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Anamika Mazumder

JUNE, 2022

MEASUREMENT OF ANTIOXIDANT CAPACITY AND CHEMICAL CONSTITUENTS OF BETEL LEAF (*Piper betle* L.) AND PRODUCING ANTIOXIDANT RICHED DAHI

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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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DEDICATED TO MY BELOVED FAMILY & TEACHERS

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List of Abbreviation

% : Percentage

& : And

ANOVA : Analysis of variance

AOAC : Association of Official Analytical Chemists

ºC : Degree Celsius

CHO : Carbohydrate

DPPH : 2,2-diphenyl-1-picrylhydrazyl

et al : *Et al*ii/ *et al*iae/ *et al*ia

etc : Et cetera

G : Gram

Kg : Kilogramme

mg : Miligram

TE : Trolox equivalent

L. : Linn

PPM : Parts per Million

m : Meter

CF : Crude fiber

CP : Crude protein

Abstract

The present study revealed that analysis of antioxidant capacity and determination of different chemical components in Dahi samples enriched with Betel Leaf (*Piper betle L.*) extracts which is more popular in Bangladesh. Although the betel leaf (*Piper betel* L.) are full of nutrients, betel leaf dahi is not a common dish in Bangladesh. The nutritional value and antioxidant capacity of betel leaf extracts was measured in the current experiment and this extract is also utilized to produce dahi. Betel leaf extract (2%,5%,7%) were used to create three distinct formulations for making Dahi respectively. To determine the level of significance at $P< 0.05$, a one-way analysis of variance (ANOVA) was conducted. The highest amount of fat, crude fiber, protein, ash were found 27.90%, 27.467%, 26.267%, 6.22% in 7% in formulated dahi respectively. A high-quality, nutrient- and antioxidant-rich product was discovered when 7% extract was added. The pH range for the samples was 4.22 to 4.39. The range of antioxidant activity following the addition of betel leaf extract was 28.28mg/100g to 30.69mg/10g. No coliform was detected in the dahi samples, and TVC were found in 2%, 5%, 7% samples $55.24\pm0.65\times10^4$, $63.45\pm0.68\times10^4$, $65.09\pm0.25\times10^4$ respectively. In terms of overall preference, sourness, color, taste, and sweetness, dahi with 2 percent betel leaf extract outperformed other varieties of dahi in terms of consumer acceptance. The statistical investigation showed that as extract quantity grew, panelist acceptability decreased. We propose that betel leaf may be utilized to enhance the qualitative attributes, and increasing antioxidant of dahi.

Keywords: Betel leaf extract, Antioxidant, Dahi, Sensory analysis.

Chapter 1: Introduction

Some 2,500 years later, interest in Hippocrates' maxim "Let food be thy medicine, and medicine be thy nourishment" has been rekindled. Natural foods have sparked a significant and expanding interest during the last ten years, especially those that are rich in and produced from vegetable sources. By highlighting the possible health advantages of certain meals, advertisements and academic studies have fueled this desire. Functional foods are those that, in addition to the regular nutrients they provide, also include a large number of biologically active substances like vitamins and antioxidants that may have specific health advantages for people (Hasler, 2002; Falk, 2004).

In India, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian countries, piper betel Linn (Piperaceae) leaves are often used as a post-meal mouth freshener. Because of its strong, spicy, and aromatic taste, betel leaves are used as masticatory by Asians. Some of its common names are betel (in English), paan (in Indian), phlu (in Thai), and sirih (in Thai) (in Bahasa Indonesian). An evergreen dioecius plant, betel thrives in many parts of India. It needs warm, moist growth conditions in order to flourish. Betel vine leaves are chewed with a variety of seasonings, including fennel, cloves, cardamom, areca nut, and areca nut (kattha) (Verma *et al*., 2004).

Due to its ability to cure, betel leaf is a well-known antibacterial that is often used to treat lesions and wounds. This characteristic opened the door for more investigation, which showed that paan extract had antibacterial and antileshmian qualities (Sarker *et al*., 2008).

The fresh juice of betel leaves is used in several ayurvedic therapies. Betel leaf pharmacology has been researched for a very long time. A nutrient-rich beverage like milk may be seen as a necessary source of nutrition for people. The food habits of the average person are, however, altering throughout this industrialization period. Given that it is a tasty and healthful cuisine, it is preferred to freshly prepared raw meals. Consequently, milk is converted into a range of milk products, including yoghurt and dahi. About 9% of the total milk supply in India is made up of fermented milk products (Singh, 2007). Fermented milk products have been connected to a number of advantages for human health because of the lactic acid bacteria found in them.

The development of colon cancer may be prevented by lactic acid bacteria, according to several experimental results (Wollowski *et al*., 2001). The most consumed fermented dairy product on the Indian subcontinent is dahi, which is consumed with almost all meals. Various recipes can also be made using it (Shekhar *et al*., 2013). Dahi is rich in nutrients, flavorful, and contains a lot of elements that support gut health (Hosono *et al*., 1986), all of which prolong human life. Due to inherent differences in the protein composition of both milks, the curd made from cow's milk and buffalo's milk had very different bodies and textures (Ganguli, 1974).

Curd has a short shelf life when kept at room or chilled temperatures because it is a product rich in moisture. To increase shelf life, preservatives are added and various packing techniques are used. The use of herbal extracts in a natural way to preserve food is gaining popularity these days (Mahfuzul Hoque *et al*., 2011).

An antioxidant is any chemical that directly scavenges Reactive Oxygen Species (ROS), upregulates antioxidant defenses, or prevents ROS formation (Khlebnikov *et al*., 2007). It shields patients against conditions including Parkinson's disease, Alzheimer's disease, Parkinson's disease, cardiovascular disease, cancer, and neurological conditions (Manch *et al*., 2004). (Di Matteo and Esposito, 2003). Antioxidant medications and foods may help to lessen oxidative damage brought on by free radicals and active oxygen species (Zhao *et al*., 1989). Synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and trolox are frequently used in the pharmaceutical and food industries (Mahfuzul Hoque *et al*., 2011).

However, it has been shown that they have harmful or mutagenic consequences. Natural antioxidants (polyphenols, tannins, and saponins) from plant species are being produced and isolated since synthetic antioxidants are hazardous. Phenolics are the most significant source of antioxidant activity in plant extracts due to their increased overall abundance (Hodzic *et al*., 2009), synergistic effectiveness as hydrogen donors, reducing agents, and free radical scavengers. A crucial factor in the area of natural antioxidants for food preservation, Wong *et al*. (2006) improved the extraction procedures for optimum total phenolic recovery.

Objectives:

1. To measure chemical constituents, antioxidant capacity which are good for human.

2. To develop unique dairy products added with betel leaf extract.

3. To determine nutritional profile of the developed products.

Chapter 2: Review of Literature

2.1 Overview of Betel Leaf

The Piperaceae family includes Piper betel, also known as Sirih (in Malaysia and Indonesia), Paan (in India and Bangladesh), Betel (in English), and Phlu (in Thailand) (Mahfuzul Hoque *et al*., 2011). In both hemispheres of the world, there are more than 700 species of Piper betel. It is widely found and grown in India, Sri Lanka, Malaysia, Indonesia, the Philippines, and other Southeast Asian and East African countries. Although it has been grown for more than 2500 years, it is believed to have originated in Malaysia (Rabiatul *et al*., 2018). Piper betel is still widely used nowadays all throughout the globe due to its well-known medicinal effects. Additionally, it is used in a variety of facets of human existence, including social, cultural, and religious facets (Guha, 2006).

The classification of betel (Piper betle L.) is as follows:

Kingdom : Plantae

Division : Spermatophyta

Sub-division : Angiosperms

Class : Magnoliopsida

Sub-class : Magnolilidae

Order : Piperales

Familia : Piperaceae

Genus :*Piper*

Species : *Piper betle* L.

Figure 1 Betel Leaf

The flat betel leaf is heart-shaped and has a long stalk. The leaf surface is green and slippery, while the tree trunk has a somewhat brownish-green hue and a rough, wrinkled skin surface. The betel fruit is a spherical, grayish-green fruit. Roots tap and have a yellowish-brown hue. An evergreen vine called betel is often utilized as a natural remedy for a number of illnesses. The Piper betel plant's leaves, roots, stems, stalks, and fruits are all used. The plant could reach a height of 10 to 15 meters with plenty of branching, but it required help to grow vertically (Kumar *et al*., 2010). The betel leaf is heart-shaped and measures between 7 and 20 cm in length and width. Yellowish green to dark green may be seen on the glossy upper surface of the leaves (Mubeen *et al*., 2014).

