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Effect of Chia or Black Seeds Supplementation on Textural, Antioxidant and Sensory Properties of Gouda Cheese

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Abstract

Gouda cheese is the most popular Dutch semihard cheese. The aim of this study was to produce and evaluate changes in the physiochemical, microbiological, and sensory qualities of Gouda cheese supplemented with 1.5 g/L black or chia seeds as an antioxidant source during three months of ripening period at 14°C. The chemical composition analysis, total phenolic content (TPC), antioxidant activity (AA) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays, and organic acid content were evaluated for all cheese treatments during the ripening period of 90 days at 14°C. Moreover, the microbiological, rheological, and organoleptic characteristics of the resulting Gouda cheese were analyzed. Black seed (BS) or chia seed (CS) addition resulted in a considerable increase in TPC (68.86±0.10-2.48±0.25 µg GAE/g), AA, measured as DPPH (30.48±0.32-42.58±0.38%), and FRAP (8.90±0.16 -11.72±0.27 mmol $FeSO_4$/g), respectively. The addition of CS caused a significant increase in the organic acids content such as lactic, propionic, acetic, succinic, and formic acids in Gouda cheese. There was a decrease in the number of yeast and mold in BS or CS Gouda cheese compared to the control. The texture profile of cheese showed that CS Gouda cheese had lower hardness and gumminess than control cheese, with opposite trends for other textural parameters. The addition of black or chia seeds improved all sensory attributes of Gouda cheese during ripening period for 90 days at 14°C.

Keywords: Antioxidant activity; Black seeds; Chia seeds; Gouda cheese; Ripening; Texture profile; Total phenols.

1. Introduction

The increasing popularity of functional foods has led to the use of dairy products as carriers to deliver phytochemicals and other nutrients, which has potential health benefits [1]. Therefore, the dairy industry requires innovative ways to enhance the functionality of conventional dairy products, which could have a considerable effect on customers. Cheese is a milk-based product that is essential to the human diet and helps the body meet its nutritional requirements [2]. Currently, the main purpose of producing and consuming cheese is to prevent hunger and provide nutrients that can have positive health effects as a result, the increasing marketing as a functional food. Since the cheese market has expanded, producers and consumers have focused on innovative cheese with distinctive flavor characteristics [3].

Gouda cheese is washed curd Dutch type cheese and is considered the most fundamental category of semi-hard cheeses coagulated with rennet, made worldwide from bovine milk, but other types of milk such as sheep's or goat's milk also applicable [4-6]. Starter cultures usually contain a mixture of mesophilic lactic acid bacteria (LAB) that are used to acidify raw or pasteurized milk to make Gouda cheese. The resulted cheese brined and covered in a plastic layer before ripening between 13 -15°C for 1 to 20 months to enhance flavor [7]. The texture varies from semi-soft to fairly firm and smooth, changing to a firmer and brittle structure as the ripening period progresses. The flavor also changes from moderate to intense with the longer ripening period. Some pea-sized spherical eyes can be seen inside the main types of cheese, and no surface flora exists. Spices and herbs are examples of acceptable additives [8,9]. Gouda cheese and related products are categorized as nutrient-dense foods because they provide an extensive range of important nutrients, including fats, fatty acids, proteins, bioactive peptides, amino acids, minerals, vitamins, that support the function of the body [6]. Each year, 40% of Egypt's food needs are imported [10]. Therefore, Gouda cheese and its various types can be introduced into the field of investment in response to local demand, as importing it is not considered a deficit in the production of cheese but rather a deficit in its manufacture. It is also possible to produce Gouda cheese by adding different types of seeds to suit consumers' different tastes to meet trends toward healthier eating habits.

