DEVELOPMENT OF SUGAR-FREE CAKES USING DRY DATE POWDER AND FORTIFYING WITH CALCIUM FROM EGGSHELL POWDER

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Roll No: 0222/21

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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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Chattogram-4225, Bangladesh

December, 2024

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List of Abbreviations

Abbreviation	Full Form
DDP	Dry Date Powder
ESP	Eggshell Powder
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
kcal	Kilocalories
mg	Milligrams
g	Grams
%	Percentage
BSF	Bakery Samples Formulation
SNR	Sensory Rating
WAI	Water Absorption Index
FAO	Food and Agriculture Organization
WHO	World Health Organization
USD	United States Dollar
CI	Confidence Interval
SD	Standard Deviation
HPLC	High-Performance Liquid Chromatography
RDA	Recommended Daily Allowance
IC50	Half maximal inhibitory concentration (for antioxidant testing)

Abstract

This study aimed to develop sugar-free cakes by replacing refined sugar with dry date powder (DDP) and fortifying them with calcium from eggshell powder (ESP), addressing dietary needs for diabetic individuals while promoting waste valorization. Four cake formulations were prepared: a control (Sample A), and samples with 25g, 50g, and 75g of DDP combined with 1%, 2%, and 3% ESP respectively (Samples B, C, D). Proximate analysis revealed significant improvements: protein increased from 4.03±0.01% in the control to 7.62±0.12% in Sample C, fiber rose from 0.92±0.01% to 2.42±0.09%, and ash from 0.72±0.01% to 1.92±0.00%. Meanwhile, carbohydrate content declined from 46.37±0.03% to 42.83±0.21%, aligning with the nutritional goal of reducing simple sugars. Mineral content improved markedly, with calcium content rising from 24.01 mg/100g in sample A to 508.35 mg/100g in Sample D. Bioactive properties were enhanced, as total flavonoid content (TFC) increased from 42.42 to 87.85 mg/100g, and total phenolic content (TPC) rose from 0.63 to 2.21 mg/100g, leading to improved antioxidant capacity (1.45 mg/100g in Sample D). Sensory evaluation showed Sample C had the highest overall acceptability (6.31±0.01), confirming that nutritional enrichment did not compromise taste. The study confirms that DDP and ESP can serve as sustainable functional ingredients in bakery products, improving nutritional and bioactive profiles while maintaining consumer preference.

Keywords: Dry date powder, eggshell powder, sugar-free cakes, calcium fortification, antioxidant capacity, sustainable food systems.

Chapter I: Introduction

1.1 Background

The intersection of food innovation, health-conscious consumer demand, and sustainability presents a compelling frontier in the food sciences. Food innovation plays a pivotal role in addressing some of the most pressing challenges in modern food systems, particularly with the growing concern over food waste and its environmental impact. One of the critical areas of food innovation today is finding ways to repurpose food waste or by-products into valuable ingredients that can be used to create new, sustainable products. This approach not only helps reduce the environmental footprint of food production but also enhances the nutritional profile of the final product.

For example, by utilizing food by-products such as fruit peels, seeds, or in this case, eggshells, we can develop new food ingredients with functional benefits. Date powder, often a by-product of date processing, is rich in natural sugars, fiber, and antioxidants, making it an ideal candidate for substituting refined sugars in food products. Similarly, eggshells, often discarded as waste in the food industry, can be processed into a fine powder rich in calcium, offering a sustainable and cost-effective way to fortify food products with essential minerals.

Innovations like this address both health-conscious consumer demands—by creating products that are lower in refined sugars and higher in nutritional value—and sustainability goals, by reducing food waste and utilizing underutilized ingredients. By transforming food waste into a resource, food innovation can play a crucial role in reducing overall waste, conserving natural resources, and contributing to a more circular food economy.

This process of upcycling food waste into value-added products not only provides environmental benefits but also supports the development of healthier, more sustainable food options, which is exactly what your research with dry date powder and eggshell calcium is aiming to achieve.

This research focuses on developing a sugar-free cake using dry date powder as a natural sugar substitute and fortifying it with calcium derived from eggshell powder, thereby offering a novel solution to meet both nutritional and sustainability goals. Addressing the needs of diabetic individuals, who face a lifelong struggle to manage blood sugar levels, alongside reducing waste through the valorization of by-products, aligns with global

health priorities and environmental objectives (Howe, 2009). Diabetes remains a formidable public health challenge worldwide, with a growing burden in lower-middle-income countries like Bangladesh. In 2021, Bangladesh had 8.4 million adults diagnosed with diabetes, projected to nearly double by 2045 (Rana et al., 2024). Globally, diabetes affects approximately 537 million adults, a number expected to surge to 783 million by 2045 (International Diabetes Federation, 2021). The dietary management of diabetes is largely centered on reducing simple sugar intake, necessitating the creation of viable alternatives for traditionally sugar-rich foods. This is particularly pertinent in a country where baked goods are increasingly becoming a staple, especially in urban areas. Alongside this, calcium deficiency is a significant public health concern, exacerbated by limited consumption of fortified foods and dairy products (Chechlacz et al., 2009).

Dry date powder offers a viable solution as a sugar replacement in baked goods. Derived from dehydrated dates, this ingredient is rich in natural sugars, primarily fructose, which has a lower glycemic index than sucrose, as well as dietary fiber and bioactive compounds like phenolics and flavonoids (Ghnimi et al., 2017). Studies demonstrate that substituting refined sugar with date powder not only reduces glycemic load but also improves the antioxidant profile of the final product, making it a functional food with added health benefits (Besbes et al., 2009). The incorporation of date powder into sponge cakes found no significant compromise in texture or flavor, even at complete (100%) replacement of refined sugar (Al-Khalili et al., 2023).

Calcium deficiency is a widespread issue in Bangladesh, affecting diverse demographic groups, including children, women of reproductive age, and the elderly. This micronutrient is critical for bone health, muscle function, and metabolic processes, yet many diets in developing countries lack sufficient calcium due to limited access to dairy products and fortified foods. Eggshell powder, with calcium carbonate as its primary component, represents a cost-effective, sustainable fortificant (Ahmed et al., 2021).

Each gram of chicken eggshell powder contains approximately 40% calcium, translating to about 401–432 mg of elemental calcium (Waheed et al., 2019). Notably, calcium from eggshells has been found to have high bioavailability, comparable to commercial calcium supplements. Incorporating eggshell powder into food products has shown promising results in addressing dietary calcium deficiencies. The addition of eggshell powder, which is rich in calcium (approximately 35.09 g/100 g), resulted in a substantial increase in the calcium levels of various baked products (Afzal, 2020). For instance, muffins fortified with 8% eggshell powder achieved a calcium content of approximately 2462.7 mg/100 g (Afzal,

2020). These findings underscore its potential as a fortificant in nutrient-enriched baked goods, particularly for populations with restricted access to conventional supplements.

The valorization of food waste into value-added products is a critical aspect of sustainable development (Sayas-Barbera et al., 2022). Municipalities and urban centers generate 3.78 million tons of waste each year (15.96% of total food waste). This study utilized the national database to analyze food waste generation and projected growth by the year 2050 (United Nations Environment Programme, 2021). Eggshells, a by-product of the poultry and food processing industries, are a prime example of underutilized waste. Globally, 8 million tons of eggshells are discarded annually, primarily in landfills, contributing to environmental pollution and resource wastage (Babalola and Wilson, 2024).

Repurposing eggshells into calcium-rich fortificants for food products addresses these waste management issues while providing a sustainable solution to malnutrition. Similarly, date powder production, which utilizes low-grade or surplus dates, reduces agricultural waste while supporting economic activities in rural areas. By integrating these by-products into mainstream food innovation, this research aligns with the principles of a circular economy, where waste is minimized, and resources are maximized.

Consumer demand for functional foods is rising globally, with the market for sugar-free and fortified products projected to grow substantially in the coming decade (Gupta et al., 2023). In Bangladesh, awareness of health-centric diets is increasing, particularly among urban, middle-class populations who are more likely to seek diabetic-friendly and nutrient-enriched products. Functional foods that address specific health conditions, such as sugar-free cakes for diabetic individuals, are thus poised to gain traction in the local market. The sensory appeal of these products, including taste, texture, and appearance, plays a crucial role in their acceptance (Gupta et al., 2023). This suggests that consumer preferences need not be compromised when integrating health and sustainability considerations into food design.

In a country where diabetes and malnutrition coexist, this research holds dual relevance. Bangladesh's under-five stunting rate of 32% underscores a critical need for calcium-enriched diets (Chowdhury et al., 2021). The development of sugar-free cakes enriched with calcium from eggshell powder and dry date powder offers an integrated solution to pressing environmental and public health issues. This study illustrates how food innovation can support sustainable development by addressing the nutritional requirements of diabetics, preventing calcium insufficiency, and encouraging waste valorization. The

results have important ramifications for Bangladesh's food sector, since the incorporation of useful, waste-derived substances into consumer goods may revolutionize regional waste management and dietary customs.

1.2 Objectives of the Study

The study seeks to:

1. Develop a sugar-free cake by utilizing dry date powder as a natural sweetener, catering to health-conscious consumers.
2. Evaluate the sensory characteristics, nutritional value, and physicochemical attributes of the formulated cakes.
3. Encourage sustainable practices by incorporating agricultural and food processing by-products to reduce waste.

Chapter II: Review of Literature

2.1. Nutritional Composition of Dry Date Powder

Dates (*Phoenix dactylifera*) are a rich source of essential nutrients, providing a wide range of vitamins, minerals, and dietary fibers. Dates are particularly high in carbohydrates, with a significant portion consisting of simple sugars like glucose, fructose, and sucrose, which are easily digestible and provide a quick source of energy (Hossain, 2019). Dates are also notable for their high fiber content, especially soluble fibers such as pectin, which are known to have beneficial effects on digestion and blood sugar control. The dietary fiber in dates has been shown to reduce the glycemic index of foods, making date powder a suitable alternative for sugar reduction in diabetic-friendly products (Hossain, 2019).

In addition to fiber, dates are rich in micronutrients such as potassium, magnesium, iron, copper, and manganese, as well as a variety of antioxidants, including flavonoids and phenolic compounds. These antioxidants are associated with reducing oxidative stress and preventing chronic diseases such as cardiovascular disease and cancer (Ayad et al., 2020). Drying the dates to produce powder concentrates these nutrients, making it a valuable ingredient in bakery products, as it not only enhances the flavor but also improves the nutritional profile of cakes and other foods. Dry date powder offers a concentrated source of nutrients with varying compositions depending on processing and cultivar. A study by Hadi et al. (2023) shows the synthesis of its key nutritional attributes in Table **2.1**:

Table 2.1: Macronutrient Profile of Dry Dates

Nutrient	Per 100g (Typical Range)	Key Features
Calories	300–360 kcal	High energy density from natural sugars
Carbohydrates	70–93%	Primarily glucose, fructose, and polysaccharides
Fiber	6–20g	Includes insoluble fiber (cellulose, hemicellulose)
Protein	2–17g	Contains essential amino acids (glutamic acid, aspartic acid)
Fat	0.15–0.44%	Unsaturated fatty acids (oleic, linoleic)

Research has also shown that date powder has a low glycemic index (GI), which makes it an ideal ingredient for sugar-free products targeted at diabetic consumers (Alsarayrah et al., 2023). GI is a measure of how quickly foods raise blood sugar levels, and date powder, with its fiber and polyphenol content, helps in reducing the speed of sugar absorption into the bloodstream, leading to more stable blood glucose levels. This makes it a healthier alternative to refined sugars, which cause rapid spikes in blood sugar levels.

2.2. Functional Properties of Dry Date Powder in Food Products

The functional properties of dry date powder go beyond just its nutritional content. One of the key reasons for incorporating date powder into bakery products is its natural sweetness and ability to improve texture and moisture retention. Unlike refined sugar, which tends to dry out cakes, date powder helps in retaining moisture, resulting in softer, more moist cakes (Hossain, 2019). The natural sugars in date powder provide a mild sweetness without overwhelming the other flavors, making it an ideal ingredient for sugar-free formulations.