The stem is strong and has a pinkish-yellow stripe. The flowers are tiny, without petals or sepals, and have two to six stigmas that are covered in a lovely, short fur. The piper betel's fragrance is distinctive and delightful (Periyanayagam *et al*., 2012). The leaves have a variety of flavors, from sweet to spicy, because they contain essential oils (Lakshmi *et al*., 2010).

In Indonesia, yards may include the plant known as betel. With an annual rainfall range of 2250 to 4750 millimeters, the ideal growing conditions are between 200 and 1000 meters above sea level. This plant flourishes in areas that are shielded from the wind and have moist, moderately damp wooded habitats(S. Dalimartha *et al*., 2008).

The betel vine is one of the most researched plants. According to phytochemical study, piper betel has a wide variety of physiologically active compounds, the concentration of which varies according on the plant type, season, and temperature. Fresh betel leaves included a high percentage of water (85–90%), protein (3–3.5%),

fat (0.4–1%), carbohydrates (0.5–6.1%), fibers (2.3%), essential oils (0.08–0.2%) tannin (0.1–1.3%) antioxidant capacity(56.67-65.88%). (Lakshmi *et al*., 2005).

Guha (2006) discovered that the leaves were high in calcium, phosphorus, potassium, iron, iodine, carotene, nicotinic acid, thiamine, riboflavin, vitamin C, and a considerable number of amino acids. Sugumaran *et al*. (2011) found saponin, phenol, alkaloids, amino acids, tannins, flavonoid, steroid, and other chemicals in the betel leaves after performing a phytochemical examination.

Guha (2006) found that the leaves contained significant amounts of calcium, phosphorus, potassium, iron, iodine, carotene, nicotinic acid, thiamine, riboflavin, and vitamin C. After conducting a phytochemical analysis, Sugumaran *et al*. (2011) discovered saponin, phenol, alkaloids, amino acids, tannins, flavonoids, steroids, and other compounds in the betel leaves.

2.2 Medicinal properties

Around the globe, a range of infectious diseases have been treated using traditional herbal medicine. Safety, accessibility, and a decreased risk of addiction and adverse effects are further benefits of employing medicinal plants as novel antibiotics (Lee s *et al*., 2003).

By acknowledging that the majority of developing nations will need to depend on more conventional medical techniques for basic health care, the World Health Organization (WHO) has undertaken a fundamental policy change (M Mahfuzul Hoque *et al*., 2011).

Betel leaf has a distinctive, warming, and spicy scent. Betel leaf extract is used to clean bad breath, halt bleeding, and cure other issues including vaginal discharge, coughing, hoarseness, and skin ulcers when the mouth is enlarged (Desai Sr *et al*., 1994).

1. Anti-diabetic activity: Betel leaf nutrients may prevent diabetes in diabetics and in those who already have it. Betel extract made with hot water may be utilized to treat type 2 diabetes patients, according to research by Bhattacharya *et al*.,2005
2. Antioxidants Activity: Polyphenols with antioxidant effects, including chatecol and allylpyrocatechol, are found in betel leaf extract. According to

Manigauha *et al*.,(2009) the methanolic extracts of betel leaves contain reducing power, DPPH radical and superoxide anion scavenging, and deoxyribose degradation activities (2009). Lei *et al*.,(2003) discovered that an aqueous extract of betel leaves could neutralize H_2O_2, superoxide, and hydroxyl radical.

3. Anti-cancer effect: Betel leaves contain bioactive substances that have been demonstrated to have anticarcinogenic properties and remove tobacco carcinogens, such as hydroxychavicol and chlorogenic bioactives. (Amonkar *et al*., 1989).
4. Antifertility effect: According to studies, betel extract can be used to reduce the weights of reproductive organs, estrogen levels in the blood, fertility, and serum glucose levels (Priya *et al*., 2012).
5. Antimicrobial: Streptococcus pyogenes, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, and other bacteria have been demonstrated to be resistant to the antibacterial effects of betel leaves. In addition, it has been shown that leaf extract has bactericidal effects against pathogenic bacteria present in the urinary system, such as Klebsiella pneumoniae, Enterococcus faecalis, Citrobacter koseri, Citrobacter freundii, and others. (Chakraborty *et al*., 2011). Antibacterial action is attributed to the bioactive molecule sterol.
6. Cardiovascular and platelet inhibition activity: Chewing betel causes the adrenal cortex to produce catecholamine, which may change our energy levels, blood sugar levels, heart rates, and brain activity. Bioactives produced from betel leaves, such as piper betol, ethylpiperbetol, and piperol, may prevent platelet aggregation. (Pisar *et al*., 2007)
7. Wound Healing: Studies have shown that betel leaves may speed up the healing of wounds. Additionally, betel leaf extract was shown to have a significant impact on burn wound healing. When oxidative stress levels are high, wound healing is hindered. The betel leaf is a rich source of antioxidants. These antioxidants help to reduce oxidative stress and hasten the healing of wounds. By increasing the rate of wound contraction and total protein content, betel leaf serves as a wound healing protector (L. Ensminger,1986).
8. Lowers High Cholesterol Levels: High cholesterol levels are related to both heart disease and stroke. Studies have indicated that betel leaf can lower high levels of triglycerides, LDL cholesterol, and VLDL cholesterol. It can also

lower high levels of total cholesterol. High-density lipoprotein (HDL) cholesterol is also produced with its assistance. The lipid-lowering effect of betel leaf is assumed to be caused by the presence of eugenol, a natural antioxidant that combats free radicals. Eugenol also inhibits the liver's production of cholesterol and reduces the gut's absorption of fat. It quickens the process of "bad" LDL cholesterol breakdown. The liver receives large quantities of triglycerides and cholesterol from the circulation, which are then excreted as bile acids (Priya *et al*., 2012).

9. Improves Oral Health: Pathogens present in the mouth are what cause dental infections and dental caries. Chewing betel leaves has been demonstrated in experiments to reduce bacterial activity and growth. Instead of using medications to treat oral and dental illnesses, betel leaves are often utilized as a breath refresher. When sugary foods and drinks are consumed, acid reacts with bacteria in tooth biofilm. Betel leaf inhibits the production of acid by salivary bacteria, preventing tooth decay .
10. Gastro Protective Activity: Studies show that chewing betel leaf is a long-used traditional treatment for stomach ulcers. The inner lining of the stomach is harmed by ulcer-causing chemicals, and less gastric mucus is produced, along with higher levels of oxidative stress. Betel leaves reduce the amount of stomach acid produced, prevents gastric ulcers, and encourages the production of gastric mucus (an essential element in protecting against ulcer-causing substances). Phytochemicals and polyphenols included in betel leaf have antioxidant and anti-ulcerogenic properties. They protect the inner layer of the stomach from toxins and other irritants, limiting overall harm (Pisar *et al*., 2007).

2.3 Impact of betel leaves on national economy

The betel plant, which thrives in tropical climates, was first domesticated in South and Southeast Asia. According to World Health Organization research, the Southeast Asian culture eats betel leaf frequently as betel quid (WHO, 1998).

- In Bangladesh, 30% of adults age 18 and over chew betel nut, compared to 10% to 20% globally. (Gupta and Warnakulasuriya 2002, Flora *et al*. 2012).

- In Bangladesh, the total area under betel leaf cultivation in 2016–17 was 23876.4529 hectares, yielding 2,14,000 MT of betel leaf, compared to 2427 acres and 5074 MT in Bagerhat (BBS, 2017).
- The regions of Bangladesh where betel leaf is abundantly cultivated include Sylhet, Moulvibazar, Jessore, Khulna, Kustia, Bagerhat, Satkhira, Narail, Bhola, Barisal, Faridpur, Rajshahi, Rangpur, Gaibandha, Pabna, Cox's Bazar, and the larger Chittagong district. (Fila *et al*. 2006).
- Additionally, it is estimated that around 20 million people, including 5 million employees, depend on the production, processing, handling, shipping, and selling of betel leaves in the nation to support themselves directly or indirectly, partially or entirely. (Jana, 1996).
- In this manner, the crop generates an annual National Revenue of tk 6000–tk 7000 million as well as tk 800-tk 1000 million in additional income.
- Currently, there is a large market for betel leaves, and Bangladesh exports high-quality betel leaves to several nations in Asia and Europe. India, Saudi Arabia, Pakistan, the United Arab Emirates, England, Germany, and Italy are the top exporters of betel leaves.
- This demonstrates the potential of the crop's foreign currency earnings, which must be enhanced for the sake of the country. This may be accomplished by doing thorough study on export systems and intelligence in addition to modifying the judgments made on export-policy.

