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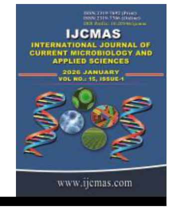

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Development of Low-Calorie Functional Foods Using Microbial Enzyme Protease and Microalgae Spirulina

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A B S T R A C T

This research explores the development of low-calorie food products utilizing microbial enzyme protease and microalgae Spirulina as functional bioactive ingredients. The focus is on four innovative formulations: peanut butter and fruit custard (developed using protease), and chocolate truffles and dark chocolate (developed using Spirulina powder). These formulations aim to provide nutritionally enhanced alternatives for health-conscious consumers by incorporating protein- digesting enzymes and antioxidant-rich algae into widely loved food matrices. Physical, microbial, and biochemical analyses were conducted to validate the nutritional and sensory quality of the products.

Introduction

Calories are units of energy derived from food, and their intake determines the human body's energy balance. A surplus—especially from high-fat and high-sugar foods—can lead to obesity, insulin resistance, and cardiovascular diseases. With the rising prevalence of lifestyle-related disorders, there is an increasing demand for low-calorie, nutrient-dense alternatives.

High-calorie diets, rich in refined sugars and saturated fats, also contribute to chronic inflammation, diabetes mellitus, and hypertension. Replacing these harmful components with nutrient-dense, low-calorie ingredients is essential for preventing metabolic diseases and improving public health outcomes (Sandrou and Arvanitoyannis, 2000). Spirulina, a blue-green microalga, is renowned for its exceptional nutrient profile as it contains plant protein, B-complex vitamins, beta-carotene, iron, and potent antioxidants (Batista *et al.,* 2013; Becker, 2007). Its bioactive compounds are effective at reducing oxidative stress and boosting immune function (Chacón-Lee *et al.,* 2010).

In parallel, microbial-derived protease enzymes enhance the digestibility and nutritional bioavailability of proteins, supporting muscle maintenance and overall metabolic efficiency (Sandrou and Arvanitoyannis, 2000). Excessive intake of calorie-dense foods can impair brain function, increase memory impairment risks, and cause hormonal imbalances affecting mood and stress levels (Rolls *et al.,* 1989). These health issues further stress the need for developing food products that are both low in calories and rich in essential nutrients. To address the growing demand for healthier food options, incorporating microalgae like Spirulina and enzymatic treatments into food products offers a sustainable solution. Spirulina's ability to improve protein digestibility and provide essential vitamins and minerals makes it a valuable ingredient in functional foods (Batista *et al.,* 2013; Becker, 2007).

The application of protease enzymes further enhances the bioavailability of proteins, making them more accessible for absorption and utilization by the body, thereby promoting better overall health outcomes (Sandrou and Arvanitoyannis, 2000). These innovative approaches not only align with the increasing shift towards healthier eating but also contribute to managing obesity, metabolic disorders, and the inflammation associated with poor diet choices (Sandrou and Arvanitoyannis, 2000).

By utilizing these ingredients, food products can maintain consumer satisfaction while supporting long-term health, thus playing a crucial role in reducing the incidence of lifestyle-related diseases such as diabetes, hypertension, and cardiovascular issues (Rolls *et al.,* 1989; Sandrou and Arvanitoyannis, 2000).

In response, this study focuses on developing low-calorie versions of commonly consumed products by incorporating protease enzymes to improve protein utilization and Spirulina powder to enhance the nutritional profile. The chosen products—peanut butter, fruit custard, chocolate truffles, and dark chocolate—were optimized to balance health benefits with consumer-preferred taste and texture.

Materials and Methods

Peanut Butter: Commercially available peanut butter.

Fruits for Custard: Assorted fresh fruits including apples, bananas, and berries.

Chocolate Ingredients: Cocoa powder, cocoa butter, and sweeteners.

Protease Enzyme: Microbial-derived protease enzyme.

Spirulina Powder: Commercially available Spirulina powder.

Enzyme-Enhanced Peanut Butter

Protease enzyme was incorporated into peanut butter at a concentration of 0.5%. The mixture was incubated at 40°C for 2 hours to allow enzymatic action, then cooled and stored at 4°C. This process aimed to improve the spreadability and protein accessibility of the product (Sandrou and Arvanitoyannis, 2000).

Enzyme-Enhanced Fruit Custard

Freshly chopped fruits were blended with a custard base. Protease enzyme was added at 0.3% (w/w) and the mixture was incubated at 37°C for 1 hour. Refrigeration followed to preserve the custard, ensuring that the enzyme's action improved nutrient bioavailability without compromising flavor (Batista *et al.,* 2013; Harun *et al.,* 2010).

Spirulina-Fortified Chocolate Truffles

A ganache was prepared using cocoa powder, cocoa butter, and a low-calorie sweetener. Spirulina powder was incorporated at 1% (w/w) into the ganache before shaping the mixture into truffles, which were then cooled until set. This formulation aimed to integrate antioxidant properties and a unique nutrient profile into the truffles (Chisti, 2007; Freitas *et al.,* 2019).

