Formulation and quality evaluation of lycopene containing tomato supplemented yoghurt

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Roll: 01-20/09

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

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

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PLAGIARISM VERIFICATION

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Dedication

Dedicated to my beloved family and teachers

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August 2022

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Abbreviation

% : Percentage

& : And

AOAC : Association of Analytical Chemists

DPPH : 2, 2-diphenyl-1-picrylhydrazyl

ANOVA: Analysis of variance

etc : Et cetera

et al : Et alii / et alia

G : Gram

Cfu : Colony forming unit

PPM : Parts per million

Mg : Miligram

Kg : Kilogram

Abstract

Since tomatoes are extremely perishable, drying them is a practical way to increase their shelf life and reduce postharvest losses. The search for foods that double as powerfully beneficial nutraceuticals for human health is currently on the global agenda. Yoghurt's ability to absorb lycopene has been demonstrated to boost its nutritional value when fortified with tomato powder. Some nutrients may breakdown while the tomato is drying, which will impact its general quality traits. Pretreatment's impact on improving drying and product quality. This study looked into the properties of lycopene containing tomato supplemented yoghurt. The goal of this study was to examine how three (3) chemical treatments affected the yield, physical-chemical makeup, and nutritional value of a cabinet dried tomato powder (KMS, $CaCl_2$ and NaCl). Using a cabinet dryer with constant air circulation at 0.7 m/s and air temperatures between 60 and 68^0C, the evaporation procedure was carried out based on previous studies that involved soaking in 0.1% kms, 2% CaCl2, and 1 molar NaCl. To ordinary yoghurt, tomato powder was added in increments of 10 ppm. Using established techniques, the contents of ash, moisture, crude protein, crude fiber, fat, and lycopene were all examined. Results indicated that fortification directly correlated with improvements in the yoghurt's ash and fat. As the enrichment with fresh tomato powder increased, so did the lycopene content. Yogurt's color and texture varied as fortification rose, and moisture levels decreased; still sample B (10 ppm tomato powder) displayed the greatest results. Tomato powder's physical characteristics and bioactive elements (total phenolic compounds, total flavonoids, anti-oxidant and lycopene) were studied. The effects of lycopene may change from person to person depending on dietary lycopene and fat intake, probiotics, genetic variances in metabolism, and other variables.

Key words: Antioxidant, lycopene, cabinet dryer and sensory evaluation.

Chapter 1: Introduction

Among the most well-known freshest produce on earth and a member of the Solanaceae family is the tomato, or (*Lycopersiconesculantum L.*) (Tambo and Gbemu, 2010). Tomatoes are a vegetable that is valued nutritionally for its high vitC concentration, as well as a significant source of lycopene (60-90 mg/kg), polyphenols (10-50 mg/kg), and trace amounts of vitamin E (5-20 mg/kg) (Charanjeet et al., 2004).Tomatoes are grown on over 4 million hectares of land in more than 150 countries (ha). The entire average annual production from 1999 to 2003 was close to 108 million tons (Surendar et al., 2018). One of the most crucial foods is the tomato. Due to the money made from exports, commercial agriculture produces veggies. A large amount of these produce is wasted because there is insufficient handling, storage, transportation, and processing centers. According to worldwide estimates, there are 20–40% overall losses in fruits and vegetables, costing close to Rs. 30000 million annually. This calls for proper preservation and processing facilities in regions where there are surpluses of produce grown. Various meal preparation techniques water removal is necessary for preservation in order to reduce water activity to a level that causes spoilage bacteria to grow more slowly. Reduced water content has other effects unwanted chemical changes that damage the food's sensory quality as well as its nutritional worth properties (Gowda, 1995). An easy way to get rid of extra water in an industrial or agricultural product is to dry it. This method of food preservation is the earliest. Food stuffs can become spoiled by bacteria and enzymes, which also lower their nutritional value. Crop moisture content, up to a particular point, reduces the impact of bacteria, enzymes, and yeast (V. Belessiotis and E. Delyannis, 2011). Tomatoes are regarded as a significant agricultural crop and a crucial component of the human diet all over the world. Despite the fact that tomatoes are frequently eaten raw, processed products like tomato juice, ketchup, and sauce account for more than 80% of all tomato consumption. The need for tomatoes is always increasing in today's society due to a growing population and its increased inclination for tomatoes. To this day, Pakistan, Bangladesh, the United Arab Emirates, Nepal, the Maldives, and Oman are the countries from where India exports the most tomatoes (Anonymous, 2001). Pomace, or leftover tomato weight from the production of products, ranges from 10 to 30 percent. 5.1% moisture, 11.9% fat, 26.8% protein, and 26.3% crude fiber make up tomato pomace (King and Zeidler, 2004). Pre-treatments with chemicals have been used to prevent undesirable alterations when tomatoes are dried and subsequently stored. Fresh vegetables that have been enzyme-treated or the use of chemicals for soliciting or salting tomatoes, such as calcium chloride or metabisulphite, as they serve a number of uses, are the most common and reasonably priced preventative methods (Roy and Choudary, 1972). Tomatoes are frequently dried in small spaces after being processed with sulfur dioxide using either sulfur gas emission from a source or sulfur cylinder burning. Another method of exposing the tomato to sulfur dioxide is by dipping it in sodium metabisulfite solutions (Valley Sun, 2000).

Previous research has suggested the potential health benefits of advantages of a diet high in tomatoes. A lycopene lacks a pro-vitamin activity, the most abundant carotenoid red tomatoes, which is thought to be the cause of their beneficial effects (Shi et al.; 1997, Rao et al.; 1998). The primary natural color of tomatoes is lycopene, which also acts as a biological antioxidant (Ibitoye et al., 2009). Lycopene, the most prevalent carotenoid in tomatoes, has been the focus of numerous studies on both fresh tomatoes and tomato products to determine its anti-oxidant properties. People may view milk as a crucial source of substances because it is nutrient-rich. The typical person's eating habits are, however, changing as a result of industrialization compared to freshly make raw meals, milk is preferred since it is a delicious and healthy dish. As a result, milk can be transformed into a variety of milk products, such as yoghurt and dahi. In India, fermented milk products make up about 9% of the total milk supply (Singh, 2013). Products made from fermented milk have been linked to several health benefits for people because they contain lactic acid bacteria. According to various experimental findings, lactic acid bacteria may inhibit the onset of colon cancer (Wollowski et al., 2001). Yoghurt, which is enjoyed with nearly all meals in India, is the most popular fermented dairy product. It can also be used to prepare other cuisines (Shekhar et al., 2013).Yoghurt with tomato lycopene is tasty, full of minerals, and loaded with substances that promote intestinal health (Hosono et al., 1992), all of which contribute to the extension of human life and reduce the risk of some cancer type. Due to inherent differences in the protein composition of both milks, the yoghurt with lycopene made using cows and bovine milk had noticeably different bodies and textures (Ganguli, 1992). Lycopene's stability is influenced by the production method and the kind of food it is added to. Lycopene in the extract remained stable for up to 37 months at 4°C and at room temperature (Takeoka et al., 2001).

The purpose of this study was to develop a novel product by incorporating tomato lycopene into the processed yoghurt for improved nutrition and health-promoting elements like antioxidant properties and other beneficial phytochemicals, as well as to produce functional processed yoghurt with high nutritional value and flavor and color to increase its public acceptance among children and older people.

Objectives:

1. To assess chemical components and antioxidant capability of lycopene containing tomato supplemented yoghurt.

2. To formulate distinctive dairy products with tomato powder.

3. To ascertain the dietary profile of the lycopene supplemented yoghurt.

Chapter 2: Review of Literature

2.1 Overview of Tomato

The tomato is an edible berry that is produced by the *Solanum lycopersicum* plant, also referred to as the tomato plant. Tomato plants are cultivated all over the world in a variety of settings. Due to their harsh climates, many regions cannot grow crops in open fields (e.g., the Netherlands, northern Europe), and other regions can, but only during certain seasons (e.g., Mexico). Because this method is very effective and has the ability to expand food production to fulfill market demands, protected production in regulated greenhouse environments is currently in vogue (Rico-Garca et al., 2009).The UN's Food and Agriculture Organization (FAO) lists the following benefits of greenhouses: Out-of-season harvests, protection from adverse weather, controlled heating, cooling, shade, and CO_2 enrichment, and improvements to crop quality; preservation of ground structure; the capacity to sow certain materials; significant Production goes up; decreased production expense; more effective use of the growing area; and lowered us to support the grower with greenhouse operations, innovative, automated technologies are being advocated (Soto-Zaraza et al., 2010).

Four types of tomatoes are available: industrial cherry, chonto, and milano (Jaramillo et al., 2007). Milano-style tomatoes are typically consumed in salads and are available in red or green mature forms (restaurants like the green mature form). Both the commercial worth and palatability of this variety are substantial. The chonto variety is used to make stews or pasta and is eaten when it is still fresh. The cherry variety contains extremely tiny fruits that are arranged in clusters of 15 or more and come in a variety of hues, including yellow, red, and orange. Fresh cherry fruit is eaten as a snack, a garnish, and in cocktails. The industrial type is preferred for processing because of its high quantities of soluble solids, which are mostly used to make sauces and pasta. The tomato fruit is a berry, and its quality depends on a variety of factors including color, ripening rate, firmness, size, shape, and composition. Fruits that are mature can be red, pink, or yellow. Capping the fruit helps to lessen this phenomena. Direct sunlight intensifies the green hue on the fruit's shoulders and, in some cases, results in a yellow color (Jaramillo et al., 2007).

Classification in scientific

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: S. lycopersicum

Binary name: Solanum lycopersicum L.

Synonyms :(L.) H. Karst's Lycopersiconlycopersicum, esculent Lycopersicon Mill.

The most crucial observable attribute of a tomato used to determine ripeness and postharvest life is color, which also plays a significant role in the consumer's buying decision. Human graders typically judge the level of ripeness visually by contrasting the tomato's color with a classification chart. Due to subjectivity, ocular stress, and human fatigue, the manual method of judging tomato ripeness usually results in errors (De Grano and Pabico, 2007). Color identification by humans is difficult because emotions like brightness, intensity, lightness, and vividness change how the primary colors (red, blue, and yellow) and their mixtures are perceived (e.g., orange, green and purple).The color sphere, characterized by three perpendicular axes: L* (from white to black), a* (from green to red), and b* (from blue to yellow), can be used to pinpoint different hues (López and Gómez, 2004). A green to red gradient can be used to determine when a tomato should be picked and consumed because color is a sign of tomato ripeness.

