NUTRITIONAL COMPOSITION, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF TWO VARIETY (LIGHT RED AND DARK RED) ROSELLE (*Hibiscus sabdariffa* L.) JAM.

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

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June 2020

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July, 2020

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DEDICATED TO MY BELOVED FAMILY & TEACHERS

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Abbreviation

%	: Percentage
&	: And
ANOVA	: Analysis of variance
AOAC	: Association of Official Analytical Chemists
TSS	: Total Soluble Solids
°C	: Degree Celsius
^{0}B	: Degree Brix
CHO	: Carbohydrate
dl	: Deciliter
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
ABTS	: 2,2-Azinobis-(3-Ethylbenzthiazolin-6-sultonic Acid)
et al	: Et alii/ et aliae/ et alia
etc	: Et cetera
G	: Gram
Kg	: Kilogramme
mg	: Miligram
TE	: Trolox equivalent
Cfu	: Colony forming unit
QE	: Quercetin equivalents
GAE	: Gallic acid equivalents
L.	: Linn
PPM	: Parts per Million
m	: Meter
DNA	: Deoxyribonucleic acid
spp.	: Species
µg	: Microgram
SPSS	: Statistical Package for Social Science

Abstract

Roselle (*Hibiscus sabdariffa* L.) calyces are rich in nutrient but the utilization of its calyces in the preparation of jam is not popular in Bangladesh. The study aimed to develop roselle jam from dark and light red varieties and to evaluate phytochemicals, antioxidant capacity, sensory and nutritional parameters. Roselle jam was processed using the open kettle method. One way analysis of variance (ANOVA) was performed to find out the level of significance at $P < 0.05$. The investigation was taken place by preparing roselle jam in addition of sugar, commercial pectin, roselle seed extract as a natural pectin and citric acid. Three different formulations for single colour variety were made from roselle calyx extract and sugar (50:50) by adding seed extract, commercial pectin and no extra pectin. The sensory score of roselle jam processed with seed extract from both varieties (sample B and sample E) were found to be the best among different formulations of two varieties. The carbohydrate, fat, protein, ash and fiber were determined at the range of 59.33% to 60.78%, 1.16% to 1.76%, 1.42% to 1.76%, 0.72% to 1.34% and 1.46 to 2.33% respectively. Energy content was found ranging from 262.94-267.112 kcal/100g. Vitamin C content in roselle jams was estimated ranging (16.87- 24.55) mg/100g. Jam from light red roselle showed higher antioxidant capacity and TPC than dark red roselle. Moreover, the anthocyanin content and total flavonoid content for light red roselle jam ranged from 0.27 to 0.51 mg TA/100 ml and 62.87 to 88.33 mg QE/100 g whereas for dark red roselle jam had 0.41 to 0.51 mg TA/100 ml and 58.66 to 79.20 mg QE/100 g. Total viable count was found in acceptable limit and fungal activity was not seen after 15 days of storage at cold (8 ± 2^0C) temperature.

Keywords: Roselle, Jam, Antioxidant, DPPH, Phytochemicals, Sensory properties

Chapter 1: Introduction

The dogma "Let food be thy medicine and medicine be thy food" given by Hippocrates nearly 2,500 years ago, is receiving renewed interest. In the last decade, there has been a marked and growing interest for natural foods, specifically those rich and made of vegetable origin. Advertisings and scientific investigations make this demand that have shown the benefits that these foods may contribute to health. Recently, these types of foods are known as functional foods which is defined as those foods that contain significant levels of active biological components, mainly vitamins, polyphenolic acids, flavonoids and anthocyanins which might provide specific health benefits to humans beyond the traditional nutrients they contain (Hasler, 2002; Falk, 2004).

Roselle (*Hibiscus sabdariffa* L.) is a member of the family Malvaceae to which okra, cotton and kenaf belong is supposed to be native of tropical Africa which has red, dark red and green types inflated calyces. The flowers of Roselle are generally small (Schippers, 2000). Roselle is popularly known as ‘mesta’ or ‘chukur’ in Indian subcontinent including Bangladesh. It is locally recognized by different names in different countries (Islam et al., 2019). Different parts of Roselle including seeds, leaves, fruits and roots are used in various foods. Among them, the fleshy red calyces are the most favored. They are used to make wine, juice, jam, jelly, syrup, gelatin, pudding, cakes, ice cream and also dried & brewed into tea, spice and used for butter, pies, sauces, tarts, and other dessert (Puro et al., 2014).

Roselle calyx is a cheap source of protein, fat and minerals. Regular consumption of roselle may minimize nutritional deficiency problems such as night blindness, scurvy and rickets (Babalola et al., 2001; Ashaye and Adeleke, 2009). It is also an important source of vitamins and bioactive compounds like organic acids, phytosterols and polyphenols. The phenolic content in the plant consists mainly of anthocyanins such as delphinidin-3glucoside, sambubioside and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like β-sitoesterol and ergoesterol (Ali et al., 2005). The most utilized part of Roselle plant is its calyces which may be green, red or dark red. The green calyces are used for making vegetable stew (Atta et al., 2013) while red and dark red ones are utilized in producing drinks, jams, jellies, sauces, chutneys, wines, preserves and tea (Delgado-Vargas and Parcedes- Lopez, 2002).

Roselle is a tropical plant of great economic potential. Its calyces have been suggested as food colorants for food industries, emulsifier for carbonated drinks, jam manufacture, juices and natural food colorants (Duangmal et al., 2004).

Peoples are becoming aware regarding the health and they are more interested to consume fruits and vegetables to maintain good health. Where, most of the time they like to intake raw and after processed as well. Now consumers are more interested in consuming nutritious food which has resulted in research aimed at determining the levels of phytonutrients and specific health benefits in a range of fruits and vegetables. However, so far we know the preparation of jam from Roselle was not familiar and nutritional assessments were not done in the context of Bangladesh.

Jam is an effective fruit preserving method having high sugar content which does not allow bacteria, yeast and moulds to grow and also prevent other spoilage. This means that the nutritional qualities of the fruits can be maintained at the same time as providing tasty products (Ashaye and Adeleke, 2009). It is an example of fruit preservation usually made from pulp and juice of one fruit (whole fruit) which is normally consumed as bread spread. The preparation of fruit jam traditionally involves the use of pectin as a gelling agent, although sugar or honey and citric acid may be added as well (Ihediohanma et al., 2014).

Pectin gels are very important in creating or modifying the texture of jams, jellies, confectionaries and in low fat dairy products. It is also used as ingredients in the pharmaceuticals industry and it also lower the glucose response of products. In order to know their type and content, pectin is separated supported their solubility by sequential extraction in water or buffer solutions, solutions of chelating agents, dilute acids, or dilute sodium hydroxide or sodium carbonate. It is also considered a safe additive with no limits on acceptable daily intake (Da Silva and Rao, 2006).

Fruits and vegetables are essential in human nutrition and commerce. However, they are seasonal and highly perishable and need to be processed into more stable forms such as jams, jellies and juice so as to derive their maximum benefits (Sinha et al., 2012). Where one attractive and effective means of fruit utilization is jam processing (Jalgaonkar et al., 2020). Considering the previous history only few studies have been conducted on roselle jam. In order to obtain more valuable data on this subject, this study was carried out to determine nutritional composition, phytochemicals, sensory

evaluation, microbial analysis, antioxidant and bioactive compounds of jam that have been made of two varieties of Roselle (Red and Dark Red).

Aim and Objectives:

i. To prepare Roselle Jam from two varieties of Roselle (red and dark red).
ii. To analyze and compare nutritional composition, phytochemicals and antioxidant capacity among the prepared jams.
iii. To compare the overall acceptability of the developed product.

Chapter 2: Review of Literature

2.1 Overview of Roselle

Roselle (*Hibiscus sabdariffa* L.) plant is an annual, erect, bushy, herbaceous sub-shrub that may grow to 8 ft (2.4 m) tall with smooth or nearly smooth, cylindrical, typically red stems. The leaves are alternate 3 to 5 in (7.5-12.5 cm) long green with reddish veins and long or short petioles. The capsule turns brown and splits open when mature and dry. The calyx stems and leaves are acidic and closely resemble the cranberry (*Vaccinium spp.*) in flavor. It is widely cultivated in tropical Africa, Asia, Australia, and Central America (Schippers, 2000). These inflated edible calyces are red or green in colour. The types can be distinguished by three different colour group named green, red and dark red (Babalola et al., 2001).

Figure2.1: Dark red, green and light red Roselle

Roselle (*Hibiscus sabdariffa* L.) is known in different countries by various common names, including roselle, razelle, sorrel, red sorrel, Jamaican sorrel, Indian sorrel, Guinea sorrel, sour-sour, and Queensland jelly plant. In English-speaking countries it is known as roselle, Jamaican sorrel, red sorrel, Indian sorrel, rozelle hemp, natal sorrel and rosella. The Japanese name is rohzelu, lalambari in Urdu and lal-ambari, patwa or laalambaar in Hindi. In French, roselle is also known as redwinged thrush. In Switzerland, the edible calyx is called karkade. The roselle fiber is called India rosella hemp, rosella fiber, rosella hemp or Pusa hemp. Vernacular names for roselle include rozelle, jelly okra, lemon bush and Florida cranberry (Mohamed et al., 2012; Salih, 2008).

Taxonomy of Roselle

Kingdom: Plantae

Division: Mangoliphta

Class: Magnoliposida

Order: Malvelas

Family: Malvaceae

Genus: *Hibiscus*

Species: *Sabdariffa*

Binomial name: *Hibiscus sabdariffa* L.

2.1.2 Origin and distribution of Roselle

Roselle is native from India to Malaysia where it is cultivated very often and must have been carried at an early date to Africa. It has been widely distributed in the tropics and subtropics of both hemispheres and in many areas of the West Indies and Central America. It has become naturalized in Kenya. However, it is not widely grown (Shruthi et al., 2016).

2.2 Utilization and economic importance of Roselle

2.2.1 Calyces

The Roselle fruit is about 2.5 cm in length with fleshy calyces containing dark brown seed (Mitra et al., 2012). Due to various uses, it has been contributing to the livelihood of people in parts of Nigeria (Arowogeo, 2008). However, its production into non - alcoholic drink (ZOBO-Nigeria) is at cottage level in Nigeria and Sudan with a very short shelf life (Omemu, et al., 2006). The red calyces surrounding the fruit can be used to brew nonalcoholic beverages and as coloring reagent for jelly, jam, beverages and foods (Wahid, 2008).

