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ORIGINAL PAPER

Effectiveness of poppy seed (*Papaver somniferum* L.) utilization in chocolate spread production

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Abstract

This study developed a novel chocolate spread incorporating yellow and blue poppy seeds as functional ingredients. Physicochemical, bioactive, textural, rheological, microstructural, FT–IR spectroscopic, and sensory properties of spreads containing 5%, 12.5%, and 20% poppy seeds were evaluated. The formulation with 12.5% blue poppy seeds exhibited desirable spreadability (1017.13 g) and oil separation (9.70%). Blue poppy seeds contributed more effectively to the bioactive capacity of the spreads. Increasing seed content significantly increased particle size distribution parameters (d(0.1), d(0.5), and d(0.9)). All samples exhibited pseudoplastic behaviour, while viscosity and consistency were strongly influenced by seed type and concentration. FT–IR multivariate analysis revealed distinct molecular patterns associated with seed type and level. Sensory evaluation showed that spreads containing 5% and 12.5% blue poppy seeds achieved higher flavour–aroma, structure–texture, and colour–appearance scores. Overall, blue poppy seeds provided a more favourable quality profile than yellow poppy seeds, with low to moderate inclusion levels offering the best balance of sensory, bioactive, rheological, and textural properties.

Keywords Poppy seed · Chocolate spread · Texture · Rheology · FT–IR · Sensory

Introduction

Increasing public awareness of the link between diet and health has encouraged the food industry to develop products that not only meet sensory expectations but also offer health benefits. Such products, known as functional foods, can support or improve body functions in addition to providing basic nutrition and have become a growing trend worldwide [1]. Oilseeds and nuts are commonly used in functional foods because they contain valuable nutrients, including healthy fats, proteins, and bioactive compounds such as antioxidants and vitamins [2].

Poppy seeds are obtained from opium poppy (*Papaver somniferum* L.) and are used as food or for the production of edible oil. Poppy seeds generally contain negligible levels of opium alkaloids; however, contamination may occur during harvesting or as a result of insect damage, due to contact with the latex of the poppy capsule containing alkaloids such as morphine, codeine, thebaine, papaverine, noscapine, and oripavine [3, 4]. Poppy seed is one of the oldest cultivated oilseed crops and has recently gained attention as a natural and functional ingredient. It has a mild, nutty flavour and is commonly used in bakery and confectionery products. The seeds contain approximately 20–25% protein and 40–50% oil, primarily composed of unsaturated fatty acids such as linoleic (66%), oleic (19.23%), and α-linolenic acids (0.5%) [5, 6]. Poppy seeds are also a good source of minerals such as calcium, magnesium, and phosphorus, and contain natural antioxidants including phytosterols, tocopherols and phenolic compounds [2, 7]. Erinç et al. [8] emphasised that poppy seeds have significant health effects due to their unique fatty acid, tocopherol and phytosterol content.

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Countries such as Türkiye, India, Australia, the Czech Republic, Spain, Hungary, France, and China permit the legal production of food–grade poppy seeds in accordance with United Nations regulations. Türkiye, the leading producer of poppy seeds, had a harvested area of 17,013 hectares and a production quantity of 8,441 tonnes in 2024. Poppy seeds display a wide variety of colours, ranging from white, yellow and brown to grey and blue [9, 10]. The compositional and sensory properties of poppy seeds vary with seed colour. In Turkish poppy varieties, blue seeds exhibit slightly higher protein content (22.7%) than yellow seeds (21.8%), whereas yellow seeds show a markedly higher oil content (49.2%) compared with blue seeds (33.6%) [11]. From a sensory perspective, blue–seeded poppy varieties are characterised by a stronger typical poppy odour and nutty flavour, while yellow seeds present a comparatively milder sensory profile [12]. An assessment of poppy seeds registered in Türkiye reveals that yellow and blue poppy seeds are particularly prevalent. Blue poppy seeds are particularly popular in baked goods and desserts.

Chocolate spreads represent a large and continuously growing market worldwide. They are commonly consumed at breakfast and are especially popular among children and young adults. Recent studies on spreadable products, such as fruit–based or reduced–sugar formulations, emphasize the importance of ingredient selection on texture, spreadability, and consumer acceptance [13]. At the same time, there is a rising interest in developing healthier alternatives with improved nutritional profiles. Incorporating poppy seeds or their oil into food products such as bakery, cakes, desserts and chocolate [14, 15] can enhance both quality and nutritional value. Previous studies have shown that replacing animal fat with poppy seed or its oil in meat, fermented meat, and bakery products reduces saturated fat levels while increasing healthier unsaturated fats, without affecting texture, flavour, or consumer acceptability [9, 16, 17]. These findings suggest that poppy seeds can serve as a functional ingredient to improve the nutritional profile of various foods. However, the use of poppy seeds in chocolate spreads has received little attention so far. Chocolate spreads are widely consumed but usually contain high levels of sugar and saturated fat. Incorporating poppy seeds into these products could improve their fat profile, enhance antioxidant content, and increase overall nutritional value. In addition, their mild nutty flavour complements cocoa well and contributes to a pleasant texture and colour.

There is growing interest in the development of new chocolate spread formulations enriched with alternative plant–based ingredients. Poppy seeds are a potential oilseed that could contribute to the nutritional value and processing properties of chocolate spreads. However, there is limited information regarding the effect of different poppy seed varieties and concentration levels on the quality characteristics of chocolate spreads. Therefore, the aim of this study was to investigate the effects of different poppy seed varieties and concentration levels on the physicochemical, textural, rheological and sensory properties of chocolate spreads. The study focuses specifically on the comparative evaluation of formulations containing poppy seeds to determine how variety and concentration affect product characteristics.

Materials and methods

Materials

The following materials were used in the production of chocolate spread with poppy seeds: yellow and blue poppy seeds (Bolvadin, Afyonkarahisar, Türkiye) and icing sugar (Dr. Oetker, İzmir, Türkiye), BES–41 S vegetable fat (Besler, İstanbul, Türkiye), skimmed milk powder (Alfasol, Kimbiotek, İstanbul, Türkiye), cocoa powder (Altınmarka, İstanbul, Türkiye), sunflower lecithin (Alfasol, Kimbiotek, İstanbul, Türkiye), ammonium phosphatite (AMP 4458) (Palsgard, İstanbul, Türkiye), vanillin (Dr. Gusto, İstanbul, Türkiye), and salt (Billur Tuz, İzmir, Türkiye).

Method

Processing of chocolate spread with poppy seed

Two types of poppy seeds (Fig. 1) were used: yellow poppy seeds (coded as A) and blue poppy seeds (coded as B). Poppy seeds were added at three different levels, 5%, 12.5%, and 20% (coded as 1, 2, and 3 respectively), in the production of chocolate spread. The experimental design of the study followed a 2×3 factorial arrangement, including two different poppy seed varieties and three concentration levels. The recipe used in the production of chocolate spread with poppy is provided in Table 1. Production was carried out using a melanger (ECGC–12SLTA, Cocoatown, USA). First, approximately 50 g of vegetable fat was placed in the melanger to lubricate the granite stones. Roasted poppy seeds were then added and ground for approximately 30 min. Subsequently, the other ingredients were gradually added to the melanger and the refining process was carried out for two and a half hours. After refining, the chocolate spread was filled into 300 g glass jars. Before analysis, the chocolate spreads were left at room temperature for one week.

