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ORIGINAL PAPER

Systematic evaluation of hemp-based dairy alternatives: how processing water shapes the quality of milk and the characteristics of developed cheese analogs

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Abstract

Growing consumer interest in health-oriented and sustainable food choices has intensified the search for nutrient-dense plant-based dairy alternatives. This study focused on the development and systematic characterization of a novel milk substitute and spreadable cheese analog derived from hemp seeds (*Cannabis sativa L.*), a sustainable raw material containing 30.41% oil and 24.09% protein. Initially, four milk substitutes were formulated using two concentrations (1:5 and 1:10 w/v) and two liquid phases (drinking water and mineral water). Samples prepared at a 1:5 ratio exhibited the highest viscosity values, ranging from 7.11 to 9.99 mPa.s, and demonstrated typical non-Newtonian shear-thinning behavior. Based on rheological stability and sensory acceptance, the 1:5 formulations were selected for developing spreadable cheese analogs. The cheese analog produced with mineral water (SCA2) exhibited a superior mineral profile, particularly in sodium (2267.73 mg/L), potassium (3089.91 mg/L), and calcium (1155.10 mg/L), while effectively reducing the 'oily mouthfeel' characteristic of plant-based matrices. Conversely, the analog made with drinking water (SCA1) demonstrated higher total phenolic content (6.66 mg GAE/mL) and antioxidant activity (~40% inhibition), as mineral interactions in the SCA2 matrix led to phenolic-metal complexation. Volatile profiling via GC-MS identified 30 aromatic compounds, where the synergistic effect of δ-octalactone and 2-heptanone contributed to a creamy, cheese-like flavor profile. Dynamic oscillatory rheology confirmed solid-like behavior (G'> G'') for all analogs, with mineral fortification in SCA2 strengthening the protein network. These findings demonstrate that hemp seeds are highly functional for sustainable dairy alternatives, with the choice of processing water serving as a key determinant in modulating bioactive stability, rheological integrity, and sensory appeal.

Highlights

- Hemp seeds with a balanced nutrient profile (30.4% oil, 24.1% protein) serve as a sustainable base for dairy analogs.
- The use of mineral water as a processing phase significantly enhances the calcium density of hemp milk.
- A 1:5 hemp-to-water ratio is critical for achieving optimal rheological stability and pseudoplastic behavior.
- Mineral-protein interactions in SCA2 analogs facilitate a robust elastic matrix with superior K and Ca retention.
- Synergy between lactones and ketones effectively mimics the complex aroma profile of traditional spreadable cheese.

Keywords Hemp seed · Plant-based milk · Dairy alternatives · Cheese analog · Mineral fortification

Introduction

The global food industry is increasingly focusing on the valorization of underutilized by-products and novel raw materials to develop nutritionally superior and functional ingredients [1]. In this context, *Cannabis sativa L.* (hemp) has recently garnered considerable attention in the domain of functional foods due to the exceptional nutritional profile

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of its seeds, offering a sustainable alternative to traditional dairy-based systems [2]. Industrial hemp seeds are characterized by a superior nutritional profile, containing approximately 25–30% protein and 35% lipids. Notably, hemp seed oil exhibits a 3:1 ratio of omega-6 to omega-3 fatty acids, which is considered ideal for human physiological functions, including metabolic enhancement and cholesterol regulation [3]. Their substantial arginine content, coupled with their naturally cholesterol-free and gluten-free properties, renders hemp seeds advantageous for cardiovascular health and a valuable dietary option for individuals with celiac disease [4]. Beyond its basic nutritional value, hemp is a rich source of phytochemicals including phenolics and cannflavins that exhibit potent antioxidant and anti-inflammatory activities. These compounds have been shown to neutralize free radicals and inhibit pro-inflammatory enzymes, thereby mitigating the risk of chronic conditions such as cardiovascular and neurodegenerative diseases [5]. These unique attributes position hemp as an ideal raw material for the production of sustainable and allergen-friendly next-generation milk alternatives. The capacity of hemp-based milk substitutes to reduce serum triacylglycerol and cholesterol levels, alongside their low allergenicity, further substantiates this potential [6]. To date, research on hemp milk has predominantly concentrated on process optimization, including enhancing emulsion stability and minimizing phase separation [7], as well as augmenting overall nutritional value [8]. However, a comprehensive and systematic investigation into the effects of the chemical composition of processing water on the stability of hemp seed–derived milk substitutes, the preservation of bioactive compounds, and the sensory attributes of the final product is currently absent in the literature. In recent years, heightened health consciousness, concerns regarding sustainability, and the increasing popularity of vegan dietary practices have catalyzed a substantial transformation within the global food system. Central to this transformation are plant-based milk alternatives, which have emerged as substitutes for traditional dairy milk. Consumers predominantly favor plant-based milk alternatives due to dietary considerations such as lactose intolerance and milk allergies, as well as functional health benefits, including reduced levels of saturated fat and cholesterol [9–11]. Furthermore, the lower carbon footprint and decreased water usage associated with these products, in comparison to animal agriculture, bolster ethical and environmental motivations [12, 13]. Although plant-based milk alternatives attract a wide consumer base, their generally lower protein and micronutrient content compared to animal milk necessitates nutritional fortification as a scientific imperative [14]. In this context, the development of sustainable alternatives derived from novel and nutrient-rich plant sources has emerged as a priority research area within food science.

Cheese analogs are alternative products developed to replace conventional cheese and are produced using plant-based ingredients. These products have gained popularity during a period of declining demand for traditional cheeses due to lactose intolerance, vegan diets, and environmental sustainability issues. Cheese analogs are produced by combining various plant-based fats and proteins, and they may exhibit diverse textural and flavor characteristics depending on the selection of ingredients and processing conditions [15]. The nutritional advantages of plant-based cheese analogs include low cholesterol and saturated fat contents and high levels of dietary fiber and phytochemicals. These products also offer sustainable food alternatives through environmentally friendly production processes [16]. Recently, cheese analogs with different formulations and ingredient compositions have been developed and characterized. In this context, the effects of soy protein in spreadable cheese analogs [17], faba bean protein/carboxymethyl cellulose combinations for ricotta-type cheese analogs [18], and fermented formulations produced using kefir grains [19] have been investigated.

The primary objective of this study was to investigate the effects of the chemical composition of processing water on the stability and preservation of bioactive compounds in hemp seed–based plant milk alternatives, as well as to evaluate their nutritional value and sensory acceptability. Additionally, the potential use of the produced hemp-based milk alternatives in the development of next-generation cheese analogs was explored. The chemical and sensory properties of hemp-based milk and cheese analogs were comparatively analyzed. In this study, the critical effects of water quality on the formulation of hemp seed–derived milk substitutes and cheese analogs were elucidated, with the aim of identifying optimal processing conditions that maximize the industrial potential of this unique raw material and provide a novel scientific contribution to the structural design of plant-based cheese analogs.

