Original Research

DEVELOPMENT OF PROTEIN-RICH FOOD PRODUCTS FROM BROWN RICE, SOYBEAN AND SESAME SEEDS IN VIETNAM

Dong Vuong Trieu[1], Nguyen Thi Kim Anh[1], Nguyen Thi Minh Nguyet[2,✉]

[1] *Department of Food Technology, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Viet Nam*

[2] *Department of Nutrition and Food Science, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Viet Nam*

ABSTRACT

Aims: This study focused on the development of a recipe for protein-rich nutrition bars (NB) using dried soybeans, puffed brown rice, and whole black sesame seeds.

Methods: The NB processing process involved binding the ingredients with a mixture of palm sugar and malt syrup, then pressing them into bars of size (8 x 4 x 1.5cm), which were packaged in aluminum film and vacuum sealed. Structural properties were determined through hardness using the Texture Profile Analysis (TPA) method on a Brookfield device (CT3 4500), and shelf life was evaluated using the thermal acceleration method (Q_{10}).

Results: The research results determined that the appropriate mixing formula was FM4, consisting of 63% dried soybeans, 17% whole black sesame seeds, and 20% puffed brown rice (by wt.). The finished NB product according to this ratio had a moisture content of 4.75 ± 0.02%. Each 100g of the product contained identified energy-generating ingredients, including 40.45 ± 0.64 g of carbohydrate, 25.68 ± 0.12 g of fat, and 22.05 ± 0.44 g of protein, with a total energy supply of 481.17 ± 0.75 Kcal. Based on the peroxide index (PoV), the study predicted that the NB could be preserved at 25 ºC for up to 113 days.

Conclusion: The NB, rich in protein, was made from abundant agricultural products in Vietnam, including soybeans, brown rice, and whole black sesame seeds, which can be a solution to utilize the available agricultural resources to create a nutritious whole grain cereal product.

Keywords: *nutritious bar, nutrition, thermal acceleration, shelf-life, whole grain, roasted soybean, puffed brown rice.*

I. INTRODUCTION

Whole grains are a major component of a high-protein diet. Globally, about 65% of food consumed is supplied by plant sources, including legumes, oilseeds and nuts (8%), and whole grains (47%). These seeds are abundant sources of protein, healthy fats, fibre, and antioxidants, as well as vitamins and minerals, etc. helping to prevent cancer, reduce cholesterol levels, lower the risk of chronic disease, and support weight management [1]. The advantages for health with the consumption of brown rice mainly come from the phytochemicals found in its bran layers. In addition, vitamins and minerals exist.

✉ Corresponding author: Nguyen Thi Minh Nguyet
Email: nguyenthiminhnguyet@iuh.edu.vn
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Brown rice contains high levels of phytic acid. Rice bran is the most nutritious part of rice and a good source of bioactive phytochemicals such as γ-oryzanol, phytic acid, etc. which have health-beneficial properties and antioxidant activity [2]. Raw sesame seeds contain many phytochemicals such as terpenoids, saponins, alkaloids, steroids, tannins, and flavonoids. It also contains other compounds such as sesamin, sesamol, sesamolin, gamma-tocopherol, and some phenolic acids such as flavonol glycosides. Furthermore, cephalin, lecithin, and free phenolic compounds are also present in sesame seeds [3]. Soybeans are rich in protein and oil content, accounting for about 60% of dry soybean weight. Many valuable vitamins, flavonoids, and polysaccharides also exist in soybeans. The soy protein content is high, containing a significant amount of essential amino acids that the human body cannot synthesize [4].

Nutrition bars (NB) are nutritional products containing grains and other high-energy components. They can be used as nutritious meals, meal replacements, or snacks, providing essential nutrients for busy consumers due to their convenience [5]. NB with 45-80 g provides about 200-300 Kcal, 3-9 g of fat, 7-15 g of protein, and 20-40 g of carbohydrates. NBs containing 20-25 g of protein per 100g can efficiently and quickly provide the necessary energy for the body to function [5]. Therefore, choosing plant-based protein sources is becoming increasingly popular as a healthy alternative solution.