2.4 Functional Food

Japan Explores the Boundary between Food and Medicine is where the phrase "functional food" originally appeared in Nature in 1993 (Swinbanks and O'Brien, 1993).

Any meal or dietary item that could provide additional health benefits to those found in its typical nutrients is referred to as a functional food. It is possible to define it as those whole, fortified, enriched, or enhanced foods that provide health advantages beyond the delivery of necessary nutrients when consumers are taken at effective amounts as part of a diverse diet on a regular basis. (Rama, 2019). The ability of functional meals to prevent illness, enhance health, and save expenditures associated with medical care (Nicoletti, 2012).

2.5 Functional foods from plant sources

A plant-based diet may lower the risk of chronic illnesses, including cancer, according to overwhelming evidence from epidemiological, in vivo, in vitro, and clinical trial data. High intakes of fruits and vegetables have been shown to be protective against a range of digestive and respiratory malignancies, according to the World Cancer Research Fund (Boffetta *et al*., 2010). Numerous epidemiological studies have shown an inverse relationship between eating fruits and vegetables and chronic illnesses including various forms of cancer and cardiovascular disease. This apparent protective effect, as reported by Schreiner and Huyskens-Keil, has been linked to phytochemicals (2006). Health practitioners are progressively becoming aware of the benefits of phytochemicals for improving health (Srivastava, 2011). The Nutrition Labeling and Education Act of 1990 (NLEA) has been passed in the USA, and it mandates nutrition labeling for the majority of foods and permits disease- or health-related information on food labels (Marietta *et al*., 1999). Atherosclerosis and hyperlipidemia are now the main contributors to cardiovascular morbidity and death in the majority of industrialized and developing nations. Elevated plasma cholesterol levels are a significant risk factor for the development of cardiovascular illnesses (Félix-Redondo *et al*., 2013). By bringing the increased serum to appropriate levels, it is essential to keep the body functioning normally. Since the advent of functional food technology, an increasing number of functional meals made from plants are being created as adjuvant therapies for certain disorders (Demigne *et al*., 1998). Anthocyanins and other phenolic compounds, which may be linked to health advantages such as a decrease in heart disease and cancer due in part to their antioxidant activity, have recently attracted more attention in study (Seeram *et al*., 2002). Diverse sources of phytochemicals are being investigated since the worldwide market for functional foods and beverages is predicted to reach $109 billion by 2010 (Watkins, 2008). Due to their favorable physiological effects on health, polyphenols are often found in drinks (Ina *et al*., 2002).

For those foods for which the diet-health linkages have not received adequate scientific validation, more study is required to support any possible health advantages.

2.5 Dahi

Humans must consume milk because it is so nutrient-dense. The food habits of the average person are, however, altering throughout this industrialization period. Fresh, raw foods are favored since they are wholesome and delicious. As a consequence, milk is transformed into a range of milk products, with yogurt being the most frequently available dairy product in India. These products include fermented milk, cheese, butter, yogurt, milk ice cream, and more. (Kamalesh Chandra *et al*.,2014).

A sizable fraction of Indians partake in the popular fermented milk product known as dahi, either as a light beverage or as a staple of their daily diet. Since the process of converting milk into dahi is essential for the creation of native butter and ghee (Sukumar dey, 2008).

According to the PFA Rules, dahi is a product manufactured from pasteurized or boiled milk that has been naturally or chemically soured by a safe lactic acid or other bacterial culture (1976).

Dahi has significant levels of calcium, phosphorus, riboflavin, vitamin B12, vitamin B5, zinc, potassium, and protein. These elements make dahi a dish that promotes health (Chowdhury *et al*.,2014).

It has been shown that acid milk is more easily digested than ordinary milk. For certain individuals, especially those who have stomach and intestinal issues, yogurt has shown therapeutic promise. The use is based on the idea that lactose in milk and bacteria that ferment acid may create digestive conditions that make it difficult for putrefactive bacteria to develop, which prevents the creation of gas and autointoxication (Sigh *et al*.,2016).

Eating fermented milk products has been associated with a range of health advantages due to the presence of lactic acid bacteria. Lactic acid bacteria may operate as a preventative measure against the development of colon cancer, according to several research. Among the main health advantages are better lactose digestion, prevention of diarrhea, immune system modulation, and a decrease in blood cholesterol.

Molds and coliforms may still be present even though fermented milk products are safer diets because disease-causing organisms cannot grow in the high acidity (Wollowski *et al*., 2001). Fruit yoghurt has historically been made using fruits such

mango, pine apple, strawberry, apricot, and blackcurrant. Indian researchers used a range of fruit liquids, including mango, papaya, pineapple, and kokun, to produce fruit yogurt (Desai, *et al*. 1994). Mustafa attempted to produce fruit yogurt (dahi) using a variety of fruit juices.

Dahi has been used for a long time as a desired nutrient, and several research have been carried out in different parts of the globe (Deasi *et al*, 1994 and Shukla *et al*. 1987 etc). Jack fruit juice and milk were combined to make dahi (Rahman, 1998), as well as mango juice and milk (Rahman, 1998). (Yasmin,1990). Mango, pineapple, strawberry, and apple are, as we all know, the most well-liked and enticing fruits. These fruits are good for you and are available in Bangladesh.

We looked into the effects of betel leaf extract (Piper betel Linn) on the physical-chemical, sensory, and textural characteristics of dahi made from cow's milk and kept at room temperature. The factors evaluated include pH, titratable acidity, textural features (firmness and consistency), and sensory analyses. The pH of the treatment maintained at 4.23 until the seventh day of storage whereas the pH of the control decreased from 4.40 to 4.03.

The treatment showed a controlled rise at the end of the time, while the titratable acidity of the control increased from the first to the seventh day of storage. Dahi prepared with 0.5 percent betel leaf extract was less hard and consistent than the control. On the sensory assessment, the two groups (control and treatment) tied (overall acceptability). The 0.5 percent aqueous betel leaf extract added to dahi was shown to be superior to the control after seven days of refrigeration. Flavor largely determines whether food items are accepted by consumers. The fruity yoghurt clearly outperformed the other options in organoleptic testing (Barnes *et al*., 1991).

It's getting more and more common to make yogurt using different fruits. Yogurt tastes better when fruit is added to it. This food item blends yogurt's healthy properties with fruit's palatable taste. Dahi made with fruit tastes better (Mahmood *et al*., 2008)

Dahi's consistency and viscosity are improved by adding sugars and pectin from the fruit, which also thickens the yoghurt (Nongonierma *et al*., 2007). Pectins are added to acidified dairy products to prevent syneresis (Tromp *et al*., 2004) They reversibly

adsorb on casein, increasing steric repulsion and lowering aggregation as a result (Nongonierma *et al*., 2007).

Because they contain natural antioxidants, regular consumption of fruits and vegetables is linked to a lower risk of illnesses including cancer and cardiovascular disease (Jang *et al*., 2010).

Oxidative damage in both food and biological systems has been connected to a number of reactive oxygen species, including hydrogen peroxide, superoxide, and the hydroxyl radical (Liu, Chen *et al*. 2005). Free radicals are thought to be the primary cause of aging (Hyun *et al*. 2006), atherosclerosis (Hyun *et al*. 2006), cancer (Kinnula and Crapo 2004), cardiovascular disease (Singh and Jialal 2006), neural disorders (Sas *et al*. 2007), Alzheimer's disease (Smith *et al*. 2000), Parkinson's disease (Bolton *et al*. 2000), and several other serious human diseases (Upston *et al*. 2003).

The body may be shielded from the damaging effects of free radicals by natural antioxidants present in food, and they can even halt the onset of many chronic disorders (Liu *et al*. 2005). Natural antioxidants are favored over synthetic antioxidants since certain synthetic antioxidants have been proved to be harmful (Liu *et al*. 2005).

