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Effect of applying beetroot juice and functional vegetable oils in the preparation of high protein nutrition bars on its physicochemical, textural and sensorial properties

Tarek N. Soliman[a], Ahmed B. Shazly[a], Said F. Hamed[b], Hamdy A. Zahran[b*]

[a]*Dairy Department, Food Industries and Nutrition Research Institute, National Research Centre, 12622 Dokki, Cairo, Egypt*

[b]*Fats and Oils Department, Food Industries and Nutrition Research Institute, National Research Centre, 12622 Dokki, Cairo, Egypt*

Abstract

High protein nutrition bars (HPNBs) can be a convenient way to add proteins, carbohydrates, vitamins and minerals to the diet. Red palm super olein (RPSO), coconut oil (CO) and black seed oil (BSO) are unique sources of essential fatty acids, medium chain fatty acids as well as natural polyphenols, tocopherols and tocotrienols that play an important role in disease prevention and human body fitness. The protein bar was formulated with 36% Whey isolate and sodium caseinate protein, 33% glucose and sorbitol, and 13% RPO, CO, and BSO to create a protein bar rich in these natural oils. The freeze-dried beetroot juice powder was added at ratio of 1% of total formula. The prepared protein bars were characterized for texture profile (TP), cutting strength, water activity and sensory evaluation. The obtained results showed that the kind of applied protein affected the texture properties and physicochemical properties. The formulated HPNB bars using sodium caseinate showed the lowest TP and cutting tests. HPNBs prepared could be regarded as a very good natural supplement of essential fatty acids needed for human beings for protecting against many diseases. The bars showed also good sensory qualities because of the contribution of the functional coconut, red palm, and black seed oils, with their high contents of phenolic compounds, natural colorants and vitamin E that could increase the shelf-life of the product.

Keywords: Beetroot, antioxidant activity, red palm super olein, protein bars, vegetable oils.

1. Introduction

High nutrition protein bars are often used in athlete's foods and in dieting since they reduce overall food intake, decrease body weight, raise energy levels, build muscle mass, and aid post-exercise recovery [1-3]. The protein bar market is driven by the growing demand for healthy, clean-label, and easy-to-eat foods with better nutrition profiles, great taste and texture, and natural ingredients. From research and markets, its sales are expected to reach $6.8 billion by 2023 [4]. Protein bars can provide health relevance through polyphenolic phytochemicals and antioxidants derived from fruits and vegetables [5, 6]. Typically, a high-protein bar comprises protein content ranges from 20 to 50% (w/w) and sugar syrups. Consequently, the type of protein or protein mix used could substantially influence the texture of the bars.

The methods for controlling the rate and/or level of hardening in protein bars include protein hydrolysis [7], anticaking agents like SiO_2 or $Ca_3(PO_4)_2$ [8], altering carbohydrate fractions [9], protein blending [10], extrusion and milling of milk protein concentrate powder and/or modify other properties of protein powder [11]. Trapping polyphenolic compounds in aggregate protein-polyphenol particles improves bio-accessibility/bio-availability during the digestion process [12], preserves hypoglycemic and antimicrobial properties, reduces potential protein allergenicity, and improves biosignatures of active polyphenol metabolites in the bloodstream [13-15]. These characteristics encourage their inclusion in food items, hence increasing dietary intake of bioactive polyphenolic components and proteins while opening up markets for new products and commercial prospects.

Beetroot juice (BRJ) is becoming more popular among athletes looking to boost their athletic performance. BRJ has a high nutritional value and is

*Corresponding author e-mail: hazahran@hotmail.com, **(Hamdy A. Zahran)**

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a good source of antioxidants, vitamins, and minerals, including a high content of nitrate, which in the human body may be converted to nitrite and then to nitric oxide (NO), a compound known to have a vasodilatory effect, resulting in lower blood pressure and enhanced oxygen and nutrition supply to active muscle [16, 17]. These findings suggest that BRJ might be useful in preventing and treating cardiovascular disease. Furthermore, BRJ intake affects oxygen transport to skeletal muscles, muscular efficiency, tolerance, and endurance, potentially improving athletic performance [16].

Red palm oil (RPO) is derived from the fruit of the oil palm (*Elaeis guineensis*) [18, 19]. It is produced before refining, and its distinctive colour is attributable to the quantity of carotenoids (500 - 700 mg/L) in the crude oil [20, 21]. Red palm oil is a rich source of vitamin E. Vitamin E refers to a group of eight different compounds: α-, β-, γ-, and δ-tocopherols and the corresponding four tocotrienols. Such high contents of vitamin E and carotenoids function as super-antioxidants [22]. Black seed oil, which is regarded as a functional oil because it is rich in essential fatty acids such as linoleic (54.0–70.0%) and oleic (15.0–24.0%), has protective and therapeutic actions against some liver diseases and also has a protective effect against oxidative stress [23, 24]. Coconut oil contains 95% from its constituents as saturated fatty acids, which about 65% are found as medium chain triglycerides. Medium-chain triglycerides (MCT) are triglycerides constructed of three fatty acids (6–12 carbon atoms) esterified to a glycerol backbone. Unlike long chain triglycerides (LCT), MCT will be broken down into glycerol and MCFAs, which will be directly absorbed into the blood stream and thereby transported to the target organs. In spite of inconsistence exists, many studies support the use of MCT as a supplement for weight loss. Studies in both human and rodents have shown that MCFA oxidation induces weight loss through increasing energy expenditure and fat oxidation and helping in the process of excess calorie burning [25].