Gluten-free NB has been successfully developed from dried fruit, seeds, oats, peanut flour, and jaggery with various levels of sweeteners [6]. There has been research on "*Whey protein-based nutrition bars*". The results obtained suggested that whey protein-based NBs have a softer initial structure and better overall homogeneity because of their highly hydrophilic nature. However, whey protein-based NBs were degraded during storage, limiting the shelf life of such products [7]. The development of NB from local grains in Vietnam to reduce imports reduce production costs, is a direction toward food security and sustainable development. There have been studies published that replacing oat flour with brown rice flour in NB production is feasible and beneficial. The resulting products have equivalent nutritional and physical parameters, meet scientific requirements, and are highly regarded by consumers. The optimal ratio of oatmeal and brown rice flour is 1:1 to get the best nutrition product [8]. However, the development of NB encounters certain difficulties such as the level of seeds and sweeteners significantly affecting the color value of the bar. Nowadays, people are gradually shifting towards a plant-based diet, so using a variety of different plant protein sources is becoming a trend. Combining diverse plant seeds can help ensure the body receives sufficient essential amino acids and other nutrients. Furthermore, seeds can also be used to replace meat in vegetarian or low-meat diets, helping to minimize health risks related to high-meat diets. NB, which is rich in protein from various seeds, is considered an effective method to gradually replace animal protein with plant protein. This study aimed to develop protein-rich NB from soybean, brown rice and sesame seeds. The specific objectives were to: (i) determine the macronutrient composition of ingredients; (ii) propose mixing formulas and choose feasible formulas; and (iii) survey the expiration date of finished products.

II. METHODS

2.1. Materials and chemicals

Puffed brown rice was purchased from Mailee Co., Ltd (Hoc Mon, Ho Chi Minh City, Vietnam). Dried soybean was the product of Kim Long Production and Trading Co., Ltd (Hoc Mon, Ho Chi Minh City, Vietnam). Whole black sesame with hulls was the product of the Tuyen Nam manufacturing facility (Ben Luc, Long An, Vietnam).

One hundred percent palm sugar syrup, in jelly form, was the product of a traditional palm sugar production facility in Tinh Bien District, An Giang Province. The malt syrup in 100 g contains more than 20 g of maltose was the product of Nhan Thuy Food Co., Ltd (Phu Cat, Binh Dinh, Vietnam).

The control sample was selected with the criterion of having the same size as the sample used to compare with the experiment and was called High-quality NB is a product of Bakalland - S.A. (Fabryczna 5 - Poland).

The following chemicals were used in research to determine raw material and product composition: crude protein (K_2SO_4 + $CuSO_4$, H_2SO_4 0.1N, $CuSO_4$, phenolphthalein, NaOH 0.1N, methyl red); Crude fat (Hexane); Crude fibre (H_2SO_4 1.25%, NaOH 1.25%); PoV (Diethyl ether, sodium sulfate, CH_3COOH: $CHCl_3$ (2:1), KI, $Na_2S_2O_3$ 0.01N) all were purchased from Hoa Nam Chemical Co., Ltd (District 11, Ho Chi Minh City, Vietnam).

2.2. Sample preparation

Formulation of nutrition bar

According to TCVN 4996-1:2011 (ISO 7971-1:2009): Cereals - Determination of bulk density, also known as "mass per 100 liters" - Part 1: Standard method, with some modifications.

Table 1. *The formulation and ingredients of the nutrition bar.*

Ingredient	Density (mL) corresponding to the weight of seeds (g) mixed with sesame seeds: brown rice									
	FM1 (1:1)		FM2 (1:2)		FM3 (2:1)		FM4 (1:3)		FM5 (3:1)	
Dried Soybean	130 mL	63 g	130 mL	63 g	130 mL	63g	130 mL	63g	130 mL	63g
Black Sesame	64 mL	35 g	43 mL	23 g	85 mL	46g	32 mL	17g	96 mL	52g
Puffed Brown rice	64 mL	14 g	85 mL	8g	43 mL	9g	96 mL	20g	32 mL	7g
Sugar translation (%)	20%		20%		20%		20%		20%	

Note: Palm sugar syrup and malt syrup (1:1) as % of total grain material.

From the obtained results of the density of each type of grain (Black sesame with ρ = 0.54 g/mL, puffed brown rice with ρ = 0.21 g/mL, and dried soybeans with ρ = 0.48 g/mL), a suitable mixing formula can be determined for the given volume of the mold. Specifically, the mixing formula with a mold volume of V_{mold} = 258 mL in this study was conducted as follows: soybeans were fixed at a volume of 130 mL to achieve a high protein content in the product and the ratio of sesame seeds to brown rice volume (mL/mL) was varied to create differences in sensory value in the product for formulas FM1, FM2, FM3, FM4 and FM5 (as presented in Table 1).

Preparation of nutrition bar

Dried soybean, puffed brown rice, and whole grain sesame were roasted using an oil-free air fryer GA-M4AS (Gaabor-Germany) at 125°C for 10 min to create a fragrant aroma in the grains [5]. The mixture of palm sugar syrup and malt syrup extract was preheated at 70°C for 30s to reduce viscosity, subsequently facilitated the mixing process [9]. The ingredients were mixed thoroughly for 1 min. This mixture was then quickly poured into the mold with a pre-lined wax paper. The mixture was then compressed tightly using a 2.74 kg brick (30 x 20 x 1.7 cm) covered by wax paper for 5 min. After shaping, the mixture was placed in an oven YXD - 40C (Southstar - China) at 170°C for 5 min and then cooled rapidly to room temperature [10]. This block was then cut using a 29 x 5 cm knife, making five clean cuts to obtain six bars, four of which have even dimensions of (8 x 4 x 1.5 cm) [11]. After cutting, the NB was allowed to cool and then vacuum-sealed (Techtongda - China) in laminated aluminum packaging and stored at room temperature for 24 hours until further analysis.

