Development and Evaluation of Bromelain-Enriched Functional Protein Bar for Management of Type-2 Diabetes Mellitus.

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***Abstract*—Type-2 diabetes mellitus (T2DM) is characterized by chronic hyperglycemia, insulin resistance, and altered lipid metabolism, necessitating safe and convenient dietary interventions to support glycemic control. Bromelain, a proteolytic enzyme complex derived from pineapple (Ananas comosus), has demonstrated anti-diabetic, anti-inflammatory, and hypolipidemic properties in preclinical and limited clinical studies, making it a promising functional ingredient in diabetic-friendly foods.In this study, bromelain-enriched functional protein bars were developed using a balanced blend of high-quality protein sources, low-glycemic carbohydrates, and selected functional ingredients, with bromelain incorporated at a defined enzymatic activity to ensure measurable bioactivity. The formulations were optimized for texture, water activity, bromelain stability, and sensory acceptability, and the bars were evaluated for physicochemical quality and storage stability under ambient conditions. In-vitro digestion experiments revealed that bromelain-enriched bars significantly reduced the rate and extent of glucose release compared with control bars, indicating a lower predicted glycemic response. Concomitantly, bromelain-mediated proteolysis enhanced the generation of bioactive peptides from the protein matrix, which may contribute to improved insulin sensitivity and metabolic regulation. These findings suggest that bromelain-enriched functional protein bars hold promise as a convenient, palatable, and physiologically beneficial dietary adjunct for the management of type-2 diabetes mellitus, warranting further clinical evaluation in diabetic cohorts.Results indicated that the bromelain-enriched protein bars exhibited significantly reduced predicted glucose release and improved peptide profile compared to control bars, suggesting potential for slower carbohydrate digestion and enhanced bioactive peptide generation. These findings support the development of bromelain-enriched functional protein bars as a convenient, palatable, and physiologically beneficial dietary adjunct for the management of type-2 diabetes mellitus. Further clinical studies are warranted to validate their efficacy on glycemic control, insulin sensitivity, and lipid profiles in diabetic patients.**

***Index Terms*—Protein bar, Type 2 Diabetes Mellitus, Bromelain, Azocasein, Protease activity.**

Abbreviations

Abbreviation	Full Form
A1C	Glycated Hemoglobin Test
ACL	Anterior Cruciate Ligament
ACLr	ACL Rupture
ADA	American Diabetes Association
ADSC	Adipose Derived Stem Cell
AKT	Protein Kinase B
BMI	Body Mass Index
FFA	Free Fatty Acid
HBOT	Hyperbaric Oxygen Therapy
HIF-1α	Hypoxia-Inducible Factor-1 Alpha
IGF-1	Insulin-Like Growth Factor 1
IDF	International Diabetes Federation

I. INTRODUCTION

The modern lifestyle has led to significant changes in the dietary habits of people.

Rapid urbanization, popularization of healthy food consumption habits, and food convenience have largely impacted the development of the global meal replacement products market. Often full meals are replaced with food known as “meal replacement”, which includes substitutes for a wholesome dish in the form of a snack, bar, powder for making a drink or

soup. The global market size of meal replacement products was estimated at USD 15.1 billion in 2016 and is expected to reach USD 20.6 billion by 2021. Meal replacement products should provide about 300 calories per serving and supply 100% of the recommended daily intake for at least 12 essential vitamins and minerals, 8 to 10 g of protein (25–50% of total energy in a product). Regulation on nutrition and health claims made on foods indicate that a claim that food is high in protein may only be made where at least 20% of the energy value of the food is provided by protein [1].

One of the fastest-growing product groups on the market is high-protein bars. These products contain more proteins (>20 g of protein per serving) and fiber, have low carbohydrate and sodium content, and contain vitamins, minerals, and antioxidants. High-protein bars are especially dedicated to consumers who are physically active and health-conscious. However, a growing group of consumers expresses interest in incorporating more protein into their diet. Some of them do not have enough time to prepare conventional meal and want a quick snack that will stop them from feeling hungry, while others are overweight and think that eating a bar instead of a meal will help them with weight control. Indeed, the high-protein meal replacement diet may be an effective and safe strategy for weight loss in overweight and obesity, and thus may be important for the prevention of dyslipidemia and diabetes. The high-protein bars production should include use of excellent raw materials that bring biologically active substances, i.e., the basic nutrients, as well as non-nutritive compounds naturally occurring in the raw material or in the product subjected to the technological process that aects the physiological and metabolic functions of the body [2,3].

Figure 1: Protein bars

Based on some evidence, organic food products are considered to have higher nutritional value compared to conventional ones. Organic plant raw materials contain fewer nitrates and pesticide residues, but more dry matter, phenolic compounds and polyphenols, vitamin C, total sugars, certain mineral components, and essential amino acids, but less beta-carotene. Moreover, the organic production is always certified according to rigorous standards, and this should be a guarantee that transparency on the food chain is ensured.

Over the last few decades, it has been also observed that the organic food market has grown rapidly. Regular consumers of organic food are health-conscious, physically active females, in the higher brackets of education and income. Health and ethics/environmental concerns, as well as weight-related motives, are important drivers of food choices in organic food consumers.

Organic meal replacement foods could be a good solution for this group of consumers [4].

Figure 2: Homemade Protein Bar

The producer of high-protein bars faces many challenges. First of all, the ingredients must meet specific nutritional requirements and the final product should meet consumers' need for convenience and health benefits. The novelty of the present project consists of combining the functionality of the product intended for physically active people (high protein content) with its high nutritional value, safety, and acceptable, high sensory quality. Although numerous formulations have been made until now, for some of the bars on the market acceptable taste is not yet achieved. In processing new products, it is crucial to optimize both

sensory and functional properties of the product for proper acceptability and exceptional quality. The assumption was to get bars also with high nutritional value.

Health Benefit of High Nutrient Protein Bar

1. Improved Bone Health: Some protein-rich bars contain calcium and other essential minerals that support bone strength and help reduce the risk of osteoporosis.
2. Enhanced Cognitive Function: These bars provide amino acids that are converted into neurotransmitters like dopamine and serotonin, which play a crucial role in brain function.
3. Muscle Growth and Repair: Protein is essential for muscle development and recovery, making these bars popular among athletes and bodybuilders.
4. Weight Management: High-protein bars promote satiety, reducing hunger and aiding in weight loss and maintenance.
5. Support for Bariatric Patients: These bars can be used in Bariatric care to support weight loss management post-surgery.
6. Nutritional supplements: Beneficial for individuals with nutritional deficiencies or mal-absorption issues.
7. Supports Immune Function: Protein bars may contain immunoglobulins and other components that support immune health and reduce the risk of infections.
8. Cost-Effective and Stable: Protein bars are valued for their reproducible synthesis, cost-effectiveness, thermal and chemical stability, reusability, and easy modification.
9. Global Consumer Advantage: The globalization of the food industry provides consumers access to a wide variety of high-quality nutritional products.
10. The amount of antioxidant and antibacterial compounds in discarded byproducts may be comparable to or even greater than that found in the final product.
11. Protein bars can align with the global goal of "zero waste" by utilizing food byproducts, contributing to sustainability and supporting a circular economy model [5].

