Chapter 01

Chapter 1: Introduction

1.1 Background

Eating "healthy" foods like fruits and vegetables provide benefits for both physical and mental health and may be a long-term investment in one's future well-being, according to research. The concept that high-calorie foods taste better, bring happiness, and improve our health status is at odds with this way of view. Inversely, studies have also shown that diets and restricted eating increase the risk of long-term weight gain and eating problems and seem to be ineffective (Mann et al., 2007). A potential change away from viewing food as just nutritional and toward one that is more positive and well-being-focused would need a new way to look at human dietary habits. In this study, Block et al. (2011) suggested a paradigm shift from "food as health" to "food as well-being.

Mandarin (*Citrus reticulata Blanco*), a medium-sized fruit tree called Blanco is utilized extensively as herbal medicine all throughout the world. According to ethnopharmacological study, the C. reticulata plant contains antioxidants including vitamin C, carotenoids, and phenolic compounds, as well as sugars, organic acids, amino acids, pectins, minerals, and volatile organic compounds. The C. Reticulata is used to treat vomiting, hiccups, gastro-intestinal distension, dyspepsia, and coughing up a lot of phlegm. The unrefined C. extracts The anti-inflammatory, anticholesterolemic, analgesic, antiasthmatic, antiscorbutic, antiseptic, antispasmodic, purgative, expectorant, and stomachic properties of reticulata fruits have been demonstrated (l-Sna AE, 2016). Citrus fruits and their byproducts are increasingly in demand as traditional medications for treatment of bacterial in developing countries as more people become nutrition-conscious.

Aloe vera (Aloe barbadensis Mill.) is a marvel and miraculous herb that has proven to be effective in the treatment of a wide range of illnesses. Despite the fact that the plant is well-known for its therapeutic properties (Sanghi, 2015), it must be proven and evaluated

in the health sector as beneficial functional food supplements. The Aloe vera plant has been utilized for generations for its health, medical, cosmetic, and skin care benefits. The term Aloe vera comes from the Arabic word 'Alloeh,' which means' shining bitter material,' and the Latin word ‘vera,' which means 'truth.' Only Aloe vera was thought to be the universal panacea by Greek scientists 2000 years ago. Aloe vera was thought to be the herb of immortality by the ancient Egyptians (as per the literature published in the Indian Journal of Dermatology). There is additional evidence that the plant has been used in countries like as China, Japan, India, Greece, Egypt, and Mexico for centuries. Egyptian monarchs Nefertiti and Cleopatra utilized the plant's leaves in their daily beauty routines. Aloe vera has been used as a laxative in the United States since the 1800s. A watershed moment came in the mid-1930s, when it was successfully employed to treat chronic and severe radiation dermatitis.

Aloe vera leaves are succulent, upright, and form a dense rosette. Aloe is known as "the herb of immortality" by the Egyptians. Many vitamins, minerals, enzymes, natural sugars, amino acids, and other nutrients are abundant in the leaves of this remarkable medicinal plant. They're also high in phytochemicals, which have emollient, purgative, antioxidant, anti-inflammatory, anti-helminthic, antibacterial, aphrodisiac, antiseptic, and cosmetic properties.

People across the globe are becoming increasingly concerned about their health. Many food companies have created aloevera supplements, such as aloevera juice, aloe desserts with milk, aloe powder, and other aloevera meals, to provide important nutrients to diabetes patients, as well as to aid in weight loss and anti-aging characteristics. 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 is concentrated to a soluble solid content of at least 65 percent. The addition of gelling agents and citric acid can compensate for inadequacies in the fruit itself. Jellies are stable because they include a significant amount of solids (sugar) and acids. jelly is intermediate moisture food, meaning it is made with a medium amount of water, gelling agent, sugar, and acids in the precise proportions for effective gelation are essential ingredients in the manufacture of jellies. (Arjun-Ringwal, 2019).

Aloe vera jelly as a food product is a completely new, novel, and nutrient-dense product produced in the laboratory of Chattogram Veterinary And Animal Sciences University's Dept. of Applied Chemistry And Chemical Technology. Mandarine was employed in this study to give the aloevera jelly a thicker consistency and a more appealing color because aloevera contains little vitamin C and stevia is merely a sweetener that is un-jelly is simple to make, eat, and preserve. Jelly is defined as a mixture of sugar and the juice or extract of one or more fruits that have sufficiently gelled (Fermandez et al., 2014). The Aloe vera jelly with peppermint flavor is suitable for people of all ages. In this study, the jelly was separated into two parts: sugared and sugar-free (stevia powder was used as a sugar substitute). This was done to observe the binding affinity of the jelly in order to achieve gel consistency; stevia has no binding affinity, but sugar does. Considering the past, just a few studies on Aloe vera-based food products have been undertaken. The purpose of this study was to look at the binding capability and nutritional content, phytochemicals, sensory assessment, microbiological analysis, and antioxidants of aloe vera jelly in order to get fresh and more significant data on this area able to bind molecules to give it a thick consistency.

Aim and Objectives:

i. To prepare an effictive Aloe vera jelly with peppermint, incorporate it with mandarin.
ii. To observe the effect of mandarin (Citrus reticulata) on jell formation in prepared jelly.
iii. To evaluate phytochemicals, antioxidant capacity, sensory and nutritional aspects in the final product.

Chapter 02

Review of literature

2.1 Mandarin

The family Rutaceae includes the big species *Citrus reticulata Blanco*, which has several variations and hybrids. Popular citrus varieties including Satsumas, Clementines, Tangerines, and the Mediterranean mandarin are among them. Although the terms "tangerine" and "mandarin" are sometimes used interchangeably, tangerine refers to a variety of orange-colored citrus fruits that are hybrids of the mandarin. Mandarins are native to Asia's subtropical and tropical regions, mainly China and Cochin-China, along with other citrus species (Tolkowsky, 1938). According to some scholars, mandarins and other citrus species developed in an area that included Vietnam, South China, India, and Japan. They are currently commonly grown in warm, temperate, and tropical regions all over the world. 10. Among the citrus species that are commercially grown, mandarins produce between 22 and 25 percent of the world's citrus crop. Mandarins are the most varied group of citrus fruits, including both monoembryonic and polyembryonic cultivars as well as numerous interspecific hybrids. However, molecular and iso-enzymatic comparisons amongst mandarin cultivars have revealed a striking resemblance. Tanaka identified 20 different species of mandarin, whereas Swingle only recognized three, one of which was C. reticulata, which included 34 different species in Tanaka's classification (Usman and Fatima, 2018).

Kingdom: Plantae

Order: Sapindales

Family: Rutaceae

Genus: Citrus

Species: *C. reticulate*

The mandarin plant is a spiky, evergreen, bushy shrub with most cultivars reaching an average height of 7.5 meters 1. The tree has a thick crown with thin branches that bear lance-shaped leaves with a distinct midrib that are dark green in color. The margins or wings of the petiole are quite little. This tree produces oval to flattened, golden fruits with pleasant flesh after its white, fragrant flowers. The mandarin fruit is spherical, oblate, orange in color, pleasant taste, and resembles other oranges in appearance. It has a thin, loose, easy-to-peel skin that can be easily influenced by cold. The fruit has segments that are simple to separate and has a diameter of up to 8 cm. Low temperatures or a water shortage in the soil may cause flowering to occur. In subtropical regions, flowering takes place in the springtime every year. In tropical regions, flowering occurs continuously and is mostly influenced by the availability of moisture from enough rain (Guerra et al., 1997).