2.2.2 Leaves and roots

In Bangladesh, roselle or mesta leaves are steamed with dried or fresh fish to make paste with garlic, onion and chilies or cooked with fish. A popular soup or dish is also prepared from roselle leaves along with prawn stock. The young leaves of roselle are consumed as dal after steaming with lentils in India. Leaves are also used to prepare Pacchadi (pesto) by mixing with spices. In Assam (India), leaves of roselle are also cooked along with chicken or fish. The leaves are widely consumed as affordable vegetable in Myanmar for poor peoples (Islam et al., 2016). Tender young leaves are eaten as vegetables especially with soup or salads and as a seasoning in curries. They

have acidic, rhubarb like flavor (Fasoyiro et al., 2005; Mungole and Chaturvedi 2011). Roselle has a tap root system. The roots are deficient of most nutrients as reported by (Ojokoh, 2006).

2.2.3 Stem

The stem is utilized in fibre extraction. Luvonga (2014) reported that the plant is grown in some regions for fibre and pulp obtained from its stem. Total yield of the dry retted fibre components from one hectare, 30 Tonnes of Green Roselle plants is 1,410 kg. China and Thailand are the largest producers in the world and control much of the global supply. Thailand invested heavily in Roselle production and their product is of high quality Whereas China's product, with less stringent quality control practices, is less reliable and reputable.

2.2.4 Seeds

The seeds are used as feed meal for fish and domestic animals (Mukhatar, 2007). There have been attempts to make condiments. Mohamadou, et al., (2007) reported a study that determined the functional potential of Mbuja, a study suggested that Mbuja was a cheap functional food that provides both antioxidants and probiotics. Mbuja production and consumption could therefore contribute to the consumer's health.

2.3 Nutritional properties

The nutritional composition of fresh roselle varies between studies, probably due to different varieties, genetic, environmental, ecology and harvest conditions of the plant. Early studies reported that in 100 g roselle calyces contain protein 1.9 g, fat 0.1 g, carbohydrates 12.3 g and fibre 2.3 g. They are rich in vitamin C 14 mg, b-carotene 300 μg, calcium 1.72 mg and iron 57 mg in 100 g (Ismail et al., 2008).

The leaves contain protein 3.3 g, fat 0.3 g, carbohydrate 9.2 g, phosphorus 214 mg, iron 4.8 mg, thiamine 0.45 mg, b-carotene 4135 μg, riboflavin 0.45 mg and ascorbic acid 54 mg in 100 g (Ismail et al., 2008).

The seeds contained crude fatty oil (21.85%), crude protein (27.78%), carbohydrate (21.25%), crude fibre (16.44%) and ash (6.2%). In terms of minerals, the most prevalent is potassium (1329±1.47 mg/100 g), followed by sodium (659±1.58 mg/100 g), calcium (647±1.21 mg/100 g), phosphorus (510±1.58 mg/100 g) and magnesium (442.8±1.80 mg/100 g). The major saturated fatty acids identified in the seed oil are palmitic

(20.84%) and stearic (5.88%) acids and the main unsaturated fatty acids are linoleic (39.31%) and oleic acid (32.06%) (Nzikou et al., 2011).

2.4 Functional properties and Phytochemicals

2.4.1 Functional foods

The term "functional food" first emerged in Nature in 1993 in an article titled "Japan Explores the Boundary between Food and Medicine" (Swinbanks and O'Brien, 1993).

Functional food is defined as any food or food substances that may provide a health benefit beyond the traditional nutrients it contains. It can be considered to be those whole, fortified, enriched or enhanced foods that ensured health benefits beyond the provision of essential nutrients when consumers are consumed at efficacious levels as part of a varied diet on a regular basis. (Rama, 2019). The potential of functional foods to mitigate disease, promote health, and reduce health care costs (Nicoletti, 2012).

2.4.2 Functional foods from plant sources

Irresistible evidence from epidemiological, in vivo, in vitro and clinical trial data indicates that a plant-based diet can reduce the risk of chronic diseases, particularly cancer. The World Cancer Research Fund reported a convincing evidence for a protective effect of high intake of fruits and vegetables against a number of respiratory and digestive cancers (Boffetta et al., 2010). There have an inverse associations between fruits and vegetables intake and chronic diseases, such as different types of cancer and cardiovascular disease, have been demonstrated in numerous epidemiological studies. Phytochemicals have been indicated to be responsible for this observed protective effect reported by Schreiner and Huyskens-Keil (2006).

Health professionals are gradually recognizing the role of phytochemicals in health enhancement (Srivastava, 2011). In USA an act has been enacted, Nutrition Labeling and Education Act of 1990 (NLEA) that requires nutrition labeling for most foods and allow disease- or health-related messages on food labels (Marietta et al., 1999).

Nowadays in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis are the leading cause of cardiovascular morbidity and mortality. A major risk factor for the development of cardiovascular diseases is the elevated levels of plasma cholesterol (Félix-Redondo et al., 2013). It is crucial to maintain the normal body functions by reducing the elevated serum to adequate levels. Since the technology

of functional food emerged, more and more functional foods are being developed from plants and regarded as the adjuvant treatment to some diseases (Demigne *et al*., 1998).

Recently there has been an increased interest in research on food components such as anthocyanins and other phenolic compounds because of their possible linkage to health benefits including reduction in heart disease and cancer, partly based on theirantioxidant activity (Seeram et al., 2002). With the global functional food and beverage market estimated at $109 billion by 2010 (Watkins, 2008), diverse sources of phytochemicals are being explored. Polyphenols in beverages are common because of their beneficial physiological effects on health (Ina *et al.*, 2002).

Additional research is necessary to substantiate the potential health benefits of those foods for which the diet-health relationships are not sufficiently scientifically validated.

2.4.3 Phytochemicals

Phytochemicals are the bioactive non-nutrient plant compounds in fruit, vegetables, grains and other plant foods have been linked to minimize the risk of major chronic diseases (Zhang et al., 2015).

A large number of Phytochemicals and bioactive components are reported to be present in foods of plant origins and have become the focus of study in functional foods. Shahidi (2004; 2008) reported that their synergistic effects are rendered by a combination of phytochemicals present in source materials and complementary nature of phytochemicals from different sources are considerable factors to figure out in the formulation of functional foods and in the choice of a healthy diet.

It is estimated that more than 5000 Phytochemicals have been identified but a large percentage still remain unknown (Shahidi, 1995) and need to be identified before their health benefits are fully understood. However, there have more convincing evidence suggests that the benefits of phytochemicals in fruits and vegetables may be even greater than is currently understood because oxidative stress induced by free radicals is engaged in the etiology of a wide range of incurable diseases (Ames, 1991).

Cells in humans and other organisms are always exposed to a variety of oxidizing agents. These agents may be present in air, food and water or they may be produced by metabolic functions within cells. The key factor is to hold down a balance between oxidants and antioxidants to sustain optimal physiologic conditions in the body.

Overproduction of oxidants can cause an imbalance, leading to oxidative stress, especially in chronic bacterial, viral, and parasitic infections (Liu and Hutchkis, 1995). Oxidative stress can cause oxidative damage to large biomolecules such as proteins, DNA, and lipids, resulting in an increased risk for cancer and cardiovascular disease (Ames et al., 1991). To prevent or slow down the oxidative stress initiated by free radicals, sufficient amounts of antioxidants need to be consumed. Fruit and vegetables contain a wide variety of antioxidant compounds (Phytochemicals) such as phenolics and carotenoids that may help to defend cellular systems from oxidative damage and lower the risk of chronic diseases (Van Breda and de kok, 2018).

Flavonoids

There has been considerable interest in the flavonoid content of foods and plants since the early 1980s when the studies of Steinmetz and Potter (1991) demonstrated a relationship between a diet rich in fruits and vegetables and a reduced risk for chronic diseases. Because reduced risk did not correlate with traditional nutrients, attention has focused on many non-nutrient, potentially bioactive compounds, of which the flavonoids constitute one family (Steinmetz and Potter, 1991).

Naturally occurring polyphenolic compounds known as plavonoids which has C6-C3-C6 backbone. This group of plant pigments which are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and can be chemically subdivided into six structural categories: flavones, flavonols, flavanones, flavanonols, flavan-3-ols (catechins), and anthocyanidins. These compounds (aglycones) are generally glycosylated (at one or more sites with a variety of sugars) and may also be alkoxylated or esterified. As a result, over 5000 different flavonoids have been identified in plant materials (Harborne and Williams, 1992). The methods that have been reported for the determination of flavonoids are based on the aluminium chloride complex formation, which is mostly used analytical procedures applied to the flavonoid content determination in different plants (Grubesic et al., 2007).

Literature survey reveals the presence of two classes of flavonoids in the extracts of *Hibiscus sabdariffa*: flavonols (gossypetin) and the anthocyanins (Bisset and Wichtl, 1994).

Anthocyanins

Anthocyanins are another group of pigments in plants. The structure of these anthocyanins differs in the types of anthocyanidins, sugar molecule and numbers, and types of acylation groups. Due to their bright color and high water solubility, anthocyanins are considered a potential natural pigment to replace artificial food colorants (Mazza and Miniati, 1993). Besides the coloring functions, anthocyanins in foods also possess potent antioxidant capacity and health promoting properties. For instance, anthocyanins in foods are believed to be able to reduce the risk ofcardiovascular diseases for people who consume wine, berry, Roselle and grape. (IUFOST, 2009)

The mechanism postulated is that anthocyanins act as antioxidants by donating hydrogen atoms to highly reactive free radicals, breaking the free radical chain reaction. Claims about health benefits of functional foods must be based on sound scientific criteria (Clydescale, 1997).

Antioxidants

Antioxidants are the compounds which are accountable to sabotage the free radicals and protect our body from various free radical associated diseases. The mechanism is associated with free radical mediated oxidative process through initiation, propagation and termination. Production of antioxidant can be occurred inside the body as well as naturally in many foods (Alam et al., 2020).

Wu et al., (2018) reported that the roselle extract is rich in anthocyanins and has good antioxidant capacity (DPPH IC50 = 4.06 mg/ml, ABTS IC50 = 3.7 mg/ml). The anthocyanins exhibited a certain degree of heat resistance and favorable color stability in an acidic environment.