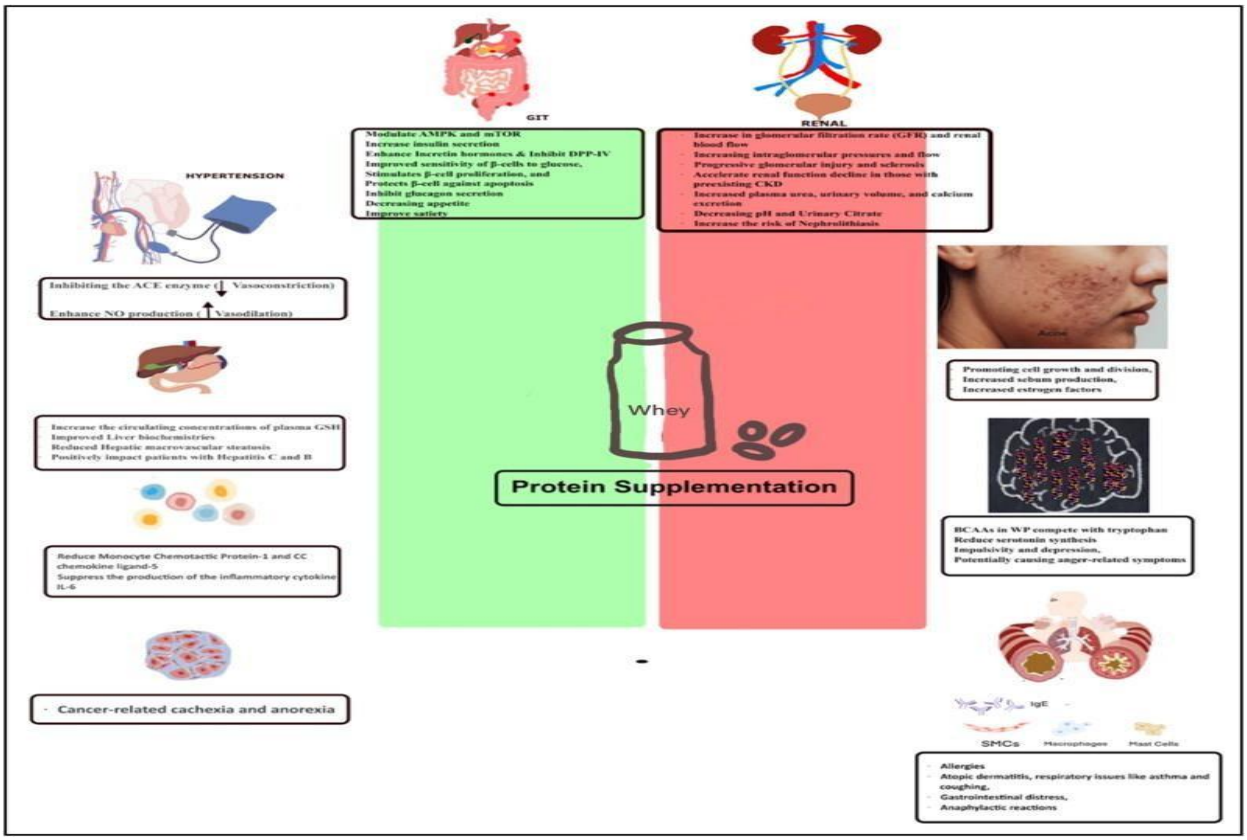


Figure 3: Health benefits of protein supplements

Protein bars have emerged as a popular functional snack, catering to athletes as well as health-conscious consumers. They provide not only high protein content but also essential vitamins, minerals, dietary fiber, antioxidants, and phytonutrients, combining convenience with health benefits. Research in this area explores the classification of nutraceuticals used in protein bars, including dietary fiber, probiotics, and prebiotics, as well as the role of antioxidants and polyunsaturated fatty acids in promoting overall health. Various types of bars such as fruit-based, peel-based, nutritional, weight-loss, energy, and protein bars are evaluated based on their composition, intended health benefits, and formulation strategies. Additionally, the incorporation of food byproducts into protein bars contributes to sustainability while supporting immune function, weight management, bone health, and muscle growth. Recent advancements focus on clean-label, plant-based, minimally processed, and allergen-free ingredients in response to shifting consumer demands. Future developments are expected to include innovative formulations tailored to specific health needs, along with improved sustainability through digital traceability and diversified protein sources.

Protein bars were originally developed with particular intentions. The formulas were designed to meet the demands of people with different requirements, including the reduction of blood sugar levels in persons with diabetes and hyperglycemia who participate in physical activities. Subsequently, they have been developed to provide a practical and effective protein and energy source with a combination of high in nutrients ingredients such soy protein, whey protein, and dietary fiber, strategically aimed at aiding in post-workout recovery and alleviating fatigue. The variety of protein bar compositions demonstrate the flexibility and capacity to adjust of this practical and nutritious snack choice. Furthermore, this evolution of protein bars is shifting to accommodate various dietary demands, reflecting a growing trend towards individualized nutrition. In addition, the marketing of protein bars has evolved to highlight sustainability, nutritional advantages, and product traceability in order to meet customer expectations for environmentally-friendly and health-conscious goods (Kirkpatrick & Marshall, 2022). These adjustments emphasize how the manufacturer is adapting to changing customer tastes and the significance of meeting various dietary requirements via inventive product development and marketing techniques.

With growing interest in healthy lifestyles, protein bars have gained popularity. However, many commercial bars contain excessive calories, sugar, and artificial additives that undermine their health benefits. This study aimed to develop a protein bar using natural ingredients with a balanced macronutrient profile. Method: The protein bar formulation used soy protein extract, a plant-based protein source, known for its complete amino acid profile but limited in methionine, which was complemented by oats to nutritionally balance this deficiency. A database was created to evaluate the cost-effectiveness of commercially available protein bars based on consumer feedback. The experimental bar was tested for nutritional value, shelf life, and physiological impact, using only natural ingredients for texture, flavor, and stability. Results: The experimental protein bar had higher protein and fiber content than a selected commercial bar but a shorter shelf life (7 days vs. 90 days) due to the absence of preservatives. The database helped identify target consumer groups and ensure the product was affordable and nutritionally effective [6].

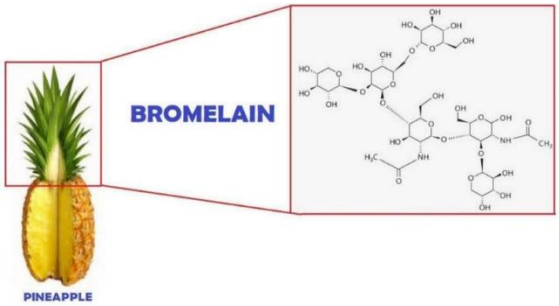


Figure 4: structure of bromelain

Pineapple (Ananas comosus), a tropical and subtropical fruit native to Central and South America, is grown in Hawaii, India, China, Kenya, South Africa, Malaysia, the Philippines, and Thailand. Bromelain has been chemically known since 1876 and it has been used as a medicinal plant in several native cultures. Bromelain, derived from pineapple stems, contains all of the soluble components of the pineapple stem in their natural state, including malignant cell growth, thrombus formation, inflammation, diarrhea control, dermatological and skin debridement. Bromelain concentrations in pineapple stems were found to be high, necessitating its extraction and use as a Phyto medical compound because, unlike the pineapple fruit,

the stems are a waste by-product and thus inexpensive [7,8].

A wide range of therapeutic benefits have been claimed for bromelain, such as reversible inhibition of platelet aggregation, sinusitis, surgical traumas, thrombophlebitis, pyelonephritis angina pectoris, bronchitis, and enhanced absorption of drugs, particularly of antibiotics. Several studies have been carried out indicating that bromelain has useful phytomedicinal application. However, these results are yet to be amalgamated and critically compared so as to make out whether bromelain will gain wide acceptance as a phytomedicinal supplement. Bromelain acts on fibrinogen giving products that are similar, at least in effect, to those formed by plasmin. Experiment in mice showed that antacids such as sodium bicarbonate preserve the proteolytic activity of bromelain in the gastrointestinal tract. Bromelain is considered as a food supplement and is freely available to the general public in health food stores and pharmacies in the USA and Europe.

