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Evaluation of black seed (*Nigella sativa* L.) cake and its protein in muffins as a valuable potential functional source for obesity control

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ABSTRACT

Background: Due to the use of high-calorie foods and reduced physical activity caused by the modern lifestyle, obesity is on the rise. Obesity occurs by the increase of fat accumulation in the body, causing different complications such as diabetes, cardiovascular diseases, cancer, respiratory disorders, blood pressure, etc., which has become a global problem.

Objectives: The production of functional food that has medicinal properties for controlling or inhibiting diseases is increasingly popular. With the expanding concerns about obesity and its related diseases, it is very important to provide natural controlling or inhibiting compounds. In this study, the functional properties of black seed cake (BSC) and its protein (BSCP) were investigated for obesity control through the inhibition of pancreatic lipase, α-glucosidase, and α-amylase enzymes as well as its antioxidant properties.

Methods: The textural properties and sensory evaluation of muffins, enzyme inhibition properties (inhibiting the effect of enzymes in the obesity process), and antioxidant properties (control or inhibition of side effects caused by body fat oxidation) of BSC and BSCP were investigated.

Results: The highest and lowest inhibitory levels of pancreatic lipase are related to black seed (*Nigella sativa*) cake protein (BSCP) (59.21%) and black seed cake (BSC) (32.90%), respectively. The highest and lowest levels of α-amylase inhibition are related to BSCP (32.51%) and black seed cake in ethanol 75% (BSC-E75%) (3.62%), respectively. The highest and lowest α-glucosidase inhibitory levels are related to BSC-E30% (71%) and BSCP (10.10%), respectively. BSC and BSCP showed high antioxidant activity. The use of BSC and BSCP in the production of muffins (a widely consumed and popular product) to replace flour and oil in the formulation of muffins caused a decrease in L*, b*, but an increase in a*. Hardness, chewiness, springiness, and cohesiveness decreased with the increase of BSC and BSCP content. Samples BSM 3%, BSM 6%, PBM 1%, and PBM 2% were similar to control muffins in sensory evaluation.

Conclusions : From the result of this study, it can be concluded that black seed cake and its protein can be considered a potential efficient source for the production of functional muffins and possibly other functional foods that aim to manage and control obesity, blood glucose, and blood fat. However, more research is needed to fully determine the effectiveness of black seed cake and its protein.

Keywords: Obesity, Black seed cake, Muffins, Diabetes, Blood pressure, Blood fat

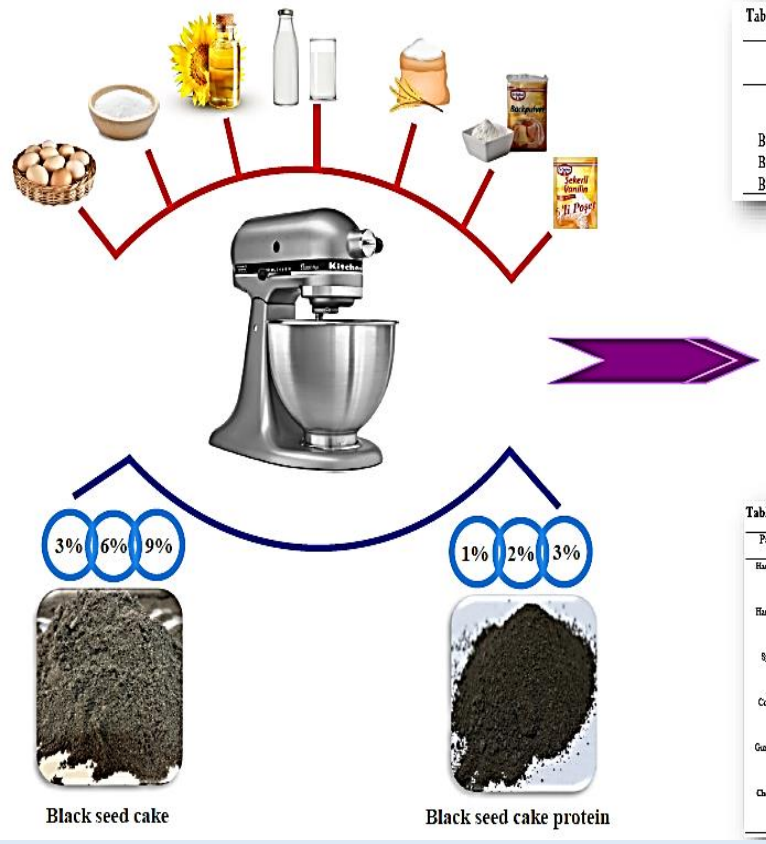


Table 3. Enzymatic inhibitory properties

No.	Pancreatic lipase inhibitory (%)	α-glucosidase inhibition (%)	α-amylase inhibition (%)
BSCP	59.21 ± 0.10^{a}	10.10 ± 0.17^{e}	32.51 ± 0.04^{a}
BSC - B	49.87 ± 0.05^{ab}	57.20 ± 0.01^{ab}	28.95 ± 0.07^{b}
BSC –E 30%	32.90 ± 0.03^{b}	71.00 ± 0.07^{a}	8.80 ± 0.09^{c}
BSC –E 50%	38.51 ± 0.04^{b}	66.70 ± 0.13^{b}	8.46 ± 0.03^{d}
BSC –E 75%	40.30 ± 0.08^{b}	57.40 ± 0.06^{ab}	3.62 ± 0.10^{e}

