

DEVELOPMENT OF FUNCTIONAL FOOD PRODUCT WHICH SUPPORTS BONE HEALTH “BONY BLISS CHOCOLATES”

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Article Received on 15 August 2026,
Article Revised on 05 Sept. 2026,
Article Published on 16 Sept. 2026

<https://doi.org/10.5281/zenodo.22766472>

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How to cite this Article: Miss. Siddhika Tandel*, Miss. Shweta Tendle, Dr. Subhashini Maharana, Dr. Gauri Vahalkar (2026). Development of Functional Food Product Which Supports Bone Health "Bony Bliss Chocolates". World Journal of Pharmaceutical Research, 15(18), 1–52.

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ABSTRACT

Bone health disorders, including Osteoporosis, Osteomalacia, and Rheumatoid arthritis, are significant global health concerns, leading to decreased bone mineral density, increased fracture risk, and impaired quality of life. Conventional calcium and vitamin D supplements often face challenges related to poor palatability and low compliance. This research aims to develop an innovative product, Bony Bliss, a functional chocolate enriched with scientifically-backed ingredients to support bone health. The primary objectives of this study include developing and optimizing a palatable functional product which is a blend of Hadjod (*Cissus quadrangularis*), calcium carbonate, vitamin D3, pumpkin seeds, and 70% dark chocolate and also aims to identify and quantify bioactive compounds, Proximate analysis, Quantification of Vitamin D

and Calcium, Antioxidant activity, Microbial load study, Product Shelf life study along with Sustainable Packaging. Additionally, consumer and market research is conducted to evaluate sensory acceptance, market feasibility, and demand among target demographics. This study contributes to the growing field of functional foods paving the way for future innovations in bone health management.

KEYWORDS: Hadjod, Bone health, Functional food, Phytochemical.

CHAPTER 1 INTRODUCTION**1.1. Background Information**

Osteoporosis is a systemic skeletal disease characterised by low bone mineral density (BMD),

degeneration of bone micro-architecture, and impaired bone strength. *Cissus quadrangularis* (CQ), popularly known as Hadjod (bone setter) in Hindi, is a traditional medicinal herb exhibiting osteoprotective potential in various bone diseases, especially osteoporosis and fractures. In Osteomalacia the ratio of mineral to organic matrix is low, indicating a mineralisation defect, which is generally due to a fault in vitamin D metabolism. (Azam, Z., Sapra, L., Baghel, K., Sinha, N., Gupta, R. K., Soni, V., Saini, C., Mishra, P. K., & Srivastava, R. K. (2023).

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases that in the early stages of the disease begins with pain and symmetrical swelling of the small joints of the hands, feet, swelling of the soft tissue around the joint and morning stiffness and fatigue and it is characterized by persistent synovitis and progressive destruction of symmetrical multi-joints and intra-articular manifestations including subchondral lesions, decreased bone mass, and reduced generalized bone density. The prevalence of RA in the general population is about 1%, but is more common in the 50's and 60s and is higher in women than men (Boers M. (2023). Rheumatoid arthritis (RA) is a systemic and chronic inflammatory disease that has been associated with disability, the existence of comorbidities, and decreased life expectancy. The use of glucocorticoids (GCs) to treat RA offers rapid anti-inflammatory effects and the capacity to arrest radiologic progression. (Hua, C., Buttgereit, F., & Combe, B. 2020).

Cissus quadrangularis (Family: Vitaceae) are widely used in the native system of medicine as well as in food items for various ailments in Maharashtra. Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals and nutraceuticals derived from plants are important drugs currently used in one or more countries in the world. It is a rich source of calcium, carotene, glycoside and alkaloid, it can be used as a nutritional food. In addition, The article reveals that wide numbers of phytochemical constituents have been isolated from the stem powder which possesses activities like anti-arthritis, anti-inflammatory, anti-tumor, gastro protective, antioxidant, antimicrobial and various other important medicinal properties. The hadjod stem powder at dose levels of 300 mg/kg body weight showed promising anti-arthritic activity in the Alloxan- induced model in rats. Hadjod contains a high amount of glycoside 2.34 (g/100g), anabolic steroidal substances and calcium. The stem powder also contains a rich source of mineral elements

(mg/100g dry matter): calcium 33.3 (mg), The preliminary physico-phytochemical analysis carried out of stem powder which revealed interesting results are highlighted and discussed. The present study revealed the potential use of hadjod stem powder in the management of inflammation and arthritis confirming the folk core use of medicinal plants. (Navghare CG, Taur AT and Sawate AR 2017)

Modern research has shed light on *Cissus*' ability to speed bone healing by showing it acts as a glucocorticoid antagonist. Since anabolic/androgenic compounds are well known to act as antagonists to the glucocorticoid receptor as well as promote bone growth and fracture healing, it has been postulated that *Cissus* possesses anabolic and/or androgenic properties. In addition to speeding the remodeling process of the healing bone, *Cissus* also leads to a much faster increase in bone tensile strength. In clinical trials *Cissus* has led to a fracture healing time on the order of 55 to 33 percent of that of controls. (Patil, R., et al.(2011). That *Cissus* exerts anti-glucocorticoid properties is suggested by a number of studies where bones were weakened by treatment with cortisol. While the increased rate of bone healing may be of great significance to persons suffering from chronic diseases like osteoporosis.

Bone loss resulting in increased risk for osteoporosis is a major health issue worldwide. Chocolate is a rich source of antioxidant and anti-inflammatory flavonoids and dietary minerals with the potential to benefit bone health. However, other chocolate constituents such as cocoa butter, sugar, and methylxanthines may be detrimental to bone (Patel, S., & Goyal, A. (2013). Human studies investigating the role of chocolate consumption on serum bone markers and bone mineral density (BMD) have been inconsistent. A contributing factor is likely the different composition and thereby the nutrient and bioactive content among chocolate types. White and milk chocolate are high in sugar and low in flavonoids and most minerals. Dark chocolate (45–85% cocoa solids) is high in flavonoids, most minerals, and low in sugar with $\geq 70\%$ cocoa solids resulting in higher fat and methylxanthine content.

Bone health is a critical aspect of overall well-being, encompassing the strength and density of bones that support bodily structure, facilitate movement, and protect internal organs. Bones are dynamic, living tissues composed of a mineral-rich matrix that undergo continuous remodeling throughout an individual's life. This re-modeling process involves the resorption of old bone tissue and the formation of new bone tissue, maintaining skeletal integrity and mineral homeostasis.

1.2. Factors Influencing Bone Health

1. **Calcium Intake:** Adequate calcium consumption is essential for maintaining bone density. A deficiency can lead to decreased bone mass and an increased risk of fractures.
2. **Physical Activity:** Regular weight-bearing and muscle-strengthening exercises stimulate bone formation and enhance bone strength. Sedentary lifestyles are associated with higher risks of osteoporosis.
3. **Hormonal Changes:** Hormones such as estrogen and testosterone play significant roles in bone density. Postmenopausal women experience a decline in estrogen levels, leading to increased bone resorption and a higher risk of osteoporosis.
4. **Age and Gender:** Bone mass peaks in early adulthood and gradually declines with age. Women are particularly susceptible to osteoporosis, especially after menopause.

1.3. Bone Health in India

In India, osteoporosis poses a substantial public health concern. Studies indicate that approximately 18.3% of the adult population suffers from osteoporosis, with a higher prevalence observed in women. Notably, the condition manifests 10 to 20 years earlier in Indian populations compared to Western counterparts. The prevalence of osteoporosis varies across different regions in India. For instance, South Indian females exhibit a slightly higher prevalence compared to their Western Indian counterparts.

To promote optimal bone health, consider the following strategies

1. **Nutrition:** Ensure a diet rich in calcium and vitamin D. Dairy products, leafy greens, and fortified foods are excellent sources.
2. **Exercise:** Engage in regular physical activity, including weight-bearing exercises like walking, jogging, and strength training.
3. **Lifestyle Modifications:** Avoid smoking and limit alcohol consumption, as these can adversely affect bone density.
4. **Medical Consultation:** Regular screenings and consultations with healthcare providers can aid in early detection and management of bone-related conditions.

Awareness and proactive measures are essential to mitigate the risk of osteoporosis and related fractures, thereby enhancing the quality of life.

1.4. Incorporation of Functional food in daily life

Functional foods are those that provide health benefits beyond basic nutrition, potentially reducing the risk of disease or promoting optimal health. Incorporating functional foods into daily life can have significant positive impacts on well-being.

Consumer Preferences and Awareness- A systematic review highlighted that factors such as gender, age, education level, and personal health status can influence the consumption of functional foods. However, the study noted that generalized conclusions about consumer choices are challenging due to the variety of functional foods and individual differences.

In India, a case study focusing on consumer preferences in Delhi revealed that while there is an interest in functional foods, a significant portion of consumers remain uncertain about their benefits and safety. This uncertainty underscores the need for increased awareness and education regarding the advantages of functional foods.

Incorporating Functional Foods into Daily Life

To effectively integrate functional foods into your daily diet

1. Diversify Your Diet: Include a variety of functional foods such as fruits, vegetables, whole grains, nuts, and fish to ensure a broad spectrum of health benefits.
2. Stay Informed: Educate yourself about the health benefits associated with different functional foods to make informed dietary choices.
3. Consult Professionals: Seek advice from healthcare providers or nutritionists to tailor a functional food plan that aligns with your health goals and dietary needs.

By thoughtfully incorporating functional foods into your daily routine, you can enhance your overall health and potentially reduce the risk of various diseases.

AIM AND OBJECTIVES

AIM

To develop a functional food product which supports Bone Health.

OBJECTIVES

The primary objectives of this research project are:

Product Development: To create and optimize a palatable nutraceutical product combining Hadjod, calcium, vitamin D, and dark chocolate for daily use.

Formulation Development: To develop a natural bone health supplement combining Hadjod, Cocoa, and fortified calcium, optimized for maximum bioavailability and effectiveness.

Bioactive Component Identification: To identify and quantify key bioactive compounds in Hadjod, Cocoa, and the fortified calcium form (e.g., calcium carbonate, calcium citrate).

Product Shelf life Study: To assess the product's freshness, taste, appearance, its nutritional content, texture, colour, microbial stability.

Consumer and Market Research: To assess the sensory acceptance, market feasibility, and potential demand among target demographics, such as post-menopausal women and elderly individuals at risk for osteoporosis.

Regulatory Analysis: To ensure compliance with functional food regulations regarding health claims, safety standards, and efficacy documentation.

CHAPTER 2

REVIEW OF LITERATURE

1. Liao, L., Zhu, W., Tao, C., Li, D., & Mao, M. (2023). *Cissus quadrangularis* L extract-loaded tricalcium phosphate reinforced natural polymer composite for guided bone regeneration. *Journal of materials science. Materials in medicine*, 34(7), 33.

