significant differences between the control and other samples in terms of antioxidant capacity which means that extracted pectin does not alter the properties and comprises with the commercial pectin (G Sultani et al., 2014).

5.3.5 Microbial Analysis

Microbiological studies (Fecal Coliform Test, yeast and mold count) were done on each of five Pomegranate Jelly samples. Yeast and mold were not found in the jelly which was shown in Table 4.8. Mold, according to (Muck 2010), is an aerobic creature that cannot develop successfully in environments when oxygen is scarce. Yeast, on the other hand, can grow in both aerobic and anaerobic environments. The acid/alkaline needs for yeast and mold growth in a wide range of food products are quite diverse, ranging from pH 2 to over pH 9. Yeast and mold growth were controlled by storing the jelly in an airtight bottle. There was no E.coli in the jelly sample (Pramanick et al., 2014) reported that the complete absence of TCCC,TFC, *E.coli, Streotococcus spp.,Vibrio spp* in jelly was an indication of better and safe quality fruit ptoduction. The utilization of high-quality raw materials is critical for producing high-quality jelly that is free of microbial contamination. The Uniform Open Dating Regulation mandates that shelf life must be included on packaging for all perishable food products (Sidhu, 2006).

5.5 Sensory Evaluation

Sensory evaluation of Pomegranate Jelly was created in order to achieve the greatest organoleptic acceptance of all jelly. According to sensory analysis results from Table 4.9, jelly with sugar (sample E) had the highest overall acceptance of 8.89±0.01. It could be because of the taste, flavor, color, consistency, and appearance. In practically all parameters, jelly with sugar (sample E) performed almost similarly to control sample A. In comparison to the control sample A, the lowest hedonic score estimated in sample C and D (with honey) was 4.8±0.01 and 4.5±0.01 respectively. It may be due to the honey which created a darker color and sour taste than control A. The composition of pectin and citric acid in jelly influenced the taste and flavor score. The texture or consistency of jelly improved as the proportion of gelatin and citric acid increased; reported by (Basu et al., 2010). According to the findings of this study, jelly with sugar (sample E) performed better organoleptically than other compositions.

CHAPTER 6
CONCLUSION

In general, the study was composed of seven sections: extraction of pectin, characterization of pectin, preparation of jelly and assessment of nutritional value,shelf life study, sensory evaluation of jelly and production cost analysis. According to this study, pomegranate peels can be used in the same way as orange and apple peels to produce pectin. The extracted pectins had high esterification degree, low ash contents, and high water-holding capabilities. Therefore, it has the potential to be employed in the food business to produce pectin on a commercial scale and the production cost is also minimal. This study also discovered that pomegranate jelly prepared with the extracted pectin had similar acceptance like control in terms of sensory perception and the proximate result was also compatible with the control. Jelly prepared with sugar has the highest acceptability and could be stored for 3months. Because of health benefits, jelly is a common food product in ready-to-eat foods. Due to unavailability in the local market commercial pomegranate jelly could not be tested in this study. Fibre isolated from pomegranate peel could improve the final product.

CHAPTER 7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

These investigations finished with promising results in the emerging field of creating new technologies for fruit value addition. It also increased its commercial value and acceptability. Recommendation of this study are-

- ✓ Sample (Fruit Peel) should be collected in a clean sampling bag and kept in a freezer to prevent microbial attack
- ✓ Over riped fruit peel cannot be used
- ✓ During hydrolysis of peel, the pH range should be 1.2-2.6
- ✓ Foam formation should not be occurred during jelly formation

Future perspectives are-

- ✓ By using this method we can achieve good quality and quantity of pectin
- ✓ Seasonally available fruits can be utilized in this way
- ✓ Fruit waste will not create environmental pollution
- ✓ It will reduce waste load in the processing industries
- ✓ Low economic extraction method
- ✓ A new variety of jelly
- ✓ Adequate actions should be taken to increase the nutritional value of commercially supplied jelly.
- ✓ Such types of research should be done for other types of fruits available in market.