Vitamins A, B1, B2, B6, C, and E, as well as minerals including potassium, magnesium, manganese, zinc, copper, sodium, iron, and calcium, are abundant in tomatoes. Because they contain proteins, crabs, fiber, folic acid, tartaric acid, succinic acid, and salicylic acid, tomatoes have a high nutritional value. The colorant lycopene, which gives tomatoes their unique red hue is abundant in tomatoes. The most potent antioxidant in tomatoes is lycopene, but they also include glutathione, which helps to rid the body of toxins and stop the buildup of heavy metals (Jaramillo et al., 2007).

In tomatoes, there is a significant amount of water (93.5%), Calcium (0.07%), and niacin, all of which are crucial for human metabolism. The vitamin A, C, and E-rich tomato aids in illness prevention (Olaniyi et al., 2010; Jaramillo et al., 2007). In addition to acceptable firmness, shape, size, composition and flavor, Customers value tomatoes as a high-end product if it has nice color and consistent ripening. Producers also take these factors into account, along with a very high nutritional value and long shelf life.

About 95% of tomatoes' weight is water. The remaining 5% is primarily made up of fiber and carbs.

Carbs

Less than 5 grams or 4% of a medium tomato's weight in raw carbohydrates (123 grams). Two simple sugars, glucose and fructose, make up more than 70% of the overall carbohydrate content.

Fiber

A typical-sized tomato has 1.5 grams of fiber, making tomatoes a healthy source of this nutrient. The tomato's fibers are mostly insoluble (87%), and include hemicellulose, cellulose, and lignin.

Vitamins and Minerals

C vitamin, this vitamin is both an antioxidant and a necessary nutrient. About 28% of the Reference Daily Intake can be obtained from one medium tomato (RDI). Potassium. Potassium is a crucial mineral that lowers blood pressure and prevents heart disease.Vitamin K, sometimes called phylloquinone, is crucial for healthy blood coagulation and bones.Folate (vitamin B9),B vitamin is essential for optimal cell formation and tissue development. Particularly crucial for expectant mothers.

Lycopene

Red tint and antioxidant lycopene's beneficial health effects have been extensively studied. Carotene is an antioxidant that frequently gives meals a yellow or orange tint and is converted by your body into vitamin A.

Functional Uses of lycopene

To use as a food color, tomato lycopene extract is used. It offers huge of color that are similar to both natural and manufactured lycopenes, ranging from yellow to red. Additionally, tomato lycopene extract is utilized as a food or nutritional supplement in goods where the presence of lycopene confers a particular benefit (e.g., antioxidant or other claimed health benefits). The item may moreover be utilized as an antioxidant in dietary supplements.

Food classifications and degrees of use

Baked goods, morning cereals, spreads, dairy products—including frozen dairy desserts, dairy product analogues, fruit and vegetable juices, soy drinks, sweets, soups, salad dressings, and other food and beverage categories—are all designed to contain tomato lycopene extract. In dahi use level is 20-40 mg/kg according to General Standard for Food Additives(GSFA).

The sponsor claims that tomato powder utilization levels, measured as the amount of lycopene added to food, vary depending on the product's intended use and can range from 2mg/1 in bottle water to 130mg/kg in ready-to-eat cereals. The formulation of food and beverage goods will ensure that each serving contains roughly 2 mg of lycopene. The proposed uses and use levels of tomato extract (represented as lycopene levels added to food) are listed in Appendix III and are organized in accordance with the Codex General Standard for Food Additives' Food Category System

2.2 Medicinal Properties

Tomatoes are beneficial to skin health

Lycopene, an ingredient used in several of the more expensive over-the-counter facial cleansers, is found in high concentrations in tomatoes. Start with eight to twelve tomatoes if you wish to try tomatoes for skin care. Place the tomato's skin on your face so that the tomato's interior touches your skin after you've peeled it. After letting the tomatoes stay on your face for at least 10 minutes, wash. Your skin will appear glossy and clean. There might be some redness, but it should go away with time.

Tomatoes protect against a number of cancers

Inflammation is among the most important elements in cancer. Thus, lycopene is being investigated in numerous both preclinical and clinical cancer investigations as one of the most effective anti-inflammatory nutraceuticals. Epidemiological research revealed a negative correlation between the serum level and the development of cancer (Nedamani et al., 2019). Additionally, increasing lycopene intake (from all sources) has been linked to a lower risk of a number of cancers, including breast, lung, ovary, prostate, stomach, and ovary (Ghadage et al, 2019).

Against Dermatologic disease

In human keratinocytes and SKH-1 mice without hair, lycopene treatment inhibited UVB-induced cell proliferation while increased apoptosis through decreasing CDK2 and CDK4 (Chen et al., 2017). Lycopene also reduced the expression of UVA1 radiation-inducible genes, including HO-1 up regulation brought on by UVA1 and UVA/B, according to a study done on volunteers (Grether-Beck et al,2017).

Crucial antioxidants can be found in tomatoes

Vitamin A and C are both abundant in tomatoes. The main reason for this is that beta-carotene and these vitamins act as antioxidants to combat dangerous free radicals in the blood. The presence of free radicals in the bloodstream is a threat because they can cause cellular damage. Keep in mind that tomatoes contain more beta-carotene the redder they are. Additionally, you need keep in mind that boiling eliminates the vitamin C, therefore eating raw tomatoes is necessary for these advantages. Antioxidant lycopene is well-known. It can guard against oxidation of lipids, proteins, and DNA. Lycopene can also interact with other free radicals, including hydroxyl, nitrogen, and hydrogen peroxide radicals (Caseiro M et al.,2020).

Tomatoes are heart-healthy

Tomatoes are good at lowering blood pressure and cholesterol because they contain potassium and Vitamin B. Nutraceutical lycopene has cardio protective properties. Numerous CVDs and atherosclerosis were shown to be protected against in several studies (Hasan T et al., 2017). Some of the strong oxidants known to be linked to atherosclerosis can be scavenged by it. Lycopene also lessens the oxidation of cholesterol. As a result, atherosclerosis' initial stages are forbidden (Sen S et al., 2019).Importantly, consuming lycopene guards the heart against Atrazine (ATZ) exposure and also stops ATZ-caused harm (Lin J et al., 2016).

Bone protective

Human's osteoblasts and osteoclasts are affected by tomato lycopene in a number of molecular and cellular ways. While it had no effect on cell survival or density, it decreased osteoclast differentiation and decreased calcium-phosphate resorption. Lycopene also increased osteoblast differentiation and proliferation (reducing apoptosis) (Costa-Rodrigues J, 2018). Additionally, postmenopausal osteoporosis can be effectively treated with lycopene supplementation (Nedamani A.R et al., 2018).By preventing oxidation, daily lycopene ingestion lowers the incidence of bone resorption in postmenopausal female patients (Mackinnon E.S et al., 2011).

Protective effect of tomato against different toxins

This activity should be briefly explored in addition to the anti-toxicity properties of lycopene that were previously highlighted. Previous research revealed that it offers strong defense against a wide range of chemical and natural poisons. Lycopene inhibits a variety of chemical agents with recognized neurotoxicity, hepatotoxicity, nephrotoxicity, and cardio toxicity. The primary characteristics of lycopene that are thought to be required for this activity include its anti-oxidative, chelating, free-radical scavenging, and ant apoptotic capabilities. This wonder pigment shields the human body against the damaging effects of metals, fluoride, pesticides, bacterial toxins, and mycotoxins (Hedayati N. et al., 2019).

Antidiabetic

Scientific proof exists for lycopene's protective effects against diabetes. Both diabetes prevention and treatment can be accomplished with its help, according to both animal research and epidemiological surveys (Zhu R. et al., 2020). Lycopene reduced blood and urine glucose levels as well as diabetes-related pancreatic damage in a diabetic rat model. The serum insulin levels were also raised (Ozmeno. et al., 2016) .Conditions similar to oxidative stress are brought on by interactions between advanced glycation end products (AGEs) and their receptors (RAGEs). Additionally, both in vitro and in vivo studies indicated that lycopene inhibits the expression of RAGE in kidneys while slowing the ribose-related formation of AGE in HK-2 cells. Reduced RAGE levels in HK-2 cells were accompanied by decreased NF-B and MMP 2 expressions (Tabrez S. et al., 2015). Supplementing with lycopene dramatically decreased serum nitrate-nitrite levels.

2.3 Perks of Tomato

2.3.1 Reduced Cholesterol

A tomato without cholesterol and a good source of nutrition; won't raise blood cholesterol levels. 9% of the fiber in a cup of tomatoes can help decrease elevated cholesterol levels. Niacin, or vitamin B3, is another substance found in tomatoes that has been used to safely decrease cholesterol levels.

2.3.2 Minimize heart disease

It has been demonstrated that tomatoes are a rich source of potassium, which lowers high blood pressure and lowers the risk of heart disease. The body needs the vitamins B6 and folate found in tomatoes to change the harmful substance homocysteine into more benevolent ones. Heart attacks are more likely to occur when homocysteine levels are high and stroke can directly harm blood vessel walls.

2.3.3 Reduce Blood Pressure

Significant blood pressure reduction is provided by tomatoes. After 8 weeks, the blood pressure top number (systolic) and bottom number (diastolic) both decreased by 10 points and 4 points, respectively, according to continued monitoring of daily tomato consumption (a tomato extract called lycopene complex).

2.3.4 Preservation against cell damage

Lycopene, an antioxidant, is found in abundance in tomatoes. By scavenging harmful free radicals that may otherwise harm cells and cell membranes, antioxidants circulate throughout the body. Atherosclerosis, diabetes problems, asthma, and colon cancer all worsen or progress more quickly as a result of free radicals. High lycopene intakes have been demonstrated to aid in lowering the risk or severity of each of these diseases.

2.3.5 Combat Acidosis

Acidosis is a major contributor to calcium loss, fatigue, headaches, insomnia, muscular aches, arteriosclerosis, acne, dermatitis, sexual dysfunction, hormone imbalance, depression, and degenerative illnesses. With a pH of 7.365, our bodies are built to maintain an alkaline equilibrium. Calcium, magnesium, potassium, and sodium are excellent sources of alkaline minerals, which assist the body naturally, maintain its alkaline equilibrium. Because they are a great source of calcium, magnesium, and potassium, tomatoes can help fend off acidosis. Reduce headaches Exceptional sources of riboflavin, which lessens migraine attacks, include tomatoes.

