

DEVELOPMENT AND CHARACTERIZATION OF BIOACTIVE-ENRICHED WHEY PROTEIN POWDER FORTIFIED WITH GUAVA, PINEAPPLE AND BLACK SEED EXTRACTS

¹ Syed Sameer, ²Dr. DT Bornare, ³Dr. SG Jaiswal

¹ M-Tech Research Scholar, ²Head & Associate Professor, ³ Assistant Professor

¹ (Food Processing Technology),

¹Maharashtra Institute of Technology, Chhatrapati Sambhaji Nagar Aurangabad, India

Abstract: Whey protein is a high-quality complete protein source, widely used in sports nutrition and dietary supplements. Whey protein is used for several purposes, such as muscle growth or recovery and performance. commonly fitness enthusiasts & bodybuilders, weight watchers or elderly peoples and busy lifestyle peoples used for good meal replacement, appetite and against old age muscle loss. whey protein is easy to digest and rich in leucine, which stimulate muscle synthesis commonly fortified in shakes, smoothies, and bars it used as quick protein source. The increasing demand for functional foods has encourages to the exploration of novel protein formulations enriched with bioactive compounds to provide both nutritional and therapeutic benefits. However, its conventional formulations are limited to macronutrient delivery and often lack additional micronutrients and antioxidants.

There is a purpose to study, the development and characterization of a functional whey protein supplement fortified with guava powder, pineapple powder (bromelain), and black seed extract (*Nigella sativa*). Guava is a natural source of vitamin C and dietary fibre, pineapple provides bromelain, a proteolytic enzyme with digestive and anti-inflammatory activity, while black seed extract delivers thymoquinone, a potent antioxidant and immunomodulator. Expected to these combinations enhance whey protein's nutritional and functional profile by improving digestion, boosting immunity, and reducing oxidative stress.

Required: The methodology and many involve formulating whey protein blends with controlled doses of guava powder, pineapple powder, and encapsulated black seed extract. Analytical evaluation will include proximate composition, mineral and vitamin analysis, bioactive compound determination (vitamin C, bromelain activity, thymoquinone), antioxidant assays, stability testing, microbiological safety, and sensory evaluation.

This research aims to produce a novel nutraceutical protein formulation that is nutrient-dense, bioactive-rich, and consumer-acceptable, with potential applications in sports nutrition, metabolic health, and immunity enhancement.

Whey protein isolate, bioactive-enriched, guava powder, pineapple powder, black seed extract, thymoquinone, bromelain, functional food, nutraceuticals, food fortification

INTRODUCTION

1.1 Whey Protein as a Functional Ingredient

Whey protein is a milk-derived protein supplement. Comes Type in Functional food / dietary supplement. And Scientific proteins inside β -lactoglobulin, α -lactalbumin, etc. Classified as Dairy protein, animal origin, functional ingredient. Its Recognition and Approved worldwide as safe, healthy, and beneficial.

Whey protein is GRAS (Generally Recognized as Safe) under FDA rules. FDA allows its use in Infant formula, Dietary supplements, Sports nutrition products and medical nutrition and functional foods. Medical nutrition and functional foods Whey protein is a high-quality protein source, containing all essential amino acids and high concentrations of branched-chain amino acids (BCAAs), leucine, which stimulates muscle protein synthesis particularly (Cava et al., 2024).

It has been shown to support muscle recovery, immune function, and satiety. A recent meta-analysis confirmed its cardiometabolic benefits, particularly reductions in LDL and total cholesterol, making it relevant not only for athletes but also for clinical populations (Prokopidis et al., 2025).

these benefits, whey protein supplementation is not without risks. High intakes may cause acne, digestive discomfort in lactose-intolerant individuals, and potential kidney strain in people with pre-existing renal disorders (Patel et al., 2023).

These risks underline the importance of fortifying whey with complementary natural ingredients that can provide antioxidants, aid digestion, and improve overall safety and functionality.

1.2 Guava Powder: Source of Vitamin C and Antioxidants

Guava is Warm climate, tropical & subtropical. Fruit Can grow in dry areas, needs well-drained soil Flowers & fruits 2–3 times a year. Best in dry season (less rain). Mostly Produced in India (largest producer: UP, Bihar, Maharashtra), also Indonesia, China, Mexico, Brazil.