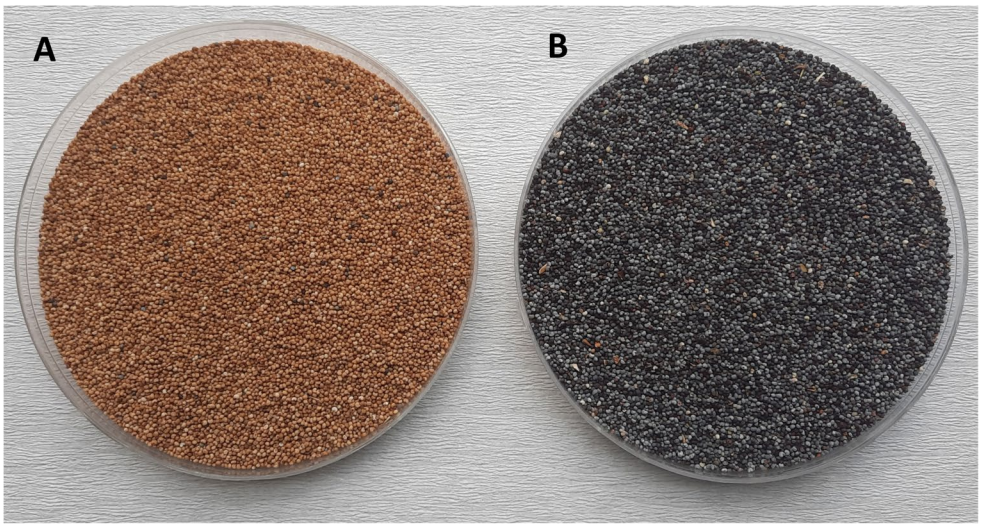


Fig. 1 Images of yellow (**A**) and blue **B**) poppy seeds

Table 1 Composition of the chocolate spreads with poppy seed

Ingredients	A1	A2	A3	B1	B2	B3
Yellow poppy seed	5	12.5	20	-	-	-
Blue poppy seed	-	-	-	5	12.5	20
Milk powder	16.5	9	1.5	16.5	9	1.5
Icing sugar	42.5	42.5	42.5	42.5	42.5	42.5
Fat	28.1	28.1	28.1	28.1	28.1	28.1
Cocoa	7	7	7	7	7	7
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5
AMP	0.2	0.2	0.2	0.2	0.2	0.2
Vanillin	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.1	0.1	0.1	0.1	0.1	0.1

Physicochemical analysis

Accelerated oil separation

Accelerated oil separation (AOS) analysis was performed according to the method provided by Aydemir [18]. A 7.5 g sample of chocolate spread was weighed into a plastic centrifuge tube and the lid was closed. It was then centrifuged at 20 °C at 20,000 *g* for 10 min. The oil separated on the surface of the tube was collected, weighed, and the result was calculated as a percentage.

Water activity

The water activity measurement was performed using the AquaLab 4 TE (Pullman, WA, USA) device.

Measurement of bioactive capacity

Extraction of phenolic compounds

To determine bioactive compounds, an extraction procedure was performed as described in [19]. Briefly, 2 g of the chocolate spread sample was mixed with 10 mL of 80% (v/v) methanol. The mixture was homogenized using a vortex for 2 min and then placed in an ultrasonic bath for 15 min. Subsequently, the samples were centrifuged at 5,000 *g* for 10 min at 4 °C. The supernatant was collected, filtered through a 0.45 µm membrane filter, and this extract was used for TPC and DPPH analyses.

Determination of total phenolic content in chocolate spreads

The total phenolic content (TPC) of the extracts was quantified following the protocol established by Singleton et al. [20]. Briefly, an aliquot of 0.5 mL of the sample was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of sodium carbonate solution (Na_2CO_3, 7.5% w/v). The resulting mixture was incubated in total darkness for 2 h. Subsequently, absorbance readings were taken at 750 nm. For quantification, a calibration curve was constructed using gallic acid concentrations ranging from 10 to 1000 µg mL^{-1} (y = -0.0002x + 0,0014, $R^2 = 0.9983$). The results were reported as milligrams of Gallic Acid Equivalents (GAE) per 100 g of sample. All experiments were conducted in triplicate.

DPPH assay

Antioxidant activity was determined by modifying the DPPH radical scavenging method developed by Brand–Williams et al. [21]. In the analysis procedure, 100 µL of the sample was added to 3.9 mL of DPPH radical solution at a concentration of 0.06 mM. The prepared mixture was incubated in the dark at room temperature for 30 min. At the end of this period, the absorbance values of the samples were measured spectrophotometrically at a wavelength of 515 nm. A calibration curve was constructed using Trolox standards prepared in the range of 0.1–1.0 mmol/mL (y = -0.0002x + 0,7327, $R^2 = 0.991$) for activity calculations. All measurements were performed in triplicate.

Particle size distribution (PSD)

The particle size distribution of the samples was determined using a Malvern Mastersizer 3000. To ensure proper dispersion and prevent agglomeration, a specific sample preparation was followed: Approximately 1 g of chocolate spread was diluted in 50 mL of sunflower oil and stirred at 1000 rpm for 5 min until a thin, homogeneous suspension was achieved. This pre-diluted mixture was then introduced into the instrument's wet dispersion unit. Sunflower oil was used as a dispersant to prevent sugar particle dissolution and ensure a stable suspension. During the analysis, the laser obscuration rate was maintained within 10–20%. Optical parameters were calculated using Mie theory. Accordingly, the particle refractive index was set to 1.590, the particle absorption index to 0.010, and the dispersant refractive index to 1.469. The results were expressed in terms of volumetric distribution; particle diameters corresponding to 10% d(0.1), 50% d(0.5) and 90% d(0.9) cumulative volume percentages, as well as the volume–weighted mean diameter D[4.3] and surface area–weighted mean diameter D[3.2] values were recorded.

Texture analysis

Texture analysis was performed using a TA.XT Plus Texture analyser (Stable Micro Systems, Godalming, UK). Chocolate spread samples were placed in the TTC spreadability rig (HDP/SR, 14580) at room temperature (24 ± 1 °C) and the analysis conditions were applied: Probe 25 mm distance preset; test speed 3 mm/s; post–test speed 10 mm/s, compression distance 23 mm.

Steady and dynamic rheological analysis

Rheological properties of the chocolate spreads were evaluated using a rotational rheometer (HAAKE MARS III, Thermo Scientific, Germany) equipped with a titanium cone–plate geometry (P35/Ti, 35 mm diameter, 1.000 mm gap). All analyses were carried out at 25 ± 1 °C after temperature equilibration, and measurements were performed in triplicate ($n = 3$) for each formulation.

Samples were sheared continuously at rates ranging from 0.01 to 100 s^{-1} in 2 min to fit the data to the Ostwald–de Waele model according to Eq. (1).

$$\tau = K.\dot{\gamma}^{n} \tag{1}$$

Where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa.s^n$), and n is the flow behaviour index (dimensionless).

The storage (G') and loss (G'') moduli were measured within the linear viscoelastic range (LVR) at a constant shear strain of 0.2 Pa and a temperature of 25 °C from a frequency range of 1–100 $rads^{-1}$. The LVR was achieved by conducting the stress sweep test. The complex modulus (G^*) and loss tangent ($tan\ \delta$) were calculated using the Eqs. (2) and (3).

$$G^* = \sqrt{\left[G\prime^{2} + G\prime\prime^{2}\right]} \tag{2}$$

$$tan\delta = \frac{G''}{G'} \tag{3}$$

Fourier transform–infrared spectroscopy (FT–IR) analysis

Bruker FT–IR Spectrometer with an Attenuated Total Reflection (ATR) apparatus was used to analyse samples. Before the analysis device is thoroughly cleaned and background zeroing was performed. Mixture samples for FT–IR analysis were directly taken from the sample containers and directly placed on the ATR diamond with full covering. At least two measurements were made from each container. Each sample was scanned more than 20 times and each analysis result is the average of these repeated measurements. This procedure was repeated to ensure reliable and repeatable FT–IR results. The FT–IR analysis was performed in 4000 1 cm^{-1} to 400 1 cm^{-1} wavenumber range. The resolution was 4 1 cm^{-1}.

Microstructural imaging

The microstructure of the chocolate spreads was determined using a Leica DM500 optical compound microscope with Leica ICC50 HD camera (Leica Microsystems, Heerbrugg, Switzerland). The samples were placed on the slide and covered by cover glass. They were then examined and imaged under a 40 × objective.

Colour measurement

The measurement of the L^*, a^*, and b^* colour parameters of chocolate spread samples was performed using a Chroma Meter (CR–400, Minolta, Osaka, Japan). Measurements were taken at three different points on each sample. Additionally, the whitening index (*WI*) value was calculated according to the Eq. (4) provided by Nourzad et al. [22].

