DEVELOPMENT OF A FUNCTIONAL PROTEIN BAR ENRICHED WITH ANTIOXIDANT COMPOUNDS DERIVED FROM CACAO (*Theobroma cacao* L.)

Sirilak Namwong
Faculty of Science and Technology, Suan Sunandha Rajabhat University
Email: sirilak.na@ssru.ac.th
Natkamol Peungsamran
Faculty of Science and Technology, Suan Sunandha Rajabhat University
Email: natkamol.pe@ssru.ac.th
Phaowana Laoongphao
Faculty of Science and Technology, Suan Sunandha Rajabhat University
Email: s64122240012@ssru.ac.th

Abstract

A high-protein functional bar was developed using a blend of pea protein, pumpkin seeds, sunflower seeds, blended peanut butter, dried cranberries, cacao powder, olive oil, and dark chocolate. Among the tested formulations, Formula II (approximately 12 g per piece) demonstrated the highest overall acceptability based on sensory evaluation. Nutritional analysis revealed that each piece contained 3.8 g of protein, 0.95 g of carbohydrate, and 0.74 g of fat, reflecting a macronutrient profile suitable for health-conscious consumers. In terms of bioactivity, Formula II exhibited strong antioxidant potential, with DPPH radical scavenging activity decreasing slightly from 89.74% at production to 87.29% after 21 days of refrigerated storage at 4 °C. These results highlight the stability and efficacy of cacao-derived bioactive compounds, particularly phenolics and flavonoids, in contributing to antioxidant function. The formulation's high protein content, low carbohydrate level, and antioxidant enrichment position it as a promising candidate for functional food applications. Furthermore, the potential incorporation of probiotic strains may enhance its health-promoting properties, supporting digestive health and overall wellness.

Keywords: Protein bar, Cacao, Antioxidant

Introduction

The increasing accessibility of energy-dense, nutrient-poor food options has contributed significantly to the rising prevalence of overweight and obesity in Thailand. Globally, obesity has emerged as a major public health concern due to its strong association with numerous noncommunicable diseases (Jitnarin et al., 2011). Among various dietary interventions, protein supplementation has gained popularity as a weight-control strategy, primarily due to its capacity to suppress appetite and stimulate the release of satiety-related gut hormones (Pang et al., 2023). Kohanmoo et al. (2020) demonstrated that daily intake of 10 g of protein supplements effectively reduced appetite markers—including hunger, desire to eat, and prospective food consumption—particularly when combined with resistance training and dietary restriction, resulting in measurable weight loss. Cocoa (*Theobroma cacao*) is a natural source of bioactive compounds, including polyphenols (mainly flavonoids), biogenic amines (e.g., tyramine and phenylethylamine), purine alkaloids (caffeine and theobromine), and essential minerals such as magnesium (Edo et al., 2023). Although chocolate is the most common product derived from cacao powder, it typically contains high levels of added sugars or sweetening agents, which may counteract its health benefits.

In recent years, there has been growing interest in functional foods that are nutrient-dense, affordable, and accessible, particularly among individuals seeking weight management solutions. Protein energy bars represent a promising category of health-promoting products. However, despite the potential of cacao as a functional ingredient, few commercially available protein bars incorporate cacao in formulations that are both high in protein and low in carbohydrates (Pang et al., 2023).

Research Objectives

1. To formulate a protein bar enriched with antioxidant compounds derived from Theobroma cacao.
2. To evaluate consumer acceptability through sensory analysis.
3. To assess the antioxidant activity of the developed product using the DPPH radical scavenging assay.

Scope of the Research

1. The study targets Thai adults aged 18 -60 who are interested in health-oriented functional foods.
2. Key variables include protein bar formulation, sensory attributes, and antioxidant activity measured by DPPH assay.
3. The research was conducted over 3 months, with antioxidant testing on day 0 and after 21 days of refrigerated storage.

Research Methodology

1. Preparation of Protein Bars Enriched with Cacao

Protein bar formulations were developed using a combination of pea protein, pumpkin seeds, sunflower seeds, dried cranberries, mixed nut butter, cacao powder, dark chocolate, olive oil, salt, and lyophilized probiotics, as detailed in Table 1. Prior to mixing, pumpkin seeds, sunflower seeds, and dried cranberries were ground to improve texture and homogeneity.

All ingredients were thoroughly blended and molded into bite-sized portions. The bars were then refrigerated at 4 °C for approximately 2 hours to allow setting and stabilization. Nutritional composition was estimated using the Thai Nutri Survey Program (TNS), providing values for macronutrients and energy content.

