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EDITED BY
Célia Quintas,
University of Algarve, Portugal

REVIEWED BY
Isabel Ratão,
University of Algarve, Portugal
Sameh Awad,
Alexandria University, Egypt

*CORRESPONDENCE
Zorica Voșgan
✉ zori_v13@yahoo.com

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The influence of different forms of black cumin (*Nigella sativa* L.) on the characteristics of sheep's curd cheese

Zorica Voșgan*, Anca Dumuța, Cristina Mihali, Thomas Dippong, Lucia Mihalescu, Monica Marian and Beatrice Mihalescu

Chemistry - Biology Department, Faculty of Sciences, Technical University of Cluj-Napoca, Baia Mare, Romania

The influence of different forms of *Nigella sativa* (seeds, powder, alcoholic extract, and oil) on the traditional sheep curd was investigated by comparison with a control simple curd considering a series of sensorial, physical–chemical, and microbiological aspects. The analysis was performed on curds freshly prepared and after 10 days of refrigeration. The sensory analysis of the curds was performed using a variety of methods such as scaling method, the method of quality describing, the method of ordering by rank, "triangle" method, and "duo-trio" method. The analyzed curds were assessed and classified according to their sensory characteristics and typicality. The *N. sativa* supplementing of the sheep curds improved their physical–chemical characteristics by raising the fat content with 0.88–2.82% and decreasing the titratable acidity with 1.42–2.32% compared to the control curd without additive. After 10 days of refrigeration, the titratable acidity increased with 1.58–3.25% and moisture decreased (8.43–13.17%). The microbiological quality of the curd samples was influenced by the addition of black cumin in different forms, with variations in the total number of bacteria (TNB) between 4.33 and 4.39 log CFU/g compared to the control sample 5.03 log CFU/g, Enterobacteriaceae (2.53–2.58 log CFU/g) compared to the control sample 2.60 log CFU/g, and coagulase-positive staphylococci (CPS) 2.30–2.68 log CFU/g compared to the control sample 2.75 log CFU/g. After 10 days of refrigeration, the number of microorganisms decreased, TNB (4.13–4.31 log CFU/g), Enterobacteriaceae (2.34–2.53 log CFU/g), and CPS (2.02–2.55 log CFU/g), while for the control sample the values increased. The most obvious antimicrobial effect was observed in the case of the cold-pressed oil addition (1%), followed by the alcoholic extract (1%), seeds (3%), and powder (3%).

KEYWORDS

black cumin, sheep curd, sensory quality, physical–chemical changes, antimicrobial activity

1 Introduction

Black cumin (*Nigella sativa* L.) is a medicinal food plant belonging to the *Ranunculaceae* family recognized for its active components which are mainly concentrated in the essential oil of the seeds. The *N. sativa* seed is black or dark brown and has a distinct angular or funnel shape, with a slightly bitter, nutty-peppery taste and a strong aroma

(Tanwar and Goyal, 2021). Numerous studies have shown that the therapeutic properties of *N. sativa* are mainly due to thymoquinone, a major biologically active constituent of the essential oil, together with other high value components such as various alkaloids (nigellicine, nigellimine, and nigellidine), volatile oils (carvone, d-limonene, and cymene), fatty acids and higher terpenoids, phenolic acids and flavonoids, saponins, aliphatic alcohols, steroids, tannin, resin, protein, sugars, and vitamins A1, A2, B1, and B2 (Morikawa et al., 2004; Paarakh, 2010; Khader and Eckl, 2014; Dubey et al., 2016; Mukhtar et al., 2019; Burdock, 2022; Dalli et al., 2022). Most of these compounds disrupt the microorganisms cell membrane and inhibit cell division and the biofilm formation (Bhatti et al., 2022), being an attractive solution for the food industry in the fight against the microbial contamination (Friedlander et al., 2019).

N. sativa has been shown to possess a wide spectrum of activities, in addition to its use as a food ingredient, presenting a series of pharmacological actions extensively investigated in the last decades. It is considered a miraculous plant, which can cure several illnesses and disorders having antihypertensive, antidiabetic, anticancer and immunomodulatory, antimicrobial, antiviral, anthelmintic, analgesic, anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective, and antioxidant properties (Ahmad et al., 2013, 2021; Dalli et al., 2022). In addition to the studies on the pharmacological effects of *N. sativa* seeds and the possible relationship with their constituents, investigations that emphasized their antimicrobial effect using different extracts were also carried out (Bakathir and Abbas, 2011). Thus, several studies have shown the possibility of the antibacterial and antifungal activity of these seeds by using their extracts or oils (Hanafy and Hatem, 1991; Morsi, 2000; Roy et al., 2006; Salman et al., 2008; Mohammed et al., 2019; Yimer et al., 2019; Shafodino et al., 2022). By studying the antimicrobial activity of some plant seeds against bacterial strains that cause food poisoning, a high potential activity of *N. sativa* seed powder was highlighted (Abu-Zaid et al., 2022).

The antimicrobial effects of *N. sativa* have been studied both *in vivo* and *in vitro* (Khan et al., 2003; Singh et al., 2005; Niakan et al., 2006; Hasan et al., 2013; Mahgoub et al., 2013; Forouzanfar et al., 2014; Rafati et al., 2014; Bakal et al., 2017) against the infections with staphylococcus, fungi, and other microorganisms such as *Escherichia coli, Listeria monocytogenes, Salmonella Enteritidis, Salmonella typhi, Shigella* spp., *Vibrio cholera, Streptococcus pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis,* and *Proteus vulgaris*. According to the results of Ogen-Shtern et al. (2021), *N. sativa* oil can be used as a safe alternative antimicrobial agent and perhaps even as a preventive caring method to maintain the balance and diversity of the microbiome. This provides new opportunities to discover alternative treatment formulas in the case of antibiotic-resistant pathogenic bacterial strains. On the other hand, the antimicrobial properties of natural products can underlie many applications, such as the preservation of raw and processed foods. This has been proven by the incorporation of alcoholic extracts in various food products (cheese, bread, and meat) (Muzolf-Panek and Gliszczyńska-Świgło, 2022) with good results regarding the antimicrobial and antioxidant effect (Burits and Bucar, 2000; Lutterodt et al., 2010; Puvača et al., 2020; Rahman et al., 2021). Although black seed extract or oil is known to possess antimicrobial activity, its full potential as an antimicrobial agent has not been fully exploited (Hasan et al., 2013) and the interactions with the natural microbial flora of complex food substrates are not completely explored (Georgescu et al., 2018b).

Also, plant essential oils could be used as flavorings in food, in addition to their role in increasing the shelf life of food products, due to their active constituents (Khorshidian et al., 2018). Black cumin seeds were and are still used as a flavoring additive for seasoning cheeses or in bread preparation due to their pleasant and slightly spicy taste (Burdock, 2022). According to the data obtained from the olfactometric analysis, the characteristic odor of the hydroalcoholic extracts of black cumin seeds was dominated by the shades of butter, cheese, and balsamic and spicy (Kesen et al., 2018). The sensory attributes are among the most important factors for the analysis of a food product, because the first contact of the consumer with the product is mediated through its sensory properties and, thus, they play a highly important role in the customer's decision to select a product. The sensory impressions of the tasters require advanced discriminative and descriptive abilities that have been quantified statistically in previous studies (Dippong, 2017). While consumers awareness of safe food consumption has increased, natural additives have become the best choice for preserving and improving the quality of food products instead of the chemical and synthetic ones. The antimicrobial activity of *N. sativa* oil has been studied, but the available data on its effectiveness on food products of animal origin (e.g. sheep curd) are limited (Abdel-Latif et al., 2021). However, there are attempts to use seeds or seed oil during the processing and preservation of various food products (e.g., cheese) to improve their stability and microbial safety (El-Sayed et al., 1994; Tarakci et al., 2005; Kokoska et al., 2008).

Curd is a traditional Romanian fresh cheese obtained by enzymatic coagulation of fresh sheep milk. It is the final product of several consecutive operations that begin by adding the rennet to milk, cutting the milk clot, draining the whey, shaping, and other operations (Derra et al., 2018) so that in the end, a product with specific sensory and nutritional characteristics is obtained (Nitu et al., 2021). In the cheeses case, flavor development is due to several complex microbial and biochemical activities that take place during processing and ripening and has a key role in the consumer choice (Hayaloglu and Karabulut, 2013). Cakir et al. (2016) hypothesize that spices addition such as black cumin could influence the proteolysis, sensory properties, and volatile compounds of the cheese.