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (4)$$

Sensory evaluation

The sensory evaluation was caried out by a semi–trained panellist group (40 people) consisting of Çankırı Karatekin University Faculty of Engineering lecturers and students using the score card with hedonic rating scale prepared in terms of flavour–aroma over 7 points (1: terrible, 2: bad, 3: just a little bad, 4: maybe bad or may be good, 5: just a little good, 6: good, 7: great), structure–texture and colour–appearance over 5 points (1: terrible, 2: bad, 3: maybe bad or maybe good, 4: good, 5: great). Sample numbers were coded as 3–digit numbers. During the texture evaluation, the chocolate spreads were also tested with the help of a knife for spreadability of the spreads on bread. Water was given to the panellists during the sample passes to cleanse their palates. Samples were presented to the panellists in a random order. Participation in the sensory evaluation was voluntary and written informed consent was obtained from all panellists prior to the study. Before the evaluation, participants were informed about the product type and the sensory attributes to be assessed. It was confirmed that none of the participants had known allergies to the ingredients used in the chocolate spread. The sensory evaluation was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical analysis

The statistical analysis of the data obtained from the three–repetition analyses was performed using SPSS statistical software version 22 (IBM, Chicago, IL, USA). Analysis of variance (ANOVA) was performed using the Tukey test for multiple comparisons among samples ($p<0.05$). Following the FT–IR scans, spectrum data was obtained as command–separated values (csv) files and imported into statistical analysis software (MINITAB 17, USA) for multivariate analysis, namely k–means clustering, hierarchical cluster analysis and principal components analysis.

Results and discussion

Physicochemical analysis

Accelerated oil separation

This test enables the free oil present in the product to rise to the surface rapidly under the effect of centrifugal force. This provides information about the amount of free oil in the product and can also indicate any oil separation that may occur during storage. Oil separation was not normally observed on the surface of the spread samples. The AOS values of the chocolate spreads ranged from 7.07% (A1) to 12.80% (A3) (Table 2). It was observed that as the quantity of poppy seeds used increased, the AOS values of the chocolate spreads also increased ($p < 0.05$). This situation arises from the poppy seed's oil content of approximately 50%. It was observed that oil separation in chocolate spreads containing 12.5% and 20% yellow poppy seeds was higher than in spreads containing blue poppy seeds. This difference was only significant for samples containing 20% poppy seeds ($p < 0.05$). However, while the poppy seed variety on AOS was found to be insignificant ($p > 0.05$), the content of poppy seed was found to be significant ($p < 0.05$). Aydemir [18] reported that the AOS values of the two most popular commercial chocolate spreads with hazelnuts in Türkiye were 7.40% and 8.87%. Samples A1, B1 and B2 are noteworthy for their proximity to these values. This highlights the 5% level for yellow poppy and the 5% and 12.5% levels for blue poppy.

Water activity

Chocolate spread is a food product with a very low a_w value, as it is a suspension in which solid particles are dispersed in an oil phase and does not contain water in its composition. The a_w values of the chocolate spread with poppy seeds produced ranged between 0.304 and 0.326 (Table 2). Although a slight increase in a_w was observed as the proportion of poppy seeds increased, this increase was not statistically significant. No difference was found between samples with yellow poppy seeds (a_w= 0.278) and those with blue poppy seeds (a_w= 0.312). The effect of both the poppy seed variety and quantity on a_w was found to be insignificant ($p > 0.05$). Aydemir et al. [23], Aydemir et al. [24] and Ertuğrul [25] found a_w values in chocolate spreads with hazelnut in the ranges of 0.299–0.337, 0.230–0.255 and 0.267–0.301, respectively. Aydemir et al. [26] reported water activity values in cocoa carob cream in the range of 0.274–0.309.

Table 2 Technological characteristics of chocolate spreads

Parameters	Chocolate spreads					
	A1	A2	A3	B1	B2	B3
Physicochemical						
AOS (%)	7.07 ± 0.07^{a}	10.43 ± 0.10^{b}	12.80 ± 0.20^{c}	7.67 ± 0.07^{a}	9.70 ± 0.10^{b}	10.73 ± 0.40^{b}
a_w	0.304 ± 0.006^{a}	0.317 ± 0.001^{a}	0.310 ± 0.010^{a}	0.304 ± 0.004^{a}	0.319 ± 0.002^{a}	0.326 ± 0.006^{a}
Bioactive						
TPC (mg GAE 100 mg^{-1})	332.13 ± 0.11^{a}	341.76 ± 3.51^{a}	375.17 ± 5.66^{ab}	391.27 ± 5.98^{bc}	431.46 ± 13.73^{c}	489.47 ± 12.66^{e}
DPPH (TEAC mg g^{-1})	7.68 ± 0.13^{a}	7.99 ± 0.07^{a}	8.94 ± 0.13^{b}	7.70 ± 0.01^{a}	7.97 ± 0.01^{a}	8.18 ± 0.10^{a}
Textural						
Firmness (g)	2817.28 ± 26.97^{d}	965.42 ± 15.01^{a}	1038.24 ± 1.23^{ab}	2838.97 ± 25.01^{d}	1556.07 ± 51.36^{c}	1235.50 ± 59.94^{b}
Work of shear (g s)	2008.36 ± 19.75^{d}	562.33 ± 1.24^{a}	647.50 ± 4.62^{ab}	2046.18 ± 32.60^{d}	1017.13 ± 21.06^{c}	806.00 ± 77.52^{b}
Stickiness (g)	-3940.38 ± 146.87^{c}	-1682.01 ± 29.35^{a}	-1717.86 ± 17.92^{a}	-4094.51 ± 50.24^{c}	-2513.66 ± 125.61^{b}	-1951.91 ± 15.09^{a}
Work of adhesion (g s)	-840.49 ± 6.13^{c}	-256.62 ± 2.18^{a}	-264.75 ± 10.82^{a}	-843.30 ± 20.44^{c}	-395.36 ± 5.89^{b}	-310.11 ± 5.31^{a}
PSD						
d(0.1) (µm)	5.19 ± 0.12^{a}	7.04 ± 0.20^{b}	6.92 ± 0.24^{b}	5.29 ± 0.10^{a}	5.93 ± 0.18^{a}	9.50 ± 0.32^{c}
d(0.5) (µm)	20.80 ± 0.75^{a}	52.3 ± 0.22^{c}	147 ± 3.48^{d}	22.80 ± 0.60^{a}	32 ± 1.18^{b}	162 ± 1.55^{d}
d(0.9) (µm)	69.10 ± 1.25^{a}	470 ± 5.43^{d}	739 ± 9.67^{e}	157 ± 3.33^{b}	349 ± 10.12^{c}	427 ± 8.72^{d}
D[3.2] (µm)	12.70 ± 0.45^{a}	20.80 ± 0.75^{d}	22 ± 0.98^{d}	13.50 ± 0.67^{b}	16.30 ± 0.90^{bc}	29.90 ± 1.15^{e}
D[4.3] (µm)	32.40 ± 1.22^{a}	382 ± 8.50^{f}	283 ± 2.5^{e}	60.20 ± 1.65^{b}	107 ± 4.12^{c}	188 ± 1.46^{cd}
Colour						
*L**	29.69 ± 0.06^{d}	28.50 ± 0.16^{c}	27.28 ± 0.13^{b}	29.40 ± 0.13^{d}	27.46 ± 0.03^{b}	26.13 ± 0.04^{a}
*a**	5.69 ± 0.05^{e}	5.30 ± 0.01^{d}	4.95 ± 0.06^{c}	5.44 ± 0.06^{d}	4.70 ± 0.03^{b}	4.00 ± 0.02^{a}
*b**	5.58 ± 0.18^{d}	5.12 ± 0.07^{cd}	4.86 ± 0.12^{bc}	5.28 ± 0.06^{cd}	4.42 ± 0.07^{b}	3.54 ± 0.03^{a}
WI	29.24 ± 0.08^{d}	28.12 ± 0.17^{c}	26.95 ± 0.14^{b}	28.99 ± 0.13^{d}	27.17 ± 0.04^{b}	25.93 ± 0.04^{a}

[a-f] Mean values within each row followed by different letters are significantly different ($p < 0.05$)

All data are represented as means ± standard deviation

Bioactive capacity

Total phenolic content

The findings demonstrate that the incorporation of poppy seeds significantly enhanced TPC of the product formulations. A clear dose–dependent relationship was observed, wherein TPC values increased linearly with higher poppy seed addition rates (Table 2). This trend confirms that poppy seeds can be effectively utilized as a functional ingredient to boost the antioxidant profile of spreadable products. The maximum TPC value of 489.47 mg GAE 100 mg^{-1} was recorded in sample B3, which contained the highest concentration (20%) of blue poppy seeds. In contrast, the minimum value of 332.13 mg GAE 100 mg^{-1} was measured in sample A1, which contained the lowest concentration (5%) of yellow poppy seeds. A comparative analysis revealed that at every equivalent addition level (5%, 12.5%, and 20%), the samples containing blue poppy seeds consistently yielded significantly higher TPC values than those with yellow poppy seeds ($p < 0.05$). This consistent superiority indicates that blue poppy seeds are inherently a richer source of phenolic compounds.