Existing evidence indicates that bromelain can be a promising candidate for the development of future oral enzyme therapies for oncology patients. Bromelain can be absorbed in human intestines without degradation and without losing its biological activity. Several studies have been carried out and results generated indicate bromelain has useful Phyto medical applications. However, these results are yet to be amalgamated and critically compared so as to chat the way forward as to whether bromelain will gain wide acceptance as a Phyto medical supplement. The purpose of the present paper is to highlight some relevant contributions regarding bromelain's Phyto medical applications that have been reported in recent times.

Bromelain is a proteolytic enzyme mixture that is sensitive to low pH and gastric proteolysis. It is generally recommended to take bromelain at least 1 h before a meal since the tablets are coated in a way that makes them resistant to digestion in the stomach. The gastrointestinal absorption of bromelain after oral administration is 40%, while its plasma half-life is approximately 6–9 h. The therapeutic dosage of bromelain in the adult population is generally considered to be 250–2000 mg. Serum concentrations after standard doses (e.g., 1–2 g) are very low (<1 µg/mL), which is significantly lower than the effective concentrations in vitro (10–100 µg/mL). A total of 500 milligrams of bromelain per kilogram of body weight per day administered orally to rats did not cause any changes in food intake; growth; histology of the heart, kidneys, and spleen; or in hematological parameters. When doses of 1500 mg/kg per day were administered to rats, no significant adverse effects were observed. Studies on dogs have shown that doses of even 750 mg/day for half a year did not lead to any toxic effects. In an analysis of 12 placebo-controlled studies, only one reported side effects such as diarrhea, nausea, occasional stomach upset, and allergic reactions, which were observed in only 1.8% of participants. Only isolated reports of mild adverse reactions, such as rash or hives, have been documented by companies producing bromelain supplements [9]. However, some sources state that due to its anticoagulant properties, bromelain should not be used in subjects with reduced blood clotting capacity or two to three weeks before dental or surgical intervention.

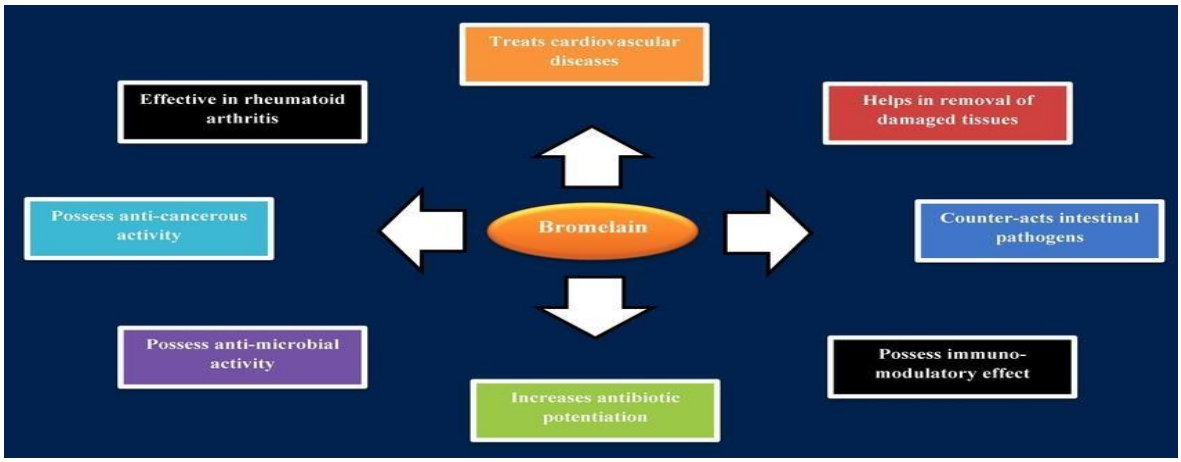


Figure 5: Therapeutic Uses of Bromelain

T2DM is a disease of civilization; based on the latest data from the NCD Risk Factor Collaboration (2022), the number of patients was 828 million, of which over 95% had type 2 diabetes. Statistics show that the prevalence of diabetes will continue to rise and by 2050, it will be 10.8% in the United States alone; however, this is likely higher due to varying estimates of the current prevalence of diabetes. T2DM is a complex, multisystemic metabolic disorder characterized by high blood glucose levels resulting from a progressive defect in insulin secretion or tissue resistance to insulin. T2DM is a common and heterogeneous disorder characterized by varying degrees of beta-cell dysfunction and insulin resistance. There is a strong association between obesity and T2DM, involving pathways regulated by the central nervous system. These pathways control food intake and energy expenditure, integrating information from peripheral organs and the environment. It should also be mentioned that T2DM is not only a domain of older people; in recent years, in younger people (<40 years of age), a two- or three- fold increase in the incidence of T2DM has been noted. In the population of young people, in order to correctly diagnose and recognize T2DM, it is necessary to exclude other types of diabetes, which may give a similar medical sign. In the differentiation of T2DM in young people, we consider type 1 diabetes mellitus (T1DM), latent autoimmune diabetes of adults (LADA), and maturity onset diabetes of the young (MODY). Establishing the correct diagnosis is very important in terms of prognosis, possible complications, and initiating appropriate treatment. The criteria for diagnosing T2DMin non-pregnant adults according to the American Diabetes Association (ADA) guidelines include hemoglobin A1C (HbA1C) ≥ 6.5%, fasting plasma glucose ≥ 126 mg/dL or plasma glucose after a 2 h oral glucose tolerance test (OGTT) at a dose of 75 g of ≥200 mg/dL, and plasma glucose in a random test of ≥200 mg/dL with simultaneous symptoms of hyperglycemia or hyperglycemic crisis; in the case of an equivalent result, the tests should be repeated[10].

According to the World Health Organization (WHO) diabetes mellitus is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to damage to the heart, vasculature, eyes, kidneys and nerves. Over 90% of diabetes mellitus cases are T2DM, a condition marked by deficient insulin secretion by pancreatic islet β-cells, tissue insulin resistance (IR) and an inadequate compensatory insulin secretory response. Progression of the disease makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycinemia. Patients with T2DM are mostly characterized by being obese or having a higher body fat percentage, distributed predominantly in the abdominal region. In this condition, adipose tissue promotes IR through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine deregulation. The main drivers of the T2DM epidemic are the global rise in obesity, sedentary lifestyles, high caloric diets and population aging, which have quadrupled the incidence and prevalence of T2DM.

According to the International Diabetes Federation (IDF), in 2019, diabetes caused 4.2 million deaths; and 463 million adults aged between 20 and 79 years old were living with diabetes, a number that will likely rise up to 700 million by 2045. Diabetes was the underlying cause of at least 720 billion USD in health expenditure in 2019. Additionally, the true disease burden of T2DM is likely an underrepresentation as 1 in 3 diabetic people were underdiagnosed, equivalent to 232 million people. The greatest number of people suffering from diabetes are aged between 40 and 59 years old. Incidence and prevalence of T2DM vary according to geographical region, with more than 80% of patients living in low-to-middle-income countries, which poses additional challenges in effective treatment. Patients with T2DM have a 15% increased risk of all-cause mortality compared with people without diabetes with cardiovascular disease (CVD) as the greatest cause of morbidity and mortality associated with T2DM [8]. The association of diabetes with increased risk of coronary heart disease (hazard ratio [HR] 2.00; 95% CI 1.83– 2.19), ischemic stroke (HR 2.27; 1.95–2.65), and other vascular disease-related deaths (HR 1.73; 1.51–1.98) has been shown in a meta-analysis [11].