2.6 Antioxidant capacity

Cells and tissues are more prone to malfunction and disease when the body's antioxidant defenses are compromised. Maintaining enough antioxidant levels while avoiding overdosing is crucial for preventing or even treating a variety of ailments. This paper goes into great detail on and discusses the use of TAC, or total antioxidant capacity test, as a biomarker of sickness in biochemistry, medicine, food, and nutritional sciences. Although it should be used with caution (choosing the right method, using other antioxidant biomarkers like cell antioxidants, genetic antioxidant-response elements (ARE), or antioxidant vitamins, and using valuable oxidative/nitrosative biomarkers), TAC may be a reliable biomarker of diagnostics and prognostics for psychiatric disorders, renal disorders, and lung diseases. TAC might be used to evaluate how diets high in TAC affect disease risk and prevention as well as anti-aging strategies (Juntachote T and Berghofer E,2005)

In a number of diseases, such as cancer, cardiovascular disease, and neurological problems, the importance of antioxidant substances in avoiding oxidative stress has been well established (Ferrari and Torres, 2003; Ferrari, 2004).

Studies have shown that consuming a diet high in fruits and vegetables causes an increase in serum TAC in this respect (Cao *et al*., 1998). For instance, consuming a lot of tomatoes may increase healthy people's total antioxidant capacity (Tyssandier *et al*., 2004). It's crucial to remember that new antioxidants (mainly polyphenolics like quercetin, luteolin, kaempferol, anthocyanins, tea catechins, and tomato licopene) have been known to reduce the risk of sickness and maintain a good physiological state since infancy (Ferrari and Torres, 2003; Ferrari, 2004).

Apple extracts, which are rich in polyphenolic compounds, had a greater TAC and stopped the development of colon cancer cells (Eber-hardt *et al*., 2000). Supplementing the diet with lyophilized apples decreased total plasma cholesterol, hepatic cholesterol, and urine MDA all while increasing serum TAC (Aprikian *et al*., 2001).

Bioactive peptides are abundant in milk proteins and are crucial components of the body's antioxidant defense mechanism (Gupta *et al*. 2009). The majority of these peptides are found in the hydrolysates of milk protein and fermented dairy products (Korhonen and Pihlanto 2006, Nagpal *et al*. 2011). Yoghurt is a well-liked, globally consumed fermented milk product. It has long been used as a healthy meal due to its excellent nutritional characteristics, health benefits, and sensory attributes (Gilliland 1989).

Lactic acid bacteria like Streptococcus thermophilus and Lactobacillus bulgaricus are used to coagulate milk to make dahi (Gahruie*et al*. 2015). By releasing several bioactive peptides and free amino acids throughout the lactic acid fermentation process, the yogurt's antioxidant activity increases (Kudoh *et al*. 2001, Korhonen 2009). By offering more bioactive peptides and amino acids that work as free radical scavengers, yogurt is coupled with different fruits, vegetables, and other natural products to increase antioxidant activity

Chapter 3: Materials and Methods

3.1 Study Area

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

3.2 Study Duration

The trial lasted three months, from November 27, 2021 until February 27, 2022.

3.3 Sample Collection

Fresh Betel leaf (Misti Paan) was collected from local market in Chattogram. Betle leaf was bought on the basis of its freshness. Other materials that were related to the experiment were collected from the laboratory stocks.

3.4 Sample preparation

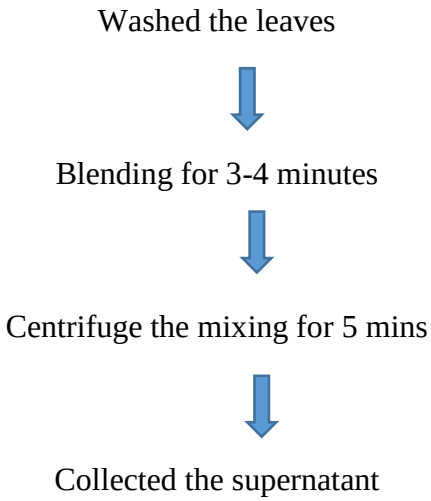


Figure 2 Preparation of Sample

3.5 Dahi Preparation

Milk was heated for 10-15 mins. Using a stirrer, the milk was thoroughly mixed while it was heating. After reaching the required temperature, the milk pan was removed from the fire and allowed to cool. The supernatant was removed from the fridge and left at room temperature for melting. The milk was split into three equal pieces when the temperature reached around 40°C. Each portion was used to prepare dahi by using betel leaf supernatant like milk without supernatant, milk with 2% supernatant, milk with 5% and milk 8% supernatant. Heated milk was chilled to 40°C before being

infected with a desired amount of culture (2%) obtained from a local store. Boiling water was used to pre-wash the plastic cups. Except for the control, supernatant was integrated with dahi at 2, 5 and 7% levels in separate cups. Incubation temperature was 37°C. The samples were kept in incubation machine for 8-12 hours for complete coagulation.

3.6 Physicochemical analysis of Betel leaf dahi

According to AVOC procedures, fresh samples of betel leaf dahi were examined for moisture, ash, pH, titratable acidity, and vitamin C (2016). Both proximate analysis and antioxidant analysis were performed on these samples.

3.6.1 Titrable Acidity

The production of an aqueous suspension of the food and titration with standard NaOH utilizing phenolphthalein indicator are required for determining the acidity in the majority of meals. The outcome represented as the dominating acid percentage.

Procedure

10ml of juice was placed in a 100ml volumetric flask

The volume was increased to 100ml by adding distilled water

10ml of the diluted juice was titrated against N/10 NaOH with phenolphthalein as an indicator

The emergence of pink color signals the titration's endpoint.

Figure 3 Determination of Titratable Acidity

3.6.2 Determination of pH

In chemistry, the pH is used to determine how acidic or basic an aqueous solution is. Technically speaking, pH is defined as the negative logarithm of the activity of the (solvated) hydronium ion, which is most often expressed as the measure of hydronium ion concentrations. The origin of the pH scale may be traced to a group of standard solutions whose pH has been universally accepted. To calculate the main pH standards, a concentration cell with transference is used to detect the potential difference between a hydrogen electrode and a standard electrode, such as the silver chloride electrode. A glass electrode and a pH meter, or indicators, may be used to measure pH, which is defined as the decimal logarithm of the reciprocal of the hydrogen ion in aqueous solutions.

3.6.3 Vitamin C

Vitamin C's chemical assay is influenced by its market-reducing properties. Vitamin C may be detected in plant or animal extracts by decreasing the dyes 2,6-dichloride phenol indophenols. In this instance, the color dye changed vitamin C into dehydroascorbic acid. At the same time, the dye is transformed into a colorless molecule. The termination point of the reaction may be easily determined. Rapid excretion and filtration are necessary because excess might be introduced into the plant product by oxidized, partly destroyed Vitamin C during sampling and grinding. Oxidation is brought on by the extraction process' usage of metaphosphoric acid. A extremely acidic solution yields the most precise result. The titration should be complete in one minute. The dye takes on a blue colour when dissolved in water. It becomes pink when diluted in an acidic solution to a colorless condition.

Procedure:

A dye solution was poured into the burette. Then, 5 mL of vitamin C solution was added to a conical flask. The conical flask, which was positioned underneath the burette, was dyed drop by drop. The titration was finished when a pink tinge appeared, persisted for 20 seconds, and then vanished. The reading was taken at least three times. The same method was used to an ascorbic acid solution with an unknown concentration. A percentage of milligrams was used to represent the outcome (mg percent).

3.6.4 Moisture content

Moisture determination is one of the most important and often used metrics in the manufacture and testing of meals. The moisture content and the quantity of dry matter in a chunk are inversely related because the amount of dry matter in a piece of food is inversely proportional to the amount of moisture it contains. By heating food to a constant weight at 105°C and normal atmospheric pressure, the moisture content of the food may be identified. Food quality and shelf life are significantly influenced by moisture. The meal's moisture is removed by vapor.