Therefore, the objective of this study was to evaluate the performance of protein-polyphenol particles created in model protein bars using whey protein isolate, sodium caseinate, or their mixtures, as well as functional vegetable oils like red palm super olein (RPSO), coconut oil (CO), and black seed oil (BSO) supplemented with polyphenol-rich beetroot juice powder. The interaction between polyphenols and protein types alterations were investigated to gain insight into the processes generating hardness in protein bars and examine the ability of protein polyphenol particles to minimize such situation.

2. Materials and Methods

2.1. Materials

Red palm super olein was kindly supplied by Malaysian Palm Oil Board (MPOB), Malaysia. Whey protein isolate (WPI, BiPro®) was obtained from Davisco Foods International, Le Sueur, MN, USA. Bovine sodium caseinate, Folin-Ciocalteu reagent, gallic acid and 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) were obtained from Sigma/Aldrich (St. Louis, MO, USA). Natural aroma (vanilla), in powder form, was received from GBD Aromaty (Warsaw, Poland). Oat was obtained from local market, Giza, Egypt. All solvents and chemicals were of HPLC grade.

2.2. Methods

2.2.1. Preparation of Beetroot juice powder (BJP)

One kilogram of beetroots was washed, peeled, cut, and then frozen at -10ºC for 24 h to destroy the cellular structure of beets. After 24 h, the frozen beets were brought out and kept at room temperature for thawing. The beetroots were minced by blender and pressed to extract the juice. The juice was filtered first through organza fabric, and afterward under vacuum through qualitative filter paper for the elimination of suspended solids. The beetroot juice (BJ) was freeze-dried to create beetroot juice powder (BJP).

2.2.2. Identification of polyphenols of beetroot juice powder (HPLC)

Separation and quantitative determination of polyphenols content of beetroot juice powder were carried out using HPLC Agilent 1260 series. The separation was carried out using the Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μl for each of the sample solutions. The column temperature was maintained at 40°C, according to Croci et al. [26]. The used standards were; gallic acid, catechin, caffeic acid, rutin, quercetin, cinnamic acid, coumaric acid, ferulic acid, naringenin and propyl gallate.

2.2.3. Oil phase preparation

Red palm super olein (RPSO), coconut oil (CO), and black seed oil (BSO) were blended at different proportions to create four admixtures, as shown in Table (1).

Table 1. Admixtures of red palm super olein, coconut oil and black seed oils (BSO).

Sample Code	RPSO	CO	BSO
	Admixtures *(v/v)*		
A	-	100	-
B	72.50	22.50	5
C	47.50	47.50	5
D	22.50	72.50	5

RPSO, red palm super olein; CO, coconut oil and BSO, black seed oil

2.2.4. Determination of Fatty acid composition

Fatty acid composition of vegetable oils and their admixtures was determined according to a modified trans-methylation method [27]. The fatty acids methyl esters (FAMEs) were separated according to Zahran and Tawfeuk [28] on an HP 6890 plus gas chromatography (Hewlett Packard, USA), using a capillary column (30.0 m × 530 µm, 1.0 µm thickness, and polyethylene glycol phase INNOWAX). Detector (FID) and the injector temperature was 250°C. The column temperature was 100°C (held for 5 min) and rose to 240°C, at rate of 4°C/min, temperature was held at 240°C for 10 min. The carrier gas was nitrogen (N_2) at flow rate 15 mL min^{-1}. Sample volume was 1µL (in *n*-hexane) and injected through a split/splitless injector at splitting ratio of 100:20. FAMEs were identified by comparing their relative and absolute retention times to those authentic standards of FAMEs (Supelco™ 37component FAME mix). Fatty acid composition was reported as a relative percentage of the total peak area.

2.2.5. DPPH radical scavenging activity (RSA %)

Free radical scavenging activity (RSA %) of the samples was measured using a modified method of Brand-Williams et al. [29]. An aliquot 100 µL of the sample solution was mixed with 2.9 mL of 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) in methanol. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a UV-visible spectrophotometer. RSA % was calculated using the following equation:

$$RSA\% = (Ab_B - Ab_S)/ Ab_B) * 100$$

Where, Ab_B and Ab_S are the absorbance values of the blank and sample, respectively. The measurements were carried out in triplicates.