2.3. Analytical methods

Moisture analysis

The moisture content of grains and grain products was determined by the method of drying to a constant weight at a temperature of 130°C according to TCVN 9306 equivalent to ISO 712:2009.

Crude protein

Protein content was determined by the Kjeldahl method: According to the Vietnamese standard (TCVN 8125:2015, ISO 20483:2013).

Crude fibre

The crude fiber was determined according to Sumczynski, et al [12], with some modifications as follows: 1g of NB sample was placed in an Erlenmeyer flask and boiled with 1.25% H_2SO_4 solution for 45 min. The sample was then filtered and washed through a sieve, using boiling distilled water to rinse the Erlenmeyer flask and sample until the solution was transparent.

The process was repeated with a dilute alkali solution: the treated sample

Crude fat

According to the standard of sector 10TCN 849:2006 on standards for agricultural and food products - Crude fat content was determined by the Ministry of Agriculture and Rural Development.

Ash

Ash content was determined by ignition method: According to Vietnamese standard (TCVN 8124:2009; ISO 2171:2007).

was boiled with 1.25% NaOH solution for 45 min and filtered and washed similarly. The sample was then dried UNB400 (Memmert - Germany) at 130°C for 1 hour, cooled in a desiccator, weighed, incinerated F48010 (Thermo - USA) at 550°C for 5 hours, cooled in a desiccator, and weighed again. The residual weight was used to calculate the crude fiber content according to the following formula:

$$\text{Crude fiber (\%)} = \frac{m_1 - m_2}{m_0} x\ 100$$

Where: m_0 is the mass (g) of the weighed sample; m_1 is the total mass (g) of the dried residue and filter flask after extraction; m_2 is the total mass (g) of the dried residue and filter flask after incineration.

2.4. Nutritional values and energy content of the product

The carbohydrate content and total energy content (Kcal) of the components and the final product sample was calculated as follows [11].

Carbohydrate (%) = 100 - (% moisture + % fat + % protein + %a sh + % fiber).

Energy value (Kcal/100g) = (4 × % carbohydrate) + (9 × % fat) + (4 × % protein).

2.5. Texture analysis

The texture of NB was determined by the method of Jo Su-Ah et al., with some modifications: The texture was measured using a texture analyzer CT3 4500 (Brookfield - USA) controlled by TexturePro CT V1.6 Build 26 software. The sample, with a rectangular size of (8 x 4 x 1.5 cm), was compressed directly on the TA-ATT fixture and cut in compression mode using the TA7 Knife Edge probe with a width of 60 mm, with the following parameters: pre-test speed of 2 mm/s, test speed of 2 mm/s and post-test speed of 2 mm/s, with an initial force of 2N, probe length of 20 mm and a holding time of 5 seconds at the midpoint between two compressions on the same cut line. The hardness (N) of the NB was recorded [13].

2.6. Peroxide value

Extracting fat from the sample according to TCVN 12940:2020, with some modifications: Weigh 25g (± 0.1g) of the sample that had been ground using a dry powder grinder SK200 (Seka, Ho Chi Minh City, Vietnam) into a 250 mL conical flask and then added 100 mL of diethyl ether and covered the flask for 2 hours. Filter the extract through filter paper into a separating funnel. Add another 50 mL of diethyl ether to the remaining part and filter the extract through the same separating funnel as before. Add 75 mL of water to the funnel and shake well. Let it settle and remove the liquid layer at the bottom. Repeat the process of adding water, shaking it, letting it settle, and removing the liquid layer at the bottom. Add an appropriate amount of sodium sulfate to remove water. Place the conical flask containing diethyl ether on a rotary evaporator (Model RV 10 - control V by IKA - Germany, 50/60 Hz, Power 1300 W) with a rotation speed of 80 rpm at a temperature of 39ºC for 60 min to evaporate the diethyl ether.

The peroxide value (PoV) of the extracted fat was determined according to TCVN 6121:2018 (ISO 3960:2017), with modifications: Accurately weigh 5.00 g of the extracted fat into a 250 mL Erlenmeyer flask with a glass stopper. Add 30 mL of CH_3COOH-$CHCl_3$ (2:1) and shake well to dissolve. Add 0.5 mL of saturated KI solution, let stand and shake occasionally for 1 min, and then add 30 mL of water. Slowly titrate with 0.01N $Na_2S_2O_3$ and shake vigorously until the yellow color of the titration disappears. Add 0.5 mL of 1% starch solution, and shake vigorously to release all I_2 from the $CHCl_3$ layer until the blue color disappears. Perform a blank determination (not more than 0.1 mL of 0.01N $Na_2S_2O_3$). Formula:

$$\text{Peroxide (meq}O_2\text{/kg oil)} = \frac{(V_1 - V_2)\ x\ N\ x\ 1000}{m}$$

Where: V_1 is the volume of the standard solution of $Na_2S_2O_3$ 0.01N used for determination (mL); V_2 is the volume of the standard solution of $Na_2S_2O_3$ 0.01N used in the blank test (mL); N is the concentration of $Na_2S_2O_3$ solution, expressed in moles per liter (mol/L); m is the weight of the sample (g).