Procedure:

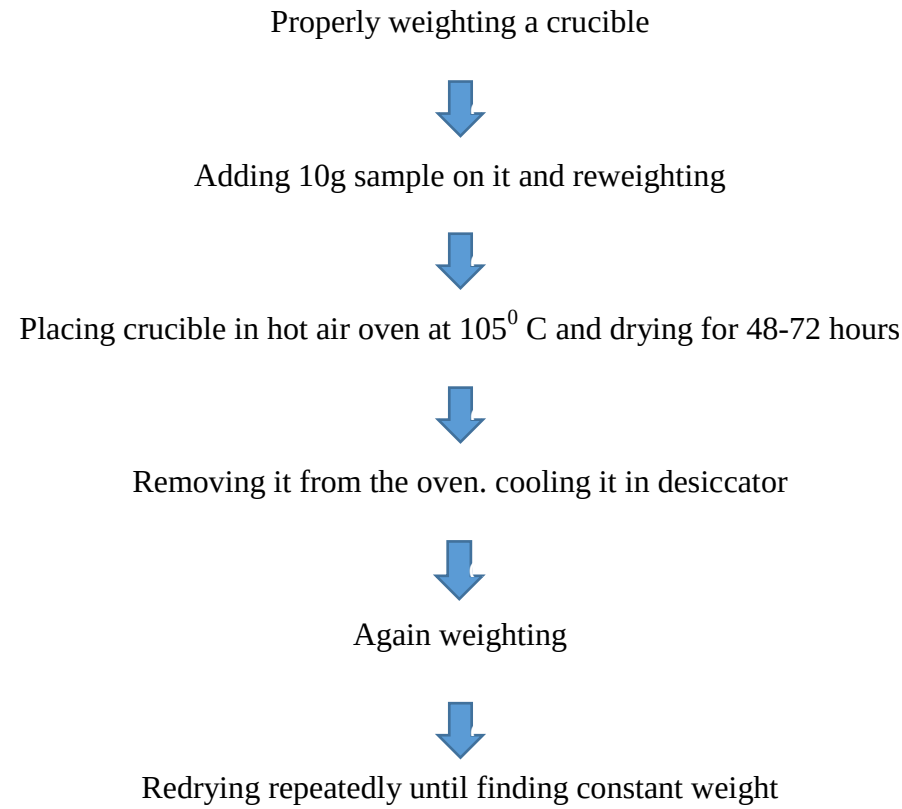


Figure 4 Determination of Moisture Content

3.6.5 Protein

The Kjeldahl technique is used to calculate the nitrogen concentration in organic and inorganic materials. To determine the protein content in meals and drinks, meat, feeds, cereals, and forages, Kjeldahl nitrogen is tested. Several normative publications, including the following, characterize it as an official procedure: (AOAC,

2016). The percentage of nitrogen multiplied by 6.25 is the protein value. A known quantity of the sample is almost always digested with H_2SO_4 while being mixed with a digestion solution. The digested sample is then distilled, and the released ammonia is contained in a solution of 2 percent boric acid after any surplus acid has been neutralized with alkali.

Procedure:

Digestion:

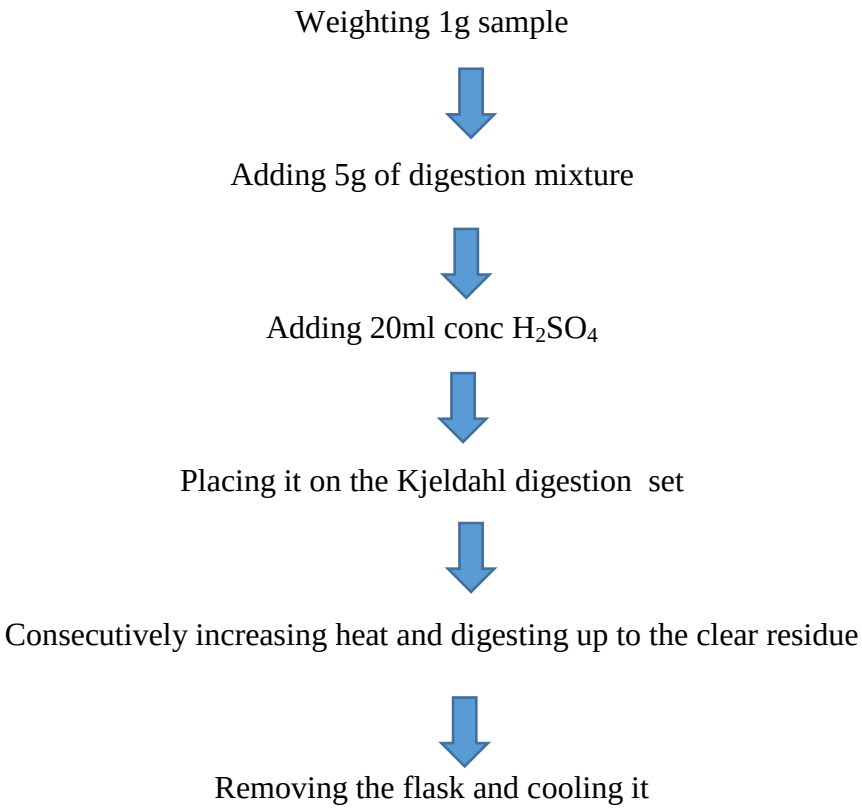
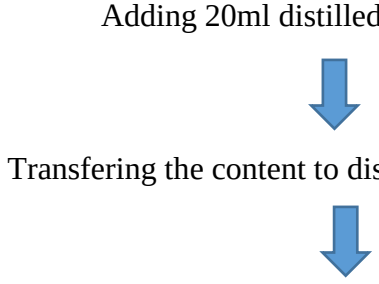


Figure 5 Digestion of Sample

Distillation:



Adding 100ml of 40% NaOH solution and setting the condenser

Adding 20ml 2% Boric acid solution and mixing the indicator in conical flask

Heating it and continuing up to collection of 100ml of distillate

Figure 6 Distillation of Sample

Titration:

Using standard N/10 HCl solution to titrate the distillate

Calculating the titration volume

Figure 7 Titration of Sample

3.6.6 Crude Fiber

When treated with (acids and bases) diluted and in specific concentrations for a brief period of time, crude fiber does not dissolve and is not digested by the digestive juices.

When different foods, such as legumes, grains, and seeds, are treated with weak acids and bases, for instance, soluble sugars and proteins are removed from the composition, leaving non-dissolved components like cellulose and hemicellulose and lignin behind (Crude fiber).

3.6.7 Ash

In the ash fraction, all of the mineral components are combined. This technique oxidizes all organic material by incinerating the weight of the leftover ash. On the other hand, high temperatures may result in the melting and fusing of mineral materials as well as the volatilization of certain elements, such as K, Na, Cl, and P.

5-10gm sample placing in crucible

Burned it up to smoke

Cooled the sample and transferred it to muffle furnace

Flamed out it at 550-600^{0}C for 6-8 hours until white ash

Cooled it at 150^{0}C and transferred it to the desiccator

Weighted the sample at mild warm

Figure 8 Determination of Ash Content

3.6.8 Carbohydrate

Calculations were made to determine how much the Nitrogen Free Extractive differed from the carbohydrate content (NFE). The value was given as the difference between 100 and the total of the other neighboring components.

3.7 Determination of Antioxidant capacity by DPPH scavenging method

Exact preparation:

A 1 gram sample was obtained and placed in a Felcon tube. Following that, 10 mL 100% methanol was added and the mixture was allowed for 72 hours. After a 4-hour gap, continuous straining was performed. The filtrate was collected after 72 hours and methanoic extract was discovered.

Procedure:

The extracts' antioxidant mobility was determined using the DPPH assay, which was modified slightly from Azlim *et al*. (2010). The methanoic DPPH solution was made by dissolving about 6 mg of DPPH in 100 mL absolute methanol. After that, 1 mL of methanoic extract was mixed with 2 mL of DPPH solution. The mixture was then lightly shaken before being left at room temperature for 30 minutes in the dark. The absorbance was measured using a UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA) at 517 nm. 1 mL methanol + 2 mL DPPH solution was used as a control, while methanol was used as a blank. The scavenging mobility of the samples

was determined by comparing their absorbance to that of the DPPH reference solution. Based on the DPPH free radical scavenging mobility of extracts, the following equation was used to assess antioxidant capability:

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

Trolox was utilized as a reference, and the calibration standard curve was made up of TEAC composite (Trolox equivalent antioxidant mobility). On a dry weight (DW) basis, the results were expressed in milligrams per 100 grams of Trolox equivalents (TE) per gram of powder.