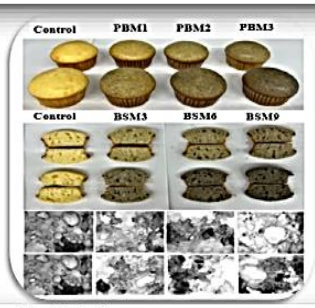


Table 6. Texture profile analysis of muffins

Parameter	Storage day	Control	BSM3	BSM6	BSM9	PBM1	PBM2	PBM3
Hardness 1 (N)	1	2.46 ± 0.20^{c}	2.21 ± 0.11^{b}	1.93 ± 0.04^{a}	1.88 ± 0.05^{a}	2.03 ± 0.09^{a}	1.88 ± 0.07^{a}	1.85 ± 0.10^{a}
	3	3.58 ± 0.18^{b}	3.28 ± 0.03^{a}	3.10 ± 0.09^{ab}	2.77 ± 0.08^{a}	3.13 ± 0.00^{ab}	2.99 ± 0.07^{ab}	2.39 ± 0.08^{a}
	7	4.40 ± 0.17^{b}	3.84 ± 0.10^{a}	3.39 ± 0.14^{a}	3.39 ± 0.13^{a}	3.88 ± 0.12^{ab}	3.51 ± 0.06^{a}	2.72 ± 0.10^{a}
Hardness 2 (N)	1	2.07 ± 0.15^{d}	1.89 ± 0.10^{c}	1.63 ± 0.01^{ab}	1.60 ± 0.06^{ab}	1.76 ± 0.07^{bc}	1.62 ± 0.06^{ab}	1.59 ± 0.07^{a}
	3	3.05 ± 0.10^{b}	2.76 ± 0.02^{d}	2.75 ± 0.08^{d}	2.34 ± 0.06^{b}	2.61 ± 0.10^{cd}	2.51 ± 0.09^{c}	1.98 ± 0.07^{a}
	7	3.65 ± 0.12^{d}	3.20 ± 0.03^{c}	2.81 ± 0.09^{b}	2.82 ± 0.10^{b}	3.28 ± 0.09^{c}	1.95 ± 0.09^{b}	2.23 ± 0.10^{a}
Springiness	1	0.74 ± 0.01^{a}	0.69 ± 0.01^{b}	0.65 ± 0.00^{c}	0.65 ± 0.01^{c}	0.70 ± 0.01^{b}	0.70 ± 0.01^{b}	0.68 ± 0.01^{b}
	3	0.65 ± 0.02^{a}	0.56 ± 0.01^{ab}	0.62 ± 0.01^{ab}	0.58 ± 0.06^{ab}	0.62 ± 0.01^{ab}	0.59 ± 0.01^{ab}	0.62 ± 0.01^{b}
	7	0.64 ± 0.03^{a}	0.58 ± 0.04^{b}	0.57 ± 0.01^{b}	0.55 ± 0.01^{b}	0.58 ± 0.06^{ab}	0.56 ± 0.00^{b}	0.57 ± 0.04^{b}
Cohesiveness	1	0.39 ± 0.01^{a}	0.37 ± 0.00^{b}	0.35 ± 0.00^{c}	0.35 ± 0.00^{c}	0.37 ± 0.01^{b}	0.36 ± 0.01^{b}	0.35 ± 0.00^{b}
	3	0.31 ± 0.02^{a}	0.30 ± 0.01^{b}	0.29 ± 0.01^{b}	0.28 ± 0.01^{b}	0.30 ± 0.01^{ab}	0.30 ± 0.00^{b}	0.31 ± 0.01^{ab}
	7	0.26 ± 0.02^{a}	0.29 ± 0.00^{a}	0.27 ± 0.01^{a}	0.28 ± 0.01^{a}	0.28 ± 0.02^{a}	0.29 ± 0.02^{a}	0.29 ± 0.01^{a}
Gumminess (N)	1	0.92 ± 0.10^{c}	0.81 ± 0.06^{b}	0.68 ± 0.00^{a}	0.66 ± 0.06^{ab}	0.75 ± 0.02^{ab}	0.68 ± 0.02^{ab}	0.65 ± 0.03^{ab}
	3	1.12 ± 0.05^{d}	0.97 ± 0.01^{c}	0.86 ± 0.02^{c}	0.79 ± 0.01^{ab}	0.94 ± 0.00^{c}	0.89 ± 0.01^{bc}	0.74 ± 0.02^{a}
	7	1.26 ± 0.12^{cd}	1.10 ± 0.07^{cd}	0.98 ± 0.02^{a}	0.94 ± 0.06^{ab}	1.13 ± 0.16^{cd}	1.09 ± 0.06^{bc}	0.78 ± 0.16^{a}
Chewiness (N)	1	0.70 ± 0.11^{d}	0.52 ± 0.05^{c}	0.44 ± 0.00^{a}	0.41 ± 0.01^{ab}	0.53 ± 0.02^{bc}	0.47 ± 0.01^{abc}	0.45 ± 0.02^{abc}
	3	0.73 ± 0.03^{c}	0.57 ± 0.00^{ab}	0.57 ± 0.01^{b}	0.45 ± 0.00^{a}	0.58 ± 0.01^{ab}	0.51 ± 0.01^{ab}	0.47 ± 0.03^{ab}
	7	0.82 ± 0.13^{d}	0.64 ± 0.07^{bc}	0.51 ± 0.01^{ab}	0.52 ± 0.06^{ab}	0.67 ± 0.16^{cd}	0.56 ± 0.03^{ab}	0.43 ± 0.10^{a}

INTRODUCTION

Black seed (*Nigella sativa* L.) which belongs to the Ranunculaceae family, is an abundant, cheap, and available medicinal plant, rich in protein, vitamins, alkaloids, thymoquinone, essential oils, and fatty acids (linoleic and oleic acids). It has many phenolic and flavonoid compounds (ferulic acid, flavon, syringic acid, apigenin, nigelflavonoid, rutin, gallic acid, vanillic acid, chlorogenic acid, catechin, quercitrin sophorotioside, kaempferol, kaempferol-3-glucoside, quercetin, quercitrin, and diosmin) that are effective against obesity, cancer, inflammation, diabetes, cellular oxidative damage, and rheumatoid arthritis [1-4].

By disturbing the balance of energy intake and energy consumption, a person becomes obese, which has become a major global health problem with the significant increase of this disorder in recent years. As a result of obesity, a person may suffer from high blood pressure, diabetes, dyslipidemia, cardiovascular disease, and stroke [5,6]. Amino acids such as phenylalanine, proline, leucine, and alanine reduce glucose absorption and insulin resistance by eliminating free radicals [7]. Aliphatic amino acids such as leucine and alanine, which are present in black seed meal and its protein, reduce fat and prevent obesity [8].

By-products of the cold press that are obtained in the food industry are excellent sources of functional ingredients. The by-products of the cold-pressed oil industry (e.g., walnut, almond, seed flour of pumpkin and defatted sunflower, etc.) are considered valuable materials due to their functionality and nutritional characteristics; these can be added to bakery products for enriching or fortifying them and as a substitution of some ingredients, including fat, flour, and sugar [9]. The use of by-products obtained from cold-press oil extraction without chemical processes, such as black beans (rich in protein, essential fatty acids, etc.) instead of being thrown away as waste or animal feed, can be considered an effective way to control obesity. [10]. In a study, the use of sorghum flour in the formulation of muffins led to the control of glucose levels and as a result, it controlled obesity [11]. In addition, the consumption of whey protein drinks with leucine and vitamin D can control blood sugar and obesity in obese and diabetic people [12]. Medicines such as Orlistat, Phentermine, Lorcaserin, Naltrexone, and Liraglutide are being used to control obesity, but have side effects such as insomnia, dizziness, nausea, pancreatitis, etc., which have increased the demand for herbal and natural compounds [13]. Studies show that antioxidant compounds can prevent the progression of obesity-related diseases (diabetes, high blood pressure, etc.) by inhibiting free radicals produced during fat oxidation in the bodies of obese people [14].

The purpose of this research was to study the anti-obesity and functional properties of black seed cake (BSC) as a valuable cold-pressed by-product, which is usually used as feed, and its extracted protein (BSCP). In addition, a functional muffin with anti-obesity effects was formulated using BSC and BSCP.

MATERIALS AND METHODS

Black seed (*Nigella sativa*) cake was kindly provided by Azarin Pishe Demor company. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbanzthiazo-line-6-sulfonate) (ABTS), Folin-Ciocalteau, ρ-nitrophenyl butyrate, orlistat, lipase (type II; from porcine pancreas), 4-nitrophenyl-α-D-glucopyranoside (pNPG), α-Glucosidase from *Saccharomyces cerevisiae*, α-amylase from *Bacillus* sp., 3,5-dinitrosalicylic acid (DNS), and acarbose were procured from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were purchased from Merck and Sigma-Aldrich or other producers with analytical grades.

Extraction of black seed (*Nigella sativa*) cake protein and phenolic compounds: The black seed cake was ground, sieved (850µm), and dried in a 40 °C oven. The obtained flour is hereafter referred to as BSC.

For extraction of protein, 25 g BSC was dispersed in 500 ml deionized water. Then the pH was adjusted to 9 using 1.0 N NaOH, and it was kept for 1 hour on the medium speed stirrer at ambient temperature, then the solution was centrifuged at 7000 x g for 15 min at 4 °C. The fat content was separated physically by filter paper. Then the pH of the supernatant was adjusted to 4.5 using 1.0 N HCl and the suspension was centrifuged again at 7000 × g for 15 min, and finally, the protein was washed two times using distilled water, freeze-dried, and kept in a freezer (-20 °C) until further analysis, this obtained protein powder is hereafter called as BSCP. Bradford's method showed a protein content of 85.29% for BSCP.