Roselle anthocyanins which are a group of natural pigments existing in the dried calyx exhibited antioxidant activity and liver protection manner. This antioxidant bioactivity in rat primary hepatocytes and hepatotoxicity was studied by Wang et al., (2000)**.** The results revealed that roselle anthocyanin's at the concentrations of 0.10 mg/ml and 0.20 mg/ml, significantly decreased the leakage of lactate dehydrogenase and the formation of malondialdehyde and the serum levels of hepatic enzyme markers (alanine and aspartate aminotransferase) significantly decreased and reduced oxidative liver

damage. An antioxidative activity was also reported in cancerous cell lines **(**Akim et al., 2011).

Phenolic compounds

Phenolic compounds are dietary constituents widely spread inplant kingdom. Phenolics include thousands of compounds with various chemical structures. Due to their influence to sensorial properties (color and astringency) their analysis in foods and beverages has been developed during last decades (Monagas et al., 2005).

Mollah et al., (2020) reported that the total phenol contents were 521.46 mg/100 g in the calyx of BJRI vegetable mesta-1. There were reports on the total phenolic contents of Roselle extracts ranged from 108 to 546 μg/g and 582 to 606 μg/g respectively. The results of various studies revealed that phenolic contents of Roselle calyx are less variable among various genotypes of Roselle.

2.5 Antimicrobial activity

Plants, particularly those prescribed against microbial infections since a long time in traditional and folk medicine from different societies could be promising sources for new antimicrobials (Abdallah, 2011). Abdullah (2016) evaluated the antibacterial potential of the calyces of the Sudanese Roselle which intensively used in Sudanese folk medicine and reported that the antibacterial test indicated that the methanol extract of roselle calyces contained effective antibacterial agent(s), revealed a considerable zone of inhibition against all tested Gram-negative and Gram-positive bacteria and it was a competitor to gentamicin and hugely higher than penicillin which showed weak or no effect.

2.6 Medicinal and health benefits

Roselle is a multipurpose plant and all above ground parts of roselle is used as traditional madicine for the treatment of several diseases in Africa, Senegal, India, Thailand and Mexico (Ngamjarus et al., 2010). Many medicinal applications of the plant parts of roselle have been reported in different countries of the world. Different reports listed which affirm the traditional health benefits of roselle extract:

i. Roselle tea reduces the blood pressure in hypertensive and pre-hypertensive persons (Faraji and Tarkhani, 1999).

ii. Due to the presence of anthocyanins found in the roselle extracts, lowers bad cholesterol (LDL) levels in the blood (Hirunpanich et al. 2006; Lin et al. 2007; Kuriyan et al., 2010).

iii. Roselle extracts exhibit anti-diabetic properties and induced sperm damage (Odigie et al., 2003).

iv. Roselle extracts is used to treat leukaemia, liver damage, hypertension and pyrexia due to its high amount of protocatechuic acid (Tseng et al., 2000).

v. The extracts abate the deposits of calcium oxalate crystal on kidneys due to its uricosuric effect with no toxicity or negative side effects (Qi et al., 2005; Prasongwatana et al., 2008).

vi. Roselle extracts are perfectly safe for use in skin conditioning treatments (Ali et al., 2005).

vii. Roselle extracts has immune-protective effects which proved based on their ability to protect human cells against cadmium-induced damage such as tumor necrosis (Sulistyani et al., 2016).

viii. Calyx extracts act as a defensive means for liver diseases by destroying radicals and conserving enzymes responsible for medicine detoxification (Duh and Yen 1997; Furuta et al., 1998).

ix. Roselle extract's has anti-nociceptive, anti-inflammatory and anti-diarrheal qualities (Ali et al., 2005).

x. Roselle extracts reduces extra fat from liver and abdomen (Cid-Ortega and Guerrero-Beltrá, 2015).

xi. Calyx extracts reduced body fat and body mass index (BMI) (Greenwood-Robinson, 1999; Ghislain et al., 2011).

xii. Roselle juice with salt, pepper and molasses is used to relieve coughs and remedy of biliousness (Mohamed et al., 2012).

xiii. Calyx extract also contain several amino acids those are important for our body (Al Wandawi, 2015).

2.7 Roselle jam

The most attractive and effective means of fruits utilization is jam processing (Ashaye and Adeleke, 2009). Jam is an intermediate moisture food which is prepared by boiling fruit pulp with sugar (sucrose), pectin, acid, and other ingredients (preservative, coloring, and flavoring materials) to a thick consistency, firm enough to hold the fruit tissues in position (Baker et al., 2005).

Farhana, (2016) used the seed mucilage as natural source of pectin to make Roselle jam in lieu of traditional method.

Pectin is usually used as gelling, thickening and stabilizing agents in foods and to a certain extent in pharmaceuticals as well. Pectin is generally used to construct the desired texture of products which result in controlling the moisture or water in the final product. The historical use of pectin was in jam and jellies preparation due to its thickening and jelling properties. Pectin has been reported as very well tolerated and safe among various food additives, thus it is grouped and acknowledge in the “Acceptable Daily Intake (ADI) levels of “not specified" products by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (Siddiqui et al., 2015).

The color of jams made from roselle calyces is red and tangy. Roselle calyces are rich with various nutrients especially vitamins (B1, B2, B3 and C), minerals and antioxidants. Roselle jam has been made since Colonial period and still obtainable in the community of Australia. It is commonly sold as preserved processed fruits or jams in Myanmar. Roselle jam and jelly are also manufactured in different countries of the world and are available in supershops (Islam et al., 2016).

Jalgaonkar (2020) reported that Jam has defined in the United States as that semi-solid food made from not less than 45 parts by weight of the fruit ingredient to each 55 parts by weight of sugar .This mixture is concentrated to 68 percent total soluble solids to achieve desired quality. Flavoring and coloring agents may be added.

Fruits, sugar (mostly sucrose), pectin and edible acids are usually the main ingredients required for jam production (Albrecht, 1994). The ingredients, which are usually combined with 65% sugar, 1% pectin, and an acid concentration of pH 3.10, are thermally treated at normal or reduced pressure to bring about a sweet jam textured product of desired pH range of 2.5-3.2 (Fasogbon et al., 2013).

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, Applied Chemistry and Chemical Technology, Department of Food Processing and Engineering, Poultry Research and Training Center, Department of Animal Science and Nutrition of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram.

3.2 Study Duration

The experiment was conducted for a period of six months from 1st September 2019 to 27th February 2020.

3.3 Collection of Sample

Fresh samples of Roselle calyces of red and dark red varieties were obtained from the local market of Chattogram district. Roselle fruits were carefully chosen in order to their color variety. Sugar, pectin and citric acid were purchased from scientific and surgical store. Other relevant materials required for the experiment were received from the laboratory stocks.

3.4 Jam Preparation

Sample A

Jam was prepared by fresh light red Roselle calyces, sugar and citric acid. Where 500 g boiled Roselle calyces, 500 g sugar and 5 g citric were used to make roselle jam remarked as control for light red roselle jam.

Sample B

Roselle jam was prepared by fresh light red Roselle calyces with seed pod, sugar and citric acid. Seeds were removed after parboiling.

Sample C

Roselle jam was prepared by fresh light red Roselle calyces, sugar, citric acid and commercial pectin. 15 g pectin was mixed with sugar properly and then added during cooking. Other elements were remained same.

Sample D

Roselle jam was prepared by fresh dark color Roselle calyces, sugar and citric acid. Roselle jam was prepared by fresh dark color Roselle calyces, sugar and citric acid. Where 500 g boiled Roselle calyces, 500 g sugar and 5 g citric were used to make Roselle jam denoted as control for dark red roselle jam.

Sample E

Roselle jam was prepared by fresh dark red Roselle calyces with seed pod, sugar and citric acid. Seeds were removed after parboiling process. 500 g boiled Roselle calyces with seed extract, 500 g sugar and 5 g citric were used.

Sample F

Roselle jam was prepared by fresh dark color Roselle calyces, sugar, citric acid and commercial pectin. 15 g pectin was mixed with sugar properly and then added during cooking.

Roselle jams from fresh and dry calyces were prepared by the open kettle methods. Fresh Roselle calyces were sorted to remove damaged ones from the clean ones. Fresh Roselle calyces for sample A, sample C, sample D & sample F and fresh Roselle calyces with seed pod for sample B & sample E were washed in water to remove dirty materials. The washed samples were partly cooked by boiling for 20 minutes. After parboiling, seeds were removed for sample B & sample E then the samples were independently blended in a high-speed blender for 10 minutes. 500 gm blended calyces were boiled to evaporate most of the water. 500 gm sugar added at intervals of 15 minutes with constant stirring while boiling continued until desired soluble solid was attained. 15 gm commercial pectin was mixed with sugar for sample C and sample D. 0.5 per cent citric acid was added as a preservatives. Roselle jam was filled hot into sterilized bottles leaving a minimum headspace. The lid was put on and allowed to cool (Ashaye and Adeleke, 2009). Whole procedure was given in Figure 3.1.

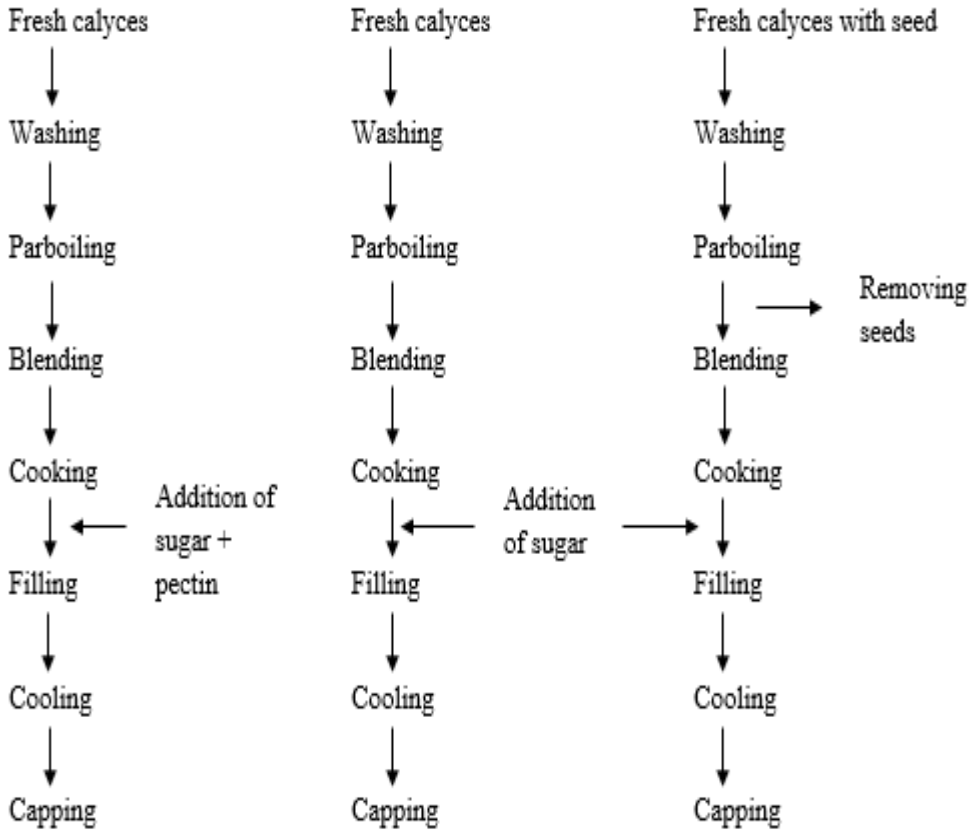

Figure 3.1: Processing steps of Roselle Jam

3.5 Physicochemical analysis of Roselle jam

Fresh samples of Roselle jam were analyzed for moisture, total solid, ash, total soluble solid, pH, titratable acidity as per the methods of AVOC (2016). These samples were also analyzed for proximate analysis, bioactive compounds analysis and antioxidant analysis.