2.2.6. Determination of total phenolic content (TPC)

The polyphenols content (TPC) of beetroot juice powder and protein bars samples were determined according to the method of Singleton, et al., [30] as a colorimetric method using the Folin-Ciocalteu reagent. The reaction mixture was prepared by mixing 0.5 mL plant extracts and 0.5 mL of 10-fold diluted Folin-Ciocalteu's reagent and 5 mL of 7.5 % Na_2CO_3 solution was added. The mixture was mixed well and left in the dark at room temperature for 90 min. A blank solution with 0.5 mL distilled water was also prepared in place of sample. The absorbance was read against the blank using spectrometer (Instrument specification) at 725 nm. A mean value of three replicates was taken for all analyses [31]. Total phenolic content (TPC) was measured through by extrapolating calibration curve construed using gallic acid solution. TPC was expressed as gallic acid equivalent (mg GAE) per gram of the dried sample.

2.2.7. Preparation of high protein bars

Whey protein isolate, bovine sodium caseinate, or combination 1:1 (36.00%, w/w) with oat (5 %, w/w) and vanilla powder (1%, w/w) were placed in a bowl and mixed using the B10A industrial mixer (Technologies 4ALL; Kępno, Poland) for 1 min at 190 rpm. Beetroot juice powder (1%, w/w) was dissolved in water (5.00%, w/w) in a separate laboratory vessel. In another vessel, soy lecithin (1%, w/w), glycerol (7% w/w) and oil phase (13.00%, w/w) were combined. Glucose syrup (33% w/w) was heated to 80 °C, poured into dry ingredients, and placed in the mixer bowl. The remaining ingredients prepared earlier were added simultaneously after pouring the syrup. The mass was mixed for 5 min at 365 rpm using the mixer. Finished processed high protein bars mass cut transversely into individual bars (95 × 30 mm, height 15 mm). The all samples were stored at 35 °C for 30 days for further analysis.

2.2.8. Characterizations of protein bars

2.2.8.1. Texture profile (TP) analysis

Texture measurements were carried out on TA-XT2i Texture Analyzer (Mult-test 1dMemesin, Food Technology Corporation, Slinfold, W. Sussex, UK) coupled with the Software Texture Expert. The test velocity was 1 mm/s. The high-protein bars were twice compressed by a 36 mm diameter probe (SMS P/36R) to achieve 70% deformation (interval between probe movements: 5 s). High-protein bar samples were evaluated for hardness (N) fracturability (N) springiness (mm) cohesiveness and gumminess (N). Analyses were carried out in five replications for each sample at room temperature. Hardness, cohesiveness, adhesiveness, springiness and gumminess were calculated from the obtained TPA according to the definition given by Małeck et al. [32].

2.2.8.2. Cutting Test

Cutting strength of high-protein bars was measured using Texture Analyzer (TA-XT2i). The blade set with knife (HDP/BSK) comprising a Warner Bratzler blade (a reversible blade with knife edge) with a slotted blade insert and a blade holder was used for the experiment. In operation, the blade was firmly held employing blade holder, which was screwed directly to the texture analyzer. The slotted blade insert was placed directly onto the heavy-duty platform and acted as a guide for the blade whilst providing support for the product. High-protein bars were placed on the metal plate. Then the blade was lowered at a speed of 2 mm/s. The cutting curve was obtained by recording the maximum force the blade needs to cut the sample completely. Five repetitions were applied for each formulation. The results were based on the maximum peak (maximum force) resulting from the shear stress [32].

2.2.8.3. Water Activity (a_w)

Water activity (a_w) was measured using the AWMD-10 water activity meter (NAGY, Gäufelden, Germany) with the accuracy of ±0.001 of a_w unit. Before measurement, the apparatus was calibrated with the dedicated humidity standard (95% HR). Measurements were performed at the temperature of 25°C. For each sample, two outliers were classified as defective and were excluded from further analysis [32].

2.2.8.4. Sensory Evaluation

A panel of 15 trained panelists was recruited from the Dairy department, National research Centre. The criteria for selection were that the panelists should be between 35 and 65 years old, regular consumers of high-protein bars, and not allergic to any raw material used. Panelists were instructed to evaluate the sensory attributes; color, aroma, consistency, and taste. A 5-point hedonic scale (1 = extremely dislike, 5 = extremely like) with significance factors (0.2—color, 0.2—aroma, 0.25—consistency and 0.35—taste) was used [32].

2.3. Statistical analyses

The analyses of prepared samples were conducted at least in triplicates; comparisons of the treatments were completed by one-way ANOVA and Tukey's tests by SPSS, ver. 16.0 statistics programs. A 95% minimum confidence level was taken for all statistical analyses.

3. Results and discussions

3.1. Bioactive components in protein bar

3.1.1. Beetroot

Total phenolics, total betaxanthins, and total betacyanins content as well as antioxidant activity, measured as DPPH radical scavenging, of beetroot juice powder are presented in Table 2. Total polyphenols, total betaxanthins, and total betacyanin content were 129.57 mg gallic acid equivalents (GAE), 85.45 mg vulgaxanthin-I equivalents (VE), and 86.29 mg betanin equivalents (BE), respectively. These results were higher or lower than those reported by Kujala et al. [33]. In addition, Georgiev et al. [34] reported different results for phenolics, beta-xanthin and beta-cyanin content in beetroot juice. These differences in our findings could be attributed to differences in beetroot varieties, climatic conditions, or extraction techniques.