2.2 Nutritional Composition

Mandarin is a great source of important minerals including calcium, potassium, phosphorus, and magnesium as well as vitamins C and A, proteins, and dietary fiber. They also include trace amounts of the vitamins B1, B2, B3, B5, B6, B9, and E8. Mandarin oranges typically contain 85% water (85.2 g), 13% carbs (13.34 g), 0.81 g protein, 0.38 g dietary fiber, and 0.31 g fat per 100g (Putnik et al., 2017). Mandarins are shown in Table 1 as having the following nutritional profile. The flavor of the fruit is influenced by the sugars, acids, carotenoids, polyphenols, limonoids, and vitamins in C. reticulata. Humans who consume the fruits and their byproducts will benefit greatly from the vitamins, fiber, and health-promoting plant substances like flavonoids.

For instance, the vitamin B complex supports cardiovascular health, energy levels, appropriate nerve function, hormone and cholesterol synthesis, cell health, and infection prevention. Mandarin fruits in particular contain high levels of cryptoxanthine, a xanthophyll with pro-vitamin A activity31. Mandarins are well-liked by customers because of their enticing flavors and rich phytochemical content. Citrus fruits like mandarins and their by-products have seen increased demand as more people become nutrition-conscious (Putnik et al., 2017).

2.3 Medicinal Applications

The portion of the raw mandarin fruit that may be eaten contains antioxidants such vitamin C, carotenoids, and phenolic compounds. Additionally, the fruit is a great source of minerals, sugars, organic acids, amino acids, pectins, and volatile chemical compounds. By defending it against chronic illnesses and giving it needed sustenance, these components are crucial for the body's normal operation 52. Mandarins' dietary fiber and phenolic chemicals are helpful in the creation of functional meals. For example, coumarins found in mandarin fruit can make skin more sensitive to sunlight (l-Sna, 2016).

The fruit's peel controls skin moisture, softens hard, rough skin, and cleanses greasy skin, while the fruit itself has been said to have laxative, aphrodisiac, antiemetic, astringent, and tonic properties56. They are used to treat and control dyspepsia, gastro-intestinal distension, cough with copious phlegm, hiccups, and vomiting. The unripened green exocarp is used to cure cirrhosis of the liver, enlarged liver and spleen, gastro-intestinal distension, and hypochondrium and chest symptoms. The seed is used to cure hernia, lumbago, mastitis, and discomfort or swelling in the testicles because it has analgesic and carminative properties (Yeung, 1985).

2.4 Pharmacological effects of mandarin

Antibacterial characteristics

With a zone of inhibition ranging from 9.16 to 27.63 mm against Escherichia coli, Listeria innocua, methicillin-resistant Staphylococcus aureus, and S. aureus, this plant's essential oil has demonstrated anti-microbial action. Zainab et al. also noted that the C. elegans peel extract contained C. reticulata demonstrated a significant zone of inhibition when exposed to S. E. aureus (28 mm), and E. coli, S. P. aeruginosa, and Typhi shown resistance to the orange extracts. Flavanones may be found in the peel of C. The effectiveness of the peel extract over the juice may be attributed to articulate. The antibacterial activity of C. reticulata var. Kinnow peel extracts against pathogenic strains of S. aureus, E. coli, P. aeruginosa, and K. pneumonia was investigated by Yashaswini and Arvind. Therefore, it was hypothesized that the volatile oil may be effective for

treating skin conditions, and that the therapy might be included into a cosmetic formulation. According to the results of this review, C. reticulata essential oil, juice, and peel extracts may contain advantageous antibacterial properties that can be used to treat unwelcome bacterial infections.

Neuropharmacological effects

The anxiolytic potentials of methanol and aqueous peel extracts of C. reticulata in Libya. The results showed that there was strong anxiolytic action in the peel extracts. Additionally, mice aged 6 to 8 weeks and weighing 30 to 35 g have been used to confirm the anxiolytic effect of naringin (Fernandez et al., 2009).

Effects of antioxidants

Boudries et al. looked into C's antioxidant properties. C. reticulata cultivar Wilking and C. reticulata clementine from Algeria utilizing decreasing power and 1,1-diphenyl-2-picrylhydrazil (DPPH). The C. elegans essential oil (EO) The most potent DPPH free radical-scavenging action was demonstrated by reticulata, followed by clementine and wilking EOs. Additionally, in a concentration-dependent way, the EO of the mandarin outperformed the EOs of the wilking and clementine. Junior et al. claim that. The skin of C. reticulata was tested for antioxidant activity, and the results showed that it has noticeable, concentration-dependent free-radical scavenging action on both reactive hydroxyl radicals and stable DPPH free radicals (Sawamura et al., 2004).

cardiovascular effect

Tangerine peel may be used to lower the risk of cardiovascular illnesses and several conditions linked to lipid oxidation (l-Sna, 2016).

Hepatoprotective action

The impact of C. Elegans essential oils as a preventive measure Investigated was the effect of mandarin on isoniazid-induced hepatotoxicity in Wistar rats. In the rats treated with isoniazid alone, as opposed to the group that did not receive isoniazid, the results

showed a significantly increased level of ALT, AST, bilirubin, and lower total protein content (Kangralkar et. al., 2010).

Anti-aging properties

In a research by Apraj and Pandita (2014), it was discovered that both hot and cold alcoholic extracts of C. The anti-aging properties of reticulata were demonstrated by its potent anti-collagenase and anti-elastase activity. To find out whether the extracts can be used as anti-wrinkle ingredients in cosmetic products, more research is necessary.

The plant of mandarin is significant since it has several nutrients and chemicals that are very crucial to one's health. The fruit is abundant in minerals, sugars, organic acids, amino acids, pectins, antioxidants, phenolic compounds, and volatile organic compounds. These compounds offer fundamental nourishment, protect the body against chronic illnesses, and are important in the creation of functional foods, all of which are necessary for the body to operate properly. According to reports, reticulata has antihypercholesterolemic, anticancer, antibacterial, antigenotoxic, neuropharmacological, hepatoprotective, and cardiovascular properties. the application of C. More study must be done on the plant's toxicity because reticulata has been demonstrated to have a wide range of uses, from nutritional supplements to the treatment of life-threatening illnesses. Further research should be done to determine if the peels can be cooked and ingested orally, define acceptable dose limits, and assess target-organ toxicity because the peels contain bioactive components of pharmacological value.

2.5 Aloe vera

The family Aloeaceae includes the herb known as aloe vera (Farnsworth et al., 1999). It is an important component of herbal therapy and has been used for hundreds of years in traditional medicine to treat a wide range of diseases. The Arabic term "Alloeh," which means "shining bitter material," and the Latin word "vera," which means "truth," are the origins of aloe vera. Egyptians referred to aloe vera as "the herb of endurance," and Greek scientists first identified it as a universal remedy 2000 years ago (Farnsworth et al., 1999; Ahlawat and Khatkar, 2011; Atherton, 1998). It primarily grows in arid regions of Asia, Europe, and North America. Despite the fact that Aloe vera is indigenous to

northern Africa. It is a natural ingredient that is frequently used in the cosmetics industry to make a variety of products with different purposes, such as face gel, night cream, sun cream, lotions, and more. To receive the health advantages from aloevera, it is essential to preserve the plant's bioactive components throughout processing (Chandegara and Varshney, 2013).

Kingdom: Plantae

Order: Asparagales

Family: Asphodelaceae

Subfamily: Asphodeloideae

Genus: Aloe

Species: A. vera (*Aloe barbadensis Mill*.)

This plant features fruits with numerous seeds, tubular yellow blossoms, and triangular, succulent leaves with serrated edges.

2.5.1 Active components and characteristics:

Luta and Anally (2005) described that, the chemical makeup of aloe vera and methods for identifying its existence in commercial goods. Shelton (1990) said that; the parts consist of Amino acids, enzymes, vitamins, minerals, lignin, saponins, salicylic acids, and saponins are a few of the aloe vera's 75 potentially active components.