For phenolic compound extraction, BSC was dissolved in four different solvents, phosphate buffer (0.1 M, pH 8), ethanol 30%, 50%, and 75%. It was then placed on a stirrer for 6 h and then centrifuged at 7000 x g for 15 min to separate the supernatant for further analysis, which is named as follows:

BSC-B: BSC in buffer

BSC-E30%: BSC in ethanol 30%

BSC-E50%: BSC in ethanol 50%

BSC-E75%: BSC in ethanol 75%

Physicochemical properties of black seed cake: BSC moisture content was measured based on the method of Martínez-Cervera et al. [15]. The BSC sample was weighed and re-weighed after 3.5 h at 105 ◦C. Moisture content was calculated by following equation:

$$Moisture\ content\ (\%) = (\frac{W1 - W2}{W1}) \times 100$$

W1: Initial weight

W2: Secondary weight

Samples 'water activity was determined by Novasina TH-500 aw meter (Switzerland).

The method of Stirk et al. [16] was used to detect the nitrogen content and consequently the amount of protein in BSC (N × 6.25).

Ash content was gravimetrically determined based on the AOAC method [17].

The fat content was determined using the Soxhlet method according to the method of Mohammadpour, et al. [18].

Minerals were identified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique [19].

Determination of Total Phenolic compound: 100 µL of Folin-Ciocalteau was added to the mixture of 20 µL of the sample (1 and 3 mg/mL) as well as 1580 µL of distilled water where it was then placed in a dark environment (4 min). After adding 300 µL of sodium carbonate (stopping the reaction) and incubating for 2 h, the absorbance of the mixture was measured at 765 nm using UV–Vis spectroscopy [20]. TPC was stated as gallic acid equivalent (mg GAE/g).

Antioxidant properties

ABTS•+radical scavenging assay: 7 mM ABTS solution was prepared with 2.45 mM potassium persulfate solution and incubated for 18 h in a dark place. The reagent was diluted to reach an absorbance of 0.70 ± 0.10 at 734 nm using a phosphate buffer. ABTS solution was added to the samples (1 and 3 mg/mL) and they were incubated for 30 min in a dark place at ambient temperature, and then the absorbance was recorded at a wavelength of 734 nm by UV–Vis spectroscopy [21,22].

Positive controls were ascorbic acid and gallic acid. Antioxidant activity was calculated by the following equation:

$$AABTS^{\cdot} \ radical \ scavenging \ activity \ (\%) = (\frac{Ablank - Asample}{Ablank}) \times 100$$

DPPH radical scavenging activity assay: 50 µL of sample (1 and 3 mg/mL), 700 µL of 50% methanol, and 750 µL of 0.4 mM DPPH were mixed; after incubation for 35 min at ambient temperature in a dark place, absorbance was recorded at a wavelength of 515 nm using a UV-Vis spectroscopy [23]. Ascorbic acid and gallic acid were used as positive controls. DPPH radical inhibition was calculated by the following equation:

$$DPPH \ radical \ scavenging \ activity \ (\%) = (\frac{Ablank - Asample}{Ablank}) \times 100$$

Pancreatic lipase inhibitory assay: First, 20 µL of substrate (ρ-nitrophenyl butyrate 10 mM dissolved in DMSO) was added to 40 µL of the sample (1 mg/ml), then 40 µL of enzyme (2.5 mg/ml dissolved in 0.1 M phosphate buffer, pH 8) was added to the mixture and after incubation for 20 min at 37 °C, the absorbance of the sample was recorded at a wavelength of 405 nm using an ELISA reader Expert 96 (Biotech, USA). Orlistat was used as a positive control [24]. Pancreatic lipase inhibitory was calculated by the following equation:

$$Pancreatic \ lipase \ inhibitory \ (\%) = (\frac{\Delta Ablank - \Delta Asample}{\Delta Ablank}) \times 100$$

Determination of α-amylase and α-glucosidase inhibitory activities: A Mixture of 70 µL of sample (1 mg/mL), 70 µL of α-amylase solution (0.6 mg/mL, dissolved in 0.1 M phosphate buffer, pH of 6.5), and 70 µL of starch solution (2 mg/mL) was incubated at 37 °C for 20 min. Then 350 µL DNS reagent was added to terminate the reaction and kept for 5 min in a boiling water bath absorbance was recorded after cooling at a wavelength of 540 nm by ELISA Reader Expert 96 (Biotech, USA) [25]. The positive control was Acarbose. The α-amylase inhibitory was calculated by the following equation:

$$\alpha - amylase \ inhibitory \ (\%) = [\frac{(Ac - At)}{(Ac)}] \times 100$$

Ac: absorbance of the control

At: absorbance of the sample

100 µL of α-glucosidase solution (0.5 U/mL, dissolved in 0.1 M phosphate buffer, pH 6.9) was added to 100 µL of the sample (1 mg/mL) and then incubation was done at 37 °C for 10 min. Then, 100 µL of pNPG (5 mM) was added to the mixture and incubated at 37 °C for 20 min, absorbance was recorded at a wavelength of 405 nm using ELISA Reader Expert 96 (Biotech, USA) [25]. Acarbose was used as a positive control. The α-glucosidase inhibitory was calculated by the following equation:

$$\alpha - glucosidase \ inhibitory \ (\%) = \left(1 - \frac{As - Ab}{A0} \right) \times 100$$

As: absorbance of the sample

Ab: absorbance of pNPG + sample

A0: absorbance of pNPG + enzyme

Preparation of muffins: Muffin production was done according to research by Gökşen and Ekiz [9] with minor changes. Muffins were prepared with different levels of 3, 6, and 9% BSC and 1, 2, and 3% of BSCP as a substitute for wheat flour, and in the samples containing BSC, in addition to flour substitute, oil was also substituted, which can be seen in Table 1 and Fig. 1.

Fifteen semi-trained panelists were selected to test the organoleptic properties of muffin samples. Muffin samples were evaluated randomly in terms of texture,

flavor, odor, color, general appearance, and overall acceptance on a hedonic scale of 5 points (1=dislike extremely, 3=neither like nor dislike, 5=like extremely) on the 1st, 3rd, and 7th days of storage. Panelists rinsed their mouths with room-temperature water before tasting each new sample.

Table 1. The muffin ingredients formulation with different levels of 3, 6, and 9% BSC (Black seed cake) and 1, 2, and 3% BSCP (Black seed cake protein).

Ingredients	Control	BSM3	BSM6	BSM9	PBM1	PBM2	PBM3
Wheat flour	100	97	94	91	99	98	97
BSC	0	3	6	9	0	0	0
BSCP	0	0	0	0	1	2	3
Egg	60	60	60	60	60	60	60
Sugar	50	50	50	50	50	50	50
Sunflower oil	50	49.35	48.71	48.06	50	50	50
Semi-skimmed milk	50	50	50	50	50	50	50
Baking powder	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vanilla	2.5	2.5	2.5	2.5	2.5	2.5	2.5

BSC: Black seed cake, BSCP: Black seed cake protein, BSM3: Muffin formulated with 3% BSC, BSM6: Muffin formulated with 6% BSC, BSM9: Muffin formulated with 9% BSC, PBM1: Muffin formulated with 1% BSCP, PBM2: Muffin formulated with 2% BSCP, PBM3: Muffin formulated with 3% BSCP.