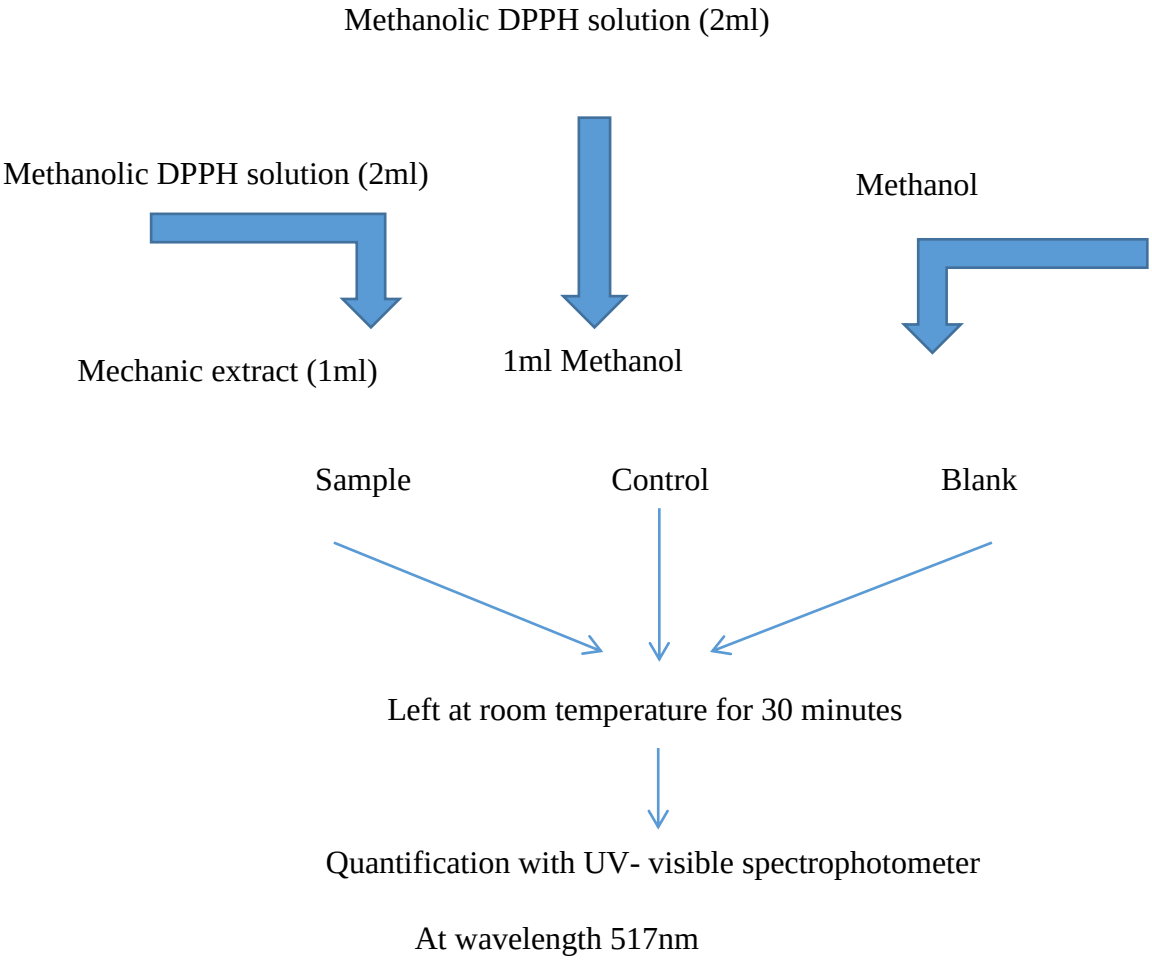


Figure 9 Determination of Antioxidant Capacity

3.8 Microbial Analysis:

3.8.1 Aerobic plate count (Bacterial plate count)

The Aerobic Plate Count is a bacterial population indicator used on a sample. Aerobic Plate Count is also known as Aerobic Colony Count (ACC), Standard Plate Count

(SPC), Mesophilic Count, and Total Plate Count (TPC) (APC). The Standard Plate Count (SPC) technique was used to calculate the total viable bacterial count (TVC). The test is based on the idea that when each cell is combined with agar containing the necessary nutrients, it will form a visible colony. It's a test for organisms that thrive aerobically at mesophilic temperatures (25 to 40°C), not for the total bacterial community. APC is unable to distinguish between various species of bacteria, but it may be used to assess organoleptic acceptability, sanitary quality, adherence to good manufacturing procedures, and as a safety indication. APC can offer information about a food's shelf life or anticipated organoleptic change (Banwart, 2012).

Sample preparation

The accuracy of the analysis and interpretation of the data is primarily determined on how the sample was collected. The sample should be an accurate reflection of the entire bulk. The product was properly blended for this reason so that the sample would be indicative of the entire bulk of the items. In a 250 ml flask, 25 g of this well-mixed dahi was placed. The sample was diluted with phosphate buffer saline (0.6 M KH_2PO_4 at pH 7.2). In the beaker, 100 mL of buffer saline was added and well mixed by to-and-fro movement. The same buffer water was used to make up the volume. All of the equipment, solutions, and other instruments should be sterilized, which means they should be heated at 1210 C for 15 minutes. The produced sample was then diluted to 10 times its original concentration (i.e. 1-10-1 dilution) and utilized as a stock solution (Andrews, 1992).

Dilution

Using 9 ml blanks, a series of dilutions were prepared as follows. The first dilution (1 ml in 9 ml) was carried out (b). In a vortex mixer, this was combined (c) 1 ml from (b) was transferred to the next tube and well mixed. It was diluted by a factor of 10-2. The dilution was increased to 10-6 times this manner.

Standard plate counts

The amount of microorganisms in the prepared and stored samples was estimated using an SPC. This information might be utilized as food quality indicators or predictions for product shelf life. 1 cc of the diluted sample was pipetted into each of the sterile empty petri-dishes with nutritional agar medium (Plate count agar) at 45°C

using a sterile pipette. Plates were swirled on a flat surface to combine them. The plates were inverted and incubated at 37 °C for 24 hours in an incubator once the media had solidified (AOAC, 1990; Sharf, 1966).

Counting and recording

Following incubation, the incubated plates were chosen for counting the bacterial colony based on the quantity of colonies and the ease with which they could be counted. It was decided to prevent the plate with separated, overlapping, and perplexing colonies. Plates with 30 to 250 colonies that were bright, clear, and countable were chosen. = average cfu plate dilution factor = colony forming unit (cfu)/g or ml. Sample preparation, sample dilution, standard plate counts, and counting and recording were all used in the viable bacterial count. The incubation period was 24 hours at 37°C (AOAC, 1990; Sharf, 1966).

3.8.2 Coliform Test

- 10ml, 1ml, 0.1ml of solution were taken from the sample solution. Then mix with the LST broth solution respectively in the three individual bottles of LST broth with Durham tubes.

- This broth bottles were put in the incubator for 24hrs at 37°C for growth**.**

3.9 Cost Analysis

The price of the betel leaf-based dahi was computed based on the total cost of the components used to make the jam. The sum was shown in taka and the cost per cup of dahi was calculated.

3.10 Sensory analysis

The overall acceptability of the finished product by customers was determined by sensory evaluation. The customer acceptability of the created product was assessed by a taste-testing panel. The panel test was conducted on the grounds of CVASU. The product was sent to 15 panelists. Sample A, sample B, and sample C were used to encode three different formulas. The panelists sampled the three samples without being told what they tasted. The panelists were asked to offer a score to each sensory quality of dahi, including appearance, color, flavor, texture, taste, sweetness, and overall acceptability. Of course, this technique does not reflect actual consumer

perception, but it does strongly suggest characteristics that a good quality product should have (Sing *et al*., 2008). They sampled four items and gave their feedback in the form of a score. Using nine-point Hedonic measures, sensory evaluation of qualitative criteria (taste, look, flavor, mouth feel, sweetness, and overall acceptability) of the four samples was performed (Larmond, 1977). The scale was set up in following way:

Ranks	Score
Like very much	5
Slightly like	4
Neither like nor dislike	3
Dislike slightly	2
Dislike very much	1

3.10 Statistical Analysis

To assess statistical analysis, data were determined and kept on a Minitab 19 spread sheet. Three replicates were used for each sample. For the proximate composition and sensory assessment of betel leaf dahi, descriptive statistics (mean and standard deviation) were used. Following that, statistical analysis was carried out. One-way ANOVA techniques were used to determine significant degree of variation at the 95 percent confidence interval for proximate composition, phytochemicals, antioxidant capacity, and sensory assessment data. To discover variance within the sample groups, a post hoc "Fisher" test was used. The statistical analysis was carried out at a significance level of 5% ($p<0.05\%$).