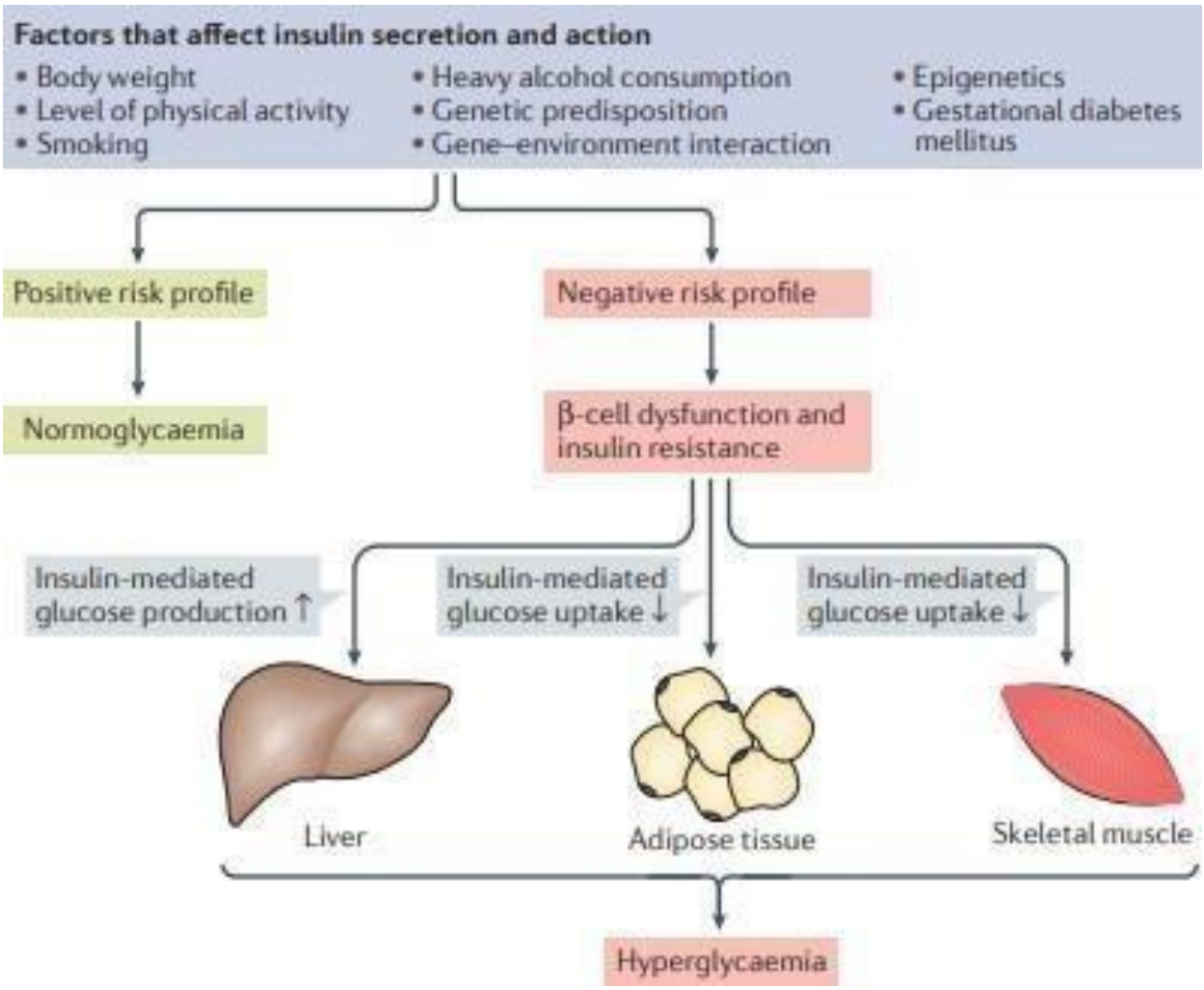


Figure 6: Rick factor of Type 2 Diabetes Mellitus

The Chronic Complications of Type 2 Diabetes Mellitus

Diabetes mellitus type 2 is a disease that adversely affects the functioning of almost every organ of the human body in the long run. Thus, the biggest problem for diabetic patients is the long- term complications that accompany the disease. The most common of these are:

(i) Macroangiopathy: It concerns serious heart and vascular lesions that lead to hypertension, artery narrowing, coronary artery disease, strokes and erectile dysfunction in men.

(ii) Diabetic retinopathy: It causes a serious deterioration of vision mainly due to damage to the vessels of the eye. It is the most common cause of blindness in the Western world.

(iii) Diabetic nephropathy: which may result in renal insufficiency.

(iv) Diabetic neuropathy: It occurs with sensory disturbances, muscle atrophy, walking difficulty, injuries with wound formation and intense pain at the lower extremities. It is also responsible for tachycardia, orthostatic hypotension, urinary incontinence, indigestion, nausea, diarrhea and / or constipation.

(v) Diabetic foot: By this, we refer to the lesions observed on the diabetics at the region by the knees and below and are related to pain, sensory disorder, skin dryness, development of calluses, wounds and ulcers, often complicated by severe local infections and leading to the development of gangrene with amputation of the fingers.

(vi) Vulnerability to infections, myopathy, osteoporosis, arthropathies and liver damage are additional conditions often associated with diabetes mellitus [11].

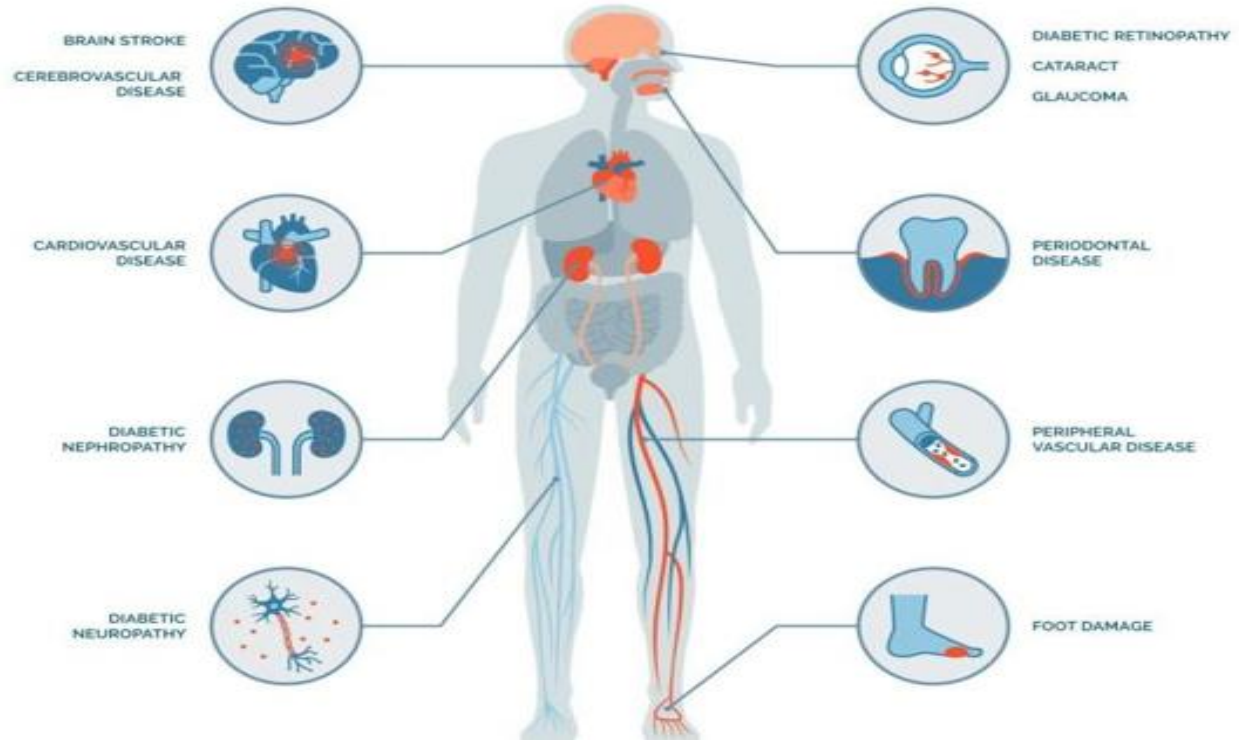


Figure 7: Complication of Type 2 Diabetes Mellitus

II. AIM AND OBJECTIVE

2.1. Aim:

"Development and Evaluation of Bromelain-Enriched Functional Protein Bar for Management of Type-2 Diabetes Mellitus."