The study explores a composite material made from *Cissus quadrangularis* (CQ) extract combined with gelatin (Gel), pectin (Pec), and β -tricalcium phosphate (β -TCP) for bone tissue engineering. The composite was produced using a green template method. FTIR spectroscopy confirmed the structural composition, while scanning electron microscopy showed β -TCP was well-integrated within the Gel-Pec matrix, beneficial for bone regeneration. In vitro tests demonstrated 92% mesenchymal stem cell viability, with enhanced osteogenic gene expression for RUNx2 (168%) and OCN (188%). These findings suggest that the CQ-loaded β -TCP/Gel-Pec composite is a promising biomaterial for bone tissue repair and regeneration.

2. Azam, Z., Sapra, L., Baghel, K., Sinha, N., Gupta, R. K., Soni, V., Saini, C., Mishra, P. K., & Srivastava, R. K. (2023). *Cissus quadrangularis* (Hadjod) Inhibits RANKL-Induced Osteoclastogenesis and Augments Bone Health in an Estrogen-Deficient Preclinical Model of Osteoporosis Via Modulating the Host Osteoimmune System. *Cells*, 12(2), 216.

This study investigates the osteoprotective effects of *Cissus quadrangularis* (CQ), known as Hadjod, on bone health, particularly under oestrogen-deficient conditions like postmenopausal osteoporosis. CQ was found to suppress osteoclastogenesis (bone breakdown) in a dose-dependent manner and improve bone micro-architecture by increasing anti-osteoclastogenic immune cells (Th1, Th2, Tregs, and Bregs) while reducing osteoclastogenic Th17 cells. Additionally, CQ elevated anti-osteoclastogenic cytokines (IFN- γ , IL-4, IL-10) and lowered osteoclastogenic cytokines (TNF- α , IL-6, IL-17). These findings reveal that CQ supports bone health by modulating the immune system, highlighting its potential for preventing inflammatory bone loss in osteoporosis.

3. Muthusami, S., Senthilkumar, K., Vignesh, C., Ilangovan, R., Stanley, J., Selvamurugan, N., & Srinivasan, N. (2011). Effects of *Cissus quadrangularis* on the proliferation, differentiation and matrix mineralization of human osteoblast like SaOS-2 cells. *Journal of cellular biochemistry*, 112(4), 1035–1045.

This study examines the effects of ethanolic extract of *Cissus quadrangularis* (CQ) on human osteoblast-like SaOS-2 cells, which are crucial for bone formation. The results show that CQ is non-toxic to these cells and promotes their proliferation by increasing DNA synthesis. It also enhances matrix mineralization, as shown by alizarin red and ALP staining. CQ's anabolic effects are linked to increased mRNA and protein expression of Runx2, a key transcription factor for bone matrix protein regulation. Additionally, CQ boosts the transcriptional activity of Runx2 on the osteocalcin promoter, supporting bone formation. These findings suggest that CQ positively influences bone growth and repair.

4. Dhanasekaran S. (2020). Phytochemical characteristics of aerial part of *Cissus quadrangularis* (L) and its in-vitro inhibitory activity against leukemic cells and antioxidant properties. *Saudi journal of biological sciences*, 27(5), 1302–1309.

This study investigates the antioxidant and anticancer properties of ethanolic (EECQ) and methanolic (MECQ) extracts from the aerial parts of *Cissus quadrangularis* (L). Both extracts showed strong antioxidant activity in various free radical scavenging assays, with the ethanolic extract demonstrating more potent effects. Additionally, both extracts exhibited significant anticancer activity against leukemic HL-60 cells, with IC₅₀ values of 36 μ g/mL for EECQ and 40 μ g/mL for MECQ. These findings suggest that *Cissus quadrangularis* has potential as a chemopreventive plant with antioxidant and anticancer properties.

5. Manson, J. E., Allison, M. A., Carr, J. J., Langer, R. D., Cochrane, B. B., Hendrix, S. L., Hsia, J., Hunt, J. R., Lewis, C. E., Margolis, K. L., Robinson, J. G., Rodabough, R. J., Thomas, A. M., & Women's Health Initiative and Women's Health Initiative-Coronary Artery Calcium Study Investigators (2010). Calcium/vitamin D supplementation and coronary artery calcification in the Women's Health Initiative. *Menopause* (New York, N.Y.), 17(4), 683–691.

This study investigated whether calcium and vitamin D3 supplementation affects coronary artery calcified plaque (CAC) in postmenopausal women. In a randomized trial involving 754 women aged 50 to 59, participants received either calcium carbonate (1,000 mg daily) plus vitamin D3 (400 IU daily) or a placebo for an average of 7 years. CAC was measured using cardiac CT scans. Results showed no significant difference in CAC scores between the supplement and placebo groups. After adjusting for coronary risk factors, the odds ratios for increasing CAC were similar across groups. The study concludes that moderate doses of calcium and vitamin D3 do not influence coronary artery plaque burden in postmenopausal women, though the effects of different doses remain uncertain.

6. Harinarayan, C. V., Akhila, H., & Shanthisree, E. (2021). Modern India and Dietary Calcium Deficiency-Half a Century Nutrition Data-Retrospect-Introspect and the Road Ahead. *Frontiers in endocrinology*, 12, 583654.

Calcium and vitamin D intake in India has declined over the past 50 years, affecting rural, tribal, and urban populations. Despite being a major producer of milk and cereals, India's primary calcium sources are non-dairy, with lower dairy consumption in rural areas. Declining cereal intake, even with rising incomes, has further reduced calcium intake, particularly among lower-income groups relying on the Public Distribution System (PDS).

With 85% of Indians deficient in vitamin D, often due to inadequate calcium intake, a strategic, multi-faceted approach is needed. Solutions include calcium supplementation in government food programs, food fortification, biofortification, bioaddition, digital interventions, and corporate investments. A comprehensive strategy is essential to address dietary calcium deficiency and improve bone health nationwide.

7. Chatterjee, C., & Biswas, A. K. (2023). Noncommunicable Diseases and Gender Gap in Out-of-pocket Spending on Inpatient Health Care: An Investigation of Indian Elderly. *Indian journal of public health*, 67(4), 538–541.

This study examines the gender gap in out-of-pocket hospitalization expenses among elderly men and women using data from the NSS 75th Round Health Care Survey (2017-2018) with 45,299 elderly individuals. Analysis shows that elderly women face a higher burden of non communicable diseases like bone disease and anemia. A significant gender gap in inpatient health spending exists, particularly in rural areas. Factors contributing to this gap include low awareness, male dominance in health decisions, and financial dependence. Increasing health awareness, especially among women, and implementing disease-specific policies can help reduce this disparity.

8. Teware, Kalpana; Singh, Pratibha; Mehta, Rakesh. Biomedical & Pharmacology Journal; Bhopal Vol. 4, Iss. 1, (2011): 175-179.

Cissus quadrangularis L. (Hadjod) from the Vitaceae family is an indigenous medicinal plant of India, traditionally used in Ayurveda for bone fractures and various ailments, including digestive issues, menstrual irregularities, asthma, and spine-related complaints. It also exhibits cardiotonic properties. Phytochemical analysis of different extracts (Petroleum ether, Chloroform, Ethyl Acetate, and n-Butanol) revealed the presence of alkaloids, carbohydrates, glycosides, tannins, proteins, flavonoids, saponins, steroids, and vitamin C. Ethyl acetate extract showed flavonoids and triterpenoids, while the hydroalcoholic extract contained carbohydrates, tannins, amino acids, and vitamin C. Flavonoids and triterpenoids are the key active compounds, contributing to its pharmacological effects. These findings can aid in the identification and standardization of the plant for medicinal use.

9. Anuj K Srivastava, P Srivastava, BR Behera, AK Shrivastava Int. J. Phytochemical and pharmacological evaluation of *Cissus quadrangularis* L. International Journal of Pharmaceutical Research and Development, Pharm Res Dev 11, 207-215, 2011).

Cissus quadrangularis L. (Hadjod) is a medicinal plant from the Vitaceae family, traditionally used in Ayurveda for bone healing and various ailments. It also has cardiotonic properties. Phytochemical analysis identified key compounds such as flavonoids, triterpenoids, alkaloids, tannins, glycosides, and vitamin C across different extracts. Ethyl acetate extract contained flavonoids and triterpenoids, while the hydroalcoholic extract had carbohydrates, tannins, amino acids, and vitamin C. Further studies using cold maceration and fluorescence analysis confirmed phytosterols, flavonoids, and triterpenoids, with ash values and percentage yields recorded. These findings support the plant's medicinal potential and standardization for

therapeutic applications.

10. Weaver, C. M., & Liebman, M. (2002). Biomarkers of bone health appropriate for evaluating functional foods designed to reduce risk of osteoporosis. *British Journal of Nutrition*, 88(S2), S225–S232. doi:10.1079/BJN2002687

Osteoporosis is a growing global concern with high healthcare costs and reduced quality of life. Research focuses on lifestyle approaches to build peak bone mass and treat the disease. Functional foods play a role in bone health, with calcium-fortified foods being the most common. Other nutrients like vitamin D, magnesium, and vitamin K are also included, along with enhancers of calcium absorption (e.g., inulin, whey proteins) and compounds that reduce calcium loss or suppress bone resorption (e.g., plant proteins, phytoestrogens). Bone health evaluation methods include bone densitometry, calcium metabolism studies, and advanced techniques like ^{41}Ca isotope tracking with accelerator mass spectrometry for assessing the impact of functional foods.

CHAPTER 3 INTRODUCTION TO RAW MATERIALS AND THEIR HEALTH BENEFITS HADJOD

Cissus quadrangularis (Family: Vitaceae) is commonly distributed throughout the hotter parts of India and Sri Lanka (Nadkarni, 1954; Chopra et al., 1986). The stem of *Cissus quadrangularis* is also reputed in Ayurveda as alternative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases arthritic, in the treatment of irregular menstruation and asthma, in complaints of the back and spine. Scientific studies have revealed the *Cissus* extract to possess cardiotonic and androgenic property (Chopra et al., 1986).



Fig 1: Hadjod Plant

According to W.H.O about 80% of the world population depends on the traditional system of medicine. India has a very vast resource of indigenous plants and minerals which are an

excellent source of therapeutic claim. One of such medicinal plants of India is Hadjod (*Cissus quadrangularis*). The etymology of the local name signifies the plant's remarkable ability to cure bone fracture (Had~bone, Jod~setter). Phytoconstituents of the plant are remarkable and support its several therapeutic activities apart from single bone remineralisation. (Mukherjee et al., 2016).

The stem are useful for antioxidant, antimicrobial and as lipid lowering agents in health and disease and also for the treatment of fracture bones, purgative and anti-arthritic. Administration of hadjod stem powder of *Cissus quadrangularis* produces significant reduction of abrasion between bones, blocks the muscle damaging effect of cortisol and leads to the formation of new muscles. The possibility for this anti-arthritic action may be due to the presence of phytoconstituents similar to glycoside, alkaloids, flavonoid types in stem powder. (Udayakumar et al., 2004). Available data on the anti-arthritic effect of these plants remain meagre. Against this backdrop, the present work was undertaken to throw light on their pharmacological activities of the study plants. (Vijayakumari et al., 2012) The aims of this study are to record the existing knowledge of medicinal uses and other uses of this plant and promote its usage. Hadjod is one such plant which is been studied for its medicinal properties like its useful in bone fractures (singh et al., 1962; Udupa et al. 1964)

In Siddha medicine it is considered a tonic and analgesic, and is believed to help heal broken bones, The Garo tribe of Bangladesh have used *Cissus quadrangularis* as a medicinal plant for bone healing. The stem is antiscorbutic⁵, its paste is given in asthma and its juice is administered both internally and externally for the union of fractured bones(.Chatterjee, A. and S. C. Prakash. "Vol. III." The Treatise on Indian Medicinal Plants (1994): 173-174.