2.7. Shelf-life

Shelf-life was determined using the Q_{10} method: Q_{10} increases the reaction rate that occurs mainly as a chemical reaction as well as the physical properties of the product in the study are also affected when the temperature increases by 10°C (18°F). Labuza developed an equation for testing frequency.

$$f_2 = f_1 \times Q_{10}^{\frac{\Delta}{10}}$$

Where: f_1 is the time between tests at the higher temperature; f_2 at the lower temperature; "delta" is the temperature difference between two tests;

$$Q_{10} = \frac{\text{The storage time at T°}}{\text{The storage time at T° +10°C}}$$ [10]

The sample was stored in two aging cabinets corresponding to two temperature levels of 40°C and 50°C:

1) Memmert cabinet (HCP 105 Humidity Chamber, USA) at 40°C with 90%RH humidity;

2) Shel Lab cabinet (FDA - S/N 04151404, USA) at 50°C with 90%RH humidity.

Every 4 to 7 days, a sample was taken to determine and evaluate the moisture content, hardness, and oxidation of the sample.

2.8. Statistical analysis

Analysis of variance (ANOVA) method and the differences between means LSD were carried out using the Statgraphics software. The statistically significant difference was considered at the level of $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

3.1. Nutrient composition of raw materials

The results of analyzing the moisture content (% wt.) in puffed brown rice (PBR) 3.86±0.00%, dried soybean (DSB) 4.82±0.04%, and whole black sesame 5.55±0.11%. From there, other nutritional components calculated as % of dry matter are presented in Figure 1. In this study, the PBR material was processed through several stages including cooking, drying, and frying in oil, while the DSB material was subjected to dry roasting. To date, there has been no published study on the effects of the frying process on the nutrient composition of brown rice grain or the effects of the drying process on the nutrients in soybeans, so there is no data available for comparison. Therefore, the interpretation can only be based on the nutrient composition of the PBR and DSB materials. Moisture content in both PBR and DSB was very low 3.86±0.00% and 4.82±0.04%, respectively, compared to the moisture content in the same ingredients reported as 10.37% [14] and 8.07% [15]. This difference in moisture content may be due to the fact that after cooking, the brown rice continues to undergo a process of drying and frying, similar to soybeans which also undergo a drying process. Heat transfer has occurred, and the heat generated during the drying and frying process will transfer to the inside, creating a rate of water evaporation from the surface of the food, thereby reducing the moisture content inside the ingredient.