3.5.1 Determination of 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).

3.5.2 Total Soluble Solids

Total soluble solids of the fruits were found out with the help of hand refractometer. Total soluble solids (TSS) were directly recorded by digital refract meter (Atago RX 1000) and the results expressed as percent soluble solids (Brix) as described in AOAC.

3.5.3 Titratable Acidity

The percentage of acidity was determined in terms of anhydrous citric acid by titrating against N/10 NaOH using phenolphthalein indicator. Every time 10ml of juice was taken in a 100ml volumetric flask and the volume was made up to 100ml by adding distilled water then 10ml diluted juice was titrated against N/10 NaOH, using phenolphthalein as indicator. The appearance of pink color indicates the endpoint of the titration. Titration was reported thrice at the average value was recorded (AVOC, 2016). Titratable acidity can be determined as below:

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

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

3.5.4 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. At the same time, the dye is reduced to the color less compound. S that end point of the reaction can easily determine. Rapid excretion and filtration are desirable as excess may be introduced in plant product by oxidized partially destroying Vitamin C during sampling and grinding. Oxidation is presented by the use of metaphosphoric acid during extraction. Strongly acidic solution will provide most accurate result. The titration should be complete within one minute. The dye has blue color in aqueous solution. Pink in acidic solution and become colorless when completely 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. 15/7.5mg of Metaphosphoric acid.
2. 40/20ml of glacial acetic acid dilutes to make 500/250 ml with distilled water.

Standard ascorbic acid solution

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

Procedure

Dye solution was taken in the burette. Then 5 ml Vitamin C solution was taken in a conical flask. The conical flask was placed under the burette and the dye was added drop wise. Titration was completed when pink color was appeared and stayed for 20 seconds and then disappeared. The reading was taken at least 3 times. The same procedure was performed for ascorbic acid solution of unknown concentration. The result was expressed as milligram percentage (mg %).

3.5.5 Moisture content

Principle: Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods. Since the amount of dry matter in a portion of food is inversely related to the amount of moisture it contains, the moisture content is of direct economic importance to the processor and the consumer. Of even greater significance, however, is the effect of moisture on the stability and quality of foods. Moisture content was determined by using the standard procedure of the Association of Official Analytical Chemists (AOAC, 2016).

Calculation: The percent of moisture was calculated as follow

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

3.5.6 Total solids

Total solid was determined by methods of AOAC (2016). Percent total solid content was calculated by using the data obtained during moisture estimation using the following formula:

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

3.5.7 Ash content

Ash content was determined by methods of AOAC (2016). Ash content is the inorganic residue remaining after destruction of organic matter. 10 gram dried jam was taken in a pre-dried weighed crucible. It was then burned to charcoal. The charcoal was then taken in a muffle furnace and heat at around 600°C for 4 hours till the charcoal was completely removed. The crucible was then taken out of the furnace. Cool it in a desiccator carefully and then weighed. Calculation: The ash content was calculated by the following expression.

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

3.5.8 Estimation of Crude Fat

Principle: Fat is estimated by dissolving food samples into organic solvents (chloroform: methanol) separating the filtrate by filtration. 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. AOAC (2016) methods using a soxhlet apparatus were used to determine the crude fat content of the samples.

Calculation: The percent of crude fat was expressed as follows expression.

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

3.5.9 Estimation of Crude Protein

Principle: The Kjeldahl method is used to determine the nitrogen content in organic and inorganic samples. The determination of Kjeldahl nitrogen is made in foods and drinks, meat, feeds, cereals and forages for the calculation of the protein content. Also, the Kjeldahl method is used for the nitrogen determination in wastewaters, soils and other samples. It is an official method and it is described in different normative such as (AOAC, 2016).

Calculations: The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. In the equations below, "N" represents 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

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

3.5.10 Estimation of Crude Fiber

Principle: Crude fiber is the water-insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose, and lignin. It is estimated through digestion of fat-free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in a weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained. The crude fiber was determined according to the AOAC method (2016). Then, ignited the residue in muffle furnace up to white ash (550-600°C, 4-6 hrs).

Calculation: Calculation of the crude fiber percentage as follows:

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

Where,

W= Weight of crucible, crude fiber and ash

W1=Weight of crucible and ash

W2= Weight of sample

3.5.11 Determination of total carbohydrate:

The carbohydrate content was determined by calculating the difference between the Nitrogen Free Extractive (NFE). It was given as the difference between 100 and a total of the other proximate components.

Calculation: Hence it was calculated using the formula below-

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

3.6 Determination of Antioxidant capacity by DPPH scavenging method

Extract preparation

1 gm sample was taken in Felcon tube. After that 10 ml absolute methanol was added and left for 72 hours. Continuous straining was done after 4 hours interval. After 72 hours, filtrate was collected and methanoic extract found.

Procedure

Antioxidant mobility of the extracts was determined using DPPH assay as the process described by Azlim et al. (2010) with slight modifications. About 6 mg of DPPH was dissolved in 100 mL absolute methanol and prepared methanoic DPPH solution.

Then 1 ml methanoic extract was diluted with of 2 ml DPPH solution. Then the mixture was mildly shaken and left for 30 min in dark at room temperature. The absorbance was read at wavelength 517 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA). Control prepared by mixing 1 mL of methanol with 2 mL of DPPH solution whilst methanol was used like a blank. The scavenging mobility was measured as the decrease in absorbance of the samples in comparison with the DPPH standard solution. Antioxidant capability based on the DPPH free radical scavenging mobility of extracts calculated using the following equation:

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

Trolox used as standard and TEAC composite (Trolox equivalent antioxidant mobility) was used for the calibration standard curve. The results were revealed in mg/ 100 g of Trolox equivalents (TE) per gram of powder on a dry weight (DW) base.

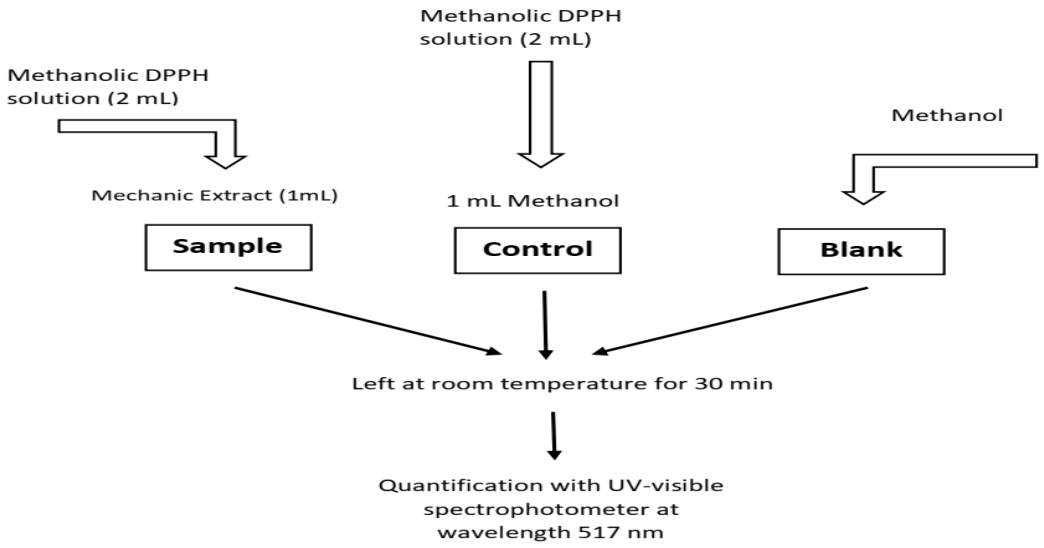

Figure 3.2: Determination of antioxidant capacity

3.7 Determination of bioactive compounds:

Extract preparation:

5 gm of sample was taken for TAC and 1 gm of sample was taken for other TPC and TFC in Felcon tube. After that 10 ml absolute ethanol was added and left for 72 hours. Continuous straining was done after 4 hours interval. After 72 hours, filtrate was collected and ethanoic extract found.