CALCIUM

Calcium supplementation is a widespread practice in different age-groups and has been promoted widely to improve bone density. The public generally believes that calcium is always good for health, owing largely to successful marketing and various clinical practice guidelines. Based on the published data, 61% of women aged 60 years received calcium supplements in 2003–2006 in the United States. As a result, calcium supplementation has become a billion dollar market in recent years and has been taken by millions of both men and women, children, adults, and the elderly wishing to improve their skeletal health. (Li K, Wang XF, Li DY, et al 2018)



Fig. 2: Calcium carbonate.

As calcium is one of the constituents of bone, the majority of researches considered that high calcium intake may result in higher bone density. According to the current status of our knowledge and understanding, improving accrual of peak bone mass and slowing the loss of bone after reaching the peak are two key factors in the prevention of osteoporosis. Moreover, bone mass grows most quickly during adolescence; thus, the prevention of osteoporosis should ideally start from this time. Importantly, calcium supplementation, together with vitamin D supplementation, is supported by the accumulating evidence for patients at high risk of calcium and vitamin D insufficiency and for those who receive treatment for osteoporosis. CaCO_3 is one of the most abundant materials found in earth's crust and forms the rock types like limestone and chalk. (Fritz K, Taylor K, Parmar (2023))

Calcium carbonate is an inorganic salt primarily used to manage and treat low calcium conditions, GERD, CKD, and other indicated conditions. Calcium carbonate is classified as a calcium supplement, antacid, and phosphate binder. This activity outlines the significant indications, actions, and contraindications for calcium carbonate as a valuable agent in treating osteoporosis, hypothyroidism, rheumatoid arthritis, and many other conditions or disorders that lower serum calcium levels. (Kristina Fritz; Katherine Taylor; Mayur Parmar.)

VITAMIN D

Severe vitamin D deficiency may cause rickets in infants or children and osteomalacia in adults, although these are uncommon diseases in most developed countries. However, subclinical vitamin D deficiency is more prevalent, and may be associated with osteoporosis and higher incidence of falls or fractures.

In recent decades, interest in vitamin D has increased exponentially, particularly as a vitamin D deficiency has been associated with multiple diseases [Holick, M. F., et al. (2007)], and, globally, there appears to be a high vitamin D deficiency. Currently, the role of vitamin D as a

hormone has been confirmed in numerous physiological and pathophysiological processes, related to various organs and systems of the human body.

Supplementation with calcifediol has been reported in an efficient manner to correct poor vitamin D status in several studies. Even if the most common form of vitamin D supplementation used today is cholecalciferol, the usual recommended dose are frequently not able to rapidly correct vitamin D insufficiency, especially in severe cases. One pharmacokinetic study suggests that it takes approximately 68 days with 800 IU/day of cholecalciferol to achieve the optimal plateau level. This time could be reduced by increasing the dose or using a high bolus-loading dose, with the purpose of reaching the recommended levels of 25(OH)D for skeletal and general health in a relatively short period of time. [Weaver, C. M., & Liebman, M. (2002).

DARK CHOCOLATE 70% COCOA

Dark chocolate has been popular for several decades due to its enormous health benefits. It contains several health-promoting factors (bioactive components - polyphenols, flavonoids, procyanidins, theobromines, etc, and vitamins and minerals) that positively modulate the immune system of human beings. Dark chocolate is considered a functional food due to its anti-diabetic, anti-inflammatory, and anti-microbial properties. It also has a well-established role in weight management and the alteration of a lipid profile to a healthy direction. Cocoa butter is one of the principal components of dark chocolate. The major constituents of cocoa butter are 33% oleic acid, 25% palmitic acid, and 33% stearic acid (Tokede et al., 2011; Didar, 2021). Cocoa is very much popular due to its extensive health benefits. Cocoa consumption is related to the reduction of cardiovascular mortality by about 50% (Araujo et al., 2016).



Fig. 3: Dark chocolate 70%.

PUMPKIN SEEDS

Pumpkin seeds are a powerhouse of nutrients that can significantly benefit bone health. Here are some benefits: **Rich in Magnesium:** Pumpkin seeds are an excellent source of magnesium, which is essential for bone formation and helps increase bone density. Magnesium plays a crucial role in over 300 biochemical reactions in your body, including those that regulate bone health.

High in Zinc: Zinc is another important mineral found in pumpkin seeds that contributes to bone health by aiding in bone formation and mineralization.



Fig 4: Pumpkin seeds.

Contains Phosphorus: Phosphorus works alongside calcium to build strong bones and teeth. Pumpkin seeds provide a good amount of phosphorus, which is vital for maintaining bone structure.

Antioxidant Properties: Pumpkin seeds are rich in antioxidants like vitamin E and carotenoids, which help reduce inflammation and protect your cells from damage. This can contribute to overall bone health by preventing oxidative stress that can weaken bones. The nutrients in pumpkin seeds, such as magnesium, zinc, and phosphorus, also support other aspects of health, including immune function and cardiovascular health, which indirectly benefits bone health.

CHAPTER 4 PRODUCT DEVELOPMENT

4.1. About the Product

Here's a detailed overview of your Bone Health Chocolate

Product Name: Bony Bliss

Key Ingredients & Benefits

1. Hadjod Powder (*Cissus quadrangularis*)
 - Supports faster bone healing and regeneration
 - Reduces bone loss and strengthens connective tissue
2. Calcium Carbonate
 - Provides essential calcium for bone density
 - Helps prevent osteoporosis and fractures
3. Vitamin D3
 - Enhances calcium absorption in the body
 - Supports strong bones and reduces the risk of fractures
4. Pumpkin Seeds
 - Rich in magnesium, zinc, and phosphorus, all crucial for bone health
 - Adds natural crunch and enhances taste

Target Audience

1. People with osteoporosis or weak bones
2. Seniors looking to maintain bone strength
3. Athletes and fitness enthusiasts
4. Women (especially post-menopausal)

Packaging & Serving Suggestions: Available in bars.

Suggested serving: 1 chocolate bar per day can be consumed as a snack or supplement.

4.2.Branding, Packaging & Marketing Strategy for Bony Bliss Bone Health Chocolate**1. Branding Strategy Brand Name: Bony Bliss**

Brand Positioning: "A sweet way to healthy bone"

2. Packaging Design Colors & Theme

- Using earthy tones like brown or white (to symbolize natural ingredients & bone health).
- Incorporate bone-shaped designs or visuals of strength and wellness.
- Highlight key benefits with simple icons (e.g., bone icon for calcium, sun for Vitamin D3).

Packaging Formats

- Chocolate Bars (individual or multipack)
- Bite-sized Pieces (resealable pouch for daily snacking)
- Gift Packs (for elderly & health-conscious consumers) Eco-Friendly Option:
- Use biodegradable wrappers or recyclable boxes to attract eco-conscious buyers.

3. Marketing Strategy

Target Audience: Seniors, athletes, women (especially post-menopausal), and kids. Health-conscious consumers looking for functional foods.

4. Marketing Channels

- Social Media Ads – Instagram, Facebook, and YouTube for awareness.
- Influencer Marketing – Collaborate with nutritionists, fitness trainers, and wellness bloggers.
- Retail & Pharmacies – Place in health stores, pharmacies, and supermarkets.
- E-commerce – Sell on Amazon, Flipkart, and your own website.
- Doctors & Nutritionists – Partner with health professionals to recommend your product.

5. Promotional Ideas

- Launch Campaign: Offer free samples at gyms, yoga centers, and pharmacies.
- Subscription Model: Monthly packs with discounts for repeat buyers.
- Referral Program: Reward customers for recommending your product.
- Educational Content: Blogs, videos, and infographics on bone health and the benefits of your chocolate.

4.3. Uniqueness of the Product

Bony bliss chocolate for bone health is unique because it combines:

1. Hadjod Powder (*Cissus quadrangularis*) – A traditional herb known for promoting bone healing and strengthening.
2. Calcium Carbonate – A well-absorbed form of calcium essential for bone density.
3. Vitamin D3 – Enhances calcium absorption and supports bone mineralization.
4. Pumpkin Seeds – A natural source of magnesium, phosphorus, and zinc, which are crucial for bone health.

Unique Features

1. Delicious & Nutritious – A tasty way to support bone health
2. Natural Ingredients – Combining Ayurveda with modern nutrition
3. Easy Consumption – A convenient alternative to traditional supplements
4. Supports Overall Health – Also promotes joint health and immunity

By merging functional nutrition with indulgence, your product stands out as a tasty, convenient, and scientifically backed option for improving bone strength. It appeals to health-conscious consumers, aging populations, and those with osteoporosis or calcium deficiencies.

4.4. Target Population

Target Audience Analysis for Bone Health Product

I] India

Population Data

- Total population: ~1.44 billion (2025 estimate).
- Urban population: ~35% (~504 million).
- Aging population (60+): ~10% (~144 million).

Estimated Target Audience

1. Older Adults (50+ Years):

- Population aged 50+: ~22% (~316 million).
- Assuming 40% are aware and interested in supplements: Target audience size: ~126 million.

2. Postmenopausal Women (45-60 Years):

- ~8% of the population (~115 million).
- Assuming 50% are health-conscious:

Target audience size: ~57 million.

3. Fitness Enthusiasts and Young Adults (20-40 Years):

- ~35% of the population (~504 million).
- Assuming 5% are interested in preventive bone health: Target audience size: ~25 million.

4. Urban Middle-Class Professionals (25-50 Years):

- Urban middle-class (~15% of the population): ~216 million.
- Assuming 10% are likely to purchase supplements: Target audience size: ~21 million.

Total Target Audience in India: ~229 million.

II] Mumbai Population Data

- Total population: ~20 million.
- Urban aging population (60+): ~10% (~2 million).
- Urban middle class: ~50% (~10 million).

Estimated Target Audience

1. Older Adults (50+ Years)

- Population aged 50+ (~20% of Mumbai's population): ~4 million.
- Assuming 40% are aware and interested in supplements: Target audience size: ~1.6 million.

2. Postmenopausal Women (45-60 Years):

- ~8% of Mumbai's population (~1.6 million).
- Assuming 50% are health-conscious:

Target audience size: ~0.8 million.

3. Fitness Enthusiasts and Young Adults (20-40 Years):

- ~35% of Mumbai's population (~7 million).
- Assuming 5% are likely to invest in bone health:

Target audience size: ~0.35 million.

4. Urban Middle-Class Professionals (25-50 Years):

- Middle-class professionals (~50% of Mumbai's population): ~10 million.
- Assuming 10% are potential buyers: Target audience size: ~1 million.