DPPH

The results obtained from the DPPH test, which measures antioxidant capacity, are shown in Table 2 and reveal a more nuanced picture than the TPC results. The highest antioxidant capacity (8.94 TEAC mg g^{-1}) was found in sample A3, which contained the highest proportion (20%) of yellow poppy seeds ($p < 0.05$). This suggests that, for antioxidant efficacy, as measured by the DPPH method, the concentration of seeds was a more decisive factor than the type of seed itself. The clear advantage of blue poppy seeds in the TPC analysis was not directly translated to the DPPH assay. This discrepancy implies that the specific phenolic compounds present in yellow poppy seeds may possess superior radical–scavenging potency per unit compared to those in blue poppy seeds. Consequently, these results highlight that a higher total phenolic content does not automatically guarantee a higher antioxidant activity, as the qualitative profile, including the chemical structure and reactivity of the individual phenolic compounds, is a critical determining factor.

PSD

The values of the PSD parameters for the chocolate spread samples are shown in Table 2. It was determined that the spreads contained particles in the range of 2.13–3080 µm (Fig. 2). The spread samples showed a bimodal distribution, except for A3. The A3 sample had a different trimodal distribution. This graph shows that the second peak is directly attributable to poppy seeds, as it exhibits growth in line

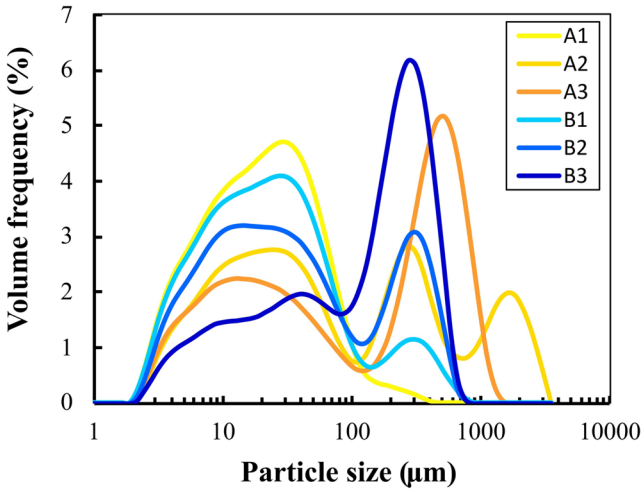


Fig. 2 Particle size distributions in the chocolate spreads

with the increasing quantity of poppy seeds. The percentage of particle diameter ranging from fine to coarse particles within the total particle volume encompasses the optimum, minimum, and maximum sizes that have a direct impact on the rheological, textural, and sensory qualities of chocolate products [27]. The value d(0.1) indicates that 10% of the total particles are below this value. The d(0.1) value ranged between 5.19 and 9.50 µm. In general, this value has increased along with the increasing content of poppy seeds. The value d(0.5) indicates that 50% of the total particles are below this value. The d(0.5) values of the samples were found to be between 20.80 and 162 µm. The d(0.5) value also increased with the rising content of poppy seeds. d(0.9) is a value indicating that 90% of the total particles are finer than this value. It was determined that the d(0.9) value of the spreads was between 69.1 and 739 µm. This value has also increased as the quantity of both poppy seed varieties increased. The values for these three important parameters, d(0.1), d(0.5) and d(0.9), were found to be higher than those in other spreads made using a melanger with different products [13, 18, 23, 28]. This indicates that poppy seeds are more difficult to fine in a melanger. However, the components of the poppy seed itself or other particles of the plant that may contaminate the seed can be ground at an industrial level using more powerful and professional equipment such as ball mill. The surface–weighted D[3.2] and volume–weighted D[4.3] mean particle size values of the samples were determined to be in the range of 12.70–29.90 µm and 32.40–382 µm, respectively. It was observed that as the proportion of poppy seed increased, the D[3.2] and D[4.3] values also rose in parallel. D[4.3] value is important in that it shows which particle size make up most of the volume. The D[4.3] value of spreads was found to be significantly high, particularly at levels where 12.5% and 20% yellow poppy seeds were added ($p < 0.05$).

Textural characteristics

The results of the parameters obtained from the textural analysis are presented in Table 2. Firmness is the maximum force on a spread that displays substantial resistance to deformation [29]. The firmness values of chocolate spreads ranged from 965.42 to 2838.97 g. It was observed that as the poppy seed ratio increased, the firmness values decreased. This can be attributed to the amount of oil contained in poppy seeds. This is because when poppy seeds are crushed during refining, the liquid seed oil leaks out. This liquid oil alters the consistency of the product. Chocolate spreads with yellow and blue poppy seeds showed similar values. A significant difference was found between samples with only 12.5% poppy seed added (A2 and B2) ($p < 0.05$). Work of shear (spreadability) is the total amount of force required to perform the shearing operation [29]. Spreadability values of the chocolate spread samples ranged from 562.33 (A2) to 2046.18 (B1) g s. The spreadability values of samples containing 5% and 20% yellow and blue poppy seeds, excluding spreads containing 12.5% poppy seeds, were found to be similar ($p > 0.05$). As a result, while the effect of the poppy seed variety on all texture parameters was insignificant ($p > 0.05$), the effect of the poppy seed quantity was found to be significant ($p < 0.05$). Aydemir [18] found that popular commercial chocolate spreads with hazelnuts have a spreadability value of approximately 1000 g s. Only sample B2, containing 12.5% blue poppy seeds, demonstrated this level of spreadability preferred by consumers. Stickiness is the maximum force necessary to overcome the attractive forces between the surface of spread and the surface of the probe [29]. The stickiness values of the chocolate spreads ranged from − 1682.01 (A2) to -4094.51 (B1) g. Except for sample A3, it was observed that the stickiness values decreased significantly as the proportion of poppy seeds increased. No difference was observed in stickiness values between the two poppy seed spreads for contribution ratios of 5% and 20% ($p > 0.05$). Work of adhesion (adhesivity) is the total amount of the force involved in the withdrawal of probe from spread [29]. The adhesivity values of the chocolate spread samples ranged from − 256.62 (A2) to -843.30 (B1) g s. It was determined that, with the exception of sample A3, the adhesivity values decreased significantly as the proportion of poppy seeds increased. This situation is directly related to the increased amount of oil derived from poppy seeds and the decreased amount of milk powder. Aydemir [18] reported that different seed oils yielded quite low spreadability and adhesivity values. The effect of two different poppy seed varieties on adhesivity was found to be insignificant at contribution rates of 5% and 20% ($p > 0.05$). Aydemir [18] found that the adhesivity values of the most popular chocolate spreads with hazelnuts were in the range

of approximately 400–500 g s. The sample showing the adhesivity value closest to this range was again B2.

Steady and dynamic rheological properties

The rheological behaviour of the poppy enriched chocolate spreads was well described by the Ostwald–de Waele model, with excellent fits for all samples (R^2>0.97). Both seed type and concentration significantly affected η_{40}, *K*, and *n* (p<0.05) (Table 3). A decline in η_{40} was observed in both A and B spreads, suggesting that poppy addition initially reduced the apparent resistance to flow. However, *K* displayed a non–linear trend: it decreased at moderate addition levels (A2 and B2) but rose again at the highest concentrations (A3 and B3). This shift indicates that low incorporation may disrupt the matrix, whereas higher poppy seed levels strengthen it through enhanced network formation.

From a practical perspective, the reduced apparent viscosity (η_{40}) and lower consistency coefficient (K) observed at moderate addition levels, particularly in A2 and B2, indicate lower resistance to flow, which facilitates easier spreading during consumption. This rheological behaviour is consistent with the sensory results, where sample B2 (12.5% blue poppy seeds) exhibited the level of spreadability preferred by consumers.