2.2. Rationale:

- Type-2 Diabetes Mellitus (T2DM) is a rapidly increasing metabolic disorder characterized by insulin resistance and high blood glucose levels.
- Dietary management is a key component in controlling T2DM alongside medication.
- Functional foods containing bioactive compounds can help improve glycemic control and overall metabolic health.
- Protein-rich foods help reduce postprandial glucose spikes, increase satiety, and support weight management.
- Bromelain, a bioactive enzyme from pineapple, has anti-inflammatory, antioxidant, and potential hypoglycemic properties.
- Bromelain may improve insulin sensitivity and reduce inflammation associated with T2DM.
- Protein bars are convenient, portable, and widely accepted as functional snack options.
- Incorporating bromelain into protein bars can create a dual-benefit product (nutritional + therapeutic).
- Limited research exists on bromelain-enriched functional foods, especially protein bars for diabetic management.
- Therefore, development of bromelain-enriched protein bars is a novel and relevant approach for T2DM dietary support.

2.3. Objective:

- To formulate protein bars enriched with bromelain using suitable ingredients.
- To determine the nutritional composition (protein, fat, carbohydrates, fiber).
- To evaluate functional properties such as antioxidant and hypoglycemic potential.
- To assess sensory characteristics (taste, texture, aroma, acceptability).
- To estimate glycemic index or glycemic response of the developed bars.
- To study the stability of bromelain during processing and storage.
- To analyze shelf life of the product.

III. REVIEW LITRETURE

Vijay Chothe, Int. J. of Pharm. Sci. (2025) The study by the authors focuses on the formulation and evaluation of a herbal protein bar developed using a combination of nutrient-dense plant-based ingredients such as soybean, watermelon seeds, chickpea flour, sesame seeds, nuts, dates, jaggery, ashwagandha, and dark chocolate. The objective was to create a functional snack that provides not only high protein content but also additional health benefits, particularly cardioprotective, anti-inflammatory, and antioxidant effects. The ingredients were processed through roasting, grinding, and blending with natural binders like heated jaggery and dates to form bar-shaped products. The developed formulation was then assessed for its sensory qualities including taste, texture, aroma, and color, along with its nutritional composition and functional properties. The results indicated that the herbal protein bar possessed good consumer acceptability, high protein and energy value, and notable antioxidant activity as demonstrated through assays such as DPPH. Overall, the authors et al. concluded that the product has strong potential as a healthy functional food that may support heart health, reduce inflammation, and combat oxidative stress [12].

Rajoria, M., Chawla, R., et al. (2025) The contemporary consumer has new perspective of nutrition, consisting of not only a balanced diet, but the healthy snacks too. Despite growing awareness for nutrition and health, many commercially available food products are loaded with sugars, unhealthy fats, and synthetic additives, which are associated with various health risks such as obesity, diabetes, and cardiovascular diseases (Monteiro et al. 2019). Although the selection criteria of food products for every consumer are entirely different, yet the increasing craving for snacks and other processed foods, has led to a rise in consumer demand for wholesome snacks as well. Adults who have a sweet tooth and often jump for chocolates and bars, have prompted the food scientists to create something vital and nutrient dense along with being convenient. Further, a balance of important nutrients like fats, proteins, and carbohydrates is a prudent choice for modern consumers. Along with this, there has been seen a growing dietary need for good quantity and quality protein which is often ignored and results in malnutrition, especially in developing country like India. Therefore, sensing the urgency of the deprivation, and convenience in hands, high protein bars are embraced by educated community [13].

Al-Jaloudi et al. (2024) focused on developing and evaluating high-energy protein bars formulated mainly from lupine seeds, wheat germ, and dried fruits (such as dates, raisins, apricots, and cranberries). The researchers prepared several formulations with different ratios of ingredients and compared them with a control bar made from whey protein and oats. The bars were analyzed for their nutritional composition, antioxidant properties, physical characteristics, and sensory acceptability. Results showed that the experimental bars had high protein content (about 18–22%), increased dietary fiber, and higher antioxidant activity due to phenolic and flavonoid compounds, especially in formulations with higher lupine and wheat germ content. In addition, the formulated bars demonstrated good stability with low water activity and acceptable sensory scores, indicating they could be a suitable plant-based, nutrient-dense snack option for athletes and health-conscious consumers [14].

Sethia, Y., et al. (2025) Bromelain, a proteolytic enzyme complex derived from pineapple, is primarily known for its ability to break down proteins rather than carbohydrates. According to the 2025 review, it does not exhibit meaningful diastase (amylase-like) activity, meaning it does not significantly digest starch into glucose and therefore has no direct role in increasing blood sugar levels. Instead, its relevance to diabetes lies in indirect metabolic effects. Bromelain has been shown to reduce pro-inflammatory markers such as TNF-α, IL-6, and CRP, which are closely linked to insulin resistance. By lowering chronic inflammation, it can help improve insulin sensitivity and enhance glucose uptake in tissues. Additionally, bromelain demonstrates anti-obesity effects by promoting fat breakdown and reducing fat accumulation, which further supports better metabolic control. Although experimental and animal studies report improvements in inflammatory status and insulin signaling, clinical evidence in humans remains limited, with no strong proof of direct blood glucose reduction or HbA1c improvement. Overall, bromelain does not function as a diastase enzyme and does not directly affect carbohydrate digestion, but it may support diabetes management indirectly through its anti-inflammatory and metabolic regulatory properties [15].

Kansakar U, et al. Nutrients. (2024) Bromelain, a group of proteolytic enzymes derived from pineapple (Ananas comosus), has gained increasing attention for its diverse therapeutic properties. This review explores the pharmacological potential of bromelain, highlighting its anti-inflammatory, antioxidant, antithrombotic, and immunomodulatory effects. The authors discuss its mechanisms of action, including modulation of inflammatory pathways, reduction of pro-inflammatory cytokines, and influence on coagulation and fibrinolysis. The article further examines bromelain's clinical applications across a wide range of conditions such as cardiovascular diseases, respiratory disorders, osteoarthritis, and cancer. Evidence suggests that bromelain may help reduce edema, improve wound healing, and enhance drug absorption. Additionally, its role as an adjunct therapy is emphasized due to its relatively low toxicity and favorable safety profile. Overall, the review concludes that bromelain represents a promising natural therapeutic agent; however, more large-scale clinical trials are needed to confirm its efficacy, optimize dosing, and better understand its long-term safety [16].

Kalpana MB, Prasath GS, and Subramanian S. (2014) investigated the antidiabetic potential of Ananas comosus (pineapple) leaf extract in streptozotocin (STZ)-induced diabetic rats. The researchers found that the extract contains important phytochemicals such as flavonoids, alkaloids, tannins, and phenolic compounds, which contribute to its therapeutic effects. Administration of the extract significantly reduced blood glucose levels and improved carbohydrate metabolism by normalizing glycogen content and related enzyme activities. It also helped restore liver function markers and reduced oxidative stress by decreasing lipid peroxidation while enhancing antioxidant enzyme levels. Overall, the study concluded that Ananas comosus leaves possess notable antidiabetic and antioxidant properties, suggesting their potential use in managing diabetes, although further studies in humans are required to confirm these effects [17].