Spirulina-Fortified Dark Chocolate

Dark chocolate was melted and homogenized with 1% Spirulina powder. The blend was tempered and molded into bars. This method ensured even distribution of Spirulina, enhancing both the nutritional and visual appeal of the dark chocolate (Li *et al.,* 2008; Rolls *et al.,* 1989).

Analytical Methods

Physical Testing

Texture analysis was conducted using a texture analyzer to measure hardness and cohesiveness. Color measurements were performed using a colorimeter.

Microbial Testing

Total plate count was determined by plating serial dilutions on nutrient agar and incubating at 37°C for 24 hours. Yeast and mold counts were assessed using potato dextrose agar.

DNSA (3,5-Dinitrosalicylic Acid) Assay

Used to quantify reducing sugars. Samples were mixed with DNSA reagent and heated at 90°C for 15 minutes. Absorbance was measured at 540 nm.

Lowry Protein Assay

Employed to determine protein concentration. Samples were treated with Lowry reagent and incubated at room temperature for 20 minutes. Absorbance was read at 750 nm.

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay

Assessed antioxidant activity. Samples were mixed with DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm.

Observation

During product development, Spirulina-based chocolate truffles and dark chocolate faced taste challenges due to Spirulina's earthy flavor, but coconut cream and vanilla essence effectively masked it, improving taste and texture. The dark chocolate's higher cocoa content paired well with Spirulina, and phycocyanin added a green tint and antioxidant boost. In protease-based products, enzymatic treatment of peanuts enhanced peanut butter's texture and protein availability, confirmed by DNSA and Lowry tests. The protease-treated fruit custard retained creaminess and improved nutrient bioavailability without affecting flavor. All products scored above average in sensory evaluation, showing successful integration of functional ingredients.

Prepared Sample/Final products

Moreover, the integration of Spirulina not only enhanced the antioxidant profile. The enzymatic action of protease improved protein digestibility, making the products more suitable for individuals with higher nutritional demands, such as athletes and the elderly. These modifications demonstrated that functional ingredients could be effectively incorporated into traditional treats without compromising consumer acceptability, thereby broadening the scope for healthier indulgent options in the food market.

Observation of microbial testing of all four products

No visible colonies indicating absence of fungal contamination

Results and Discussion

Physical Testing Results

Peanut Butter and Fruit Custard

The protease-treated peanut butter exhibited a light brown color, smooth texture, and an improved protein content with minimal oil separation. Its sensory attributes and shelf-life (15–30 months) met the expected quality standards (Sandrou and Arvanitoyannis, 2000).

The custard appeared creamy and smooth, retained a pleasing pink hue, and maintained a pH of The enzymatic treatment retained its creaminess while enhancing nutrient bioavailability (Patel *et al.,* 2020; Rolls *et al.,* 1989).

Dark Chocolate & Truffles

The Spirulina dark chocolate was dark, aromatic, and had a melting point of approximately 80°C. The chocolate truffles, infused with Spirulina, showed an earthy flavor masked by additional ingredients such as coconut cream and vanilla essence, ensuring both taste and visual appeal (Batista *et al.,* 2013; Becker, 2007; Chacón-Lee and González-Mariño, 2010).

Microbial Testing

Peanut Butter & Custard

Microbial analysis revealed no growth of pathogens such as *Staphylococcus aureus* or *Escherichia coli*, indicating the product's safety and proper enzymatic treatment (Batista *et al.,* 2013).

Chocolate Products

The absence of unwanted microbial growth was confirmed in both Spirulina-based products, validating the formulation process used to maintain quality and safety.

Prepared Sample/Final products

Figure.1 Proteanut	**Figure.2** Spirudelight truffles
	

Figure.3 and 4

Image no :- 3 Custea

Image 4:- spriumelt chocolate

Figure.5 Microbial testing on nutrient and potato dextrose agar plates

Figure.6 Confirms hygienic handling

Figure.7 No microbial growth

Figure.8 Without any microbial colonies

Figure.9 Absence of contamination

Microbial testing of all developed products, as shown in the attached images, confirmed that they were free from harmful microbial contamination and safe for consumption. The absence of bacterial and fungal growth indicates effective hygiene and stability throughout the production process.