Materials and methods

Hemp seed analyses

Hemp (*Cannabis sativa L.)* seeds were obtained from Türkiye. The proximate composition of the hemp seeds, including moisture, ash, crude protein, and crude oil content, was determined according to AOAC standard methods [20].

The fatty acid composition of hemp seed oil samples was characterized using an Agilent 7890B GC system coupled with a 7010B MS detector (Agilent Technologies, Santa Clara, CA, USA). For the analysis, fatty acid methyl esters (FAMEs) were prepared via a trans-esterification procedure using sodium methoxide, following the methodology described by Manis et al., [21] with slight modifications. Chromatographic separation was achieved using a DB-WAX capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was employed as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set to 250 °C, and samples were injected with a 20:1 split ratio. The mass spectrometer facilitated the identification of FAMEs by comparing mass spectral profiles with the NIST library.

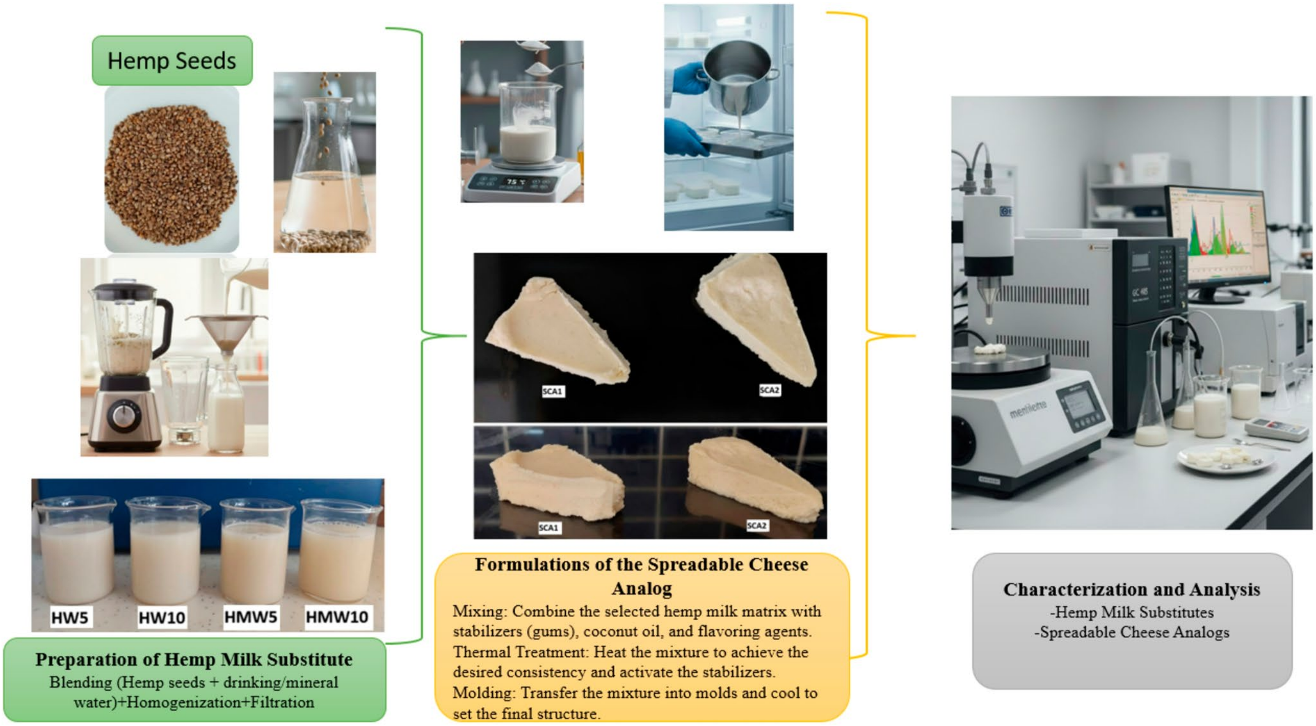
Preparation of hemp-based milk substitutes

Hemp seed milk substitutes were produced according to the method described by Awad and Mortaş [7] with some modifications. Two different liquid phases (drinking water and degassed mineral water) were employed at two seed-to-liquid ratios (1:5 and 1:10, w/v). For the extraction process, the hemp seeds were blended with the respective processing liquid and homogenized for 5 min using a high-speed blender (Vestel Mix, Türkiye). The resulting slurries were filtered through a double-layered cheesecloth to remove large insoluble particles. To investigate the specific effect of water composition, a second set of samples was prepared using degassed mineral water (Beypazarı, Türkiye) following the same homogenization and filtration procedures. The formulated milk alternatives were coded based on the water type and concentration as follows: HW5 (drinking water, 1:5), HW10 (drinking water, 1:10), HMW5 (mineral water, 1:5), and HMW10 (mineral water, 1:10). All samples were stored at 4 °C until further analysis (Fig. 1).

Production of spreadable cheese analogs

Based on preliminary sensory screening, hemp-based milk substitutes prepared at a 1:5 (w/v) ratio using either drinking water or degassed mineral water exhibited the highest scores in terms of hemp aroma and consistency; therefore, these formulations were selected for producing spreadable cheese analogs.

The spreadable cheese analogs were developed by modifying the formulation principles described by Sanders et al. [22] to suit the specific characteristics of hemp-based systems. Two different formulations were produced. For the first formulation (SCA1), xanthan gum (1 g) and locust bean gum (2.5 g) (Tito, Türkiye) were added to 100 mL of hemp-based milk substitute (drinking water, 1:5 w/v). Subsequently, pre-melted coconut oil (50 mL; Naturlife, Türkiye) and lecithin (4 g; Tito, Türkiye) were incorporated as emulsifiers to facilitate homogenization. After adding cheese flavor and sodium ascorbate (Tito, Türkiye), the mixture was homogenized until uniform. The resulting blend was heated to 75 °C, poured into molds, cooled, and stored under refrigeration (4 °C). The second formulation (SCA2) was produced following the same procedure using the hemp-based milk substitute prepared with degassed mineral water (1:5 w/v) (Fig. 1).



Preparation of Hemp Milk Substitute
Blending (Hemp seeds + drinking/mineral water)+Homogenization+Filtration

Formulations of the Spreadable Cheese Analog
Mixing: Combine the selected hemp milk matrix with stabilizers (gums), coconut oil, and flavoring agents.
Thermal Treatment: Heat the mixture to achieve the desired consistency and activate the stabilizers.
Molding: Transfer the mixture into molds and cool to set the final structure.