2.3.6 Enhance Immunity

Tomato eating helps people avoid the flu and colds, especially men. There is a widespread belief that these prevalent diseases are caused by carotenoid deficiencies, namely by low levels of lycopene and beta carotene in our bodies. Tomato juice consumption helps to strengthen the body's immunity to colds and the flu.

2.3.7 Enhanced Bones

18% of the recommended daily intake of the bone-healthy vitamin K is found in a serving of tomatoes. The main non-collagen protein in bone, osteocalcin, is activated by vitamin K. Calcium molecules within the bone are mineralized by osteocalcin.

2.3.8 Procedure for Vasodilatation

Vitamin C has successfully caused normal blood channel dilation in conditions such as atherosclerosis, congestive cardiac failure, high cholesterol, unstable angina, and high blood pressure. Vitamin C supplementation has been demonstrated to enhance blood vessel dilatation.

2.3.9 Lead Poisoning

Child lead toxicity is a serious health issue, particularly in metropolitan areas. Children exposed to lead have been reported to have abnormal growth and development. They experience behavioral issues, learning difficulties, and low IQ. In adults, it may cause renal damage and raise blood pressure. Supplemental vitamin C lowers the level of lead in the blood. Thus, lead toxicity in youngsters can be decreased by eating tomatoes. Thus, children's exposure to lead toxicity can be decreased by eating tomatoes. Therefore, because tomatoes are high in vitamin C, they can help to reduce this risk factor.

2.3.10 Eye Illness

The most frequent cause of visual issues is cataracts. A rise in cataracts is caused by a decline in vitamin C levels in the human eye's lens. Consuming more vitamin C-rich tomatoes improves blood flow to the body's visual regions and aids in the treatment of this eye condition.

2.3.11 Cancer

It has been discovered that a higher consumption of fresh produce is linked to a lower risk of developing different malignancies. A higher intake of vitamin C is linked to a lower risk of malignancies of the mouth, throat, colon, stomach, and esophagus, as well as of the lung, mouth, vocal chords, and body. Fruits high in vitamin C include tomatoes, oranges, and pomegranates.

2.3.12 Fighting Strokes

Ascorbic acid, which is a kind of vitamin C found in tomatoes, aids in lowering the risk of stroke, a type of cardiovascular illness. A diet rich in fruits and vegetables generates a significant amount of vitamin C, which helps to maintain a healthy blood pressure level. It may be because it shields the body from free radicals due to the stroke.

2.3.13 Mood

The tomato's vitamin C is essential for the synthesis of the neither neurotransmitter nor epinephrine. Vitamin C is essential for the healthy functioning of the brain and if there is a deficiency, it might alter a person's mood.

2.3.14 Wound Healing

The Vitamin C in tomatoes, which is consumed in the right quantity each day, aids in the body's ability to heal wounds. It promotes connective tissue growth, which aids in the process of our body's wounds healing.

2.4 Functional Foods

As a working definition, a food can be deemed functional if it contains an ingredient (whether or not it is a nutrient) that benefits one or a small number of body processes in a selective fashion that is pertinent to either the state of health and wellbeing or the reduction of disease risk (Bellisle et al., 1998), or if it has metabolic or psychological effects aside from the traditional nutritional effect (Fergus, 1997).At the conclusion of a three-year project funded by the European Union and overseen by the International Life Science Organization Europe, a group of European experts adopted the following clear definition: "A food can be regarded as functional if it is adequately demonstrated to affect fruitfully one or more goal functions in the body, beyond proper dietary effects in a way that is relevant to e (Diplock et al., 1999).A functional dietary component is a macronutrient that has particular physiological benefits, such as resistant starch or n-3 fatty acids. A micronutrient is considered important if it is consumed in excess of the daily recommendations. A food element that, while having some nutrient benefit, is not required (like some oligosaccharides) or even has no nutrient benefit at all could potentially fit this description. In actuality, in addition to its nutritious (metabolic requirements) worth and role of delivering pleasure, a diet offers clients elements capable of both modifying body systems and reducing the risk of specific diseases.

2.5 Functional foods from plant/vegetable sources

A diet rich in plants or vegetables may lower the incidence of chronic diseases, particularly cancer, according to epidemiologic, in vivo, in vitro, and clinical research data. A range of digestive and respiratory malignancies have been shown to be prevented by consuming a variety of fruits and vegetables, according the World Cancer Research Fund (Falk M, 2004).Several epidemiological studies have shown a negative link between eating fruits and vegetables and developing chronic illnesses like various cancer types and cardiovascular disease. According to Schreiner and Huyskens-Keil (2006), phytochemicals were responsible for this ostensible protective effect. The benefits of phytochemicals for improving health are becoming more widely known among medical practitioners.

In the USA, the Nutrition Labeling and Education Act of 1990 (NLEA)approved, requiring nutrition labels on most products and allowing information on illnesses or health to appear on packaged foods (Ferrari,2003). In most industrialized and developing countries, atherosclerosis and hyperlipidemia are now the leading causes of cardiovascular illness and mortality. According to Felix-Redondo et al. (2013), elevated plasma cholesterol levels significantly increase the chance of developing cardiovascular diseases. To keep the body operating regularly, it is crucial to bring the elevated serum to the proper levels. Since the development of functional food technology, more and more plant-based functional meals are being developed as adjuvant treatments for various diseases (Jiang et al., 2020).Recent research has placed more emphasis on phenolic and antioxidant compounds, which may provide health advantages such a decrease in cardiac disease and cancer because of their antioxidant capacity (lobo et al., 2010). Polyphenols are frequently included in beverages due to their positive physiological impacts on health (Cori et al., 2002).Any possible health benefits for such foods for which the nutrition linkages have not yet attained sufficient scientific validation require more study.

2.6 Yoghurt

Milk is consumed by individuals due to its high nutritional value and completeness as a food source for young animals. Its constituent parts are all advantageous to human health. Milk contains all the nutrients you require, excluding water, including protein, lipids, carbohydrates, vitamins, and minerals. It also contains a number of bioactive compounds, such as immune globulins, hormones, cytokines, and nucleotides. Caseins, b-lactoglobulin, and a-lactalbumin, three of the most common dietary allergens, have reportedly been found in milk. To reduce or eliminate milk allergies, process milk using a variety of methods, including heat treatment, enzymatic hydrolysis, and fermentation by lactic acid bacteria. Research aimed at creating milk that is hypoallergenic will be pursued in the future (Widyastuti and Febrisiantosa).

Yoghurt, a famous fermented milk product, is consumed by a sizeable portion of Indians as either a light drink or as a component of their regular meal.Yoghurt must be made from milk in order to make local butter and ghee. The Prevention of Food Adulteration (PFA) Rules define yoghurt as a product made from pasteurized or boiling milk that has been safely soured by lactic acid or another culture of bacteria through natural or chemical means (1976).Significant amounts of calcium, phosphorus, riboflavin, vitamin B12, vitamin B5, potassium, zinc, and protein can be found in yoghurt. These components make yoghurt a healthy food (Chowdhury et al., 2014).

It has been demonstrated that acid milk is easier to digest than regular milk. Yogurt has demonstrated therapeutic promise for some people, particularly for those who have digestive and intestinal problems. Considering the theory that lactose in milk and bacterium that ferment acid may create gastrointestinal requisite that make it challenging for fermentative bacteria to proliferate, This application is created to avoid the release of gases and autointoxication.Due to the lactic acid bacteria present, eating milk products that have undergone fermentation has been linked to a number of health benefits. According to various studies, lactic acid-producing bacteria may act as a shield against the emergence of colon cancer. Better lactose digestion, diarrhea prevention, immune response modulation, and a reduction in inflammation are a few of the key health benefits.

Even though cultured dairy goods are safer for diets as a result of the high acidity, which prevents disease-causing microorganisms from growing, molds and coliform bacteria may still be present (Wollowski et al., 2001). Historically, fruits like mango, pine apple, raspberry, apricot, and blackcurrant have been used to make fruit yogurt. Fruit yogurt was created by Indian researchers using a variety of fruit juices, such as mangoes, papaya, pineapple, and kokun (Desai et al., 1994). Using a mixture of fruit liquids, Mustafa tried to make fruit yogurt (dahi) (1997).

Long utilized as a desirable nutrient, yogurt has been the subject of numerous studies. Yogurt was made using jack fruit juice and milk as well as mango juice and milk (Rahman, 1998). The most popular and alluring fruits are, as we all know, the mango, pineapple, strawberries, and apple. These fruits are healthy and can be found in Bangladesh.

The chemical-physical, visual, and tactile characteristics of yoghurt prepared from bovine's milk and stored at ambient temperature were examined in relation to the impacts of tomato powder. Textural characteristics (firmness and consistency), as well as sensory analyses, are evaluated.

In comparison to the control, dahi made with 10 ppm tomato powder was harder and more reliable. Both the control and treatment groups tied on the sensory evaluation (overall acceptability). Consumer acceptance of food products is strongly influenced by flavor. Organoleptic tests showed that the fruity yoghurt performed better than the others (Barnes et al., 1991).

Using various fruits to make yogurt is becoming more and more prevalent. Fruit added to yogurt enhances its flavor. This cuisine combines the nutritious qualities of yogurt with the tasty flavor of fruits. Yogurt with fruit is more delicious (Mahmood et al.; 2008). Adding sugars and fruit pectin to yogurts improves its consistency and viscosity while also thickening the curd (Nongonierma et al., 2007). To avoid syneresis, products made from acidified dairy are given pectins. (Tromp et al., 2004)

They chemically adsorb on casein, resulting in an increase in steric repulsion and a decrease in aggregation (Nongonierma et al., 2007). Regular fruit and vegetable eating is associated with a lower risk of diseases including cardiovascular disease and cancer because they contain antioxidant compounds (Jang et al., 2010).

There have been studies linking a range ofoxygen radicals in reaction, including such hydrogen peroxide, superoxide, and the hydroxyl radical, to oxidative damage in both dietary and biological systems (Liu, Chen et al., 2005). Ageing (Hyun et al., 2006), atherosclerotic (Hyun et al., 2006), cancers (Kinnula and Crapo, 2004), and heart disease (Singh and Jialal, 2006) are all believed to be primarily caused by free radicals, Parkinson's (Bolton et al., 2000), neurological abnormalities (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000), and numerous other severe human ailments (Upston et al., 2003).