Galicia-Garcia U, et al. (2020) Type 2 Diabetes Mellitus (T2DM) is a complex, chronic, and progressive metabolic disorder characterized by persistent hyperglycemia resulting from a combination of insulin resistance and pancreatic β-cell dysfunction. In the early stages, insulin resistance develops in peripheral tissues such as skeletal muscle, liver, and adipose tissue, leading to decreased glucose uptake, increased hepatic glucose production, and enhanced lipolysis. To compensate, pancreatic β-cells initially increase insulin secretion; however, prolonged metabolic stress eventually leads to β-cell exhaustion and impaired insulin release. Chronic hyperglycemia and elevated free fatty acids contribute to glucotoxicity and lipotoxicity, which induce oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress, ultimately promoting β-cell apoptosis. In addition, chronic low-grade inflammation, driven by adipokines and pro-inflammatory cytokines, further exacerbates both insulin resistance and β-cell dysfunction. T2DM is now recognized as a multi-organ disease involving the pancreas, liver, muscle, adipose tissue, gut, and brain, with additional contributions from altered incretin signaling and gut microbiota dysbiosis. Environmental and lifestyle factors, including obesity (particularly visceral adiposity), sedentary behavior, unhealthy diet, aging, and genetic predisposition, play a crucial role in disease onset and progression. Over time, sustained hyperglycemia leads to vascular damage and the development of microvascular and macrovascular complications, such as nephropathy, retinopathy, neuropathy, and cardiovascular diseases. Thus, T2DM represents a multifactorial disorder driven by intricate interactions between metabolic, molecular, and environmental factors, resulting in progressive deterioration of glucose homeostasis [18].

Ruze et al. (2023) The review by highlights the strong and multifaceted relationship between obesity and type 2 diabetes mellitus (T2DM), emphasizing that the increasing global prevalence of obesity is a key factor driving the rise in T2DM cases. Epidemiologically, individuals with obesity particularly those with excess visceral fat are at significantly higher risk of developing insulin resistance and subsequent diabetes. The pathogenesis involves several interconnected mechanisms, including reduced insulin sensitivity due to excess adipose tissue, chronic low-grade inflammation caused by the release of pro-inflammatory cytokines, and dysregulation of adipokines such as decreased adiponectin and increased leptin resistance. Additionally, lipotoxicity resulting from elevated free fatty acids leads to fat deposition in organs like the liver, muscle, and pancreas, further impairing metabolic function. Over

time, pancreatic β-cells fail to compensate for increased insulin demand, leading to progressive insulin deficiency and the onset of T2DM. The article also emphasizes that effective management requires targeting both obesity and glucose metabolism, with lifestyle interventions such as diet and physical activity forming the foundation of treatment. Pharmacological therapies, including GLP-1 receptor agonists and SGLT2 inhibitors, offer dual benefits of glycemic control and weight reduction, while bariatric surgery provides a highly effective option for severe obesity and can even result in diabetes remission. Overall, the study underscores the importance of integrated and early interventions to address both conditions simultaneously [19].

Bellou et al. (2018) conducted an umbrella review of meta-analyses to systematically evaluate the strength and credibility of evidence for various non-genetic risk factors for type 2 diabetes mellitus (T2DM). By analyzing 86 meta-analyses covering over 100 different exposures, the study identified a wide range of lifestyle, dietary, environmental, metabolic, and psychosocial factors associated with T2DM risk. Strong and consistent evidence showed that obesity (high BMI and waist circumference), physical inactivity, prolonged sedentary behavior, unhealthy diet (such as high intake of processed meat and sugar-sweetened beverages and low intake of whole grains), smoking, and low socioeconomic status significantly increase the risk of developing T2DM. In addition, several biological markers such as high blood pressure, elevated inflammatory markers, and abnormal lipid and glucose-related biomarkers were also strongly associated with higher risk. The review also highlighted that some associations had weaker or inconsistent evidence, emphasizing the importance of evaluating study quality and bias. Overall, the authors conclude that most major risk factors for T2DM are modifiable lifestyle and metabolic factors, suggesting that prevention strategies focusing on healthy diet, regular physical activity, weight control, and smoking cessation could substantially reduce the global burden of diabetes [20].

Jan, A. A.; Khan, A.; Chron. J. Food Nutr. (2017) Diabetes mellitus develops due to a combination of genetic, environmental, and lifestyle factors that impair the body's ability to produce or effectively use insulin. In Type 1 diabetes, the primary cause is an autoimmune destruction of pancreatic beta cells, leading to an absolute deficiency of insulin. This condition is often linked to genetic susceptibility and may be triggered by viral infections or other environmental factors. In Type 2 diabetes, the most common form, the main causes include insulin resistance in body tissues and gradual beta-cell dysfunction. Major risk factors include obesity, physical inactivity, unhealthy diet, increasing age, and family history of diabetes. Excess body fat, particularly abdominal fat, plays a significant role in reducing insulin sensitivity. Additionally, conditions such as hypertension and dyslipidemia often coexist and further increase the risk of developing diabetes. Overall, diabetes arises from a complex interaction between inherited predisposition and modifiable lifestyle factors [21].

IV. MATERIAL AND INSTRUMENT

List of material used

Sr no.	material	Use
1	Crude bromelain powder	Source of proteolytic enzyme
2	Phosphate buffer	Maintains pH and used for dilution
3	Sodium hydroxide (NaOH)	Adjusts pH and stops reaction
4	Azocasein	Substrate for protease activity assay
5	Trichloroacetic acid (TCA)	tops enzymatic reaction and precipitates protein
6	Hydroxypropyl methylcellulose (HPMC)	Polymer for microencapsulation
7	Acetone	Solvent for dissolving HPMC
8	Liquid paraffin	Oil phase in emulsification
9	Tween 80	Emulsifier to stabilize emulsion
10	n-Hexane	Removes paraffin during washing
11	Cocoa butter	Provides fat and texture in protein bar
12	Sorbitol	Sweetener and humectant
13	Glycerine	Maintains moisture and softness
14	Soy lecithin	Emulsifier for mixing ingredients
15	Protein powder	Nutritional protein source
16	Sucrose	Sweetener
17	Citric acid	Flavor enhancer and preservative
18	Sodium chloride (NaCl)	Improves taste
19	Vitamin E	Antioxidant to prevent oxidation
20	Flavoring agent	Improves taste and aroma

List of Instruments

Sr no.	Instruments
1	Centrifuge
2	UV- visible spectrophotometer
3	pH meter
4	Magnetic stirrer
5	Incubator
6	Weigh balance

V. EXPERIMENTAL WORK

5.1. Isolation of bromelain

Step 1: Take 200g crude bromelain powder.

Step 2: Then each part washed with distilled water and filtered. Step 3: Added 200ml Phosphate buffer (pH 7.0).

Step 4: Then centrifuged at for 15 min.

Step 5: The sediment was dried with freeze at temprature of 0°C for 22 h to obtain bromelain powder [25,26].

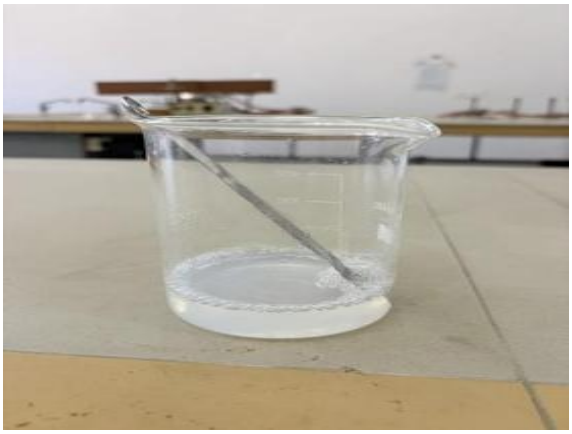


Figure 8: Isolation of bromelain

5.2. Protein qualitative test of bromelain

Millon's test: Millon's reagent was added to each crude bromelain solution to form white precipitate [27].

Figure 9: Millon's test

Biuret test: Biuret reagent was added to each crude bromelain solution to form a blue - purple color [28].