The increase in *K* at elevated concentrations can be associated with the protein and fibre rich nature of poppy seeds, which promotes denser particle packing and stronger particle–fat interactions within the chocolate matrix. Similar viscosity reinforcement has been reported in other semi–solid systems containing protein and fibre rich plant materials [30, 31]. This behaviour is also consistent with previous findings on poppy seed cake, where stronger networks improved the rheological stability of emulsified foods [32].

Shear stress (τ) increased nonlinearly with shear rate ($\dot{\gamma}$) for all samples, confirming non–Newtonian flow. B formulations showed a steeper rise, indicating stronger resistance to deformation, whereas A2 exhibited the weakest response, supporting that flow behaviour depends on particle characteristics as well as concentration (Fig. 3). Consistently, η decreased with increasing $\dot{\gamma}$, demonstrating shear–thinning behaviour across formulations. B group samples retained higher viscosity throughout the shear range, while sample A2 showed the most rapid viscosity decay, further highlighting the weaker structural integrity at moderate inclusion levels (Fig. 3). All formulations showed pseudoplastic (shear–thinning) behaviour (n < 1), meaning the structure becomes easier to spread when shear is applied. In A group samples, *n* values remained similar at lower levels (A1–A2) but dropped noticeably at 20% (A3), indicating that stronger shear–thinning appears only when the matrix becomes highly loaded with particles. In B group samples, *n* remained statistically unchanged, suggesting that their deformation behaviour is driven more by the inherent characteristics of blue poppy particles rather than the concentration level. This shear–thinning pattern is in line with reports showing that coarse or suspended plant particles disrupt Newtonian flow by breaking apart into weaker aggregates under shear [33, 34]. In our chocolate spreads, rigid poppy seed fragments appear to create a similar effect, reducing n and enabling smoother spreadability without loss of structural stability, particularly in sample A3 and the formulations B.

Dynamic viscoelastic data of the spreads are shown in Fig. 3, depicting the variation of complex modulus (G^*) and loss tangent ($tan\ \delta$) with angular velocity (ω). All samples behaved as weak gels, indicated by rising G^* and $tan\ \delta$<1, showing that elasticity dominated over viscous flow. The B formulations, particularly B2 and B3, exhibited markedly higher G^* and lower $tan\ \delta$ than the A samples, indicating a stronger and more organized particle–fat network. In contrast, A2 showed the weakest response, while A3 displayed only moderate improvement, remaining substantially less elastic than the B series. These findings show that viscoelastic reinforcement depends not only on poppy seed concentration but also on the interaction capacity and structural performance of the particles. The superior elastic response of the B formulations suggests more effective particle–fat associations, whereas the weak behavior of sample A2 indicates that moderate addition may disturb network continuity rather than support it. Although sample A3 showed partial improvement, only samples B2 and B3 formed a cohesive, solid–like system.

Table 3 Apparent viscosity (η_{40}) and Ostwald–de Waele model parameters (*K* and *n*) of chocolate spreads formulated with yellow (A) and blue (B) poppy seeds at three addition levels (5, 12.5, and 20%)

Sample	η_{40} (Pa·s)	K (Pa·s^n)	*n*
A1	16.62 ± 0.46^{aB}	143.00 ± 5.37^{bA}	0.46 ± 0.01^{aA}
A2	9.64 ± 2.38^{cB}	61.83 ± 7.10^{cA}	0.49 ± 0.02^{aA}
A3	14.49 ± 0.69^{bA}	152.17 ± 3.18^{aA}	0.41 ± 0.02^{bA}
B1	18.20 ± 0.13^{aA}	250.20 ± 19.23^{aB}	0.30 ± 0.03^{aB}
B2	15.00 ± 0.30^{bA}	133.85 ± 3.75^{cB}	0.43 ± 0.01^{aB}
B3	15.25 ± 0.33^{bA}	209.10 ± 10.47^{bB}	0.34 ± 0.02^{aB}

Values are expressed as mean ± SD (n=3). Different lowercase letters indicate significant differences among concentrations within the same seed type, while different uppercase letters indicate differences between seed types at the same concentration (p<0.05, two-way ANOVA followed by Tukey's test)

FT–IR results

Figure 4 shows the FT–IR spectra of the chocolate spread samples. Several characteristic peaks in the spectra are visible. These peaks were identified and assigned to functional groups. The broad peak between 3650 and 3050 cm^{-1}

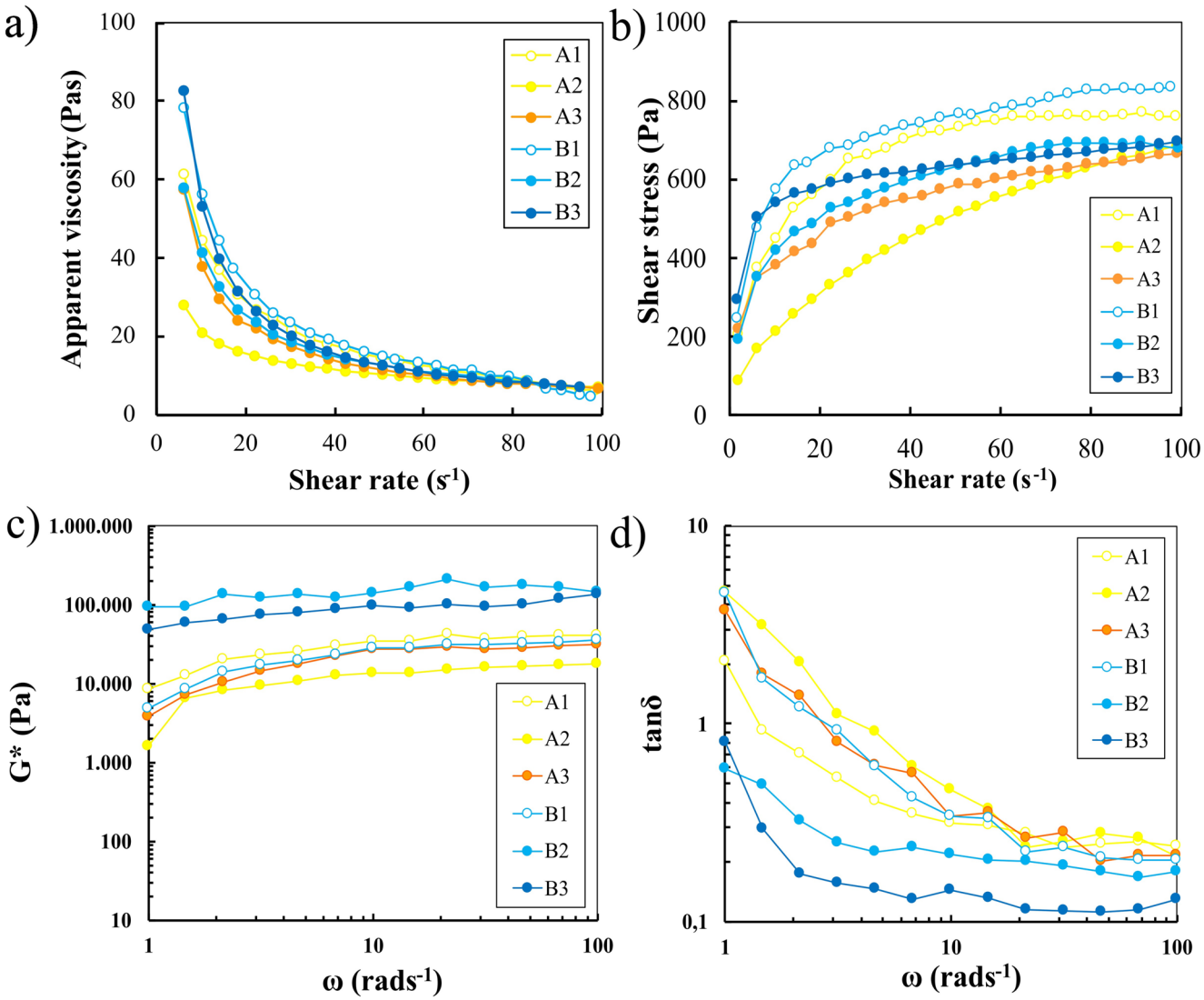


Fig. 3 Apparent viscosity–shear rate plots (**a**), flow curves (**b**) dependence of the complex modulus on frequency (**c**), and dependence of the loss tangent on frequency (**d**) of the chocolate spreads