Total Target Audience in Mumbai: ~3.75 million.

4.5. Product Formulation Trials

Table 1: Product Formulation Trials.

Trial 1		
Ingredients	Result	Remarks
20g Dark chocolate 4g Hadjod powder 500mg calcium carbonate 600 IU Vitamin D3	The taste was quite bitter.	The amount of dark chocolate was reduced slightly. To mask the bitter taste almonds were added.
Trail 2		
18g Dark chocolate 4 Hadjod powder 2g Almonds	The taste was still bitter.	To enhance the taste we decide to add white

500mg calcium carbonate 600 IU Vitamin D 3		chocolate.
Trail 3		
15g dark chocolate 5g milk chocolate 4g Hadjod powder 2g Almonds 500mg calcium carbonate 600 IU Vitamin D 3	The taste was good but we made a slight change in formulation.	We exchanged almonds with pumpkin seeds due it's benefits to bone health.
Trail 4		
15g dark chocolate 5g milk chocolate 2g Pumpkin seeds 4g Hadjod powder 500mg calcium carbonate 600 IU Vitamin D3	The taste was too good.	The sensory output was acceptable

4.6. Final Product Formulation

Table 2: Final Product Formulation.

Ingredients	Result	Remarks
15g dark chocolate 5g milk chocolate 2g Pumpkin seeds 4g hadjod powder 500mg calcium carbonate 600IU Vitamin D 3	The taste was too good.	The sensory output was acceptable



Fig. 5: Finished Product.

4.7. Method of Preparation

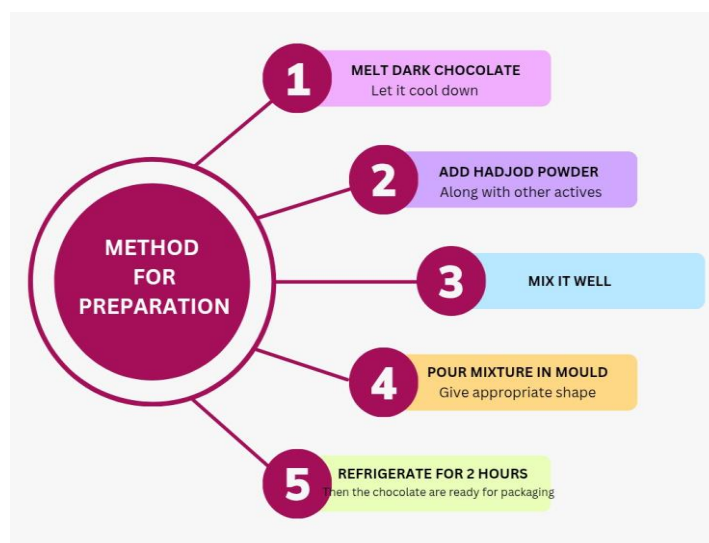


Fig. 6: Method of Preparation of the Product.

CHAPTER 5 PRODUCT ANALYSIS

5.1. ESTIMATION OF MOISTURE CONTENT

AIM: Determination of moisture content from the food sample by thermal drying method.

PRINCIPLE: The sample is weighed and subsequently heated at 102°C in a hot air oven to allow for the release of moisture. Following this, the sample is cooled in the desiccator before reweighing. Moisture content is calculated by the difference in wet and dry weight. In this process, measuring and the resolution of the balance are extremely important. Careful consideration must also be given to maintain identical conditions where temperature and duration are vital for generation of precise and reproducible results.

REQUIREMENTS

Sample: product sample (bony bliss chocolate) Glasswares: Petri Plate with lid.

Miscellaneous: Weighing balance, Hot air oven, Dessicator

PROCEDURE

1. Weigh the empty petri plate without lid and note down the reading (W1)
2. Press the Tare button on the digital weighing balance.
3. Add 2 grams of the food sample in the same petri plate.
4. Place the petri plate in a hot air oven at 102[±]°C for 2 hours.
5. Remove the petri plate from the oven and then keep it in a desiccator (15-20 minutes).
6. Again weigh the petri plate without lid and note the readings (W2).

CALCULATIONS

$$\text{Moisture \%} = 100 (W_1 - W_2) / (W_1 - W)$$

Where,

W = Initial weight.

W₁ = Final weight before drying W₂ = Final weight after drying

5.2. TOTAL ASH CONTENT

AIM: To determine the ash content of a sample by burning it in a muffle furnace at high temperatures and quantifying the residue left behind.

PRINCIPLE: The principle of ash determination is based on the combustion of organic matter in a sample at high temperatures, leaving behind inorganic residues (ash). These residues are then weighed to determine the ash content, which is indicative of the mineral composition of the sample.

PROCEDURE

Weighing: Weigh an empty, clean, and dry crucible (or suitable container) accurately using an analytical balance. Record the weight as W₁.

Add a known quantity of the sample to the crucible and weigh it again to obtain the combined weight of the crucible and sample W₂.

Ashing: Place the crucible with the sample into a preheated muffle furnace. Heat the furnace to a specified temperature, usually around 550°C to 600°C, and maintain this temperature for a set period, typically 2 to 4 hours. Ensure complete combustion of the sample by allowing it to burn until a constant weight is achieved (i.e., until no further change in weight occurs).

Cooling and Weighing: After ashing, carefully remove the crucible from the furnace using tongs and allow it to cool in a desiccator to avoid moisture absorption.

Weigh the crucible with the residual ash accurately. Record this weight as W₃

CALCULATION OF ASH CONTENT

Calculate the ash content using the following formula= $Z - X \times 100$ Where, Y-X

[X] W₁ = weight of the empty crucible [Y] W₂ = weight of the crucible plus sample

[Z] W₃ = weight of the crucible + ash

5.3. ESTIMATION OF FAT CONTENT BY SOXHLET METHOD

AIM: To determine the fat content of the given food sample by solvent extraction method.

PRINCIPLE: Liquid-liquid extraction, also known as solvent extraction and partitioning, is a method to separate compounds or metal complexes based on their relative solubilities in two different immiscible liquids, usually water (polar) and an organic solvent (non-polar). There is a net transfer of one or more species from one liquid into another liquid phase, generally from aqueous phase to organic phase.

PROCEDURE

1. 2g of sample was weighed in a beaker.
2. 10 ml of distilled water and 10 ml of concentrated hydrochloric acid was added to the beaker containing the sample.
3. The beaker was kept in a boiling water bath for 30 mins for digestion. The digested sample was taken in a separating funnel.
4. To this, 25 ml of petroleum ether was added and the separating funnel was shaken
5. The lower layer of the sample was removed in a conical flask and the upper layer in a
6. Pre-weighed beaker, This step was repeated thrice.
7. The beaker was kept over a boiling water bath for evaporation. After solvent evaporation the beaker was weighed again.

CALCULATION

Total fat% $W_2 - W_1 / W_1 \times 100$

W₁ = weight of empty beaker W = weight of sample

W₂ = weight of beaker + fat content

5.4. PROTEIN ESTIMATION

AIM: To determine the protein content of the sample

THEORY: Biuret is a small compound that forms when urea is heated allowing two urea molecules to join. Urea molecules fused in this manner produce amide groups (-NH) at the centre of the molecule which bind to cupric ions at a basic pH. The copper complexes that result from this interaction produce a strong blue color that can be measured with a spectrophotometer. Proteins also contain amide groups. When an amino group and a carboxyl group join to form a peptide bond, the amino group (-NH₂) becomes an amide group (-NH). At basic pH, the proteins form complexes with copper ions. Because this reaction was first

observed with biuret, it is called the biuret reaction (Fig. 2.1), and when this reaction is used to measure protein concentrations, it is called the Biuret Protein Assay. Thus, in this assay you will combine protein samples with Biuret Reagent which contains copper ions in a basic solution. The copper ions will combine with the amide groups in the proteins to create a blue color that will be measured using a spectrophotometer. The intensity of the color formed is directly proportional to the number of peptide bonds participating in the reaction and thus in the amount of protein present.

REQUIREMENTS

1. Biuret reagent: Dissolve 1.5 gm of CuSO₄ and 4.5 gm of Sodium
2. BSA stock (standard) solution (10 mg/ml): Dissolve 50 mg of BSA in 5 ml distilled water
3. 0.2 N NaOH
4. Distilled water

PROTOCOL

1. Sample preparation: Powder of Heart-fit powder (1 g) was dissolved in 9 ml distilled water and kept in rotary shaker for 10 min, filtrate was used as sample by making aliquots of 0.5, 0.75, 1.0 ml for further quantification.
2. Standard calibration curve was prepared from BSA working standard in concentration range of 2-10 mg/ml. Distilled water was used as a blank.
3. In 2 ml of all the solutions including both the samples and blank, 3ml of Biuret reagent was added.
4. Contents of the tubes were mixed properly and kept for incubation at room temperature for 10 minutes.
5. Absorbance was recorded at 540 nm against blank. 6. A standard curve of protein concentration (X-axis) was plotted against absorbance at 540 nm (Y-axis). From this standard curve the concentration protein was calculated in g/100g for the given samples.

5.5. TOTAL CARBOHYDRATE CONTENT

AIM: To determine the total sugar in the given food sample by difference method.

PRINCIPLE: Carbohydrate rich foods are the primary source of energy for all body functions. Our body breaks down carbohydrates into fuel for use by our cells and muscles -that's why eating a moderate amount of carbohydrates is necessary for most people. There are two types of carbohydrates- sugar and starches. Sugar are simple carbohydrates that can be easily digested

by body and include foods like cake, soda, candy, jellies and fruits. Starches are complex carbohydrates that take longer to be digested and include foods such as breads, grains, pasta, tortillas, noodles, fruits and vegetables.

FORMULA: Carbohydrates (in grams) = $100 - [\text{Proteins (in grams)} + \text{Fats (in grams)} + \text{Moisture (in grams)}]$

5.6 DETERMINATION OF TOTAL ENERGY CONTENT

AIM: To determine the energy content of the developed product.

PRINCIPLE: The energy density of food refers to the calorie content per weight of food. We know that we tend to consume a fairly consistent weight of food from day to day. But some foods have more calories, more energy per weight, than others do. One component that might influence energy density for instance would be the water content of food. The body functions on the basis of survival when you take in excess energy, the body saves it for a time when nutrients may not be available

FORMULA

Energy (Kcal) = $[\text{Carbohydrates (in grams)} \times 4 \text{ Kcal/g}] + [\text{Proteins (in grams)} \times 4 \text{ Kcal/g}] + [\text{Fats (in grams)} \times 9 \text{ Kcal/g}]$

5.7. BRIX REFRACTOMETER

AIM: To estimate total solids or sugar in the given sample.

PRINCIPLE

A refractometer is an instrument that optically measures the density of a liquid. Light passes through the sample and is deflected to varying degrees in relation to the density of the sample. The instrument is calibrated in terms of refractive index and also usually contains a scale in terms of degrees °Brix. Some instruments also can automatically convert between °Brix and °Baume units.