Date powder also plays a role in extending the shelf life of bakery products. The antioxidants in date powder, particularly phenolics, can help prevent oxidation and spoilage in cakes and other baked goods, which are typically prone to going stale. Studies have shown that the addition of date powder to cakes can improve their shelf life, maintaining both flavor and texture for longer periods compared to products without it (Alsarayrah et al., 2023).

Furthermore, date powder contributes to the dietary fiber content of cakes, which is essential for digestive health. Fiber has long been recognized for its role in improving bowel regularity, lowering cholesterol levels, and controlling blood sugar. The soluble fibers in date powder can also promote satiety, which is beneficial for weight management (Thamer et al., 2022). As dietary fiber intake continues to be lower than recommended levels in many populations, incorporating fiber-rich ingredients such as date powder can contribute to a healthier diet, particularly in sugar-free and low-calorie formulations.

2.3. Eggshell Powder as a Source of Calcium

While calcium carbonate is a well-known supplement used to improve bone health, eggshell powder offers an alternative, cost-effective, and sustainable source of calcium, which is often under-consumed in many diets (Waheed et al., 2019). The calcium found in eggshell powder is highly bioavailable, meaning it can be easily absorbed and utilized by the human body. This makes eggshell powder an effective means of fortifying foods to prevent calcium deficiencies, which are common in populations with limited access to dairy

products or fortified foods (Arnold et al., 2022). 1 gram of broiler chicken eggshell can satisfy 50% of an adult female's daily calcium needs, as it contains approximately 380 mg of calcium per gram (Oginni et al., 2018).

Eggshells contain approximately 40% calcium carbonate by weight, with each gram containing 380–401 mg of calcium (Oginni et al., 2018). Approximately half of an eggshell, weighing around 1 to 1.5 grams, has the potential to fulfill 50% to 100% of an adult's recommended daily calcium intake, which is about 1,000 mg. Studies have shown that calcium derived from eggshells may be more bioavailable than conventional purified calcium carbonate. This enhanced absorption is likely due to the presence of additional components in the eggshell, such as proteins and trace minerals like strontium and magnesium, which can facilitate calcium uptake (Oginni et al., 2018).

Table 2.2: Calcium requirements by age and reproductive status

Category	**Life Stage**	**Calcium RI (mg)**
Infant & Children	0–6 months	300
	7–12 months	450
	1–3 years	500
	4–6 years	550
	7–9 years	700
Males	10–18 years or above	1,000
Females	19 years to menopause	750
	Pregnant	800
	Lactating	750

[Note: The recommended intake (RI) of "WHO/FAO theoretical calcium allowance for animal protein intake 20–40 g" (World Health Organization, 2003) is approximated by 1 gram of chicken eggshell]

Source: (World Health Organization, 2004)

Calcium is essential for the development and maintenance of bones and teeth, and its deficiency can lead to various health issues, such as osteoporosis, especially in vulnerable groups like children, pregnant women, and the elderly (Zhu and Prince, 2012). Given that eggshells are primarily discarded as waste, converting them into a functional ingredient for food fortification can be an effective strategy for reducing food waste while simultaneously

addressing public health concerns related to calcium intake. The use of eggshell powder in food products is especially significant in regions where calcium-rich foods are scarce or too expensive for large segments of the population.

In addition to calcium, eggshell powder contains other trace elements such as magnesium, phosphorus, and zinc, which further enhance its nutritional profile and contribute to bone health and metabolic function (Zhu and Prince, 2012). Research has demonstrated that eggshell powder can be used effectively to fortify foods like bread, cakes, and biscuits, helping to address calcium deficiencies while improving the overall nutritional value of these products.

2.4. Benefits of Fortifying Foods with Eggshell Powder

Fortifying foods with eggshell powder presents several advantages, particularly in addressing calcium deficiencies and improving bone health. The high bioavailability of calcium from eggshell powder ensures that it is absorbed efficiently by the body, and studies have shown that it can be just as effective as other calcium sources, such as dairy or synthetic calcium supplements (Shahnila et al., 2022). In a study conducted by Shahnila et al. (2022), it was found that eggshell powder can significantly increase the calcium content in fortified foods, improving bone density in both young and elderly individuals.

The potential for eggshell powder to be incorporated into sugar-free cakes is particularly beneficial for individuals with diabetes or lactose intolerance, as they may have difficulty obtaining sufficient calcium from traditional sources like milk. The addition of eggshell powder not only fortifies these cakes with calcium but also provides additional trace minerals that are beneficial for overall health. Moreover, the powder is fine and odorless, which makes it easy to incorporate into various bakery products without affecting their flavor or texture (Shahnila et al., 2022).

In addition to bone health, calcium is essential for several physiological processes, including nerve function, muscle contraction, and blood pressure regulation. Diabetic individuals, who are at an increased risk for osteoporosis and hypertension, may particularly benefit from the inclusion of eggshell powder in their diets, as it can help mitigate these risks (Arnold et al., 2021).

2.5. Health Benefits of Dry Date and Eggshell Powder

Both dry date powder and eggshell powder offer significant health benefits for diabetic individuals. Dates, with their low glycemic index and high fiber content, can help regulate blood sugar levels and prevent the rapid spikes typically associated with refined sugar

consumption. The fiber in date powder slows down the absorption of glucose, leading to a more gradual increase in blood sugar levels, which is crucial for individuals managing diabetes (Alsarayrah et al., 2023).

The combination of dry date powder and eggshell powder in sugar-free cakes offers a comprehensive health solution, addressing both blood sugar control and bone health. Diabetes is often associated with increased risks of osteoporosis, as high blood sugar can lead to decreased bone density over time. By fortifying sugar-free cakes with both date powder and eggshell powder, it is possible to create a product that supports healthy bones while also helping to regulate blood sugar levels, providing a functional and nutritious option for diabetic patients (Arnold et al., 2021).

Furthermore, the antioxidant properties of date powder may help reduce oxidative stress, which is a contributing factor to the development of chronic complications in diabetic individuals, including cardiovascular disease and neuropathy (Arnold et al., 2022). The combination of antioxidants and essential minerals in date powder and eggshell powder creates a synergistic effect that can offer multiple health benefits to diabetic consumers.

2.6. Pharmacological Activity

Pharmacological activity refers to the effects that a substance or compound has on the body when administered. These effects can be beneficial, neutral, or harmful, and they are often studied in relation to the treatment of diseases or conditions. The pharmacological properties of various natural compounds, such as those found in date powder and eggshell powder, can include their antioxidant, antimicrobial, anti-inflammatory, analgesic, and other therapeutic activities. These natural products can provide a range of health benefits and are often used in alternative medicine or as ingredients in functional foods. Natural products, especially those derived from plants and minerals, have gained attention in pharmacology due to their diverse pharmacological activities. For instance, dates have been shown to possess antioxidants, anti-inflammatory, antimicrobial, and immunomodulatory properties, making them useful in treating or preventing chronic diseases like diabetes, cardiovascular disease, and cancer (Alsarayrah et al., 2023).

The research field is highly interested in the discovery of effective anti-tumor agents due to the numerous adverse effects of current cancer treatments, including chemotherapy and radiotherapy. Anti-tumor activities have been associated with the functional properties of fruits' phytochemicals, including flavonoids and other phenolic compounds. The bioactive compounds rutin and quercetin, which are derived from Ajwa dates, have been shown to

have a strong anti-tumor potential. For example, the bioactive compounds were orally administered to breast tumor mutagenic mice at a dosage of 5 mg kg−1 BW for 11 days in a synergistic manner with an injection of doxorubicin, a chemotherapeutic agent that is frequently used against breast cancer and is known to cause cardiotoxic effects (Barakat and Alfheeaid, 2023). The doxorubicin injection's alleviation of weight loss and a substantial decrease in the levels of plasma cardiac troponin-I, a protein biomarker used to diagnose heart attacks, were observed by Dasgupta and Wahed (2014). They discussed cardiac markers in clinical chemistry as favorable effects of this intervention.

Similarly, eggshell powder, rich in calcium and other trace minerals, has demonstrated beneficial effects on bone health and may be utilized to combat conditions like osteoporosis (Zhu et al., 2012).

2.6.1. Types of Antioxidants

Antioxidants are compounds that protect the body from oxidative stress by neutralizing free radicals—unstable molecules that can damage cells and tissues, contributing to aging and disease. There are two main types of antioxidants:

- **Enzymatic antioxidants**: These include enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which are produced by the body and work to neutralize free radicals and reactive oxygen species (ROS) in cells (Sadak et al., 2023).
- **Non-enzymatic antioxidants**: These are molecules obtained from food or supplements that act as scavengers of free radicals. They include vitamins like Vitamin C and E, carotenoids like beta-carotene, polyphenols found in plants, and flavonoids. These antioxidants are crucial in preventing cellular damage caused by oxidative stress (Sarhat et al., 2023).

2.6.2. Functions of Antioxidants

Antioxidants play several key roles in the human body, protecting cells from oxidative damage and supporting various physiological processes. Some of the major functions of antioxidants include:

- **Neutralizing Free Radicals**: The primary function of antioxidants is to neutralize free radicals and prevent cellular damage, which could otherwise lead to chronic diseases like cancer, heart disease, and neurodegenerative disorders (Kattappagari et al., 2015).

- **Preventing DNA Damage**: By reducing oxidative stress, antioxidants help prevent damage to DNA, which is a precursor to genetic mutations and cancer (Singh et al., 2017).
- **Supporting Immune Health**: Antioxidants like Vitamin C and E help strengthen the immune system by supporting the function of immune cells, such as T-cells and macrophages, which are essential for fighting infections (Kattappagari et al., 2015).
- **Reducing Inflammation**: Many antioxidants, particularly flavonoids and carotenoids, exhibit anti-inflammatory properties, which can help in reducing chronic inflammation associated with diseases like arthritis, diabetes, and cardiovascular conditions.

2.6.3. Antioxidant Activity of Dry Date Powder and Eggshell Powder

Antioxidant activity refers to the ability of a compound to neutralize free radicals, which can cause oxidative damage to cells and tissues. The antioxidant activity of natural ingredients like dry date powder and eggshell powder is of particular interest in the formulation of health-promoting, sugar-free cakes.

- **Dry Date Powder**: Dates are rich in antioxidants, particularly flavonoids, carotenoids, and phenolic compounds, which have been shown to possess strong free radical scavenging abilities. Research has highlighted that the phenolic compounds in date fruit can reduce oxidative stress by neutralizing reactive oxygen species (ROS) (Sarhat et al., 2023). The drying process further concentrates these beneficial compounds, making dry date powder a potent antioxidant agent. Studies have indicated that the inclusion of date powder in cakes could help maintain their freshness and shelf life by preventing oxidative degradation.
- **Eggshell Powder**: While eggshell powder is primarily known for its calcium content, it has also demonstrated antioxidant properties. The minerals found in eggshell powder, such as magnesium and zinc, play a role in enhancing antioxidant activity within the body (Zhu and Prince, 2012). Moreover, calcium carbonate in eggshell powder has been shown to help prevent oxidative damage by supporting various enzymatic antioxidants in the body.

2.6.4. Sources of Antioxidants in Sugar-Free Cakes

Incorporating antioxidant-rich ingredients like dry date powder and eggshell powder into sugar-free cakes enhances both their nutritional value and health benefits. Date powder

serves as a natural sweetener and a source of antioxidants, while eggshell powder contributes to bone health through its calcium content and supports antioxidant enzyme activity.

- **Fruits and Vegetables**: To further enrich the antioxidant profile of sugar-free cakes, fruits like berries (e.g., blueberries, strawberries) and vegetables (e.g., spinach, kale) can be added, offering additional sources of flavonoids and carotenoids (AlFaris et al., 2021).
- **Nuts and Seeds**: Almonds, walnuts, and chia seeds provide healthy fats and polyphenolic compounds that complement the antioxidant properties of date and eggshell powders (Torabian et al., 2009).