Figure.10

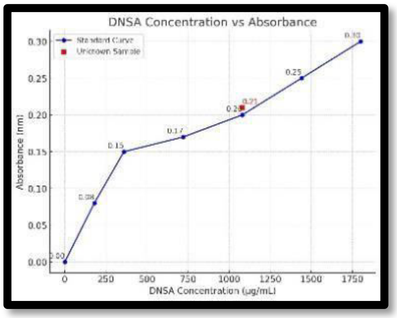


Figure.11

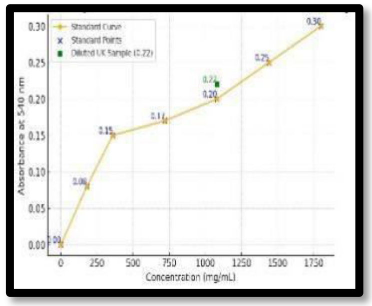


Figure.12

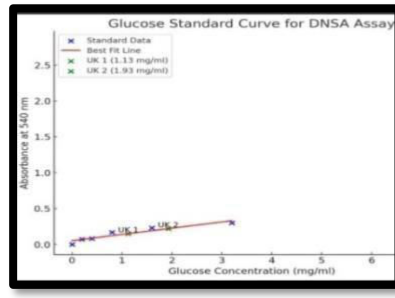


Figure.13 Proteanut

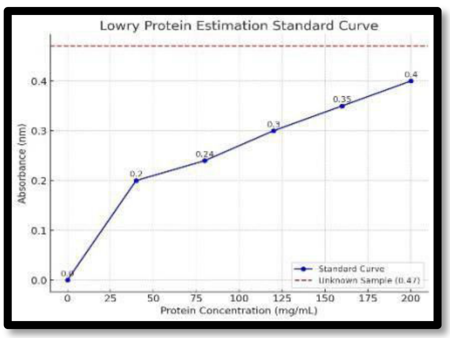


Figure.14 Spirudelight truffles

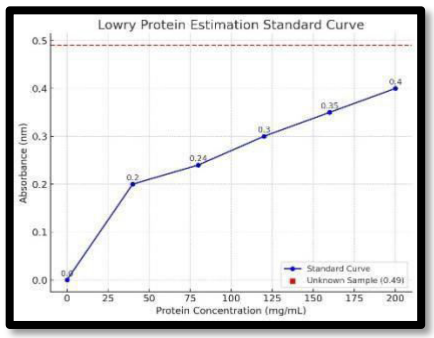
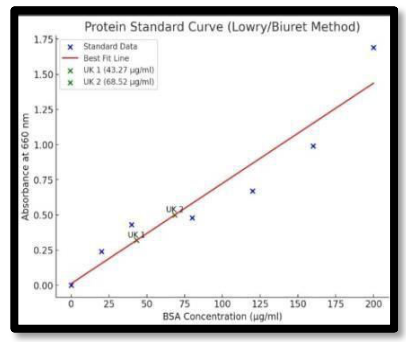


Figure.15 Custea/ spriumelt



DNSA method

The DNSA assay confirmed reduced sugar content in the stevia-based food product. In one trial, the sample showed an absorbance of 0.21, corresponding to ~1200 µg/mL sugar. In another, the original sample absorbance was 0.44, requiring 1:2 dilution (Abs = 0.22), estimating sugar content at ~2200–2600 µg/mL.

Both results indicate significantly lower sugar levels compared to conventional products, validating stevia's effectiveness in sugar reduction.

Both samples show relatively low glucose concentrations compared to the standard range, indicating successful formulation of low-calorie variants.

Fruit custard (UK 1) has a lower glucose content than dark chocolate (UK 2), confirming that enzymatic treatment (likely by protease) and Spirulina integration effectively reduced sugar content while maintaining nutritional value (Rolls *et al.,* 1989).

Lowry method result

The Lowry assay revealed significant protein content across all four low-calorie products. Fruit custard (43.27 µg/ml) and dark chocolate (68.52 µg/ml) showed moderate protein levels, while peanut butter and chocolate truffle exhibited very high protein content, with absorbance values (0.49 and 0.47 respectively) exceeding the standard curve range. This indicates that all products, while being low in calories, are nutritionally enriched with protein, making them healthy and balanced alternatives (Batista *et al.,* 2013; Becker, 2007; Caporgno and Mathys, 2018).

DPPH results

The DPPH scavenging activity results show strong antioxidant potential in all four samples. Peanut butter (68.18%), chocolate truffles (72.73%), dark chocolate (82.95%), and fruit custard (62.5%) demonstrated significant free radical neutralization. This indicates the presence of bioactive compounds like polyphenols, phycocyanin, and enzymes, contributing to their health-promoting, oxidative stress-reducing properties (Chacón-Lee and González-Mariño, 2010; Chisti, 2007; Freitas *et al.,* 2019).

The incorporation of Spirulina and microbial protease into food products like peanut butter, fruit custard, chocolate truffles, and dark chocolate has resulted in nutritionally enhanced, safe, and palatable low-calorie options. These formulations address current health challenges while preserving consumer taste preferences. Future work may focus on shelf life extension and clinical validation of health benefits.

Author Contributions

Vidisha Vinay Mishra: Investigation, formal analysis, writing—original draft. Riya shigwan: Validation, methodology, writing—reviewing. Suman Satyarum:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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