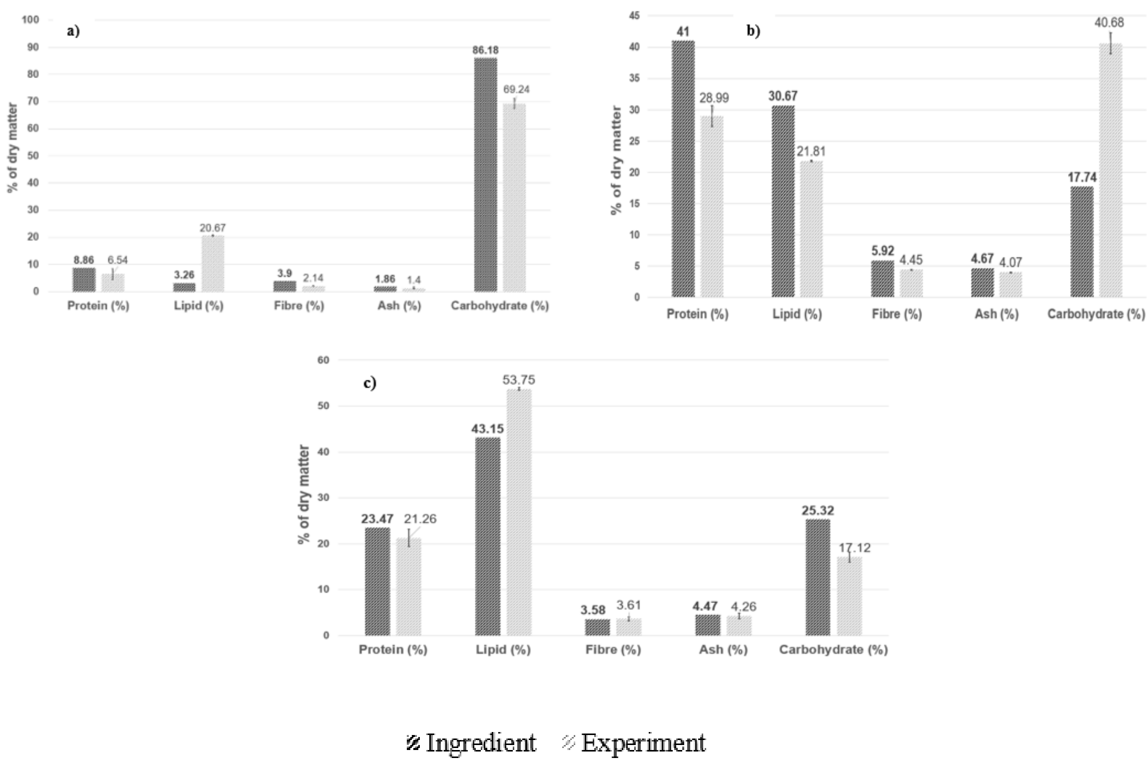

Figure 1. *Nutritional components in each type of ingredient.*
a) Puffed brown rice; b) Dried soybean; c) Whole black sesame seeds.

As observed in (Figure 1.a), the low protein content in PBR decrease compared to the corresponding raw materials may be due to the heat process that denatures the protein or may differ based on seed source, conditions growth, etc. On the contrary, the increase in lipid content in PBR is due to the frying process, which leads to the creation of a surface texture due to water loss, allowing the fat to be absorbed and increase lipid content. For DSB (Figure 1.b), both lipid and protein content decrease compared to the corresponding raw materials. This phenomenon can be explained based on the drying process of the seeds, where high drying temperatures cause some lipids to oxidize.

In addition, it could also be due to the storage time leading to lipid oxidation. The crude fiber and ash content in DSB and PBR are also lower than in the raw materials. This can be explained by the image processing affecting the components of brown rice and soybeans due to high temperatures and the time leading to their alteration and decomposition. Furthermore, frying and drying processes can also cause some minerals and vitamins to be lost due to the effects of temperature and oxidation. When considering the carbohydrate content in PBR, it was also lower, which can be explained as follows: carbohydrate is the main nutritional component in brown rice and when fried, most carbohydrates remain intact, but some sugars may be oxidized, leading to a decrease in their content inside. In contrast, in DSB, when the drying temperature increases, both protein and fat decrease while carbohydrate increases [16], which is also observed in the experimental sample at 40.68±1.66%, higher than the reported carbohydrate content of 17.74% in the raw material. Sucrose, stachyose, and raffinose are the main soluble sugars in soybeans [17]. Therefore, it can be inferred that when the drying temperature increases, the moisture content of the seeds decreases, increasing the levels of sucrose, raffinose, and stachyose in the seeds. The protein in sesame seeds (Figure 1.c) is a complete protein with a very important ratio of essential amino acids for the human body [18]. A genome-wide association study on sesame seed coat color found that the protein content of sesame seeds increases as the seed coat color becomes darker [19]. The lipid content in the analysis varies from 37% to 63% compared to the reported values for sesame [18] and in this study, the determined lipid content was at a moderate level (53.75±0.26%). The amount of fat also affects the size and color of the seeds, with decreasing oil content as the seed coat color darkens [18]. Overall, the differences in fat content can be explained based on seed source or other factors such as growing season, and harvest time [18] and may also be affected by the method of fat extraction.

3.2. Proposed blending formula and selection of feasible formula.

Figure 2. *Illustrates the nutrient bars with blending ratios according to formulas FM1, FM2, FM3, FM4, and FM5 (from left to right).*

Product development was carried out according to the blending formula presented in Table 1 and the sample preparation method described in section 2.2.2. The NBs obtained are illustrated in Figure 2. Due to the varying nutrient contents of each ingredient, their influence on the product quality was different (see Table 2).

Table 2. *Effects of ingredients composition on product quality.*

Formula	Hardness (N)	Protein (g/100g)
Control	$8.77^{a} \pm 0.23$	6.50
FM1	$21.04^{cd} \pm 1.87$	22.58 ± 0.18
FM2	$19.44^{bc} \pm 1.07$	22.22 ± 0.34
FM3	$22.71^{d} \pm 0.83$	23.03 ± 0.06
FM4	$18.49^{b} \pm 1.64$	22.05 ± 0.44
FM5	$23.10^{d} \pm 0.45$	23.16 ± 0.10

The NB formulas 1, 2, 3, 4, and 5 with different ratios of soybean, sesame, and brown rice. The average value with the different characters (a, b, c, d) in the same column are significantly different (p>0.05). The Mean ± SD values were represented for 3 repetitions.