2.6.5. Antioxidant Mechanism

The human body's antioxidant defense system involves both enzymatic and non-enzymatic antioxidants that work together to mitigate oxidative stress. Dry date powder and eggshell powder contribute to these defense mechanisms.

- **Endogenous Antioxidants**: The body produces antioxidants like glutathione and superoxide dismutase (SOD) to combat ROS. These antioxidants work synergistically with dietary antioxidants, such as those from date and eggshell powder, to neutralize oxidative damage (Adwas et al., 2019).
- **Supporting Enzyme Activity**: The minerals in eggshell powder, such as calcium, can support enzymatic antioxidants like glutathione peroxidase, which help break down harmful peroxides in the body (Adwas et al., 2019).

2.7. Antimicrobial Activity in Sugar-Free Cakes with Dry Date Powder and Eggshell Powder

Antimicrobial activity refers to the ability of a substance to inhibit or kill microorganisms such as bacteria, viruses, fungi, and parasites. Both dry date powder and eggshell powder have demonstrated antimicrobial properties, making them valuable additions to sugar-free cakes.

- **Dry Date Powder**: Dates contain phenolic compounds that have been shown to exhibit antimicrobial activity against various pathogens, including *Escherichia coli* and

Staphylococcus aureus. These compounds inhibit bacterial growth by disrupting the microbial cell membrane and interfering with cellular processes (Raya et al., 2023). By incorporating date powder, sugar-free cakes may help provide some level of protection against microbial contamination, particularly during storage.

- **Eggshell Powder**: Eggshell powder has also demonstrated antimicrobial activity, especially against certain fungi and bacteria. This is attributed to the calcium carbonate content, which can disrupt bacterial cell walls, inhibiting growth and reproduction (Raya et al., 2023). Moreover, the alkaline nature of eggshell powder can make cakes less hospitable to the growth of some microorganisms, adding a layer of protection.

2.8. Products Developed from Dry Date Powder and Eggshell Powder

The functional and nutritional attributes of dry date powder and eggshell powder have supported their growing application in a variety of food products, health supplements, and functional foods. These ingredients offer a natural and health-promoting alternative for consumers seeking nutrient-enriched options, especially those with specific dietary requirements or a focus on overall wellness.

Dry date powder, rich in natural sugars, dietary fiber, antioxidants, and essential minerals, serves as a wholesome alternative to refined sugar. It has been widely incorporated into baked goods, energy bars, breakfast cereals, and health drinks. In addition to enhancing taste and texture, it contributes to better digestive health and blood sugar regulation due to its high fiber content and low glycemic index.

Eggshell powder, on the other hand, is an excellent source of bioavailable calcium along with other trace minerals such as magnesium and strontium. It has been utilized to fortify baked products, dairy alternatives, and nutritional supplements. Its inclusion not only boosts calcium levels in food but also supports bone health and helps prevent calcium-deficiency-related disorders, making it particularly beneficial for children, the elderly, and individuals with increased calcium needs.

Together, these ingredients exemplify an innovative approach to food development that aligns with global priorities around health, sustainability, and food waste reduction. By converting nutrient-rich by-products into value-added components, dry date powder and eggshell powder contribute to a circular food economy while enhancing the nutritional quality of the final products.

2.8.1. Sugar-Free and Functional Cakes

One of the most popular products developed using dry date powder is sugar-free cakes. Dates, as a natural sweetener, provide not only sweetness but also a host of bioactive compounds such as phenolic compounds, flavonoids, and antioxidants. Dry date powder serves as an ideal substitute for refined sugars, making cakes healthier while retaining moisture and improving texture.

Table 2.3: Synergistic Benefits in Cakes

Ingredient	Key Contributions
Dry Date Powder	Natural sweetener: Replaces refined sugars with glucose/fructose.
	Fiber and antioxidants: Adds polyphenols and dietary fiber.
Eggshell Powder	Calcium: Provides ~380–401 mg calcium per gram, enhancing bone health.
	Bioavailability: Proteins and magnesium in eggshells improve calcium absorption.

The inclusion of eggshell powder in sugar-free cakes adds further value, as eggshell powder is a rich source of calcium, essential for bone health and other metabolic processes (Barakat and Alfheeaid, 2023). Such cakes are beneficial for individuals with diabetes, lactose intolerance, or those on calorie-controlled diets.

2.8.2. Energy Bars and Snack Bars

Dry date powder is commonly used in the production of energy bars and snack bars. These products often cater to consumers who are looking for healthy, convenient snacks that provide sustained energy. Dates, being high in natural sugars like glucose and fructose, offer a quick energy boost while providing fiber and essential minerals such as potassium and magnesium. When combined with eggshell powder, these products gain added nutritional value, particularly in terms of calcium, which is crucial for bone health and muscle function (Barakat and Alfheeaid, 2023). Energy bars with dry date powder and eggshell powder are also rich in antioxidants, supporting immune function and reducing oxidative stress.

2.8.3. Nutritional Supplements

The powdered forms of both dry date powder and eggshell powder have found applications in the production of nutritional supplements. Dry date powder is often used as a base for natural multivitamin formulations, while eggshell powder is increasingly used as a source of calcium in dietary supplements aimed at improving bone density and strength (Al-Khalili et al., 2023). These supplements are especially beneficial for individuals with osteoporosis, arthritis, or for the elderly, who are at higher risk of calcium deficiency. Additionally, the combination of these powders in supplements may have synergistic effects, promoting overall health through their antioxidant and anti-inflammatory properties.

2.8.4. Protein Powders and Smoothies

Another innovative product utilizing dry date powder is protein powder. Protein powders are popular in the fitness industry for their role in muscle repair and recovery. Combining dry date powder with plant-based or whey protein creates a natural, nutrient-dense protein powder. The natural sweetness of dates means these protein powders often require no added sugars, making them suitable for those following low-sugar or sugar-free diets. Adding eggshell powder to these protein powders provides an additional calcium boost, benefiting consumers who require extra calcium in their diet, such as post-menopausal women or individuals with bone health concerns (Barakat and Alfheeaid, 2023).

Smoothies made with dry date powder and eggshell powder have also become a health trend, providing a delicious and nutritious way to supplement one's diet (Day et al., 2022). These smoothies can be further enhanced with other bioactive ingredients like flaxseeds, chia seeds, and spinach, offering a complete nutritional profile for maintaining optimal health.

2.9. Important Bioactive Compounds in Dry Date Powder and Eggshell Powder

Dry date powder and eggshell powder are rich in several bioactive compounds that contribute to their therapeutic properties. These compounds are responsible for many of the health benefits of these ingredients, including antioxidants, anti-inflammatory, antimicrobial, and antidiabetic effects.

2.9.1. Flavonoids in Dry Date Powder

Flavonoids are a group of polyphenolic compounds that have a wide range of biological activities, including antioxidants, anti-inflammatory, and anticancer properties. Flavonoids are known to scavenge free radicals, reduce oxidative stress, and modulate signaling pathways involved in inflammation.

Dry date powder is an excellent source of flavonoids, including kaempferol, quercetin, and rutin, which are responsible for many of its health benefits. These flavonoids have been shown to exert protective effects on the cardiovascular system, improve circulation, and reduce blood pressure (Mistrello et al., 2014). Additionally, flavonoids can help regulate blood sugar levels by increasing insulin sensitivity, making them beneficial for diabetic individuals.

The antioxidant capacity of flavonoids in dates has been well-documented in several studies. For instance, quercetin, a potent flavonoid found in dates, has been shown to reduce the oxidative damage caused by free radicals, thus protecting cells from premature aging and degeneration (Mistrello et al., 2014). Furthermore, flavonoids play a role in improving gut health by promoting the growth of beneficial bacteria and reducing inflammation in the digestive tract.

2.9.2. Phenolic Compounds in Dry Date Powder

Phenolic compounds are another significant class of bioactive molecules found in dry date powder. These compounds, such as phenolic acids and tanins, are known for their powerful antioxidant properties (Hossain et al., 2014). The phenolic compounds in dates contribute to their high antioxidant capacity, which helps protect the body from oxidative damage, inflammation, and chronic diseases such as cancer, diabetes, and heart disease.

One of the most well-researched phenolic compounds in dates is gallic acid, which has shown strong antioxidant and anti-inflammatory activities. Gallic acid can help scavenge reactive oxygen species (ROS), protecting cells from oxidative (Soto-Hernandez et al., 2017). Other phenolic compounds, such as ferulic acid and caffeic acid, are also present in dates and have been shown to have antioxidant and anti-inflammatory effects, making them beneficial for individuals with inflammatory conditions (Hossain et al., 2014).

Phenolic compounds in eggshell powder contribute to its antioxidant activity as well. Eggshell powder contains trace amounts of polyphenolic compounds that are released during processing, which can contribute to oxidative stress reduction in the body. The presence of these compounds further enhances the health benefits of eggshell powder when used in dietary supplements and functional foods.

2.9.3. Anthocyanins in Dry Date Powder

Anthocyanins are a group of flavonoid pigments responsible for the red, purple, and blue colors of many fruits and vegetables. These compounds are well known for their potent antioxidant activity and have been widely studied for their health benefits, including their

ability to protect against cardiovascular diseases, cancer, and cognitive decline (Hemmateenejad et al., 2015).

While dates are not as rich in anthocyanins as other fruits such as berries, certain varieties of dates, especially those with darker skin, do contain measurable amounts of anthocyanins. The anthocyanins in dates, particularly in Medjool dates, exhibit strong antioxidant activity that can help reduce oxidative stress, promote heart health, and improve metabolic function (Jarrar et al., 2023).

In addition to their antioxidant properties, anthocyanins have been found to have anti-inflammatory, anticancer, and neuroprotective effects. Studies have shown that anthocyanins can help regulate blood sugar levels, reduce inflammation in the body, and improve cognitive function by protecting neurons from oxidative damage (Cao et al., 2018).

The presence of anthocyanins in dry date powder contributes to the health benefits of products like energy bars, smoothies, and cakes. Their antioxidant and anti-inflammatory effects complement the other bioactive compounds found in dates, making these products not only delicious but also highly nutritious.

2.10. Sustainability and Waste Minimization

The use of dry date powder and eggshell powder in food products not only enhances the nutritional value of the cakes but also contributes to waste minimization. Dates, which are often discarded when overripe, can be dried and processed into powder, thus reducing food waste and providing a valuable ingredient for sugar-free formulations. Similarly, eggshells, which are typically discarded by the food industry, can be converted into a functional ingredient for food fortification. This approach aligns with the principles of sustainability and circular economy, where waste products are transformed into valuable resources for food production (Ghosh et al., 2016).

In regions like Bangladesh, where food security is a significant concern, the development of such products can play a role in improving nutritional intake while also reducing environmental impact. By utilizing food byproducts such as dry date powder and eggshell powder, it is possible to create functional, nutrient-dense products that contribute to both public health and environmental sustainability.

Chapter III: Materials and Methods

3.1. Study Area and Sample Collection

The research was conducted over six months, starting on July 1, 2024, and concluding on November 1, 2024. The primary focus of the study was the analysis and development of products using dry dates (*Phoenix dactylifera)* and eggshell powder, targeting nutritional enhancement and functional properties.

3.1.1 Sample Collection and Preparation

Dry Date Samples:

Recently harvested and processed dry date samples were obtained from various local markets in the Chattogram district, Bangladesh. To ensure consistency, only fully mature, high-quality dry dates were selected for the study. These dates were visually inspected for uniformity in size, ripeness, and absence of physical damage or contamination. Once collected, the dates were transported to the Applied Human Nutrition Laboratory at the Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). The samples were then stored in air-tight plastic containers to preserve their quality and minimize exposure to external contaminants.

Eggshell Powder Formulation:

Eggshells, a byproduct of the food industry, were collected from local sources in Chattogram. The collected shells underwent an extensive cleaning process, which included washing with hot water to remove membranes and impurities, followed by drying at a controlled temperature. The dried shells were then finely ground into a uniform powder using a laboratory-grade mill. The formulated eggshell powder was stored in sealed containers and kept in a dry environment at the Applied Human Nutrition Laboratory to maintain its stability and avoid contamination.