Characterization and Analysis
-Hemp Milk Substitutes
-Spreadable Cheese Analogs

Fig. 1 Schematic overview of the development, formulation, and analytical characterization of hemp-based dairy alternatives

Characterization of hemp-based milk substitutes and spreadable cheese analogs

Stability, pH, and total soluble solids analysis

To monitor serum separation and syneresis, 15 mL aliquots of the hemp-based milk substitute samples were transferred into graduated plastic tubes immediately after production. The samples were stored at 4 °C for 15 day, and phase separation was visually monitored during storage. The analysis was performed with some modifications to the method [23].

The total soluble solids content (°Brix) of the plant-based milk substitutes was measured using a digital refractometer (ATAGO RX-7000i, Japan). The pH values were determined using a portable pH meter (WTW pH/Cond 3320; Weilheim, Germany).

Proximate composition analysis

The ash, moisture, crude protein, and crude fat contents of the spreadable cheese analogs were determined to characterize the nutritional composition of the final products. All analyses were performed according to the official AOAC methods, and the results were expressed as weight percentages [20].

Color analysis

Color measurements of both hemp-based milk substitutes and spreadable cheese analogs were performed using a colorimeter (Konica Minolta, Osaka, Japan). Color parameters were recorded according to the CIELAB color space, including lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) values of the samples [17].

Total phenolic content and antioxidant activity analysis

The total phenolic content of hemp-based milk alternatives was determined using the Folin–Ciocalteu method with a UV–VIS spectrophotometer (GENESYS 10 S). Briefly, 400 µL of methanol-diluted samples were mixed with 2 mL of Folin–Ciocalteu reagent, which was previously diluted 1:9 with distilled water. Subsequently, 1.6 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark for 1 h. Absorbance was measured at 765 nm, and the total phenolic content was calculated as mg gallic acid equivalents (GAE) per 100 g of sample using a gallic acid standard curve [24].

The antioxidant activity of the hemp-based milk alternatives was assessed using a modified DPPH radical scavenging assay [25]. Briefly, 800 µL of methanol-diluted sample was mixed with 3.2 mL of 0.1 mM DPPH solution and incubated in the dark for 30 min. The absorbance was measured at 517 nm.

Rheological analysis

The rheological properties of the hemp-based milk alternative samples were measured using a rheometer (Malvern Kinexus Pro, United Kingdom) equipped with a cone-plate system. Measurements were performed at 25 °C using a Peltier-controlled plate over a shear rate range of 0.10–100 s^{-1}. The apparent viscosity was expressed as a function of the shear rate.

The rheological properties of the cheese analog samples were determined using dynamic rheological analysis owing to their structured nature. Measurements were conducted using the same temperature-controlled rheometer, and the storage (G') and loss (G'') moduli were recorded over a frequency range of 0.1–10 Hz [17].

Mineral content analysis

The mineral content (Na, Mg, K, Ca, and Zn) of hemp-based milk alternatives and spreadable cheese analogs was determined using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific iCAP Q, Germany). The analytical procedure was adapted from the method described by Chen et al. [26] with some modifications. Samples were subjected to acid digestion by adding 6 mL of HNO_3 and 2 mL of H_2O_2 and heating at 200 °C using a Milestone Ethos SK 10 microwave digestion system. After digestion, the samples were diluted to 50 mL with ultra-pure water. Subsequently, 5 mL of each diluted sample was spiked with an internal standard (50 ppb) and diluted to 10 mL. To ensure the elemental concentrations fell within the instrument's linear range, the resulting solutions were further diluted 100-fold prior to ICP-MS analysis. The concentrations of Na, Mg, K, Ca, and Zn were automatically calculated by the instrument software, accounting for the initial sample volume and all subsequent dilution factors.

Analysis of volatile compounds

The volatile profile of the hemp-based spreadable cheese analogs was analyzed using an Agilent 7890B gas chromatograph coupled with an Agilent 7010B mass spectrometer (GC-MS). The extraction of volatile compounds was performed via solid-phase microextraction (SPME), based on the principles described by Mefleh et al. [27] with specific modifications optimized for the hemp-based matrix and the analytical configuration of the Agilent system.

Briefly, a 3 mL sample was placed in a headspace vial and equilibrated at 60 °C for 15 min. Volatile compounds were then adsorbed using a 75 µm CAR/PDMS-coated fiber for 30 min at the same temperature. Following extraction, the analytes were thermally desorbed in the injection port at 250 °C (split ratio 1:10) and separated using a DB-Wax capillary column (60 m

× 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed to start at 40 °C, increasing to 240 °C with optimized ramps to achieve maximum resolution. The mass spectrometer was operated in electron impact (EI) mode at 70 eV, with a scan range of 30–600 m/z. Compound identification was performed by comparing the obtained mass spectra with the NIST library.

Sensory analysis

Sensory evaluation of the hemp-based milk alternatives and spreadable cheese analogs was conducted using a panel of 20 non-smoking individuals aged 18–35 years. The panelists assessed the samples using a 9-point hedonic scale to evaluate the color and appearance, taste and aroma, odor, mouthfeel, texture, homogeneity, and overall acceptability.

Statistical analysis

Statistical evaluation of the analytical data was performed using SPSS Statistics 17.0 software (IBM Corp., Armonk, NY, USA). All experiments and analyses were conducted in triplicate, and the results are expressed as mean values ± standard deviation. To determine the significant differences between groups, a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was employed. Statistical significance was defined at a confidence level of $p < 0.05$.

Results and discussion

Chemical composition and fatty acid profile of hemp seeds

The chemical composition of the hemp seeds used in this study was determined to be 30.41% oil, 24.09% protein, 5.78% moisture, and 4.43% ash (Table 1). These values are in good agreement with the industrial standards for *Cannabis sativa L.* reported in the literature [5, 28]. While our findings align with the general nutritional profile of edible hemp seeds, slight dissimilarities in oil and protein yields compared to previous reports [29, 30] can be primarily attributed to the influence of environmental factors, such as the geographical origin, soil quality, and climatic conditions of the cultivation period [31]. The fact that our moisture and ash contents are within the expected ranges confirms the high quality and purity of the hemp seeds used as a raw material for food processing.