Natural antioxidants found in food can protect the body from the harmful effects of free radicals and potentially delay the onset of many chronic illnesses (Liu et al.; 2005). Since some substances have been shown to be detrimental, natural antioxidants are preferred over synthetic ones (Liu et al., 2005).

2.7Antioxidant capacity

When the body’s natural antioxidant defenses are weak, cells and tissues are more vulnerable to malfunction and disease. It's essential to keep antioxidant levels at a healthy level while avoiding overdoing in order to prevent or even treat a variety of illnesses. The application of total antioxidant capacity, or antioxidative ability test, as a indicator of disease in biochemistry, medicine, food, and dietary sciences is extensively covered in this work. Total antioxidant capacity may be are liable indicator of respiratory illnesses, kidney diseases, and psychiatric issues for diagnosis and prognostics, but while It should be handled with care(choosing the proper method, using other free radical scavenging genetic markers like cell antioxidant compounds, genes that respond to antioxidants, or levels of antioxidants, and using valuable oxidative indicator). TAC could be utilized to assess the effects of high-TAC diets.

The significance of antioxidant compounds in preventing oxidative stress in a range of disorders, including cancer, heart disease, and neurological issues, has been extensively documented (Ferrari and Torres, 2003)

Analyses have demonstrated that a diet rich in fruits and veggies raises serum TAC in this way (Cao et al., 1998). For example, eating lots of tomatoes may boost a healthy person's overall antioxidant property (Tyssandier et al., 2004). It's important to keep in mind that since childhood, fresh antioxidants (mostly polyphenolics like flavonoid, luteolin, kaempferol, anthocyanins, tea catechins, and tomatoes licopene) have been recognized to lower the risk of illness and maintain a healthy physiological state (Ferrari and Torres, 2003; Ferrari, 2004).

Apple liqueurs had a higher TAC and prevented the colorectal cancer cell expansion because they are high in polyphenolic chemicals (Eber-hardt et al., 2000). Lyophilized apples were added to the diet to increase serum TAC while lowering total plasma levels, hepatic cholesterol, and pee MDA (Aprikian et al., 2001).

Milk proteins are rich in bioactive peptides, which are vital components of the bodies natural antioxidant protective mechanism (Gupta et al., 2009). The proteolytic enzymes of protein content and fermented dairy items include the bulk of these peptides (Korhonen and Pihlanto 2006; Nagpal et al., 2011). A popular and often eaten fermented milk product is dahi. Due to its great nutritional value, benefits for health, lengthy history of using it as a nutritious food, and sensory attributes (Gilliland, 1989).

Yoghurt is made by coagulating milk with lactic acid bacteria such as *Streptococcus thermophilusand Lactobacillus bulgaricus*. Yoghurt's antioxidant activity rises as a result of the lactic acid fermentation process' release of numerous bioactive peptides and liberated amino acids (Korhonen, 2009). Yogurt is used with various fruits, vegetables, and other natural substances to boost antioxidant performance by providing more bioactive components and amino acids that function as hunters for free radicals.

2.8 Conclusion

Despite finding "very limited evidence" to support an association between tomato consumption and decreased risks of prostate, ovarian, gastric, breast, or pancreatic cancers, the FDA concluded that there is "no credible evidence" to support an association between lycopene intake and any of these cancers (Kavanaugh et al., 2007).The effects of lycopene may change from person to person depending on dietary lycopene and fat intake, probiotics, genetic variances in metabolism, and other variables. The absence of readily available, precise illness indicator to calculate the impact of a treatment on a disease process is another constraint of human studies. Our daily diets must include this delectable dairy product, also known as dahi or yogurt, because it is so thick, creamy, and satisfying. Yoghurt, one of the world's super foods that offers a variety of health advantages, is made from living dahi cultures and has recently evolved into different avatars. In addition, lycopene yoghurt is well-liked for its high protein content. According to the results of this investigation, lycopene has excellent antioxidant capacity and might be used in dairy products to improve quality and shelf life.

Chapter 3: Materials and Methods

3.1 Study Area and Duration

The whole experiment was conducted at the laboratory of Applied Food Science and Nutrition, Applied Chemistry and chemical Technology, Food Processing and Engineering and Animal Science and Nutrition at Chattogram Veterinary and Animal Science University (CVASU).This study was conducted from February to July 2022.

3.2 Design of experiment

First, tomatoes were collected. After the sample were taken, they were used to powder up ripe tomatoes. Yoghurt was then prepared utilizing this powder. Following processing, the tomato powder's proximate analysis (dry matter, moisture, ash, crude fiber, crude protein, ether extract/fat), lycopene levels, and antioxidant activity were all assessed.

Consumer acceptance tests were conducted for each product category (A= sample 1, B=sample 2). Bioactive compound, proximate analysis, microbiological load and consumer approval tests were performed for each product category. Analyses of a similar nature were conducted one of the control.

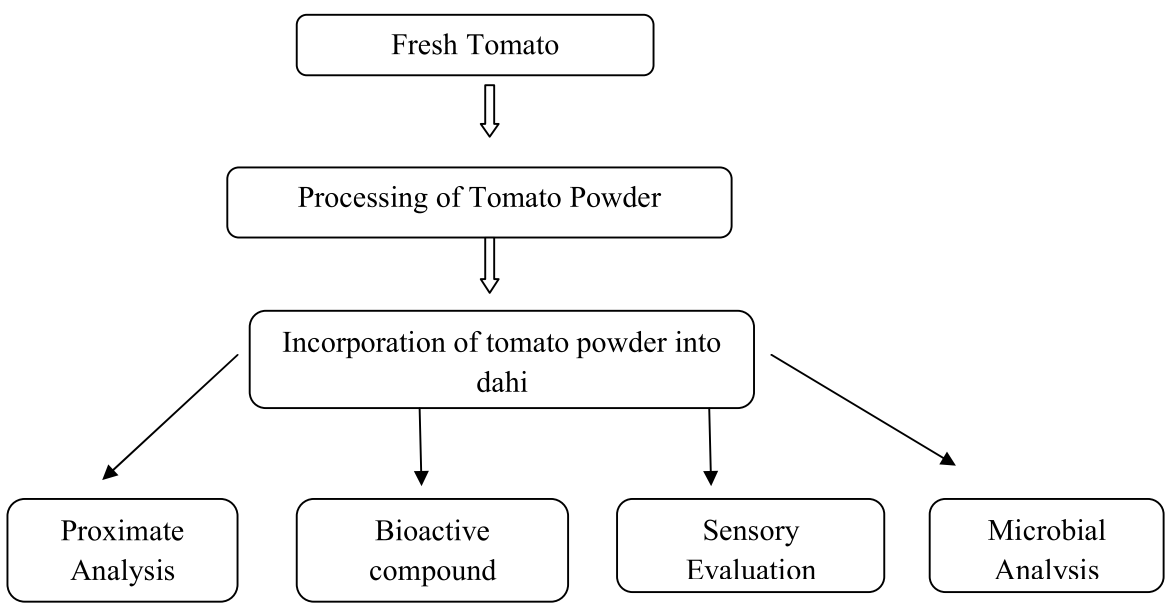

Figure 1: Design of Experiment

3.3 Sample collecting

Sample of milk and tomatoes were collected from Supermarket (Basket) in the Chattogram metropolitan, khulshi, that is the neighborhood provided samples of milk and fully ripened, freshly harvested tomatoes. To preserve their freshness and guard against any deterioration, the samples were put in the fridge as soon as they were collected.

3.4 sample preparation

Sand and other contaminants were removed from the tomatoes using skilled cleaning techniques. The tomato was then divided into 5 mm-thick pieces and dried in a cabinet dryer for almost 24 hours at 65°C to 68°C. Then use a grinder to ground it. The finely milled material was kept in air tight sealed glass container before use.

Process for preparation of tomato powder with cabinet dryer

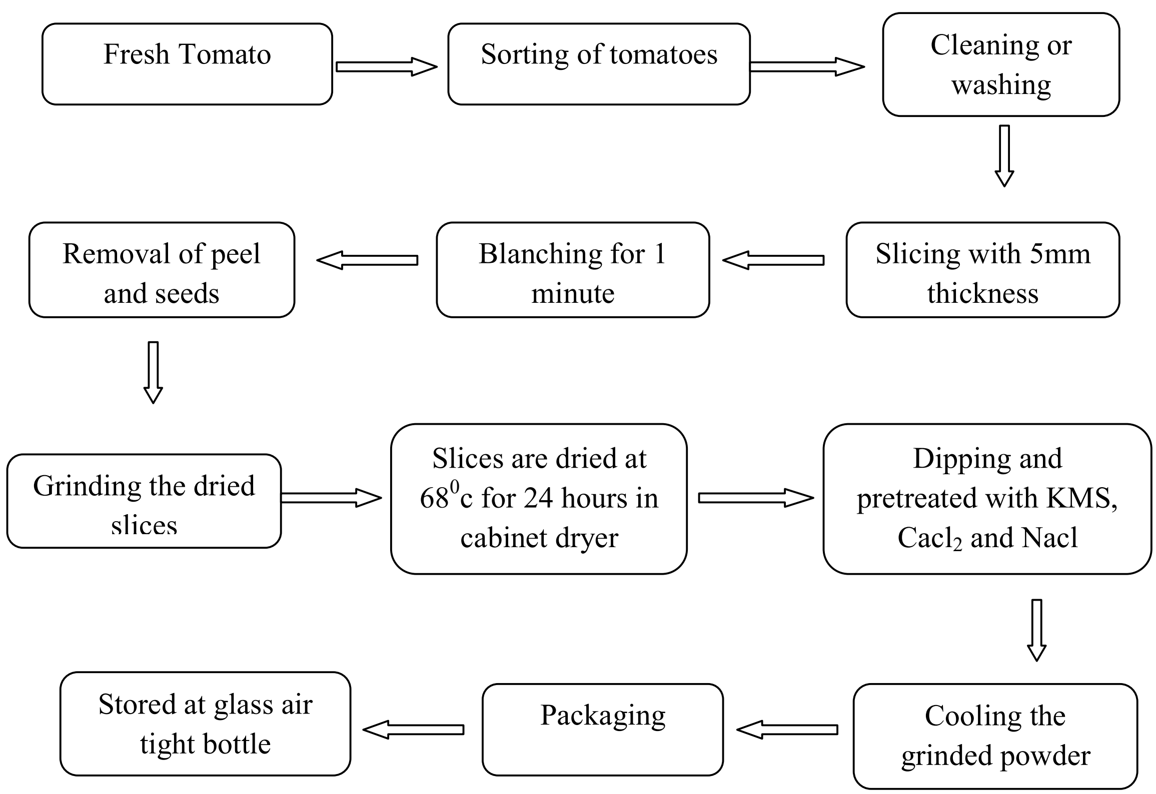

Figure 2: Process for preparation of tomato powder by cabinet dryer.