Chapter 4 Result

4.1 Composition of Betel leaf

This table shows the chemical constituent of betel leaf.

Table 1 Composition of betel leaf :

Component	Values (%)
Moisture	84.465
Protein (P)	3.288
Fiber (F)	2.212
Fat	0.4563
Antioxidant capacity	35.544

4.2 Physicochemical Characteristics of Dahi

When it comes to dahi, the pH of the jam is crucial. In table (4.1) Sample C had the lowest pH (4.319±0.0151) while sample A had the highest (4.3967±0.00153).There was no significance different in sample B and D in pH. For vitamin C sample A had no vitamin C. Sample D contained highest vitamin C while sample B contained lowest vitamin C. Sample C had the highest acidity value (0.704±0.0121%), while sample A had the lowest (0.633±0.00577%).

Table 2 Physicochemical Properties

Sample	pH	Vit C	Total acidity
A	$4.3967\pm.00153^{a}$	Not found	0.6333 ± 0.00577^{c}
B	4.2200 ± 0.01609^{b}	1.0500 ± 0.0500^{c}	0.6900 ± 0.0100^{b}
C	4.3190 ± 0.01510^{c}	1.4100 ± 0.0854^{b}	0.70400 ± 0.01217^{a}
D	4.3717 ± 0.0254^{b}	1.9500 ± 0.0500^{a}	0.7100 ± 0.0100^{a}

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

Sample A: Dahi (control)

Sample B: dahi with 2% betel leaf extract

Sample C : Dahi with 5% betel Leaf Ectract

Sample D: Dahi with 7% betel leaf extract

4.3 Nutritional Composition

Table (4.3) showed the nutritional composition of dahi. Almost every sample differs significantly. Dahi cantains highest amount of moisture. Sample A had maximum moisture and sample C had least amount of moisture. But in fat measurement Sample A had lowest value and sample D had highest value. Highest amount of crude fiber, ash and crude protein found in sample B.

Table 3 Nutritional Composition

Sample	**Moisture**	**Fat**	**Crude Fibre**	**Ash**	**Protein**
A	81.560±0.529[a]	21.4467±0.0503[d]	25.0833±0764[c]	4.5133±0.0808[c]	19.5500±0.055[c]
B	81.1933±0.0252[b]	26.4833±0.0764[c]	27.88±0.0721[a]	6.2500±0.0265[a]	26.2467±0.0252[a]
C	80.0833±0.0764[d]	27.1567±0.0603[b]	26.800±0.0265[b]	6.0833±0.0764[b]	25.15±0.1323[b]
D	80.75±0.1323[c]	27.90±0.0391[a]	27.4670±0.451[a]	6.2233±0.0929[a]	26.267±0.0306[a]

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

4.4Antioxidant Capacity

In table (4.4) Antioxidant capacity was found to be substantially higher in sample A (56.7464±0.036 mg TE/100 g) and significantly lower in sample B (10.2810±0.001 mg TE/100 g).

Table 4 Antioxidant Capacity

Antioxidant capacity	mg/100g
A	10.746 ± 0.0306^{d}
B	28.2810 ± 0.0010^{c}
C	29.2767 ± 0.0015^{b}
D	$30.6967\pm.1376^{a}$

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

4.5 Microbial analysis

The total number of live cells (microorganisms) present in the prepared dahi was determined by TVC of a yogurt sample. Due to the inclusion of jackfruit juice, which may increase the amount of acidity thus plain yogurt's TVC was greater in type A than the other (B, C, and D) samples of dahi (A type dahi). As a result, it was thought that any kind of dahi made from jackfruit juice was safe for ingestion.

The hazardous bacteria known as coliform is often found in nature and may result severe diarrhea. In every kind of prepared yogurt sample, TCC was zero. As advised by Bangladesh Standards Testing Institute, the final goods should contain coliform fewer than 10cfu/ml (BSTI). However, in this investigation, the coliform count of every kind of prepared dahi sample remained zero, as shown in Table 3. Four prepared yogurt samples (A, B, C, and D) were deemed to be safe for ingestion as a result.

Table 5 Microbiological Evaluation

Sample	TVC/g($\times10^{4}$)	Coliform/g
A	67.17±1.25	0
B	55.24±0.65	0
C	63.45±0.68	0
D	65.09±0.25	0

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

4.6 Sensory analysis

Sample B achieved the greatest acceptance rate across all metrics. Sample D, on the other hand, received the least amount of acceptance when compared to the other samples.

Table 6 Hedonic Rating Test for Sensory

Sample	Color	Taste	Smell	Consistency	Overall acceptability
A	4.600 ± 0.516^{a}	4.400 ± 0.516^{a}	4.500 ± 0.707^{a}	4.700 ± 0.483^{a}	4.600 ± 0.516^{a}
B	4.400 ± 0.516^{a}	3.900 ± 0.568^{b}	4.100 ± 0.738^{a}	4.300 ± 0.483^{a}	3.700 ± 0.483^{b}
C	4.300 ± 0.675^{a}	3.300 ± 0.483^{c}	3.800 ± 0.919^{a}	3.500 ± 0.527^{b}	3.200 ± 0.789^{b}

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

4.7 Cost analysis:

Heads	Tk./Kg	Quantity Used(gm/kg)	Total Tk (sample product)	Total Tk(control)
1)Expenditure Raw materials	80	20	50	
Liquid milk	70	1 ½	105	105
culture	95	2%	2	2
Sub total			157	107
Processing cost @ 15% of raw material			23.55	16.07
Bottling cost(10 cups)			40	40
Total			220.55	163.07

In the table control sample having without leaf extract. But formulation product contains betel leaf extract.

By following this recipe, we can prepared 1 kg dahi. So, price of per cup dahi is:

Formulated dahi= 220.55/10 tk

= 22.06 tk

For control= 163.07/10tk

=16.31tk

Chapter 5: Discussion

5.1 Physiological properties

For dahi the pH is crucial. Low pH in food also inhibits microbial development. Samples A had higher pH value of 4.3967±.00153, whereas Sample C had the lowest value of 4.3190±0.01510 in this investigation. Addition of fruit slightly decreased the pH value of Dahi. It is well known that when pH value decreases then acidity increases. In this experiment acidity of betel leaf juice fortified Dahi increased which might be due to decreased pH values. The result of present findings agreed with the work of Islam *et al*. who found that pH of plain Dahi was 4.25. Kosikowski27 also reported that the pH of normal Dahi samples should be approximately.

All of the values are in the range of 4.2-4.39; Sivakumar G. M. and Dhanalakshmi, B. also found comparable results (2015). Almost all of the samples in this investigation exhibited no significant change in total titratable acidity content of dahi. The variations in acidity % between treatments were substantial, according to statistical analysis. The addition of leaf extract enhanced the acidity. The acidity of Dahi samples agrees with the findings of Desai *et al*., who discovered that adding fruit juice/pulp considerably boosted the titratable acidity of fruit Dahi. Mustafa made Dahi with several types of seasonal juices and discovered that adding fruit juice to Dahi boosted the acidity content of the dish.

The addition of betel leaf extract to milk in the preparation of dahi offers essential nutrients such as protein, fat, fiber, and ash. Table 2 shows the approximate composition of three varieties of dahi. Highest moisture found in sample A while lowest value was in sample C. Moisture is a significant component that influences product shelf life and freshness. Foods with a high moisture content have a shorter shelf life. Dah with betel leaf has a moisture level of 78 to 85 percent, according to Ashaye and Adeleke (2009). The moisture content of dahi may vary depending on how it is stored (Broomes and Badrie, 2010). Sample D had high amout of fat while sample A had low amount of fat. The fat contents of A, C, B, and D type Dahi differed significantly, according to statistical research. The findings backed with the findings of Desai *et al*., who discovered that fruit yogurt had less fat than plain yogurt. Mustafa also came up with similar results. Cliff *et al*. evaluated the fat percent of plain sweet Dahi, and the fat percent of plain Dahi in our experiment is virtually identical to their findings. In C and D, the protein content was higher, but in A, it was

lower. The protein composition of the A, C, B, and D Dahi samples differed significantly, according to statistical analysis. The findings corroborated those of Islam *et al*., who discovered that betel Dahi had a greater protein content than ordinary Dahi. Islam *et al*. likewise came up with a similar finding. This study showed that sample B contained highest crude fiber (27.88±0.072) and ash (6.2500±0.026). The ash content of the A, B, C, and D Dahi samples differed significantly ($p<0.05$) according to statistical analysis. This study's findings are consistent with those of Mustafa and Desai *et al*. Both researchers discovered that adding extract to Dahi enhanced the ash percentage. For life to exist, ascorbic acid is required. Highest vitamin C found in sample D while plain dahi did not contained any vitamin C.