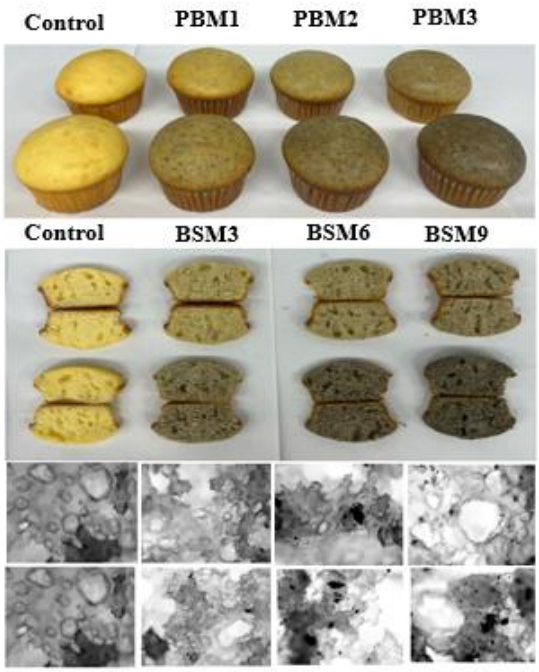


Fig 1. Pictures of muffins produced with different levels of BSC (3%, 6%, and 9%) and BSCP (1%, 2%, and 3%)

Color analysis: Muffin crust and crumb color were analyzed by Hunter Lab ColorFlex, A60-1010-615 model colorimeter (HunterLab, Reston, VA) with three-dimensional color scale L* (lightness), a* (greenness to redness), and b* (blueness to yellowness) [26]. The color of BSC and BSCP in the muffin was ignored. The samples were evaluated on the first, third, and seventh days of storage.

Texture measurements: Texture profile analysis (TPA) was carried out using a Texture Analyzer (CT3, Brookfield, USA), with a test speed of 1.0 mm/s. The test was performed using a cylindrical stainless-steel probe. The samples were monitored on the first, third, and seventh days of storage [9].

Physicochemical properties of muffins: Muffin moisture content was measured according to the method of Shih et al. [27]. The sample was weighed and re-weighed after 24 h at 105 ◦C. Moisture content was calculated by the following equation:

$$Moisture\ content\ (\%) = (\frac{W1 - W2}{W1}) \times 100$$

W1: Initial weight

W2: Secondary weight

Muffin height (the distance between the lowest and the highest point) was measured using a digital caliper.

Muffins were cut into 2 x 2 x 2 cm and were placed in a container with a specified volume and the container was filled with rapeseed (specified volume). The volume of the muffin was obtained from the difference between the volume of the container and the volume of rapeseed. Specific volume was determined based on the ratio of volume to weight [28].

Statistical analysis: All experiments were carried out in triplicate. Statistical analysis was performed through one-way ANOVA by Minitab software (version 18) and means were compared using Duncan's test with SPSS software (version 16).

RESULTS AND DISCUSSION

Physicochemical properties of black seed cake: BSC has 29.00% protein, 21.47% fat, and 38.02% carbohydrates, and contains useful minerals (Table 2). Carbohydrate is calculated based on the following equation:

$$Carbohydrate\ = 100 - (moisture\ content\ + ash + protein\ +\ fat)$$

Due to its high antioxidant properties, black seed has an effective effect on diabetes, obesity, inflammation, and cancer [29].

Total Phenolic compound: The TPC values of the samples in two concentrations of 1 and 3 mg/mL is shown in Fig 2. As the concentration increased, the amount of TPC increased. Black seed contains many phenolic compounds such as thymoquinone, gallic acid, etc., in which the extraction method is very important [30]. Based on the results of the present research, a reduction in the number of phenolic compounds in the solvent was observed but may be due to the use of ethanol and the solubility of some phenolic compounds in water. The type of solvent has a significant effect on the amount of TPC ($p<0.05$).

Table 2. Overall characterization of black seed cake

Characterization	Unit	Parameter	Black seed cake
Compositional parameters	-	aw	0.26 ± 0.05
	(%)	Moisture content	4.75 ± 0.20
		Ash	6.76 ± 0.13
		Protein (CP)	29.00 ± 0.13
		Lipid	21.47± 0.09
		Carbohydrate	38.02
Elements by ICP-MS	(mg/100 g)	K	1066.32
		Ca	875.79
		Na	716.84
		P	445.26
		Mg	329.47
		Fe	48.84
		Mn	12.59
		Zn	5.62
		Cu	0.81

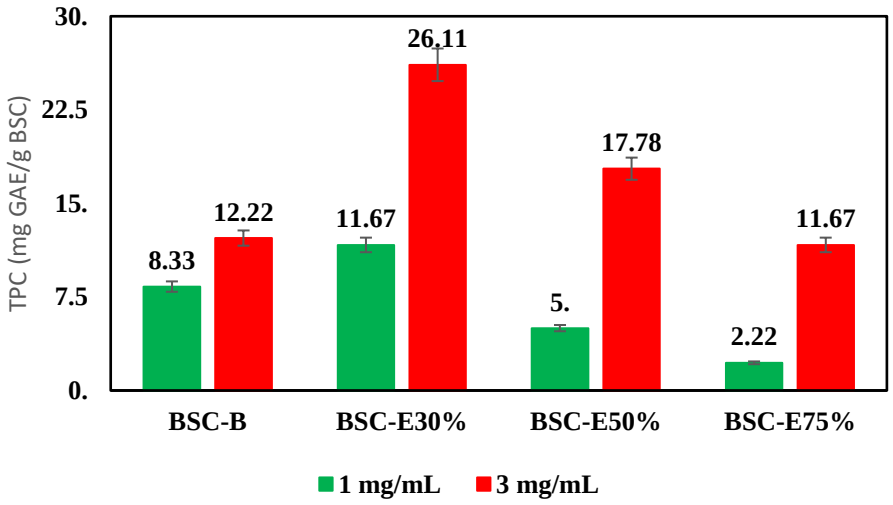
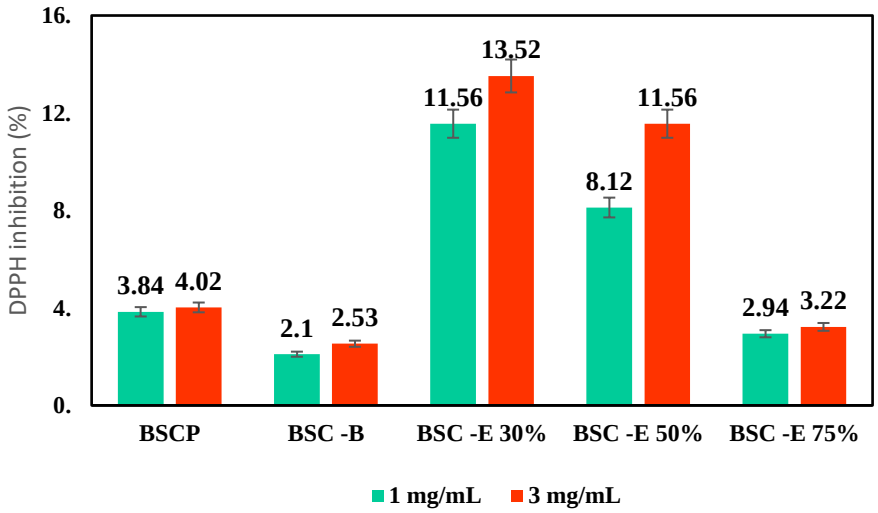


Fig 2. Total phenolic content (TPC) (presented as gallic acid equivalent). The results were expressed as mean ±SD (n=3). Different small letters indicate a significant difference at p< 0.05 between samples based on ANOVA and Duncan method at the 95% level. BSC-B (Black seed cake) in buffer, BSC-E30%: BSC in ethanol 30%, BSC-E50%: BSC in ethanol 50%, BSC-E75%: BSC in ethanol 75%.