3.1.2 Study Location

The experimental work and sample analysis were carried out at the Applied Human Nutrition Laboratory, located at the Faculty of Food Science and Technology, CVASU. The university, situated in the Khushi area of Chattogram, provided the necessary infrastructure and advanced facilities for sample preparation, chemical analysis, and

product development. This location was ideal due to its accessibility to local markets for sourcing raw materials and its specialized facilities designed for food science and nutrition research.

3.2. Experimental design

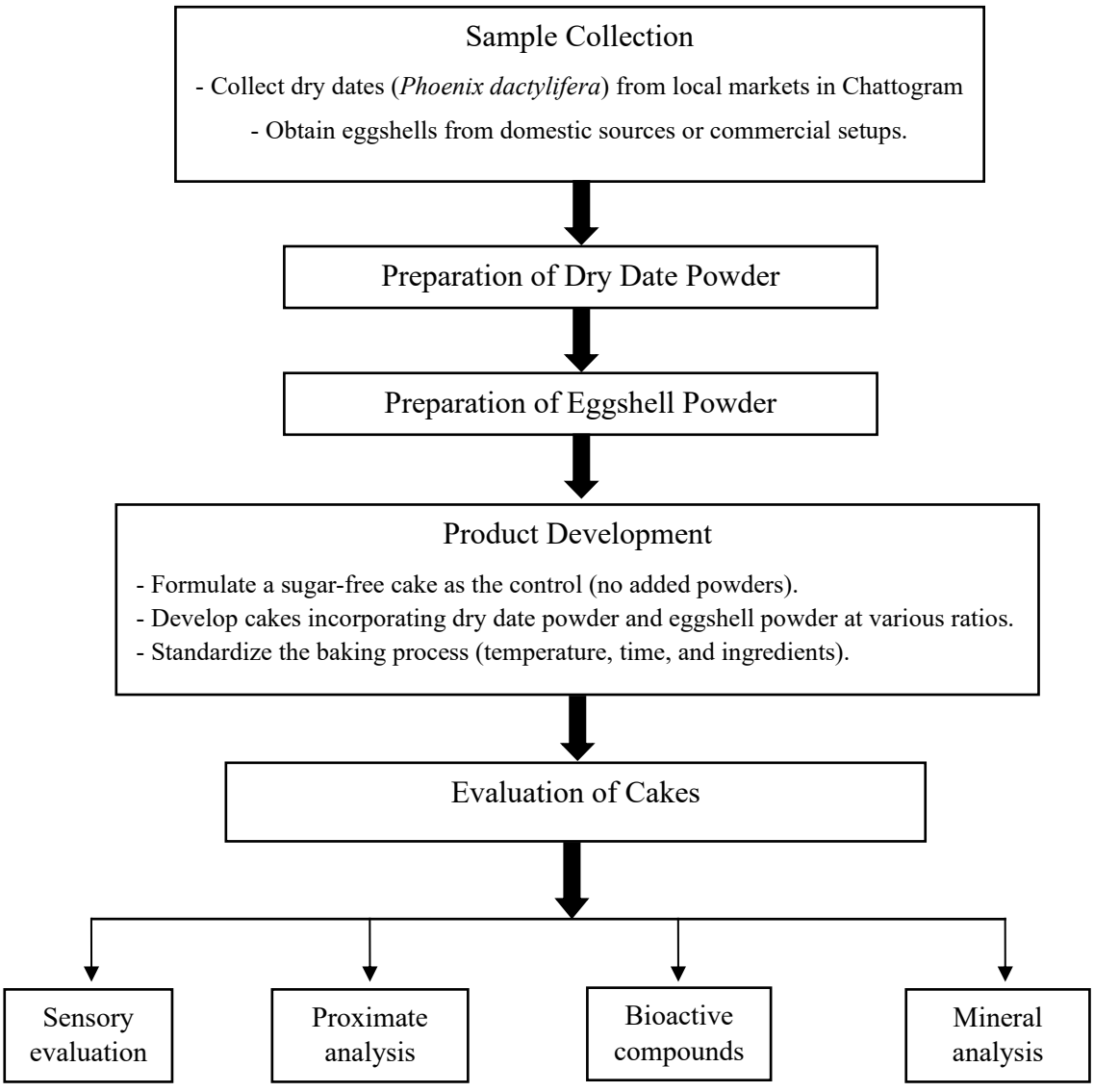

Figure 3.1: Experimental design

3.2. Dry Date Powder Preparation

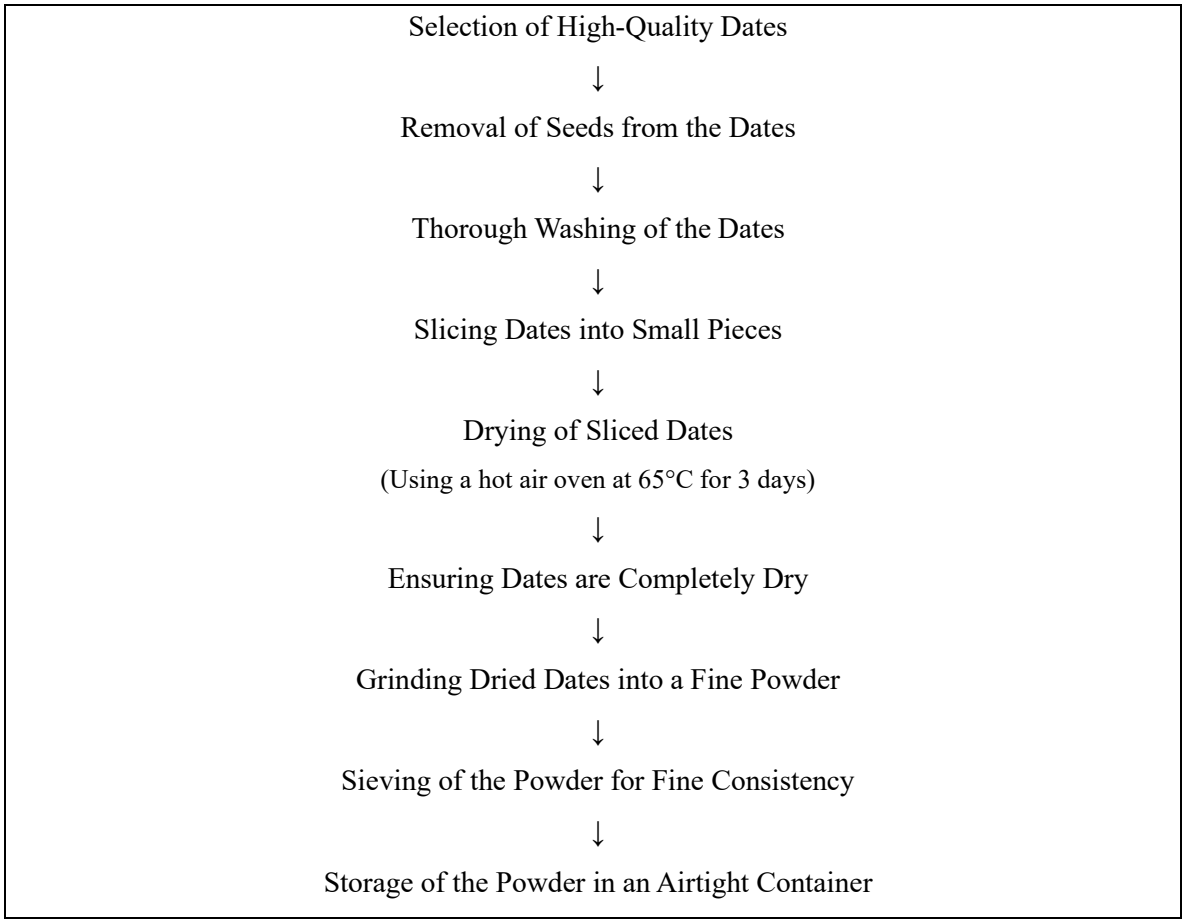

Figure 3.2: Dry Date Powder Preparation

3.3 Preparation of Eggshell Powder

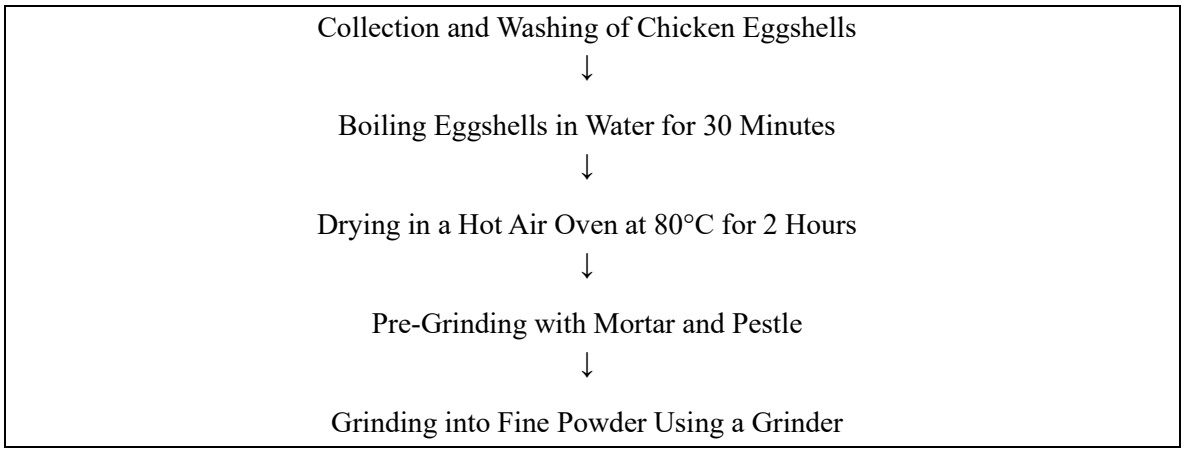

Figure 3.3: Preparation of Eggshell Powder

3.4. Formulation of cake

Ingredient	Sample A (0% ESP, 0g DDP)	Sample B (1% ESP, 25g DDP)	Sample C (2% ESP, 50g DDP)	Sample D (3% ESP, 75g DDP)
Wheat Flour (g)	100	99	98	97
Dry Date Powder (DDP) (g)	0	25	50	75
Eggshell Powder (ESP) (g)	0	1	2	3
Butter (g)	50	50	50	50
Egg (unit)	1	1	1	1
Baking Powder (g)	0.5	0.5	0.5	0.5
Salt (g)	0.5	0.5	0.5	0.5
Water (mL)	100	100	100	100
Sugar (g)	100	0	0	0
Flavor (mL)	2	2	2	2

ESP: Eggshell Powder; DDP: Dry Date Powder

Table 3.1: Preparation of Cake using formulation

Table 3.1 outlines the formulation of four cake samples incorporating dry date powder (DDP) and eggshell powder (ESP) at varying levels. Sample A serves as the control, containing no DDP or ESP, while Samples B, C, and D include increasing proportions of these ingredients. The formulations maintain consistent quantities of butter, eggs, baking powder, salt, water, sugar, and flavoring across all samples. The DDP content increases from 25g to 75g, and the ESP from 1% to 3% in Samples B to D, respectively. This design evaluates the impact of these nutrient-rich powders on the cakes' sensory, nutritional, and functional properties.