Fresh cheese is considered one of the most traded cheeses worldwide; however, being characterized by a higher moisture percent and a lower salt content makes this type of cheese very susceptible to rapid deterioration through microbial alteration that shortens its shelf life, as well as to contamination with various pathogenic microorganisms harmful for human consumption (Oliveira et al., 2016).

Therefore, the aim of this study was to investigate the effects of seeds, powder, alcoholic extract, and oil of *N. sativa* on the traditional sheep curd which is a fresh cheese easily alterable. The objectives of this study were as follows: (I) to study the influence of the *N. sativa* seeds, powder, extract, and oil addition on the traditional sheep curd acceptability of the consumers; (II) to study the changes of the physical–chemical curd characteristics as a result of *N. sativa* addition in different forms compared to the control curd both fresh and after the refrigeration period; (III) to study the effect of different forms of *N. sativa* on the growth and survival of total bacteria, enterobacteria, and coagulase-positive staphylococci in the sheep curds fresh and after a period of 10-days of refrigeration compared to the control curd.

2 Materials and methods

2.1 *Nigella sativa* seeds (black cumin) and the preparation of the extract/oil

Nigella sativa seeds were purchased from specialized natural pharmacies; packaged by Solaris Plant S.R.L, Bucharest, Romania; country of origin: Turkey. A part was grounded with a grinder, and the black cumin powder was obtained. The Velp solvent extractor was used to obtain the black cumin extract, as follows: 5 g of ground *N. sativa* seeds and 60 mL of ethyl alcohol were introduced in the extractor cylinders. The extractor was programmed for 180 min at the extraction temperature of 200°C (Nicula et al., 2012). In this way, the black cumin alcoholic extract was obtained. To produce the oil, the *N. sativa* seeds were mechanically pressed, without any heating treatment. The crushed seeds were kept overnight at room temperature (20°C) to separate the oil phase from the fibers, and then, the oil was filtered using the Whatman filter paper and a glass funnel (Mohammed et al., 2016).

2.2 Preparation of the traditional sheep curd

The curd was obtained using local sheep milk from the Maramures region, in the North-West of Romania. The cheese was made immediately after the milking process (homemade) without any heat treatment, so this traditional obtaining method limits a lot of hygiene measures during the processing of the final product. A total of 10 liters of freshly milked milk, filtered through the cheesecloth, were used to prepare the curd. The milk was heated to 35°C (Figure 1A) and manually mixed with 25 mL natural rennet (8 g rennet from lamb's stomach in 250 mL warm water) (Georgescu and Raita, 2019). After dispersing the rennet in the whole milk mass, the mixture was left at room temperature (25°C) for 30–40 min for coagulation. When the milk clot was obtained, it was cut into four parts and left to rest for approximately 10 min. The clot must be shaken well from top to bottom until it is well crushed (Figure 1B). The curd started slowly to form, by lifting the clot from the bottom-up and releasing the whey. The curd was formed when the clot has turned into a well-bound mass and the resulting whey no longer contained cheese particles (Figure 1C).

After the curd formation and the whey draining using the cheesecloth filtration method, it was divided into five parts, one of which was kept as a control curd without *N. sativa* addition, while the plant elements (seeds, powder, alcoholic extract, and oil) were incorporated into the other parts. The fresh obtained curds, in three repetitions, were left to further solidification for 6 h to receive the specific shape of the traditional Romanian curd (Figure 2).

2.3 Sample description

The experiment was based on the use of five types of curds (Figure 3), one of which served as a control curd (without addition), and the others were enriched with different forms of black cumin.

The presentation of each type of curd and their assigned symbolization were as follows:

- Sheep curd (control sample) – C1.
- Sheep curd with *N. sativa* seeds (3% w/w) – C2.
- Sheep curd with *N. sativa* seed powder (3% w/w) – C3.
- Sheep curd with alcoholic extract from *N. sativa* seeds (1% w/w) – C4.
- Sheep curd with cold-pressed oil from *N. sativa* seeds (1% w/w) – C5.

The choice of the percentage value for black cumin additions was made based on the scientific documentation from other studies related to the testing of cheese (Alsawaf and Alnaemi, 2010; Abdel-Latif et al., 2021). For each experimental curd, the evaluation of the sensory, physical–chemical, and microbiological quality was carried out according to the described methodology both after processing as well as after 10 days of storage under refrigeration conditions (4°C). This time interval of keeping the curds in the refrigerator was chosen based on the consideration that it is the maximum period during which the product can be consumed without being depreciated.

2.4 Sensory analysis

2.4.1 Scaling method

The sensory analysis of the curd samples was performed according to the scoring quality assessment method (Dippong et al., 2014;

FIGURE 1
Stages of the traditional sheep curd preparation by heating the milk **(A)**, crushing the clot **(B)**, forming the curd, and releasing the whey **(C)**.

FIGURE 2
Curd filtration through the cheesecloths.

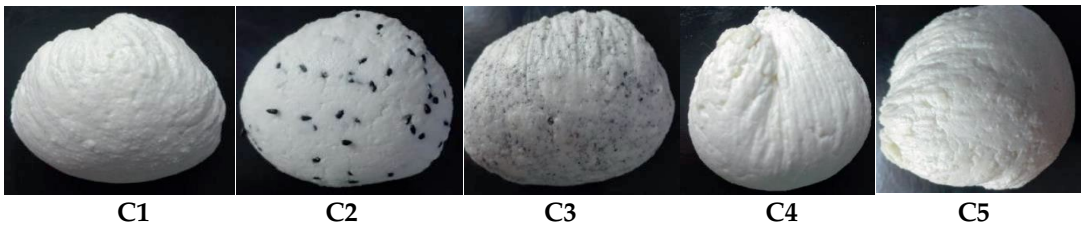

FIGURE 3
Final products subjected to the experimental analyses: C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%).

Dumuta and Vosgan, 2015). The analysis was carried out by a group of 10 taster volunteers from the Chemistry-Biology Department of the Technical University of Cluj-Napoca, Romania, five women and five men aged between 35 and 50 years. The tasters examined the characteristics of the sheep curds by comparison with the scoring scale of 20 cumulated points for fresh cheese according to the sensory analysis standards (Dippong et al., 2014). The curds were evaluated for appearance and shape, consistency, color, smell and taste after production, as well as after 10 days of refrigeration, using scores ranging from 0 (very poor) to 5 (excellent). Samples refrigerated at 4°C were allowed to equilibrate at room temperature for 30 min before tasting.

2.4.2 The paired sample method

In this method, samples are presented to the taster in pairs to be compared based on a defined characteristic; tasters are given one or more pairs of coded samples (A and B) that are presented to them in a random order or at random. The two samples of each pair can be identical or different, and the examiner must indicate whether the samples are similar or different based on one or more specified characteristics (Dippong, 2017). The method of paired samples is applied to distinguish the smallest sensory differences that can exist for a sensory characteristic between the two samples of the pair, to establish the difference in intensity between two samples (sweeter or less sweet, more bitter, or more slightly bitter, etc.), respectively, to establish the consumer's preference towards one of the two samples (Dippong, 2017). The method is used in product quality control to determine if a change in the technological process (change in raw material, addition of additives, and use of new equipment) is accompanied by detectable changes in sensory characteristics. The paired sample method is frequently used in the testing and selection of tasters (Dippong, 2017).

2.4.3 The duo-trio method

This method consists in receiving the information from the tasters regarding the set of samples examined by them, and among these, one is a reference sample, and the other is paired with the reference one (Dippong, 2017). Tasters must determine which of the two coded samples is paired with the reference sample. The tasters are informed that they are given a set of samples which include a reference sample and two coded samples, one of which is paired with the reference test (Dippong, 2017). Additionally, they are informed that the tasting starts from the left, and that the sample located on the left-hand part is the reference one. From the randomly received combination set, the taster must determine which of the two encoded samples is paired with the reference one, which is noted with an X (Dippong, 2017). Tasters register the results in a form in which there is the following information "One of the two other samples is the same as the reference, and the other is different." The purpose of this method is the identification of the sample, by combining the five types of curds (C1–C5) after the preparation and the five curds (C1–C5) after 10 days of refrigeration for the 10 tasters.