Enzymes & Amino Acids: Alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulase, lipase, and peroxidase are the other eight enzymes found in it. Bradykinase, among other enzymes, aids in the digestion of sugars and fats while others serve to avoid excessive inflammation when administered topically to the skin. Aloevera contains amio acids such as L- Isoleucine, L-Valine, L-Proline, L-Phenylalanine, L-Arginine, L-Asparagine, L-Leucine, L-Histidine, Aspartic Acid, and L-Tyrosine.

Vitamins: The antioxidants vitamin A (beta-carotene), vitamin C, and vitamin E are present. Choline, folic acid, and vitamin B12 are other ingredients in this supplement. Antioxidants can squelch free radicals.

Minerals: Among the minerals found in it are Ca, Cr, Se, Mg, Mn, K, Na, and zn. Only a small portion of them are antioxidants, yet they are necessary for the proper operation of numerous enzyme systems in different metabolic pathways.

Sugars: It includes polysaccharides (glucomannans/polyominoes) as well as monosaccharides (glucose and fructose). Mucopolysaccharides are polysaccharides produced by the mucilage layer of the plant. The most prevalent monosaccharide is mannose-6-phosphate, while the most prevalent polysaccharide is glucomannans [beta-(1,4)-acetylated mannan]. Additionally, the well-known glucomannan acemannan was found. Recently, a glycoprotein with anti-allergic properties was found (Lawless and Allen, 2000).

Fatty acids: It contains plant steroids such as cholesterol, campesterol, sitosterol, and lupeol. They are all anti-inflammatory, and lupeol also possesses antibacterial and analgesic properties. (Hutter et al., 1995)

Hormones: Wound healing is aided by the anti-inflammatory hormone's auxin and gibberellin. Other advantages include the presence of seven out of ten essential amino acids and 20 of both the 22 amino acids that humans require. There is also salicylic acid, which has anti-inflammatory and antimicrobial properties. Topical therapies benefit from the use of lignin, an inert substance, as it aids in the other ingredients' deeper skin penetration. About 3% of the gel is made up of saponins, or soapy compounds, which offer cleaning and hygienic characteristics

2.5.2 Mechanism of actions

Robert, (1997) "Aloe vera: a scientific approach"; in this study, he provided an overview of the role that aloe vera leaves play.

Healing capabilities: Following topical and systemic Aloe vera treatment, the growth hormone gibberellin and the mannose-rich polysaccharide glucomannan interact with the

fibroblast's growth factor receptors to increase the fibroblast's activity and proliferation. This significantly increases collagen formation. Hyaluronic acid and dermatan sulfate generation in the inflammatory cells of a healed lesion has been seen to be increased by oral or topical therapy. (Palve et al., 2013).

Effects of aloe vera on skin: Aloe vera gel has been demonstrated to protect the skin from radiation damage. Lowering the production of immunosuppressive cytokines from skin keratinocytes, like interleukin-10, prevents Photothermal suppression of dedelayed-type hypersensitivity (IL-10). (Panesar et al., 2012). Mucopolysaccharides help to bond moisture balance, making it more moisturizing and youthful-looking. The skin becomes more elastic and wrinkle-free as a result of aloe's promotion of collagen fibers formation. (Sasikumar et al., 2013.)

Anti-inflammatory action: By blocking the cyclooxygenase pathway, aloe vera reduces the production of prostaglandin E2 from arachidonic acid. Recently, the novel anti-inflammatory molecule C-glucosyl chromone was isolated from gel extracts.

Effects on the immune system: Alprogen has an impact on the immune system because it prevents calcium from entering mast cells, which in turn stops those cells from producing histamine and leukotriene in response to antigen-antibody interactions.

Laxative effects: The numerous anthraquinones found in latex have laxative effects. It promotes mucus secretion, increases intestinal moisture contents, and speeds up intestinal peristalsis.

Antiviral and carcinogenic activity: Both direct or indirect mechanisms may be responsible for the activity. Whereas anthraquinones get a direct impact, the immune response is boosted indirectly. The anthraquinone aloin renders influenza, varicella-zosterzoster, and herpes simplex viruses inactive. (Kim et al., 1997)

2.6 Aloe vera gel

Aloe vera is a shrub that can only be grown in dry environments, and it is widely utilized to produce a wide range of skin medications. The bitterness of the extract may be heightened by the leaf, skin, and yellow portion of the aloe gel, among other components.

Aloe vera gel can be used as a natural antioxidant and flavoring agent in some dishes (Panesar and Shinde, 2012; Sasi. et al., 2013, Palve et al., 2013; Rahman et al., 2015).

As a result of the aloe vera plant's effectiveness in treating all acne-related problems as well as some skin infections, dermatologists strongly advise using it to cure burns and soften the skin (Richardson et al., 2005; Dal' Belo et al., 2006). For stomachaches and cramps, motion sickness, electrolyte disturbances, diarrhea, renal problems, cirrhosis, muscular weakness, and cardiovascular problems, aloe latex is crucial (Bottenberg et al., 2007).

2.7 Aloe vera is used as a nutritional supplement

2.7.1 Aloe juice concentrate

In a study titled "Rheological Properties of Aloe Vera (Aloe barbadensis Miller) Juice Concentrates," Swami et al. (2014) found that aloe juice may be concentrated, just like other fruit juices, to increase its shelf life and for potential industrial uses in food and pharmaceuticals. According to Qian (2002), aloe vera juice extract can be used to create a powder, jam, jellies, and squash. It can also be blended with other fruit juices and water to create aloe juice. Aloe juice helps treat gastrointestinal diseases and reduces inflammation in the body, including that caused by rheumatic, ear inflammation, and arthritis. Several number of fruit beverages are created by combining amla juice, other citrus fruits, and aloe vera. A variety of fruit juices can be combined with aloe vera juice to create mixed ready-to-serve (RTS) drinks. (Boghani et al., 2012). Dessert, Dahi, yogurt, lassi, as well as culinary items like RTS and edible coatings, now contain aloe vera gel or juice (Keerthi et al., 2016).

2.7.2 Powdered aloe gel

Ramachandra and Rao (2008) and Gautam and Awasthi (2007) conducted a study on "nutrient content and physicochemical properties of Aloe vera (Aloe barbadensis) powder" and "processing of aloe vera leaf gel: A review." By lyophilizing gel fillets or concentrates, tray drying pulp, or dehydrating gel fillets in a controlled humid environment, this study's findings suggest that Aloe vera gel powder can be produced. This study led to the creation of aloe juice. Before being placed in a heated air chamber

with controlled humidity and temperature, aloe vera gel fillets are cleaned to get rid of any remaining aloin. Aloe vera gel powder is made from dried fillets following processing (Ramachandra and Rao, 2008). The most effective way to reduce aloe vera while preserving its bioactivity, flavor, and color is through freeze-drying. After being dried for 65 hrs at 88 °C and 0.01 mm Hg pressure, the aloe gel fillet is crushed to create aloe powder with a moisture content of less than 4%. Aloe vera gel concentrates were made using reverse osmosis and ultra-filtration, and it was then freeze-dried dried.

Gautam and Awasthi (2007), showed that Aloe vera gel extract was made using reverse osmosis and ultra-filtration, and it was then freeze-dried. To create aloe vera leaf powder, powdered aloe vera leaf pulp was dried for 12 hours at 50°C in a tray dryer. The substance is then ground from the powder.

2.7.3 Making yogurt and desserts with aloe vera

Buffalo or cow milk, Lactobacillus bulgaricus, and Streptococcus thermophilus microorganisms are used to make yogurt, together with or without any other foods that are allowed (Tamime and Robinson, 1994). Cultured buttermilk that has been supplemented with Aloe vera juice (5–20%) maintains its consistent acidity and pH.