3.5 Physiochemical analysis :

Fresh samples were tested for lycopene content, ash crude fibre, crude fat, and protein using AOAC (2016) protocols. Additionally, proximate analysis, lycopene content analysis, and bio active compound analysis were performed on these samples.

3.5.1 Determination of lycopene content of tomato powder:

Lycopene-rich extract prepared from the ripe fruits of tomato *(Lycopersicon esculentum L.)*. Utilizing low volume hexane extraction method (LVHEM) (Fish et al. 2002), tomato powder is converted into lycopene extract.

3.5.2 Spectroscopic Analysis:

As in, the technique for extracting hexane at low volume (LVHEM) will be used. Two 40 ml amber screw-top vials containing 5 ml of 0.05% (w/v) butylate dhydroxy toluene (BHT) in acetone, 5 ml of 95% USP grade ethanol, and 10 ml of hexane will each contain 0.6 g (determined to the nearest 0.01 g) of duplicate samples. During sampling, purees will be agitated using a magnetic stirring plate.. Samples will be using an orbital shaker to extract for 15 minutes at 180 RPM. The samples will be shaken for an additional 5 minutes after which 3 ml of deionized water will be added to each vial. To allow for phase separation, the vials were then left at room temperature for 5 minutes. In a quartz cuvette with a 1 cm path length and a 503 nm blanked with hexane, the absorbance of the top hexane layer will be measured.

Calculation:

Lycopene (mg/kg) = $\frac{A503}{g\ tissue} \times 31.2$

Here,

G tissue = Tomato powder, A_{503}= Absorbance

3.6 Yoghurt preparation:

It took 10-15 minutes to heat the milk. The milk was constantly stirred with a stirrer as it heated. The milk pan was taken from the fire once it reached the proper temperature and left to cool. The supernatant was taken out of the refrigerator and let to melt at room temperature. When it was about 40°C, the milk was split into two equal shares. One portion of the milk was used to make yoghurt without tomato powder, and the other portion of the milk was used to make yoghurt with 10% tomato powder. After being heated, milk was cooled to 40°C and then inoculated with the necessary quantity of culture from a nearby shop. The Pre-washing was done on plastic cups in boiling water 37°C was the making temperature. For complete coagulation, the samples were left in the dahi maker for 6-8 hours (kanawjia and S.K., 2006)

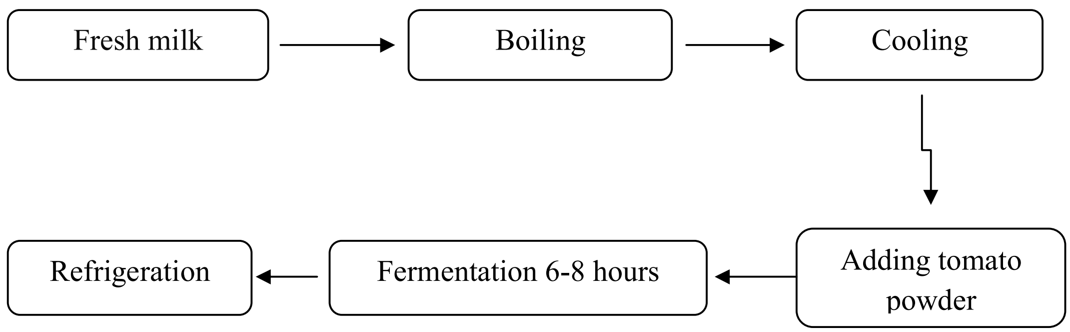

Figure 3: Preparation of yoghurt

3.7 Proximate analysis of yoghurt:

3.7.1 Moisture content

In the preparation and testing of meals, moisture measurement is one of the most crucial and frequently utilized metrics. The water content and the quantity of dry matter in a chunk are related in an inverse manner because the amount of dry mass in a food item is inversely proportional to the quantity of moisture it contains..Food can be heated to a uniform mass at 105°C and standard the atmosphere's force to determine its moisture content. Moisture has a big impact on food quality and shelf life. Vapor takes the moisture out of the food. A single, spotless crucible is selected and accurately weighed. After that, 10 grams of sample were added, and the weight was adjusted. After that, dry the crucible in the hot air oven for 48 to 72 hours at 105°C. using desiccators to fully chill it after removing it from the oven Weigh the sample once more. Redrying repeatedly until a consistent weight is found.

Calculation:

% moisture $=\frac{W-W1}{W} \times 100$

Here,

W=The sample's fresh/air-dried weight, W1 = Weight of the dried sample

3.7.2 Estimation of Ash

The ash fraction contains an equal mixture of all the mineral components. It would be more beneficial to know the concentrations of several different chemicals. However, it does allow for the computation of Nitrogen-Free-Extract (by difference) from dry matter and the estimation of contamination (due to too much soil or too much salt applied).This method entails weighing the ash after completely oxidizing a weighed material sample. Low-carb fish and fish products, among other things, may be made with it. High temperatures can also result in the volatilization of a number of elements in addition to the melting and fusing of solid materials (especially K, Na, Cl, and P).An empty crucible was first scrubbed clean and dried in a hot air furnace. Before being weighed, it was placed in desiccators and given time to cool. Placing the sample, which weighs 2 to 5 grams, in the crucible. The sample was allowed to burn until there is no more smoke at all. The crucible was chilled before being heated for 6-8 hours at 550–600°C in the muffle furnace. The process is finished when white ash forms. Before being put in a desiccators, it was cooled to 150°C.When the temperature had decreased to a comfortable, warm level, the weight was taken.

Calculation:

% of Ash $= \frac{W-w1}{w2} \times 100$

Here,

W = weight of the crucible with ash, W_1 = Weight of the empty crucible, W_2 = Weight of the sample

3.7.3 Estimation of crude fiber:

The bulk of crude fiber, or the part of carbohydrates that are insoluble in water,is composed of cellulose, hemicelluloses and lignin. It is calculated by first cooking a known amount of fat-free meal was placed in a mild acid solution for 30 minutes.(1.25 percent H_2SO_4), then cooking a constant volume of food for 30 minutes in a diluted alkali solution(1.25 percent NaOH), and finally subtracting the ash from the left over residue. The AOAC method was used to calculate the crude fiber (2016). Then, the residual substance was warmed to between 550 and 600 °C and burned for four to six hours in a muffle furnace, resulting in white ash.

Calculation:

% of crude fibre = $\frac{W-w1}{w2} \times 100$

Here,

W = Weight of crucible, crude fibre and Ash, W_1 = Weight of crucible and ash, W_2 = Weight of sample

3.7.4 Estimation of crude protein

Calculating the nitrogen content in organic and inorganic materials uses the kjeldahl method Kjeldahl nitrogen testing is done to find out how much protein is in foods and beverages, meat, feeds, cereals and forages. It is described as an official method in a number of normative publications, including the one listed below: (AOAC, 2016). The protein value is calculated by multiplying the nitrogen percentage by 6.25. Almost always, H_2SO_4 is used to digest a predetermined part of the sample while it is immersed in a digestive solution. After distilling the digested sample and neutralizing any excess acid with alkali, the liberated ammonia is then put into a solution of 2 percent boric acid.

Using circles of #1 Whatman filter paper, weigh out 1 g of forage samples. To the nearest 0.1 mg, weigh the object. In Kjeldahl flasks, wrap filter paper around the forage. Run one blank after each set. To assess the run's acceptability, a forage laboratory reference sample should also be taken. Place a Kjeldahl package and two to three boiling chips in the flask along with the sample. Sulfuric acid is added in 30 ml. Set the Kjeldahl unit's water aspirator to on. To extract fumes, turn on the ventilation fan as well. Burners on high, digest for two hours on the Kjeldahl unit. After 30 minutes of digestion, rotate flasks by a half. After two hours, switch off the burners and leave the flasks alone for five to ten minutes. Flasks should be removed from burners, promptly sealed with rubber stoppers, and set on a Kjeldahl rack. Disconnect the water aspirator. Give the flasks at least 30 minutes to cool. After that distillation was done by this way where, each sample should have 50 ml of boric acid placed in

500 ml Erlenmeyer flasks. Place these flasks on the distillation Kjeldahl apparatus. Turn on the water for condensing and make sure the burettes for the sodium hydroxide and water are full. On high, turn the burners. 250 ml of distilled water, 3–4 mossy zinc chips, and 100 ml of sodium hydroxide should be added gradually in that sequence. Connect to the condenser right away. Once there is 200 ml of liquid, turn off the burners and remove the flasks from the condenser tubes. About 30 minutes pass during distillation. once you're done, turn off the water and wash the Kjeldahl flasks. Then, titrate the liquid in the Erlenmeyer flask with 0.1N sulfuric acid up until the blue liquid turns purple pink again. Don't use too much sulfuric acid while adding it. Read the number of ml of sulfuric acid used on the burette and note it.

Calculation:

$$\frac{\mathrm{A} \times \mathrm{B} \times 0.04}{W} \times\ 6.25 \times 100$$

Here,

A = Quantity of standard, B = Normality of HCl , W = Mass of the sample

3.7.5 Estimation of crude fat

Organic solvents (such methanol or chloroform) are used to dissolve food samples before the filtrate is separate the filtration was spilt into funnels, drying the final mixture produced the extracts, and the fat content was calculated. Utilizing a soxhlet apparatus, the samples amount of crude fat was examined in line with AOAC (2016) guidelines. All glassware should be washed in petroleum spirit, drained, dried at 102°C for 30 minutes, and then cooled in a desiccators. A layer of cotton balls should be placed at the bottom of a 100 mL beaker. An extraction thimble should have cotton wool in the bottom. Place the thimble in the beaker. In the thimble, precisely weigh 5 g of the sample. Mix the sample with 1–1.5 g of sand together using a glass rod. Put some cotton wool in the top of the thimble and use it to clean the glass rod. The sample should be dried in a 102°C oven for five hours. In the analysis of meat meal, the drying phase may be skipped. In desiccators, let the sample cool. Take the piece of cotton wool out of the beaker's bottom and insert it into the thimble's top. A Soxhlet liquid/solid extractor should have the thimble in it. 90 ml of petroleum spirit should be added to a 150 ml round bottom flask after precisely weighing the flask. Over a water bath or an electric heating mantle, assemble the extraction device. The flask's solvent should be heated until it boils. Set the heat source such that around six drops of solvent drip into the sample chamber every second from the condenser. Continually extract for six hours. Disconnect the extractor and condenser from the extraction unit and the heat source, respectively. Put the flask back on the heat source to burn off the solvent. (The solvent may be collected and distilled.) Dry the contents of the flask until a steady weight is attained (1-2 hours) by placing it in an oven set to 102°C. Weigh the flask and contents after letting the flask cool in desiccators.