Table 2. Bioactive components and DPPH radical scavenging activity of beetroot juice powder

Items	Values
Total phenolic content (GAE/100 g)	129.57 ±4.045
Total betaxanthins content (VE/100 g)	85.45 ±2.09
Total betacyanins content (BE/100 g)	86.29 ±2.21
DPPH radical Scavenging Activity (%)	75.98 ±2.00

Table 3 shows the individual phenolic components and water-soluble vitamin content in BJP as determined by HPLC methods. It was revealed that cinnamic acid, propyl gallate, naringenin, caffeic acid, and catechin were the major components found in BJP. According to Desseva et al., [35] the HPLC examination of the native red beet juice's phenolic acids showed that it had amounts of chlorogenic, caffeic, pcoumaric, and sinapic acids that were, respectively, 16.99, 22.21, 52.69, and 19.86 µg/g dw. The bioaccessibility of sinapic acids remained at 16.15 µg/g dw, or 81% recovery. Regarding the water-soluble vitamin's concentrations, the decreasing order in BJP was folic acid > B_2> B12> C.

Table 3. HPLC analysis for polyphenols and water-soluble vitamins of beetroot juice powder.

Identified compounds	(µg/g)
Catechin	8.87
Coffeic acid	1.22
Ellagic acid	2.72
Naringenin	1.06
Propyl Gallate	2.07
Querectin	2.42
Cinnamic acid	3.17
Vitamin B_{12}	513.70
Vitamin B_2	241.50
Vitamin C	200.10
Folic acid	586.40

3.1.2. Vegetable oils under investigation

3.1.2.1. Fatty acid composition

The FA composition of red palm super olein (RPSO), coconut oil (CO) and black seed oils (BSO) and their admixtures were analyzed and given in Table 4. All FA constituents seemed to be within the normal ranges for the three oils according to *Codex Alimentarius Standards* [36]. Red palm oil as it is a mildly processed crude palm oil retains many useful components such as antioxidants and carotenes that are lost in physical or chemical palm oil refining [37]. Palm olein is a liquid fraction produced through palm oil fractionation, involving crystallization under controlled temperature and filtration to remove crystals. RPSO is a more unsaturated fraction obtained by further fractionation of olein. As shown in Table 3, RPSO contains approximately 40% saturated fat, 33% of which is palmitic acid (16:0) and 60% unsaturated fatty acids. Oleic acid (C18:1), is the major unsaturated (monounsaturated) fatty acid that constituted about 46% of total FA composition. While polyunsaturated fatty acids are represented by linoleic acid (C18:2) with about 13% share. Such high unsaturated fatty acid content in RPSO was reflected on its melting point (18–20°C) and therefore it is a liquid at room temperature (25°C). Therefore, super olein has better clarity, stability, and lower tendency to turn cloudy compared to normal olein [38].

Table 4. Fatty acid composition of red palm super olein (RPSO), coconut oil (CO), black seed oil (BSO) and their admixtures.

Fatty acid	RPSO	CO (A)	BSO	B	C	D
	Area (%)					
Caproic acid (C6:0)	ND	0.50	ND	ND	ND	ND
Caprylic acid (C8:0)	ND	7.73	ND	ND	2.56	2.64
Capric acid (C10:0)	ND	6.98	ND	ND	3.05	3.65
Lauric acid (C12:0)	0.50	51.86	ND	10.22	24.01	34.12
Myristic acid (C14:0)	1.00	16.86	ND	4.29	8.64	12.30
Palmitic acid (C16:0)	33.82	7.16	12.87	26.69	20.54	14.31
Palmitoleic acid (C16:1)	ND	0.05	0.32	ND	ND	ND
Stearic acid (C18:0)	5.03	3.01	3.25	4.25	3.67	5.42
Oleic acid (C18:1, n9)	46.00	4.45	26.57	38.73	27.05	19.24
Linoleic acid (C18:2, n6)	13.25	0.93	53.88	15.82	10.48	8.32
Linolenic acid (C18:3, n3)	ND	ND	0.45	ND	ND	ND
Arachidic acid (C20:0)	0.40	0.47	0.54	ND	ND	ND
Gadoleic acid (C20:1)	ND	ND	2.57	ND	ND	ND
SFA	40.75	94.57	16.66	45.45	62.47	72.44
USFA	59.25	5.43	83.34	54.55	37.53	27.56
PUFA	13.25	0.93	54.33	15.82	10.48	8.32