Antioxidant properties: DPPH and ABTS as antioxidant assays are typically classified as electron transfer-based methods, although they can also function in hydrogen atom transfer reactions [31]. Fig 3 shows DPPH and ABTS

inhibitory activity of BSCP and BSC in different solvents. In both concentrations (1 and 3 mg/mL), BSC-E30% and BSC-E50% samples have significant differences from other samples in DPPH inhibition ($p<0.05$). The protein has the most inhibitory properties of ABTS compared to other samples in a concentration of 1 mg/mL, but in a concentration of 3 mg/mL, BSC-B ($p>0.05$) is comparable with protein. The type of solvent in BSC had a remarkable effect on the antioxidant inhibitory activity ($p<0.05$). ABTS inhibition test is more suitable for hydrophilic and highly pigmented systems (similar to black seed) than the DPPH method [32]. Considering the high level of inhibition of ABTS compared to DPPH, it can be concluded that this inhibition can be caused by thymoquinone, flavonoid, and water-soluble phenolic compounds.

a)



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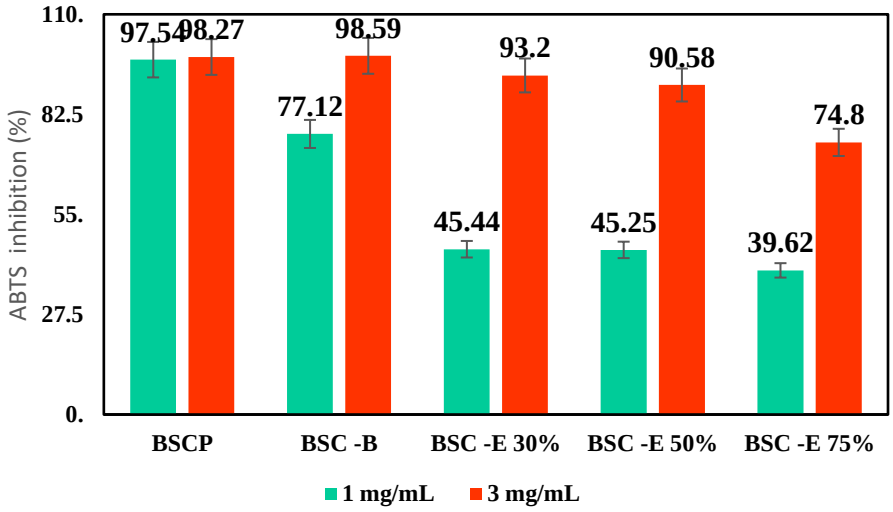

Fig 3. Antioxidant properties, (a) DPPH inhibition and (b) ABTS inhibition. The results were expressed as mean ±SD (n=3). Different small letters indicate a significant difference at $p< 0.05$ between samples based on ANOVA and Duncan method at the 95% level. BSCP: Black seed cake protein which dissolved in buffer, BSC-B (Black seed cake) in buffer, BSC-E30%: BSC in ethanol 30%, BSC-E50%: BSC in ethanol 50%, BSC-E75%: BSC in ethanol 75%.

Pancreatic lipase inhibition assay: The most effective approach to prevent obesity is widely believed to be reducing the digestion and absorption of fat. Pancreatic lipase plays a crucial role in fat absorption by hydrolyzing dietary fat [33]. Inhibiting pancreatic lipase results in a decrease in the absorption of dietary lipids in the digestive system, which can lead to sustained weight loss over time. Due to the increase in complications caused by chemical drugs (heart attack, stroke, liver damage, etc.), the demand for natural and herbal compounds to control and curb diseases such as obesity is increasing [6]. By inhibiting the activity of the lipase enzyme, BSC and BSCP may help control obesity and even cause weight loss in the long term. The highest and lowest inhibitory activity of pancreatic lipase is related to BSCP (59.21%) and BSC in buffer (49.87%) (Table 3). The amount of inhibition of BSCP and BSC in the buffer was significantly different from other samples ($p<0.05$). Proteins significantly inhibit pancreatic lipase activity, and thus, inhibit the digestion of triglycerides into absorbable components in the body and prevent obesity [34]. The type of solvent in BSC had a significant effect on the inhibition of pancreatic lipase enzyme activity ($p<0.05$). Antioxidant activity (ABTS and DPPH) and TPC had a significant effect on the inhibition of pancreatic lipase activity ($p<0.05$). Antioxidant compounds such as flavonoids are compounds effective in inhibiting pancreatic lipase enzyme activity [35]

α-amylase and α-glucosidase inhibition: Inhibiting the activity of carbohydrate digestive enzymes by delaying the hydrolysis of carbohydrates and the production of glucose in the body can control obesity and diabetes [25]. α-amylase, which is present both in the mouth and pancreas, breaks the internal chains of dietary starch, causing the production of compounds such as maltose, maltodextrin, etc. These compounds are eventually turned into glucose in the intestine, which causes blood glucose, obesity, and diabetes [36]. BSC and BSCP both had α-amylase inhibitory activity, the highest and lowest inhibitory activity was related to BSCP (32.51%) and BSC-E75% (3.62%), respectively (Table 3). Protein can inhibit α-amylase [37], according to the results of the present study, and has a greater ability to inhibit α-amylase than BSC compared to α-glucosidase.

α-glucosidase produces glucose from disaccharides, which can control obesity, blood glucose, and diabetes by delaying or inhibiting its activity [38]. The α-glucosidase inhibition rate of BSC and BSCP can be seen in Table 3. The use of phosphate buffer and ethanol with different percentages causes the release of phenolic compounds from BSC; phenolic compounds have a high inhibitory effect against α-glucosidase [39]. The highest and lowest inhibitory rate was for BSC-E30% (71%) and BSCP (10.10%), respectively. The inhibition rate of BSCP was significantly different ($p<0.05$) compared to BSC samples.

Hence, our results showed the same as earlier studies in which lower inhibitory activity on α –amylase than α- glucosidase was obtained for plant extract [40].

Table 3. Enzymatic inhibitory properties

No.	Pancreatic lipase inhibitory (%)	α-glucosidase inhibition (%)	α-amylase inhibition (%)
BSCP	59.21 ± 0.10^{a}	10.10 ± 0.17^{c}	32.51 ± 0.04^{a}
BSC - B	49.87 ± 0.05^{ab}	57.20 ± 0.01^{ab}	28.95 ± 0.07^{b}
BSC –E 30%	32.90 ± 0.03^{b}	71.00 ± 0.07^{a}	8.80 ± 0.09^{c}
BSC –E 50%	38.51 ± 0.04^{b}	66.70 ± 0.13^{b}	8.46 ± 0.03^{d}
BSC –E 75%	40.30 ± 0.08^{b}	57.40 ± 0.08^{ab}	3.62 ± 0.10^{e}

The results were expressed as mean ±SD (n=3). Different small letters indicate a significant difference at p< 0.05 between samples based on ANOVA and Duncan method at the 95% level. BSCP: Black seed cake protein, BSC-B: Black seed cake,

Physicochemical properties of muffins: The moisture content of the muffin makes it fresher and gives it a better texture. The addition of BSC and BSCP in the muffin formulation had a significant effect on the moisture level (p<0.05). The highest and lowest of moisture content is related to PBM3 (24.46%) and BSM3 (19.22%). The addition of BSC and BSCP has a significant effect on the moisture content of the muffin (p<0.05). The increase in muffin moisture was accompanied by an increase in the amount of BSC and BSCP, which can be caused by the binding of protein with water molecules through hydrogen bonding and water retention during the baking process [41].