Calculation:

% of fat = $\frac{W1}{W2} \times 100$

W_1= The extract's weight, W_2 = The sample's weight

3.8 Bioactive Analysis of yoghurt

3.8.1 Determination of antioxidant capacity by DPPH scavenging method

A felcon tube was used to hold the a sample of 1 gram was taken.10 ml of 100% methanol was then together with the addition was let to sit for 72 hours. Continuous straining was done after a 4 hour break. After 72 hours, methanoic extraction was discovered when the supernatant was taken. The DPPH assay, which was significantly modified from azlim et al, was used to ascertain the antioxidant mobility of the extracts (2010). To create the methanoic DPPH solution, 6 mg of DPPH were dissolved in 100 ml of pure methanol. Then, 1 ml of methanoic extract and 2ml of DPPH solution were combined. After giving the mixture a gentle shake, it was allowed to sit at ambient temperature in the dark for 30 minutes. A UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA) set to 517 nm was used to measure the absorbance. The blank was made of methanol and 1ml methanol +2 ml DPPH as a control. By contrasting the samples absorbance with that of the DPPH reference solution, the scavenging mobility of the samples was identified. The antioxidant potential of extracts was determined using their mobility in DPPH free radical scavenging, the equation below:

% of inhibition =$\frac{Blank\ Absorbtion - Sample\ absorbtion}{Blank\ absorbtion}$

Trolox was used as a reference, while TEAC composite made up the calibration standard curve (Trolox equivalent antioxidant mobility). The results were given in milligrams per 100 grams of Troloxequivalents(TE) per gram of powder on a dry weight (DW) basis.

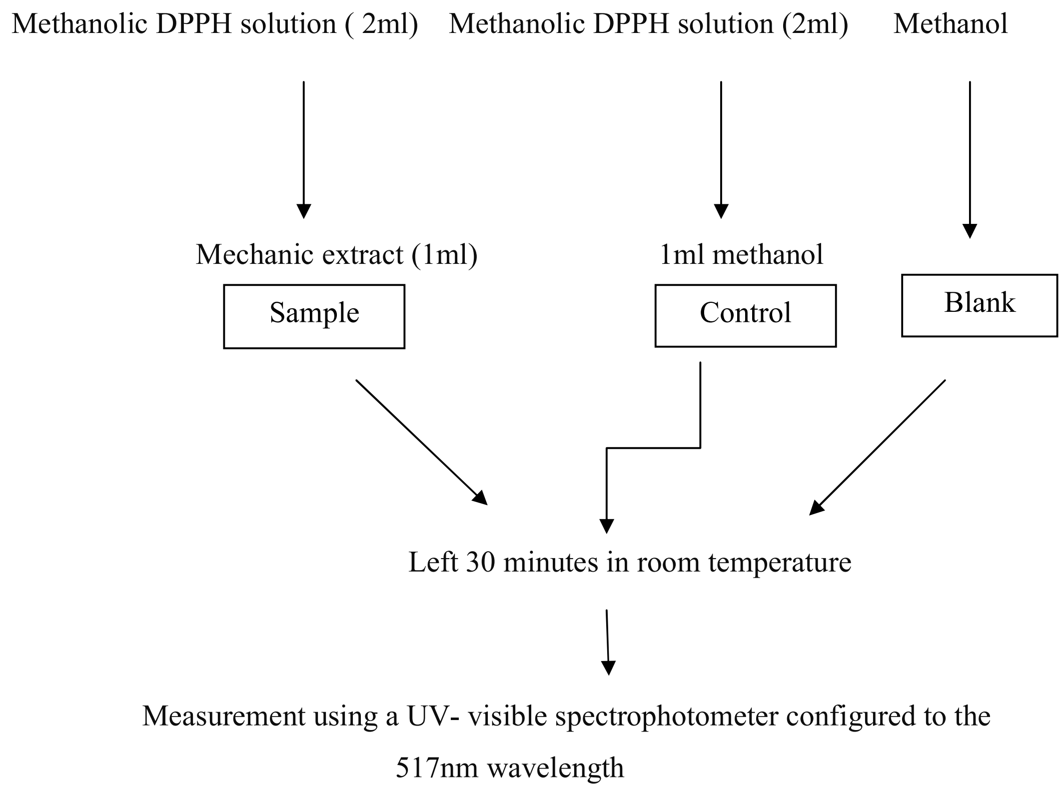

Figure 4: Determination of antioxidant capacity

3.8.2 Determination of phenolic compound

After weighing a 0.5g ground, freeze-dried sample, phenolic chemicals were extracted using 50 ml of 80% aqueous methanol on an ultrasound bath for 20 minutes. The extracts were ultracentrifuged for five minutes at 1400 rpm in an aliquot (2 ml).

Using the folin-ciocalteu test, Fruits and veggies' combined total phenolic was assessed.(V.L.Singleton 1965, et al). Gallic acid extracts or standard solutions (20, 40, 60, 80, and 100 mg/l) were added in the amounts of one milliliter (1 ml) to a volumetric flask that held 25 milliliters (9 ml) of distilled deionized water (dd H_2O). It was created a reagent blank using dd H_2O. The mixture was then mixed with one milliliter of folin-ciocalteu's phenol reagent. In five minutes, 7% Na_2CO_3solution dissolved in 0 ml was added to the mixture. With ddH_2O, the solution was diluted to volume (25ml), then blended. Using a UV-vis spectrophotometer Lamda 5, the absorbance against a produced reagent blank was assessed during a 90-minute incubation at room temperature. Fruits and vegetables' total phenolic content was measured as mg gallic acid equivalent (GAE)/100 g fresh weight. Duplicate analyses of each sample were conducted.

3.8.3 Determination of flavonoid

A 0.5g pulverized, freeze-dried sample was weighted, and 50 ml of 80% aqueous methanol was used to extract the flavonoid components over the course of a 20-minute ultrasonic bath. The extracts were ultra centrifuged for five minutes at 1400 rpm in an aliquot (2 ml).

The aluminum chloride colorimetric assay was used to determine the total flavonoid concentration (J. Zhishen 1999, et al).Adding an aliquot (1ml) of catechin extracts or standard solutions (20, 40, 60, 80, and 100 mg/l) to a 10 ml volumetric flask with 4ml of deionized water. 0.3ml of 5% $NaNo_2$ was added to the flask. 0.3ml of 10% $AlCl_3$ was added after 5 minutes. At the six-minute mark, 2 ml of 1 M NaOH were added, and dd H_2O was used to bring the total volume to 10 ml. After thoroughly blending the solution, the absorbance was measured at 510 nm against a prepared reagent blank. Fruits and vegetables' total flavonoid content was calculated as mg catechin equivalents (CE)/100 g of fresh bulk. Duplicate samples were analyzed.

3.9 Microbial analysis

3.9.1 Aerobic plate count (bacterial plate count)

The bacterial population that was used on a sample is determined by the aerobic plate count. Standard plate count and aerobic colony count are the alternate names for the aerobic plate count (SPC), total plate count (TPC), and mesophilic count, respectively (APC). Typical plate count (SPC). The total number of viable bacteria was counted using a certain technique (TVC). The test is based on the hypothesis that, when paired with agar that has the required nutrients; each cell will develop into a clear colony. It exclusively evaluates microorganism that can survive aerobically at mesophilic temperature (25 to 40^{o}c), disregarding the complete bacterial habitat. Even while APC cannot distinguish between various bacterial species, it can be used to assess overall acceptability, sanitary quality, compliance with excellent requirements, and as a safety warning. APC can offer details about a food's potential for long-term preservation or expected organoleptic development (Banwart, 2012).

3.9.2 Sample preparation

The sample process's approach has a big influence on how accurately the data are analyzed and interpreted. The sample must accurately reflect the entire volume. In order to guarantee that the sample was accurate for the present batch of items, the mixture was completely blended. 25 g of this fully combined yoghurt were added to a 250ml conical flask. Phosphate buffer saline (0.6 M KH_2PO_4 at PH 7.2) was used to dilute the samples. 100 ml of buffered saline was added to the pipette, which was then vigorously shaken back and forth. The volume is being covered by buffer water that is nearly identical. All of the instruments, solutions and other materials must always be heated to 1210^{o}c for 15 minutes to sanitize them. After being diluted to ten times its original concentration (1-10-1 dilution), the produced sample was then used as a stock solution (Andrews, 1992).

The following dilutions were created using 9 ml blanks. First, a dilution was made (1 ml in 9ml) (b) combining these (c) and 1ml from (b) was transferred to the following tube and fully combined. It was 10-2 time less concentrated. In this way, the dilution was raised to 10-6.To determine how many microbes were in the prepared and stored samples, an SPC was employed. This data can be analyzed to predict product shelf life or provide feedback on the nutritional value of foods. A pipette is used to pipette 1 cc of the dissolved material onto any of the sterile, empty Petri dishes with Plate Count Agar as the feeding agar medium at 45°C. On a flat surface, the dishes were pressed together. After the medium had developed, the plates were changed, rather than being incubated for 24 hours at 37 °C (AOAC, 1990).

The quantity of colonies and ease of counting were taken into consideration when selecting the incubated plates for total bacterial counting after incubation. It was determined to avoid creating a plate with jumbled-up, exceeding, and confusing colonies. Plates with 30 to 250 countable, bright, and distinct colonies were selected.. Colony forming unit (cfu)/g or ml, which is the average dilution factor for cfu plates. The viable bacterial count involved the following steps: sample preparation, sample dilution, standard plate counts, counting and recording. At 37°C, the incubation period lasted for 24 hours (AOAC, 1990; Sharf, 1966).

3.10 Cost Analysis

Based on the overall cost of the ingredients required to manufacture the dahi, the price of the tomato powder yoghurt was calculated. The total was displayed in taka, and the price per cup of yoghurt was determined.