3.7.1 Total Phenolic Content (TPC)

TPC of the extracts were determined according to the Folin-Ciocalteu reagent method described with slight modifications (Al-Owaisi et al., 2014). Total polyphenol content (TPC) of the roselle jam determined according to the Folin-Ciocalteu method reported by Vergani et al. (2016) with slight modifications. 1 ml ethanoic extract was taken in a falconer tube and added 1.5 ml of FC reagent and left for 3 mins at room temperature. Then 1.5 ml Na_2CO_3 (7.5%) was added into the mixture and left for 60 minutes. The absorbance was read at wavelength 765 nm using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA) and C_2H_5OH was used as the blank. TPC was calculated and revealed as mg of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

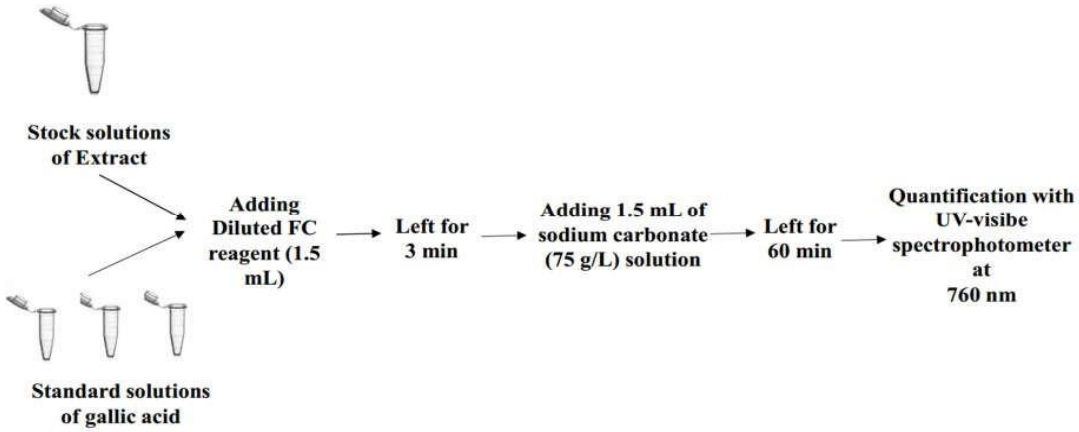

Figure 3.3 Determination of Total phenolic content (TPC)

3.7.2 Total Anthocyanin content (TAC)

Stock solutions of 10mg/mL of extracts were prepared. Extract solution (3mL) was pipetted into a cuvette. The intensity of the extract color was measured at wavelength 520nm using UV-VIS spectrophotometer. Ethanol was used as a blank TAC will be calculated and expressed as milligrams per 100 ml (mg/100 ml) using the following equation:

TAC = Absorbance of sample × DF×100/M×E

Where,

DF stands for dilution factor, M means weight of sample used to make stock solution. E refeers to extinction coefficient (55.9) (Giusti and Wrolstad, 2001).

3.7.3 Total flavonoid content (TFC)

Total flavonoids content (TFC) of the samples was determined by using the aluminum chloride colorimetric process reported by Chang et al. (2002) with slight modifications. Stock solution (1 mg/mL) of extracts was prepared and aliquots of 0.5 mL of diluted extract diluted with 1.5 mL of 95% C_2H_5OH in a cuvette. Then 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water were added to the immixture in the cuvette. The immixture left at room temperature for 30 min. The absorbance was read at wavelength 415 nm in UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA) and 10% aluminum chloride substituted with $D.H_2O$ of the same quantity were used as the blank. Total flavonoids amount in the

sample was calculated by comparing absorbance of the sample extracts with a quercetin standard curve. TFC estimated and revealed as mg quercetin equivalents (QE) per gram of extract (mg QE/g).

3.8 Microbiological analysis

3.8.1 Aerobic plate count (Bacterial plate count)

The Aerobic Plate Count is used as an indicator of bacterial populations on a sample. Aerobic Colony Count (ACC), Standard Plate Count (SPC), Mesophilic Count and Total Plate Count (TPC) are different names of Aerobic Plate Count (APC). Total viable bacterial count (TVC) was determined through the Standard Plate Count (SPC) technique.

The test is based on an assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. It is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40°C), not a measure of the entire bacterial population. APC cannot differentiate types of bacteria can use to gauge organoleptic acceptability, sanitary quality, adherence to good manufacturing practices and as an indicator of safety. Information regarding shelf-life or impending organoleptic change in a food can be provided by APC (Banwart, 2012).

Sample preparation

The reliability of the analysis and interpretation of the results depend largely on the correct manner in which the sample was taken. The sample should be a true representative of the whole mass. For this purpose the product was thoroughly mixed so that sample would be the representative of the whole mass of the products. 25 g of this well mixed Roselle jams were taken in 250 ml flask.

Phosphate buffer saline (0.6 M KH_2PO_4 of pH 7.2) was used for dilution of the sample. About 100 ml of the buffer saline was added to the beaker and mixed well by to-and-fro movement. The volume was made up with the same buffer water. All the apparatus, solutions and other tools used should be sterilized i.e. heated at 121^0 C for 15 minutes. The prepared sample was then diluted to 10 times i.e. 1×10^{-1} time's dilution and used as stock solution (Andrews, 1992).

Dilution

A series of dilution were made as follows using 9 ml blanks. The initial 1/10 dilution (1 ml in 9 ml) was performed (b). This was mixed in a vortex mixer (c) 1 ml from (b) was taken, added to the next tube and mixed well. It was become 10^{-2} time's dilution. In this way, the dilution was made up to 10^{-6} times.

Standard plate counts

A SPC was used to estimate the level of microbes in the prepared & stored samples. This data could be used as the indicators of food quality or predictors for the shelf life of the product. Using a sterile pipette, 1 ml of the diluted sample was then taken into each of the sterile empty petri-dishes having nutrient agar media (Plate count agar) at a temperature of 45°C. Plates were mixed by swirling on a flat surface. After solidification of the media the plates were inverted and incubated at 37 °C for 24 hours in an incubator (AOAC, 1990; Sharf, 1966).

Counting and recording

After incubation the incubated plates were selected for counting the bacterial colony based on the number and easy of counting of the colony. The plate containing segregated, overlapping and confusing colonies was avoided. The plates containing 30 to 250 bright, cleared and countable colonies were selected.

Number of colony forming unit (cfu)/g or ml. = average cfu plate × dilution factor. The viable bacterial count was done through the steps of sample preparation, sample dilution,standard plate counts and counting and recording. The incubation was performed at 37°C for 24 hours (AOAC, 1990; Sharf, 1966).

3.8.2 Fungal analysis in jam

Media Preparation

Sabouraud Dextrose Agar (SDA) is a selective medium primarily used for the isolation of dermatophytes, other fungi and yeasts but can also grow filamentous bacteria such as Nocardia. The acidic pH of this medium (pH about 5.0) inhibits the growth of bacteria but permits the growth of yeasts and most filamentous fungi. Antibacterial agents can also be added to augment the antibacterial effect. The SDA media is comprised of enzymatic digest of casein and animal tissues which provide a nutritious

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 used were prepared according to the manufacturer's instructions and sterilized in the autoclave at 121°C for 15 minutes. Although many selective agars exist for the cultivation and determination of mold and yeast cultures, a majority of them do not depend on strict nutritive requirements for growth. Many fungal strains will grow on Sabouraud Dextrose Agar. Methods and technique are followed here as described by Chen and Gu (2000), FSSAI (2012) and APHA (1996).

Procedure for preparation of media

At first 65 g of the medium was suspended in one liter of purified water. Then heated with frequent agitation and boiled for one minute to dissolve the medium completely. Autoclaved at 121°C for 15 minutes. Then cooled to 45°C to 50°C and poured into petri-dishes. For processing of specimen, the specimen was streaked onto the medium with a sterile inoculating loop in order to obtain isolated colonies. Then the plates were incubated at 25-30°C in an inverted position (agar side up) with increased humidity. Cultures were examined weekly for fungal growth and were held for 4-6 weeks before being reported as negative (Aryal, 2015).

Interpretation

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Examine plates for fungal colonies exhibiting typical color and morphology. Additional procedures should be performed to confirm findings. Yeasts will grow as creamy to white colonies. Molds will grow as filamentous colonies of various colors (Aryal, 2015).

3.9 Energy estimation

The energy content of the Roselle jam was determined by calculating the amount of protein, fat and carbohydrate of respective food items and by using the following equation (Baer et al., 1997).

Energy = (Protein × 4.1) + (Fat × 9.2) + (Carbohydrate × 4.1)

3.10 Cost analysis

Cost of the jam made from light red and dark red Roselle calyces were calculated from the overall ingredients cost which were utilized for the preparation of the jam. The amount was presented in taka and analyzed for the price of per kg of jam.

3.11 Sensory evaluation

Sensory evaluation was performed for the determination of overall acceptability of the final product by the consumers. A taste-testing panel evaluated the consumer's acceptability of developed product. The panel test was done in the CVASU premises where the panelists were both the teachers and students of CVASU. Panelists of 15 persons were given the product that has been developed from the Roselle calyces. There were six formulations which were encoded with sample A, sample B, sample C, sample D, sample E and sample F. The six samples were tasted by the panelists without informing them the formulations. The panelists were requested to assign appropriate score for sensory attributes of appearance, color, flavor, texture, taste, sweetness and overall acceptability of jam. This method does not, of course, reflect actual consumer perception, but it does strongly indicate attributes which a good quality product should possess (Sing et al., 2008). They tasted six samples expressed their opinion giving score about. Sensory evaluation of qualitative parameters (taste, appearance, flavor, mouth feel, sweetness and overall acceptability) of the six samples was carried out using nine point Hedonic scales (Larmond, 1977). The scale were arranged such that:

Table 3.1: Rating Scale for sensory evaluation

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

Dislike very much	2
Dislike Extremely	1

3.12 Statistical Analysis

Data were determined and stored in Microsoft Excel 2013 spread sheet to evaluate statistical analysis. All samples were in three replicates. Descriptive statistics (mean and standard deviation) were done for proximate composition and sensory evaluation of Roselle Jam. Data were sorted, coded and recorded in IBM SPSS Statistics 25. After that statistical analysis were conducted. Proximate composition, phytochemicals, antioxidant capacity and sensory evaluation data were analyzed by using One-way ANOVA procedures to assess significant level of variation at 95% confidence interval. Post hoc "Tukey" test was conducted to identify the variation within the sample groups. The statistical analysis was conducted for at 5% level of significant ($p \leq 0.05$).

Chapter 4: Results

4.1 Physicochemical properties of jam

pH of jam is an important factor for optimum gel condition. In table 4.1, lowest (2.83±0.06) pH found in sample C and highest (3.07±0.06) in sample A and sample D. TSS (total soluble solids) was highest (69 degree brix) in sample B, sample C, sample E and sample F and lowest in (68 degree brix) in sample A and sample D. The maximum value (0.79±0.04%) of acidity obtained in sample B and the least value (0.71±0.02%) found in sample D.

Table 4.1: Physicochemical properties of roselle jam.

Variety	Formulation	pH	TSS (^{0}B)	Acidity (%)
Light Red	Sample A	3.07±0.06^a	68±0.00^a	0.78±0.02
	Sample B	2.97±0.06	69±0.00^{ab}	0.79±0.04^a
	Sample C	2.83±0.06^{ab}	69±0.00^{ab}	0.72±0.01
Dark Red	Sample D	2.93±0.06	68±0.00^b	0.71±0.02^a
	Sample E	3.07±0.06^b	69±0.00^{ab}	0.76±0.02
	Sample F	2.98±0.10^b	69±0.00^{ab}	0.75±0.06

Legends: Means ± SD and values in the same column with the same superscripts are statistically significant (P<0.05).