Working of Refractometer - When light enters a liquid at an angle, it changes direction. This phenomenon is called refraction. Light will refract more when traveling through a liquid with dissolved or suspended solids. Therefore, refraction can be used to measure the concentration of dissolved or suspended solids within a solution. Refractometers are scientific instruments that measure refraction angles and correlate them to an already established refractive index

(nD).

Brix Percentage - Degrees Brix (symbol °Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. If the solution contains dissolved solids other than pure sucrose, then the °Bx only approximates the dissolved solid content.

REQUIREMENT

1. Brix refractometer.
2. Distilled water
3. Product sample (Chocolate)
4. Plastic pipette or dropper
5. mortar and pestle

PROCEDURE

1. Open the daylight plate of the Refractometer and wipe the prism.
2. Place one to two drops of sample (if solid sample then dilute it with water) on the prism
3. Close the lid and let it spread and also remove excess drops with the help of soft tissue etc.
4. Hold the refractometer up to the light and look into it.
5. Focus the eyepiece, if necessary.
6. Take notice of where the line in the viewer fall
7. The viewer also shows a distinct line, usually a split between blue or grey and white, which is the Brix number of the sample.
8. The Brix rating of the sample directly affects where the color changes. So the exact spot where the color changes is the Brix rating.

ANALYSIS

Refractometer measured up to 9 on the Brix scale. The color changes from blue to white on the line marked 9, Hence 9 is the liquid's Brix rating.

CALIBRATION

Calibrate to 0% SSC using distilled water at the beginning of use and depending on how many samples are measured, periodically throughout measurements Contrast line should cross the scale at zero If not the scale needs adjustment using a calibration screw.

5.8. QUALITATIVE ANALYSIS OF PHYTOCHEMICAL

AIM: To qualitatively estimate the presence of the phytochemicals in the product sample.

PRINCIPLE

Phytochemical screening is a scientific process analyzing, examining, extracting, experimenting and thus identifying different classes of phytoconstituents present in various parts of the plants as bases for the discovery of drugs, the active components could be further taken for investigation and research. Preliminary screening of phytochemicals qualitatively is a valuable step, in the detection of the proactive components present in test medicinal plants. Phytochemical testing can be used in New Product Development for understanding the phytochemical constituent of the plant-based products which can also help in making claims regarding the product. The test can be used to make sure and check that the botanicals obtained have not been adulterated and so identical to the predetermined standards. Therefore, making it practical a tool for qualitative estimation of the product.

REQUIREMENTS

- Sample
- Chemicals: 10% lead acetate solution, 10% ferric chloride solution, 10% NaOH solution, chloroform, Acetic anhydride, Conc. H₂SO₄, Mayer's Reagent, magnesium ribbons, pyridine, Sodium-nitropruside, absolute alcohol, Distilled water.
- Glassware: Clean and dry test tubes, Beakers, 2ml Pipette, 1ml pipette, Boiling.

PROCEDURE

Table 3: Phytochemical Analysis.

TEST	OBSERVATION	INFERENCE
Alkaloids Test : Mayer's Test - 0.5 ml sample extract + 2 drops of Mayer's reagent along the sides of test tube	A creamy white precipitate	Alkaloids present / absent
Glycosides Test: Libermann's Test - 0.5ml sample extract 2 ml chloroform + 2 ml acetic acid (mixture was kept in ice water bath) and add Conc.H ₂ SO ₄	Color change from Violet to blue to Green	Glycosides present/absent
Flavonoids Test: Shinoda test - 0.5ml sample extract dissolved in alcohol + few fragments of magnesium	A pink to crimson coloured solution.	Flavonoids absent /present

+few ribbon drops of cone HCL.		
Saponins Test: Foam Test - 0.5ml sample extract + 2ml D/W.	Persistent foam for 10 minutes.	Saponins absent/ present
Terpenoids Test: Salkowski Test - 0.5ml sample extract + 2ml chloroform + 3ml sulphuric acid	Reddish Brown ring at interface.	Terpenoids present/absent
Gums and Mucilage: Alcohol Test - 0.5ml sample extract + 10ml D/W+2ml absolute solution (constant stirring)	White or Cloudy precipitate	Gums and mucilage present/ absent
Phenolic Compounds: Ferric Chloride Test - 0.5ml sample extract + 5ml D/W + few drops of 10% ferric chloride solution.	Dark green precipitate or bluish green colour	Phenolic compound Present/ absent
Gelatin Test- 0.5ml sample extract + 5ml D/W + 2ml of 1% Gelatin solution.	White precipitate	
Lead Acetate Test - 0.5ml sample extract + 2ml D/W + 3ml lead acetate solution.	White precipitate	

5.9. DETERMINATION OF ANTIOXIDANT BY DPPH METHOD

AIM: To utilize UV Visible Spectrophotometer for determining Antioxidant activity of sample by α, α -diphenyl- β -picryl hydrazyl (DPPH) assay.

PRINCIPLE: Spectrophotometers are used to measure the concentration of known substances in a solution. It is done by passing light through the substance and measuring light intensity as a function of wavelength. For measuring both ultraviolet and visible light, two lamps are usually required:

- 1) Tungsten filament lamp which produces wavelength in the visible region;
- 2) Hydrogen / Deuterium lamp which is suitable for ultraviolet regions.

There are two kinds of optical arrangements: i) Single beam [A single beam spectrophotometer uses a reference standard to standardize or blank the instrument before taking measurements.] ii) Double beam [A double beam spectrophotometer splits the beam of light in two different paths; one passes through sample, other passes through reference standard.]. UV Visible Spectrophotometer follows Beer Lambert Law which states that there is a linear relationship between the concentration and the absorbance of solution, which enables the concentration of solution to be calculated by measuring the absorbance.

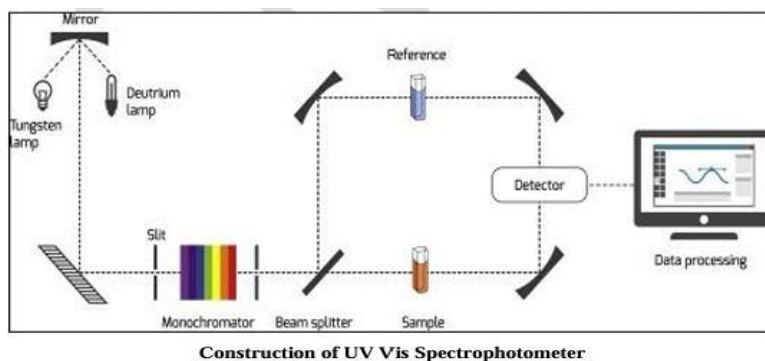


Fig 6: Construction of UV VS Spectrophotometer.

Antioxidant Activity Determination by DPPH method

The DPPH assay method was developed by Blois (1958) to determine the antioxidant activity by using a stable free radical α, α -diphenyl- β -picryl hydrazyl (DPPH). The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine.

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, like most other free radicals. The delocalisation also gives rise to the deep violet colour. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour.

Preparation of standard dilutions & Unknown for DPPH assay (for Antioxidant activity).

1. Stock solution of ascorbic acid was prepared. Dissolve 10mg of Ascorbic acid in 100ml of Ethanol making 0.1 mg/ml solution.
2. Dissolve 10mg of DPPH in 250ml of Ethanol to make 0.004% DPPH solution.
3. In the test tube, dilution of ascorbic acid was made with different volume from the stock by mixing appropriate amount of ascorbic acid stock and water
4. 4 ml of DPPH is added to all the test tubes.
5. In another test tube, 1 ml of unknown sample is taken and 4 ml of DPPH is added to it
6. Incubate all test tubes in a dark room at room temperature for 30 minutes.
7. Then plot the standard curve by taking concentration of ascorbic acid along the X-axis and absorbance at 517 nm along the Y-axis using the UV Vis Spectrophotometer.

5.10. ESTIMATION OF CALCIUM

AIM - To quantify amount of Calcium present in the product.

THEORY

Calcium is a chemical element with the symbol Ca and atomic number 20. It is an essential nutrient for living organisms, particularly in cell physiology, where its movements into and out of the cytoplasm function as a signal for many cellular processes. Calcium is also a key component of bones and teeth, providing structural support and strength.

Physical and Chemical Properties: Appearance- Silvery-white metal; Group: Alkaline earth metals (Group 2 of the periodic table); Density: 1.55 g/cm³; Melting Point: 842 °C (1548 °F); Boiling Point: 1484 °C (2703 °F)

Biological Role

- **Bone Health:** Calcium is the primary mineral in bones and teeth, providing structural integrity.
- **Muscle Function:** It plays a crucial role in muscle contraction and relaxation.
- **Nerve Transmission:** Calcium ions help transmit nerve impulses.
- **Blood Clotting:** It is involved in the clotting process to prevent excessive bleeding.
- **Cell Signaling:** Acts as a secondary messenger in various cellular activities.

Sources of Calcium

- **Dairy Products:** Milk, cheese, yogurt
- **Leafy Greens:** Kale, spinach, broccoli
- **Fortified Foods:** Some cereals, plant-based milk alternatives
- **Fish:** Sardines, salmon (with bones)

Health Benefits

- **Prevents Osteoporosis:** Adequate calcium intake helps maintain bone density.
- **Supports Cardiovascular Health:** Plays a role in maintaining heart rhythm.
- **Aids Metabolic Functions:** Involved in enzyme activity and hormone secretion.

Recommended Daily Intake - Adults: 500-1000 mg per day

Calcium Compounds

- Calcium Carbonate (CaCO_3): Commonly used as a dietary supplement and antacid.
- Calcium Chloride (CaCl_2): Used in food preservation and as a de-icing agent.
- Calcium Hydroxide (Ca(OH)_2): Known as slaked lime, used in construction and food processing.

Understanding calcium's role in various biological and chemical processes highlights its importance in maintaining overall health and well-being.

PROCEDURE

Followed inhouse method for heavy metal (calcium) which is SOP-CHM-27-00 Closed vessel digestion in which we take

1. Approximately weight 0.3 to 0.5 gm weight
2. Add 05 ml supra pure 65 % nitric acid keep for digestion it take time as per method
3. Transfer in to tarson tube and make up to 25 ml with ultra pure water
4. Run the sample on ICP- OES against known concentration of standards Instrument use - ICP-OES (Agilent 5100)

5.11. ESTIMATION OF VITAMIN D3

AIM: To quantify amount of Vitamin D3 present in the product.

THEORY: Vitamin D is a crucial nutrient for bone health, immune function, and beyond. Adequate sun exposure, a balanced diet, and, if necessary, supplementation can help ensure optimal level. The role of vitamin D in overall health is still a topic of research. While it's clear that vitamin D is essential, the exact amounts needed for optimal health and the benefits of supplementation in various populations continue to be explored.

Synthesis and Absorption - Sunlight Exposure: The primary source of vitamin D is sunlight. When UVB rays from the sun strike the skin, they convert 7-dehydrocholesterol to pre-vitamin D3, which then becomes vitamin D3 (cholecalciferol). **Dietary Sources:** Apart from sunlight, vitamin D can be obtained from certain foods. There are two main dietary forms

- Vitamin D2 (ergocalciferol): Found in some mushrooms.
- Vitamin D3 (cholecalciferol): Found in fish, liver, egg yolk, and fortified foods.