Table 1: Protein bar ingredients

Ingredients	Protein bar	
	Formula I (g)	Formula 2 (g)
Pea protein	25-30	15-20
Pumpkin seed	5-10	10-15
Sunflower seed	5-10	10-15
Dried cranberries	5-12	5-12
Mixed nut butter	5-12	5-12
Cacao	2-6	2-6
Dark chocolate	12-18	12-18
Salt	1-3	0.2-1.0
Olive oil	6-8	1-3

2. Sensory Evaluation

Protein bar samples were stored at 4 °C prior to sensory evaluation. Acceptability was assessed across five attributes: appearance, color, taste, texture, and overall acceptability. A 9-point hedonic scale was employed, ranging from 1 (dislike extremely) to 9 (like extremely), with 5 representing a neutral response (neither like nor dislike). Thirty panelists participated in the evaluation. Each sample was coded with a randomized three-digit number and presented in pairs to each panelist to minimize bias (Lusk et al., 2015).

3. Determination of Antioxidant Capacity by (DPPH) Assay

The antioxidant activity of the protein bars was determined using the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging assay, following the protocols of Silva et al. (2024) and Thongkao et al. (2020), with minor modifications. A 200 µM DPPH stock solution and sample solution were prepared in absolute ethanol at a 1:1 ratio. The assay involved mixing 40 µL of DPPH stock with 1120 µL of sample solution, followed by incubation for 24 hours at room temperature. Absorbance was measured at 517 nm using a spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

% DPPH radical scavenging activity = [(ABlank – Asample)/ ABlank] x 100 (1)

ABlank - Absorbance of the control sample (absolute ethanol+ DPPH in absolute ehanol)

ASample - Absorbance of the sample (experimental samples in absolute ethanol + DPPH in absolute ethanol)

Research Results

1. Preparation of Protein Bars Enriched with Cacao

The protein bars were formulated into uniform bite-sized pieces measuring 2.5 × 2.5 × 2.5 cm (Figure 1). The primary macronutrient was protein (ranging from 3.01 to 3.82 g per piece), derived from pea protein, pumpkin seeds, and sunflower seeds, as detailed in Table 1. Minor ingredients included sources of fat—such as mixed nut butter, cacao powder, olive oil, and dark chocolate—and carbohydrates, primarily from dried cranberries.

Figure 1: Protein bars enriched with cacao

Table 2 summarizes the macronutrient content of the two protein bar formulations, as calculated using the Thai Nutri Survey Program (TNS). Formula II demonstrated a higher protein concentration (3.82 g per piece) compared to Formula I (3.01 g), indicating its potential as a more protein-dense option suitable for individuals seeking muscle maintenance or satiety enhancement. Conversely, Formula I contained slightly higher levels of carbohydrates (1.10 g) and fat (0.84 g) than Formula II, which provided 0.95 g of carbohydrates and 0.74 g of fat per

piece. These differences may be attributed to variations in ingredient ratios, particularly the inclusion of dried cranberries and nut-based components, which contribute to carbohydrate and lipid content.

The comparative nutrient profile suggests that Formula II may be more appropriate for consumers aiming to increase protein intake while minimizing carbohydrate and fat consumption. Such characteristics align with current dietary trends favoring high-protein, low-carbohydrate functional foods for weight management and metabolic health.

Table 2: The estimated nutrition of protein bars

Nutrition	Protein bars	
	Formula I (g)	Formula II (g)
Protein	3.01	3.82
Carbohydrate	1.1	0.95
Fiber	0.39	0.34
Sugar	0.15	0.11
Fat	0.84	0.74
Energy	27.49	26.07

2. Sensory Analysis

Sensory evaluation was conducted to compare the acceptability of two protein bar formulations, Formula I and Formula II. As illustrated in Figure 2, Formula II received higher scores across all evaluated attributes, including appearance, color, odor, texture, taste, and overall acceptability. These results indicate that Formula II was more favorably perceived by the panelists. A total of 30 untrained panelists participated in the evaluation. Each sample was coded with a randomized three-digit number and presented in pairs under controlled conditions to minimize bias. The assessment employed a 9-point hedonic scale, ranging from 1 ("dislike extremely") to 9 ("like extremely"), with 5 representing a neutral response ("neither like nor dislike").

The superior performance of Formula II may be attributed to its balanced flavor profile, smoother texture, and higher protein content. These characteristics align with consumer preferences for health-oriented products, particularly those with reduced sodium and enhanced nutritional value (Pang et al., 2023).