The height of the control muffin was 4.60 cm and reached 4.74 cm in BSM3 and PBM3 muffins with the increase of BSC and BSCP content, which was consistent with the results of Shevkani and Singh [42]. There was no significant difference in the height of the produced muffins (p>0.05).

Specific volume and height are necessary and important checks for muffin quality. The specific volume is directly related to the amount of wheat gluten [28]. In the present study, by reducing the amount of wheat flour by replacing it with BSC and BSCP, the specific volume of muffins decreased compared to the control muffin, but in muffins with BSCP, this parameter increased with the increase of BSCP, which was consistent with the results of Ammar et al. [43] who used whey protein. The specific volume of the muffins had a significant difference compared to the control muffin (p<0.05).

Table 4. Physicochemical properties of muffins

Parameter	Control	BSM3	BSM6	BSM9	PBM1	PBM2	PBM3
Moisture content (%)	20.64 ± 0.06^{f}	19.22 ± 0.02^{g}	20.96 ± 0.04^{e}	22.30 ± 0.05^{d}	22.71 ± 0.03^{c}	24.34 ± 0.14^{b}	24.46 ± 0.03^{a}
Height (cm)	4.60 ± 0.07^{a}	4.66 ± 0.08^{a}	4.72 ± 0.03^{a}	4.74 ± 0.01^{a}	4.60 ± 0.07^{a}	4.73 ± 0.09^{a}	4.74 ± 0.05^{a}
Specific volume (g/mL)	2.14 ± 0.07^{a}	1.89 ± 0.17^{b}	1.44 ± 0.08^{c}	1.35 ± 0.05^{cd}	1.20 ± 0.03^{d}	1.29 ± 0.05^{cd}	1.38 ± 0.06^{c}

The results were expressed as mean ±SD (n=3). Different small letters indicate a significant difference at p< 0.05 between samples based on ANOVA and Duncan method at the 95% level. BSM3: Muffin formulated with 3% BSC (Black seed cake), BSM6: Muffin formulated with 6% BSC, BSM9: Muffin formulated with 9% BSC, PBM1: Muffin formulated with 1% BSCP (Black seed cake protein), PBM2: Muffin formulated with 2% BSCP, PBM3: Muffin formulated with 3% BSCP.

Muffin color: Fig. 1 shows the muffins produced with different levels of BSC and BSCP. The Maillard reaction during muffin baking between protein and sugars due to reducing the level of water content is the factor affecting the brown color, flavor, and texture in the crust more than the crumb [44]. The color value (L*, a*, b*) of the crust and crumb color of muffins are presented in Table 5. By increasing the amount of BSC and BSCP, the lightness (L*) and yellowness (b*) of the crust and crumb decreased while the redness (a*) increased. These results are related to the dark color of the BSC and BSCP. The darkest muffin (lowest L*) belonged to sample BSM9,

which is significantly different from the control muffin ($p<0.05$). ΔE is the difference in the color of the muffins with the control muffin. The highest and lowest amount of ΔE is related to BSM9 and PBM1.

Table 5. Color parameters of muffins

	Storage day	L*		a*		b*		ΔE	
		crust	crumb	crust	crumb	crust	crumb	crust	crumb
Control	1	89.62 ± 0.03^{a}	89.14 ± 0.06^{b}	1.24 ± 0.05^{a}	0.65 ± 0.05^{a}	33.45 ± 0.09^{a}	23.44 ± 0.05^{a}	*	*
	3	88.25 ± 0.05^{b}	89.37 ± 0.03^{b}	1.63 ± 0.42^{a}	0.32 ± 0.07^{a}	34.71 ± 0.08^{a}	26.75 ± 0.30^{a}	*	*
	7	88.34 ± 0.02^{b}	89.16 ± 0.02^{a}	1.84 ± 0.21^{b}	0.28 ± 0.01^{ab}	38.25 ± 0.01^{a}	25.76 ± 0.02^{a}	*	*
BSM3	1	89.14 ± 0.04^{a}	90.22 ± 2.04^{ab}	2.49 ± 0.03^{c}	2.38 ± 0.06^{c}	23.52 ± 0.06^{e}	16.15 ± 0.03^{d}	10.02	7.57
	3	88.27 ± 0.92^{bc}	89.72 ± 1.13^{ab}	2.62 ± 0.46^{b}	1.37 ± 0.13^{c}	27.67 ± 0.13^{c}	16.82 ± 0.30^{d}	7.11	10.0
	7	80.75 ± 0.07^{d}	89.74 ± 0.07^{a}	2.17 ± 0.02^{b}	0.72 ± 0.24^{bc}	27.66 ± 0.24^{e}	15.66 ± 0.02^{cd}	13.04	10.12
BSM6	1	74.47 ± 0.71^{b}	77.20 ± 1.71^{c}	3.58 ± 0.04^{d}	7.18 ± 0.04^{g}	24.23 ± 0.04^{d}	17.51 ± 0.10^{c}	17.89	14.85
	3	67.02 ± 0.04^{d}	73.13 ± 0.04^{c}	7.92 ± 0.03^{e}	0.40 ± 0.53^{ab}	25.64 ± 0.53^{d}	17.70 ± 0.03^{c}	23.93	18.6
	7	62.82 ± 0.02^{f}	74.65 ± 0.02^{c}	7.42 ± 0.56^{c}	0.22 ± 0.00^{a}	28.33 ± 0.00^{d}	14.41 ± 0.96^{de}	27.95	18.42
BSM9	1	63.35 ± 0.68^{c}	68.77 ± 0.68^{d}	6.43 ± 0.09^{e}	6.84 ± 0.02^{f}	29.19 ± 0.02^{b}	18.57 ± 0.09^{b}	27.12	21.84
	3	62.27 ± 0.21^{e}	68.34 ± 0.21^{d}	8.69 ± 0.20^{f}	2.18 ± 0.26^{d}	25.20 ± 0.26^{e}	21.99 ± 0.20^{b}	28.55	21.65
	7	50.63 ± 0.18^{g}	63.12 ± 1.15^{d}	13.30 ± 0.03^{d}	1.53 ± 0.05^{e}	31.76 ± 0.05^{b}	22.58 ± 0.03^{b}	39.95	26.26
PBM1	1	89.74 ± 1.66^{a}	91.21 ± 1.51^{a}	1.61 ± 0.03^{b}	5.11 ± 0.17^{e}	29.29 ± 0.17^{b}	15.67 ± 0.03^{d}	4.17	9.2
	3	89.29 ± 0.05^{ab}	90.68 ± 0.07^{a}	2.71 ± 0.30^{b}	0.71 ± 0.07^{b}	29.92 ± 0.07^{b}	17.75 ± 0.30^{c}	5.02	9.1
	7	88.80 ± 0.05^{a}	88.63 ± 0.05^{b}	2.38 ± 0.02^{b}	0.53 ± 0.05^{c}	30.20 ± 0.05^{c}	22.58 ± 0.02^{b}	8.08	3.23
PBM2	1	89.60 ± 0.73^{a}	90.28 ± 0.33^{ab}	6.86 ± 0.10^{f}	3.38 ± 0.33^{d}	25.88 ± 0.33^{c}	17.39 ± 0.10^{c}	9.43	6.74
	3	89.50 ± 0.99^{a}	90.25 ± 1.00^{a}	3.36 ± 0.07^{c}	1.68 ± 0.01^{c}	27.73 ± 0.01^{c}	15.29 ± 0.03^{f}	7.31	11.57
	7	87.79 ± 0.05^{c}	88.93 ± 0.05^{b}	0.80 ± 0.36^{a}	0.54 ± 0.05^{c}	30.18 ± 0.05^{c}	13.60 ± 0.96^{e}	8.15	12.16
PBM3	1	89.09 ± 1.15^{a}	89.73 ± 1.16^{ab}	6.84 ± 0.01^{f}	1.64 ± 0.16^{b}	20.63 ± 0.16^{f}	17.39 ± 1.01^{c}	14.0	6.16
	3	87.71 ± 0.92^{c}	89.26 ± 0.07^{ab}	6.84 ± 0.02^{d}	0.78 ± 0.02^{b}	23.36 ± 0.02^{f}	15.93 ± 0.02^{e}	12.5	10.83
	7	74.23 ± 0.03^{e}	88.88 ± 0.03^{a}	1.33 ± 0.09^{a}	0.38 ± 0.04^{bc}	20.59 ± 0.04^{f}	15.86 ± 0.09^{c}	22.62	9.9