5.2 Antioxidant

To boost the antioxidant activity of dairy products, several extracts can be used (Premalatha *et al*., 2016). The findings indicate that betel leaf extract is an effective ingredient for boosting the antioxidant activity of the dahi. DPPH was used to determine the antioxidant capacity of samples. After testing, the results showed that betel leaf extract increased the antioxidant capacity. Table 3 shows that there is a substantial variation in antioxidant capacity among all samples. DPPH was a widely used substrate for determining antioxidant activity, particularly in the study of biological and chemical compounds' free radical scavenging abilities. The lowest antioxidant activity was found in a control sample of 10.746±0.0306 mg/100g, whereas it rose in the other samples when the amount of sample extract was increased. The bioactive components in the enhanced dahi with antioxidant characteristics absorb radicals, resulting in strong antioxidant activity. Highest value contained sample D 30.6967±.137. Sigh *et al*.,2016, found similar results for the loss of antioxidant activity in traditional dahi milk enhanced with strawberry extract. Dahi milk is a type of yogurt or fermented milk made from buffalo or goat milk that originated in India.

5.3 Microbial

A, B, C, D varieties of dahi had total viable counts of $67.17\pm1.25\times10^4$, $55.24\pm0.65\times10^4$, $63.45\pm0.68\times10^4$, $65.09\pm0.25\times10^4$ per g, respectively Table(4). The statistical analysis revealed that there were substantial differences between the samples. This data

demonstrates that adding apple juice to the mix increases the total viable count. The results of this study correspond with those of Rahman (1998), who discovered that the average total viable count of flavoured dahi drinks was 120.22(2.51) 104/g. The current findings are also consistent with those of Nahar *et al*. (2007), who discovered that the average total viable count per gram of yoghurt sample was 75×10^4.

Coliform bacteria were not detected in any of the dahi samples. The presence of coliform bacteria implies unsanitary dahi preparation circumstances. There was no coliform count in this investigation, which might be attributable to the stringent hygienic conditions utilized during milk collection and processing, as well as the use of a good bacterial starter culture.

5.4 Sensory analysis

Dahi samples containing 2%, 5%, and 7% betel leaf extract scored 4.400±0.516, 3.900±0.56, 3.300±0.483 respectively, in terms of taste . Statistical analysis revealed that taste scores of different types of dahi differed significantly. On the other hand, dahi with 7% fat content had the highest lowest taste score. The addition of 7% extract reduces the taste of dahi, according to the results of this investigation. The results of this trial contradict Mustafa's (1997) findings, which claimed that adding fruit juice to dahi enhanced the fragrance and flavor. White also found similar findings (1991). The consistency scores of dahi samples containing 2%, 5% and 7% apple juice were 4.700±0.48, 4.300±0.48 and 3.500±0.527 respectively. Table (4.5)summarizes the findings. The body and consistency score of different varieties of dahi samples differed significantly, according to statistical analysis. In the instance of dahi containing 2% and 5% extract, the highest body and consistency score was achieved. This experiment's findings are consistent with those of Desai *et al*. (1994) and Mustafa *et al* (1997). Statistical study revealed that the color and texture scores of several types of dahi samples differed significantly. The dahi with 7 percent apple juice received the highest color and texture score. This investigation backs up the findings of Desai *et al*. (1994), who found that adding fruit juice to dahi increased the color and texture score. Shukla *et al*. (1987) proposed for the use of stabilizers and chemicals in yogurt to improve the textural features. Similar findings were reported by Rahman (1998), who discovered that adding fruit juice to dahi increased the color and texture score. The caliber of the raw ingredients utilized in the production of

prepared yogurt also had an impact on flavor. Table 4 and Figure 1 both include the physical ratings for the general flavor of several kinds of prepared dahi. The prepared dahi samples A, B and C had an average overall taste rating of 4.600±0.516,3.700±0.483, and 3.200±0.789 respectively. Hedonic analysis revealed that prepared dahi samples A was superior than samples B and C. However, sensory study found that dahi with up to 2% betel leaf juice was acceptable. The panelists did not approve the extract dahi at its greatest concentration (7percent or Csample) based on any sensory rating. Due to its greater acidity, C sample had a somewhat bitter flavor. Finally, the research demonstrated that panelists preferred and accepted samples A (2 percent), and B (5 percent).

Chapter 6: Conclusion

On the basis of organoleptic, chemical, and microbiological analyses, the research was carried out to compare various levels (2%, 5%, and 7%) of betel leaf extract dahi to plain plain (0% betel leaf extract). The experiment demonstrates that dahi may be made from milk by adding betel leaf extract, which has a distinct flavor and gives consumers additional alternatives. Additionally, it has been shown that integration up to 7% is pretty excellent. The microbial load rises with the addition of additional extract. Without taking into account microbial health, general consumers often pick fruit dahi with superior physical and chemical properties. Betel leaf extract added to the dahi may boost the microbial burden. From the perspective of public health, controlling the microbiological condition of dahi made with betel juice may aid in reducing the microbial count. Commercially speaking, making dahi with milk will lower production costs. Therefore, adding betel juice to the process of making dahi from skim milk may be welcomed by both producers and customer. In comparison to 7 percent betel juice yogurt, 2 percent and 5 percent of the extract containing dahi had improved look, color, taste, and texture on a five-point hedonic scale of organoleptic features. Additionally, microbial quality All items are made safe by guidelines, and in the case of TVC, CC were within permissible limits. Therefore, all of the dahi samples that had been produced could be eaten. The antioxidant activity was also rising with the addition of betel leaf extract. Regular consumption of acceptable standards When betel leaf extract is added to dahi, it will satisfy our nutritional needs and serve as a healthy source of additional carbohydrates. When compared to ordinary dahi, it is very digestible.

Chapter 7: Recommendations and Future Perspectives

People are becoming increasingly interested in plant-based treatments and alternative dietary sources. In Bangladesh, these fruits naturally grow everywhere. Given the nutritional aspects, this may be an inexpensive source of nutrients for individuals living in rural areas of our nation. We've achieved some encouraging outcomes in the domain of betel leaf-based dahi production. As a consequence, it also has a higher commercial value and better marketability. The current food sector may employ the methods from medium and large scale manufacturing. On the basis of the recent inquiry, the following recommendations and possibilities for further study are made**.**

1. The current research might be expanded upon to corroborate the experimental results.
2. Additional compositional alterations might occur.
3. In future research, flavor could be introduced.
4. Because betel leaf and betel nut are a very common food combination among the locals, the influence of the nutritional content of betel leaf when combined with them may be tasted.
5. It could be advantageous for individuals who are economically disadvantaged.
6. The discoveries will be helpful from a therapeutic perspective since they have medicinal potential.
7. The high sample size allowed for statistical comparisons between the analytical findings. Our conclusion should be considered with caution due to the limited number of analyzed samples, and the results should be confirmed in a larger research.

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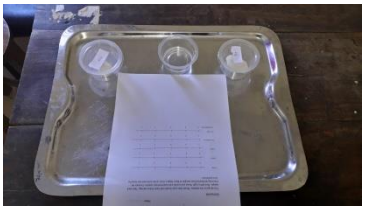
Appendices

Product

Fat & crude fiber detemination



Sensory evaluation

Antioxidant measurement

pH and Acidity measurement

Brief Biography

Anamika Mazumder passed the Secondary School Certificate Examination in 2011 from Zorargonj Buddho High School, Chattogram, and then Higher Secondary Certificate Examination in 2013 from Chattogram Govt Girls College, Chattogram. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness among people about food safety and quality.