Mudgil et al. (2016) reported that the aloe vera juice increases the viscosity and decreases phase separation in buttermilk enriched with aloe vera. The best sensory acceptance is achieved when buttermilk is combined with 10% aloe vera juice (Mudgil et al., 2016).

According to the report, aloe vera is employed in confectionery items (Anonymous, 2008). Sweetener candies, jellies, chocolates, and other confectionaries are acceptable. Various fruit juices are utilized to flavor items such as jelly; orange juice is used to enhance the jelly flavor.

Palve et al. (2013) investigated the 'development, sensory, and chemical features of the jelly created by mixing Aloe vera gel in pineapple juice' and discovered that blending aloe vera gel with pineapple fruit juice in a 40:60 proportion generates a high-quality jelly with high nutritional content. The methodology is applied to optimize all of the ingredients used for the preparation of Aloe vera jelly (Jayabalan and Karthikeyan, 2013).

2.8 Peppermint

In Europe and North America, peppermint also referred to as *mentha Piperita*, is a herb. Peppermint oil has been used to relieve headaches, common colds, neuralgia, and other diseases from time immemorial. The antispasmodic benefits of peppermint oil are the topic of this review. Peppermint oil smells like menthol, is colorless to light yellow in color, and has a watery consistency. India is the world's top producer and exporter of mint oil. Mint oil and its components and derivatives are used in the food, medicinal, fragrance, and flavoring sectors. Peppermint can be taken as a tea, tincture, oil, or extract, or used externally as a rub or liniment. It really is commonly used to treat abdominal discomfort, digestive problems, nausea, and the symptoms of colds in youngsters (Blumenthal, 1998). It also has anti-inflammatory, anti-microbial, and anti-carcinogenic properties. The plant contains polyphenols, which are highly potent antioxidants that are less hazardous than manufactured ones (Barbalho et al., 2011).

Because phenolic compounds slow the oxidative destruction of lipids, they are extremely beneficial to the food industry by improving the quality of food and nutritional content (Roblova et al., 2016). Its medicinal properties, such as reduced, anti-inflammatory, antibacterial, and antioxidant properties, are also of great interest in medicine. Flavonoids with anti-allergic characteristics include eriocitrin, narirutin, hesperidin, luteolin-7-O-rutinoside, isorhoifolin, diosmin, rosmarinic acid, and 5, 7-dihydroxycromone-7-O-rutinoside. JP de Sousa (Guedes et al., 2016).

2.8.1 Leaf Structure

The extract of the leaves of M. Piperita contains menthol, menthone caffeic acid, acetaldehyde, amyl alcohol, menthyl esters, limonene, pinene, cardiac glycosides, phellandrene, cadinene, pugelone, and dimethyl sulfide. Among the contents are alpha-pinene, sabinene, terpinolene, ocimene, diterpenes, gamma-terpinene, steroids, fenchene, alpha- and beta-thujone, coumarin, citronellol, carotenes, tocopherols, betaine, choline, saponin, tannins, and others. (Johari et al., 2015). The main ingredient in peppermint; menthol, is primarily responsible for the antispasmodic properties of the drug. limonene (1.0-5.0%), cineole (3.5-14.0%), menthone (14.0-32.0%), menthofuran (1.0-9.0%), isomenthone (1.5-10.0%), methyl acetate (2.8-10.0%), isopulegol (0.2%), menthol

(55.0%), pulegone (4.0%), and carvone are some of the elements of peppermint (max. 1.0 percent). Additionally, it has 84 percent hydration, 1.6% ash, 4.1% protein, 5.6% CHO, 0.9% fat, and 0.9% vitamin C. (26 mg).

2.8.2 The antispasmodic effect of peppermint

Prior studies have demonstrated that different varieties of mint are efficient in lowering muscle soreness, muscle relaxation, and fatigue. Numerous research has been done to date on how well certain natural supplements can help athletes perform. The herb mint possesses antioxidant, decongestant, anti-inflammatory, antispasmodic, and antispasmodic effects. Menthol and menthone are the two major ingredients in peppermint essential oil. Humans who had external applications of peppermint extract had higher pain thresholds. Anxiety observed physiological effort, as well as mental task, effort, and effort, were all decreased by the scent of peppermint.

2.9 Stevia

The Stevia rebaudiana plant, a part of the Asteraceae family and a native of Paraguay and Brazil is used to make stevia by drying its leaves and roots. Other names for it include candy leaf, honey leaf, and a sweet leaf of Paraguay. The leaf has no carbohydrates nor calories but is 10 times sweeter than processed sugar. A diterpenoid glycoside with 300 times the sweetness of sucrose is called steviol. Steviol is a safer sweetener to use. It is appropriate for those who are fat or diabetic. With the progression of type 2 diabetes, it might be advantageous. Additionally, it was shown to have qualities that were antibacterial, antifungal, anti-inflammatory, anti-fertility, hypoglycemic, diuretic, and cardiotonic. Among other skin diseases, it has shown success in treating eczema, dermatitis, and acne.

According to Ranjan et al. (2007), steviol modulates blood glucose levels in insulin-deficient rats by boosting both insulin production and utilization. Additionally, it functions as a source of fiber. It is expected to give optimism to diabetics who enjoy sweet things.

Sharma et al. (2006) observed that, fesh stevia leaves contain between 80 and 85 % moisture, the most frequent constituents were glycosides like stevioside, steviol, and

rebaudioside A and B. Stevia contains several nutrients including ascorbic acid, b-carotene, chromium, cobalt, magnesium, iron, potassium, phosphorous, riboflavin, thiamin, tin, and zinc. Additionally, it contains apigenin, austroinulin, avicularin, b-sitosterol, caffeic acid, compes-terol, caryophyllene, centaureidin, chorogenic acid, chlorophyll, cynaroside, daucos-terol, di-terpene glycoside, dulcosides A and B, foeniculin, formic acid, gibberellin.

2.9.1 Functional use

i. The non-caloric sweetener stevia is popular throughout the world. Unprocessed stevia leaf powder is 20–30 times sweeter than sugar cane, incredibly safe to take, and has no calories. Numerous studies support the use of stevia in the food, soft drink, and beverage, as well as household product industries. work as a cavity preventative and aid in weight loss.

ii. The liver mitochondria's oxidative phosphorylation was inhibited by stevioside, steviolbioside, isosteviol, and steviol. It was determined that S. rebaudiana natural compounds may potentially operate as oxidative phosphorylation uncouplers in addition to their inhibitory actions (Bracht et al., 1985). Stevia reduces blood pressure while enhancing the diuretics and the method uses different effects on users.

iii. Blood sugar levels are affected: It was also found how stevioside and its aglycon steviol affect human insulin secretion and the mechanism by which stevioside lowers blood sugar levels (Jeppesen et al., 2000).

Finally, we can state that stevia is used as a contraceptive, to treat diabetes and hypertension, and for other purposes in traditional medicine. It is suitable for those with diabetes, PKU (phenylketonuria), and obesity who desire to lose weight by cutting out sugar additions from their diet. It doesn't seem to cause any allergic reactions149. Stevia has been associated with harmful effects on the kidneys, urinary system, and cardiovascular system. Healthy individuals (aged 24 to 40) observed bradycardia and a small decline in arterial blood pressure (approximately 9.5%) after receiving stevia leaf tea for 30 days (Geuns, 2003).

Chapter 03

Materials and Methods

3.1 Study Area

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

3.2 Study Duration

The research was carried out during a three-month period, from January to March 2022.

3.3 Collection of Sample

Fresh samples of Mandarin and Aloe Vera obtained from the local market of Chattogram district. Mandarin, fresh Aloe Vera leaves, peppermint were carefully chosen in order to their color and size variety. Sugar, gelling agent were purchased from scientific and surgical store. Other relevant materials required for the experiment were received from the laboratory stocks.