corresponds to O–H stretching vibrations. This peak likely arises from water in the formulation. The hydroxyl groups of carbohydrates in the formulation are likely overlapped by water [35]. Peaks between 3030 and 2775 cm^{-1} correspond to C–H stretching. These peaks likely originate from the fatty acids. The sharp peak at 1744 cm^{-1} is attributed to C = O stretching of ester bonds in triglycerides [36]. In the 1700–1400 cm^{-1} region, peak overlap is expected in food formulations. Even so, this region can still indicate the presence of functional groups in the spread formulations. The small peak near 1650 cm^{-1} may arise from H–O–H bending of water and amide I vibrations of milk proteins. Peaks at 1460, 1450, 1440, and 1415 cm^{-1} may arise from CH_2/CH_3 bending vibrations. These are likely arisen from of lipid acyl chains [36, 37]. Several peaks in the 1200–900 cm^{-1} region (including 1160, 1070, 1010, and 980 cm^{-1}) may correspond to C–O–C, C–O, and C–O–H vibrations. These peaks likely arise from the carbohydrates in the spreads [38, 39].

It should be noted that these spectra belong to formulations. Therefore, they mainly reflect the spectral properties of the major components. Spectral features of minor ingredients are probably hidden by overlapping bands. Nonetheless the spectra provide useful information about the dominant chemical groups in the formulations. The spectra of the different formulation samples appear very similar to the naked eye. This is expected since the ingredient compositions of the spreads are very similar. Thus additional analysis might be helpful to observe similarity and differences between spectra. FT–IR spectra of A and B samples were analysed using principal component analysis (PCA), k–means clustering, k–medians clustering, and hierarchical cluster analysis (HCA) using Camo Unscrambler X 10 software. These

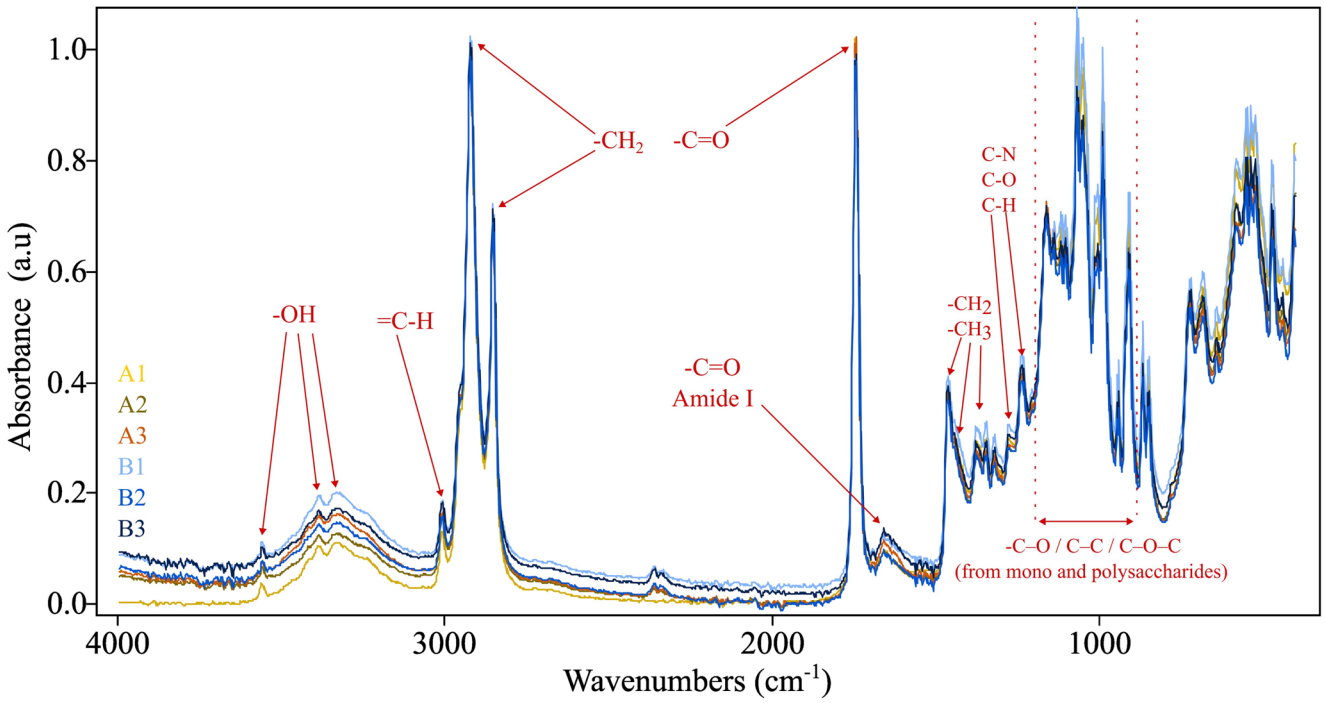


Fig. 4 FT–IR spectra of the chocolate spreads

approaches allowed us to reduce data dimensionality and to examine whether samples tend to cluster according to their FT–IR spectral characteristics. Baseline correction and mean centring were applied to the FT–IR spectra. PCA was performed on the using singular value decomposition (SVD) and cross–validation. Two principal components (PC1 and PC2) were calculated. They explained 97.70% of the total variance together (PC1: 73.69%; PC2: 24.01%). Since the total explained variance was greater than 90%, these two components can be considered to provide a compact representation of the original FT–IR spectra [40].

A scatter plot was drawn using the PC1 and PC2 scores of the samples. Although PCA is not a clustering method, the scatter plot helps to display natural grouping tendencies among the samples based on their FT–IR signatures. Hotelling's T^2 ellipse at the 95% confidence level was overlaid on this plot to detect potential outliers. The score plot is shown in Fig. 5. All samples lay inside the 95% confidence region, and no outliers were detected. The PCA score plot show some natural groupings k–means and k–medians clustering was applied to the PC scores to objectively explore natural groupings. Clustering was performed with K = 2, 3, and 4 values. When K was selected as 2, first cluster included A1 and B1. All other samples were included in the second cluster. When K was selected as 3, A1 formed the first cluster, B1 formed the second cluster and the remaining samples formed the third cluster. When K was selected as 4, A1 formed the fourth cluster and B1 formed the first cluster. The remaining samples were grouped as B2–B3 in second cluster and A2–A3 in third cluster. The analyses were repeated with k–medians method for each K value, and the same cluster memberships were obtained. This agreement between k–means and k–medians provide some support that the clustering patterns are consistent under the tested conditions of this small data set. These clustering results show that A1 and B1 are consistently separated from the remaining samples. A1 contains yellow poppy seed and B1 contains blue poppy seed at the lowest ratio. In this data set, spreads with different seed types at low seed ratios therefore occupy different regions in the multivariate FT–IR space. Moreover, samples with higher seed ratios (A2, A3, B2, B3) broadly group according to both seed type and ratio. In all clustering configurations, A and B samples were separated, which is consistent with the formulation differences between yellow and blue poppy seed spreads.