2.4.4 The triangle method

The triangle method consists of the distribution, to each taster, of three coded samples that were arranged in all the possible combinations. Tasters receive the information and instruction: Two of the samples are identical and one is different, and they should select

the unpaired sample (Dippong, 2017). A total of 10 tasters performed this task, each of them receiving a total of three coded samples, which were arranged into six possible combinations: ABB, BAA, AAB, BBA, ABA, and BAB. The tasters were informed that two samples are identical, and one is different; the examination must be done from left to right and that they should select the unpaired sample (Dippong, 2017). Tasters will complete a form with the identified or unidentified combination of samples. The triangle method has a limited use in the sensory assessing of curd because tasters might experience sensory fatigue (Dippong, 2017). The purpose of this method is the identification of the sample, by combining the five types of curds (C1–C5) after the preparation and the five curds (C1–C5) after 10 days of refrigeration.

2.4.5 The ordering by rank method

This sensory method consists in receiving of the coded samples by the testers in a previously established sequence (Dippong, 2017). The ordering by rank method can be applied to the five types of curds (C1–C5) after the preparation and the five curds (C1–C5) after 10 days of refrigeration, as follows: A series of samples is presented to classify them according to a certain sensory characteristic (spicy to pepper, in this case). This test by itself does not show the magnitude of the difference that can exist between samples. Tasters receive the coded samples simultaneously, in a particular order and arrange them according to the considered criterion (Dippong, 2017).

2.4.6 The method of describing the quality

This method is comprised of a systematic description of the flavor of tested samples and the assessment of the intensity of this attribute using grades. First, the tasters agree on the used attributes, and, for each attribute (10–40), a scale is used, where possible, following the reference standards (Dippong, 2017). After testing the curd sample, the tasters give marks to quantify the attribute intensity; the results are then used to compute the sensory profile of the sample (Dippong, 2017). The flavor profiles of two or more samples can be compared by using mathematical statistics.

The sensory tests performed in the current study for all five curds were made with five repetitions in similar humidity and temperature conditions.

2.5 Physical–chemical analysis

From the physical–chemical point of view, the samples were analyzed for acidity, moisture, and fat content in dry matter. In total, 10 g of each curd and 30–50 mL of distilled water were used to determine the titratable acidity and homogenized until a fine suspension was obtained. This suspension was transferred quantitatively into an Erlenmeyer vessel, and 1 mL of phenolphthalein (2%) was added; then, the mixture was titrated with 0.1 N sodium hydroxide solution, until the pink color appeared and persisted for 1 min. The titratable acidity was expressed as % lactic acid which is the main acid in curd samples in mass percentage (Dumuta and Vosgan, 2015). The moisture content was measured using the oven-drying method at 105°C (Dumuta and Vosgan, 2015).

To determine the fat content, 3 g of the well-shredded cheese sample and sulfuric acid were introduced into the butyrometer glass. After the complete dissolution of the proteins in the water bath at 65–70°C (for 30 min), 1 mL of isoamyl alcohol was introduced, stirred for 3 s, and completed with sulfuric acid. The butyrometer was inserted again into the warm water bath at 65 ± 2°C (5 min), then shaken, and centrifuged for 10 min at 1000–1200 rotations/min. After centrifugation, the butyrometer was inserted again into the water bath, at 65–67°C, for 5 min, and then, the number of divisions occupied by the fat column was read on the butyrometer column.

The curd fat content, related to the dry substance and expressed in percentage, was determined with the formula:

$$\%G = \frac{G}{100 - A} \times 100$$

G = the fat content, determined with the butyrometer (%); A = the water content of the product (ISO 3432:2008, 2008; Dumuta and Vosgan, 2015).

2.6 Microbiological analysis

From the microbiological point of view, the total number of bacteria (TNB) was determined according to the method of successive dilutions, whose principle is based on the counting of living aerobic mesophilic germs capable of developing on the culture medium, at temperatures of 30–37°C (ISO 4833-2:2013/AMD 2022, 2022). In total, 10 g of each curd type was first homogenized in 90 mL of sterile saline solution (0.85%) for 60 min. Later, the appropriate dilutions were carried out up to 10^{-5} and the inoculation took place in Petri dishes on a Plate Count Agar medium. These microorganisms were cultured at a temperature of 30°C for 72 h. In addition, curd samples were processed according to ISO 21528-2:2017 (2017) to determine the *Enterobacteriaceae* colony counts. They were isolated on the Violet Red Bile Agar with Glucose (VRBG) medium at 37°C for 24 h. The international standard method ISO 6888-1:2021 (2021) was used to carry out the bacteriological analyses regarding the presence of *Staphylococcus aureus* and other species (coagulase-positive staphylococci, CPS). The inoculum was transferred to Petri dishes with Baird-Parker agar and incubated for 24–48 h at 37°C. After 48 h of incubation, the colonies suspected of *S. aureus* were subjected to the coagulase test through an agglutination reaction using rabbit plasma. After incubation under the specified conditions, the bacterial colonies were counted, and the average values obtained were converted into number of colony-forming units (CFU) per 1 g of product.

2.7 Statistical analysis

The results of the sensory, physico-chemical, and microbiological evaluation of the traditional curds with *N. sativa* addition were processed in Excel (Microsoft Office 2021, Microsoft Corporation, Washington, USA) and by using Statgraphic CS Technologies software (Centurion 19, 2023, Virginia, USA). Statistically significant differences in the sensory, physical–chemical, and microbiological results were tested by Fisher's least significant difference (LSD) test. The analysis of variance was applied testing the effect of additives on the sensory, physical–chemical, and microbiological characteristics for the five fresh prepared curds. The effect of 10-days storage on the sensory, physical–chemical, and microbiological characteristics of

each curd sample was also statistically tested. Logarithmic transformations were applied for all values obtained in the case of the microbiological analysis. The mean values ± standard deviation of the microbial tests was given in log CFU/g. All determinations were made with 5 multiples for reproducibility testing.

3 Results and discussion

3.1 Sensory analysis

The sensory evaluation of the curds is necessary to determine the relative merits of the manufacturing procedures and the influence exerted by the composition on specific sensory characteristics, as well as to assess the consumer's acceptability.

3.1.1 The scaling method

The scaling method was employed to achieve tests aiming to quantify the sensory characteristics (appearance, consistency, smell, and taste) of the five types of curd (C1, C2, C3, C4, and C5). The mean ± standard deviation for the curds samples shows variable values depending on the assortment (control sample, curd with seeds, powder, alcoholic extract, and oil) and the testing period, relative to control sample (Table 1). The least differences between the samples were observed in terms of appearance, respectively, consistency both after preparation and after 10 days of refrigeration storage. The greatest variability was recorded in the typicality of taste and smell. The highest average scores were recorded for the control curd and the one with seed addition, being the most appreciated by the tasters. Lower scores were recorded in terms of taste and smell for the samples with the addition of powder, alcoholic extract, and oil compared to the control sample (Table 1).

In the second testing stage, 10 days after refrigeration, the average values calculated for all parameters were slightly reduced (Table 1). Sensory changes regarding the taste and smell of the curd samples were observed, being depreciated compared to the previous testing stage. The most obvious sensory change of all parameters was observed for the curd with addition of black cumin powder, followed by the curd with alcoholic extract and oil, especially for taste and smell. Considering the total score, the tasters gave the highest score to the control curd (C1) and the lowest score to the curd with oil (C5), when the fresh samples were analyzed, while to the refrigerated samples for 10 days, the highest score was registered for the curd with seeds (C2) and the lowest score for the curd with powder (C3). Thus, tasters' preferences for fresh products were C1 > C2 > C4 > C3 > C5, and after 10 days of refrigeration, the order of tasters' preferences was C2 > C1 > C4 > C5 > C3. According to the qualifications, only fresh curds C1 and C2 fall into the category of very good products, and the others fall into the category of good products. In the case of products after 10 days of refrigeration, they all fall into the category of good products.

Considering the general evaluation of the curd assortments both in the first testing stage (after preparation) and after 10 days of refrigeration, the highest scores were recorded for the control curd and the curd with *N. sativa* seeds, while the lowest scores were for the curd samples with powder, alcoholic extract, and oil.