Hardness is an important parameter of protein-rich NB [20]. The difference in hardness of the produced protein bars was significantly different from the commercial control bars ($p \leq 0.05$). NB (FM3, FM5) had the highest hardness value ($22.71^{d} \pm 0.83$ N, $23.10^{d} \pm 0.45$ N), while the control formula had the lowest hardness value ($8.77^{a} \pm 0.23$ N). This indicates that when different levels of protein are used, the bars may have better or worse than expected texture [21]. However, this difference can be explained by the different ratios of the ingredients used, uneven distribution of the mixture, compression force on the particles, and different aging processes that create the texture of NB. Moreover, the hardness of NB is significantly affected by the level of sweeteners and flaxseeds [22].

The highest protein content was found in FM5 (23.16±0.10%), while the lowest protein content was found in FM4 (22.05±0.44%) and all formulas met the value of protein-rich NB containing 20-25g protein per 100g [5]. The protein content in the study samples was significantly higher than that in the control bar (6.50%), which may be due to the use of different raw materials containing different levels of protein.

FM4 was considered feasible because the hardness value had a significant difference but was closest to the control sample ($p \leq 0.05$), so it was proposed to further investigate the physical characteristics such as texture, moisture, and chemical properties through the peroxide value (PoV) when storing the samples at different temperatures. The results of determining the components and calculating the energy of NB obtained according to FM4 are presented in Table 3.

Table 3. *Nutritional composition of nutrition bars mixed according to FM4.*

Chemical composition	Unit	Content
Moisture	g/100g	4.75 ± 0.02
Crude protein	g/100g	22.05 ± 0.44
Crude fat	g/100g	25.68 ± 0.12
Crude fibre	g/100g	3.66 ± 0.05
Ash	g/100g	3.40 ± 0.12
Total carbohydrate	g/100g	40.45 ± 0.64
Energy	Kcal/100g	481.17 ± 0.75

The mean ± SD values were represented for 3 repetitions.

Depending on specific needs, NB can be focused on carbohydrates, fats, and proteins and sometimes serve as a complete meal [5]. Carbohydrates are the highest component (40.45±0.64%), followed by fats (25.68±0.12%), protein (22.05±0.44%) and the energy content is 481.17±0.75 Kcal/100g in NB processed according to FM4. The results of this nutrient content are quite similar to the study of Eke-Ejiofor J, Okoye C, when processing bars from various grains and oats, with carbohydrate, fat, and protein content of 38.8%, 29.4%, and 21.3%, respectively, with a total energy of 505 Kcal [23]. There was also a study on energy bars with carbohydrate content of 55.6–60.8%, fat 12.3–22.4%, protein 7.5–9.8%, and total energy 354–468 Kcal [24]. The nutrient composition of this blended formula showed a significantly high content of fat and protein, which explains why this type of food provides more calories. However, the presence of lipid content in NB is mostly unsaturated fats in oily grains, which is beneficial for health [25]. This significant difference may also be related to the different unavoidable compositions of raw materials [23]. Therefore, NB mixed in this study has a nutrient composition similar to some other studies, while still maintaining the inherent nutritional value of this product line.

3.3. Investigation of shelf-life

Shelf-life survey uses the heat acceleration method (Q_{10}) to accelerate the aging process of the product. In particular, the factors that greatly affect the product are moisture, hardness and PoV. However, during the survey process, whichever factor appears first will stop the survey and select that factor to determine the product shelf-life.

Moisture changes and texture of NB over storage time

The changes in moisture content and hardness of NB blends prepared according to FM4 over time are shown in Table 4.

Table 4. *Changes in moisture content (%) and hardness (N) of nutrition bars over time at storage temperatures of 40°C and 50°C.*

Storage time (days)	Moisture (%)		Hardness (N)	
	40°C	50°C	40°C	50°C
1	$6.26^{cx}\pm0.82$	$6.26^{cx}\pm0.82$	$18.49^{ABC}\pm0.66$	$18.49^{ABC}\pm0.66$
4	$5.18^{cx}\pm0.11$	$6.18^{cy}\pm0.28$	$23.54^{CD}\pm1.17$	$27.02^{CD}\pm0.32$
10	$3.88^{bx}\pm0.32$	$5.52^{by}\pm0.19$	$23.83^{DE}\pm0.68$	$32.03^{DE}\pm1.15$
17	$3.81^{ax}\pm0.11$	$3.66^{ay}\pm0.14$	$33.78^{E}\pm1.79$	$34.85^{E}\pm2.01$
24	$3.16^{ax}\pm0.09$	$3.62^{ay}\pm0.08$	$23.67^{D}\pm1.32$	$29.25^{D}\pm0.65$
31	$3.01^{ax}\pm0.26$	$3.31^{ay}\pm0.26$	$22.03^{BCD}\pm2.54$	$23.77^{BCD}\pm2.19$
38	$2.85^{ax}\pm0.10$	$3.18^{ay}\pm0.30$	$20.89^{ABC}\pm2.14$	$15.96^{ABC}\pm1.67$
45	$2.80^{ax}\pm0.11$	$3.01^{ay}\pm0.36$	$17.77^{AB}\pm0.70$	$15.37^{AB}\pm0.87$
52	$2.78^{ax}\pm0.52$	$2.90^{ay}\pm0.09$	$15.95^{A}\pm0.52$	$10.94^{A}\pm1.95$

The average values of different indices (a-c), (A-E) in rows, and (x-y) in columns are significantly different ($p \leq 0.05$). The mean ± SD values of three replicates were presented.