As a complementary approach to partitioning methods, hierarchical cluster analysis (HCA) was applied to the PCA scores as well. Complete–linkage was used as the agglomeration method and Euclidean distance as the similarity measure, which are common choices for spectral data and tend to produce compact, well–separated clusters. The results were presented as a dendrogram to visualise how samples group together across different similarity levels. The HCA dendrogram was given in Fig. 5. A1 and B1 have the lowest similarity to each other (around 40%) and very low similarity to the other samples. They are located separate from the remaining samples on distinct branches. This agrees with their different seed types at the lowest ratio. A2 and

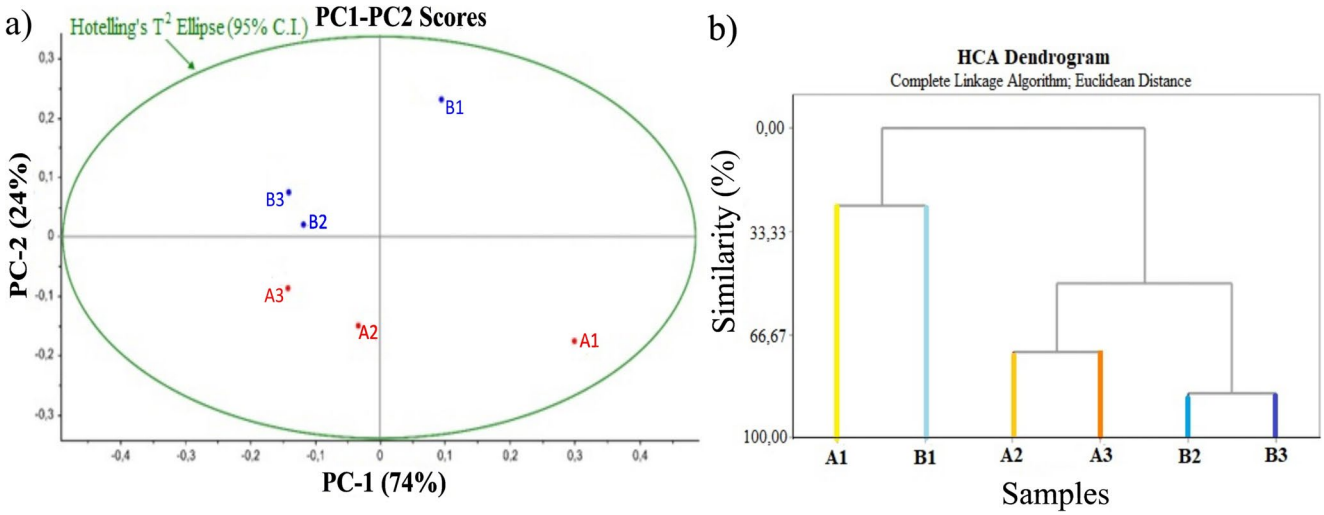


Fig. 5 Principal component analysis (PCA) scores plot of the chocolate spreads (**a**) and dendrogram of the chocolate spreads by HCA using complete linkage clustering algorithm (**b**)

A3 show higher similarity to each other (around 70%) and intermediate similarity to B2 and B3 (around 50%). B2 and B3 are the most similar pair (around 80%). These relationships are consistent with effects of both poppy seed type and ratio given all other ingredients are similar in the spreads.

Overall, PCA reduced the high-dimensional FT–IR data to two components that captured most of the spectral variance. Although PCA is not a clustering technique, the PC1–PC2 score plot still showed similarities and differences between spectra. In this plot, A1 and B1 (low seed ratios) have positive PC1 scores. The higher seed ratio samples (A2, A3, B2, B3) have negative PC1 scores. All B samples have positive PC2 scores, whereas all A samples have negative PC2 scores. These patterns suggest that seed type and inclusion level have subtle but detectable effects on the FT–IR spectra in this data set.

Microstructural properties

Light microscope images and photographs of chocolate spreads with poppy seeds of different varieties and quantities are presented in Fig. 6. From the photographs, in fact, the samples have a fairly homogeneous structure and have not shown any fat or sugar bloom. It was also observed that the samples had a slightly rough/sandy texture due to poppy seed shell particles. It can be seen that as the proportion of poppy seed in the samples increases, softening occurs due to the increase in poppy seed oil in the structure. The small brown particles visible in the microscope images of chocolate spread samples are cocoa powder, while the shiny parts are powdered sugar and solid fat [41]. No sharp–angled saturated fat crystals were observed. It was observed that as the proportion of poppy seed increased, the gaps in the structure decreased and the colour gradually darkened. Blue poppy seed resulted in a darker coloured structure compared to yellow one. This situation can be attributed to the increased seed colour agents and, in particular, oil of poppy seeds. Apart from the colour difference, there was not much difference between samples A and B in terms of microscopic appearance.

Colour characteristics

The colour parameters of the chocolate spreads are presented in Table 2. The L^* value of the samples ranged from 26.13 (B3) to 29.69 (A1). For both seed varieties, as the poppy seed content increased, the L^* value decreased significantly ($p < 0.05$). The decrease in the L^* value indicates that poppy seeds darken the spread colour. Again, in terms of the effect on the chocolate spread colour, there was no difference between the two seeds for 5% poppy seed additions ($p > 0.05$). However, this effect became significant as the poppy seed addition rate increased. For 12.5% and 20% poppy seed additions, blue poppy seeds yielded lower L^* values than yellow poppy seeds ($p < 0.05$). The a^* value of chocolate spreads decreased significantly for both seed types as the poppy seeds content increased ($p < 0.05$). The decrease in a^* value indicates that poppy seeds reduced the red colour tone of the spread. In terms of its effect on the colour of the chocolate spread, for all three contribution ratios, blue poppy seeds significantly reduced a^* value more than yellow poppy seeds ($p < 0.05$). The b^* value of chocolate spreads decreased for both seed types as the poppy seed content increased. This decrease was significant only for blue poppy seeds at each seed inclusion rate ($p < 0.05$). The decrease in the b^* value indicates that poppy seeds reduced the yellow colour tone of the spread. For the 12.5% and 20% addition ratios, blue poppy seeds significantly reduced the

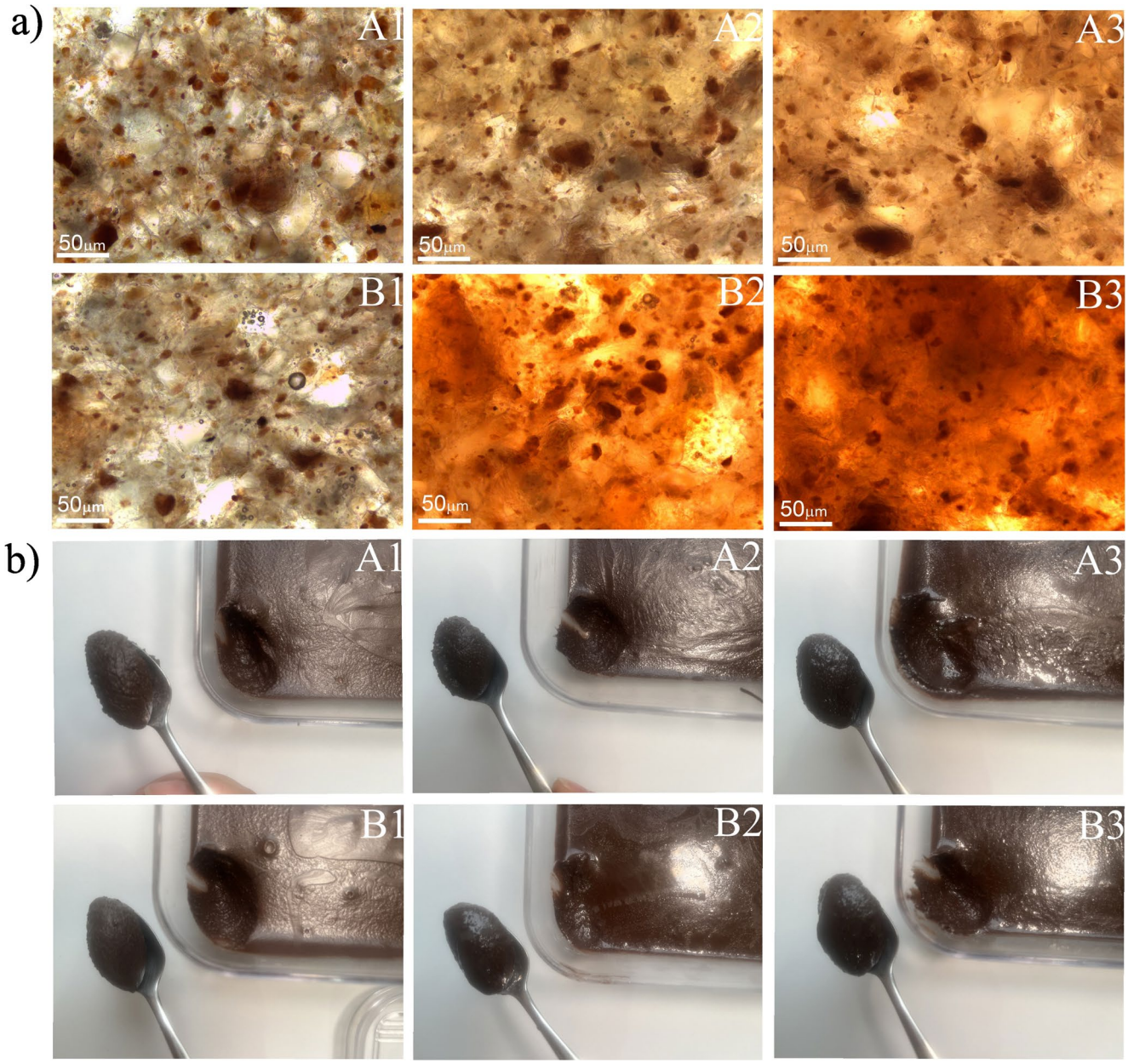


Fig. 6 The microstructure and visual evaluation of the chocolate spreads. Light microscope images of the chocolate spreads (**a**), visualization images of the chocolate spreads (**b**). The scale bar is 50 µm