Sample A- Jam form light red Roselle without additional pectin
Sample B- Jam form light red Roselle with seed extract
Sample C- Jam form light red Roselle with commercial pectin
Sample D- Jam form dark red Roselle without additional pectin
Sample E- Jam form dark red Roselle with seed extract
Sample F- Jam form dark red Roselle with commercial pectin

4.2 Nutritional Composition

Nutritive value of Roselle jam is shown in Table 4.2, almost all samples are significantly different. Where sample E contained the highest percentage of crude fiber (2.33±0.01%) & crude fat (1.76±0.03%) and Sample B contain the most abundant percentage of crude protein (1.76±0.03%). The lowest percentage of crude fiber (34.75±0.03%), crude fat (1.16±0.02%) and crude protein (1.42±0.02%) found in sample A, sample C and sample F respectively.

Table 4.2: Nutritional composition of roselle jam

Variety	Formulation	Moisture%	Crude Fiber %	Ash%	Crude Fat %	Crude Protein %	CHO %	Vitamin C %
Light Red	Sample A	34.75±0.03^{a}	1.46±0.02	0.72±0.03	1.19±0.02^{a}	1.65±0.03^{a}	60.23±0.06	21.75±0.02
	Sample B	34.59±0.03^{a}	1.80±0.03	0.83±0.02^{a}	1.27±0.03	1.76±0.03	59.74±0.03	17.17±0.03
	Sample C	33.88±0.05^{b}	1.54±0.02	1.03±0.02	1.16±0.02^{a}	1.60±0.02^{ab}	60.78±0.03	16.87±0.02
Dark Red	Sample D	34.80±0.02^{a}	1.93±0.03^{a}	0.88±0.03^{ab}	1.44±0.02^{b}	1.56±0.03^{b}	59.39±0.78^{a}	24.55±0.03
	Sample E	33.61±0.34^{b}	2.33±0.01	0.91±0.02^{b}	1.76±0.03	1.62±0.04^{ab}	59.58±0.03	18.84±0.03
	Sample F	34.46±0.02^{a}	1.96±0.02^{a}	1.34±0.03	1.48±0.03^{b}	1.42±0.02	59.33±0.03^{a}	18.28±0.03

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

4.3 Phytochemical composition of roselle jam

The results of bioactive compounds (TAC, TFC and TPC) are presented in table 4.3. There have a significantly different values found among all samples. Sample C carried the highest value of total anthocyanin content (0.51±0.002 mg TA/100 mL) and total flavonoid content (88.33±0.18 mg QE/100 g) where sample A contain the highest value of total phenolic content (5.29±0.02 mg GAE/100mL). Lowest value of total flavonoid content (62.04±0.17 mg QE/100 g) and total phenolic content (1.08±0.02 mg GAE/100mL) found in sample E**.**

Table 4.3: Phytochemical composition of roselle jam

Variety	Formulation	Total Anthocyanin Content (TAC)(mg TA/100 mL)	Total Flavonoid Content (TFC) (mg QE/100 g)	Total Phenolic Content (TPC) (mg GAE/100mL)
Light Red	Sample A	0.31±0.002	62.87±0.08	5.29±0.02
	Sample B	0.27±0.003	65.22±0.20	4.97±0.02
	Sample C	0.51±0.002[a]	88.33±0.18	5.13±0.01
Dark Red	Sample D	0.41±0.003	58.66±0.15	2.85±0.02
	Sample E	0.51±0.002[a]	62.04±0.17	1.08±0.02
	Sample F	0.51±0.002[a]	79.20±0.13	2.66±0.02

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

4.4 Antioxidant capacity

From the table 4.4, it was observed that antioxidant capacity was significantly highest (2.45±0.03 mg TE/100 g) in sample B and significantly lowest (1.64±0.02 mg TE/100 g) in sample D.

Table 4.4: Antioxidant capacity of roselle jam

Variety	Formulation	Total Anti-oxidant Capacity (TAC) (mg TE/100 g)
Light Red	Sample A	2.13±0.02[a]
	Sample B	2.45±0.03
	Sample C	2.22±0.01
Dark Red	Sample D	1.64±0.02
	Sample E	1.72±0.02
	Sample F	2.08±0.01[a]

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

4.5 Microbial analysis

Table 4.5 revealed total viable count and fungal count also determined from 0 to 15 days after preparation of the jam. Samples were stored in 4^0C temperature for 15 days for the evaluation. The presence of yeast and mold were not exist when the products were produced and after 15 days their presence had not been identified.

Table 4.5: Microbiological evaluation of Roselle jam

Variety	Formulation	TVC (cfu/ml)		Mold and Yeast	
		0 day	15 days	0 day	15days
Light Red	Sample A	2.8×10^1	9.3×10^1	No growth	No growth
	Sample B	3.6×10^1	1.8×10^2	No growth	No growth
	Sample C	2.8×10^1	9.8×10^1	No growth	No growth
Dark Red	Sample D	2.6×10^1	1.3×10^2	No growth	No growth
	Sample E	3.6×10^1	1.4×10^2	No growth	No growth
	Sample F	3.6×10^1	1.3×10^2	No growth	No growth

4.6 Cost analysis

Table 4.6: Production cost of Roselle Jam

Heads	**Tk./Kg**	**Quantity used (kg/2kg products)**	**Total Tk** (for sample C and sample F)	**Total Tk** (for sample A, sample B and sample D and sample E)
1)Expenditure				
Raw materials				
Fresh Roselle	40	3	120.00	120.00
Sugar	60	1	60.00	60.00
Pectin	12000	0.015	180.00	0
Citric acid	180	0.060	11.00	11.00
Sub total			371.00	180.00
2) Processing cost @ 15% of raw material			55.65	27.00
3) Bottling cost 2 piece	25 Tk./piece		50.00	50.00
Total production cost of 2kg Roselle Jam			**476.65**	**268.00**

In the table, Sample A- Jam form light red Roselle without additional pectin, Sample B- Jam form light red Roselle with seed extract, Sample C- Jam form light red Roselle with commercial pectin, Sample D- Jam form dark red Roselle without additional pectin, Sample E- Jam form dark red Roselle with seed extract and Sample F- Jam form dark red Roselle with commercial pectin

By following this recipe, we can prepared 2 kg roselle jam. So, price of per kg jam is:

Sample C and sample F, per kg jam is = 476.65/2 tk

= 238.33 tk

Sample A, sample B and sample D and sample E, per kg jam is = 268/2 tk

= 134 tk.

4.7 Energy content

From the figure 4.1, Energy content in sample E was calculated in highest amount (341.94 kcal/100g) and lowest (299.6 kcal/100g) in sample D.

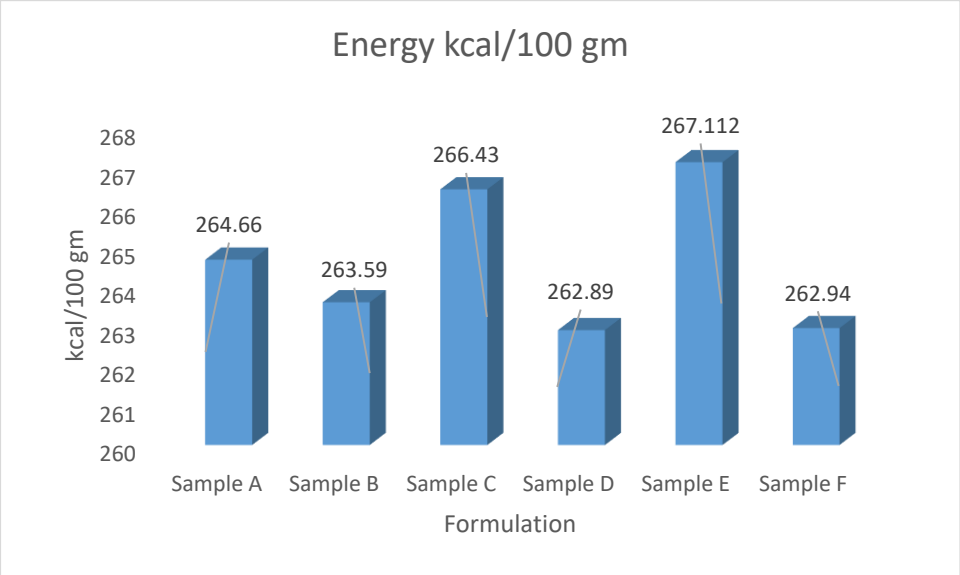

Figure 4.1: Comparison of energy content among six roselle jam

4.8 Sensory evaluation

There was not a significant difference ($p<0.05$) in all the sensory parameters assessed (table 4.8). In all the parameters sample B had the highest acceptance rate. However, sample D scored least acceptance compared to other samples.

Table 4.7: Hedonic rating test for sensory evaluation of roselle jam.

Formulation	Taste	Sweetness	Mouth feel	Flavor	Appearance	Overall Acceptability
Sample A	7.07 ± 0.46^{a}	8.33 ± 0.49^{ab}	8.00 ± 0.48^{a}	7.87 ± 0.64^{ab}	7.60 ± 0.51^{ad}	8.00 ± 0.53^{ab}
Sample B	7.73 ± 0.70^{b}	8.60 ± 0.51^{ab}	8.60 ± 0.51^{b}	8.20 ± 0.68^{ab}	8.47 ± 0.52^{bc}	8.60 ± 0.51^{a}
Sample C	7.73 ± 0.46^{b}	8.20 ± 0.56^{ab}	7.87 ± 0.52^{a}	7.93 ± 0.59^{ab}	8.27 ± 0.70^{bcd}	7.93 ± 0.80^{b}
Sample D	7.80 ± 0.66^{bc}	8.07 ± 0.47^{a}	7.87 ± 0.35^{a}	8.00 ± 0.65^{ab}	7.87 ± 0.35^{acd}	7.73 ± 0.59^{b}
Sample E	8.40 ± 0.51^{c}	8.67 ± 0.49^{ab}	8.60 ± 0.51^{b}	8.53 ± 0.52^{b}	8.13 ± 0.35^{bcd}	8.33 ± 0.49^{b}
Sample F	7.80 ± 0.68^{bc}	7.93 ± 0.46^{c}	8.00 ± 0.38^{a}	8.07 ± 0.26^{ab}	7.93 ± 0.46^{acd}	7.80 ± 0.41^{b}

Legends: Means ± SD and within the column bearing different superscripts (a, b, c) are not significantly different ($P< 0.05$).

Chapter 5: Discussions

5.1 physicochemical properties of roselle jam:

The pH of jam is an important factor for optimum gel condition. Microbial growth is also prevented by low pH in food. In this study Sample A and Sample E were found higher pH value 3.07±0.06; while Sample C had the lowest value 2.83±0.06.