3.4 Juice extraction

The blending procedure was used to make mandarine, aloevera, and peppermint, Different percentages of citrus fruit mandarin were used in the treatment, at 20%, 30%, and 40% mandarin juice. To remove any extraneous particles or ions, the samples were washed with deionized water.

3.4.1 Sample Preparation

In this study, the aloevera jelly prepared on the basis of different percentages of mandarin juice. Sample A was prepared by mandarin juice, fresh aloe vera, peppermint, stevia powder. Where 500g aloevera, 100g peppermint, 5g stevia powder and 20% mandarin juice were used to make Aloevera jelly. Sample B was prepared from 500g aloevera,

100g peppermint, 500g sugar, 20% mandarin juice. Similarly Sample C & Sample D were made with 30% & 40% mandarin juice, other aspects remained unchanged.

3.5 Preparation of jelly

First, fresh aloe vera, peppermint leaves, and mandarin were weighed and carefully rinsed in cold water. Using a steel knife, cut the washed aloe vera into thin slices. The peppermint leaves were cut from the stalk. These were then mixed to obtain the juice. At medium heat, aloe vera and sugar/stevia powder were simmered for 5 minutes with continuous stirring. After that, gelatin and peppermint were added, followed by mandarin to adjust the pH level. The heating was maintained while stirring. According to the formulation, the amount of aloe vera juice, peppermint extract, gelatin, and sugar/stevia was estimated. After 20 minutes the endpoint was indicated at 65-67^0 Bx 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.

Figure 3.1: Processing steps of Aloe vera jelly

3.6 Physicochemical analysis of Aloe Vera jelly with mandarin

3.6.1 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).

3.6.2 Total Soluble Solids

The total soluble solids of the fruits were calculated using a hand refractometer. Total soluble solids (TSS) were measured with a digital refractometer (Atago RX 1000), and the result were expressed as percent soluble solids (Brix) in accordance with AOAC standards.

3.6.3 Titratable Acidity

The percentage of acidity was determined in terms of anhydrous citric acid by titrating against N/10 NaOH using phenolphthalein indicator. As 10 ml of juice was poured into a 100 ml volumetric flask and the volume was diluted to 100ml by adding distilled water, 10ml of the diluted juice was titrated against N/10 NaOH using phenolphthalein as an indicator. The appearance of pink color indicates the titration's endpoint. Titration was reported 3 times, and the average value was recorded each time (AVOC, 2016).

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

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

3.6.4 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 $NaHCO_3$ 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 dilutes to make 500/250 ml with distilled water.

Standard ascorbic acid solution

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

Procedure

- Dye solution was taken in the burette up to 0 marks,
- Then 5 mL of Vitamin C solution was added in a conical flask.

- The dye was added dropwise to the conical flask by using a burette.
- Ttitration was completed when the pink color was appeared and stayed for 20 seconds before fading.
- The reading was taken at least three times.The same procedure was performed for ascorbic acid solution of unknown concentration.
- The result was expressed as milligram percentage (mg %).

3.6.5 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

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

3.6.6 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:

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

3.6.7 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.

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

3.6.8 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. The crude fat content of the samples was determined using AOAC (2016) procedures and a soxhlet equipment.

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

Fat % = Weight of the extract × 100 / Weight of the sample

3.6.9 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

Nitrogen % = (ml standard acid-ml blank) × N of acid × 1.4007/ Weight of sample in gram

3.6.10 Estimation of Crude Fiber

Principle: The water-insoluble fraction of carbohydrates termed crude fiber mainly consists of cellulose, hemicellulose, and lignin. It is measured by boiling a fat-free known amount of food sample in a weak acid solution (1.25 % H_2SO_4) for 30 minutes, followed by boiling in a weak alkali solution (1.25 % NaOH) for 30 minutes at a constant volume, and then deducting 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:

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

Where,

W= Weight of crucible, crude fiber and ash

W1=Weight of crucible and ash

W2= Weight of sample

3.6.11 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-

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

3.7 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

The antioxidant mobility of the extracts was determined using the DPPH test, which was slightly modified from the procedure reported by Azlim et al. (2010). 6 mg of DPPH was dissolved in 100 mL of 100% methanol to develop a methanoic DPPH solution.

The methanoic extract was then diluted with a 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:

% of inhibition = Blank absorbance - Sample absorbance× 100 / Blank absorbance

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

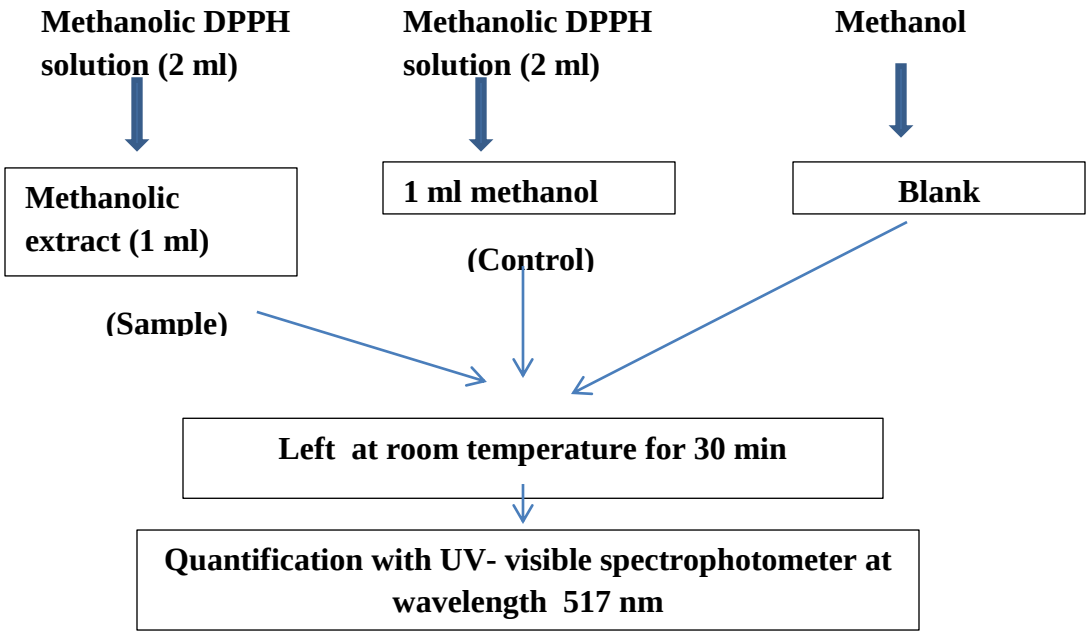

Figure 3.2: Determination of antioxidant capacity.

3.8 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.

3.8.1 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). TPC of Aloe vera jelly was determined by using the Folin-Ciocalteu method described by Vergani et al. (2016), with slight adjustments. In a falconer tube, 1 mL of ethanoic extract was added to 1.5 mL of FC reagent and kept for 3 minutes. The mixture was then incubated with 1.5 ml Na2CO3 (7.5%) for 60 minutes. A UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA) was used to measure absorbance at 765 nm, with C2H5OH functioning as a blank. TPC was determined as mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g).

3.8.2 Total flavonoid content (TFC)

The total flavonoid content (TFC) of the samples was determined using a slightly modified version of the aluminum chloride colorimetric method reported by Chang et al. (2002). Extract stock solution (1 mg/mL) was made, and aliquots of 0.5 mL of diluted extract were diluted in a cuvette with 1.5 mL of 95 percent C2H5OH. The immixture in the cuvette was processed to 0.1 mL of 10% AlCl3, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water. For 30 minutes, the immixture was left at room temperature. The absorbance was read at 415 nm usinguse a UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA), and a blank of 10% aluminum chloride substituted with the same quantity of D.H2O was used. The quantity of total flavonoids in the sample was calculated by comparing the absorbance of

the sample extracts to a quercetin standard curve. TFC as estimated and presented as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g).