b^* value of the chocolate spread more than yellow poppy seeds ($p < 0.05$). Consequently, while the effect of the poppy seed variety on all colour parameters was insignificant ($p > 0.05$), the effect of the poppy quantity was found to be significant ($p < 0.05$). The whitening Index (*WI*) is essentially a parameter used to measure fat crystallisation, which is a cosmetic defect typically caused by fat migration and recrystallisation during chocolate storage, resulting in a transformation from the original brown colour (low *WI*) to a whitened colour (high *WI*) [42, 43]. No fat bloom was observed on the chocolate spreads surface. It was observed that *WI* values decreased in proportion to the increasing poppy seed content, similar to L values ($p < 0.05$). This situation is thought to be primarily related to the decrease in the amount of milk powder used.

Sensory properties

For the purpose of assessing chocolate quality, consumers focus on three key characteristics. These are flavour, texture, and appearance [27]. The analysis results for the sensory parameters of the chocolate spread samples are presented in Fig. 7. The flavour–aroma scores of the samples ranged from 4.25 (A3) to 5.77 (B1). For both poppy seed varieties, the

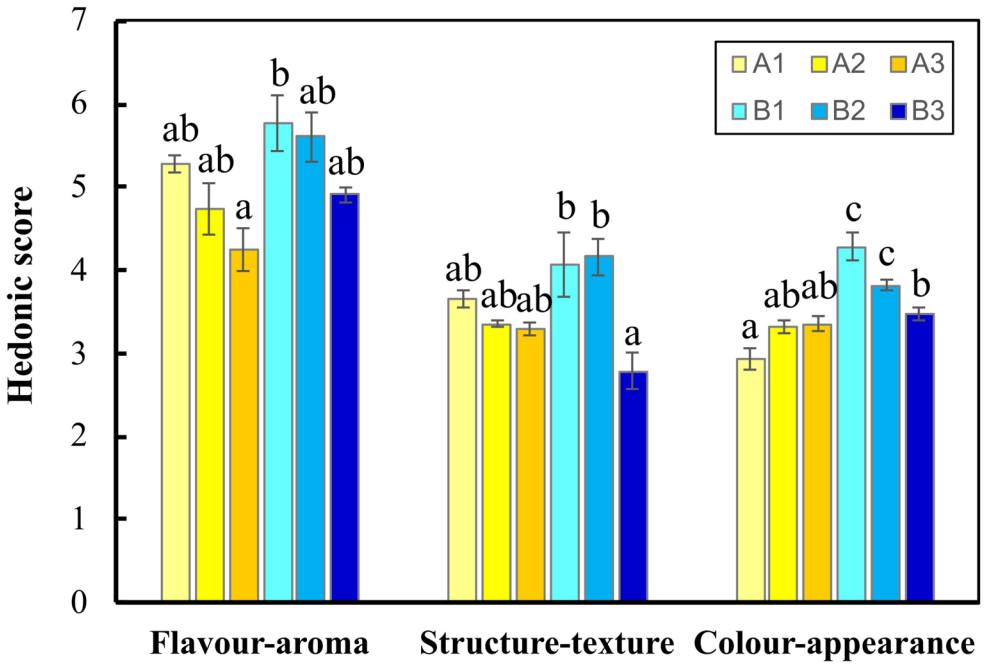


Fig. 7 Sensory scores of the chocolate spreads

flavour–aroma score of the chocolate spreads decreased as the seed quantity increased ($p < 0.05$). In white chocolate production, the use of 4% whole poppy seeds has received higher taste ratings and overall approval than the use of conched or ground seeds [15]. The flavour–aroma scores of chocolate spreads with blue poppy seeds were higher, but these differences were not statistically significant ($p < 0.05$). The panellists awarded the highest flavour–aroma scores to samples B1 and B2, respectively. Gupcsó et al. [12] reported that blue poppy seeds have a higher aroma and flavour preference than white poppy seeds. Emir et al. [44] reported that roasting the poppy seeds prior to cold pressing improves the flavour of the oil, and that roasted yellow (53.55%) and blue (36.66%) poppy seeds oil is more favourably perceived than unroasted and white seed oil. The structure–texture scores of the spreads decreased as the proportion of poppy seeds increased, except for sample B2. Spreads with blue poppy seeds were preferred more in terms of structure–texture, except for those with a 20% poppy seed ratio. The samples with the highest structure-texture scores were B2 and B1, respectively. As the poppy seed ratio increases, panellists noted a sandy/rough texture defect in the spreads. The colour–appearance scores of the spreads ranged from 2.93 (A1) to 4.28 (B1). Blue poppy seed spreads had higher colour–appearance scores, but this difference was only significant at a 5% poppy seed addition. The colour–appearance scores of samples with yellow poppy seeds showed a very slight increasing trend with increasing addition rate, while the scores of spreads with blue poppy seed decreased. Similarly, Zay and Gere [15] reported that colour preference scores for white chocolate decreased as the poppy seed content increased. Sample B1 received a higher colour–appearance score than all other samples except B2. Ultimately, it was observed that the poppy seed variety had a significant effect on flavour–aroma and colour–appearance, while the proportion of poppy seeds was found to have a significant effect only on structure–texture ($p < 0.05$). When the results of the textural analysis parameters are analysed, it is seen that the use of 5% blue poppy seeds in chocolate spread production is preferred, closely followed by spreads with 12.5% blue poppy seeds. The panellists rated the spreads B1 and B2 as 'good' in terms of all three sensory parameters.

Conclusions

This study showed that poppy seeds noticeably affect the technological and sensory characteristics of chocolate spreads. Higher seed levels increased oil separation, particle size and texture, and also produced a darker colour. Blue poppy seeds stood out with their higher phenolic content, stronger rheological structure and more favourable sensory scores compared with yellow seeds, although very high seed levels caused coarse particle effects that reduced textural and sensory quality. FT–IR multivariate analyses demonstrated that seed type and concentration created measurable molecular differences in the formulations. Sensory evaluations indicated that blue poppy spreads at low to moderate levels provided the most appealing flavour, texture and appearance. Overall, poppy seeds, particularly blue variety, show promise as functional ingredients in chocolate spreads, and their amount should be carefully controlled to preserve desirable product quality.

Limitations

The present study did not include a control formulation without poppy seed. The experimental design was primarily intended to compare the effects of different poppy seed varieties and concentration levels within chocolate spread formulations. Therefore, the results mainly represent the relative differences among poppy-seed-containing samples rather than the absolute effect of poppy seed addition compared to a poppy-seed-free spread. Although the inclusion of a control formulation could provide additional insight into the specific contribution of poppy seed incorporation, the current design still allows a meaningful evaluation of how poppy seed variety and concentration influence the physicochemical, textural, rheological, functional and sensory characteristics of chocolate spreads. Future studies may further explore this aspect by including a poppy-seed-free control formulation.

Although the present study focuses on the formulation and technological characterization of chocolate spread with poppy seeds, the possible presence of opium alkaloids in poppy seeds should be acknowledged. The concentration of these compounds may vary depending on cultivation conditions, harvesting practices, and post-harvest processing. Since the analysis of alkaloid content was outside the scope of the present study, future research should evaluate the potential presence of these compounds in similar formulations to further ensure product safety.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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