Table 1 Oil, protein, moisture, ash contents and fatty acid composition of hemp seeds

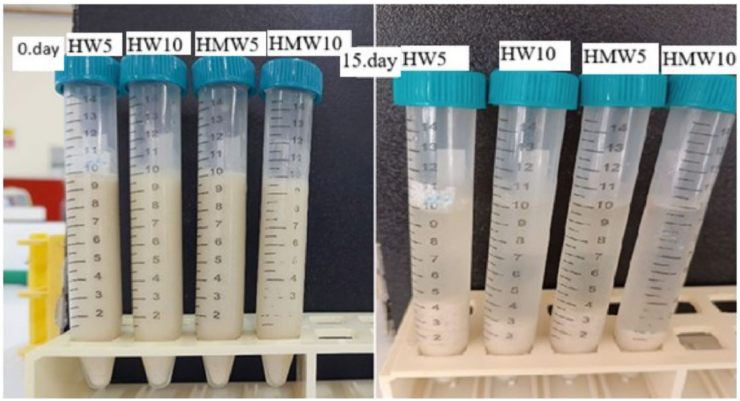
		Hemp seed
Oil Content (%)		30.41 ± 0.50
Protein Content (%)		24.09 ± 0.05
Moisture Content (%)		5.78 ± 0.05
Ash Content (%)		4.43 ± 0.01
Fatty acid composition	Palmitic Acid (C16:0) (%)	6.03 ± 0.18
	Stearic Acid (C18:0) (%)	3.16 ± 0.09
	Oleic Acid (C18:1) (%)	13.83 ± 0.07
	Linoleic Acid (C18:2) (%)	58.0 ± 0.11
	Linolenic Acid (C18:3) (%)	16.38 ± 0.14

Fatty acid profile analysis revealed that the predominant fatty acids were linoleic acid (58.00%), α-linolenic acid (16.38%), oleic acid (13.83%), palmitic acid (6.03%), and stearic acid (3.16%). The high proportions of linoleic and α-linolenic acids highlight the richness of hemp seed oil in polyunsaturated fatty acids (PUFAs). The linoleic to α-linolenic acid ratio in our samples (approx. 3.5:1) closely follows the ideal 3:1 nutritional balance identified in different global ecotypes [29]. This stability in the fatty acid distribution across different studies [30, 31] emphasizes that regardless of the total oil yield variations caused by environmental stressors, the high-quality PUFA profile essential for functional food applications remains a consistent characteristic of *Cannabis sativa L.* seeds. Overall, the results confirm that the hemp seed used in this study is a nutrient-dense and safe raw material, characterized by a high PUFA content and low levels of saturated fatty acids.

Stability, pH, and total soluble solids (Brix) analysis

The total soluble solids (°Brix) values of the hemp-based milk substitutes ranged from 0.86 to 1.45, depending on the initial hemp seed concentration (Table 2). As expected, increasing the hemp-to-water ratio in the HW5 and HMW5 samples significantly increased the amount of dissolved solids in the aqueous phase. While the water type did not significantly affect °Brix values ($p > 0.05$), it had a profound impact on pH and physical stability. In contrast, increasing the hemp seed concentration led to higher pH values, and the use of mineral water resulted in a further significant increase in the formulation pH (7.55–7.85). In a similar study, plant-based milk alternatives were reported to exhibit near-neutral pH values ranging from 6.8 to 6.9 [32], which is consistent with our findings. This near-neutrality is critical for colloidal stability, as it is driven by the electric charge resulting from the dissociation of functional groups within the protein complexes [33]. The stability of hemp-based milk samples was significantly influenced by both the water type and hemp seed concentration during the 15-day storage period. Samples prepared with degassed mineral water (HMW) exhibited a higher degree of phase separation than those prepared with drinking water (HW). This behavior can be attributed to the presence of divalent

Table 2 Stability, pH and water-soluble dry matter values of hemp-based milk substitutes

	HW5	HW10	HMW5	HMW10
Stability				
Brix (20 °C)	1.45±0.03^{a}	0.86±0.03^{b}	1.32±0.03^{a}	0.87±0.03^{b}
pH	6.08±0.02^{b}	6.13±0.01^{b}	7.55±0.01^{a}	7.85±0.02^{a}

*Different lowercase letters in the same row indicate a statistically significant difference (p<0.05). ±; standard deviation

ions (such as Ca^{2+} and Mg^{2+}) in mineral water, which screen the electrostatic repulsive forces between protein particles, thereby promoting aggregation and accelerating sedimentation [34–36]. According to the model proposed for milk-like systems, these divalent ions reduce the system's repulsive charges and trigger coagulation [33]. Specifically, Ca^{2+} and Mg^{2+} ions facilitate the formation of aggregates and complexes, significantly increasing the separation and sedimentation rates compared to ion-free systems [37]. Furthermore, the transformation from a colloidal suspension to larger flocs in HMW samples suggests that these ions promote protein coagulability by overcoming the stabilizing effect of Brownian motion [38]. Consequently, increasing the hemp seed concentration from 5% to 10% resulted in a more pronounced sediment layer, as the gravitational forces acting on the larger protein-lipid aggregates outweighed the stabilizing forces.

Color characterization of milk substitutes and cheese analogs

Color parameters play a crucial role in sensory perception by influencing the perceived flavor intensity and consumer preference [39]. In this study, the hemp-to-water ratio (1:5 and 1:10) had a statistically significant effect ($p < 0.05$) on the optical properties of hemp-based milk substitutes. Samples produced with a higher hemp concentration (HW5 and HMW5) exhibited significantly higher lightness (L^*) values than those prepared at a 1:10 ratio. This phenomenon can be attributed to the higher concentration of suspended particles, such as proteins and lipid droplets, which increase light scattering and result in a brighter appearance. As the hemp content decreased, the L^* values declined, resulting in a darker and more matte appearance (Table 3).

The green hues originating from the natural pigments of hemp seeds were reflected in the negative a^* values observed for all milk samples. Notably, in the 1:10 formulations with higher water content, both the intensity of the green hue increased and the lightness decreased, contributing to a more matte and grayish-green visual appearance. This suggests that as the system becomes more dilute, the masking effect of the white emulsion base (protein-lipid complexes) diminishes, making the underlying seed pigments more visually dominant. Similar color characteristics have been reported for pistachio and Siirt pistachio-based milk substitutes, with L^* values of 56.62 and 59.22, a^* values of − 2.88 and − 2.47, and b^* values of 8.68 and 11.92, respectively [40]. Similarly, kidney bean-based milk alternatives have been reported to exhibit higher L^* values, negative a^* values, and lower b^* values [41].