ND: not detectable; SFA: saturated fatty acids; USFA: unsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Regarding coconut oil (CO), Table 3 indicates that it consists of about 95% saturated fatty acids while unsaturated fatty acids were about 5% only. The medium chain fatty acid lauric acid constituted about 51 % of FA profile of coconut oil. Numerous reports have been published on the antimicrobial properties of lauric acid and monolaurin both in vitro and in vivo [39]. Also, they revealed activities against a number of viruses and fungi [40-42]. Detailed study [43] has shown that the majority of ingested lauric acid is transported directly to the liver where it is directly converted to energy and other metabolites rather than being stored as fat. Such metabolites include ketone bodies, which can be used by extrahepatic tissues, such as the brain and heart, as an immediate form of energy. Black seed oil (BSO) fatty acid composition, on the other hand revealed that the unsaturated FA content represented more than 80% of the total FA composition of which the essential polyunsaturated fatty acid, linoleic, accounted for more than 50% (Table 4). This essential PUFAs play a vital role and may act as mediator (s) for the nervous and immune systems by regulating the gene expression, altering the membrane structure, and influencing prostaglandin [44-47]. As shown in Table 4 we tried to tailor three admixtures of these functional oils to reach the most promising fatty acid formulation to be used as the fat base of our targeted protein bar.

3.2. Effect of polyphenols of BJP and oil source on texture profile analysis

As shown in Table 5, the total content of polyphenols in the protein bar produced from whey protein isolate, BJP, and coconut oil was 57.23 mg/100 g, and it increased as more red palm oil was added, reaching 81.5 mg/100 g .The antioxidant activity continued to follow the same pattern with the addition of 95% red palm oil. The functional characteristics of food items and the enjoyment of eating are typically impacted by the interplay of polyphenols and protein constituents. The effectiveness of protein-polyphenol conjugates in food products is enhanced by their stronger antioxidant and better rheological behavior. However, the conjugation of proteins and polyphenols from various food sources results in the development of new food ingredients or products with better nutritional, functional, sensory, and bioactive properties [48].

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Table 5: Total phenolic content and antioxidant activity for HPN bars.

Samples	Total phenol content (mg/100g)	Antioxidant activity (%)
T_1 A	57.23^{d}	35.3^{e}
T_1 B	68.22^{b}	41.28^{de}
T_1 C	74.72^{ab}	46.05^{d}
T_1 D	81.5^{a}	54.94^{c}
T_2 A	50.36^{e}	43.23^{d}
T_2 B	58.63^{d}	48.76^{cd}
T_2 C	64.87^{cd}	53.81^{b}
T_2 D	71.7^{b}	60.51^{a}
T_3 A	58.51^{d}	32.52^{e}
T_3 B	69.53^{b}	39.73^{de}
T_3 C	76.03^{ab}	44.99^{d}
T_3 D	84.94^{a}	53.91^{b}

- ***T_1**, whey protein isolate (WPI); **T_2**, Bovine sodium caseinate (NaCas); **T_3**, WPI: NaCas (1:1);*
- ***A**, 100% coconut oil (CO);*
- ***B**, 72.5% coconut oil (CO) + 22.50% red palm super olein (RPSO) + 5% BSO;*
- ***C**: 47.5% CO + 47.5% red palm super olein (RPSO) + 5% black seed oil (BSO); and*
- ***D**: 22.5% red palm super olein (RPSO) + 72.5% coconut oil (CO) + 5% black seed oil (BSO).*
- *Mean values with different letters in the same column are significant ($p \leq 0.05$)*

Table 6 shows the texture profile analysis of HPNBs fortified with 1000 mg beetroot juice powder (BJP) and red palm oil. In general, protein type had a significant effect on the protein bar's texture profile. The protein bar made from whey protein concentrate had the highest hardness, but that made from bovine sodium caseinate had the least; their combination produced a medium hardness. The results showed that the HPN bar to which coconut oil was added revealed the highest hardness, followed by treatment "D" which contains red palm oil, then (treatment c) consisted of 47.5% Coconut oil: 47.5% red palm oil: 5% black seed oil. The lowest degree of hardness was shown with (treatment B) which consisted of 71.25% coconut oil: 22.50% red palm oil: and 5% black seed oil. This result was attributed to the ability of BJP and RPO to bind to the glycation sites of WPC and NaCas by reacting with active carbonyl groups, thereby blocking the sites available for glycation.

The hardness of HPNBs was associated with the generation of advanced glycosylation end products (AGEs) [49]. Polyphenols such as cinnamic acid, catechin, quercetin, and propyl gallate were used as AGE inhibitors in bread and cookies [50, 51]. Microencapsulated mulberry polyphenols (MMPs) considerably decreased the insolubility, aggregation, and sugar oxidation of HPNBs during late-term storage, according to Khalifa et al. [52]. Specifically, after 45 days of storage. This conclusion was ascribed to MMPs' capacity to attach to whey protein glycation sites by interacting with active carbonyl groups, inhibiting glycation sites. Consequently, Millard reactions involving lysine residues were avoided, causing the hardening process to delay. After 43 days of storage at 32°C, Diaz et al. [53] found that HPNBs created with cranberry juice was substantially softer (firmness range of 0.09-0.85 kPa) than HPNBs prepared without cranberry juice (firmness range of 0.17-85 kPa). This might be due to the inclusion of polyphenols, which inhibited interactions between proteins and other molecules in the HPNB matrix. Noncovalent interactions allow one protein molecule to bind one or more polyphenols to create stable protein-ligand complexes [54]. Similarly, Schneider et al. [55] discovered that whey protein-polyphenol aggregates formed as phenolic concentration increased which improved HPNB stability and decreased textural variance.