The *N. sativa* seeds have been used for years as a spice and food preservative (Hassanien et al., 2015) because they are less spicy, contributing to the taste improvement of the food products (Ramadan, 2007). This fact was also observed for the results obtained after the sensory evaluation of the curd with seed addition. The other powder, alcoholic extract, and oil forms added to the food product do not have such a high degree of acceptability. There were differences compared to the control for the curds with alcoholic extract and black cumin oil addition regarding the taste and smell values, a situation also presented by Abdel-Latif et al. (2021) which showed that a high concentration, from 1 to 3%, was not accepted by the panel members during the cheese's storage period. This was also presented by Georgescu et al. (2018a) who found that higher levels of black cumin oil concentration could cause unwanted changes for color and taste of the final product. It is considered that for the consumer acceptability of the cheese, the percentage of the added oil in the product should not exceed 0.6% (Abd Elmontaleb et al., 2020). The consistency values of the analyzed curds decreased after the storage period (10 days), especially for those with powder, alcoholic extract, and *N. sativa* oil; this aspect is due to the overconcentration of the different black cumin forms added to the product (Abdel-Latif et al., 2021).

3.1.2 Method of paired samples

The method of paired samples was applied to all five types of curds (C1–C5) after preparation and refrigeration for 10 days. For the 10 tasters (T1–T10), there were prepared 25 pairs of coded samples, and without knowing the coding, they identified correctly ($\surd$) or incorrectly (x) the similar or different reference samples in each pair, using only the taste analyzers, olfactory, visual, and tactile. This method was carried out to observe differences and sensory similarities between the samples of freshly prepared curds and after refrigeration, but on the other hand, the efficiency of the tasters was also tested. A total of five successive

TABLE 1 Sensory analysis by scaling method of C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%), after the preparation and after 10 days of refrigeration.

Sensory attribute	Fresh prepared*					After 10 days of refrigeration*				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
Appearance	4.8 ± 0.5^{a}	4.4 ± 0.5^{a}	3.7 ± 0.4^{a}	$4.6 \pm 0.5^{a,b}$	4.2 ± 0.4^{a}	$4.5 \pm 0.4^{a,b}$	4.1 ± 0.4^{a}	$3.5 \pm 0.3^{a,b}$	4.3 ± 0.4^{a}	$4.0 \pm 0.3^{a,b}$
Consistency	$4.5 \pm 0.4^{a,b}$	$4.6 \pm 0.4^{a,b}$	$4.6 \pm 0.5^{a,c}$	4.4 ± 0.4^{a}	4.3 ± 0.4^{a}	$4.4 \pm 0.5^{a,b}$	$4.5 \pm 0.5^{a,b}$	4.2 ± 0.5^{a}	$4.3 \pm 0.4^{a,b}$	4.1 ± 0.4^{c}
Smell	4.9 ± 0.5^{a}	$4.7 \pm 0.6^{b,c}$	$4.5 \pm 0.5^{a,b}$	$4.4 \pm 0.3^{a,c}$	3.8 ± 0.3^{b}	4.1 ± 0.4^{a}	$4.4 \pm 0.4^{a,b}$	$4.0 \pm 0.4^{a,b}$	$3.8 \pm 0.3^{a,c}$	$3.7 \pm 0.3^{a,b}$
Taste	$4.8 \pm 0.6^{a,b}$	4.9 ± 0.5^{a}	$4.5 \pm 0.4^{a,b}$	$4.2 \pm 0.3^{a,b}$	$3.9 \pm 0.2^{a,b}$	$4.4 \pm 0.5^{a,c}$	$4.6 \pm 0.5^{a,c}$	$3.4 \pm 0.3^{a,b}$	3.9 ± 0.2^{a}	3.6 ± 0.2^{a}
Total	$19.0 \pm 0.5^{a,b}$	$18.6 \pm 0.5^{a,b}$	$17.3 \pm 0.5^{a,b}$	$17.6 \pm 0.4^{a,b}$	$16.2 \pm 0.4^{a,b}$	$17.4 \pm 0.4^{a,b}$	$17.6 \pm 0.4^{a,b}$	$15.1 \pm 0.4^{a,b}$	$16.3 \pm 0.3^{a,b}$	$15.4 \pm 0.3^{a,b}$

*Values are mean ± standard deviation ($n = 5$).
Different letters $^{a, b, c}$ indicate statistically significant differences for Fisher's LSD test and $p < 0.05$.

repetitions were carried out, for all 10 tasters on the 25 pairs, and the majority answers from the five testers are presented in Table 2. The method of paired samples showed that, from a sensory point of view, sample C4 is like C5 both after preparation and 10 days after refrigeration. This can be seen in Table 2 by the failure to identify the five varieties.

The T1 taster managed to identify all pairs of curds both after fresh prepared and after 10 days of refrigeration. Tester 4 identified all pairs in the samples after preparation but failed to identify three pairs of the refrigerated samples after 10 days. In total, taster T2 failed to identify many pairs of freshly prepared curd samples, and tasters T2, T6, and T10 did not identify many pairs after refrigeration for 10 days. It can be concluded that the sensory analysis of the paired samples showed the similarities between the five samples and the decrease in sensory quality after refrigeration.

3.1.3 Duo-trio method

The duo-trio method was applied to all five samples of curds (C1–C5) after preparation and refrigeration for 10 days. For the 10 tasters (T1–T10), 20 configurations of coded curds were prepared, and without knowing the coding, they identified correctly ($\surd$) or incorrectly (x) the similar or different five samples in each configuration, using only the taste analyzers, olfactory, visual, and tactile. A total of five successive repetitions were carried out, for all 10 tasters on the 20 different configurations, and the majority answers from the five testers are entered in Table 3. The duo-trio method showed that, from a sensory point of view, samples C4 and C5 are similar after preparation but also after 10 days of refrigeration. In addition, the C2 and C3 samples likened after the 10-days storage period, and the tasters had a harder time differentiating these samples. Overall, tasters T6 and T10 failed to identify most pairs of freshly prepared curd samples, and tasters T2, T5, T6, and T10 did not identify most pairs after refrigeration for 10 days.

The duo-trio method has the advantage that the reference sample is presented without creating confusion. The disadvantage consists of the fact that the test is ineffective when the product leaves a pronounced aftertaste in the mouth.

3.1.4 Triangle method

The 10 tasters performed the triangle method for the five fresh prepared curds and the five refrigerated curds. The samples were coded with A and B, two samples were identical and one different, resulting in 20 different configurations grouped into six categories of three samples each (ABB, BAA, AAB, BBA, ABA, and BAB). With $\surd$, they marked the recognized identical curds samples from the group of 3, and the wrong answers were marked with x. The examination was carried out from left to right. After processing the individual data from each taster, according to the method of paired and duo-trio samples, the majority positive ($\surd$) or negative (x) answers were centralized in Table 4. The testing was repeated five times by every taster. It can also be observed in this case that C1 control curd and C2 curd with seeds, respectively, C3 with powder were very different from a sensory point of view both at the time of preparation and after 10 days of refrigeration. At the opposite pole, C4 and C5 were very similar, and the tasters did not manage to delimit them even by the triangle method. Instead, C3 was well separated from C4 and C5 by most tasters. It was also found that even with the triangle method, the curd samples were more difficult to identify than after refrigeration for 10 days.

In the case of the triangle method, the analysis of the results is based on the comparison of the number of correct identifications with the number of correct answers that would be obtained according to probability theory when there are no differences between samples (Dippong, 2017). Tasters will complete a form with the identified or unidentified combination of samples. The triangle method has a limited use in the sensory assessing of the curd because tasters might experience sensory fatigue (Dippong, 2017).

3.1.5 The ordering by rank method

This ordering by rank method by itself does not show the magnitude of the difference that can exist between samples, based on certain spicy sensory characteristic. Each subject evaluated the coded and placed samples in a set order and then classified them in the order of the evaluated spicy sensory characteristic increasing, and the results are listed in Table 5. Tasters receive the coded curds simultaneously, in a particular order and arranged them according to the considered criterion. The intensity of the curds spicy was investigated through the ordering by rank method. After processing data, it was found that the curd with oil (C5) had the strongest flavor, fatty, and corresponded to a high-quality curd, after preparation and after 10-days refrigeration. Interestingly, control curd (C1), was very weak in terms of spicy, while in the score scale method, it was declared as the best from the sensory point of view; on the other hand, the results about the very strong spicy curd with oil (C5) were consistent across another four methods, retaining its very weak character. The ranking method is faster, easier to perform, and cheaper, especially when the number of samples is small and when a specific sensory characteristic of the products is of particular interest (Dippong, 2017).