In this study, all NB samples were stored in vacuum-sealed aluminum packaging, so the moisture changes can be considered insignificant. According to the obtained data, the initial moisture content before storage was $6.26^{cx}\pm0.82$%. NB was stored at two temperatures of 40°C and 50°C and on the first day of storage, the moisture content was $5.18^{cx}\pm0.11$% and $6.18^{cy}\pm0.28$%, respectively. After 52 days, the moisture content significantly decreased ($p\leq0.05$) at both temperatures and storage times (days). However, there was no difference between samples stored from day 1 to day 4 and from day 17 to day 52. The moisture loss over time has also been reported in other research when conducting experiments on low-calorie cereal bars. The authors suggested that the moisture loss was due to a decrease in water activity. Some moisture may have evaporated from the surface, leading to a decrease in moisture during storage [13].

Most NBs will change in taste and texture during storage, with changes in hardness being the most significant factor [26]. According to Table 4, the initial hardness before storage was $18.49^{ABC}\pm0.66$ (N). After 17 days of storage, the obtained data showed that there was an increase in hardness at both temperatures, but it was not significant ($p>0.05$). However, when compared over time (days) of storage, significant differences in hardness values were observed between samples on day 1 and day 17 ($p \leq 0.05$). This result was similar to the study by S.M. Loveday et al. when investigating protein bars containing sugar, milk protein concentrate (MPC), glycerol, and cocoa butter. These authors also acknowledged that the increase in hardness values of NB during storage may be due to thiol-disulfide exchange reactions leading to the synthesis of protein crosslinks and network formation. Hardness can also be due to the second-order texture of proteins being more ordered and having lower surface hydrophobicity of protein particles [27]. Published research has found that moisture migration is an important factor in food products and the quantity and distribution of water significantly affect the quality and stability of the food [28].

Additionally, protein containing cross-links through the Maillard reaction between reducing sugars and residual lysine also plays a role in stiffening NB, and the NB matrix contains reducing sugars and proteins that are prone to Maillard reactions during heating, this can decrease the quality characteristics of NB, resulting in flavor, color, and stiff texture loss [28]. On the 24th day of storage, the hardness decreased gradually from $33.78^{E}\pm1.79$ (N) to $23.67^{D}\pm1.32$ (N) at 40°C and from $34.85^{E}\pm2.01$ (N) to $29.25^{D}\pm0.65$ (N) at 50°C. The reason for this phenomenon may be due to the effect of sweeteners. There were published acknowledged that the hardness of NB is significantly affected by the ratio of sweeteners added [22]. Therefore, in this study, the FM4 samples (excluding the control sample) used sweeteners such as corn syrup and molasses, which were reasonable for the hardness decrease after prolonged storage. It is also possible that storage time and temperature increase the rate of lipid oxidation in the sample, leading to structural damage.

Peroxide value of the nutrition bar

The storage time and temperature significantly affect the PoV in cereal bars as they contain lipids, which accelerate the oxidation process through reactions that generate free radicals and/or oxidative species. The oxidation of fats in the sample leads to a decrease in flavor, aroma, color, texture, and loss of beneficial polyunsaturated lipids [29]. The PoV value of NB (FM4) significantly increased after 52 days of storage at 50°C compared to 40°C (Figure 3).

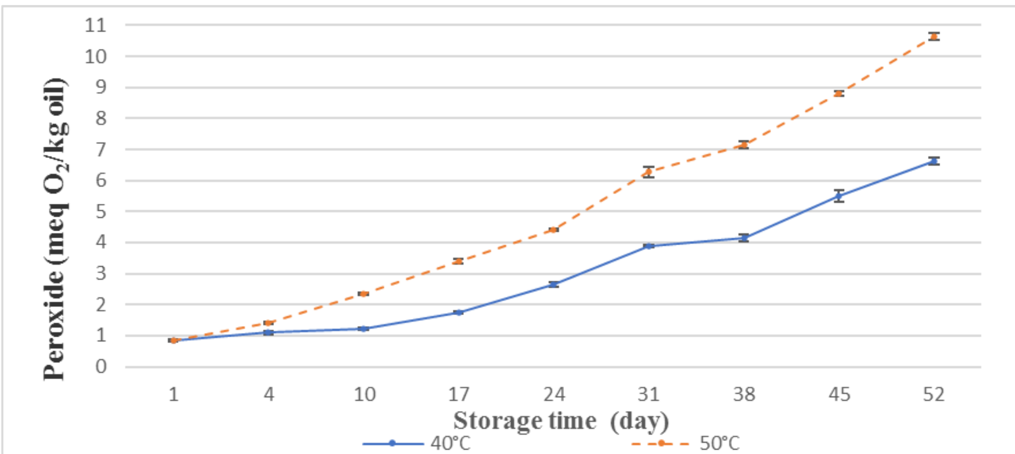

Figure 3. *Effect of temperature and storage time on peroxide change of NB (FM4).*