Table 6. Texture profile analysis of HPN bar fortified with BJP, and different vegetable oils.

Samples	Hardness (N)	Fracturability (N)	Springiness (mm)	Cohesiveness	Gumminess (N)
T_1 A	95.47	7.47	0.11	0.12	11.08
T_1 B	73.91	8.66	0.13	0.09	6.83
T_1 C	75.23	6.30	0.16	0.12	8.68
T_1 D	90.09	6.14	0.27	0.11	9.52
T_2 A	19.41	8.32	0.10	0.04	0.72
T_2 B	10.58	12.11	0.35	0.04	0.40
T_2 C	12.39	16.50	0.34	0.04	0.47
T_2 D	16.35	11.42	0.13	0.01	0.24
T_3 A	77.54	5.08	0.17	0.13	9.73
T_3 B	50.32	6.24	0.15	0.09	4.73
T_3 C	55.98	4.29	0.40	0.05	2.57
T_3 D	57.22	2.66	0.16	0.10	5.99

- ***T_1**, whey protein isolate (WPI); **T_2**, Bovine sodium caseinate (NaCas); **T_3**, WPI: NaCas (1:1);*
- ***A**, 100% coconut oil (CO);*
- ***B**, 72.5% coconut oil (CO) + 22.50% red palm super olein (RPSO) + 5% BSO;*
- ***C**: 47.5% CO + 47.5% red palm super olein (RPSO) + 5% black seed oil (BSO); and*
- ***D**: 22.5% red palm super olein (RPSO) + 72.5% coconut oil (CO) + 5% black seed oil (BSO).*

Conjugation with tannic acid, gallic acid, and epigallocatechin gallate (EGCG) decreased surface hydrophobicity and α-helix content while increasing myofibrillar protein solubility. These polyphenols that interact with proteins by hydrogen bonding have a larger binding site or affinity to the proteins than quercetin and quercitrin, which engage mostly through electrostatic contact. The phenolic chemicals unfold the tertiary structure at lower concentrations, while the proteins retain their -helix shape, resulting in increased surface hydrophobicity and decreased conjugate solubility [56]. Furthermore, the presence of polysaccharides reduces polyphenol-protein interaction, probably owing to phenolic compound binding to polysaccharides [57]. Furthermore, the protein's molecular flexibility varies based on the protein/phenolic ratio. Because of unsaturated complexes, a higher protein/phenolic ratio increases flexibility and unfolding, resulting in increased surface area and solubility of the protein, whereas a high phenolic concentration causes saturation of the protein binding sites, which negatively affects protein functionality [58].

3.3. Cutting Test

The cutting test reveals a product's firmness or hardness. If the top front teeth were pulled from their natural curvature and made into a straight line, they would resemble a "knife edge," one may think. An accurate biting or cutting motion simulation may be achieved using a knife blade [59]. From Fig. 1, the whey protein concentrate bar had the most significant cutting resistance (51.81 N). However, high protein bars consisting of WPC: bovine caseinate were revealed the most vulnerable to this impact (23.68 N). Combining various proteins in the specified quantities significantly lowered the resistance to cutting in these bars. Additionally, the softness of all bars made from several types of proteins is influenced by polyphenols and red palm oil. The findings are consistent with the hardness study and support the hypothesis that the force required to cut a bar depends on its hardness.