All the results fall within the range between 2.82 to 3.74; similar results were also observed by Ashaye and Adeleke (2009). Different variety of roselle extract provided different pH value, may be the main cause behind the significant change of pH value (Morales-Cabrera et al., 2013).

The results in table 4.1 showed that the pH value had a significant change among all samples, ranged between 2.83±0.06 to 3.07±0.06 and these values were within the range of typical jams. Broomes et al., (2010) reported that the pH of jam roselle calyx extruct was 2.90.

One of the most common causes of jam failure is due to the presence of insufficient acid content. The pH value of jam should be taken when the jam is concentrated sufficiently to pour. If the pH is above 3.3, citric acid should be added to reduce the pH to the range of 3.0 to 3.4 where at a pH of 3.2 - 3.4 and in the presence of a high concentration of sugar, has the property of forming a viscous semi-solid. Adding the citric acid at the end of the boiling period may be provided better control of the pH and minimizes pre-gelling of the batch and hydrolysis of the pectin. Different extract will require different amounts of additional acid, depending upon the original acidity of the extract and the buffering capacity of the extract. The pH may be adjusted to attain optimum flavor, to control or modify the rate of setting and to modify the degree of sugar inversion (Eke-Ejiofor and Owuno, 2013).

In this present study, almost all samples showed no significant change in total titratable acidity content of jam. The maximum value (0.79±0.04%) obtained in sample B and the least value (0.71±0.02%) found in sample D. Addition of citric acid during jam making may be the main reason for Acidity. In this study, titratable acidity of roselle jams fall within the range of five different varieties of apple and olive fruit blended jam, ranged from (0.68 – 0.83%) reported by Shah et al., (2015).

The least and most noteworthy TSS of various Roselle jam tests was recorded 0.68 and 0.69. Increment in TSS was presumably because of the hydrolysis of polysaccharides. According to Shah et al. (2015), the TSS of apple and olive jam was found nearly 0.69 and increasing with storage. They also reported that, the increasing in total soluble solid of the apple olive jam might be due to the degradation of polysaccharides in the presence of acid.

5.2 Nutritional composition of jam:

Combination of pectin and seed extract with the roselle calyx in development of roselle jam provides the basic nutrients like protein, fat, fiber, CHO and vitamins. The proximate composition of six types of jam was shown in table 4.2. Sample B and sample C contained lower amount of moisture than control sample A whereas sample E and sample F had lower amount of moisture compared to control sample D. It may be due to addition of pectin and seed extract during processing for both varieties. Siddiqui et al., (2015) reported that pectin is mostly used to construct the desired texture of products which result in controlling the moisture or water in the product. Moisture is an important factor which affects the shelf life and freshness of products. Food Products having high moisture content display short shelf life. According to Ashaye and Adeleke (2009), moisture content of roselle jam from dark red and light red variety was 31.39% to 36.13%. Moisture content of roselle jam may be varied due to storage condition and type of pectin used during cooking (Broomes and Badrie, 2010).

In this present study, sample E carried the most abundant value of crude fiber (2.33±0.01%), crude fat (1.76±0.03%), crude protein (1.62±0.04%) and CHO (59.58±0.03%) whereas sample F contained highest ash content (1.34±0.03%) when compared with control sample D. On the other hand, almost similar variation found in jam from light red roselle. Here, crude fiber (1.80±0.03%), crude fat (1.27±0.03%) and crude protein (1.76±0.03%) found in extensive amount in sample B whereas CHO and ash content was higher in sample C compared to control sample A. This significant change in nutritional composition for both varieties roselle jam may be due to addition of commercial pectin or seed extract. According to Ismail et al., (2008), roselle seeds are rich in nutritional composition specially proteins, crude fat and dietary fiber. Pectin was a kind of polysaccharide which enrich CHO profile of product (Brejnholt, 2009). Ash content gives an indication of minerals composition of food products (khan et al.,

2012). In present study showed higher nutritional content than roselle jam prepared from roselle calyx extract, reported by Mordi et al., (2011).

Ascorbic acid is fundamental for life. Vitamin C content of both light and dark varieties of roselle jam with no extra pectin denoted as sample A and sample D was higher (21.75±0.02) mg/100g and (24.55±0.03) mg/100g in respectively than other samples. It may be due to long time heat treatment during processing. High processing temperature reduced ascorbic acid content, reported by Martinsen et al., (2020). The ascorbic acid content extended from 14.16 to 28.82 mg/100 gm in roselle jam prepared from light and dark red roselle calyces (Ashaye and Adeleke, 2009). High processing temperature reduced ascorbic acid content, reported by Martinsen et al., (2020). The consumption of fruit rich in vitamin C is associated with the prevention of cardiovascular diseases and obesity (GonzálezMolina et al., 2010). Thus, the supplement of these nutrients from dietary intake of fruits and vegetables is vital, since the human body is unable to synthesize them (Leong and Oey, 2012).

5.3 Phytochemicals

Besides the basic nutrition, bioactive compounds exert an important role in biological functions for humans, such as chronic diseases prevention and maintenance of immune system (Liu, 2004, 2013). Thus, the quantification of these compounds is of utmost importance and the results of bioactive compounds content found in roselle jam shown in Table 4.3.

Total anthocyanin content and total flavonoid content of sample C (0.51±0.02 mg TA/100 ml and 88.33±0.18 mg QE/100 g) was higher than the control sample A where sample A, B and C are formulated from light red roselle calyx. Similar variation found in dark red roselle jam. Sample F possessed intensive amount of TAC and TFC (0.51±0.02 mg TA/100 ml and 79.20±0.13 mg QE/100 g) compared to control sample D for dark red roselle jam. Total phenolic content is almost similar to control and jam with extra pectin for both varieties, valued near 5 GAE/100ml for light red variety and 2.75 GAE/100 ml for dark red variety. The variation of the value of bioactive compounds in same variety jam may be due to change in pectin concentration in the final product (Poiana et al., 2012). Borrás-Linares et al., (2015) reported the phytochemical profile (TPC, TFC and TAC) of the ethanolic extracts of a collection of 25 Mexican roselle varieties with different calyx color intensities, from green to deep

red, cultivated in the same condition. Rababah et al., (2011) reported in their study that the phenolic content of different fruit jam ranged from (1.30 GAE/100 ml) to (12.24 GAE/100 ml). Present findings was within the range.

5.4 Antioxidant capacity:

There have a significant difference in antioxidant capacity among the all samples showed in table 4.4. DPPH was an extensively used substrate to evaluate antioxidant activity especially for investigating the free radical scavenging activities of biological as well as chemical substances. Antioxidant capacity was higher (2.45±0.03 mg TE/100 g) in sample B compared to control sample A for light red roselle jam. This may be due to seed extract used in sample B because roselle seeds are a good source of lipidsoluble antioxidants (Mohamed et al., 2007). Whereas sample F carried maximum antioxidant capacity (2.08±0.01 mg TE/100 g) compared to dark red roselle variety control sample D. It may be due to addition of commercial pectin because Ogutu et al., (2017) observed that high pectin concentration increase the antioxidant capacity. Heat and pH influence the antioxidant capacity of Roselle and its product (Wu et al., 2018) though 65% antioxidant capacity were retained after processing fruits into jams (ceron et al., 2014).

5.5 Microbial Analysis:

Microbiological analyses (Total viable count, yeast and mold count) were performed for each of six samples of roselle jam. Yeast and mold were not found in the roselle jam showed in Table 4.5. Muck (2010) reported mold is aerobic organisms and cannot grow well under conditions where oxygen is limited. Yeast, on the other hand, can grow in aerobic and anaerobic conditions. Acid/alkaline requirements for yeast and mold growth in a wide range food product are quite broad, ranging from pH 2 to above pH 9. Due to preserving jam in air tight bottle yeast and mold growth were inhibited.

TVC found in roselle jam ranged from 2.8×10^1 to 3.6×10^1 cfu/ml. Bacterial count increased after 15 days stored in cold temperature (8 ± 2^0C) and ranged between 9.3×10^1 to 1.8×10^2 cfu/ml. Mordi et al., (2011) showed that the roselle jam had a total plate count of 4.0×10^1 cfu/gm while the coliforms and E-coli values were nil. Microbiological analyses revealed that the roselle jam showed the highest overall acceptability presented a total number of microorganisms, yeasts and molds less than 10 cfu/g (Wongchalat and Chatthongpisut, 2017).

5.6 Sensory Evaluation:

Sensory analysis roselle jam was done to obtain the most organoleptic ally acceptable proportion among all jams. Sensory analysis data from Table 4.8 shows that jam from light red roselle calyx with seed extract (sample B) scored highest in overall acceptability of 8.60±0.51. It may be due to mouth feel, sweetness and appearance. Whereas jam from dark red roselle calyx with seed extract (sample E) scored almost similar to sample B in all parameters. The overall acceptability level of sample B was nearest with the value of sample E. The mouth feel score was affected by the composition of pectin and citric acid in jam. The texture score of jam increased with increase of percentage of pectin and citric acid. Similar propensity was reported by Basu et al., (2010). Highest mean score of acceptability 8.60 in sample B in hedonic rating scale denoted "Like Very Much". From present investigation, it was concluded that roselle jam with seed extract in both variety was found to be better organoleptically than other compositions. The sensory scores of Roselle jam processed from both varieties were generally high. Roselle jams prepared from either dark or light red varieties and stored for six weeks at cold temperature are still acceptable (Ashaye et al., 2009).

Chapter 6: Conclusion

Jam is a popular food product in ready-to-eat foods because of its health benefits. This study revealed that Roselle jam from two varieties has the greatest acceptability in terms of sensory perception. The physicochemical test was performed for Roselle jams which showed significant differences. In proximate analysis, Roselle jam was rich in carbohydrate and also a good source of protein, fat and vitamins. It is considered as functional food because of having ample quality of phytochemicals like antioxidant and bioactive compounds. Due to the unavailability in the local markets, commercial Roselle jam was not tasted in the current study. It was observed that the nutritional values were good which helped to improve nutritional status. Due to cost effective and easy jam making procedure, consumer can grab this method. This study points out a prosperous probability of processing of jam from two varieties of Roselle for the advantage of the growers, processors and the consumers in Bangladesh. It may also be observed that by exporting the best quality jam of international standard may earn foreign exchange that may positive contributions in the national economy of Bangladesh. Further study is important for research with other necessary ingredients for trial with different types of fruits for preparation of jam.