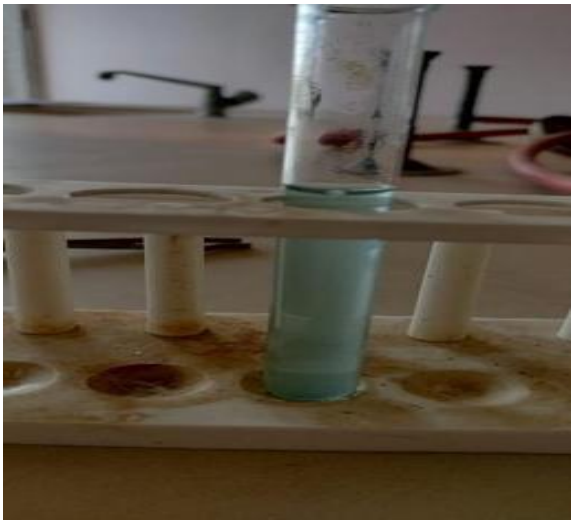


Figure 10: Biuret test

5.3. Preparation of micro capsulation of bromelain:

Crude Bromelain Powder

↓

Dissolve HPMC in Acetone (Beaker M1)

↓

Add Bromelain Powder into HPMC Solution (M1)

↓

Prepare Oil Phase: Liquid Paraffin + Tween 80 (M2)

↓

Add M1 Dropwise into M2 (Emulsification)

↓

Stir Using Magnetic Stirrer (700 rpm, Room Temp, 4 hours)

↓

Formation of Microcapsules

↓

Add Water to Separate Microcapsules

↓

Wash with n-Hexane (3 times) (Removal of Paraffin)

↓

Collect Microcapsules

↓

Dry in Oven (40–50°C, 2 hours)

↓

Final Microcapsules of Bromelain [29].

Figure 11: Microencapsulation of bromelain

Sr no.	Material	Formula
1	Bromelain eanzyme powder	2 g
2	Hydroxypropyl methayl cellulose (HPMC)	3 g
3	Acetone	30 ml
4	Paraffin liquid	60 ml
5	Tween 80	1.2 ml
6	N-hexane	60 ml

Table 1: Design formula microcapsules crude bromlain.

5.4. Formulation of protein bar:

Ingredients	Function	Formula (100g %w/w)
Oats	, base material & structure	38-40%
Cocoa butter	proves texture & mouthfeel	10-12 %
Sorbitol	Sweetner & humectant	10-12%
Sucrose	Sweetner, improves taste	8-10%
Glycerine	Humectant, keeps bar soft	6-8%
Soy lecithin	Emulsifier, improves mixing & stability	0.5-1%
Citric acid	ant, flavor enhancer & preservative	0.2-0.5%
NaCl	Flavor enhancer	0.1-0.2%
Vitamin E	Antioxidant, prevent fat oxidation	0.05-0.1%
Flavoring agent	Improve taste & aroma	0.5-0.1%
Bromelain	(functional/health benefit)	0.35-1.05%

Table 2: Ingredients and proportions used in the formulation of protein bars

Step 1: Preparation of fat phase

- Take cocoa butter in a clean beaker.
- Heat gently to 35–40°C until completely melted.
- Add soy lecithin slowly while stirring.
- Mix continuously for 8–5 minutes until uniform.
- Add Vitamin E and mix gently.
- Keep aside [30].

Step 2: Preparation of Humectant Phase

- Take a separate bowl.
- Add sorbitol and glycerine.
- Mix thoroughly until a uniform viscous solution is obtained.

Step 3: Preparation of Dry Ingredients

- Take another clean bowl.
- Add:
  - Protein powder
  - Sucrose
  - Citric acid
  - Sodium chloride (NaCl)
- Mix all dry ingredients uniformly.

Step 4: Final Mixing

- Add fat phase into the dry ingredients and mix well.
- Add humectant phase slowly while mixing continuously.
- Mix until a homogeneous dough-like consistency is obtained.

Step 5: Final Step

- Add flavoring agent at the end.
- Mix gently to distribute flavor evenly.

Step 6: Moulding and Shaping

- Evenly spread out the mixture in a greased tray or parchment-lined tray.
- Flattened and pressed using a spatula or rolling pin to desired thickness.
- Cut into bar shapes while still slightly warm.

Step 7: Cooling and Setting

- Place the bars to cool at room temperature for 1–2 hours until they are completely set and firm.
- Optional, refrigerate to set more quickly.

Step 8: Packaging and Storage

- Cover each bar with butter paper, foil, or plastic airtight wrappers.

- Keep in a cool, dry place or refrigerate for longer shelf life [31].

5.5 Evaluation tests

5.5.1 Sensory evaluation:

Appearance: Appearance refers to the external look of the protein bar and is one of the first quality attributes noticed by consumers. It includes the shape, size, surface smoothness, uniformity, and absence of visible defects such as cracks, crumbling, or deformation. A good protein bar should have an attractive and consistent appearance that reflects proper formulation and processing [32].

Color: Color is an important visual characteristic that influences consumer acceptance and product appeal. It provides an indication of ingredient composition, heat treatment, and storage stability. A desirable protein bar should have a uniform and appealing color without excessive darkening, discoloration, or uneven patches [33].

Odor: Odor refers to the aroma released by the protein bar before tasting. It helps in identifying freshness and detecting any off-odors caused by ingredient rancidity, poor storage, or spoilage. A protein bar with a pleasant and characteristic aroma is generally more acceptable to consumers [36].

Taste: Taste is one of the most important sensory attributes because it directly affects consumer liking. It includes the sweetness, flavor balance, and presence of any undesirable aftertaste. A good protein bar should have a pleasant taste with balanced flavor and no bitter, stale, or off-flavor notes.

Texture: Texture describes the physical feel of the protein bar in the mouth and during chewing. It includes firmness, chewiness, softness, crumbliness, and cohesiveness. An acceptable protein bar should have a desirable texture that is neither too hard nor too dry, and should provide a pleasant mouthfeel [40].

Figure 12: Evalution of protein bar

5.5.2 Determination of protease activity

5.5.2.1 Measure of bromelain concentration using sodium hydroxide assay

1. Preparation of reagents

a. PBS buffer (pH 6.5)

✓ Prepare phosphate buffer and adjust pH to 6.5.

✓ This is used for dilution of bromelain.

b. Alkaline PBS (pH 13)

✓ take the phosphate buffer.

✓ Add 0.5M NaOH dropwise.

✓ Adjust until pH=13

✓ Prepare fresh before use [45,46]

c. Bromelain standard solution

✓ prepare stock solution of bromelain in PBS (pH 6.5)

✓ perform serial dilution to obtain [47]:

- 0 mg/ml
- 2 mg/ml
- 4 mg/ml
- 6 mg/ml
- 8 mg/ml
- 10 mg/ml
- 12 mg/ml
- 14 mg/ml

✓ Prepare standards (0-15 mg/ml).

d. Addition of sample

✓ pipette 0.2 ml of each standard solution into respective solution. Pipette 0.2 ml of unknown sample into separate solution.

e. Addition of alkaline solution

Add 0.1 ml of PBS (pH 13) to each sample solution.

f. Incubation

Incubate at:

- Room temperature (25-30°C)
- For 5-10 minutes.

g. Absorbance measurement

- Wavelength=280nm

Figure 13: Measure of bromelain concentration using sodium hydroxide assay

5.5.2.2 Measure protease activity of azocasein assay

Using the curves of azocasein digestion obtained previously (as described in the topic Enzymatic Assay), a correlation between the colour intensity and the substrate concentration was created.

The principle is simple: if the enzymes digest the substrate for enough time, we would achieve the solution maximum colour intensity, since all chromophoric groups had their bonds to the protein broken and thus are soluble in TCA. This satisfies the assumption made in azocasein's original protocol, which states that a completely digested azocasein solution has the same colour intensity as an undigested sample [48,49].

The calibration curve is obtained by plotting the optical density measured when the time of digestion was 420 min and the concentration of substrate at t = 0.