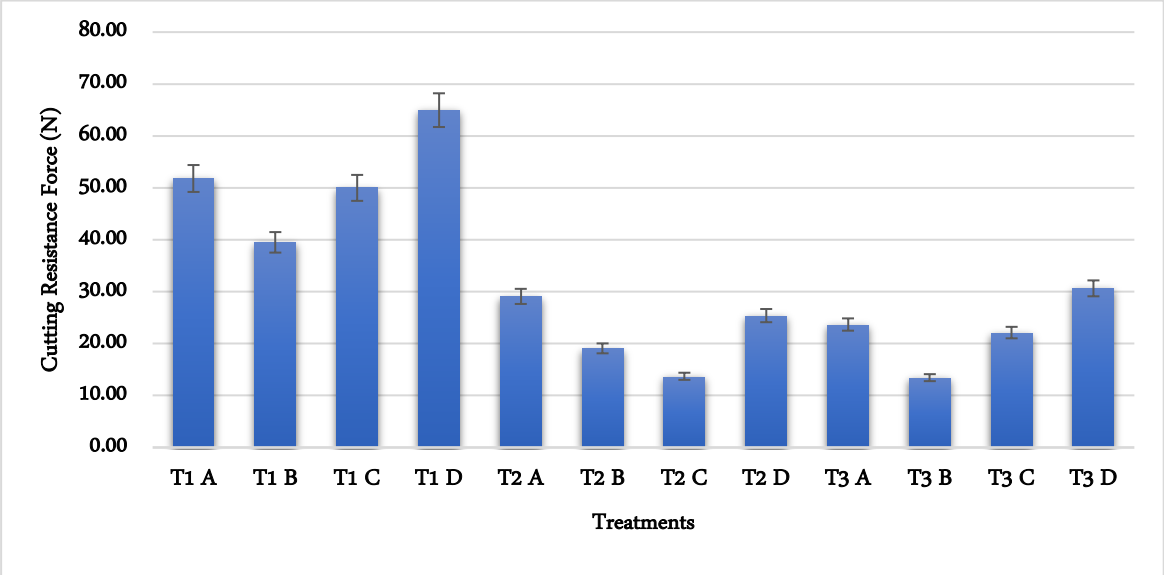

Fig 1. Effect of different vegetable oils and BJP on high-protein bars cutting resistance.

- ***T*$_1$***, whey protein isolate (WPI);* ***T*$_2$***, Bovine sodium caseinate (NaCas);* ***T*$_3$***, WPI: NaCas (1:1);*
- ***A****, 100% coconut oil (CO);*
- ***B****, 72.5% coconut oil (CO) + 22.50% red palm super olein (RPSO) + 5% BSO;*
- ***C****: 47.5% CO + 47.5% red palm super olein (RPSO) + 5% black seed oil (BSO); and*
- ***D****: 22.5% red palm super olein (RPSO) + 72.5% coconut oil (CO) + 5% black seed oil (BSO).*

3.4. Water activity (a_w)

Water activity is the difference between the water vapour pressure produced by free or non-bound water in food and the water vapour pressure produced by pure water. The water activity value is a vital sign of how it can affect the food shelf life. The water activity readings for meals with intermediate moisture content (IMF) range from 0.6 to 0.9. The danger of microbial deterioration is essentially eliminated for foods with intermediate moisture levels. Additives with high water binding capabilities (humectants) are ideal options that may increase the food's shelf life by reducing water activity. Ordinary salt, glycerol, sorbitol, and sucrose have potential as humectants. Additionally, some of these substances like sorbitol and glycerol may serve as sweets and are not suitable for use in many meals at the levels necessary to regulate water activity from consumers' perspective [60]. Fig. 2 shows that the water activity values are less than the intermediate moisture content. The highest values showed with the whey protein isolate prepared with coconut oil. In contrast, the lowest values were noticed with the mixture of whey protein isolate and caseinate in the presence of red palm oil at a concentration of 47.5%. Since water activity represents the ideal, i.e., highly diluted solutions in the thermodynamic equilibrium state, it is not the sole signal to consider when determining foods with low water content storage life. Food's physical characteristics must also be regarded as better forecast shelf life since phase transition, which describes the impact of water contact and hydrophilic components during storage, depends on them. Water activity is not the sole signal to consider when determining the storage life of foods with low water content since it depicts the ideal, i.e., extremely dilute solutions in thermodynamic equilibrium. To better forecast shelf life, the physical qualities of food must be included in addition to a phase transition, which explains the influence of water contact and hydrophilic components during storage. We find that the water activity values in all samples increase with storage at 35°C for 30 days. Still, this increase is proportional to the values with which each treatment started.

Food content, temperature, and storage period all influence the physical condition of foods. For example, depending on the temperature, the phases might be glassy, rubbery, or very viscous, and if the hydrophilic components of food are hydrated, the food phase transforms to plastic. As a result, the water content influences the temperature thermal gravimetric analysis (phase transition temperature) [61- 63].