Function and Importance

- **Calcium and Phosphorus Regulation:** Vitamin D plays a pivotal role in regulating the absorption of calcium and phosphorus from the gut, essential for maintaining healthy bones and teeth.
- **Immune System:** It supports the immune system, helping the body fend off infections.
- **Cell Growth and Repair:** Vitamin D is involved in cell growth, neuromuscular function, and inflammation reduction.

Deficiency and Health Implications - 1] Rickets: In children, severe vitamin D deficiency can lead to rickets, a condition characterized by weak or soft bones. 2] Osteomalacia: In adults, deficiency can result in osteomalacia, causing bone pain and muscle weakness. 3] Other Implications: There's ongoing research into the potential links between vitamin D deficiency and conditions such as cardiovascular diseases, diabetes, and certain cancers.

Recommended Intake and Supplementation -

- **Daily Intake:** The recommended daily allowance (RDA) for vitamin D varies by age, sex, and life stage. For most adults, it's around 600-800 IU (International Units).
- **Supplements:** For those who have limited sun exposure or dietary intake, vitamin D supplements can help maintain adequate levels.

PROCEDURE

Sample preparation for Vitamin D3

1] 1g of sample in 10ml of n-hexane.

2] Sonicate for 30 minutes.

3] Filter the solution through a 0.22micrometre syringe filter. Vitamin D3 – HPLC Instrument is used for quantification of sample

Column: Shim-pack GIS C18-P (250 mm x 4.6 mm I.D., 5 µm), P/N : 227-30557-07

Mobile phase: Acetonitrile

Flow rate: 1.5 mL/min

Column temp. : 40 °C

Detection: UV 265 nm

Injection vol.: 200 µL

5.12. MICROBIAL ANALYSIS

AIM: To study the microbial load present in the chocolate sample by total viable count method.

PRINCIPLE: A viable count method consists of determination of the number of live and actively growing organisms in a given food sample. It is of two types-spread plate and pour plate. In spread plate technique serial ten-fold dilutions (1:10) of the sample are made and spread on the medium to obtain a number of colonies between 30-300. The nutrient agar is used which is a non-selective, general purpose medium and supports growth of all non-fastidious organisms. The Sabouraud's agar is used for the growth of fungal culture. However, certain limitations to these techniques are as follows:

Autotrophic microorganisms, sulphate reducers, etc. cannot be cultured on nutrient agar medium as well as Sabouraud's agar. Obligate anaerobes cannot be cultured. Symbiotic organisms are difficult to culture as one cannot survive without the other.

MEDIA COMPOSITION: NUTRIENT AGAR

Table 4: Composition of Nutrient Agar.

Sr.no	Components	Quantity	Significance of the components
1	Peptone	0.5g	Source of nitrogen
2	NaCl	0.5g	Maintain osmotic balance
3	Beef extract	0.15g	Source of carbon
4	Yeast extract	0.15g	Source of carbon
5	Agar	2.5g	Solidifying agent
6	Distilled water	100ml	
7	pH=7.2±0.2	-	

5.13. SENSORY EVALUATION

METHOD: Hedonic Rate Testing.

SIGNIFICANCE

Sensory evaluation is defined as scientific discipline used to measure, analyse and interpret reactions to those characteristics of food and materials as they are perceived by the sense of light, smell, taste. Touch, colour and hearing. Appearance can be judged by eye e.g colour, size, shape etc. Absence of defects is of importance in food selection.

A number of quality assurance procedures are used to examine and maintain quality of food products. The testing starts from reception of raw materials to close examination of finished product.

These tests are physical, chemical, microbiological and sensory evaluation is very important in product evaluation on account of following advantages:

- It is a simple analytical tool.
- It identifies the presence or absence of perceptible difference in terms of flavour, texture, colour and appearance.
- These important quality attributes are measured in fast and quantifiable manner using sensory techniques.
- The use of chemical and instrumental methods for examining sensory attributes are time consuming, complicated, and expensive.
- Sensory evaluation ensures that the consumer gets a non-defected and enjoyable product.

HEDONIC RATE TESTING

This test is used to measure the degree of pleasurable or unpleasurable experience with the foods. In general, this test measures the overall quality of the foods and is used in consumer analysis for a new product acceptability. In pilot consumer analysis, the most commonly used hedonic tests include: Facial hedonic test and Numerical hedonic test.

METHOD FOR HEDONIC TEST

Each panelist is presented with one or more samples in one session. The panelists are asked to rate the acceptability of each sample on a scale of 1 to 9 ranging from "Dislike extremely" to "Like extremely".

For the product, following attributes were accessed:

- Colour
- Texture
- Taste
- Odour
- Mouth sensation
- Overall acceptability

RATING SCALE

Table 5: Rating Scale for Hedonic Test.

Score	Remarks
1	Dislike extremely
2	Dislike very much
3	Dislike moderately
4	Dislike slightly
5	Neither like/dislike
6	Like slightly
7	Like moderately
8	Like very much
9	Like extremely

Sensory Evaluation Questionnaire -

Sensory Evaluation of Bony bliss

Name of participant :

Date:

Product Name	Bony bliss		
	Scale	Remarks	Any suggestions
Colour/Appearance			
Odour			
Taste			
Texture			
Mouthfeel			
Overall acceptance			

Scale description

1-Extremely dislike,2-Dislike very much,3-Dislike moderately,4-Dislike slightly, 5-Neither like Nor dislike,6-Like slightly,7-Like moderately,8-Like very much ,9-Extremely Like

Specific Remarks

Colour:-very light/Light/dark/very dark(mention specific colour e.g.light brown/pale yellow etc).

Odour:-milky/ cocoa/buttery/moderate/mild/sweet/pleasant non-significant/any other (mention specific odour e.g.flour/milk powder/cocoa.

Taste:-Sweet/Bitter/ Perfect sweet.Any other(mention specific taste e.g.milk/cereal flour/fruit etc.).**Texture:**-smooth/soft/creamy/coarse/granular/fine/hard/anyother

Mouthfeel:-Smooth/lumps/granular/creamy/any other.

Fig 8: Sensory Evaluation Questionnaire.

5.14. SHELF LIFE STUDY

Shelf life is a study of physical, microbiological and chemical processes, triggered by any one of a multitude of contributing factors. Product characteristics, including the quality and consistency of ingredients, the moisture content and acidity levels, all play a part, as do external factors like storage, transport and packaging materials ensuring that the quality remains acceptable and safe for consumers. By performing shelf-life analysis, you can define accurate

dates for your products, ensuring that the quality remains acceptable and safe for consumers. Accurate and reliable shelf life testing will reduce the risk of product recalls and identifying the causes of reduces shelf life will help you to improve the products, processes and ultimately the profitability.

Determining accurate shelf-life data for foods is essential for assuring food quality and protecting consumers from the effects of degradation. With a proper balance of theory and practical examples, Shelf-Life Assessment of Food presents the essential criteria and current methodologies for obtaining accurate and reliable shelf-life dating.

All products undergo changes, depending on the way that they are stored after they are manufactured, and herbal medicinal products (HMPs) are no exception, the World Health Organization has issued Guidelines for Assessment of Herbal Remedies (WHO, 1991), which suggest that all herbal remedies should also be assessed for their stability or shelf-life. In India regulatory requirements for traditional medicines, i.e., Ayurvedic, Unani and Siddha, do not make it mandatory for manufacturers to label the products with an expiry date or shelf-life period. However, in 2006, the government of India issued draft notification in the Gazette of India providing for proposed shelf-lives to be marked on products Ayurveda, Unani and Siddha systems, which is currently under scrutiny from academia and industry.

INTERACTION OF FOOD WITH PACKAGING MATERIAL

Most food products rely on their particular packaging to achieve their expected shelf life. In some circumstances the desired shelf life can be a major factor in the selection of packaging material.

In either case, the expected shelf life will be dependent on the integrity of the package seal to maintain atmosphere within the package (beyond any expected gas transmission across the packaging film) for nominated shelf life.

Interactions between foods and packaging can be detrimental to quality and/or safety. Careful consideration must be given to those factors affecting such interactions when selecting packaging materials in order to maximize product quality, safety, and shelf-life while minimizing undesirable changes

Polymer considerations include type of polymer and processing method, volume or mass of polymer to product ratio.

PROCEDURE

1. Pack the product properly.
2. The product in its packaged form was stored in cool & dry condition for 6 months and also at room temperature for 2 months.
3. Analyze the product at regular intervals for any change in the appearance, color, taste.

CHAPTER 6 PACKAGING OF THE PRODUCT

6.1. ALUMINIUM WRAPPER (As Primary packaging material)

Aluminium wrappers are quite the multitaskers in the world of primary packaging materials. Below are few compelling reasons as follows as



Fig 9: Aluminium Wrappers.

1. Barrier Properties: Aluminium provides excellent protection against light, oxygen, moisture, and other contaminants, which helps preserve the freshness and quality of the products inside.
2. Durability: It's strong and resistant to punctures and tears, ensuring that the packaged goods remain intact during transportation and handling.
3. Recyclability: Aluminium can be recycled indefinitely without losing its properties, making it an eco-friendly choice for packaging.
4. Versatility: It can be easily molded into various shapes and sizes, accommodating different packaging requirements.
5. Heat Conductivity: Aluminium's ability to conduct heat makes it ideal for packaging items that need to be heated, such as ready-to-eat meals.

6.2. GLOSSY PAPER (As Secondary packaging material)

Glossy paper can certainly make packaging stand out with its sleek and attractive appearance. It's often used for premium products to convey a sense of quality and luxury. Here are some key aspects of using glossy paper as packaging material:



Fig. 10: Glossy Papers.

1. **Durability:** Glossy paper is often coated, which can provide some level of protection against moisture and physical damage. Often more resistant to wear and tear, glossy paper can withstand handling better than matte options.
2. **Premium Appeal:** The shiny, smooth finish gives a high-end look that enhances the perceived value of the product.
3. **Vivid Colors:** Glossy paper can display vibrant and sharp colors, making the packaging visually striking and attractive.
4. **Moisture Resistance:** The coating on glossy paper provides a certain level of protection against moisture, which can be beneficial for maintaining the quality of the packaging.
5. **Enhanced Readability:** The reflective surface can make text and images more readable and appealing under good lighting conditions.
6. **Marketing Impact:** Eye-catching packaging can attract more consumers and create a stronger brand impression.

6.3. CARDBOARD PAPER BOX AND TIN BOX (As Tertiary packaging material)

Cardboard as a tertiary packaging material is quite an interesting choice. It's commonly used for storage purposes to provide an extra layer of protection to goods during transportation. It's durable, recyclable, and can be easily customized to fit various products, which makes it a popular choice in logistics and supply chain management.



Fig 11. Cardboard box.

Cardboard tertiary packaging usually involves the use of corrugated boxes, which consist of a fluted corrugated sheet and one or two flat liner boards. This structure gives the boxes their strength and ability to withstand pressure, making them suitable for heavy or delicate items.