3.1.6 The simple descriptive method

The simple descriptive method of describing the quality of curds combined with the method of establishing the aroma profile had the aim of achieving a systematic description of the aroma that is tested to evaluate the quality control or for the training and improvement of the tasters. The method was applied to a group of five curd samples that were compared by 10 tasters. The tasters agreed on the examined attributes and used a 5-point scale, with the help of reference standards, and then, the results were used to create the sensory profile of the sample (Dippong, 2017). The results of individual tasters for the method of flavor profiling are summarized in a final report (Table 6), where there are no significant discrepancies between tasters. In this case, fresh prepared, control curd (C1) obtained the highest scores and curd with oil (C5) the lowest ones. Similarly, after 10 days of refrigeration, curd with seeds (C2) obtained the highest scores and curd with powder (C3) the lowest ones.

Thus, fresh prepared control curd (of high quality) had the most intense flavor and corresponded to the curd type. After 10 days of refrigeration, the flavor of the curds was formed due to maturation, and the curd with seeds was considered of high quality. Overall, our combination of the scaling sensory analyses with sorting methods (triangle method and duo-trio method) was powerful as these methods were complementary to each other and allowed us to uncover additional information about sensory properties of curds and their typicality.

3.2 Physical–chemical properties

Curd samples were analyzed both fresh and after a period of refrigeration storage (10 days) regarding titratable acidity (% lactic acid), moisture (%), and fat in dry matter (%). The results of the physical–chemical analysis are presented in Table 7.

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TABLE 2 Sensory analysis by paired sample method of C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%), fresh prepared and after 10 days of refrigeration.

Sample	Paired	Identifying combinations for fresh prepared										Identifying combinations after 10 days of refrigeration										
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Majority	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Majority
C1	C1-C1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	C1-C2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√	√
	C1-C3	√	√	x	√	√	√	√	√	√	√	√	√	x	x	√	√	√	√	√	√	x	√
	C1-C4	√	x	√	√	√	√	√	√	√	√	√	√	x	√	√	√	x	√	√	√	x	√
	C1-C5	√	x	√	√	√	x	√	√	√	√	√	√	x	√	√	√	x	√	√	√	x	√
C2	C2-C1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√	√
	C2-C2	√	√	√	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√	√	√	√
	C2-C3	√	√	√	√	√	√	√	x	√	√	√	√	√	x	√	√	x	√	√	x	x	√
	C2-C4	√	√	x	√	√	√	√	√	√	√	√	√	x	√	√	√	x	√	√	√	x	√
	C2-C5	√	x	√	√	√	√	√	x	√	√	√	√	x	√	√	√	x	√	√	√	x	√
C3	C3-C1	√	√	x	√	√	√	√	√	√	√	√	√	x	x	√	√	√	√	√	x	√	√
	C3-C2	√	√	√	√	√	x	√	√	√	√	√	√	√	x	√	x	x	√	√	√	x	√
	C3-C3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	x	√	√
	C3-C4	√	x	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	x	√	√	√	√
	C3-C5	√	x	√	√	√	x	√	√	√	√	√	√	x	x	√	√	x	√	x	√	√	√
C4	C4-C1	√	x	√	√	√	√	√	√	√	x	√	√	x	√	√	√	x	√	√	√	x	√
	C4-C2	√	√	x	√	√	√	x	√	√	√	√	√	√	x	√	√	√	x	√	√	x	√
	C4-C3	√	x	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	x	√	√	√	√
	C4-C4	√	x	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√	√	√	√	√
	C4-C5	√	x	x	√	√	x	x	√	x	x	**x**	√	x	√	x	x	x	√	x	√	x	**x**
C5	C5-C1	√	x	√	√	√	√	√	√	√	√	√	√	√	√	√	x	x	√	√	√	x	√
	C5-C2	√	x	√	√	√	√	√	x	√	√	√	√	x	√	x	√	x	√	√	√	x	√
	C5-C3	√	x	√	√	√	x	√	√	√	x	√	√	x	√	√	x	x	√	√	√	x	√
	C5-C4	√	x	x	√	x	x	√	x	√	x	**x**	√	x	√	x	√	x	√	x	x	x	**x**
	C5-C5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	x	x	√
Majority		√	**x**	√	√	√	√	√	√	√	√		√	**x**	√	√	√	**x**	√	√	√	**x**	

T1–T10 – Testers; √ – identified sample, x – unidentified sample.

TABLE 3 Sensory analysis by duo-trio method of C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%), fresh prepared and after 10 days of refrigeration.

Reference	Samples	Identifying combinations for fresh prepared										Identifying combinations after 10 days of refrigeration										
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Majority	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	Majority
C1	C1-C1-C2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	C1-C1-C3	√	√	√	√	√	x	√	√	√	√	√	√	x	√	√	x	√	√	√	√	√	√
	C1-C1-C4	√	√	√	x	√	x	√	√	√	x	√	√	x	√	√	x	x	√	√	√	x	√
	C1-C1-C5	√	√	√	x	√	x	√	√	√	x	√	x	√	√	√	x	x	√	√	√	x	√
C2	C2-C2-C1	√	x	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√	√	√	√	√
	C2-C2-C3	√	x	√	√	√	x	√	√	√	x	√	√	x	√	x	x	x	x	√	√	x	**x**
	C2-C2-C4	√	√	√	√	√	x	√	√	√	√	√	√	√	√	√	√	x	√	√	√	x	√
	C2-C2-C5	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√	x	√	√	√	√	x	√
C3	C3-C3-C1	√	√	√	√	√	√	√	√	√	x	√	√	x	√	√	x	√	√	√	√	√	√
	C3-C3-C2	√	√	√	√	x	√	√	√	√	x	√	√	x	√	x	x	x	√	x	√	x	**x**
	C3-C3-C4	√	x	√	√	√	√	√	√	√	√	√	√	√	√	√	√	x	√	√	√	√	√
	C3-C3-C5	√	√	√	√	√	x	√	√	√	√	√	√	x	√	√	√	x	√	√	√	√	√
C4	C4-C4-C1	√	x	x	√	√	x	√	x	x	x	√	√	x	√	x	√	x	√	√	√	√	√
	C4-C4-C2	√	√	√	√	√	x	√	√	√	x	√	√	x	√	√	√	√	√	√	√	x	√
	C4-C4-C3	√	√	x	√	√	√	√	√	√	√	√	√	√	√	√	√	x	x	√	√	√	√
	C4-C4-C5	√	x	x	√	x	x	√	√	x	x	**x**	√	x	**√**	x	x	x	x	x	√	x	**x**
C5	C5-C5-C1	√	√	√	√	√	x	√	√	√	x	√	√	x	√	√	x	√	x	√	√	x	√
	C5-C5-C2	√	x	√	√	√	√	√	√	√	√	√	√	√	√	√	x	x	√	√	√	√	√
	C5-C5-C3	√	√	√	√	√	x	√	√	√	√	√	√	x	√	√	√	√	√	√	√	x	√
	C5-C5-C4	√	x	√	x	√	x	x	√	x	x	**x**	x	√	x	x	x	x	x	√	√	x	**x**
Majority		√	√	√	√	√	x	√	√	√	x		√	x	√	√	x	x	√	√	√	x	

√ – identified sample, x – unidentified sample.

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TABLE 4 Sensory analysis by triangle method of C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%), fresh prepared and after 10 days of refrigeration.

Sample		Identifying combinations for fresh prepared						Identifying combinations after 10 days of refrigeration					
A	B	ABB	BAA	AAB	BBA	ABA	BAB	ABB	BAA	AAB	BBA	ABA	BAB
C1	C2	√	√	√	√	√	√	√	√	√	√	√	√
C1	C3	√	√	√	√	√	√	√	√	√	√	√	√
C1	C4	√	√	√	x	√	√	√	x	√	x	√	√
C1	C5	√	√	√	x	√	√	√	√	√	x	√	x
C2	C3	√	√	√	x	√	√	√	√	√	x	√	x
C2	C4	√	√	√	√	√	√	√	√	√	x	√	x
C2	C5	√	√	√	√	√	√	√	√	√	√	√	√
C3	C4	√	√	√	√	√	√	√	√	√	√	√	√
C3	C5	√	√	√	√	√	√	√	√	√	x	√	√
C4	C5	√	√	√	x	√	x	√	x	√	x	√	x

√ – identified sample, x – unidentified sample.
The opinion of majority of the tasters was considered.

TABLE 5 Sensory analysis of ordering by rank method of C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%), fresh prepared and after 10 days of refrigeration.