3.11 Sensory evaluation

The total degree of satisfaction of customers with the final piece was evaluated sensory. A panel test was held on the CVASU campus. Each of the 15 panelists that participated received a product. The sample A and B were the first two different formulae that were encoded. The panelists tasted the two samples without being informed of what they were doing. The panelists evaluated each dahi sensory attribute, including appearance, color, taste, consistency, sweetness, and general approval. Even if it is obvious that this technique does not correspond to actual customer perception, it expressly implies characteristics that items of high caliber should have (Sing et al., 2008). The four things they tested were each evaluated separately. The five-point Hedonic measurement was used to assess the four samples' qualitative qualities (taste, appearance, flavor, mouth feel, sweetness, and overall acceptability) (Larmond, 1977).

Table 1: The scale was configured as follows:

Ranks	Score
Like very much	5
Slightly like	4
Neither like or dislike	3
Dislike slightly	2
Dislike very much	1

3.12 Statistical analysis

In order to assess data study, data were collected and recorded on a Minitab 19 spread sheet. For each sample, there were three repetitions used. To examine the precise composition and sensory assessment of lycopene yoghurt, descriptive statistics (mean and standard deviation) were used. After that, a statistical evaluation was carried out.

The primary degree of variance for data on proximate composition, bio-active compound and sensory evaluation at the confidence interval of 95% was determined using one way ANOVA methods. Using a post hoc “Fisher” test, variance within the sample groups was sought after. The statistical analysis (P=0) was performed with a significant threshold of 5%.

Chapter 4: Result

In our experiment, we make lycopene-infused yoghurt. For the creation of lycopene-incorporated items, we prepare two types of products—one control and the other lycopene substitute for each group as yoghurt. Lycopene content was determined. Here, we also perform a microbiological analysis and examine the proximate analysis and the capacity for bioactive compounds. Additionally, sensory evaluation was carried out to assess people's compliance.

4.1 Determination of lycopene content

Different tomatoes were chosen to measure the amount of lycopene in them. To test the lycopene concentration of tomatoes, two varieties—*Lycopersicon esculentum* (local or deshi tomatoes) and *Lycopersicum cerasiforme* (cherry tomatoes)—were chosen. Table 1 displays the results. Cherry tomatoes have the lowest lycopene concentration, whereas local tomatoes have the greatest, at 97.234 ppm and 273.418 ppm, respectively. Temitope et al. reported similar findings in 2009. To make it translucent and in accordance with the process, tomato samples are dilated as needed. Take the absorbance at 503 nm after that.

We use this variety of tomato for the lycopene extraction method since our research indicates that local tomatoes have the highest level of lycopene. To determine their lycopene concentration, three commercial tomato sauces and two foreign sauces were chosen. The results are displayed in table 2. Due to the fact that imported products have higher lycopene content than local products. Since imported products contain high or equivalent lycopene contents to raw tomatoes, concentrated product processing and production must result in greater lycopene content. Here also dahi with tomato powder shown significantly higher lycopene content than tomato without lycopene content. However, choosing unripe tomatoes for processing can result in lycopene products with low levels. Moreover, lycopene levels in tomatoes may drop after thermal processing (Temitope et. al., 2009).

Table 2: Lycopene content of different tomatoes and tomato produscts

Sample	Absorbance	Lycopene content (mg/kg) ppm
Local Tomato *(Lycopersicon esculentum)*	4.408	273.197±0.22
Cherry Tomato *(Lycopersicum cerasiforme)*	1.873	97.234±2.1
Tomato sauce (local)	0.780	40.507±1.6
Tomato sauce (imported)	2.508	130.45±1.2
Dahi with tomato powder	1.411	74.151±.19
Dahi without tomato powder	0	0

Legends: Data are mean± standard deviation. Values are not significant in the same column with the same superscripts (P<0.05).

4.2 Proximate Analysis of Dahi

Two formulas for proximate analysis were used in the dahi preparation (shown in table). Three replication procedures were used to collect sample data. In this investigation, we discovered that the content of protein and moisture decreased significantly ($p < 0.05$) while the level of fat increased ($p < 0.05$). Fiber content increased by 16%, while ash content also increased. Since the addition of lycopene increase the amount of fiber in the fortified product.

Table 3: Proximate analysis lycopene dahi

Sample	Crude Protein (%)	Crude Fiber (%)	(fat) (%)	Ash (%)	Moisture (%)
Dahi without tomato	19.033 ± 0.152^{a}	22.866 ± 0.057^{b}	2.736 ± 0.015^{b}	4.536 ± 0.015^{b}	70.00 ± 0.100^{a}
Dahi with tomato	17.466 ± 0.057^{b}	26.466 ± 0.057^{a}	3.156 ± 0.015^{a}	8.596 ± 0.015^{a}	66.440 ± 0.052^{b}

Legends: Data are mean± standard deviation. Values are not significant in the same column with the same superscripts (P<0.05).

4.3 Bioactive Compound capacity

Antioxidant capacity was determined to be significantly lower in sample A(1.48600 mg/100g) and significantly higher in sample B(1.92100 mg/100 g). Total flavonoid content was found to be substantially higher in Sample B(15.1740 mg/100g) and lower in sample A (14.0593 mg/100g).It was discovered that sample A's total phenolic content was significantly lower while sample B's was significantly greater in table (3).

Table 4: Bioactive Compound analysis of yoghurt

Sample	Antioxidant Capacity (mg/100g)	Total flavonoid content (mg/100g)	Total phenolic content (mg/100g)
A	1.48600± 0.00458^{b}	14.0593± 0.0319^{b}	0.9070± 0.001^{b}
B	1.92100± 0.00346^{a}	15.1740± 0.0395^{a}	0.9153± 0.005^{a}

Legends: A= Control yoghurt B = Lycopene containing tomato supplemented yoghurt. Data are mean± standard deviation. Values are not significant in the same column with the same superscripts ($P<0.05$).

4.4Microbial analysis

The safety, quality, and storage stability of processed lycopene yoghurt are determined by its microbiological properties. Total viable bacterial count in a sample is determined by how many bacteria can develop and form calculable colonies on nutrient agar after being incubated at37°Cfor 24 hours. Standard plate count methodology was used to conduct this investigation. Colony-forming unit (cfu) multiplied by dilution number yielded the overall amount of viable bacteria. In table 5, the total numbers of live bacteria in samples A (control) and B (tomato powder yoghurt) are displayed (Ademosun et al., 2019).

Table 5: Total Viable content

Parameter Dahi sample	Dahi without tomato (N=3)	Dahi with tomato (N=3)	P(1-ANOVA)
Total Viable count (cfu/gm)	86×10^{7}	144×10^{7}	<0.001

Legends: N=3 no. of replicates, cfu/gm= coloni forming unit/gm

4.5 Sensory Evaluation

Across all criteria, Sample B had the highest acceptance rate. Comparing Sample A (smell) to the other samples, Sample A received the least degree of acceptance in table (4).

Table 6: Hedonic rating test for sensory

Sample	Color	Smell	Taste	Consistency	Overall acceptance
A	3.600 ± 0.507^{b}	3.733 ± 0.704^{a}	3.467 ± 0.640^{b}	3.267 ± 0.594^{b}	3.533 ± 0.516^{b}
B	4.600 ± 0.507^{a}	3.333 ± 0.617^{b}	4.200 ± 0.775^{a}	4.000 ± 0.845^{a}	4.400 ± 0.632^{a}

Legends: A= Control yoghurt B = Lycopene containing tomato supplemented yoghurt. Data are mean± standard deviation. Values are not significant in the same column with the same superscripts (P< 0.05).

4.6 Cost analysis

Raw materials	Tk/kg	Quantity used (gm/kg)	Total tk (sample product)	Total tk (control)
Tomato powder	60	36	62	0.00
Liquid milk	80	1000	80	80
Sugar	95	50	5	5
Culture	300	40	12	12
Subtotal			159	97
Processing cost @ 15% of raw materials			24	15
packaging Cost (10 cups)	2 tk/piece		20	20
Total			203	132

The control sample in the table is the one without tomato powder. However, the formulation product includes tomato powder. This recipe allows us to create 1 kilogram of yoghurt. Thus, the cost of a cup of yoghurt is:

Formulated yoghurt = 203/10 tk

= 20 tk

Control yoghurt = 132/10 tk

= 14tk

Chapter 5: Discussion

5.1 physiological properties:

Numerous epidemiological studies have revealed a favorable role for tomato consumption in the prevention of several serious chronic diseases, including some cancers and cardiovascular disease (Sesso et al. 2003; Benner et al. 2007). Lycopene, a significant phytochemical found in tomato products that helps prevent cancer (Frohlich et al. 2006). Processing tomatoes increases lycopene's bioavailability, which is good for human health (Shi and Le Maguer 2000). According to Thompson et al. (2000), higher pH and maturity are directly associated to lycopene levels, which may help to explain why the amount of lycopene found in raw tomatoes varies so widely. Two different variations of the lycopene concentration of tomatoes and tomato products were examined. The range of lycopene contenteis from(74.15 ± .19 to 273.19 ± 0.22). The lycopene content of the raw samples varied significantly ($p<0.05$) among the two variations examined. The varying growing circumstances, cultivars, and tomato ripening stages are likely to blame for the diversity in lycopene content of tomatoes collected from various regions of the world.The variance in lycopene levels observed among investigations may be explained by these factors(Thompson et al. 2000; Takeoka et al. 2001). In (table 2) dahi with lycopene has the signicantly higher lycopene content than the control dahi.But local tomato powder contain the highest amount of lycopene content (273.19 ± 0.22). This study demonstrates that heat increases lycopene content decline and that lycopene oxidation by light and heat is the primary source of lycopene degradation. This is consistent with Shi et al (2003) findings on the effects of heat and light exposure on the stability of lycopene. By rupturing cell walls and enabling the extraction of lycopene from the chromoplasts, where it is contained in fresh tomatoes, lycopene is more bioavailable after being heated.(Stahl and Sies 1996).Additionally, the majority of the lycopene in fresh tomatoes is in the trans-isomeric form. Tomato juice's isomerization to cis-isomers was demonstrated to be enhanced by heat processing, making it more bioavailable (Stahl and Sies 1992).