Cardboard Packaging has its Benefits

1. **Cost-Effective:** Cardboard is relatively inexpensive to produce and purchase.
2. **Eco-Friendly:** It's recyclable and biodegradable, which helps reduce environmental impact.
3. **Light-weight:** Despite its strength, cardboard is light, which helps save on transportation costs.
4. **Versatile:** It can be easily cut, folded, and printed on to suit various needs.

Benefits of Tin as Packaging Material

1. **Corrosion Resistance:** Tin's natural resistance to corrosion makes it ideal for packaging perishable items.
2. **Non-Toxic:** Tin is non-toxic, ensuring that it doesn't contaminate the contents of the package.
3. **Malleability:** Tin can be easily shaped into various forms, making it versatile for different packaging needs.
4. **Barrier Properties:** It acts as an excellent barrier to light, oxygen, and moisture, preserving the quality and freshness of the product inside.
5. **Recyclability:** Tin is 100% recyclable, which makes it an environmentally friendly choice.



Fig. 12: Tin Box.



Fig. 13: Packaging of the Product.

CHAPTER 7 LABELING OF THE PRODUCT

The labelling regulations you're referring to fall under the Food Safety and Standards (Labelling and Display) Regulations, 2020. These regulations were established by the Food Safety and Standards Authority of India (FSSAI) to ensure that food products are labelled accurately and transparently, providing consumers with essential information about the product.

7.1. Objectives of the Regulations

- **Consumer Protection:** Ensure that consumers have access to clear, accurate, and relevant information about the food products they purchase.
- **Transparency:** Promote transparency in labelling to prevent misleading information and ensure informed consumer choices.
- **Health and Safety:** Provide essential information to consumers about the nutritional content, ingredients, and potential allergens, contributing to better health and safety

7.2. General Labelling Requirements

1. Name of Food: Should accurately represent the product.
2. List of Ingredients: Listed in descending order of weight/volume.
3. Nutritional Information: Energy value, protein, carbohydrate, sugar, fat, and other relevant nutrients.
4. Veg/Non-Veg Declaration: Identifying the product as vegetarian or non-vegetarian.
5. Manufacturer Information: Name and address of the manufacturer, packer, or importer.
6. Net Quantity: Clearly stated quantity of the product.
7. Lot/Batch Identification: Lot, code, or batch number.
8. Dates: Manufacturing date, packing date, best before/use by date.
9. Country of Origin: Origin of the product.
10. Usage Instructions: Instructions for use, if necessary.
11. Warnings: Any relevant warnings and advisory statements.
12. Nutritional Information: Must be provided per 100 grams or milliliters. Includes energy value (calories), protein, carbohydrate (including sugar), and fat content. Additional information on vitamins and minerals if applicable.
13. Allergen Information: Mandatory declaration of potential allergens present in the product. List of commonly known allergens like peanuts, tree nuts, soy, milk, eggs, fish, shellfish, and wheat.
14. Font Size and Legibility: Ensure labels are printed in a legible font size and contrast against the background.
15. Language: Information must be in English or Hindi, with the option to include other languages.
16. FSSAI Logo and License Number: The FSSAI logo and the license number must be displayed on the label.

7.3. Enforcement and Compliance

- Implementation Date: The regulations came into effect on July 1, 2021.
- Compliance: Food businesses are required to comply with these regulations and ensure their labels meet all specified criteria.
- Penalties: Non-compliance may lead to penalties and legal actions as per the Food Safety and Standards Act.

These regulations aim to empower consumers with the right information, enabling them to make safe and informed food choices.

INGREDIENTS:
Dark chocolate (55%), milk chocolate (19.47%),
Hadjod powder (15%), Pumpkin seeds,
Calcium carbonate, vitamin d3.

Serving size:- One chocolate per day (25g per)

Nutrient	Per 100g	Per serve	%RDA
Energy (kcal)	418.58	104.64	20.93
Carbohydrates (g)	40.192	10.048	13.40
Sugar(g)	24.34	6.085	48.68
Protein (g)	8.30	2.075	16.61
Total fat(g)	29.06	7.26	41.51
Saturated fat(g)	18.94	4.73	94.70
Trans fat(g)	0.074	0.18	3.70
Sodium (mg)	38.66	9.66	1.68
Calcium (mg)	2000	500	200
Vitamin D	600 IU	150 IU	
Potassium(mg)	783.8	195.9	16.67

BONY BLISS

a sweet way to healthy bones...♡

CHOCOLATE

enriched calcium fortified vit D supports bone health antioxidant

SUPPORTS BONE HEALTH.
FORTIFIED WITH CALCIUM & VitD.
HELPS TO IMPROVE BONE HEALTH.

Storage conditions:-
Store in cool and dry temperature.
Keep away from sunlight.

ALLERGEN- CONTAINS MILK SOLIDS.

"Indulge in Health:
Our bone-strengthening chocolate blends the goodness of dark and milk chocolate with Hadjod powder, calcium carbonate, and vitamin D for a delicious boost to your bone health. Nourishment never tasted this good!"

Yes I'm a recyclable & sustainable packaging

FUNCTIONAL FOOD

Manufactured & Packed by,
creative industry 1st flr
floranic pvt ltd parel Mumbai -400012

COUNTRY OF ORIGIN INDIA

CUSTOMER CARE, P.O. BAG2, Mumbai -110001
bonybliss@in.floranic.com
180001921947

MFD:- Jan 2025
USE BY:- June 2025
LOT NO:- 2705
MRP:- 40/-
USP:- 1.6 Rs per g

25g

0 00035 54562 0

Fig 14: Labeling of the Product.

CHAPTER 8

OBSERVATIONS & CALCULATION

8.1. TOTAL MOISTURE CONTENT

Formula: Moisture % = $100 (W_1 - W_2) / (W_1 - W)$ Where,

W = Initial weight.

W1 = Final weight before drying W2 = Final weight after drying

Table no. 6: Observation Table for Moisture Content.

Sample	Weight of sample taken	% moisture	Mean
Sample 1	1.039 g	0.38%	0.39%
Sample 2	1.030g	0.41%	
Sample 3	1.003g	0.40%	

8.2. TOTAL ASH CONTENT

Formula: Calculate the ash content using the following formula= $\frac{Z-X}{Y-X} \times 100$

Where,

Y-X

[X] W1 = weight of the empty crucible

[Y] W2 = weight of the crucible + sample

[Z] W3 = weight of the crucible + ash

Table No. 7: Observation Table for ASH Content.

Sample No	Weight of sample taken	W1=Wt of empty crucible = sample in [g]	W2=Wt of crucible after drying [g]	% ASH
Sample 1	2.013g	20.549g	18.694g	7.84%

8.3. TOTAL FAT CONTENT

Formula: Total fat% $\frac{W2-W1}{W1} \times 100$ W1= Weight of empty beaker

W2=Weight of the beaker and fat after drying W=Weight of sample

Total fat content (%) = $\frac{110.700-108.992}{110.700} \times 100 = 34\%$

5.002

8.4. TOTAL PROTEIN CONTENT

Table No. 8: Readings obtained from Colourimeter.

Concentration of BSA (ug/ml)	Absorbance at 660 nm
Blank	0.0
1	0.04
2	0.10
3	0.12
4	0.13
5	0.15
Unknown	0.04

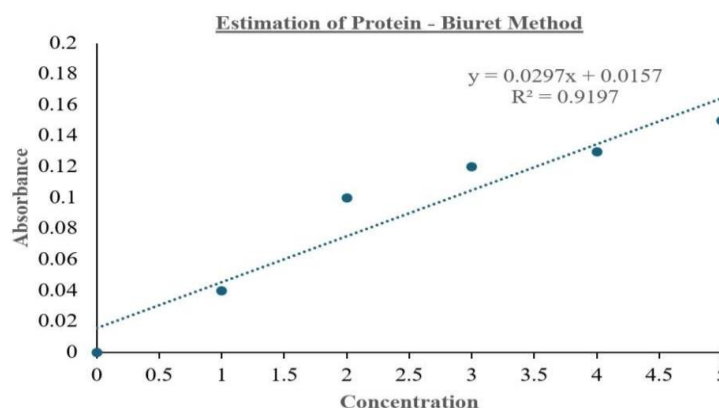


Fig 15: Standard Graph for Estimation of protein by Biuret Method.

Calculations: Now solving for x using,

$$y = 0.0297x + 0.0157$$

$$x = \frac{y - 0.0157}{0.0297}$$

By Substituting the value of unknown in the above equation,

$$x = \frac{y - 0.0157}{0.0297} = \frac{0.04 - 0.0157}{0.0297} = \frac{0.0243}{0.0297} = 0.8181 \text{ mg/ml}$$

Now converting 0.8181 mg into g = 0.008181 g

If for 1 g of sample = 0.008181 g

Then 100g of sample = 'x' g

Therefore, **x = 8.181g** of protein content in 100g of sample

8.5. TOTAL CARBOHYDRATE CONTENT

Formula: Carbohydrates (in grams) = 100 - [Proteins (in grams) + Fats (in grams) + Moisture (in grams)]

Total carbohydrates = 40.192g (in 100 grams)

8.6. DETERMINATION OF TOTAL ENERGY CONTENT

FORMULA

Energy (Kcal) = [Carbohydrates (in grams) x 4 Kcal/g] + [Proteins (in grams) x 4 Kcal/g] + [Fats (in grams) x 9 Kcal/g]

$$= [40.192\text{g} \times 4 \text{ Kcal/g}] + [8.181 \text{ g} \times 4 \text{ Kcal/g}] + [34 \text{ g} \times 9 \text{ Kcal/g}]$$

$$= 160.768 + 32.724 + 306$$

$$= \mathbf{499.492 \text{ Kcal/g}}$$

8.7. BRIX REFRACTOMETER

BRIX = 15%

8.8. QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Table No. 9: Observations for Qualitative Analysis of Phytochemicals.

Phytochemical	Observation	Inference
Flavonoids	A pink to crimson color observed	Presence of Flavonoids
Alkaloids	A creamy white precipitation	Presence of Alkaloids
Terpenoids	Reddish brown ring at interface	Presence of Terpenoids
Phenolic Compound	White precipitate	Presence of Phenols

8.9. DETERMINATION OF ANTIOXIDANT BY DPPH METHOD

Table no. 10: Results of Antioxidant Activity by DPPH method.

Sr. No.	Test parameter	Test method	Result	Unit
1	Antioxidant activity	DPPH method	35.07	%

8.10. ESTIMATION OF CALCIUM

Table No. 11: Results of Calcium Test.

Sr. No.	Parameters	Units	Methods	Results of Analysis
1	Calcium	mg/100g	SOP-CHM-27-01 (Part A)	1030.13

8.11. ESTIMATION OF VITAMIN D3

Table No. 12: Results of Vitamin D3 quantification by HPLC.