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APPENDICES

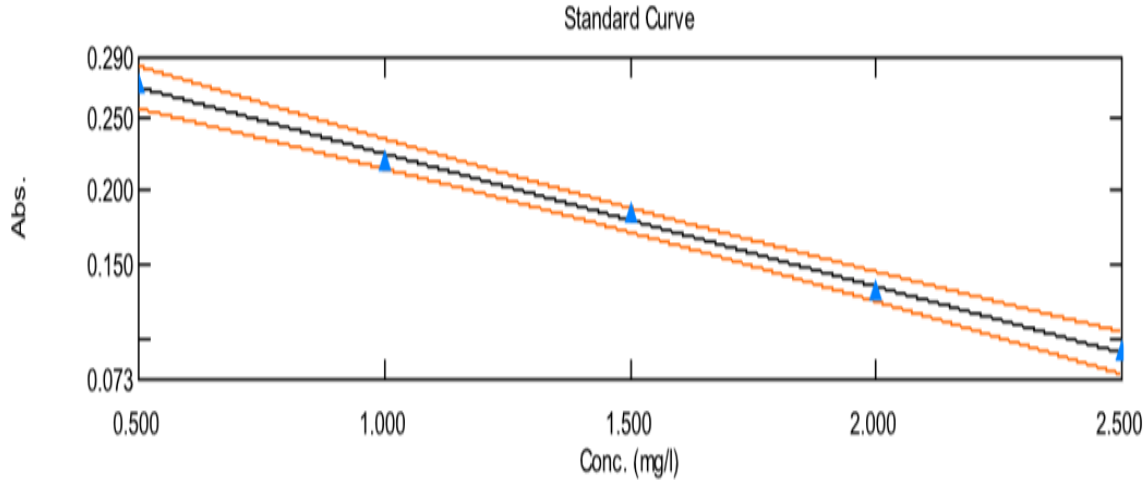
Appendix A:

Antioxidant capacity

Standard Table of Trolox:

	Sample ID	Type	Conc(ppm)	WL517.0
1	Std1	Standard	0.500	0.272
2	Std2	Standard	1.000	0.221
3	Std3	Standard	1.500	0.185
4	Std4	Standard	2.000	0.133
5	Std5	Standard	2.500	0.092

Standard Curve:

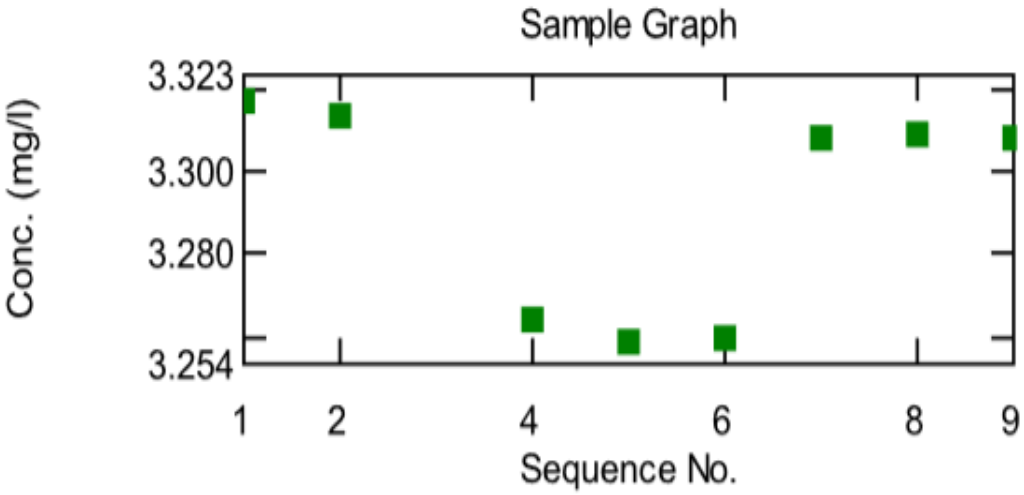


y = - 0.0894539 x + 0.314536
r2 = 0.99735

Sample Table

Sample No.	Sample ID	Type	Conc (mg/100g)	WL517.0	Comments
1	S1.1	Unknown	3.317	0.018	Dilution Factor 2
2	S1.2	Unknown	3.314	0.018	Dilution Factor 2
3	S1.3	Unknown	3.317	0.018	Dilution Factor 2
4	S2.1	Unknown	3.254	0.024	Dilution Factor 2
5	S2.2	Unknown	3.256	0.024	Dilution Factor 2
6	S2.3	Unknown	3.250	0.024	Dilution Factor 2
7	S3.1	Unknown	3.234	0.021	Dilution Factor 2
8	S3.2	Unknown	3.232	0.021	Dilution Factor 2
9	S3.3	Unknown	3.230	0.021	Dilution Factor 2
10	S4.1	Unknown	3.264	0.023	Dilution Factor 2
11	S4.2	Unknown	3.259	0.023	Dilution Factor 2
12	S4.3	Unknown	3.260	0.023	Dilution Factor 2
13	S5.1	Unknown	3.308	0.019	Dilution Factor 2
14	S5.2	Unknown	3.309	0.019	Dilution Factor 2
15	S5.3	Unknown	3.308	0.019	Dilution Factor 2

Sample Graph:



Appendix B: Questionnaire for Hedonic test

Name of the Taster: …… Date: …….

Please taste these samples and check how much you like or dislike each one on seven sensory attributes such as color, taste, 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

Appendix C: Photo Gallery

Raw Pomegranate

Collection and weighing of peel

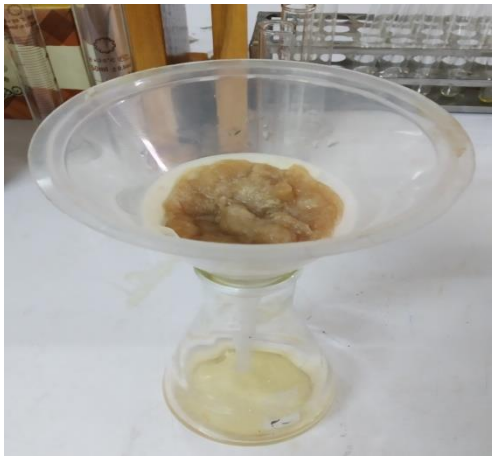
Drying

Pomegranate Peel Powder

Adjusting the pH of peel powder

Heating with continuous stirring

Pectin Extract+ Ethanol



Filtration of pectin

Drying

Final Pectin

FTIR analysis of Pectin

Aril of Pomegranate

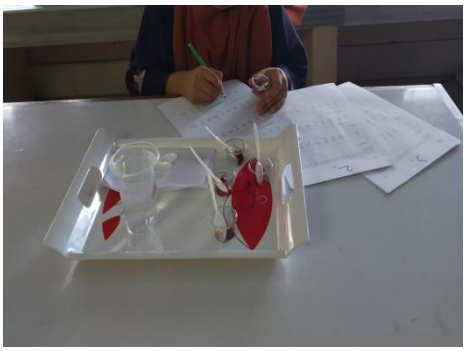
Pomegranate juice +raw materials

Heating

Determination of TSS

Sterilization of jars

Final product (Jelly)

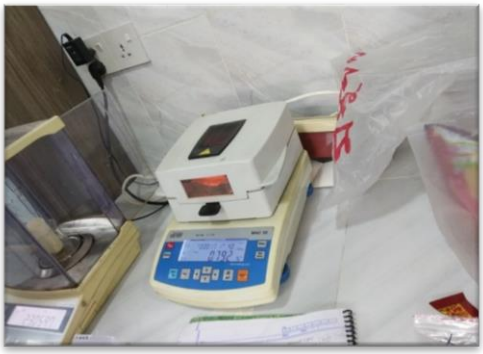


Sensory Evaluation of Jelly

Fat determination by Soxhlet apparatus

Protein determination by Kjeldahl method

Determination of Ash



Determination of Moisture Content

BRIEF BIOGRAPHY

Trishna Roy passed the Secondary School Certificate Examination in 2012 from Bakalia Government Laboratory High School, Chattogram, and then Higher Secondary Certificate Examination in 2014 from Kapasgola City Corporation Mohila 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 (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.