Chapter 7: Recommendations and Future Perspectives

Nowadays, more than half of the people suffer malnutrition in our country, in these situations roselle jam could be a good source of the nutrients and energy as these are available in rural areas of Bangladesh. We have been concluded with good findings in the area of developing roselle jam. It is also resulted with its commercial value and better marketability. Modern food industries can adopt the procedure form medium and large scale of production. On the basis of present investigation, the following suggestions and prospects are made for the further research work.

a) The present studies may be repeated for confirmation of the experimental findings.

b) The composition may be modified further and may try for making mixed jam with various recipes with different ratio of fruit.

c) Since it is easy to prepare. It can be also kept up to long time and recommended for off season. On the other hand, it will be helpful from economic point of view for those people who come under economically weaker section.

d) Such types of research should be done for other fruits like papaya, mango etc. available in markets especially for off season.

e) Modern packaging and storage condition would be developed for the betterment of roselle jam.

f) The findings will be helpful from therapeutic point of view as it has medicinal value.

g) Although the sample size was sufficient to perform statistical comparisons between analytical data. Our conclusion should be considered with caution because of the small number of analyzed samples and results would need to be confirmed with another larger study.

h) Sufficient steps should be taken to enrich commercially available jam with more nutritional value.

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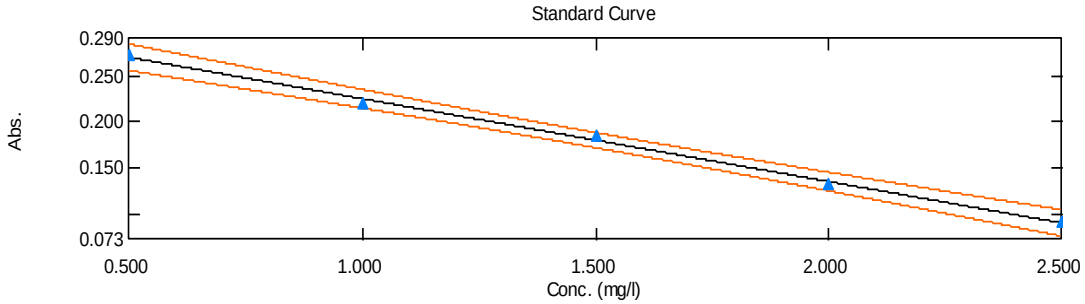
Appendices

Appendix A: Antioxidant capacity of roselle jam

Standard curve of Trolox



y = - 0.0894539 x + 0.314536
r2 = 0.99735

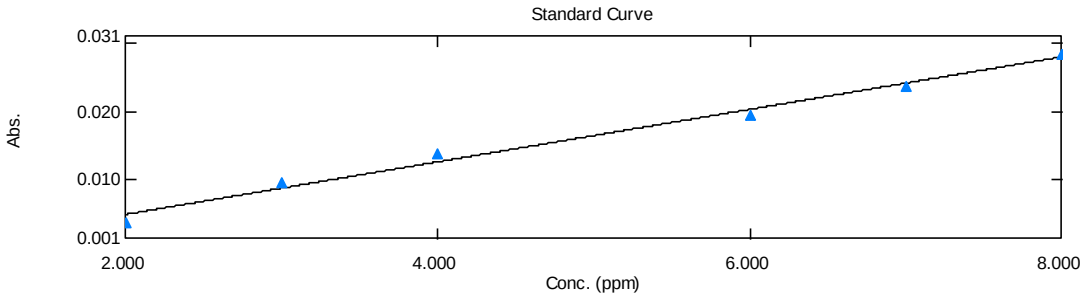
Descriptives

Antioxidant capacity

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Sample A	3	2.1333	.01823	.01053	2.0880	2.1786	2.12	2.15
Sample B	3	2.4450	.02921	.01686	2.3724	2.5176	2.42	2.48
Sample C	3	2.2193	.01270	.00733	2.1878	2.2509	2.21	2.23
Sample D	3	1.6390	.02107	.01217	1.5867	1.6913	1.62	1.66
Sample E	3	1.7217	.01856	.01071	1.6756	1.7678	1.70	1.74
Sample F	3	2.0813	.01443	.00833	2.0455	2.1172	2.07	2.10
Total	18	2.0399	.28809	.06790	1.8967	2.1832	1.62	2.48

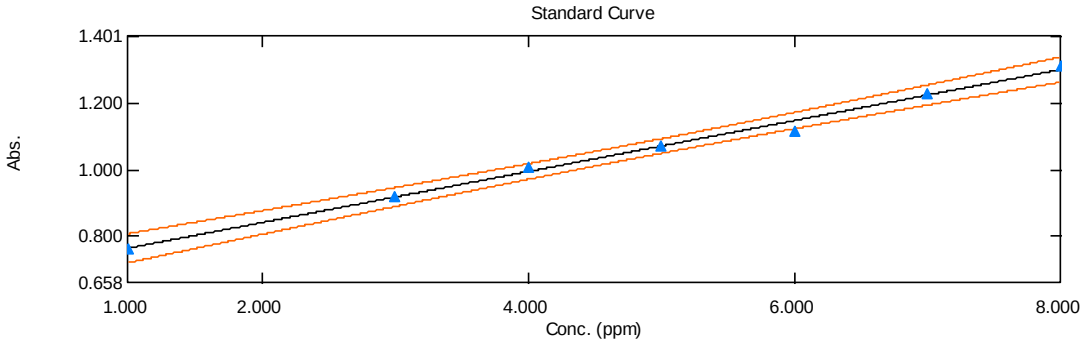
Appendix B: Bioactive compounds of roselle jam

Standard curve of TFC



y = 0.00385110 x - 0.00271158
r2 = 0.98868

Standard curve of TPC



y = 0.0768527 x + 0.687090
r2 = 0.99301

Descriptives

Bioactive compounds

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
TAC	Sample A	3	.3067	.00208	.00120	.3015	.3118	.31	.31
	Sample B	3	.2667	.00252	.00145	.2604	.2729	.26	.27
	Sample C	3	.5087	.00208	.00120	.5035	.5138	.51	.51

	Sample D	3	.4103	.00306	.00176	.4027	.4179	.41	.41
	Sample E	3	.5093	.00153	.00088	.5055	.5131	.51	.51
	Sample F	3	.5100	.00173	.00100	.5057	.5143	.51	.51
	Total	18	.4186	.10324	.02433	.3673	.4700	.26	.51
TPC	Sample A	3	5.2910	.01609	.00929	5.2510	5.3310	5.27	5.31
	Sample B	3	4.9697	.01762	.01017	4.9259	5.0134	4.95	4.99
	Sample C	3	5.1323	.01328	.00767	5.0993	5.1653	5.12	5.14
	Sample D	3	2.8530	.01552	.00896	2.8144	2.8916	2.84	2.87
	Sample E	3	1.0753	.01762	.01017	1.0316	1.1191	1.06	1.09
	Sample F	3	2.6607	.02250	.01299	2.6048	2.7166	2.64	2.68
	Total	18	3.6637	1.62017	.38188	2.8580	4.4694	1.06	5.31
TFC	Sample A	3	62.8730	.07967	.04600	62.6751	63.0709	62.78	62.92
	Sample B	3	65.2170	.20159	.11639	64.7162	65.7178	65.01	65.42
	Sample C	3	88.3280	.18460	.10658	87.8694	88.7866	88.15	88.52
	Sample D	3	58.6583	.15233	.08795	58.2799	59.0367	58.49	58.79
	Sample E	3	62.0423	.16884	.09748	61.6229	62.4618	61.88	62.22

Sample F	3	79.2023	.13741	.07933	78.8610	79.5437	79.12	79.36
Total	18	69.3868	10.98721	2.58971	63.9230	74.8506	58.49	88.52

Appendix C: Questionnaire for Hedonic test of roselle jam

Name of the Taster: …… **Date: …….**

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

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

Here,

A- Jam form light red Roselle without additional pectin.
B- Jam form light red Roselle with seed extract.
C- Jam form light red Roselle with commercial pectin.
D- Jam form dark red Roselle without additional pectin.
E- Jam form dark red Roselle with seed extract.
F- Jam form dark red Roselle with commercial pectin.

Hedonic	Taste						Flavour						Mouth feel						Sweetness						Appearance						Overall Acceptability					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Like Extremely																																				
Like very much																																				
Like moderately																																				
Like slightly																																				
Neither like or dislike																																				
Dislike slightly																																				
Dislike moderately																																				
Dislike very much																																				
Comments																																				

Appendix D: Photo Gallery

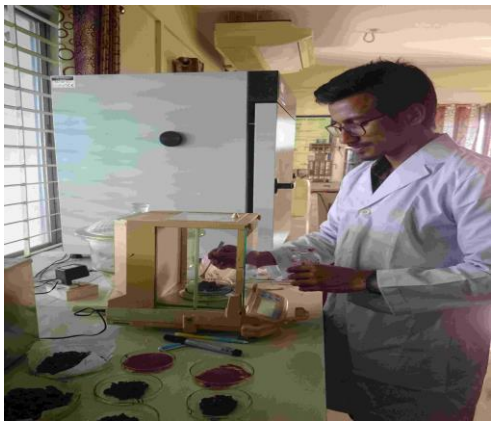
Seperating Seeds from Roselle fruits

Boiling

Roselle jam

pH determination

Acidity determination



Weighing dry jam samples

Crude fiber determination

Protein digestion

Fat determinaion

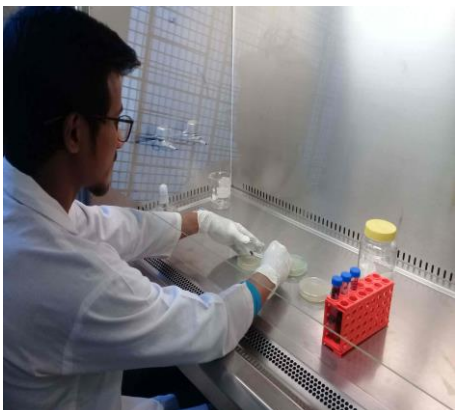
Filtering Ethanoic extract

Ethanoic extract preparation

Preparing samples for Spectrophotometric analysis

Working in UV spectrophotometer

Sensory Evaluation



Microbial Analysis

Brief Biography

Md. Kaium Khan passed the Secondary School Certificate Examination in 2009 from Cumilla Housing Estate School and College, Cumilla, and then Higher Secondary Certificate Examination in 2011 from Cumilla Government College, Cumilla. He obtained his 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, he is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). He 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.