Tester	Identifying combinations for fresh prepared					Identifying combinations after 10 days of refrigeration				
	Intensity of spicy with cruds codification									
	1	2	3	4	5	1	2	3	4	5
	very week	week	moderate	strong	very strong	very week	week	moderate	strong	very strong
Tester 1	C1	C2	C4	C3	C5	C1	C2	C3	C4	C5
Tester 2	C2	C1	C3	C5	C4	C2	C1	C3	C5	C4
Tester 3	C1	C2	C4	C3	C5	C1	C2	C3	C4	C5
Tester 4	C1	C2	C4	C3	C5	C1	C2	C3	C4	C5
Tester 5	C1	C2	C3	C4	C5	C1	C3	C2	C4	C5
Tester 6	C1	C4	C3	C2	C5	C1	C2	C3	C5	C4
Tester 7	C1	C2	C4	C3	C5	C1	C2	C3	C4	C5
Tester 8	C1	C2	C4	C3	C5	C1	C2	C3	C4	C5
Tester 9	C1	C2	C4	C3	C5	C1	C2	C3	C4	C5
Tester 10	C3	C1	C2	C5	C4	C2	C1	C3	C4	C5
Majority	**C1**	**C2**	**C4**	**C3**	**C5**	**C1**	**C2**	**C3**	**C4**	**C5**

The acidity of fresh prepared curds varied in the range of 0.756–0.774% lactic acid and increased to 0.768–0.794% lactic acid after 10 days of refrigeration. The addition of the whole seeds of *N. sativa* (C2 curd sample) influenced the most the titratable acidity of curds which was lower by 2.32% compared to control curd C1 due to the inhibition of the reproduction of bacteria (antimicrobial effect). The same behavior was observed for C3, C4, and C5 for which the measured acidity was lower with 1.42, 1.94, and 1.80%, respectively, in comparison to control curd without additives. During the storage period of 10 days, the titratable acidity of all the curds samples increased by 1.58–3.25% compared to the fresh prepared curds. The lowest increase of titratable acidity of 1.58% was observed in the case of C2. The highest increase of titratable acidity (3.25%) was registered for C3, the curd with *N. sativa* powder. The increase in acidity of the curd samples was due to lactic fermentation with acid lactic generation and to fatty acids derived from the hydrolysis of curd lipids and for C2–C5 curds, the lipids contained in the *N. sativa* seeds (whole or powder), *N. sativa* cold-pressed oil, or ethanol extract of *N. sativa* seeds. In the whole *N. sativa* seeds, the lipids are included in the cells being protected by their membrane but in the seed powder, ethanolic extract, and cold-pressed oil, the lipid is spread in the curd and directly exposed to the curd environment containing water and lipases (lipolytic enzymes were not inactivated as the milk was not subjected to pasteurization in the process of curd preparation). In the ripening process of curds, part of lipids suffered hydrolysis with free fatty acids generation. The increase in the titratable acidity during the 10 days of storage could be attributed mainly to progressive conversion of lactose to organic acids, mainly lactic acid by the activity of lactic bacteria

TABLE 6 Sensory analysis by simple descriptive method with number of awarded points (0–5) of C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%), fresh prepared and after 10 days of refrigeration.

Tester	Identifying combinations for fresh prepared					Identifying combinations after 10 days of refrigeration				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
Tester 1	5	5	4	5	3	4	5	4	4	3
Tester 2	4	5	4	3	4	3	5	3	3	3
Tester 3	5	4	3	4	3	4	4	3	3	4
Tester 4	5	5	3	4	3	5	5	2	4	2
Tester 5	5	4	4	4	3	4	4	3	3	3
Tester 6	5	3	4	4	4	4	3	3	4	4
Tester 7	5	4	3	3	3	4	4	3	3	3
Tester 8	5	4	4	4	3	4	4	2	4	2
Tester 9	5	5	3	4	4	4	4	3	3	4
Tester 10	4	5	4	4	4	3	4	3	4	3
Average score ± SD	4.8 ± 0.1^{a}	4.4 ± 0.1^{a}	3.6 ± 0.2^{c}	3.9 ± 0.2^{b}	3.4 ± 0.2^{c}	3.9 ± 0.1^{a}	4.2 ± 0.2^{b}	2.9 ± 0.2^{b}	3.5 ± 0.2^{c}	3.1 ± 0.2^{a}

Values are means ± standard deviation ($n = 5$).
Different letters $^{a, b, c}$ indicate statistically significant differences for Fisher's LSD test and $p < 0.05$.

TABLE 7 Physical–chemical analysis of fresh prepared assortments of different forms of black cumin curd C2–C5 compared to control C1 and after 10 days of refrigeration; C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%).

Physical–chemical indicator	Sample code of curd	Fresh prepared*	After 10 days of refrigeration*
Titratable acidity, % lactic acid	C1	$0.774 \pm 0.005^{*b**, i***}$	$0.794 \pm 0.007^{c, j}$
	C2	$0.756 \pm 0.006^{a,i}$	$0.768 \pm 0.005^{a,j}$
	C3	$0.763 \pm 0.004^{a\ i}$	$0.788 \pm 0.008^{bc,j}$
	C4	$0.759 \pm 0.004^{a,i}$	$0.780 \pm 0.007^{ab,j}$
	C5	$0.760 \pm 0.005^{a,i}$	$0.777 \pm 0.008^{ab,j}$
Moisture, %	C1	$55.87 \pm 0.92^{c,i}$	$49.27 \pm 1.00^{bc,j}$
	C2	$54.69 \pm 0.65^{bc,i}$	$48.98 \pm 0.60^{b,j}$
	C3	$54.92 \pm 0.72^{bc,i}$	$50.29 \pm 0.48^{c,j}$
	C4	$54.21 \pm 0.82^{a,i}$	$47.32 \pm 0.61^{a,j}$
	C5	$53.76 \pm 0.79^{ab,i}$	$46.68 \pm 0.75^{a,j}$
Fat, in dry matter%	C1	$49.85 \pm 0.31^{a,i}$	$49.94 \pm 0.52^{a,i}$
	C2	$50.32 \pm 0.61^{ab,i}$	$50.43 \pm 0.86^{ab,i}$
	C3	$50.29 \pm 0.46^{ab,i}$	$50.26 \pm 0.58^{ab,i}$
	C4	$50.81 \pm 0.32^{bc,i}$	$50.89 \pm 0.73^{ab,i}$
	C5	$51.26 \pm 0.33^{c,i}$	$51.29 \pm 0.54^{b,i}$

*Values are means ± standard deviation ($n = 5$).
**Different letters $^{a, b, c, d, e}$ in the same column of the same mean value of a physico-chemical indicator indicate statistically significant differences at $p = 95\%$ according to Fisher's least significant difference (LSD) procedure.
***The letters ^{i} and ^{j} indicate statistically significant differences at $p = 95\%$ between the physical–chemical indicators for each variant of curd sample fresh prepared and after 10 days of refrigeration.

(Ahmad et al., 2014; Hoxha et al., 2023) and to a lesser extent on the hydrolysis of lipids contained in the *N. sativa* powder, ethanolic extract of *N. sativa* or cold-pressed oil, as well as the hydrolysis of curd lipids derived from milk. The hydrolysis of added lipids or of lipids from milk generates free fatty acids and increases the acidity of curds samples. Mahgoub et al. (2013) measured 0.54% acidity for fresh dominated cheese and the lower values of 0.52 and 0.50% for the supplemented cheese with 0.1 and 0.2% black cumin seed oil. During the storage, the acidity increased but the supplemented cheese with black cumin seed oil showed lower increases. The black cumin seed oil controlled the development of titratable acidity. This might be due to the continuous fermentation of lactose to lactic acid as well as the gradual increase of degradation process in the product.

The moisture of the fresh prepared curds varied in the range of 53.76–55.87%. The highest moisture was registered for the C1, the control curd sample (55.87%), while the lowest moisture (53.76%) was found in the case of C5 curd sample with addition of cold press oil of *N. sativa*. During the 10-days storage, the moisture of curd samples decreased by 8.43–13.17%. The lowest decrease was found in the case of C3 curd with *N. sativa* powder seeds addition, while the highest decrease of 13.17% was registered for C5 curd sample with cold-pressed oil of *N. sativa*. The decrease of the curd moisture during storage was due to the syneresis process with whey removal and also to the water evaporation. A shrinkage of the curd took place during storage due to the lactic fermentation process with lactic acid generation which helped to expel the whey from curd mass (Hassanien et al., 2014). The lower trend of moisture decrease for C3 curd sample with *N. sativa* as powder can be attributed to the water holding capacity of seed powder. Similar behavior was reported by Tarakci et al. (2011) for curd supplemented with garlic.