3.5. Preparation of cake with Dry date powder and Eggshell powder

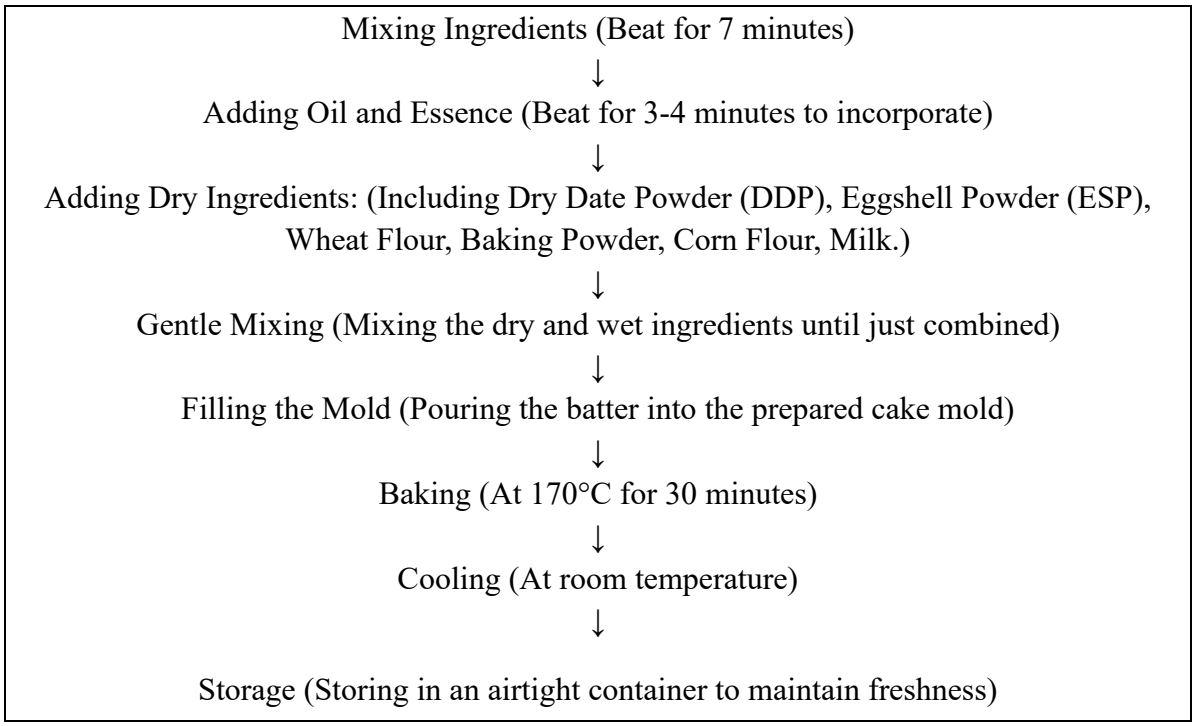

Figure 3.4. Preparation of cake with Dry date powder and Eggshell powder

3.6. Proximate Analysis of Cakes Fortified with Dry Date Powder (DDP) and Eggshell Powder (ESP)

3.6.1. Moisture Content

The moisture content of cakes fortified with dry date powder (DDP) and eggshell powder (ESP) was determined following the standard method outlined by the AOAC. To determine the moisture, a 2g sample of the fortified cake was placed in a pre-weighed crucible and dried in an oven at 105°C for three hours. After drying, the crucible was cooled in a desiccator and weighed again. The difference in weight before and after drying was used to calculate the percentage of moisture content, a key parameter in assessing the shelf life and quality of the cakes.

3.6.2. Crude Protein

Crude protein content was determined using the Kjeldahl method (method number 920.87), as per standard procedures. A 1g sample was digested with sulfuric acid and a Kjeldahl catalyst to release nitrogen. The nitrogen was then distilled and absorbed in boric acid, followed by titration with a standard hydrochloric acid solution. This procedure allowed

for the quantification of nitrogen, which was then used to calculate the protein content in the sample.

3.6.3. Dietary Fiber

The dietary fiber content in the fortified cakes was measured using AOAC method 920.86 (Delwiche, 2003). A 1g sample of the cake was subjected to acid digestion with sulfuric acid, followed by alkali digestion with potassium hydroxide. After digestion, the remaining material was washed, dried, and then combusted in a muffle furnace at 525°C to determine the weight of the fiber content. This method provides an estimate of the indigestible carbohydrate portion in the cakes, contributing to their dietary fiber content.

3.6.4. Crude Fat

Crude fat content was measured using the Soxhlet extraction method, as described by AOAC (2000). The dried cake samples were placed in a Soxhlet thimble, and petroleum ether was used to extract the fat content for a period of 16 hours. The extracted fat was separated, and its weight was determined by the difference in weight of the beaker before and after evaporation of the petroleum ether. This process is widely recognized for providing accurate fat content determination in food products.

3.6.5. Antioxidant Capacity (DPPH Scavenging Method)

The antioxidant capacity of the fortified cakes was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. For this, a sample of the fortified cake was extracted with methanol, and the extract was then mixed with a DPPH solution. The scavenging ability of the antioxidants was assessed by measuring the decrease in DPPH absorbance at 760 nm using a UV-VIS spectrophotometer. The percentage of inhibition of the

DPPH radical was calculated using the formula:

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

This method quantifies the cakes' ability to neutralize free radicals, which is important for understanding their potential antioxidant properties. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) to provide a standardized comparison of antioxidant activity.

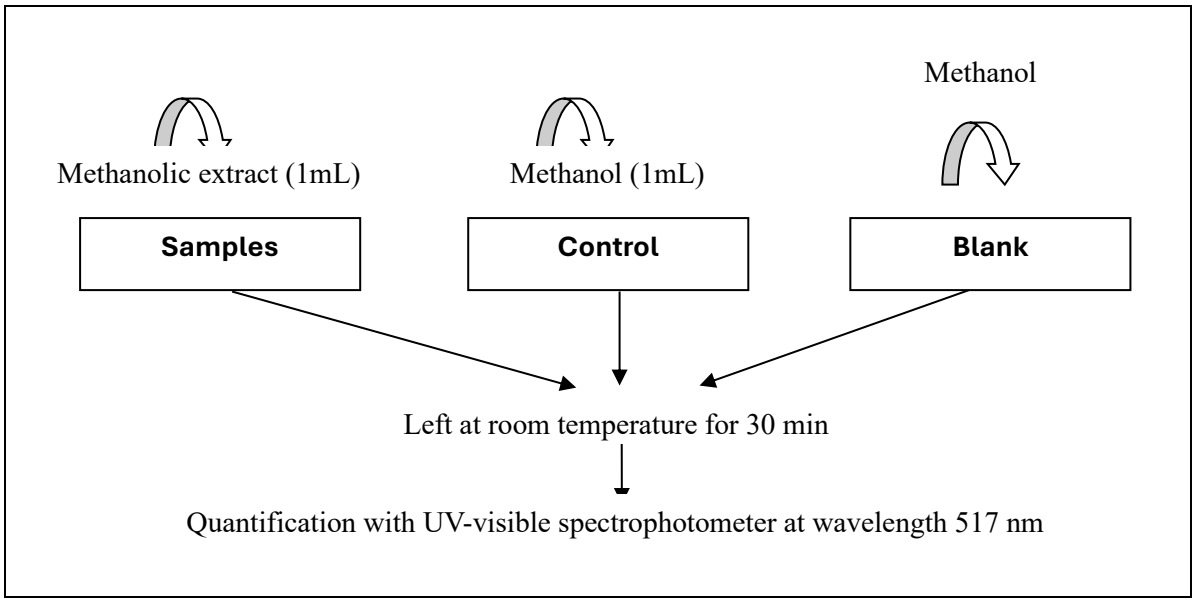

Figure 3.5: Determination of antioxidant capacity

3.7. Determination of Bioactive Compounds

Extract Preparation

For the determination of bioactive compounds, 5 grams of the TAC sample, along with 1 gram of both TPC and TFC samples, were placed in a Falcon tube. To this mixture, 10 milliliters of 100% ethanol was added. The tube was left undisturbed for 72 hours to allow for the extraction of the bioactive compounds. During this period, the mixture was strained every 4 hours to ensure continuous extraction. After 72 hours, the resulting filtrate was collected, which gave rise to an ethanolic extract that was used for subsequent analysis of bioactive compounds.

3.7.1. Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) of the extracts was determined using the Folin-Ciocalteu reagent method with slight modifications, as described by Al-Owaisi et al. (2014). The procedure followed the methodology outlined by Vergani et al. (2016) to quantify the total polyphenol content in the sample. In this method, 1 milliliter of ethanolic extract was mixed with 1.5 milliliters of Folin-Ciocalteu reagent in a Falcon tube. The mixture was allowed to stand at room temperature for 3 minutes. Then, 1.5 milliliters of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the mixture was left to rest for 30 minutes to allow for full reaction. The absorbance of the resulting solution was measured at 765 nm using a UV-VIS spectrophotometer (UV2600, Shimadzu Corporation, USA). Ethanol was used as the blank in the experiment to calibrate the spectrophotometer. The TPC was calculated in terms of milligrams of Gallic Acid Equivalent (GAE) per gram of extract.

3.7.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method, as described by Chang et al. (2002). This technique is widely used for assessing flavonoid levels in plant-based materials due to its accuracy and relevance in evaluating the nutritional and antioxidant potential of the samples. Flavonoids are bioactive compounds known for their antioxidant, anti-inflammatory, and potential anti-cancer properties, making their quantification crucial in the context of health-promoting foods.

To determine the TFC, 5 mL of the diluted extract was mixed with 1.5 mL of 95% ethanol. The mixture was transferred to a cuvette, followed by the addition of 2.8 mL of distilled water, 0.1 mL of 10% aluminum chloride ($AlCl_3$), and 0.1 mL of 1 M potassium acetate. The cuvette was allowed to stand for 30 minutes at room temperature for the reaction to occur. A blank sample, prepared by replacing aluminum chloride with an equivalent volume of distilled water, was used to calibrate the spectrophotometer. After the incubation period, the absorbance of the mixture was measured at 415 nm using a UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA).

The total flavonoid content was calculated by comparing the absorbance of the sample with a standard quercetin curve. The results were expressed as milligrams of quercetin equivalent per gram of sample (mg QE/g). This method provides a reliable quantification of flavonoids, contributing to a better understanding of the nutritional and medicinal properties of the samples.

3.8. Mineral Content Analysis

The mineral content of the fortified biscuits, which encompassed calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), and zinc (Zn), was assessed through a digestion process that utilized nitric and perchloric acids. This digestion method enables the extraction of minerals from the sample matrix, thereby facilitating their detection and quantification (Manchanda et al., 2024). The sample was subjected to a temperature-controlled digestion procedure, during which a mixture of perchloric and nitric acids degrades the sample, releasing the minerals in a soluble form. A chromogen-containing reagent was introduced to the metabolized solution in order to ascertain the concentration of particular minerals. A colored complex is formed when the chromogen reacts with the mineral of interest. The mineral's concentration is directly proportional to the intensity of the color generated, which enables its quantification.

3.9. Estimation of soluble carbohydrate

The carbohydrate content was assessed by calculating the nitrogen-free extract value (NFE), typically derived as the complement of the sum of the remaining proximate components subtracted from 100. Hence, it was calculated using the formula below:

$$\% \text{ Soluble carbohydrate} = 100 - (\text{Moisture\%} + \text{Ash\%} + \text{Crude protein\%} + \text{Crude fat\%} + \text{Crude fiber\%})$$

Estimation of Energy content

For each sample, energy content was determined by applying the following equation:

$$\textbf{Energy} = (\text{Crude protein} \times 4.1) + (\text{Crude fat} \times 9.2) + (\text{Soluble Carbohydrate} \times 4.1)$$

3.10. Sensory Analysis

The sensory evaluation of the DDP and ESP fortified cakes was conducted to assess the overall acceptability of the product based on various organoleptic qualities such as appearance, taste, texture, and aroma. The panel consisted of 20 untrained assessors, equally divided between male and female participants, from the Chattogram Veterinary and Animal Sciences University (CVASU). The participants were asked to evaluate the three different formulations of the fortified cake using a 7-point Hedonic scale, where they indicated their level of preference for each sample.

The scale ranged from a score of 7 (extremely like) to 1 (extremely dislike), with intermediate scores representing varying levels of preference. The evaluation was conducted in a controlled environment to ensure the accuracy of results. The panelists rated the cakes according to their sensory properties including texture, taste, and overall flavor. The scores were then recorded and analyzed to determine the preferred cake formulation.