Sr. No.	Test parameter	Test method	Result	Unit
1	Vitamin D3	HPLC	350	IU

8.12. MICROBIAL ANALYSIS

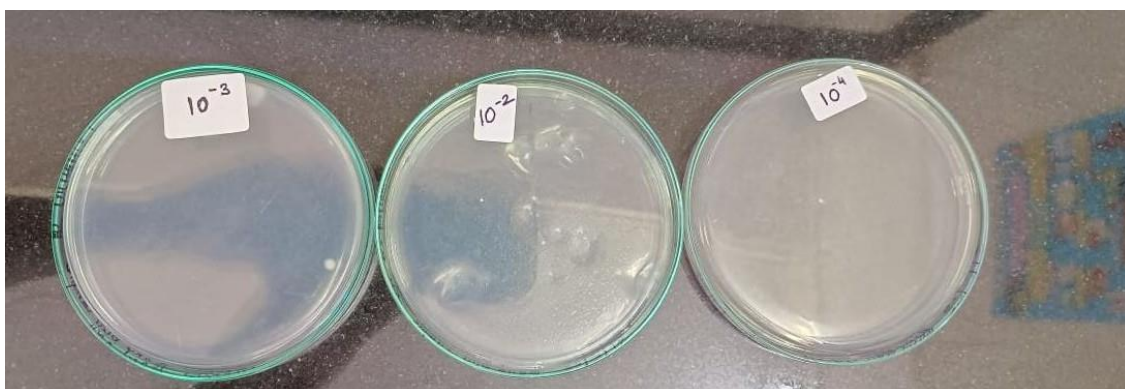


Fig. 16: Observations for Total Viable Count method (TVC).

Table 13: Calculations for Microbial load Analysis.

Serial no.	Dilutions	Observations count/ml	Count xDF	Average
1	10-2	1	>30 CFU/GM	Too few to count
2	10-3	0		
3	10-4	0		

8.13. SENSORY EVALUATION

Table 14: Average Response Score of Sensory Test.

Parameters	Score
Colour /Appearance	9
Taste	7
Odour	6
Mouthfeel	8
Texture	7
Overall acceptance	8

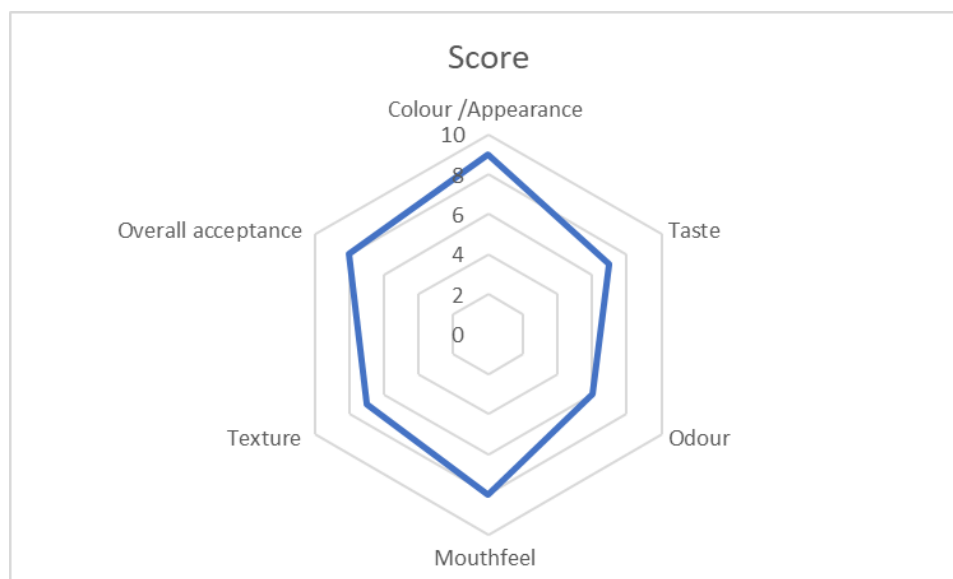


Fig. 17: Sensory Analysis (Radar Graph).

8.14. SHELF LIFE STUDY

The product was kept at two Environmental conditions

1] Refrigerated conditions for 6 months

2] Room temperature for 2 months

And was routinely observed at a 10 days interval of time.

Table 15: Observations of the product shelf life at Refrigerated conditions for 6 months.

DAYS	OBSERVATIONS
10th day	No changes observed in colour, taste, texture, odour
20th day	No changes observed in colour, taste, texture, odour
30th day	No changes observed in colour, taste, texture, odour
40th day	No changes observed in colour, taste, texture, odour
50th day	No changes observed in colour, taste, texture, odour
60th day	No colour changes, taste is also good
70th day	No as such changes observed
80th day	In good condition and no changes observed
90th day	No changes observed in colour, taste, texture, odour
100th day	No changes observed in colour, taste, texture, odour
110th day	No changes observed in colour, taste, texture, odour
120th day	No changes observed in colour, taste, texture, odour
130th day	No changes observed in colour, taste, texture, odour
140th day	No changes observed in colour, taste, texture, odour
150th day	Mild odour changes observe, no colour and taste changes
160th day	Mild odour changes but acceptable, no colour and taste changes
170th day	Mild odour changes but acceptable , no colour and taste changes
180th day	Mild odour changes but acceptable , no colour and taste changes

Table 16: Observations of the product shelf life at Room temperature for 2 months.

DAYS	OBSERVATIONS
10th day	No changes observed in colour, taste, texture, odour of product
20th day	No changes observed in colour, taste, texture, odour of product
30th day	No changes observed in colour, taste; mild odour changes of product and texture
40th day	No changes observed in colour, taste ; mild odour changes of product and texture
50th day	Taste, colour of product is good but changes in texture and odour
60th day	Taste, colour of product is good but changes in texture and odour

CHAPTER 9 RESULTS AND DISCUSSION

SUMMARY OF RESULTS

Table no. 17: Summary of Results.

Test parameters	Result (per 100g of product)
Total moisture content	0.396%
Total ash content	7.84%
Total fat content	34 g
Total Carbohydrate	40.192g
Total Protein content	8.181 g
Total Energy Content	499.492 Kcal
Qualitative analysis of phytochemicals	Flavonoids, Terpenoids, Phenolic compound, Alkaloids Present
Antioxidant by DPPH	35%
Vitamin D3	350IU/22g of sample
Calcium	1030.13mg
Shelf life study	Product was observed to be good at time period of 6 months in refrigerated conditions and at RT till 2 months some little changes in texture and odour were observed
Sensory evaluation	Overall acceptable score was 8 out of 10
Brix	15 % total solids
Microbial load	Very few to count >15 CFU/mL

CHAPTER 10

CONCLUSION

Bone health is a growing concern, and nutrition plays a vital role in maintaining strong bones and preventing conditions like osteoporosis. Functional foods offer an innovative and convenient way to support bone health, especially for consumers seeking alternatives to traditional supplements.

Bony Bliss is a unique, science-backed functional chocolate that combines essential nutrients like calcium, vitamin D3, Hadjod, and pumpkin seeds with the indulgence of dark chocolate.

By offering a delicious yet effective bone health solution, BonyBliss stands out in the expanding functional food market.

Qualitative and quantitative analysis like bioactive compounds, Proximate analysis, Quantification of Vitamin D and Calcium, Antioxidant activity, Microbial load study, Product Shelf life study along with Sustainable Packaging were done.

With increasing consumer awareness and demand for health-conscious products, BonyBliss has the potential to revolutionize the way people approach bone care—making it both enjoyable and beneficial.

CHAPTER 11

FUTURE SCOPE

Synergistic Effects Study: To investigate the synergistic effects of Hadjod, Cocoa, and Calcium on bone health, particularly on bone mineral density, fracture healing, and anti-osteoporotic effects.

Bioavailability Study: To evaluate the bioavailability of calcium and other minerals when combined with Hadjod and Cocoa.

Long-Term Efficacy Studies: Further research could explore the long-term effects of the product on bone health, especially in preventing osteoporosis and fractures in younger populations.

Expansion to Other Health Benefits: Investigating the potential benefits of Hadjod and Cocoa in other areas of health, such as joint health, anti-inflammatory effects, or cardiovascular benefits.

Further Ingredient Research: Exploration of additional bone-supporting nutrients, such as magnesium and collagen, could enhance the product's efficacy and broaden its consumer appeal.

Clinical Trials Expansion: Additional trials focusing on different populations, such as athletes and post-menopausal women, could broaden the product's applications to muscle recovery and joint health.

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CHAPTER 13

ANNEXURE

Annexure 1: Results for Calcium Test

Testing Lab Address : Equinox Center, R65,
TTC, Rabale, Navi Mumbai, Maharashtra
400701



TEST REPORT

Report Issue Date : 04-Mar-2025
Reference Number : 001:FT:25:02:07361
Report Number : EQNX:001:FT:25:02:07361

Information Provided by Customer

Client Name : Siddhika Tandel Sampling Location : NA
Address : Tandel house , Chendani Koliwada near Sample Sub Group : Coffee And Cocoa Products
mumbaikar apartment near railway station
thane East,Mumbai,Maharashtra,India-
400603
Contact Person : Siddhika Tandel Sample Name : Chocolate

Particulars of Sample Analysed

Sampling Protocol : NA Sample Collected by : Client

Quantity & Condition : Approx -30 GM of sample in a client packaging is intact without any leaks or breaks.

Date of Sampling	Date of Receipt	Start Date of Analysis	End Date of Analysis
NA	24-Feb-2025	26-Feb-2025	03-Mar-2025

----- Result of Analysis -----

Discipline : Chemical Group : Food and Agricultural Products

Sr.No	Parameters	Units	Methods	Results of Analysis
1	Calcium	mg/100g	SOP-CHM-27-01 (Part A)	1030.13

Note:

- This Report is Valid for the tested sample only
- Test report shall not be reproduced except in full & with written approval of Equinox Labs Private Limited.
- This report should not be used for advertisement / judicial purpose.
- The samples has been provided by the customer and results applied to the sample as received by the lab.
- The information provided by customer can affect the validity of results

Dr. Manisha Kharade
Quality Manager
(Reviewed & Authorised By)



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----- End Of Report -----

Page 1 of 1

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Annexure 2: Results for Vitamin D3 (HPLC) and Antioxidant activity (DPPH).



CIN - U71200RJ2024PTC094749

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ADHRYSA LAB & SOLUTIONS

Test Report Number: ALS/08/02025	Location: Mumbai
Name Of Customer: Siddhika	
Address: Guru Nanak Khalsa College, Matunga Mumbai	Date of Sample Received: 26/02/2025
	Date of Test Start: 27/02/2025
	Date of Test Completion: 28/02/2025
	Date of Reporting: 28/02/2025
Product Category: --	DATA PROVIDED BY CUSTOMER:
Name of Sample: Chocolate	Brand Name: N.A
Nature of Sample: solid	Batch No: N.A
Packaging Condition: Intact	Production Date: N.A
Quantity: 22g	Exp. Date: N.A.
Pick up & drawn: Sample not drawn by laboratory	Remark if any: N.A.
	Mfg. by: N.A.

Results:

Discipline: Chemical			Group: Food	
No.	Test parameter	Test method	Result	Unit
1	Vitamin D3	HPLC	350	IU
2	Antioxidant activity	DPPH method	35.07	%

Verified By:

Technical Director
Dr. Minal Jain



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