3.9 Microbiological analysis of Aloevera Jelly with mandarin

3.9.1 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. As APC cannot differentiate different types of bacteria, can be used to assess organoleptic acceptability, sanitary quality, adherence to good manufacturing practices, and as a safety indicator, 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 guava jelly were taken in 250 ml flask.

Phosphate buffer saline (0.6 M KH2PO4 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 1210 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 the media had settled, the plates were inverted and incubated in an incubator at 37 °C for 24 hours (AOAC, 1990; Sharf, 1966).

Counting and recording

The incubated plates were selected for counting the bacterial colony based on the number and ease of counting of the colony after incubation. The plate with isolated, overlapping, and perplexing colonies was avoided. Plates of 30 to 250 bright, clear, 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).

3.9.2 Fungal analysis in jelly

Media Preparation

Sabouraud Dextrose Agar (SDA) is a selective medium that can grow filamentous bacteria such as Nocardia and also dermatophytes, different fungi, and yeasts. The acidic pH of this medium (about 5.0) inhibits the growth of bacteria but permits yeasts and most filamentous fungi to grow. To improve the antibacterial effect, antibacterial agents can be

added. The SDA media is made of an enzymatic digest of casein and animal tissues, which works as a rich supply of amino acids and nitrogenous compounds for the growth of fungi and yeasts. For 1 liter SDA media, 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. All media were used to 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).

3.10 Sensory evaluation

Sensory evaluation was carried out in order to get the highest acceptability of the final product by consumers. A taste-testing panel determined whether the developed product

was acceptable. The panel test was held on the CVASU campus, and the panelists were both CVASU teachers and students. The product developed from Aloe Vera Jelly was given to a panel of 15 people. The panelists tasted four formulations which were encoded with sample A, sample B, sample C, & sample D without it being told of the formulations. The panelists were asked to assign appropriate scores for the sensory attributes of jelly's appearance, color, flavor, texture, taste, and overall acceptability. Of course, this method does not reflect actual consumer perception, but it does strongly indicate attributes that a high-quality product should have (Sing et al., 2008). They tasted four samples and reported their feedback in terms of a score. Sensory evaluation of the four samples' qualitative parameters (taste, color, flavor, consistency, and overall acceptability) was performed using nine-pointpoint Hedonic scales (Larmond, 1977). The scale was set up in such a way that:

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.

3.11 Statistical Analysis

Data were collected and stored in a Microsoft Excel 2013 spreadsheet for statistical analysis. All samples were repeated three times. Descriptive statistics (mean and standard deviation) were calculated for proximate composition and sensory evaluation of Aloe Vera Jelly. IBM SPSS Statistics 25 is also used to sort, code, and record data. 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. A posthoc "Fisher" test was used to determine variation within the sample groups. The statistical analysis was performed at a 5% level of significance ($p \leq 0.05$).

Chapter 04

Results

4.1 Physicochemical properties of Aloe Vera jelly with mandarin

pH of Jelly is an important factor for optimum gel condition. In table 4.1, lowest (2.75±0.02) pH found in sample A and highest (3.02±0.01) in sample B. TSS (total soluble solids) was highest (0.67 ^{0}B) in sample B and lowest in (0.62 ^{0}B) in sample C. The maximum value (1.03±0.01%) of acidity obtained in sample B and the least value (0.60±0.01%) found in sample D.

Table 4.1 Physicochemical analysis test result of Aloe Vera jelly

Sample Formulation	**P^{H}**	**TSS (^{0}B)**	**Acidity (%)**
Sample A	2.75±0.02^{d}	0.66±0.01^{ab}	0.87±0.02^{b}
Sample B	3.02±0.01	0.67±0.01	1.03±0.01
Sample C	2.95±0.02^{b}	0.62±0.01^{c}	0.64±0.01^{c}
Sample D	2.81±0.01^{c}	0.65±0.01^{b}	0.60±0.01^{d}

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

4.2 Proximate analysis of Aloe Vera jelly with mandarin

Nutritive value of Aloe vera jelly is shown in Table 4.2, almost all samples are significantly different without the crude fiber; Where sample B contained the highest percentage of crude fiber (1.16±0.02%) & CHO (66.15±0.01%) and The lowest percentage of crude fat (0.44±0.01%) & the highest percentage of crude protein (33.64±0.01%) and vitamin C (0.62±0.01%) found in sample A. The lowest value of vitamin C is found in sample C (0.22±0.01%).

Table 4.2 Proximate analysis test result of Aloe Vera jelly

Parameters / Sample Formulation	Aloe vera Jelly (Sample)			
	A	**B**	**C**	**D**
Moisture (%)	64.19±0.01[a]	33.82±0.01[c]	34.88±0.01[b]	34.87±0.02[b]
CHO (%)	35.79±0.01[d]	66.15±0.01[a]	65.11±0.01[b]	65.08±0.01[c]
Ash Content (%)	1.63±0.01[a]	0.41±0.01[d]	0.52±0.01[c]	0.55±0.01[b]
Crude Fiber (%)	1.11±0.01	1.16±0.02	1.14±0.04	0.75±0.55
Crude Protein (%)	33.64±0.01[a]	0.73±0.01[b]	0.51±0.01[c]	0.64±0.01[d]
Crude Fat (%)	0.44±0.01[d]	0.54±0.01[c]	1.12±0.02[b]	1.33±0.01[a]
Vitamin C (mg)	0.62±0.01[a]	0.42±0.02[b]	0.22±0.01[d]	0.35±0.01[c]

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

4.3 Anti-oxidant and Bioactive compound of Aloe Vera jelly with mandarin

From the table 4.3, it was found that antioxidant capacity was significantly highest (3.055±0.001 mg TE/100 g) in sample A and significantly lowest (2.622±0.001 mg TE/100 g) in sample D.

The results of phytochemicals (TFC and TPC) are presented in table 4.3. There have a significantly different values found among all samples. The highest value of total phenolic content (3.02±0.01mg TA/100 mL) and total flavonoid content (3.23±0.01mg QE/100 g) is found in sample A and sample B respectively. Lowest value of total flavonoid content (2.68±0.07 mg QE/100 g) and total phenolic content (2.68±0.01 mg GAE/100mL) found in sample B.

Table 4.3 Anti-oxidant and Bioactive compound analysis test result of Aloe Vera jelly.

Sample Formulation	Anti-oxidant capacity (DPPH)	Total phenolic content (TPC)	Total flavonoids content (TFC)
Sample A	3.055 ± 0.001^{a}	3.02 ± 0.01^{a}	3.12 ± 0.01^{b}
Sample B	2.924 ± 0.003^{b}	2.96 ± 0.06^{a}	3.23 ± 0.01^{a}
Sample C	2.626 ± 0.001^{c}	2.76 ± 0.01^{b}	2.77 ± 0.01^{c}
Sample D	2.622 ± 0.001^{c}	2.68 ± 0.07^{b}	2.68 ± 0.01^{d}

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

4.4 Microbial analysis test result of Aloe Vera jelly with mandarin

Total viable count and the fungal count were determined from 0 to 15 days after jelly preparation, that according to Table 4.4. For the evaluation, specimens were stored at 400 ° C. for 15 days. Yeast and mold were not found when the products were produced, and their presence was not found after 15 days.

Table 4.4 Microbial analysis test result of Aloe Vera jelly.

Sample Formulation	TVC (ml CFU)	Yeast and mold
Sample A	4.7×10^{1}	No growth
Sample B	6.8×10^{2}	No growth
Sample C	5.3×10^{1}	No growth
Sample D	5.2×10^{2}	No growth

4.5 Sensory Quality Evaluation

There was not a significant difference ($p<0.05$) in all the sensory parameters assessed (table 4.5). In all the parameters sample B had the highest acceptance rate. However, sample A scored least acceptance compared to other samples.