Grading System for Sensory Evaluation

Rank	Score
Like extremely	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike extremely	1

3.11. Statistical Analysis

Minitab Version 21 software was employed to conduct statistical analysis of experimental data. The differences between the numerous biscuit formulations were assessed using a one-way analysis of variance (ANOVA). Tukey's post hoc test was employed to evaluate the significance of the differences between groups, which is a method that identifies specific differences between sample means when the aggregate ANOVA indicates significant variation. The significance level was established at $p<0.05$, a standard that is frequently employed in food science research to ascertain the statistical significance of the observed differences. The conclusions derived from the experimental data are guaranteed to be reliable and robust as a result of this rigorous statistical approach.

Chapter IV: Result

4.1. Nutritional properties

The nutritional analysis of the formulated cakes was conducted to evaluate the impact of dry date powder (DDP) and eggshell powder (ESP) on their overall composition. This assessment is essential to determine improvements in protein, fiber, and mineral content while maintaining desirable sensory and functional qualities.

4.1.1. Proximate Composition of the formulated cakes

The proximate composition of the formulated cakes varied significantly depending on the levels of dry date powder (DDP) and eggshell powder (ESP) incorporated. Carbohydrate content ranged from 42.83±0.21% in Sample B to 46.37±0.03% in Sample A, with Sample D (45.99±0.01%) showing no significant difference from Sample A. The crude protein content increased with the addition of DDP and ESP, reaching the highest value in Sample C (7.62±0.12%) compared to the control (4.03±0.01%). Similarly, crude fat content was highest in Sample B (14.15±0.07%) and lowest in Sample A (11.25±0.07%). The cake's proximate composition values, expressed as mean percentages with standard deviations (ME±SD), can be found in **Table 4.1**.

Table 4.1: Proximate Composition

Sample	Carbohydrate (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)
A	46.37±0.03^{a}	4.03±0.01^{a}	11.25±0.07^{a}	0.92±0.01^{a}	0.72±0.01^{a}	36.72±0.06^{a}
B	42.83±0.21^{b}	5.30±0.00^{b}	14.15±0.07^{b}	1.42±0.01^{b}	1.60±0.01^{b}	34.70±0.14^{b}
C	43.48±0.04^{c}	7.62±0.12^{c}	13.10±0.14^{c}	1.66±0.07^{c}	1.74±0.01^{c}	32.40±0.00^{c}
D	45.99±0.01^{a}	6.65±0.00^{d}	12.28±0.15^{d}	2.42±0.09^{d}	1.92±0.00^{d}	30.75±0.07^{d}

Means ± SD and Values in the same column with the same superscript are not significantly different $p<0.05$)

Crude fiber content increased significantly with higher DDP inclusion, with Sample D containing the highest amount (2.42±0.09%) compared to the control (0.92±0.01%). Ash

content also increased from 0.72±0.01% in Sample A to 1.92±0.00% in Sample D due to the mineral contribution of ESP. Moisture content showed a decreasing trend as DDP and

ESP levels increased, with the highest value in Sample A (36.72±0.06%) and the lowest in Sample D (30.75±0.07%), indicating that higher DDP levels reduced moisture retention in the cakes.

4.1.2 Energy content of formulated cakes

Table 4.2 presents the energy content of the formulated cakes, showing significant variations among the samples ($p<0.05$). The control sample (Sample A) had the lowest energy content (310.12±0.56 kcal/100 g), while Sample C exhibited the highest (330.03±0.95 kcal/100 g). The increased energy content in Samples B, C, and D is attributed to the incorporation of dry date powder (DDP) and eggshell powder (ESP), which contributed additional macronutrients.

Table 4.2: Energy Content of Cakes (Kcal/100 g)

Sample	Energy Content (Kcal/100 g)
Sample A	310.12 ± 0.56^{a}
Sample B	327.51 ± 0.22^{b}
Sample C	330.03 ± 0.95^{c}
Sample D	328.75 ± 1.42^{bc}

Means ± SD and Values in the same column with the same superscript are not significantly different $p<0.05$)

The results highlight that the substitution of refined wheat flour with DDP and ESP enhances the nutritional density of the cakes while maintaining an optimal energy balance.

4.2. Analysis of Mineral Content of the Formulated Cakes

The mineral composition of the formulated biscuits showed a significant increase in calcium, potassium, magnesium, and phosphorus with the incorporation of dry date powder (DDP) and eggshell powder (ESP).

Table 4.3: Mineral Content of Formulated Cakes (mg/100 g)

Sample	Calcium (mg)	Potassium (mg)	Magnesium (mg)	Phosphorus (mg)
Sample A	24.01±0.01[a]	60.45±0.07[a]	28.02±0.01[a]	96.35±0.07[a]
Sample B	306.15±0.07[b]	130.25±0.07[b]	36.60±0.14[b]	150.40±0.14[b]
Sample C	460.35±0.07[c]	151.35±0.07[c]	49.25±0.07[c]	280.45±0.07[c]
Sample D	508.35±0.07[d]	172.40±0.14[d]	60.20±0.00[d]	336.40±0.14[d]

Means ± (SD) followed by different superscripts (a, b, c, d) in the same column are significantly different ($p<0.05$)

Calcium content increased from 24.01 mg/100 g in Sample A (control) to 508.35 mg/100 g in Sample D, highlighting the contribution of ESP as shown in **Table 4.3**. Similarly, potassium levels ranged from 60.45 mg/100 g in the control to 172.40 mg/100 g in Sample D. Magnesium and phosphorus content also exhibited notable enhancement, with Sample D containing the highest values (60.20 mg/100 g and 336.40 mg/100 g, respectively). The significant differences ($p<0.05$) across all samples confirm the nutritional enrichment due to DDP and ESP incorporation.

4.3. Bioactive Compounds of the Cakes

4.3.1 Total flavonoid content (TFP):

The total flavonoid content (TFC) of the formulated cakes showed a significant increase with the incorporation of dry date powder (DDP) and eggshell powder (ESP). The control sample (Sample A) had the lowest flavonoid content, averaging around 42.42 mg/100g. Sample B exhibited a moderate increase (≈61.15 mg/100g), while Sample C showed a substantial rise (≈82.54 mg/100g). The highest TFC was observed in Sample D (≈87.85 mg/100g), indicating that higher DDP inclusion contributed significantly to flavonoid enrichment. The statistical analysis confirmed significant differences ($p<0.05$) between the samples, demonstrating the positive effect of date powder on the antioxidant properties of the cakes.

Table 4.4 Total Flavonoid Content (TFC) of Cake

Sample	Total Flavonoid Content (mg/100g)
Sample A	42.42±0.40[a]
Sample B	61.15±0.15[b]
Sample C	82.54±0.06[c]
Sample D	87.85±0.09[d]

The ANOVA test showed a significant difference ($p<0.05$) in the total flavonoid content among the samples

4.3.2 TPC (Total Phenolic Content) of the Cakes

The total phenolic content (TPC) of the formulated cakes varied significantly with the inclusion of dry date powder (DDP) and eggshell powder (ESP), as shown in **Table 4.5**.

Table 4.5 Total Phenolic Content (TPC) of the Cake Samples

Sample	Total Phenolic Content (mg/100g)
Sample A	0.63±0.01[a]
Sample B	0.72±0.01[b]
Sample C	1.86±0.00[c]
Sample D	2.21±0.00[d]

*Means ± SD with different superscripts in the same column indicate significant differences ($p<0.05$) *

The control sample exhibited the lowest TPC (0.63 mg/100g), while Sample B showed a slight increase (0.72 mg/100g). A more substantial increase was observed in Sample C (1.86 mg/100g), and the highest TPC was recorded in Sample D (2.21 mg/100g). The statistical analysis confirmed significant differences ($p<0.05$) among the samples, indicating that the incorporation of DDP significantly improved the phenolic content, contributing to the antioxidant potential of the cakes.

4.3.3 Antioxidant Capacity of Sample Cakes

The antioxidant capacity of the formulated cakes, as presented in **Table 4.6**, increased with the inclusion of dry date powder (DDP) and eggshell powder (ESP). The control sample exhibited the lowest antioxidant capacity (1.08 mg/100g), while Sample B showed a

moderate increase (1.13 mg/100g). A substantial rise was observed in Sample C (1.43 mg/100g), and the highest antioxidant activity was recorded in Sample D (1.45 mg/100g).

Table 4.6 Antioxidant Capacity of Cake Samples

Sample	Antioxidant Capacity (mg/100g)
Control	1.08 ± 0.002^{a}
Sample B	1.13 ± 0.008^{b}
Sample C	1.43 ± 0.002^{c}
Sample D	1.45 ± 0.003^{c}

*The statistical analysis ($p<0.05$) confirmed significant differences among the samples, highlighting that the incorporation of **DDP** enhanced the antioxidant properties*

4.4. Sensory Evaluation

The sensory attributes of the formulated cake samples, including color, appearance, smell, texture, taste/flavor, and overall acceptability, were evaluated to assess the impact of dry date powder (DDP) and eggshell powder (ESP) incorporation. The results, as presented in **Table 4.7**, indicate significant differences ($p<0.05$) among the samples, suggesting that variations in formulation influenced sensory perception.

Table 4.7: Sensory Evaluation of Cake Samples

Sample	Color	Appearance	Smell	Texture	Taste/Flavor	Overall Acceptability
Sample A	6.06 ± 0.01^{a}	6.01 ± 0.01^{a}	5.05 ± 0.01^{a}	5.71 ± 0.01^{a}	6.05 ± 0.01^{a}	6.02 ± 0.01^{a}
Sample B	6.14 ± 0.01^{b}	5.75 ± 0.01^{b}	5.61 ± 0.01^{b}	5.91 ± 0.01^{b}	5.03 ± 0.01^{b}	5.02 ± 0.01^{b}
Sample C	6.85 ± 0.07^{c}	6.78 ± 0.01^{c}	6.61 ± 0.01^{c}	6.66 ± 0.01^{c}	6.69 ± 0.01^{c}	6.31 ± 0.01^{c}
Sample D	6.36 ± 0.01^{d}	6.85 ± 0.07^{c}	6.65 ± 0.01^{c}	5.73 ± 0.01^{a}	6.60 ± 0.00^{c}	6.27 ± 0.01^{c}

means ± SD with different superscripts in the same column indicate significant differences ($p<0.05$) among the cake samples

Sample C exhibited the highest scores across most parameters, particularly in color (6.85 ± 0.07), appearance (6.78 ± 0.01), texture (6.66 ± 0.01), and taste/flavor (6.69 ± 0.01),

leading to its highest overall acceptability (6.31 ± 0.01). Sample D also received relatively high ratings, especially in appearance (6.85 ± 0.07), smell (6.65 ± 0.01), and taste/flavor (6.60 ± 0.00), although its texture score (5.73 ± 0.01) was lower than that of Sample C.

In contrast, Sample B recorded the lowest scores, particularly in taste/flavor (5.03 ± 0.01) and overall acceptability (5.02 ± 0.01), suggesting a less favorable sensory profile. Sample A, which served as the control, demonstrated moderate sensory ratings but remained significantly different from the other formulations. These findings suggest that the inclusion of DDP and ESP improved the sensory properties of the cakes, particularly in terms of color, taste, and overall acceptability, making them more appealing to consumers.

Chapter V: Discussion

The present study assessed nutritional composition, bioactive properties, and sensory attributes of cakes formulated with varying levels of dry date powder (DDP) and eggshell powder (ESP). The results demonstrated significant improvements in proximate composition, mineral content, antioxidant properties, and sensory acceptability in the fortified cakes compared to the control sample. These findings highlight the potential of DDP and ESP as functional ingredients in bakery products to enhance both nutritional quality and health benefits.

Proximate Composition

The incorporation of DDP and ESP induced notable modifications in the proximate composition of the cakes, particularly in carbohydrate, protein, fat, fiber, and ash contents. A significant reduction in carbohydrate content was observed with increasing levels of DDP, which can be attributed to the partial substitution of refined wheat flour with fiber- and protein-rich DDP. This is in agreement with previous findings, which reported a decline in carbohydrate content in bakery products supplemented with fiber-rich ingredients (Dhankhar et al., 2021).