The fat contents of curd samples as % of dry matter varied between 49.85 and 51.26%. The lowest fat content as % of dry matter was found for C1 curd sample and the highest fat content was measured for C5 curd sample due to the fat addition as cold-pressed oil of *N. sativa*. The supplementation of curd with *N. sativa* contributed to the increase of fat content of curd by 0.88–2.82% of dry matter. During the 10 days of storage, the fat content of the curd samples registered a slight increase due to the decrease in humidity during the curd ripening (Mohammed et al., 2016). The addition of *N. sativa* as seeds, powder, alcoholic

extract, and cold-pressed oil increased the nutritional value and health benefits of the curds due to their content of monounsaturated and polyunsaturated fatty acids, tocopherols, and polyphenols (Forouzanfar et al., 2014).

3.3 Microbiological characteristics

Total number of bacteria (TNB), *Enterobacteriaceae,* and coagulase-positive staphylococci (CPS) were determined in the five types of curds (C1, C2, C3, C4, and C5) both fresh and after 10 days of storing the products at 4°C. Microbiological quality of the curd samples (obtained from un-pasteurized milk) was influenced by the addition of black cumin in various forms, as well as by the time interval of performing the analyses. The lack of heat treatment in the production of the traditional Romanian sheep curd shows the fact that careful monitoring of the microbiological parameters is necessary to ensure the consumers safety.

This fact has also been noted in other studies on cheeses produced in small farms using traditional methods without the pasteurization process or advanced technologies (Knysz et al., 2018), which showed a highly variable microbiological quality and nutritional value.

The values of total bacterial count (the means ± standard deviation) registered in the case of curd samples were converted into logarithmic colony-forming units (CFU/g) and are presented in Table 8, being reported to the maximum limit allowed for cheese.

In the first stage (fresh prepared) of the TNB determination of curds, an exceeding of the maximum values allowed according to the International Microbiological Criteria for Dairy Products (Council Directive, 1992) was registered only for the control curd, and the other curds enriched with different forms of *N. sativa* were within the limit of normal values. The best aerobic mesophilic germ inhibition was found in sample C3 with addition of ground seeds, followed by sample C5 with black cumin oil, C4 with alcoholic extract, and C2 with black cumin seeds. In addition, after 10 days of refrigeration storage, the effects of inhibiting the total number of bacteria were observed for the curds with the addition of oil, alcoholic extract, and seeds. On the other hand, for the curd with seed powder (C3), a microbial growth was recorded, but the value was below that recorded for the control curd where the increase was the most obvious.

Even if, following the TNB determinations in the fresh state, the curd with powder (C3) showed a marked antimicrobial effect (3.92 log CFU/g), after ripening, an increase in the total number of bacteria was found (4.31 log CFU/g), even more than in the sample with seeds. This is explained by the fact that the active principles from the *N. sativa* seeds were eliminated in the product and exerted their antimicrobial effect over time. The seeds store these active principles better and have a superior preservation capacity of the dairy product over time. Instead, the powder disperses better in the product from the beginning, but over time the antimicrobial effect decreases under the action of lactic bacteria that intervene in the ripening process.

After the ripening period, the most pronounced antibacterial effect was observed in the case of cold-pressed oil added in a proportion of 1% when 4.13 log CFU/g were counted, followed by alcoholic extract (1%) with 4.15 log CFU/g and seeds (3%) with 4.28 log CFU/g compared to the control sample where 5.23 log CFU/g were recorded.

TNB test results agree with those described by Alsawaf and Alnaemi (2010) who evaluated the effect of *N. sativa* (seeds and oil) on TNB (CFU/g) during manufacturing and storage of soft white cheese at refrigeration temperature. Their results showed that there was a significant decrease of TNB and other pathogenic bacteria in cheese samples treated with *N. sativa* seeds (1 and 3%) and oil (0.3 and 1%), the inhibition of germs being dependent of the form in which *N. sativa* was added, in contrast to the control curd in which there was a significant increase in the number of bacteria.

The addition of cold-pressed oil had the most pronounced antimicrobial effect compared to the other added forms, for the curds analyzed in this research. This indicates a significant improvement of the microbiological properties of the curd, a fact demonstrated also in the research made by Georgescu et al. (2018b), which showed that the antimicrobial activity of *N. sativa* seeds oil may be selective, as beneficial lactic acid bacteria strains were not inhibited.

Enterobacteriaceae were determined in the curd samples as indicator organisms for the hygienic quality assessment, considering the manual milking of sheep's and the traditional processing practices. Average values result ± standard deviation is presented in Table 9, being reported to the reference limit value which must fall between 1 and 3 log CFU/g cheese (Commission Regulation, 2005).

Enterobacteriaceae presence in the examined samples should raise safety issues, as was also reported by Nosir et al. (2014) because this

TABLE 8 Microbiological analysis (TNB) of fresh prepared assortments of different forms of black cumin curds C2–C5 compared to control C1 and after 10 days of refrigeration; C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%).

Microbiological indicator	Code of the curd sample	Fresh prepared* log CFU/g	After 10 days of refrigeration*, log CFU/g	Limit log CFU/g
Total number of bacteria (TNB)	C1	5.03 ± 0.07*c**, i***	5.23 ± 0.12 c,j	5
	C2	4.39 ± 0.15 b, i	4.28 ± 0.08 ab, i	5
	C3	3.92 ± 0.14 a, i	4.31 ± 0.19 b, j	5
	C4	4.35 ± 0.21 b, i	4.15 ± 0.10 a, i	5
	C5	4.33 ± 0.39 b, j	4.13 ± 0.09 a, i	5

*Values are means ± standard deviation ($n = 5$).

**Different letters a, b, and c in the same column of the same mean value of a TNB indicator indicate statistically significant differences at $p = 95\%$ according to Fisher's least significant difference (LSD) procedure.

***The letters i and j indicate statistically significant differences at $p = 95\%$ between the TNB indicators for each variant of curd sample fresh prepared and after 10 days of refrigeration.

high sample contamination rate is an indicator of direct or indirect milk fecal contamination and of the appropriate hygiene measures neglection. The number of colony-forming units falls within appropriate limits for all samples examined both in the first stage of the determinations and in the second stage; the values for the curd samples with the addition of *N. sativa*, in different forms, are below the values of the control sample.

The addition of different *N. sativa* forms had inhibitory effects on the number of *Enterobacteriaceae* in the curds C2–C5, compared to the control curd C1.

In the fresh state, the fastest effect of *Enterobacteriaceae* reduction was manifested by the ground seed powder, but this potential decreased after keeping the samples for 10 days. The oil from the seeds, respectively, the alcoholic extract from the black cumin seeds, inhibits most effectively the enterobacteria development during the period of storage by refrigeration, with a decrease in the number of colonies of approximately 2.34–2.36 log CFU/g. This fact was also demonstrated by other research in which the spices addition was considered to improve the microbiological quality of the cheese (Ibrahim and Abdel-Hakiem, 2015); thus, the cumin oil presented a stronger antimicrobial effect than the aqueous extract. *N. sativa* seeds have an antimicrobial effect to a lesser extent on the curd kept for 10 days by refrigeration, and these findings were also reported by Dormans and Deans (2008) which showed the fact that the essential oils have stronger effects than the spices themselves or their aqueous extract.

Black cumin seeds oil was also tested for its inhibitory effect against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes,* and *Salmonella Enteritidis* in soft cheese during cold storage (Hassanien et al., 2014), and the results showed the best antibacterial activity against *S. aureus*. Based on the obtained results, this effect could also be observed in the case of the traditional sheep curds, for which lower values of CPS were encountered even on the first day of analysis (2.30 log CFU/g of curd with 1% oil). After 10 days of refrigeration, the effect is even more pronounced, being recorded 2.02 log CFU/g. In addition, the alcoholic extract added in a proportion of 1% had a good effect against CPS, and the values from the first day, respectively, after the tenth day were 2.38–2.09 log CFU/g curd with extract. This reveals the fact that extracts from *N. sativa* seeds contain a variety of chemical components and functional structures related to their antimicrobial properties, which supports the possibility of their use as natural precursors of nutraceutical products (Shafodino et al., 2022).