Table 3 Color values of hemp-based milk substitutes and spreadable cheese analogs

	L*	a*	b*
Hemp-Based Milk Substitutes			
HW5	64.26 ± 0.03^{a}	-0.51 ± 0.01^{b}	13.92 ± 0.02^{a}
HW10	57.67 ± 0.02^{b}	-1.03 ± 0.02^{a}	9.73 ± 0.01^{b}
HMW5	63.25 ± 0.01^{a}	-0.48 ± 0.01^{b}	13.03 ± 0.00^{a}
HMW10	55.49 ± 0.02^{b}	-1.06 ± 0.02^{a}	9.42 ± 0.01^{b}
Spreadable Cheese Analogs			
SCA1	82.60 ± 0.03^{a}	0.36 ± 0.01^{b}	13.25 ± 0.02^{b}
SCA2	76.15 ± 0.01^{b}	0.91 ± 0.03^{a}	16.82 ± 0.01^{a}

*Different lowercase letters in the same column indicate statistically significant differences within the product groups (p < 0.05). Data are presented as mean ± standard deviation

In the case of spreadable cheese analogs, significantly higher lightness values (76.15–82.60) were achieved compared to the corresponding milk matrices. Among all the formulations, SCA1 exhibited the brightest white appearance. This substantial increase in lightness during the transformation from milk to cheese analog can be explained by the formation of a more compact and dense matrix during the coagulation and heating processes, which significantly enhances light reflectance. Statistically significant differences ($p < 0.05$) were also observed in the a^* and b^* values among the cheese analogs. These color shifts are consistent with previous studies, highlighting the influence of plant-based milk sources on the optical properties of cheese analogs. For example, Oyeyinka et al. [42] reported that cashew milk enriched formulations exhibited higher lightness and yellowness than soy milk based cheese analogs. Similarly, the present findings demonstrate that variations in lightness and yellowness, driven by the hemp-based matrix structure and formulation ratios, confirm the decisive role of raw material composition in the optical and sensory characterization of spreadable cheese analogs. Similarly, the present findings demonstrate that the shifts in lightness and yellowness are driven by the structural arrangement of the hemp-based matrix, confirming that the raw material ratio and the resulting physical structure are the primary determinants of the optical and sensory characterization of spreadable cheese analogs.

Total phenolic content and antioxidant activity

Hemp seeds and their protein extracts exhibit high phenolic content and antioxidant capacity, supporting the strong natural bioactive potential of hemp-based milk substitutes [43, 44]. The total phenolic content (TPC) of the hemp-based milk samples varied significantly depending on both the liquid phase and the seed concentration (Table 4). The TPC values for HW5, HW10, HMW5, and HMW10 ranged from 11.07 to 17.16 mg GAE/mL. Samples prepared with drinking water exhibited significantly higher phenolic contents than those prepared with mineral water. This decrease in HMW samples can be attributed to the high concentration of divalent cations (like Ca^{2+} and Mg^{2+}) in mineral water, which readily chelate with phenolic O- groups to form insoluble metal–phenolic complexes [45, 46]. The formation of these complexes effectively reduces the accessibility of free hydroxyl groups during the Folin-Ciocalteu assay, leading to lower measured TPC values. Consistent with these results, Jemaa et al. [32] reported that hemp milk is a superior source of phenolic compounds, surpassing almond and oat-based alternatives. Antioxidant activity, expressed as percentage inhibition, also varied according to the water type and seed ratio. The highest antioxidant activity was observed in the HW5 sample (32.58%), highlighting that raw material concentration plays a decisive role in determining the bioactive profile.In the spreadable cheese analogs (SCA1 and SCA2), although the TPC values were lower (5.17–6.66 mg GAE/mL) than those of the corresponding milk samples, the antioxidant inhibition capacities reached approximately 40%, which is notably higher than the values observed in the milk formulations. This intriguing shift a decrease in TPC alongside an increase in antioxidant activity reflects the bioactive modifications occurring during the cheesemaking process. The structural transformation of the hemp-protein matrix during heating and coagulation may have released bioactive peptides with high radical scavenging activity [47]. Furthermore, the synergistic effects of added functional ingredients, such as sodium ascorbate and lecithin, likely provided stronger oxidative protection, outweighing the loss of free phenolics. While phenolic compounds were the dominant contributors to antioxidant activity in the milk samples, stronger oxidative protection was achieved in the cheese analogs owing to the synergistic effects of added ingredients. Notable variations observed in the spreadable cheese analogs were consistent with literature reports describing the effects of processing on bioactive components. Moraes Filho et al. [47] reported that during the production of Petit Suisse cheese from black soybeans, both total phenolic content and antioxidant activity partially decreased due to fermentation and processing steps [48]. The present findings demonstrate that while processing steps and mineral interactions influence the phenolic concentration, the hemp-based cheese matrix maintains a competitive and robust bioactive profile compared to other plant-based formulations.

Table 4 Phenolic and antioxidant values of hemp-based milk substitutes and spreadable cheese analogs

	Total phenolic content (mg GAE/ml)	Inhibition capacity (%)
Hemp-based milk substitutes		
HW5	17.16 ± 0.08^a	32.58 ± 0.08^a
HW10	12.67 ± 0.07^b	28.42 ± 0.07^c
HMW5	12.67 ± 0.11^b	30.12 ± 0.11^b
HMW10	11.07 ± 0.06^c	28.67 ± 0.06^c
Spreadable Cheese Analogs		
SCA1	6.66 ± 0.02^a	40.01 ± 0.01^a
SCA2	5.17 ± 0.04^b	38.03 ± 0.03^b

*Different lowercase letters in the same column indicate statistically significant differences within the product groups ($p<0.05$). Data are presented as mean ± standard deviation

Mineral composition of milk substitutes and cheese analogs

The mineral composition of the hemp-based milk substitutes was significantly influenced by both hemp seed concentration and the type of liquid phase (drinking water vs. mineral water) ($p < 0.05$) (Table 5). The use of mineral water resulted in an enriched mineral profile in the HMW series compared to the HW series, particularly through increased concentrations of sodium (Na), magnesium (Mg), and zinc (Zn). Potassium (K) levels reached their highest values (1244.14–1297.90 mg/L) in the 1:5 ratio samples, regardless of the water type, reflecting the contribution of the raw material. This high potassium content is a characteristic feature of hemp seeds, and its retention in the aqueous phase underscores the potential of hemp-based milks to serve as a significant source of intracellular electrolytes.

As expected, the 1:5 formulations (HW5 and HMW5) exhibited higher mineral concentrations across all measured parameters than the 1:10 samples (HW10 and HMW10) owing to the lower dilution factor. Notably, the calcium (Ca) content of the HMW5 sample (398.79 mg/L) was approximately twice that of the HW5 sample (211.81 mg/L). Elevated concentrations of divalent ions, particularly Ca and Mg, are considered to play a decisive role in physical stability by promoting protein aggregation through ion–protein interactions. Specifically, these divalent cations act as bridge-formers between negatively charged protein subunits, leading to the formation of larger, insoluble complexes that overcome the Brownian motion and accelerate gravitational settling [33, 38].