Protein content exhibited a significant increase, particularly in Sample C (7.62±0.12%), which contained the highest levels of DDP and ESP. This enhancement can be attributed to the inherent protein content of dates and the contribution of ESP, which has been shown to provide a calcium-protein matrix that supports protein enrichment in food products.

Fat content varied across the samples, with Sample B exhibiting the highest value (14.15±0.07%). This increase is likely due to the lipid content of dates and their capacity to bind fat, leading to improved moisture retention. Saeed et al. (2021) examined the impact of incorporating date pit flour in biscuits as a fat replacer. Crude fiber content showed a substantial increase, reaching its highest level in Sample D (2.42±0.09%). The fiber-enriching effect of DDP aligns with prior studies demonstrating that date-derived ingredients enhance the dietary fiber content of food products (AlFaris et al., 2021). Similarly, the ash content, which serves as an indicator of overall mineral composition, increased significantly from 0.72±0.01% in the control to 1.92±0.00% in Sample D,

confirming the mineral-enriching properties of ESP. A notable decline in moisture content was observed with increasing levels of DDP and ESP.

Energy Content

The energy content of the formulated cakes was significantly influenced by the incorporation of DDP and ESP. The control sample exhibited the lowest energy value (310.12±0.56 kcal/100g), while Sample C recorded the highest (330.03±0.95 kcal/100g). This increase is attributable to the higher protein and fat contents, which contribute to overall caloric density.

Mineral Composition

The mineral analysis revealed substantial enhancements in calcium, potassium, magnesium, and phosphorus levels, with ESP serving as a particularly effective source of calcium. The calcium content exhibited a marked increase from 24.01 mg/100g in the control to 508.35 mg/100g in Sample D. This result supports previous studies that identified ESP as a bioavailable and cost-effective calcium fortificant in bakery products (Khan et al., 2020). Potassium, magnesium, and phosphorus levels also increased with higher DDP inclusion, which is consistent with reports that highlight dates as an excellent source of essential minerals (AlFaris et al., 2021).

Bioactive Compounds and Antioxidant Capacity

Total flavonoid content (TFC) and total phenolic content (TPC) demonstrated significant increases with the incorporation of DDP. Sample D exhibited the highest TFC (87.85 mg/100g) and TPC (2.21 mg/100g), confirming that date powder is a rich source of bioactive compounds with antioxidant potential. These results corroborate existing literature indicating that dates contain high concentrations of flavonoids and phenolic compounds, which contribute to their functional properties (Sarhat et al., 2023).

Antioxidant capacity exhibited a similar trend, with Sample D displaying the highest value (1.45 mg/100g). The significant differences observed among samples underscore the potential health benefits of DDP incorporation, particularly in enhancing the functional properties of baked goods through increased antioxidant activity.

Sensory Evaluation

The sensory properties of the formulated cakes were significantly influenced by the addition of DDP and ESP. Sample C received the highest overall acceptability scores, particularly excelling in color, appearance, texture, and taste. The natural caramelization effect of DDP likely contributed to the enhanced color and flavor perception, while the fiber content may have positively influenced textural attributes. This aligns with findings by

Jahan et al. (2023), who reported that date powder enhances the sensory characteristics of bakery products.

Conversely, Sample B received the lowest sensory scores, particularly in taste and overall acceptability. This may be attributed to an excessive amount of ESP, which could have introduced a chalky texture or modified the flavor profile unfavorably. These findings suggest that while ESP is highly beneficial for mineral enrichment, its incorporation level should be carefully optimized to maintain desirable sensory attributes.

Chapter VI: Conclusion

The present study demonstrated the successful development of sugar-free cakes using dry date powder (DDP) as a natural sweetener and eggshell powder (ESP) as a sustainable calcium fortificant. The primary objectives of researching refined sugar content, enhancing nutritional and functional value, and promoting sustainable practices through food waste valorization—were fully achieved through scientific formulation and evaluation.

The incorporation of DDP and ESP led to significant improvements in the nutritional profile of the cakes. Protein content increased from 4.03±0.01% in the control to 7.62±0.12% in Sample C, dietary fiber rose from 0.92±0.01% to 2.42±0.09%, and calcium content surged more than twentyfold, from 24.01 mg/100g to 508.35 mg/100g in Sample D. These enhancements are particularly meaningful in addressing the dual burdens of diabetes and calcium deficiency prevalent in many populations, including Bangladesh.

From a functional perspective, the cakes exhibited substantial enrichment in antioxidant bioactive compounds. Total flavonoid content and total phenolic content increased significantly, resulting in enhanced antioxidant capacity, which is critical for reducing oxidative stress—a key contributor to chronic diseases. These findings highlight the potential of such functional foods in preventive nutrition strategies.

Sensory analysis showed that moderate levels of DDP and ESP (Sample C) produced the most acceptable formulation, with the highest scores in taste, texture, and overall appeal. This indicates that nutritional improvements need not compromise consumer acceptability when formulations are optimized carefully. However, over-fortification (Sample D) had a minor negative effect on textural attributes, suggesting the need for further refinement in future product development.

Importantly, the study exemplifies how food by-products—typically discarded as waste—can be transformed into high-value, health-promoting ingredients. This aligns with the global goals of sustainable food systems and circular economy practices, as advocated by the United Nations and numerous food security organizations. Eggshells, often treated as waste, were converted into a bioavailable calcium source, while dry dates, typically underutilized or discarded due to over-ripeness, served as an effective natural sweetener rich in functional compounds.

Moreover, the successful integration of these ingredients into bakery products has significant implications for the food industry. As consumer demand grows for clean-label, diabetic-friendly, and functional foods, this formulation offers a scalable solution for commercial bakeries, health food brands, and community nutrition programs. It can help reduce reliance on imported supplements and refined sugar, fostering greater self-reliance and nutritional resilience in local food systems.

In conclusion, the development of DDP and ESP-enriched sugar-free cakes not only meets critical nutritional needs but also presents a sustainable, consumer-friendly, and economically viable food innovation. Future research should explore long-term shelf life, bioavailability in human trials, and broader consumer market testing. With further validation, such products could play a pivotal role in addressing public health challenges while promoting environmental stewardship through food waste minimization.

Chapter VII: Recommendation and Future Perspectives

The findings of this study highlight the potential of dry date powder (DDP) and eggshell powder (ESP) as functional fortificants in bakery products. Based on the results, the following recommendations and future perspectives are proposed:

1. **Optimization of Formulation:** Further studies should focus on refining the optimal levels of DDP and ESP to maximize nutritional benefits without compromising sensory acceptability. A balanced formulation would ensure both enhanced bioactive properties and consumer preference.
2. **Exploring Other Bakery Applications:** The incorporation of DDP and ESP could be extended to other bakery products such as biscuits, muffins, and bread. Investigating their effects on different matrices would provide insights into their broader application in functional food development.
3. **Shelf-Life and Storage Stability:** Future research should evaluate the storage stability of DDP- and ESP-fortified cakes, assessing factors such as moisture retention, microbial safety, and antioxidant degradation over time.
4. **Bioavailability and Digestibility Studies:** While the current study confirms enhanced mineral content and antioxidant properties, further research should explore the bioavailability and digestibility of these nutrients in fortified cakes to ensure effective nutrient absorption.
5. **Consumer Acceptance and Market Feasibility:** Large-scale sensory trials and consumer preference studies should be conducted to assess market viability and potential commercial adoption of DDP- and ESP-fortified bakery products.
6. **Sustainable and Cost-Effective Production:** Investigating cost-effective production methods for DDP and ESP could support their large-scale application, promoting sustainability and reducing food waste by utilizing by-products such as eggshells.

Overall, future studies should integrate nutritional, functional, and economic aspects to enhance the applicability of DDP and ESP in the food industry, ensuring their role in the development of sustainable and health-promoting bakery products.

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APPENDICES

Appendix A

Photo gallery of the experiment

Eggshell Powder Preparation




Dry Date Powder Preparation

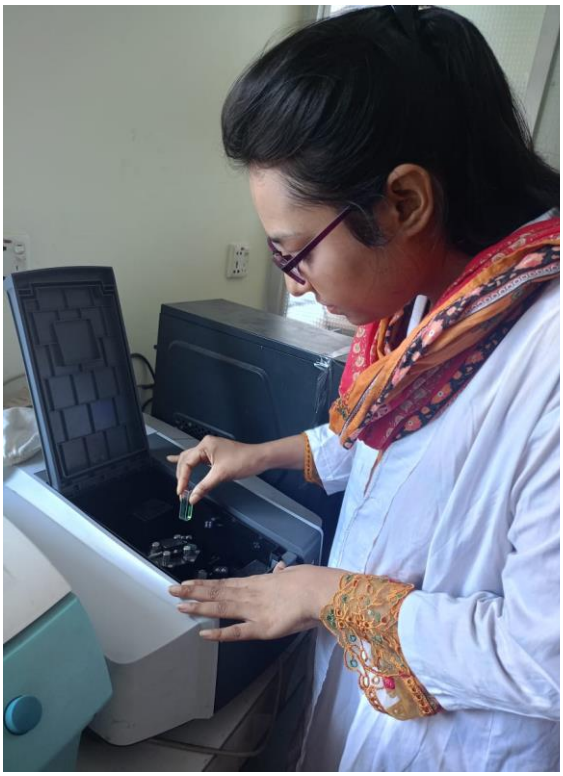
Sample A

Sample B

Sample C

Sample D

Sample Formulation



Instrumental Analysis

Sensory Evaluation

Appendix B

Sensory research using a 7-point hedonic scale (Questionnaire)

Sensory evaluation form for Banana peel powder cake

Taste the sample and tick (✓) how much you like or dislike each of the characteristics

Name:

Sample no:

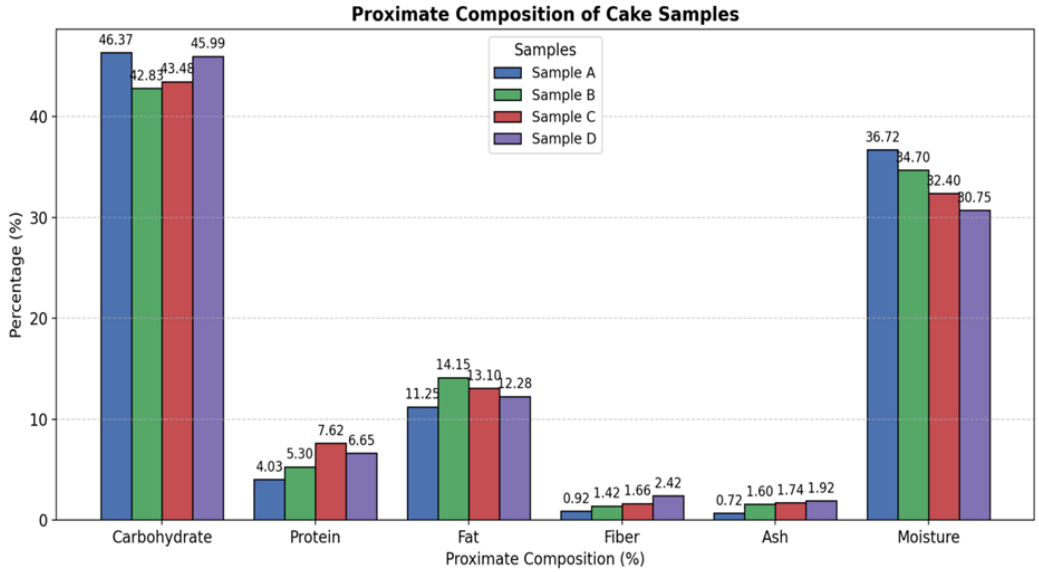
Sample name:

Panelist Hedonic Rating	**Liking score**	**Color**	**Appearance**	**Smell**	**Texture**	**Taste**	**Overall acceptability**
Like extremely	7						
Like moderately	6						
Like slightly	5						
Neither like or dislike	4						
Dislike slightly	3						
Dislike moderately	2						
Dislike extremely	1						