The seeds, respectively, the powder from ground black cumin seeds, have a reduced effect of inhibiting pathogenic germs, while the ripening time (of 10 days) contributes to obtaining lower values of CPS compared to the first day of analysis. The coagulase-positive staphylococci colonies developed on Baird-Parker medium both after the preparation of the curds and after 10 days of refrigeration.

The results for CPS logarithmic values (mean ± standard deviation) recorded for the analyzed curds are presented in Table 10 being reported to the reference limit value according to Commission Regulation (2005). The presence of *S. aureus* (some having coagulase positive) in cheese samples generally reflects the hygiene conditions during production and the lack of heat treatment to eliminate microorganisms (Haddad and Yamani, 2017).

3.4 Cluster analysis

Cluster analysis is a method of grouping elements with the greatest similarity. Cluster analysis was used in the research about cheese making to discriminate between different assortments of cheese (Eroglu et al., 2015; Bittante et al., 2024).

Cluster analysis showed the similarity between samples based on multiple characteristics. Cluster analysis, performed for the five curd samples fresh and after 10 days of storage based on their physical–chemical and microbiological characteristics, is shown in Figure 4. Two main clusters were generated: the cluster A formed of control curd fresh and after 10 days of storage C1-F and C1-10 and the cluster B that contained the treated curds samples. Cluster B is formed of two elements: B1 and B2. B1 is composed of six elements: the pair C2-F and C3-F (the curd with *N. sativa* seed addition whole and as powder) linked by the pair C4-F and C5-F (fresh curd with alcoholic extract and cold-pressed oil). B2 is composed of two elements: C2-10 and C3-10, the curds with *N. sativa* seeds addition whole and as powder after 10 days of storage. The highest similarity was observed between C4 and C5 fresh curds containing alcoholic extract of *N. sativa* seeds and cold-pressed oil of *N. sativa*. A high similarity between these curds samples was maintained after 10 days of storage, and the distance between C4–10 and C5–10 was also low.

Cluster analysis of curd assortments was performed also considering the sensory scores (Figure 5) to highlight the curd samples with the most similar sensory characteristics.

TABLE 9 *Enterobacteriaceae* of fresh prepared assortments of different forms of black cumin curds C2–C5 compared to control C1 and after 10 days of refrigeration; C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%).

Microbiological indicator	Code of the curd sample	Fresh prepared* log CFU/g	After 10 days of refrigeration*, log CFU/g	Limit log CFU/g
Enterobacteriaceae	C1	2.60 ± 0.17*a**, i***	2.73 ± 0.23 b, i	3
	C2	2.58 ± 0.20 a, i	2.39 ± 0.06 a, i	3
	C3	2.50 ± 0.21 a, i	2.53 ± 0.39 ab, i	3
	C4	2.55 ± 0.18 a, i	2.36 ± 0.24 a, i	3
	C5	2.53 ± 0.17 a, i	2.34 ± 0.17 a, i	3

*Values are means ± standard deviation ($n = 5$).

**Different letters a and b in the same column of the same mean value of a *Enterobacteriaceae* indicator indicate statistically significant differences at $p = 95\%$ according to Fisher's least significant difference (LSD) procedure.

***The letter i indicates statistically significant differences at $p = 95\%$ between the *Enterobacteriaceae* indicators for each variant of curd sample fresh prepared and after 10 days of refrigeration.

TABLE 10 Coagulase-positive staphylococci (CPS) of fresh prepared assortments of different forms of black cumin curds C2–C5 compared to control C1 and after 10 days of refrigeration; C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seed powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%).

Microbiological indicator	Code of the curd sample	Fresh prepared* log CFU/g	After 10 days of refrigeration*, log CFU/g	Limit log CFU/g
Coagulase-positive staphylococci (CPS)	C1	2.75 ± 0.23*c**, i***	2.66 ± 0.12 b, i	3
	C2	2.68 ± 0.24 bc, i	2.53 ± 0.25 b, i	3
	C3	2.64 ± 0.30 bc, i	2.55 ± 0.41 b, i	3
	C4	2.38 ± 0.23 ab, j	2.09 ± 0.08 a, i	3
	C5	2.30 ± 0.08 a, j	2.02 ± 0.08 a, i	3

*Values are means ± standard deviation ($n = 5$).
**Different letters a, b, and c in the same column of the same mean value of a CPS indicator indicate statistically significant differences at $p = 95\%$ according to Fisher's least significant difference (LSD) procedure.
***The letters i and j indicate statistically significant differences at $p = 95\%$ between the CPS indicators for each variant of curd sample fresh prepared and after 10 days of refrigeration.

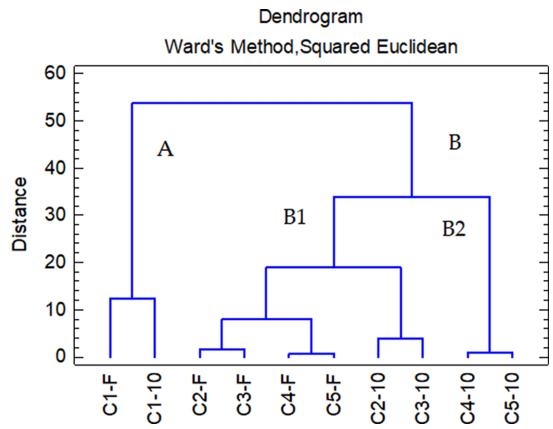

FIGURE 4
Cluster analysis of curds based on the physical–chemical and microbiological characteristics: C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seeds powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%); F – indicates fresh sample, 10 – indicates the curd sample after 10 days of storage.

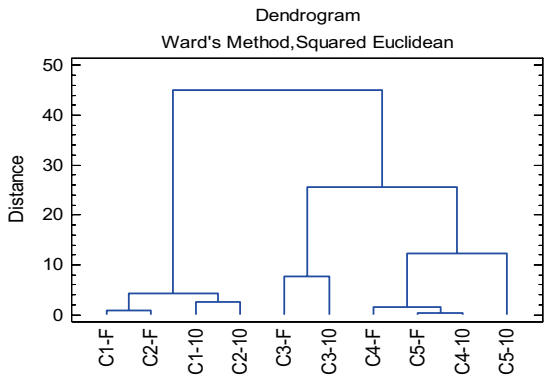

FIGURE 5
Cluster analysis of curds based on the sensory scores: C1 – control curd; C2 – curd with seeds (3%); C3 – curd with seeds powder (3%); C4 – curd with alcoholic extract (1%); C5 – curd with oil (1%); F – indicates fresh sample, 10 – indicates the curd sample after 10 days of storage.

The high similarity between C1-F and C2-F showed that the sensory characteristics of the curd with whole *N. sativa* seeds were less modified compared with the curd without additives. In addition, the curd C4–10 showed a high similarity with C5-F (curd with cold-pressed oil) and C4-F (curd with alcoholic extract), suggesting the fact that both the alcoholic extract and the oil change the specific sensory characteristics of the curds but confer the highest degree of preservation, the characteristics of the fresh curd being best preserved in their case.

4 Conclusion

The present research was mainly focused on exploring the potentials of black cumin seeds, powder, extract, and oil for the development of innovative functional foods. The addition of a natural spice (black cumin) to traditional sheep's cheese contributed to the improvement of the sensory and physico-chemical properties of the product, stabilized the microbial alteration over time, and offered promising perspectives for food safety.

Each sensory method gives useful and thorough information about the sensory attributes of the curds and, also, about its individuality, typicality, and maturation. Considering the sensory analyses, it can be concluded that in the case of freshly prepared curd, the control sample was the most liked by the tasters, whereas after 10 days of refrigeration, the most liked product was the curd with seeds. The products that were most disliked by the tasters were the curd with oil and the curd with powder.

The addition of *N. sativa* in all the forms to the curds contributed to the increase of fat content in dry matter and to a decrease of titratable acidity compared to the curd without the supplement.

In terms of antimicrobial activity against pathogens, the oil and alcoholic extract were found to be more beneficial than the spice itself, inhibiting the development of microorganisms even at lower concentrations. This potential of *N. sativa* in its various forms has contributed to maintaining a good microbiological quality of the curd during storage.

Obtaining a safe curd through traditional practices without pasteurization reinforces the idea of using natural alternatives for instance black cumin as an antimicrobial and flavoring compound.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ZV: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AD: Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. CM: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. TD: Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. LM: Resources, Validation, Visualization, Writing – original draft. MM: Resources, Validation, Visualization, Writing – review & editing. BM: Formal analysis, Validation, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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