During the transition from milk substitutes to spreadable cheese analogs (SCAs), minerals become more concentrated within the product matrix. The SCA2 sample, produced using mineral water and a 1:5 milk matrix, exhibited the highest mineral content among all samples, with sodium (2267.73 mg/L), potassium (3089.91 mg/L), and calcium (1155.10 mg/L) content. This enhanced mineral retention can be attributed to the ability of the three-dimensional network formed by xanthan gum and locust bean gum to entrap minerals within the matrix. The synergistic interaction between these hydrocolloids creates a dense structural mesh that reduces the mobility of free ions, thereby “locking” them into the final product during the coagulation phase. In addition, the elevated sodium and calcium levels observed in SCA2 likely resulted from the combined effects of the inherent mineral load of the mineral water and incorporation of sodium ascorbate.

The mineral profile of hemp-based products is distinct from that of bovine milk and other plant-based alternatives. While hemp milk has been reported to contain iron levels nearly 70 times higher than those of bovine milk, its native calcium content (12.03–20.25 mg/100 g) is generally lower. In the present study, however, the use of mineral water (HMW5) partially mitigated this limitation by increasing the calcium level to approximately 40 mg/100 mL. Compared with other plant-based beverages, adzuki bean based milks have been reported to contain higher calcium and iron levels than mung bean milks, whereas peanut–oat–chickpea blends may provide up to 74.7 mg/100 mL of calcium and 45.6 mg/100 mL of magnesium content [49]. Furthermore, Vashisht et al. [50] highlighted soy milk as a notable source of magnesium (49 mg/100 g) and potassium (364.29 mg/100 g) content. In this context, while many plant-based substitutes require synthetic fortification to match the mineral density of dairy products, our findings demonstrate that the strategic use of mineral water as a liquid phase provides a natural and effective alternative for mineral enrichment. Consequently, the hemp-based cheese analog (SCA2) demonstrates considerable potential as a mineral-dense functional food, outperforming many plant-based alternatives in terms of potassium and magnesium density.

Rheological properties

Rheological measurements of the hemp-based milk substitutes revealed that the viscosity of all samples decreased with increasing shear rate, indicating typical pseudoplastic (shear-thinning) flow behavior (Fig. 2A). This non-Newtonian flow characteristic is commonly observed in plant-based milk due to the progressive orientation of protein molecules and lipid droplets along the flow field, which reduces the internal resistance of the system. At a shear rate of $50\ s^{-1}$, representing oral shear conditions during consumption, the highest viscosity values were observed for the HMW5 (mineral water, high hemp ratio) and HW5 (drinking water) samples. In contrast, a pronounced decrease in viscosity was detected in the lower concentration (1:10) samples, HMW10 (2.90 mPa·s) and HW10 (2.36 mPa·s), respectively. This reduction is attributed to alterations in

Table 5 Mineral values of hemp-based milk substitutes and cheese analogs

	Na (mg/L)	Mg(mg/L)	K(mg/L)	Ca(mg/L)	Zn(mg/L)
Hemp-based milk substitutes					
HW5	1004.90 ± 0.12^{b}	538.17 ± 0.12^{b}	1244.14 ± 0.12^{a}	211.81 ± 0.24^{b}	1004.90 ± 0.11^{c}
HW10	1075.21 ± 0.21^{b}	323.81 ± 0.23^{d}	730.61 ± 0.09^{b}	182.05 ± 0.13^{c}	1075.21 ± 0.12^{c}
HMW5	1357.20 ± 0.23^{a}	662.36 ± 0.22^{d}	1297.90 ± 0.12^{a}	398.79 ± 0.08^{a}	1357.20 ± 0.21^{a}
HMW10	1298.72 ± 0.13^{a}	425.24 ± 0.31^{c}	692.26 ± 0.11^{b}	387.93 ± 0.07^{a}	1298.73 ± 0.09^{b}
Spreadable Cheese Analogs					
SCA1	1144.54 ± 0.18^{b}	325.98 ± 0.09^{b}	1045.09 ± 0.14^{b}	914.86 ± 0.09^{b}	1144.54 ± 0.13^{b}
SCA2	2267.73 ± 0.21^{a}	426.86 ± 0.23^{a}	3089.91 ± 0.13^{a}	1155.10 ± 0.08^{a}	2267.73 ± 0.27^{a}

*Different lowercase letters in the same column indicate statistically significant differences within the product groups ($p < 0.05$). Data are presented as mean ± standard deviation

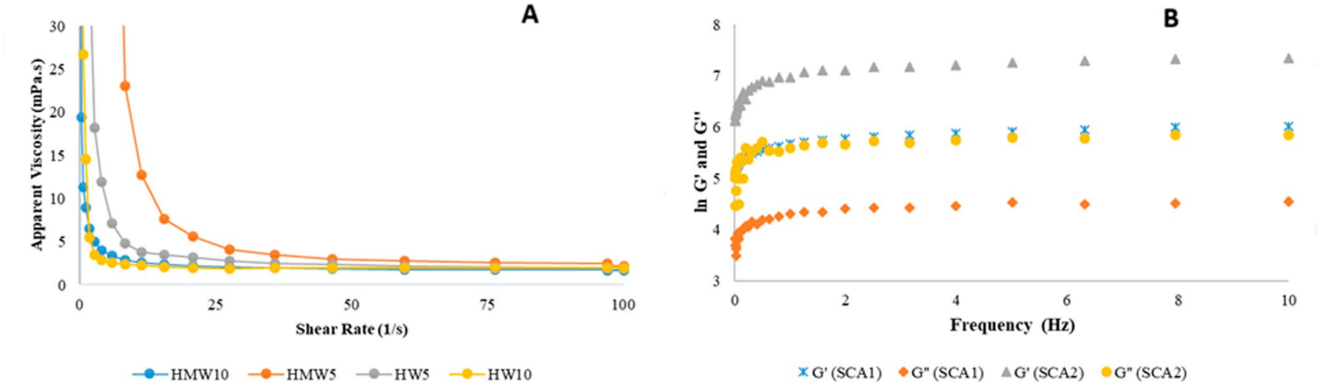


Fig. 2 Rheological properties of hemp-based products: (**A**) steady shear flow curves of milk substitutes and (**B**) dynamic frequency sweep of spreadable cheese analogs

the hydrodynamic structure of proteins and the weakening of protein-protein interactions as the collision frequency between suspended particles diminishes in more diluted systems. Previous studies have highlighted the influence of raw material variety on viscosity, as reported by Tang et al. [51] for red kidney bean system. Unlike some commercial alternatives where thickeners are essential to maintain body, the high viscosity in our 1:5 hemp formulations suggests that hemp proteins possess significant inherent thickening capacity. In the present study the slightly higher stabilization of viscosity observed in mineral water based formulations through modification of the rheological matrix is consistent with the findings of Sánchez-Quezada et al. [52] regarding the effects of additives on plant-based systems. This can be further explained by the presence of divalent ions in mineral water, which slightly increase the effective volume of protein aggregates through hydration and ion-binding, thereby offering greater resistance to flow.