Table 4.5 Hedonic scale scoring test results for Aloe Vera jelly.

Parameters / **Sample Formulation**	**Aloe vera Jelly (Sample)**			
	A	**B**	**C**	**D**
Color	6.153 ± 0.005^{c}	8.40 ± 0.01	6.66 ± 0.01^{b}	6.23 ± 0.02^{c}
Flavor	6.176 ± 0.015^{c}	7.87 ± 0.02	6.13 ± 0.01^{d}	6.25 ± 0.02^{b}
Taste	7.13 ± 0.01^{b}	8.87 ± 0.01	7.03 ± 0.01^{bc}	6.90 ± 0.01^{c}
Consistency	6.07 ± 0.02^{d}	8.86 ± 0.02	7.73 ± 0.01^{b}	7.68 ± 0.02^{c}
Overall acceptability	6.67 ± 0.06^{d}	8.89 ± 0.01	7.76 ± 0.01^{b}	7.68 ± 0.02^{c}

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

4.6 Cost analysis

Table 4.6: Production cost of Aloe Vera jelly

Heads	**Tk./Kg**	**Quantity used (kg/1kg products)**	**Total Tk** (for sample A)	**Total Tk** (for sample B, C & D)
1)Expenditure Raw Materials Aloe vera	50	4	200	200
Peppermint	60	1	60	60.00
Sugar	60	1		60.00
Stevia powder	4500	0.065	292.5	
gelatin	1000	0.060 0.072 (stevia)	72	72
Mandarin	220	0.3	66	66
Sub total			630.5	524
2) Processing cost @ 15% of raw material			94.57	78.6
3) Bottling cost	40 tk/piece	4 piece	160	160
Total production cost of 1 kg aloe vera jelly			885.07	762.6

According to table 4.6, Sample A contains stevia and the price per kg jelly is 885.07tk, hence the price per 250gm jelly is 221.25 tk.

Similarly, Sample B, C, and D per kg jelly are762.5 tk.,hence the price per 250gm jelly is 190.5 tk.

The market price for mango or orange jelly is Tk. 440/kg. In this study, jelly with stevia powder costs more than processed jelly but provides more beneficial health effects, while samples B, C, and D cost less and provide more health benefits.

Chapter 05

Discussions

5.1 physicochemical properties of Aloe Vera Jelly with mandarin:

P^H

The pH of the jelly is crucial for maintaining the best gel state. The correct pH level in food also inhibits microbial development. The existence of insufficient acid content is one of the most frequent causes of jam failure. When the jelly is sufficiently concentrated to pour, the pH value should be measured. If the pH is higher than 3.3, citric acid should be added to bring it down to the range of 3.0 to 3.4, 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 pH can be changed to achieve the best flavor, to regulate or change the rate of setting, and to change the level of sugar inversion (Eke-Ejiofor and Owuno, 2013).

The results in table 4.1 showed that the pH value had a significant change among all samples, ranged between 2.75±0.02 to 3.02±0.01 and these values were within the range of typical jellys. Since aloe vera naturally has a pH level of 4.5 to 5.5, mandarin juice was used in this study to lower the pH level.

Total Soluble Solid (TSS) & Titratable Acidity

Aloe vera 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 and to be rising with storage. 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.

Nearly all samples in the current investigation demonstrated a considerable change in the jelly's total titratable acidity. The highest value (1.03±0.01%) was identified in sample B, and the lowest value (0.60±0.01%) was discovered in sample D. The main cause of acidity may be the addition of citric acid during the jelly-making process.

The main cause of acidity may be citric acid addition during jelly production. The TSS and TA levels of fruits during storage periods did not significantly differ between oranges that had been covered with aloe and those that had not. Coated oranges have a higher ascorbic acid concentration than uncoated fruits, it was discovered (Arowora et al., 2013).

5.2 Nutritional composition of aloe vera jelly with mandarin:

Table 4.2 shows the approximate composition of jelly. Samples B, C, and D contained less moisture than sample A, but sample A (64.19±0.01%) is produced with stevia and the others with sugar. Sample B has the lowest moisture value (33.82±0.01), and there is no significant difference between samples C and D. It could be owing to the inclusion of gelling chemicals or gelatin during the manufacturing process. According to Siddiqui et al. (2015), pectin or gelatin is mostly utilized to create the appropriate texture of products, which results in managing the moisture or water in the product. Moisture is a key component that influences product shelf life and freshness. Foods with a high moisture content have a short shelf life.

There is no significant difference in crude fiber (percent) between the samples in this investigation. Because Aloe vera is high in protein and the processed jelly contains stevia powder and a high amount of gelatin, sample A had the highest value of crude protein compared to control C. Sample A (stevia) had the lowest CHO value (35.79±0.01%) while Sample B had the highest CHO value (66.15±0.01%). This large shift in nutritional makeup for jellies could be attributed to the inclusion of commercial pectin or gelatin. According to Brejnholt (2009), pectin and gelatin are examples of substances that improve the CHO profile of a product. Ash content indicates the mineral composition of food products (khan et al., 2012). The most abundant ash content was observed in sample A (1.630.01%) compared to the control sample C, while the least amount of ash was found in sample B (0.410.01%).

Ascorbic acid is essential to life. Sample A had a greater vitamin C content (0.620.01) mg/100g than the other samples. It could be the result of a lengthy heat treatment during processing. According to Martinsen et al., high processing temperatures reduced ascorbic acid concentration (2020). Consumption of vitamin C-rich fruits has been linked to a lower risk of cardiovascular disease and obesity (González Molina et al., 2010). Because the human body is unable to synthesis these nutrients, they must be obtained through dietary intake of fruits and vegetables (Leong and Oey, 2012).

5.3 Phytochemicals and Antioxidant capacity of aloe vera jelly with mandarin:

Apart from basic nutrition, bioactive chemicals play a significant role in human biological activities such as chronic illness prevention and immune system maintenance (Liu, 2004, 2013). Peppermint and aloe vera are both high in bioactive compounds and have therapeutic properties. As a result, quantifying these substances is essential, and the results of bioactive component content in jelly are reported in Table 4.3.

Sample A, which contained stevia powder, had a greater total phenolic content (3.02±0.01 mg QE/100 g) than the control sample C. Sample fact, sample A and sample B statistically do not contain substantial variations in TPC value, in comparison to sample C and sample D. Sample B contained the greatest total flavonoid concentration (3.23±0.01 percent). A difference in the amount of pectin or gelatin in the finished product could explain the variation in the value of bioactive chemicals in the same variety of jelly (Poiana et al., 2012).

Table 4.3 shows that there is a substantial difference in antioxidant capacity among all samples. DPPH was a widely used substrate for testing antioxidant activity, particularly for evaluating the free radical scavenging capabilities of biological and chemical compounds. Sample A has a higher antioxidant capacity (3.055±0.001 mg TE/100 g) than control sample C. It could be because of the inclusion of commercial gelling agents, as Ogutu et al. (2017) discovered that high concentrations of it increase antioxidant capability. Antioxidants are abundant in aloe vera and peppermint. The antioxidant capacity of jelly and its product is influenced by heat and pH. (Wu et al., 2018).

5.4 Microbial Analysis of aloe vera jelly with mandarin:

Microbiological studies (total viable count, yeast and mold count) were done on each of four Aloe Vera Jelly samples. Yeast and mold were not found in the jelly shown in Table 4.4. 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.