The nutrition of guava and guava powder is recognized by international food authorities such as USDA, WHO/FAO Codex, ICMR-NIN (India), and FSSAI (India), and measured using AOAC official laboratory methods. These recognitions establish guava as a safe, beneficial, and healthy food ingredient, rich in vitamin C, dietary fibre, minerals, and antioxidants, supporting its use in functional foods and health-promoting diets." the richest natural sources of vitamin C are Guava (*Psidium guajava*) and abundant in dietary fibre, carotenoids, and polyphenols. guava's potential in enhancing immunity, improving digestion, and reducing oxidative stress. Its powder form is widely studied for use in food fortification. Khan et al. (2024).

there is a crucial role in nutrient retention, is Processing methods. Yousaf et al. (2024) reported that freeze-drying guava preserves significantly higher amounts of vitamin C and phenolic compounds compared to spray-drying, showed that freeze-dried guava powder not only retained antioxidants but was also better accepted by consumers in instant beverages. making it the preferred method for functional formulations. Similarly, Rafiqzaman et al. (2025)

Given its nutrient density, even small additions of guava powder in whey protein formulations can boost antioxidant activity and provide immune benefits, thereby addressing some of the limitations of plain whey protein.

1.3 Pineapple and Bromelain Enzyme

Pineapple is Hot & humid tropical climate. Needs sunlight and rainfall but not too much water. Fruit Takes 18–24 months to fruit. Harvest season depends on country, usually in warm months. Mostly produce in Philippines, Indonesia, Costa Rica, Brazil. In India: Assam, Kerala, West Bengal.

Pineapple and its powder are acknowledged by leading food authorities including USDA, WHO/FAO Codex, ICMR-NIN (India), and FSSAI (India) as safe and Valuable components of the human diet, with their nutrients routinely measured by AOAC official laboratory methods. Recognized for their richness in vitamin C, manganese, dietary fibre, natural antioxidants, and the digestive enzyme bromelain, pineapple products contribute to immune support, digestive health, and overall wellness. These qualities justify their wide application in functional foods, beverages, and nutraceutical formulations as safe and health-promoting ingredients."

Pineapple (*Ananas comosus*) Bromelain has been documented as an effective digestive aid, anti-inflammatory compound and it's valued for its vitamin C content and its unique proteolytic enzyme, bromelain, which has multiple therapeutic properties and immune modulator Its addition to whey protein formulations is particularly attractive because it improves protein digestion and reduces gastrointestinal discomfort. (Kansakar et al., 2024).

bromelain is highly sensitive to processing. Colletti et al. (2021) noted significant losses in enzyme activity during conventional drying and storage. Advanced technologies such as encapsulation are being explored to overcome this issue. Tang et al. (2022) developed a microencapsulation system that significantly enhanced bromelain stability, while Ataide et al. (2021) demonstrated successful nanoencapsulation in chitosan nanoparticles for controlled release. Chiarelli et al. (2024) further highlighted sustainable methods of bromelain recovery from pineapple by-products, indicating its potential in cost-effective nutraceutical production.

These findings support the inclusion of pineapple powder or bromelain extract in fortified whey protein products, provided that protective processing such as freeze-drying or encapsulation is used.

1.4 Black Seed (*Nigella sativa*) and Thymoquinone

Black Seed comes in Herb/Spice Grows in dry/semi-arid climate. Cannot tolerate too much moisture. Needs well-drained soil. Planted in cool season, harvested in dry season when seeds mature (late spring/summer).

Mostly produce India, Pakistan, Egypt, Turkey, Syria, Saudi Arabia. Egypt is famous for high-quality seeds. Black seed (*Nigella sativa*), along with its powder and standardized extracts, is officially recognized within international food and herbal standards, including Codex Alimentarius (WHO/FAO), USDA databases, ICMR-NIN (India), and FSSAI (India). Their nutritional and bioactive components are validated through AOAC official analytical methods, ensuring scientific accuracy in labelling and safety assessment. Rich in thymoquinone, essential fatty acids, protein, fibre, and antioxidants, black seed products are valued not only as safe food ingredients but also for their traditional and evidence-based roles in supporting immunity, metabolic health, and overall well-being. Because of these qualities, black seed and its derivatives are increasingly incorporated into functional foods, dietary supplements, and wellness formulations worldwide." Black seed extract has been traditionally used for its medicinal properties, Thymoquinone its main bioactive compound, exhibits strong antioxidant, anti-inflammatory, anti-diabetic, and immunomodulatory activities and modern research validates its wide range of therapeutic effects. (Alberts et al., 2024; Trivedi et al., 2024). Clinical studies confirm its safety at low doses. Thomas et al. (2022) reported that thymoquinone-rich black cumin oil is safe at up to 200 mg/day in healthy adults, supporting its use in fortified foods. Nevertheless, its strong taste and instability during storage present formulation challenges. Encapsulation technologies have been proposed to solve these issues. Santiworakun et al. (2022) demonstrated that microencapsulation significantly improved the stability and reduced the bitterness of black seed oil, while Rahim et al. (2022) reviewed various oil encapsulation techniques suitable for food applications.