The viscoelastic properties of the spreadable cheese analogs were evaluated over a frequency range of 1–10 Hz. In all samples, the storage modulus (G') exceeded the loss modulus (G'') (G'> G''), confirming the presence of a solid-like, elastic gel matrix rather than a fluid structure (Fig. 2B). Notably, the SCA2 sample produced with mineral water exhibited higher G' values than SCA1, indicating that minerals, particularly calcium and magnesium, enhanced the strength of the protein network, resulting in a more resilient matrix. Mechanistically, these divalent cations act as cross-linking agents that bridge negatively charged carboxylic groups of hemp proteins and hydrocolloids (xanthan and locust bean gum), reinforcing the junction zones of the gel network. Marangoni and Mattice [53] reported similarly high storage modulus values in zein protein based cheese analogs, emphasizing the critical role of protein network formation in textural strength. Moreover, the elevated G' and G'' values reported by Fan et al. [54] for starch based cheese analogs further support the present findings. However, a notable dissimilarity observed in our hemp-based matrix is the higher degree of frequency independence of G', suggesting a more stable and covalently-reinforced elastic structure compared to purely starch-based analogs. This demonstrates that the energy storage capacity of the hemp-based matrix exceeds energy dissipation and confers structural properties comparable to, and in some cases more robust than, those of commercial cheese analogs.

Volatile aroma compounds profile

Aroma plays a decisive role in the sensory perception and consumer acceptance of plant-based cheese analogs. The type and combination of volatile compounds vary depending on the target cheese variety and the desired complexity of the flavor profile; therefore, aroma is a critical research and development focus in plant-based cheese formulation [15]. In the hemp-based cheese analogs (SCA1 and SCA2), 30 volatile aroma compounds belonging to the aldehyde, ketone, and alcohol groups were identified, with peak area percentages exceeding 0.1% (Table 6). This diversity highlights the richness of hemp-based raw materials in terms of volatile aroma constituents. Consistent with these findings, previous studies have reported alcohols, aldehydes, and ketones as the dominant aroma compounds in hemp seed milk based products [55].

In the SCA1 sample, δ-octalactone, hexanal, d-limonene, and 2-heptanone were identified as the primary aroma compounds, and 3-carene, naphthalene, and δ-amylvalerolactone also contributed substantially to the aroma profile. The high abundance of lactones, particularly δ-octalactone and δ-amylvalerolactone, indicates the significant contribution of coconut oil to the overall aroma composition. Lactones impart creamy and milk-like sensory notes, reinforcing cheese-like aroma attributes in the final product. Additionally, the detection of compounds such as octanoic acid, 2-heptanone, and nonanone is noteworthy, as

Table 6 Volatile aroma profiles of hemp-based cheese analogs

Aroma compounds	Retention time (min)	SCA1 (%)	SCA2 (%)
Aldehydes			
Hexanal	13.24	13.77	11.05
Pentanal	9.11	1.21	1.03
Nonanal	28.30	2.06	2.21
Benzaldehyde	33.03	1.22	1.07
Octanal	34.98	-	0.25
Ketones			
2-Heptanone	18.23	6.35	5.39
2-Nonanone	28.10	2.51	2.34
2-Undecanone	37.24	1.39	1.25
Terpenes & Terpenoids			
dL-Limonene	19.39	10.53	9.31
3-Carene	16.90	2.86	2.84
o-Cymene	22.74	2.69	2.98
α-Pinene	10.97	0.84	-
β-Pinene	14.85	-	0.28
Linalool	34.41	-	0.41
Lactones & Acids			
δ-Octalactone	49.02	18.74	-
δ-Amylvalerolactone	54.55	3.67	-
Octanoic acid	50.99	7.44	1.31
Hexanoic acid	45.29	2.91	-
Alcohols & Others			
1-Pentanol	21.50	1.97	1.81
1-Hexanol	26.26	1.40	1.54
(S)-(+)-1,2-Propanediol	35.81	-	8.76
Toluene	11.38	1.31	1.25
Naphthalene	42.19	4.71	-

these volatiles are commonly associated with fresh cheese. Their presence suggests that plant-based analogs can successfully mimic the aroma characteristics typically derived from fatty acid and ketone metabolism in conventional cheeses. Similar observations have been reported for plant-based cheese analogs formulated using different protein and fat sources [56, 57].

In the SCA2 sample, hexanal (11.05%), D-limonene (9.31%), 1,2-propanediol (8.76%), and 2-heptanone (5.39%) were the dominant volatile components. This formulation contains several aldehydes, including hexanal, pentanal, and nonanal, which are commonly associated with "green" and "herbal" aroma notes [58]. Notably, the hexanal content in SCA2 was more pronounced compared to SCA1. This increase can be attributed to the catalytic effect of certain minerals present in the mineral water, which may slightly accelerate the lipoxygenase activity or autoxidation of hemp seed polyunsaturated fatty acids during processing.

Overall, the results demonstrated that cheese-like aroma attributes were successfully established in hemp-based cheese analogs through the presence of lactone and ketone compounds. The additional detection of D-limonene, characterized by its fresh citrus notes, indicates a balanced coexistence of creamy and fresh aroma perceptions. A distinct dissimilarity from traditional dairy cheese is the presence of terpene compounds like 3-carene and D-limonene, which are intrinsic to the hemp botanical profile, providing a unique "botanical-fresh" character that distinguishes hemp-based analogs from soy or nut-based alternatives. The synergistic interactions among these volatile compounds contribute to a complex and sensorially appealing aroma profile.

Sensory analysis

The sensory profiles of the hemp-based milk substitutes and cheese analogs are shown in Fig. 3. No significant differences were observed in the color, general appearance, or homogeneity among the milk substitutes. However, in terms of taste, aroma, and mouthfeel, HW5 prepared with drinking water and a higher hemp ratio received the highest overall acceptability score. The use of mineral water tended to negatively affect the mouthfeel in the liquid matrix, likely due to the increased mineral-induced protein aggregation which can impart a subtle "gritty" or "sandy" sensation on the palate. Sharma et al. [59] reported that high protein content in plant-based beverages can result in "beany" or "chalky" textures, reducing consumer preference. Similarly, the natural aromatic intensity of hemp may be perceived as "foreign" by some panelists compared to conventional bovine milk, as noted by Vashisht et al. [50] for soy and oat milk. Nevertheless, the high HW5 scores indicate that hemp-specific aromas can be successfully balanced through appropriate formulation.