TVC levels in jelly varied from 5.2×10^{2} to 4.7×10^{1} cfu/ml. Bacterial load in 120 days above the standard limits (105 cfu/ml) stated by by Zealand, (2001) in guidelines for the microbiological examination of ready to eat food. The utilization of high-quality raw materials is critical for producing high-quality jelly that is free of microbial contamination. According to the Uniform Open Dating Regulation, the shelf life of a perishable food product must be specified in terms of a sell by date (Sidhu, 2006).

5.5 Sensory Evaluation of aloe vera jelly with mandarin:

Sensory evaluation Aloe Vera Jelly was created in order to achieve the greatest organoleptically acceptable proportion of all jelly. According to sensory analysis results from Table 4.5, jelly with sugar (sample B) 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 D) performed almost similarly to control sample C. In comparison to the control sample C, the lowest hedonic score estimated in sample A (with stevia) was 6.67±0.06. The composition of pectin or gelatin 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). The highest mean acceptability score of 8.89 in sample B on the hedonic rating scale designated "Like Very Much." According to the findings of this study, jelly with sugar (sample B) performed better organoleptically than other compositions.

Chapter 6

Conclusion

Jelly is a common food product in ready-to-eat foods due to its health benefits. This study discovered that sugar- based Aloe vera jelly has the highest acceptability in terms of sensory perception. The Aloe Vera jelly physicochemical test and consistency revealed considerable changes due to the impact on mandarin. In a proximate study, Aloe vera jelly was high in carbohydrates and low in protein, fat, and vitamins. It is classified as a functional meal due to its high concentration of phytochemicals such as antioxidants and bioactive substances. It was discovered that the nutritional values were enough, which aided in the improvement of nutritional status. Customers can benefit from this method because it is inexpensive and simple to make jelly. This study indicates a promising future for the production of jelly from two types of sweeteners for the benefit of producers, processors, and consumers in Bangladesh. It may also be recognized that exporting the top-grade international standard jelly may generate foreign exchange, which may have a favorable effect on Bangladesh's national economy. Further research with additional necessary elements for experimenting with other types of fruits for jelly making is required.

Chapter 7

Recommendations and Future Perspectives

In our country, more than half of the people are malnourished; in these cases, Aloe vera jelly could be an excellent source of nutrients and energy because it is available in rural areas of Bangladesh. We have reached a satisfactory conclusion in the development of Aloe vera jelly. It has also increased its commercial value and marketability. Modern food enterprises can use the process on a medium and large scale of production. The following suggestions and opportunities for future research effort are given based on the current investigation.

a) The current research could be replicated to corroborate the experimental findings.

b) The composition can be tweaked further, and you can try making mixed jelly with other recipes and fruit ratios.

b) Because it is simple to prepare. It can also be kept up for a long period and is ideal for the off season. On the other side, it will be beneficial from an economic standpoint for individuals who are economically disadvantaged.

d) Similar study should be conducted on other fruits accessible in markets, such as papaya and mango, especially during the off season.

e) Aloevera jelly might benefit from modern packaging and storage conditions. The discoveries will be beneficial in terms of therapy because they have medicinal value.

g) Despite the fact that the sample size was adequate for statistical comparisons between analytical results. Because of the small number of tested samples, our conclusion should be viewed with care, and the results should be validated in a bigger study.

h) Adequate actions should be taken to increase the nutritional value of commercially supplied jelly.

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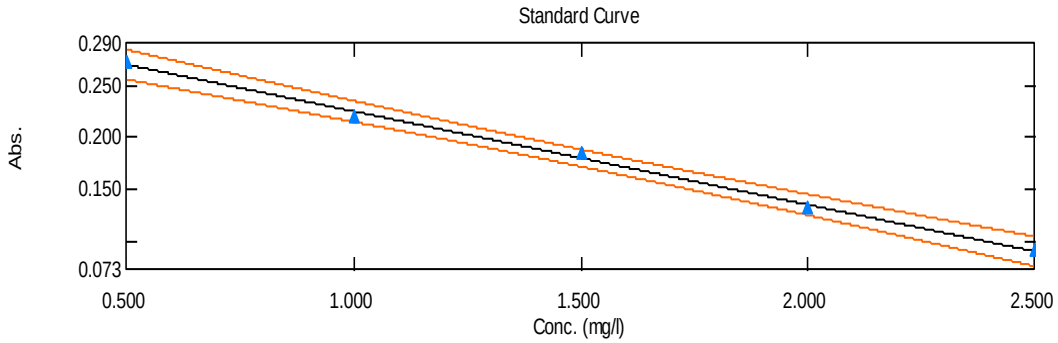
Appendices

Appendix A: Antioxidant capacity of Aloe Vera Jelly

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	std4	Standard	1.500	0.185
4	std5	Standard	2.000	0.133
5	std6	Standard	2.500	0.092

Standard Curve:

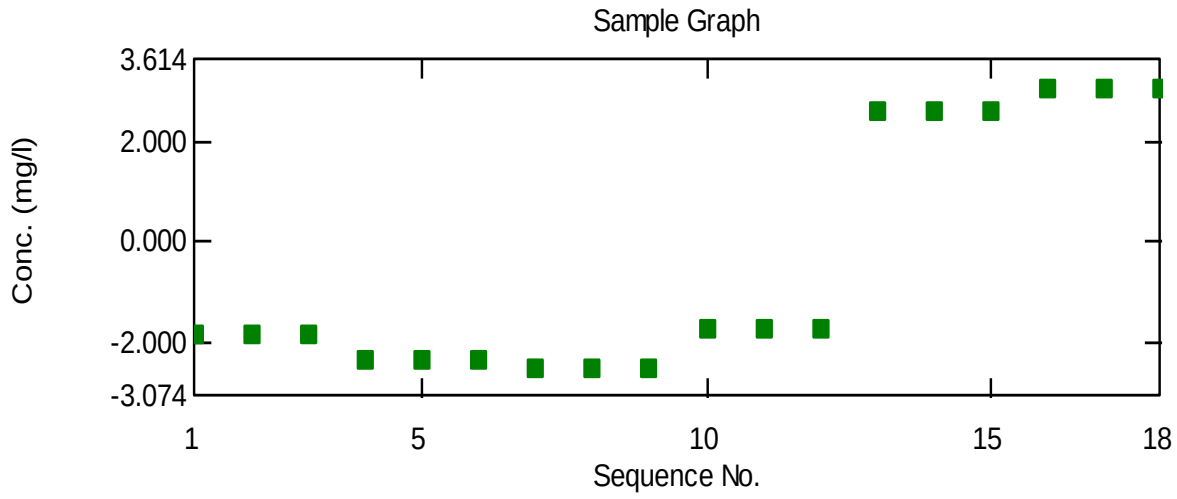


y = - 0.0894539 x + 0.314536
r2 = 0.99735

Sample Table:

Sample ID	Type	Comments		Conc (mg/100g)	WL517.0	comments
1	Sample A		Unknown	3.056	0.080	
2	Sample A		Unknown	3.056	0.079	
3	Sample A		Unknown	3.056	0.079	
4	Sample B		Unknown	2.627	0.041	
5	Sample B		Unknown	2.630	0.041	
6	Sample B		Unknown	2.630	0.041	
7	Sample C		Unknown	2.625	0.052	
8	Sample C		Unknown	2.624	0.051	
9	Sample C		Unknown	2.625	0.052	
10	Sample D		Unknown	2.623	0.053	
11	Sample D		Unknown	2.625	0.052	
12	Sample D		Unknown	2.622	0.051	

Sample Graph:



Appendix B: Questionnaire for Hedonic test of Aloe Vera Jelly

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.

Hedonic	Color				Flavor				Taste				Consistency				Overall Acceptability			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Like Extremely																				
Like very much																				
Like moderately																				
Like slightly																				
Neither like or dislike																				
Dislike																				

slightly																				
Dislike moderately																				
Dislike very much																				