In the context of whey protein, controlled addition of black seed extract offers added antioxidant capacity and therapeutic value. However, dosage must remain within safe limits, and encapsulation should be considered to improve palatability and stability.

1.5 Processing and Stabilization of Functional Ingredients

Careful selection of processing techniques requires the integration of fruit powders and herbal extracts for whey protein formulations. Freeze-drying has consistently been shown preserve bioactive in guava and pineapple (Yousaf et al., 2024; Rafiquzzaman et al., 2025). Similarly, carrier agents such as maltodextrin and gum Arabic can improve powder flow, reduce hygroscopicity, and protect sensitive compounds during storage (Pujapanda et al., 2025).

For bioactive-rich extracts such as bromelain and thymoquinone, encapsulation (micro-or nano scale) ensures controlled release, improved bioavailability, and minimized side effects. This combination of freeze-drying and encapsulation represents the most promising strategy for developing stable, consumer-acceptable fortified whey protein products.

NEED OF THE STUDY.

2.1 Growing demand for natural functional supplements

Consumers increasingly prefer clean-label, natural, and plant-based ingredients instead of artificial additives, motivating the development of enriched whey protein with fruit and herbal bioactive.

2.2 Conventional whey protein lacks micronutrients and bioactive

Normal whey protein provides mainly macronutrients (protein) but has low antioxidants, no digestive enzymes, and minimal micronutrients, creating a clear need for improvement.

2.3 Strong scientific evidence for benefits of guava, bromelain, and black seed

Each ingredient offers proven health benefits —Guava: vitamin C & antioxidants

Bromelain: digestion & anti-inflammatory

Black seed: thymoquinone & immune support

This motivates combining them with whey to enhance nutritional value.

2.4 Potential to improve digestion and absorption

Bromelain is known to support protein breakdown, which can help reduce digestive discomfort associated with whey protein, providing a strong reason to explore fortified formulations.

2.5 Increasing interest in immunity-boosting foods

Post-pandemic health awareness has driven demand for foods rich in antioxidants and immune-supporting compounds — which guava and black seed naturally provide.

2.6 Lack of multifunctional protein supplements in the market

Most commercial whey supplements focus only on muscle building.

There is very limited availability of multi-nutrient, multi-bioactive whey products, creating a strong motivation to innovate.

2.7 Scientific gap in combining multiple bioactive

There is no existing formulation or research combining guava, bromelain, and black seed with whey protein. This uniqueness motivates the need to investigate such a comprehensive blend.

2.8 Potential applications in sports nutrition and wellness

A well-designed fortified whey protein can support:

Athletes

Elderly individuals

People with poor digestion

Immunity-conscious consumers

This broad applicability motivates the study.

2.9 Opportunity to create a nutrient-dense, health-promoting product

Combining fruits and herbal extracts with whey protein may produce a more balanced and complete supplement, offering benefits beyond traditional protein powders.

2.10 Academic and industrial relevance

research contributes both: Scientifically by addressing a real research gap, commercially by suggesting a practical, marketable product

RESEARCH METHODOLOGY

3.1 Materials

Whey Protein Isolate (WPI) / Hydrolysed Whey Protein

Guava Powder (freeze-dried)

Pineapple Powder (freeze-dried)

Black Seed Extract (encapsulated)

Carrier agents: maltodextrin, gum Arabic

Chemicals & standards (DPPH, Folin–Ciocalteu reagent, ascorbic acid, assay kits, etc.)

3.2 Formulation Development

3.2.1 Base Formula

Whey Protein: 30 g/serving

3.2.2 Fortification Levels

Guava Powder: 1–2 g

Pineapple Powder: 0.5–1 g

Encapsulated Black Seed Extract: 0.1–0.15 g

3.2.3 Blending

All ingredients homogenized using a mixer/blender with carrier agents.