Regarding spreadable cheese analogs, the scores for general appearance and consistency were notably high. The SCA2 sample, prepared with mineral water, received the highest overall acceptability, which was attributed to its more balanced mouthfeel. Interestingly, while mineral ions negatively impacted the liquid milk's texture, they appeared to enhance the spreadability and structural integrity of the cheese analog by reinforcing the gel network. The panelists found the texture of the products to be satisfactory, although the taste and aroma were identified as areas for further improvement. These observations align with those of Falkeisen et al. [60], who emphasized that consumers often find plant-based cheeses visually appealing but perceive lower satisfaction in terms of flavor and aroma compared to traditional dairy cheeses. The textural success of hemp-based cheeses is consistent with the high structural appeal of cashew- and coconut-based yogurts [48]. A notable dissimilarity from previous soy-based cheese studies is that hemp-based SCA2 exhibited a less "beany" aftertaste, which may be due to the unique terpene profile of hemp seeds providing

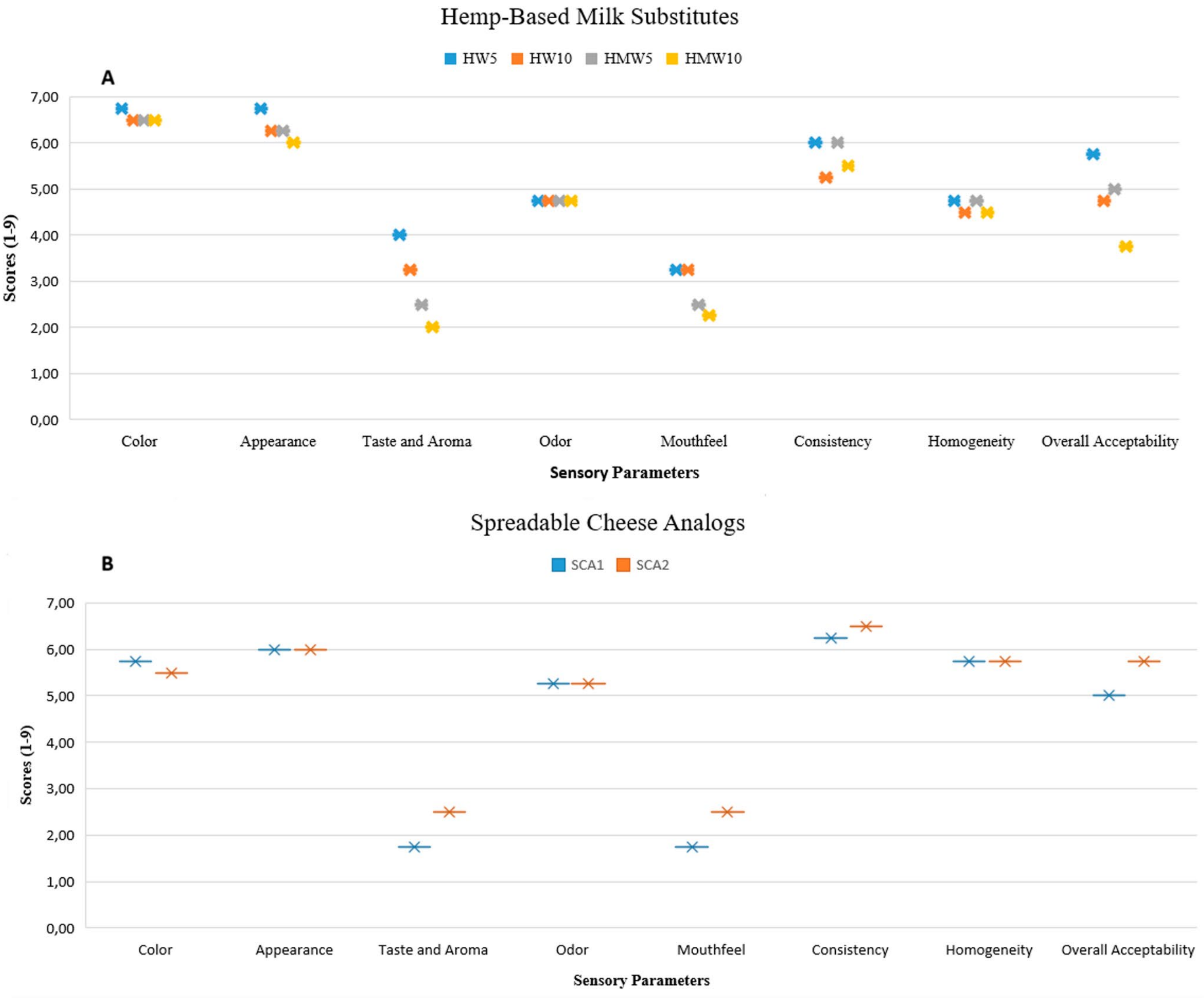


Fig. 3 Sensory profiles of hemp-based products: (**A**) hemp-based milk substitutes and (**B**) spreadable cheese analogs

a fresher sensory note. Overall, hemp-based matrices, particularly mineral-enriched formulations such as SCA2, represent a competitive alternative from both functional and structural perspectives. Furthermore, the challenges identified in taste and aroma perception are consistent with recent reports on underutilized plant protein sources, where flavor masking and sensory optimization have been highlighted as critical requirements for increasing consumer acceptance of novel dairy alternatives [61].

Conclusion

This study demonstrates that hemp seeds, as a sustainable raw material, offer significant nutritional and technological potential for producing plant-based milk substitutes and cheese analogs. Hemp-based milk maintains a favorable lipid profile rich in linoleic (58%) and linolenic (16%) acids, which are crucial for cardiovascular health, while also exhibiting functional food characteristics through high antioxidant capacity. Rheological analysis confirmed that the cheese analogs exhibited dominant elastic behavior (G' > G''), indicating that hemp proteins can form a resilient gel network. The use of mineral water enriched the mineral density of the cheese analogs and optimized their textural balance by stabilizing the "oily mouthfeel" often associated with plant-based products. However, in milk substitutes, mineral water accelerates phase separation due to divalent ion interactions and reduces free antioxidant content through chelation with phenolic compounds. Consequently, a 1:5 (w/v) dilution with drinking water was identified as a more stable formulation for milk substitutes. Volatile compound analysis identified 30 different components, highlighting the synergistic harmony between the creamy notes contributed by lactones and the natural aromatic profile of hemp seeds. This study successfully demonstrated the formulation of hemp-based analogs. Future research should focus on the oxidative stability during storage and bioavailability of bioactive compounds within the complex matrix using advanced models to optimize these products for industrial applications.

Author contributions Conceptualization: TT, MGS; Methodology: TT; Formal analysis: TT; Investigation: TT; Resources: MGS; Writing—original draft preparation: TT, MGS; Writing—review and editing: MGS; Visualization: TT; Supervision: MGS; Project administration: MGS. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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