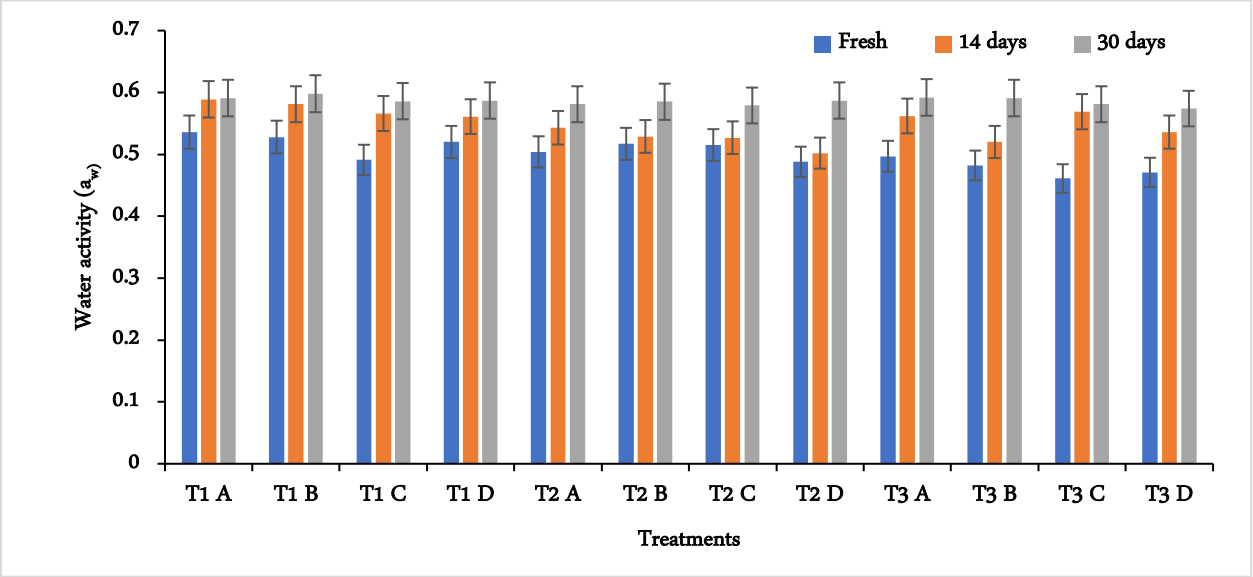

Fig. 2: Water activity of formulated HPN bars using different vegetable oils.

- ***T*₁, *whey protein isolate (WPI); T*₂, *Bovine sodium caseinate (NaCas); T*₃, *WPI: NaCas (1:1);***
- ***A**, 100% coconut oil (CO);*
- ***B**, 72.5% coconut oil (CO) + 22.50% red palm super olein (RPSO) + 5% BSO;*
- ***C**: 47.5% CO + 47.5% red palm super olein (RPSO) + 5% black seed oil (BSO); and*
- ***D**: 22.5% red palm super olein (RPSO) + 72.5% coconut oil (CO) + 5% black seed oil (BSO*

3.5. Sensory Acceptability

Results of the sensory evaluation of the tested high-protein bars are presented in Figure 1. The analysis revealed that the highest scores for the T_3B treatment were made up of WPI and sodium caseinate bars (1:1). The panelist judge appreciated the external appearance, color and taste sensations the most in the highest-rated bars. According to the judges, high ratings for these proteins are associated with a pleasant consistency, taste and color. The worst-rated bar (WPI) had too high hardness, but the aftertaste and the color gave it a high score. Also, all treatments of bars had high scores for odor, and color & appearance, due to the odor of coconut butter and the colour of red palm olein and BJP.

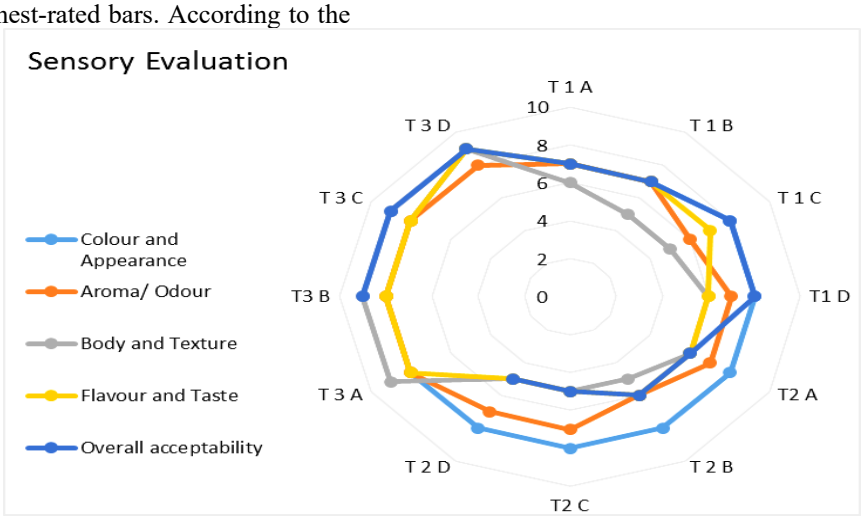

Fig. 3: Sensory evaluation of the tested high-protein bars with BJP and different vegetable oils.

- ***T*₁, *whey protein isolate (WPI); T*₂, *Bovine sodium caseinate (NaCas); T*₃, *WPI: NaCas (1:1);***
- ***A**, 100% coconut oil (CO);*
- ***B**, 72.5% coconut oil (CO) + 22.50% red palm super olein (RPSO) + 5% BSO;*
- ***C**: 47.5% CO + 47.5% red palm super olein (RPSO) + 5% black seed oil (BSO); and*
- ***D**: 22.5% red palm super olein (RPSO) + 72.5% coconut oil (CO) + 5% black seed oil (BSO).*

4. Conclusion

HPNB hardening is mitigated by using polyphenols and red palm super olein, according to this research. The experiment results showed that the kind of protein utilized considerably impacts textural, nutritional, and physicochemical properties. When comparing texture profile (TP) and cutting force findings, the bars constructed with sodium caseinate were shown to have the lowest TP and cutting tests. It is common practice to substitute raw ingredients and add polyphenols to prevent the development of disulfide bonds and the Millard process. These kinds of measures have a significant impact on HPNB hardness and have the potential to maintain HPNB nutrition and quality during storage and transportation.

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