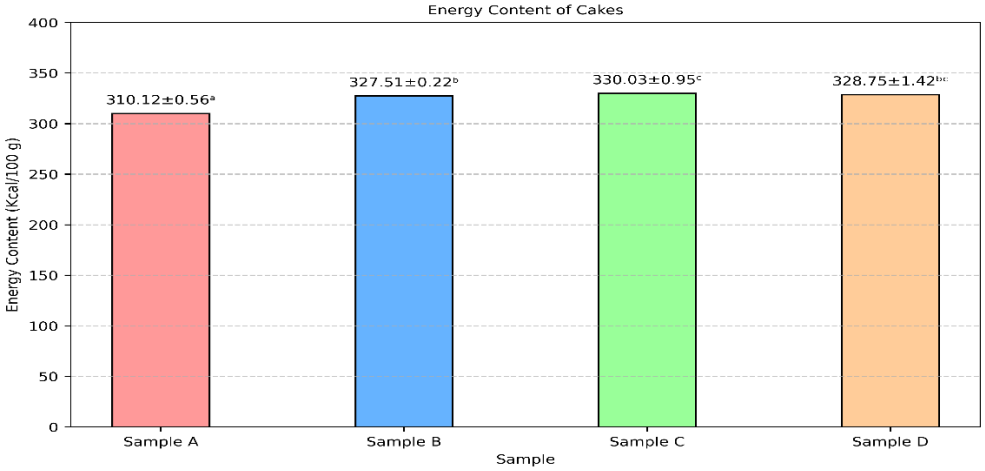
Appendix C

Standard curve and sample curve

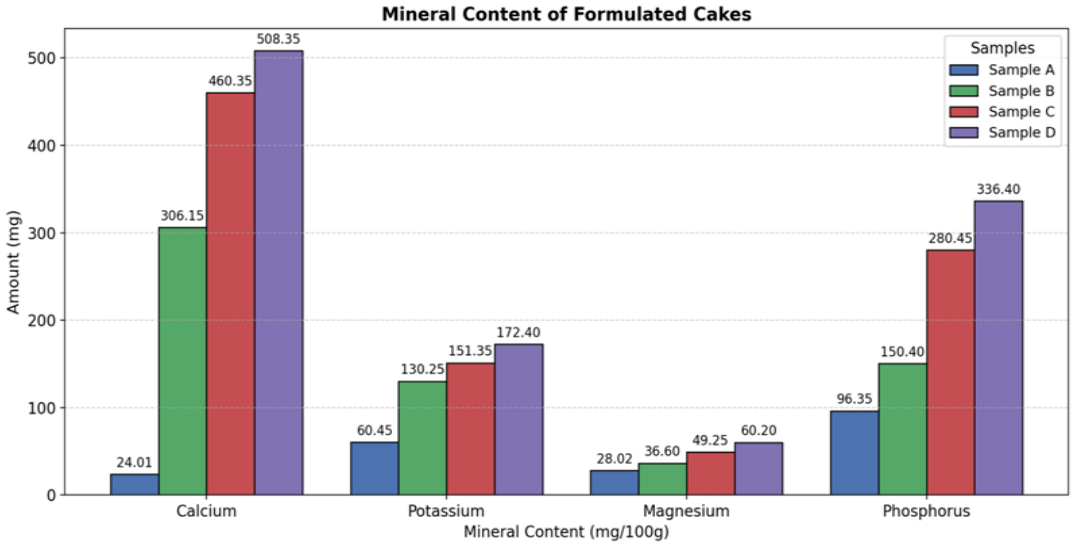
Proximate Analysis of Cake Samples



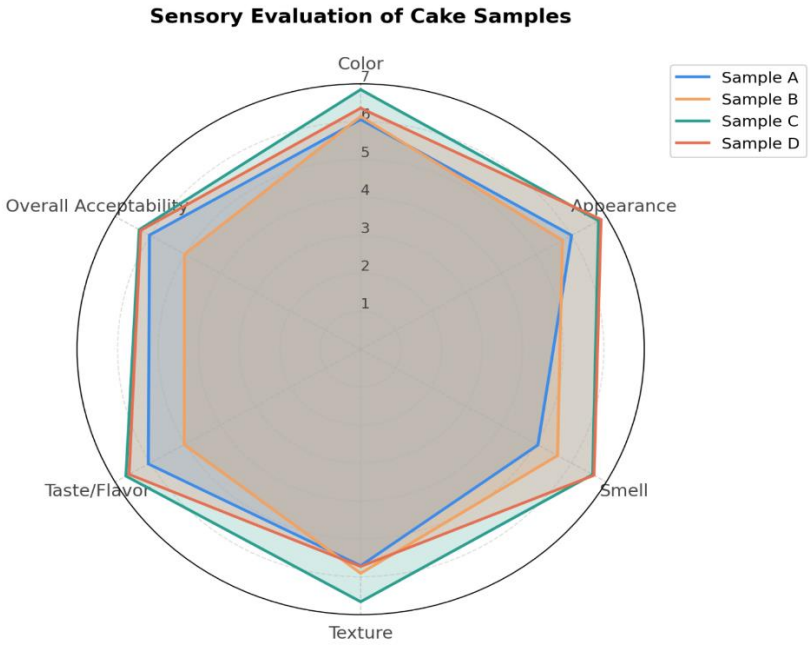
Energy Content of Cakes (Kcal/100 g)



Mineral Content Analysis

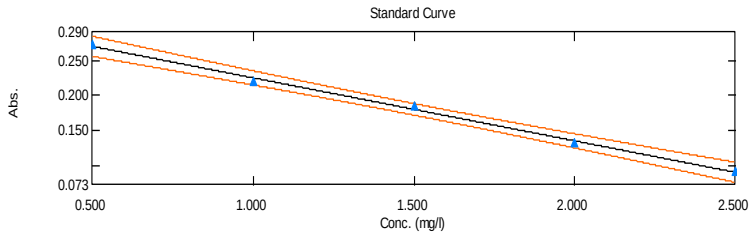


Sensory Analysis



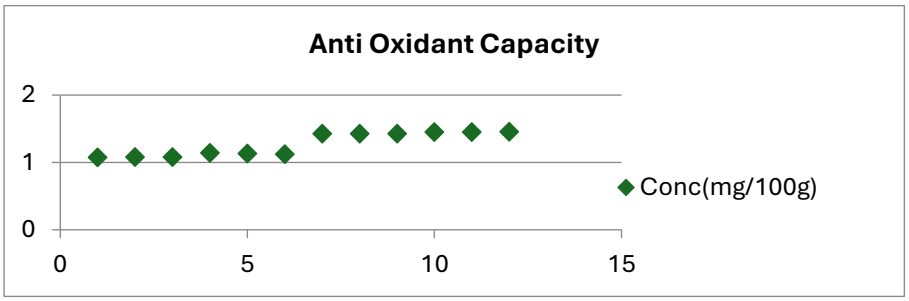
Antioxidant Capacity of Cake

Standard Curve:



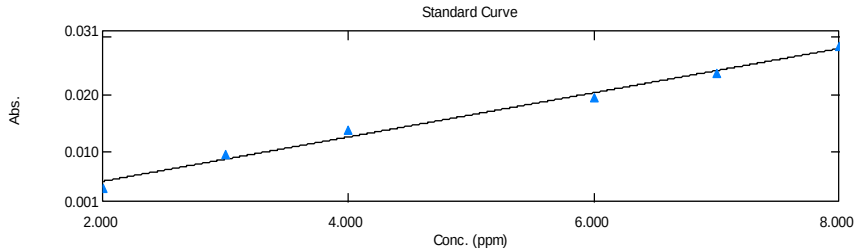
y = - 0.0894539 x + 0.314536
r2 = 0.99735

Sample Graph:



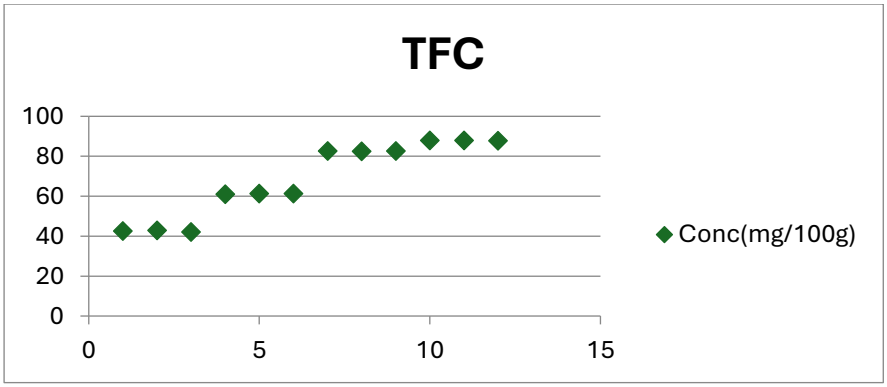
TFC (Total Flavonoid Content) of Cake

Standard Curve:



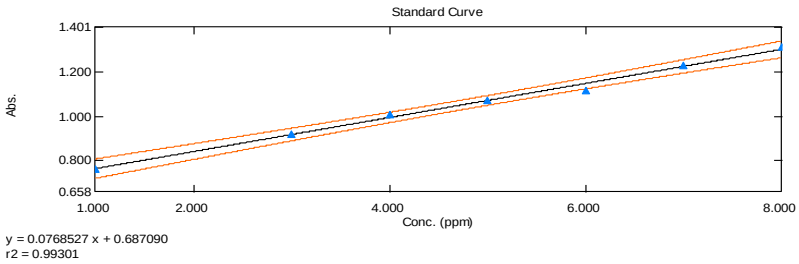
y = 0.00385110 x - 0.00271158
r2 = 0.98868

Sample graph:

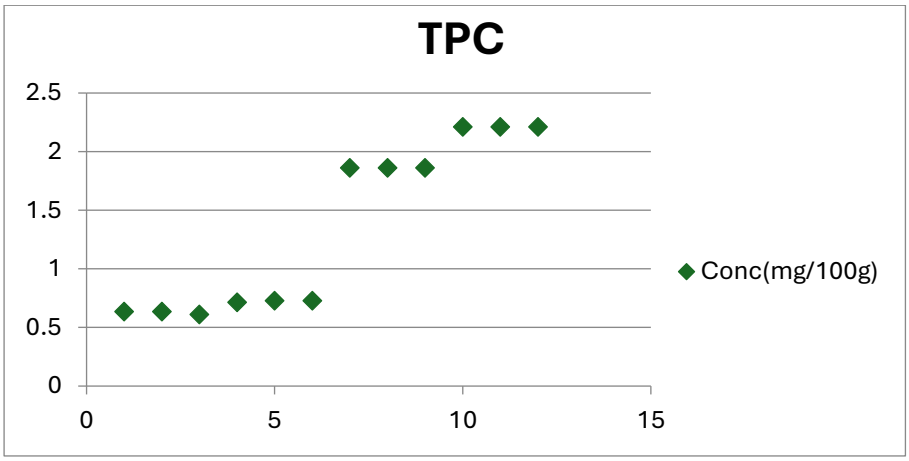


TPC (Total Phenolic Content) of Cake

Standard Curve:



Sample Graph:



Brief biography

Mobasharin Zannat is a dedicated and aspiring nutrition professional with a solid academic background in food science and a strong commitment to advancing public health through evidence-based dietary interventions. She commenced her formal education with distinction, successfully completing the Secondary School Certificate (SSC) examination in 2013 from Chattogram Government Girls' High School, followed by the Higher Secondary Certificate (HSC) examination in 2015 from Chattogram Government City College. Driven by a keen interest in the science of nutrition and its implications for human health, she pursued a Bachelor of Science with Honours in Food Science and Technology under the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University (CVASU). Currently, she is furthering her academic and professional expertise through a Master of Science in Applied Human Nutrition and Dietetics at the same institution. Her academic and research interests primarily lie in the fields of clinical and therapeutic nutrition, with a particular focus on the development of personalized nutritional strategies for individuals affected by acute and chronic health conditions.