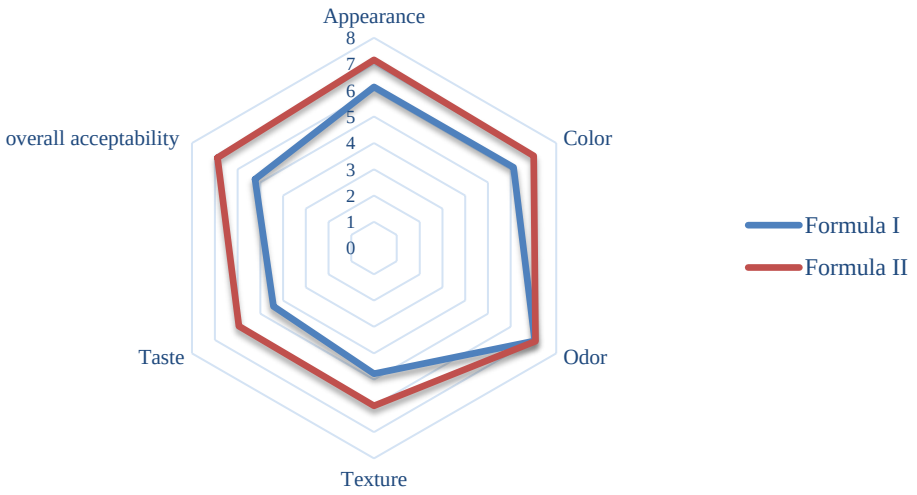

Figure 2: Sensory analysis diagram for formula I and II according to the appreciation levels

3. Determination of Antioxidant Capacity Using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Assay

Protein bar Formula II, which contained cacao powder as a key antioxidant source, was selected for evaluation of antioxidant capacity using the 1,1-Diphenyl-**2**-Picrylhydrazyl (DPPH) radical scavenging assay. The assay is based on the principle that DPPH, a stable free radical with a deep violet color, undergoes reduction upon interaction with antioxidant compounds, resulting in a color change from purple to yellow (DPPH-H), indicating the neutralization of free radicals (Yamin et al., 2021). As illustrated in Figure 3, the protein bar demonstrated strong antioxidant potential, with an initial DPPH radical scavenging activity of 89.74% on the day of production. This high level of activity reflects the presence of bioactive compounds in cacao, such as polyphenols and flavonoids, which are known for their free radical neutralizing properties.

During storage at 4 °C over a period of 21 days, the antioxidant activity showed a slight but consistent decline, reaching 87.29% by the end of the storage period. This reduction may be attributed to the gradual degradation or transformation of antioxidant compounds over time, influenced by factors such as moisture migration, oxidation, and interactions with other ingredients in the matrix. Despite the minor decrease, the protein bar maintained a relatively high level of antioxidant activity throughout the storage period, suggesting its potential as a functional food product with health-promoting properties. The stability of antioxidant capacity under refrigerated conditions further supports its suitability for commercial application in health-oriented snack formulations.

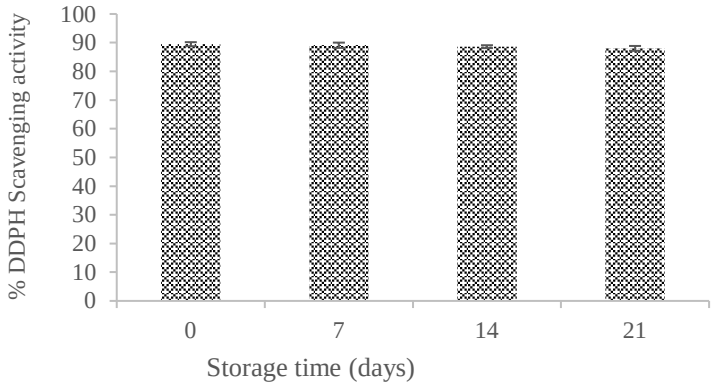

Figure 3: The percentage of DPPH radical scavenging activity of formula II

Discussion

The developed protein bar demonstrated a favorable nutritional profile, providing low energy (26.07 kcal per piece), high protein (3.82 g), and low carbohydrate content (0.95 g), consistent with dietary strategies for weight management and metabolic health (Pang et al., 2023). Protein-enriched products are known to suppress appetite and enhance satiety, contributing to reduced energy intake and obesity prevention. The inclusion of cacao powder significantly enhanced the antioxidant potential of the formulation, as evidenced by high DPPH radical scavenging activity. Cocoa (*Theobroma cacao* L.) is a rich source of bioactive compounds, including phenolics and flavonoids, which exhibit antioxidant, anti-inflammatory, antihypertensive, and insulin-sensitizing properties (Edo et al., 2023). These compounds may help mitigate oxidative stress and support cardiovascular and metabolic health.

In addition to its nutritional and functional benefits, the protein bar represents a value-added product by integrating health-promoting ingredients into a convenient, shelf-stable format. This approach aligns with current consumer demand for affordable, functional foods that contribute to preventive health and offer commercial potential in the expanding health food market.

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