The results were expressed as mean ±SD (n=3). Different small letters indicate a significant difference at p< 0.05 between samples based on ANOVA and Duncan method at the 95% level. BSM3: Muffin formulated with 3% BSC (Black seed cake), BSM6: Muffin formulated with 6% BSC, BSM9: Muffin formulated with 9% BSC, PBM1: Muffin formulated with 1% BSCP (Black seed cake protein), PBM2: Muffin formulated with 2% BSCP, PBM3: Muffin formulated with 3% BSCP.

Evaluation of texture properties: The result of TPA analysis is shown in Table 6. By increasing the amount of BSC and BSCP, the hardness of the muffins decreased compared to the control. Texture is an important factor in consumer acceptance. The amount of BSC and BSCP had a significant effect on the hardness, springiness, cohesiveness, gumminess, and chewiness of the muffins (p<0.05). By increasing the amount of BSC as well as BSCP, the amount of hardness (necessary force to compress the muffin) decreased. During the storage time, the highest and lowest hardness was related to the control muffin and PBM3, respectively.

On day 1, all muffins were significantly different from the control muffin (p<0.05). After 3 days, muffins BSM9, PBM1, PBM2, and PBM3 had a significant difference from the control muffin (p<0.05), but other samples had no significant difference (p>0.05). On the 7th day of storage, the hardness of the muffins compared to the control muffin showed a significant difference (p<0.05). The amount of oil is the same in all the samples, with the difference that in the formulation of muffins containing BSC, due to the presence of BSC fat, we reduced the amount of sunflower oil, based on which it can be said with reference to the control sample, that the fatty acids of BSC is placed in the carbohydrate and protein chains and has reduced the hardness [45]. Increasing the amount of protein in the formulation increases the absorption of water and its storage during the shelf life and reduces the hardness of the muffin [46]. During the storage period of 7 days, the hardness of all samples increased, which can be attributed to the decrease in moisture content during storage and the crystallization of amylopectin. The amount of amylopectin in the control muffin is higher than in other samples, and this can be a reason for increasing its hardness [47].

Springiness on day 1, all muffins had a significant difference compared to the control muffin (p<0.05). On day 3, BSM9 had a significant difference from the control (p<0.05), but other muffins had no significant difference from the control muffin (p>0.05). On the 7th day of storage, the springiness of the control muffin showed a significant advantage over the muffins (p<0.0). According to research by Matos et al. [48], adding protein to the muffin formulation increased springiness, while in our produced muffins, a decreasing trend was observed. Springiness has a direct relationship with quality. During the storage period, this parameter decreased due to the decrease in humidity [49].

Cohesiveness is a parameter that indicates the internal bonds of the muffin, which is the result of the proportionality between the force of compressing the muffin in the second stage and the first stage [50]. Substituting BSC and BSCP instead of wheat flour and replacing oil with BSC did not have a positive effect on cohesiveness, and the reason for the decrease in cohesiveness could be due to porosity. Over time, the cohesiveness of the muffins was no longer significantly different from the control muffin (p<0.05).

Due to protein interaction with muffin flour during production, viscoelasticity and the number of trapped air increases, which is directly related to the height of the muffin [28]. As a result, increasing the amount of protein reduces gumminess. The gumminess of the muffins was similar to the control muffin on the first day, but with time, a significant difference was created with the control muffin (p<0.05).

Based on the results of Bala et al. [41], adding egg

white protein and whey protein to the muffin formulation reduced the hardness and chewiness, which is in accordance with our result. The chewiness of PBM2 and PBM3 showed a significant superiority over the control muffin over time ($p<0.05$).

Table 6. Texture profile analysis of muffins

Parameter	Storage day	Control	BSM3	BSM6	BSM9	PBM1	PBM2	PBM3
Hardness 1 (N)	1	2.46±0.20^{c}	2.21±0.11^{b}	1.93±0.04^{a}	1.88±0.05^{a}	2.03±0.09^{a}	1.88±0.07^{a}	1.85±0.10^{a}
	3	3.58±0.18^{b}	3.28±0.03^{a}	3.30±0.09^{ab}	2.77±0.08^{a}	3.13±0.09^{ab}	2.99±0.07^{ab}	2.39±0.08^{a}
	7	4.40±0.17^{b}	3.84±0.10^{a}	3.39±0.14^{a}	3.39±0.11^{a}	3.88±0.12^{ab}	3.51±0.06^{a}	2.72±0.10^{a}
Hardness 2 (N)	1	2.07±0.15^{d}	1.89±0.10^{c}	1.63±0.05^{ab}	1.60±0.08^{ab}	1.76±0.07^{bc}	1.62±0.06^{ab}	1.59±0.07^{a}
	3	3.03±0.10^{e}	2.76±0.02^{d}	2.75±0.08^{d}	2.34±0.06^{b}	2.61±0.10^{cd}	2.51±0.09^{c}	1.98±0.07^{a}
	7	3.65±0.12^{d}	3.20±0.05^{c}	2.81±0.09^{b}	2.82±0.10^{b}	3.28±0.09^{c}	2.95±0.09^{b}	2.21±0.10^{a}
Springiness	1	0.74±0.01^{a}	0.69±0.01^{b}	0.65±0.00^{c}	0.63±0.01^{c}	0.70±0.01^{b}	0.70±0.01^{b}	0.68±0.01^{b}
	3	0.65±0.02^{a}	0.59±0.01^{ab}	0.62±0.01^{ab}	0.58±0.00^{ab}	0.62±0.01^{ab}	0.59±0.01^{ab}	0.62±0.01^{b}
	7	0.64±0.05^{a}	0.58±0.04^{b}	0.57±0.01^{b}	0.55±0.01^{b}	0.58±0.06^{ab}	0.56±0.00^{b}	0.55±0.04^{b}
Cohesiveness	1	0.39±0.01^{a}	0.37±0.00^{b}	0.35±0.00^{c}	0.35±0.00^{c}	0.37±0.01^{b}	0.36±0.01^{b}	0.35±0.00^{b}
	3	0.31±0.02^{a}	0.30±0.01^{b}	0.29±0.01^{b}	0.28±0.01^{b}	0.30±0.01^{ab}	0.30±0.00^{b}	0.31±0.01^{ab}
	7	0.29±0.02^{a}	0.29±0.00^{a}	0.27±0.01^{a}	0.28±0.01^{a}	0.29±0.02^{a}	0.29±0.02^{a}	0.29±0.05^{a}
Gumminess (N)	1	0.92±0.10^{c}	0.83±0.06^{b}	0.68±0.00^{a}	0.66±0.00^{ab}	0.75±0.02^{ab}	0.68±0.02^{ab}	0.65±0.03^{ab}
	3	1.12±0.05^{d}	0.97±0.01^{c}	0.96±0.02^{c}	0.79±0.01^{ab}	0.94±0.00^{c}	0.89±0.01^{bc}	0.74±0.02^{a}
	7	1.26±0.12^{d}	1.10±0.07^{cd}	0.90±0.02^{a}	0.94±0.08^{ab}	1.13±0.16^{cd}	1.00±0.06^{bc}	0.78±0.16^{a}
Chewiness (N)	1	0.70±0.11^{d}	0.52±0.05^{c}	0.44±0.00^{a}	0.41±0.01^{ab}	0.53±0.02^{bc}	0.47±0.01^{abc}	0.45±0.02^{abc}
	3	0.73±0.03^{c}	0.57±0.02^{ab}	0.57±0.01^{b}	0.45±0.00^{a}	0.58±0.01^{ab}	0.52±0.01^{ab}	0.47±0.03^{ab}
	7	0.82±0.13^{d}	0.64±0.07^{bc}	0.51±0.02^{ab}	0.52±0.05^{ab}	0.67±0.16^{cd}	0.56±0.03^{ab}	0.43±0.10^{a}