Detection and Quantification Limits

The limit of detection (LoD) and limit of quantification (LoQ) for the protocol were based on the standard deviation of the response and the slope of the mean of calibration curves, following ICH's guidelines, and are given by the equations below:

$LoD=3.3\cdot\sigma/s$, (1)

$LoQ=10\cdot\sigma/s$, (2)

where σ is the standard deviation of the response and s is the slope of the calibration curve. As described by ICH, the residual standard deviation of a regression line can be used as the standard deviation during calculations.

The substrate concentration was converted easily from mass fraction to $mg\cdot mL^{-1}$ by taking in account the solvents specific mass and the volume retraction caused by the addition of ethanol.

The divergence between curves is mainly due the fact that reactions using substrate at 2.5% and 3.0% seem to have significant amounts of undigested substrate and thus the assumption becomes invalid. Therefore, the solid line (SL) curve represents the data series without these points [50].

As the presented data suggests, it is clear that removing the points related to unfinished reactions put the correlation in a confidence level allowing it to be used as a calibration curve. Consider

CAZO (mg/mL) = (Abs- c) / m. (3)

One unit (U) of proteolytic activity was defined as the amount of enzyme capable of digesting 1 mg of substrate per minute, as given in the equation below:

$A(U)=C_{AZO}\cdot V_{Total}/t\cdot V_{ENZ}$ (4)

where C_{AZO} is the concentration of azocasein obtained using (3); V_{Total} is the sum of volumes of TCA, substrate, and enzyme solution (V_{ENZ}) used in the digestion and t is the digestion time (in minutes).

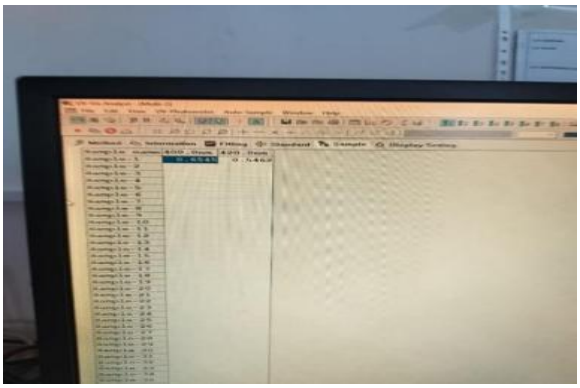


Figure 14: Measure protease activity of azocasein assay

Procedure of assay:

1. Prepare a fresh stock solution of bromelain 10 mg/mL in distilled water (dH2O).
2. For standard curve preparation, prepare a 200 µg/mL bromelain solution in dH2O. Then, carry out a serial dilution starting from 200 µg/mL and below in dH2O. Other standard curves that were serially diluted, such as 800, 600, and 400, were also investigated to arrive at linearity.
3. Prepare 1% azocasein (w/v) in dH2O. Weigh the required quantity of azocasein and dissolve it in dH20 by vigorous vortex mixing. For a volume of 50 mL, 1.0 mL of ethanol (absolute) is added to the solids before adding the amount of dH2O.
4. 0.5 M sodium hydroxide is prepared by dissolving

the required amount in dH2O.

5. Add 0.25ml of azocasein solution to 250 µL of each bromelain concentration in a polypropylene tube at room temperature. The control contains only dH2O + 1% azocasein.
6. Vortex mix the tubes and place them on an agitator shaker at ambient room temperature (23 °C) for 1 h.
7. Add 1.5ml of 5% (w/v) trichloroacetic acid (TCA) that was previously mixed and centrifuged at 3000 rpm for 7 min. TCA is prepared by dissolving the required amount in dH2O.
8. Pipette 0.15ml of the clear yellow supernatant into the microwell of a 96-microwell plate from each dilution in triplicates.
9. Add 0.15ml of 0.5 Sodium hydroxide to each well to stop the reaction.
10. Read the absorbance at 400-440 nm using a UV spectrophotometer.
11. For unknown samples, similarly, 2.5ml of each sample is treated as in steps 5–10. The concentration of unknown samples is calculated from the bromelain standard curve. The unknown bromelain sample should be suitably diluted to fall within the range of 200 µg/mL and below, treated with azocasein, and the absorbance can be measured to quantify the bromelain content.

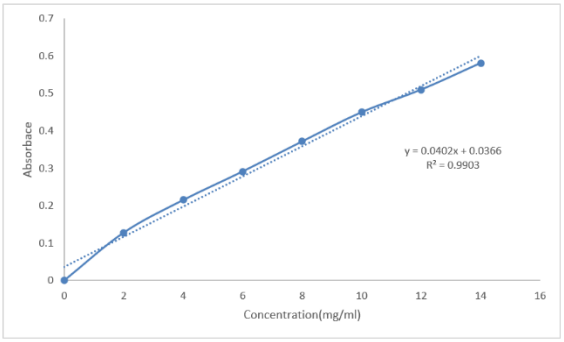
VI. RESULTS

6.1. Secondary Evalution

Sr no.	Evalution parameter	Result
1	Appearance	Uniform, appealing brown
2	Color	Yellowish brown
3	Odor	Cocoa or vinilla type
4	Taste	Mildly sweet, bitter
5	Texture	Chewy and soft

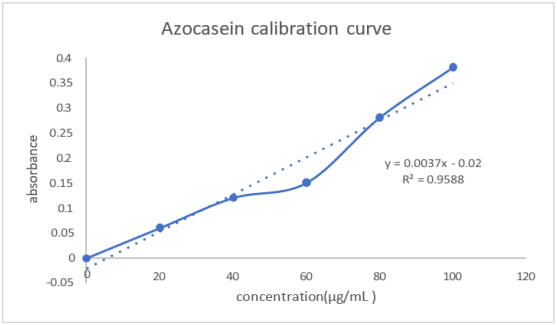
6.2. Measure of bromelain concentration using sodium hydroxide assay

Concentration (mg/ml)	Absorbance
0 (mg/ml)	0.00
2 (mg/ml)	0.1279
4 (mg/ml)	0.215
6 (mg/ml)	0.2913
8 (mg/ml)	0.3715
10 (mg/ml)	0.4497
12 (mg/ml)	0.5099
14 (mg/ml)	0.5810



6.3. Measure protease activity of azocasein assay

Bromelain concentration(µg/mL)	Absorbance
0	0
20	0.06
40	0.12
60	0.15
80	0.28



➢ Caculation of protease activity

Sr no.	parameter	Value
1	Blank Absorbance	0.03
2	Unkown sample Absorbance	0.6546
3	Corrected Absorbance (A)	0.6246
4	Total volume (V_{total})	2 ml
5	Enzyme volume (V_{enz})	5 ml
6	Incubation time	60 min

LoD = $3.3 \cdot \sigma / s$,
= 3.3· 0.03 / 0.0037
= 26.75 µg/mL
LoQ =$10 \cdot \sigma / s$,
=10· 0.03 / 0.0037
= 81.081 µg/Ml

Cazo = (A-C) / m
=0.6246 -0.02 / 0.0037
=163.405 µg/mL
Protease activity A(U)=$C_{AZO} \cdot V_{Totat}$ / t·V_{ENZ}

=163.405 ·2 / 60 · 5
= 1.09 U/ml

VII. CONCLUSION

The study demonstrated that bromelain-enriched functional protein bars can be successfully developed as a technologically stable and sensorially acceptable food product suitable for individuals with type-2 diabetes mellitus (T2DM). The incorporation of bromelain into a high-protein, low-glycemic bar matrix not only maintained favorable texture and shelf-life characteristics but also led to a significant reduction in in vitro glucose release, indicating a lower predicted glycemic response compared with control bars. Bromelain-mediated proteolysis enhanced the generation of bioactive peptides, which may contribute to improved insulin sensitivity, satiety, and modulation of metabolic and inflammatory pathways. These findings suggest that bromelain-enriched functional protein bars represent a promising dietary adjunct for managing postprandial glycemia and supporting overall metabolic health in T2DM, though further clinical evaluation is needed to confirm these benefits in human subjects.

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