3.2.4 Storage

Samples stored at:

25 °C (ambient)

4 °C (refrigerated)

3.3 Processing Techniques

Freeze-drying of guava and pineapple powders

Microencapsulation (spray drying / freeze drying) for black seed extract

Powder standardization: sieving to <250 µm particle size

3.4 Analytical Methods

3.4.1 Proximate Composition (AOAC methods)

Moisture (AOAC 925.09)

Ash (AOAC 923.03)

Protein (Kjeldahl, AOAC 981.10)

Fat (Soxhlet, AOAC 991.36)

Carbohydrates (by difference)

Fiber (AOAC 985.29)

3.4.2 Mineral Analysis

ICP-OES / AAS for Ca, Mg, Fe, Zn, K, P

3.4.3 Vitamin & Bioactive Analysis

Vitamin C (UV-Vis)

Total Polyphenols (Folin–Ciocalteu)

Bromelain Activity (casein digestion assay)

Thymoquinone (HPLC/GC-MS)

3.4.4 Functional Properties

Antioxidant Activity (DPPH, FRAP)

Solubility (%)

Foaming capacity & stability

Emulsifying activity

3.4.5 Stability Studies

Storage: 25 °C & 4 °C

Duration: 30, 60, 90 days

Parameters: moisture uptake, vitamin C retention, bromelain activity, thymoquinone retention, colour, solubility

3.4.6 Safety Analysis

Microbiological: TPC, coliforms, Yeast & Mold

3.4.7 Sensory Evaluation

Panel size: 10 semi-trained

Attributes: taste, aroma, colour, mouthfeel, overall acceptability

Scale: 9-point Hedonic

3.5 Statistical Analysis

Triplicate experiments

Mean ± SD reporting

ANOVA for comparison

Significance: $p < 0.05$

IV. RESULTS AND DISCUSSION

4.1 The Fortification: A whey with guava, pineapple, and black seed extract addresses the specific micronutrient and enzyme deficiencies found in plain whey supplements. Guava Fortification: Nutritional Density Data analysis confirms that guava is a superior fortifying agent for addressing the micronutrient gap in standard whey supplements.

Fortification Variable	Plain Whey Isolate (control)	Fortification Result (Reported Data)	Supporting Reference
Guava (Vitamin C)	Trace/ Negligible	15-40 mg/ 100g	Yousaf et al. (2024)
Guava Fiber	0g	1-1.5 per 30g serving	Sahal (2025)
Pineapple (Bromelain)	Not proteolytic activity	>75% retention (Encapsulated)	Tang et al. (2022)
Black Seed (Thymoquinone)	No antioxidant synergy	Safe up to 200 mg/day	Thomas et al. (2022)
Sensory Acceptance	standard	>7.5/9.0 (Hedonic Scale)	Wang et al. (2025)

4.2 Bioactive Stability and Retention Results: Established data suggests that processing methodology is the primary determinant of bioactive efficacy.

Bromelain Retention: Standard studies show that microencapsulation maintains >75% of proteolytic activity, protecting the enzyme from denaturation during storage.

Thymoquinone Stability: Encapsulation techniques significantly reduce the natural bitterness of black seed oil while preserving its antioxidant capacity.

4.3 Sensory and Safety Parameters

Sensory Scores: A fortified blends consistently score higher on the 9-point Hedonic scale for "overall acceptability" (>7.5/9.0) as tropical acidity masks protein aftertastes.

Safety Thresholds: Thymoquinone dosages up to 200 mg/day are safe. Clinical data confirm that supporting the proposed use of 0.1-0.15 g of extract per serving.

4.4 DISCUSSION

Optimization of Bioactive Stability

The review confirms that specifically freeze-drying processing techniques and microencapsulation are the most effective strategies for maintaining quantitative levels of sensitive compounds like Vitamin C, Bromelain, and Thymoquinone etc. The use of carrier agents such as maltodextrin or gum Arabic ensures high solubility and protects the enzyme structure from thermal degradation during storage.

4.5 Synergistic Health Benefits and Safety

Antioxidant Synergy: Vitamin C (from Guava) and Thymoquinone (from Black Seed), which collectively enhances the antioxidant capacity of the protein powder more than single-ingredient supplements.

Improved Digestibility: The Bromelain directly addresses the common digestive discomfort associated with high whey protein intake in athletes and clinical populations.

Safety Thresholds: Clinical data from recent trials (Thomas et al., 2022) confirms that the proposed inclusion levels (0.1–0.15 g for black seed) fall well within the established safety limit of 200 mg/day for human consumption.

5.3 Identification of the Scientific Research Gap

whey protein: The fortification of guava, pineapple, and black seed extract shows a significant "Scientific gap" in the micronutrient and enzyme deficiencies found in conventional whey. By proposing this specific multi-bioactive blend in whey this study identifies a novel pathway for developing high-performance, functional supplements in the sports nutrition and wellness market.

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