The results were expressed as mean ±SD (n=3). Different small letters indicate a significant difference at $p< 0.05$ between samples based on ANOVA and Duncan method at the 95% level. BSM3: Muffin formulated with 3% BSC (Black seed cake), BSM6: Muffin formulated with 6% BSC, BSM9: Muffin formulated with 9% BSC, PBM1: Muffin formulated with 1% BSCP (Black seed cake protein), PBM2: Muffin formulated with 2% BSCP, PBM3: Muffin formulated with 3% BSCP.

Sensory evaluation of muffins: The addition of different levels of BSC and BSCP in muffins had an effect on the interest of trained panelists. The results of the sensory characteristics of the muffins on the 1st, 3rd, and 7th days of shelf life is shown in Fig 4. On the 1st day of the shelf life of the muffins, the overall scores of the BSM 3%, PBM 1%, and PBM 2% samples did not have a significant difference ($p<0.05$) from the control sample, but the other samples had a significant difference with the control ($p<0.05$). On the 3rd day of the shelf life of the muffins, the highest overall scores were related to the samples of BSM 3%, BSM 6%, PBM 1% and PBM 2%, which were significantly different from the control sample ($p<0.05$), but the other samples, except BSM 9%, that were different from the control did not have a significance difference ($p<0.05$).

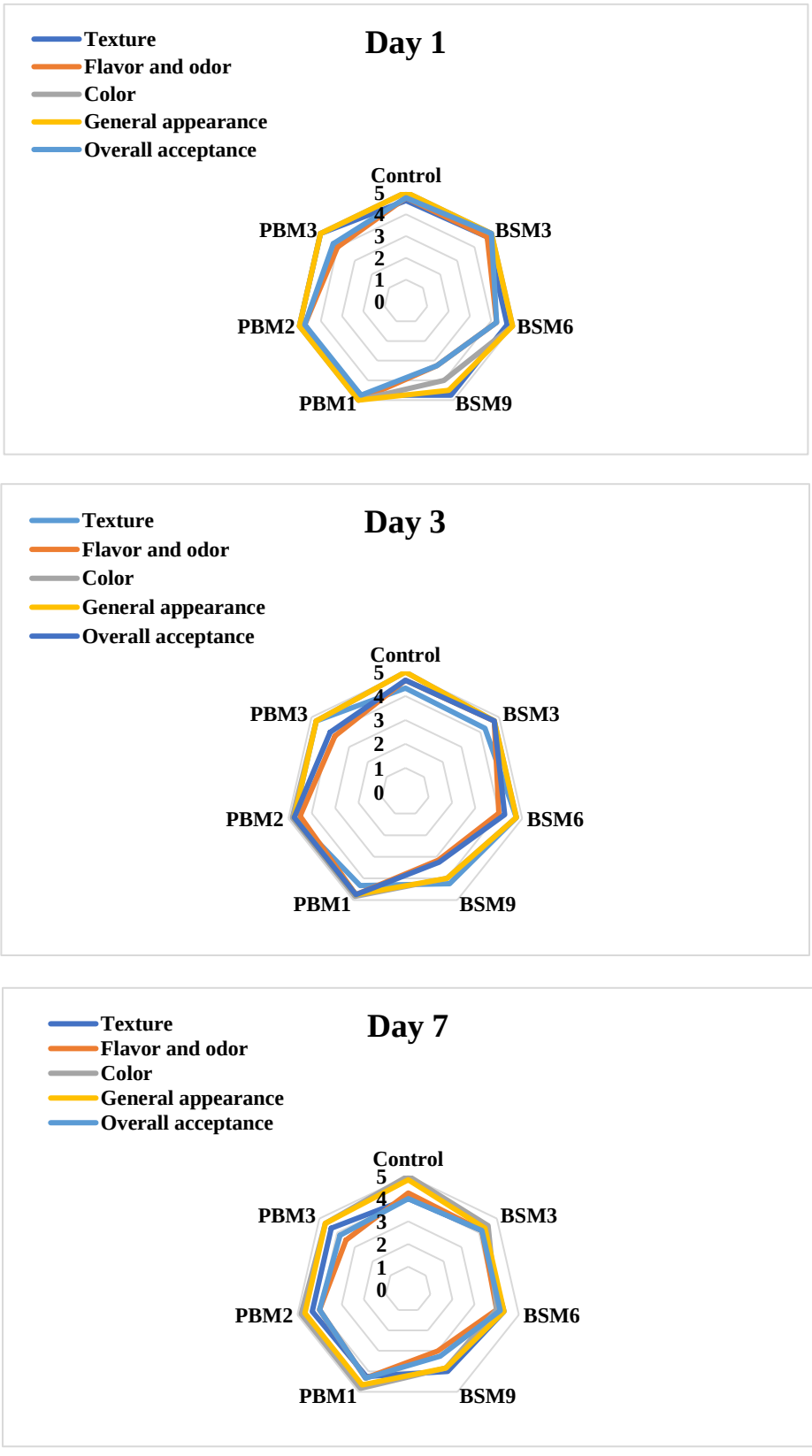

Fig 4. Mean sensory evaluation scores for muffins with different formulations on three different storage days (1, 3 and 7 days). BSM3: Muffin formulated with 3% BSC (Black seed cake), BSM6: Muffin formulated with 6% BSC, BSM9: Muffin formulated with 9% BSC, PBM1: Muffin formulated with 1% BSCP (Black seed cake protein), PBM2: Muffin formulated with 2% BSCP, PBM3: Muffin formulated with 3% BSCP.

CONCLUSIONS

With the increase in obesity in recent years and the diseases caused by obesity as well as the side effects of obesity drugs, the use of natural and herbal compounds such as BSC will be very desirable. Our research indicated that incorporating black seed cake into foods can have a positive impact on controlling obesity. Considering the inhibition of pancreatic lipase, α-amylase and α-glucosidase enzymes by BSC and BSCP, it can be concluded that these ingredients have a high potential for preventing obesity although further *in vivo* studies are needed. The high antioxidant properties of BSC and BSCP to reduce free radicals show promising effects for the prevention of fat oxidation in the body of an obese person. The superior textural properties of the formulated muffins compared to the control muffins showed that the use of BSC and BSCP as a substitute for wheat flour and sunflower oil in muffins containing BSC is desirable.

Abbreviations: ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), DMSO: Dimethylsulfoxide, DNS: 3,5-dinitrosalicylic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, pNPG: 4-Nitrophenyl-α -D-glucopyranoside, TPA: Texture profile analysis, TPC: Total Phenolic compound

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