5.2 Nutritional Composition of lycopene yoghurt:

Protein, fat, fiber, and vitamins are all delivered when tomato powder is combined with milk to make fruit yoghurt. It was discovered that the components of two distinct yogurts were comparable. Compared to the control sample, lycopene yoghurt exhibited decreased moisture content. The findings of the proximate analysis of the yogurt and the fortified samples are displayed in Table 3.The impact of moisture on a product's freshness and shelf life is important. Foods that contain a lots of moisture have a lower shelf life. According to Ashaye and Adeleke (2009), the moisture content of powder enhanced dahi is between 75% and 80%. The amount of powder used during preparation and storage conditions might affect how wet fruit yogurt is (Broomes and Badrie, 2010).The lycopene yoghurt had the highest levels of crude fiber (26.50%), crude fat (3.17%), and crude ash (8.60%) when compared to the control, whereas the lycopene yoghurt had the lowest protein level (17.50%) and moisture level (66.42%).This result concurred with those of Ademosun et al., (2019), and Ajanaku et al., (2019). Nutritionally, tomato powder is very rich, especially in dietary fiber. Ash concentration in food products reveals the meal's mineral makeup (khan et al., 2012). Another fruit peel powder added to yogurt in this study had a higher nutritional content, according to Mordi et al (2011).

5.3 Bioactive compound of lycopene yoghurt:

Several extracts can be utilized to increase dairy products' antioxidant activity (Premalatha et al., 2016). The results show that lycopene extract is a useful additive for increasing the antioxidant activity of dahi. The ability of samples to act as antioxidants was evaluated using DPPH. Lycopene extract was found to boost the antioxidant capacity after testing. Between control and lycopene dahi samples, there is a significant difference in antioxidant capacity, as shown in Table 4. Particularly in the investigation of the free radical scavenging properties of biological and chemical substances, DPPH was an extensively employed substrate for assessing antioxidant activity. Antioxidant activity increased in the other samples, but was lowest in the control sample (1.486±0.004).Strong antioxidant activity is produced as a result of radicals being absorbed by the bioactive components in the increased dahi with antioxidant properties. Nguyen and Hwang,2016 investigated that in all treatments, the ratio of juice was directly proportional to antioxidant activity. Therefore, when compared to control, husk tomato juice with yoghurt exhibited considerably higher antioxidant activity (p 0.05) (70.59%). The phenolic chemicals in Husk Tomato Juice may be the cause of this activity. Antioxidant activity decreased more rapidly in control samples than in treated samples, whether they were fresh or preserved.

There are several different phenolic derivatives found in plants. These substances are necessary for plant development and reproduction. Additionally, phenolic compounds are organic antioxidants that can be found in all plant components and serve as natural insecticides and antibiotics (V. K. Gupta et al., 2014).The total phenol content of the tested sections from the control and lycopene dahi is displayed in (Table 4). Between two varities of dahi, lycopene dahi (0.9153± 0.005) has the highest amount of phenolic content .The difference between the TPC of the lycopene dahi and the control dahi was statistically significant ($p < 0.05$). (Table 4).The polyphenol content of tomatoes was examined in another investigation by (Valleverdu-QueraltA et al., 2011). Naringenin was found to have a concentration range of 0.5 to 6.9 mg/kg fresh weight, and chlorogenic acid content ranged from 0.79 to 21.8 mg/kg fresh weight. Our findings are in line with those previously published by (Toor et al., 2004), who noted that concentrated canned tomato products had greater TPC and antioxidant values than canned tomato and liquids because they contained more dry matter.

The majority of total phenolics are made up of flavonoids, which have potent antioxidant properties that greatly enhance their health benefits (Frusciante, L et al., 2007). The widespread use of tomatoes has led to their recognition as a significant dietary source of flavonoids (Slimestad, R, 2008). We found that the tomato fruit variety had an impact on the flavonoid concentration. This variance may be caused by genetic variations, various environmental stressors, and agricultural practices that alter the chemical makeup of plants. Here, the flavonoid content was tested from control and lycopene yoghurt. Lycopene yoghurt (15.1740± 0.0395) shows the more flavonoid content than control (14.0593± 0.0319). A statistically significant difference ($p < 0.05$) existed between the TFC of control and lycopene dahi (Table 4).The concentration of flavonoids varied between the nine tomato types examined, according to (Barman et al., 2014). When Toor and Savages investigated the effects of semi-drying on tomato antioxidant components, they discovered flavonoids ranging from 197-211 mg RE 100 g1 DM for tomatoes of three varieties.

5.4 Microbial Analysis:

Yoghurt with lycopene had a total viable count of 144 $\times 10^7$ per g, while yoghurt without lycopene had a total viable count of 86×10^7 per g. The statistical analysis showed that the samples differed noticeably from one another. This data showed that adding tomato powder to the solution enhanced the total number of viable cells. The present results accord with those who made the discovery that the average number of viable per gram of yoghurt experiment was 75×10^4(Nahar et al.,2007). The typical number of viable organisms per dahi drink was 120.22 (2.51) 104/g (Rahman., 1998). Inadequate storage conditions may still allow microbes to grow, as yogurt has bactericidal properties that depend on its pH and low storage temperature (Kotz et al.,1990).

5.5 Sensory Analysis:

Through sensory evaluation of all yogurt samples, the highest organoleptically acceptable fraction of fruit yogurt was identified. The lycopene dahi with tomato powder has the highest overall acceptance 4.400± 0.632, as shown by the sensory analysis data in Table. It might be as a result of how the food seems, tastes, and is consistency. Yoghurt sample containing tomato lycopene extract scored 4.200± 0.775 in terms of taste. According to statistical analysis, the taste ratings of several dahi varieties varied greatly. Conversely, yoghurt devoid of lycopene earned the lowest taste rating. In terms of smell, lycopene yoghurt achieved fewer score than control dahi. The consistency score of lycopene yoghurt containing is 4.000± 0.845 which is more than control dahi. Moreover according to hedonic rating test color also had the more acceptance than control dahi. The score of lycopene color is 4.600± 0.507. Excellent sensory ratings were given to yoghurt prepared with tomato powder (lycopene). After four days of storage at room temperature, lycopene dahi produced from tomato powder is still acceptable (Ashaye et al., 2009).

Chapter 6: Conclusion

Dahi is a common ingredient in prepared food recipes because of its nutritional advantages. According to this experiment, fruit yogurt produced with tomato powder (lycopene) has the best level of sensory acceptability. A test for measuring lycopene in tomato powder dahi indicated significant variances. In short term, lycopene dahi offered a plentiful source of fiber, fat and vitamins. Since it has a high concentration of phytochemicals including antioxidant, flavonoid and phenolic content, it is categorized as functional foods. Due to the lack of commercial lycopene dahi in the area markets, it was not tasted during the current experiment. The nutritional results were found to be satisfactory, which enhance the stat of health. Furthermore, regulations ensure the safety of all objects based on their microbiological quality, and in the case of TVC, the result was within acceptable limits. Because of its low price and ease of use, that method may be advantageous for consumers. It is much easier to digest than regular yoghurt as it has more fiber. This study forecasts a promising future for dahi production in Bangladesh utilizing tomato powder, to the advantages of farmer, processors and consumers. It's also crucial to keep in mind that exporting products that adhere to the highest international standards could result in revenue for Bangladesh's economy. To develop yoghurt, tests with various types of fruit waste need to be conducted in conjunction with other essential components. The current study provided evidence that lycopene has significant antioxidant capacity and should be included in fatty foods to improve quality and shelf life. However, using pure lycopene as an antioxidant source is the only way to make practical uses conceivable. Overall acceptance of dahi with lycopene supplementation was comparable to treatments with natural antioxidants. This proves that natural antioxidants and dahi are equally acceptable. The idea of natural antioxidants will help produce functional foods while also extending the shelf life of food products.

Chapter 7: Recommendations and Future Perspectives

In Bangladesh, where more than 50% of the population is undernourished, yoghurt, which is widely available though out the nation, may be a fantastic source of the essential nutrients. Alternative nutritional sources and treatments based on plants are gaining popularity. These fruits grow naturally all over Bangladesh. Given the nutritional qualities, this could be a reasonably priced nutrient source for people living in our country's rural areas. In the area of making yoghurt using tomato powder as the major ingredient, we have seen some positive results. Making lycopene containing tomato powder yoghurt from leftover yoghurt has shown some encouraging effects, according to our research. Its market ability and commercial potential have both risen. Modern food companies can exploit the transition from intermediate to large- scale production. The following recommendations and research projects are put forth in light of the investigation's results.

a) Additional study could be done to support the experimental findings.
b) Additional compositional changes could take place.
c) Flavor might be added in further studies.
d) Similar research should be done on other commercially available fruits, like papaya and mango.
e) Modern packaging and storing conditions will be developed to improve storage circumstances.
f) The research will be advantageous from a therapeutic perspective because it has medical importance.
g) Even if the sample size might be used to statistically compare the analytical findings. Our result should be considered with caution due to the small number of samples analyzed, and the results need to be confirmed in a larger study.
h) Enough efforts should be made to provide commercially sold yogurt more nutritional value.

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Appendices

Appendices 1: pictorial presentation processing of yoghurt

Fresh Raw tomato

Slicing of tomato

Removal of peel and seeds

Dipping with KMS , Cacl$_2$ and Nacl

Slices are set in tray

Drying at cabinet

Tomato powder

Preparation of dahi

Final product

Appendices 2: Pictorial presentation of lab work

Magnetic Stirer

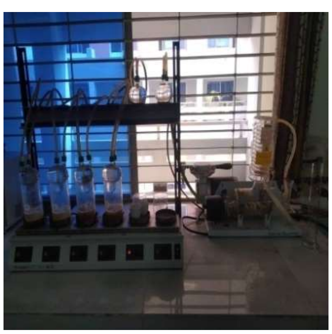
Lycopene Absobance

TVC count of control sample

TVC count (10^{-5}) of control sample

TVC count of lycopene dahi sample

TVC count (10^{-5}) of lycopene dahisample



Crude fiber detremination

Protein determination

Ash determination

Flavonoid determination

Antioxidant determination

Phenolic content determination

Appendices 3: Pictorial presentation of sensory evaluation

Brief biography of the author

Jannatul Adan earned a B.Sc. (Hons.) in Food Science and Technology from the Chattogram Veterinary and Animal Sciences University (CVASU), in Chattogram, Bangladesh, with a 3.39 grade point average (CGPA). She is currently applying to the Department of Applied Food Science and Nutrition at CVASU for the MS in Applied Human Nutrition and Dietetics degree. She is really interested in working on the creation of yogurt using tomato powder (lycopene) and identifying its quality criteria.