The experimental results showed that the PoV increased gradually from the early storage days to day 52, corresponding to different storage temperatures and both temperature levels and storage time had significant statistical differences ($p\leq0.05$). This is because the oxidation process increased rapidly due to the influence of humidity, temperature, and oxygen. In addition, the product had a high-fat content of up to 25.68±0.12% which affects the shelf-life of the product because the high-fat content increases the risk of rancidity of the product. Based on the results of Afifah et al., every 10°C increase in temperature doubles the rate of oxidation [30]. This may explain why heat treatment methods such as frying in oil and drying raw materials such as PBR and DSB mainly undergo oxidation on the surface, showing that the quality of frying oil can greatly affect the overall stability during storage [29]. Even in dry roasted grains, the surface oxidation process is the determining factor because endogenous oil moves to the surface of the grain and interacts with oxygen in the atmosphere [29]. The increase in peroxide content over storage time and temperature has also been reported in the shelf life study of protein-rich mixed cereal grains [31] Granola cereal bars rich in Omega-3 [32]. In summary, cereal grains stored at high temperatures and for longer periods will undergo lipid oxidation more rapidly due to the susceptibility of unsaturated fats in the cereal grains to change, leading to the production and accumulation of more peroxides that damage the product.

Predict the shelf-life

Physical properties changes such as hardness, moisture content, and PoV during storage can significantly affect the shelf-life and quality of food products, of which PoV is one of the significant physical properties monitored during the thermal acceleration method to determine the shelf-life of protein-rich NB. The experimental results showed that the PoV values of NB samples increased significantly before spoilage began. The time of appearance of a slight off-odor in the samples was 52 days at 40°C and 31 days at 50°C. The Q_{10} value was calculated using the following formula:

$$Q_{10} = \frac{52}{31} \approx 1.68$$

The storage time of NB at a temperature of 25°C will be:

$$f_2 = f_1 \times Q_{10}^{\frac{\Delta}{10}} = 52 \times 1.68^{\frac{40-25}{10}} \approx 113.23 \text{ days}$$

The predicted result shows that the NB sample packaged in the vacuum-sealed aluminum composite film has a shelf-life of about 113.23 days, which is quite reasonable compared to previous publications. Specifically, there has been research reported that cereal bars packaged in polypropylene have a shelf-life of 3 to 6 months in aluminum polyethylene, metalized polyester, and vacuum-sealed packaging at ambient and 37°C conditions [31]. In addition, reported that cereal bars made from the fruit peel and baru nut were a source of protein, fiber, and fat, especially unsaturated fatty acids such as oleic and linoleic acids. The cereal bars exhibited stability when stored in vacuum-sealed packaging for approximately 120 days [33].

IV. CONCLUSION

The NB, rich in protein, was made from abundant agricultural products in Vietnam, including soybeans, brown rice, and whole black sesame seeds, which can be a solution to utilize the available agricultural resources to create a nutritious whole grain cereal product. The initial ingredient composition plays a particularly important role in the quality of the product. In the study, both soybean and brown rice ingredients underwent heat treatment, and proximate analysis showed nutritional changes, such as protein, lipid, and carbohydrate content being affected by heat. Additionally, varying the ingredient ratios in the formulas leads to differences in physicochemical properties and structure. Significant differences have been observed in texture and protein content between the control sample and the NB formulas, but all formulas are rich in protein, and the proposed protein-rich NB from soybeans, brown rice, and sesame seeds formula is as follows: 63g of dried soybeans, 17g of whole black sesame seeds, 20g of puffed brown rice, and 20% palm sugar syrup and malt syrup (1:1). Physical properties of protein-rich NB such as hardness value and moisture changed over time during storage, while the PoV when stored at 40°C for 52 days remained within the permissible limits according to the Vietnamese standard (TCVN 10127:2013), with less than 10 meq O_2/kg oil, whereas storage at 50°C exceeded the permissible limit after 52 days. The final product was packaged in a vacuum-sealed aluminum laminated bag with a predicted shelf-life of 113 days. Further studies were needed to evaluate the sensory aspects of the formulations and implement microbiological criteria on the products or to learn more about the effects of sweeteners such as palm sugar on the texture of the protein-rich NB during storage.

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