Nigella sativa, popularly referred to black seed or black cumin, is a medicinal plant used worldwide and is a part of the Ranunculaceae family. It is originally native to the regions bordering the Mediterranean and southwest Asia [11,12]. Several studies on the chemical composition of *N. sativa* have revealed that its quinone ingredient, also known as thymoquinone (the primary component of essential oils), is primarily responsible for the plant's medical usefulness and has various pharmacological

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properties, including hepatoprotective [13-15], anti-inflammatory [16,17], antibacterial [18], fungicidal [19], antioxidant [20], nephroprotective [21], and anticancer properties [17,22,23]. In addition, *N. sativa* seeds contain high concentrations of unsaturated fatty acids such as oleic, linoleic and palmitic acid [24]. The seeds also contain vitamins, flavonoids, indazole-type alkaloids, saponins, and cardiac glycosides. A few essential elements are also present, including calcium, phosphorus, and iron [25]. *Salvia hispanica* L., commonly referred to as "chia," is a plant in the family Lamiceae that originates in Mexico and certain regions of South America. Chia seeds are highly nutritious because of their high fiber content and polyunsaturated fatty acids (PUFA), including oleic acid, linolenic acid, and α-linolenic acid. Moreover, it has natural antioxidants that protect against specific cancers and cardiovascular diseases, like quercetin, kaempferol, caffeic acid, rosmarinic acid, and chlorogenic acid. The seeds also have a sufficient amount of protein, macro- and microelements, and vitamins. Furthermore, it is considered an especially rich source of Ca and Mg [26-31]. Therefore, the aim of this research was to produce Gouda cheese with antioxidant activity through black or chia seeds supplementation and to evaluate the total phenolic content, antioxidant capacity and organic acids of black or chia seeds Gouda cheese. In addition, the physicochemical, microbiological, rheological, and organoleptic properties of the resulting Gouda cheese were studied during the 90-day ripening period at 14 °C.

2. Materials and Methods

2.1. Materials

The cow milk (11.75% total solids, 3.2% fat, 3.3% protein, 4.42% lactose, 0.67% ash, 6.78 pH) was obtained from the dairy unit at Cairo University's Faculty of Agriculture in Giza, Egypt. A direct vat set (DVS) mesophilic aromatic culture (CHN-22) of freeze-dried lactic culture was obtained from Chr. Hansen in Denmark. Cheese wax and calf rennet were purchased from CSK Food Enrichment CV in the Netherlands. The calcium chloride E509 originated from Gebr Rademaker in the Netherlands. Sodium chloride was purchased from El-Gomhouria Company, Egypt. Black and chia seeds were provided from Imtenan Company, Egypt, and their chemical composition is as shown in Table 1. Analytical-grade chemicals were utilized for all other analyses.

Table 1. Chemical composition of black and chia seeds

Component %	Black seeds	Chia seeds
Moisture	4.82	5.10
Protein	20.96	23.20
Fat	38.61	30.50
Total carbohydrates	28.90	22.00
Crude fiber	6.98	16.10
Ash	3.70	2.57

2.2. Methods

2.2.1. Gouda cheese making

Three batches of Gouda cheese were made using the procedure outlined by Kosikowski and Mistry [32]. 75 kg of cow milk were heated to 72 °C for 15 seconds, cooled to 5 °C, and then heated to 30 °C. The milk was mixed with 2% of a freeze-dried mesophilic lactic culture and 0.20 g/kg CaCl2, which were let to rest for 45 minutes at 30°C. After adding the appropriate amount of calf rennet (0.25 ml/kg of milk), the mixture was left to coagulate for 45 minutes at 32–33°C. The curd was sliced into 1 × 1 × 1 cm pieces after coagulation, allowed to rest in the whey for 20 to 30 minutes, and then agitated. The whey temperature was gradually increased to 36 °C for 30 minutes while stirring. A third of the whey was removed and substituted with an equivalent volume of warm water (55 °C) in order to lower the lactose content and control the generation of lactic acid throughout the production process. The whey was drained after the temperature was kept at 36°C for 30 minutes. The curd was divided into three parts; each BS- or CS-Gouda cheese was pre-soaked and boiled before having 0.15% black seed (BS) or chia seed (CS) added. The third portion was served as a control Gouda cheese. All treatments were packaged into a wheel-shaped plastic mold and pressed at 300 kPa for 20 h (2~3 h interval). Finally, the all-fresh cheese treatments were salted in a 16% brine solution at 14°C for 24 h and stored at 14°C with a relative humidity of 85% for 3 months for ripening. Samples were analyzed at regular intervals on fresh, 30, 60, and 90 days of ripening (Figure 1).

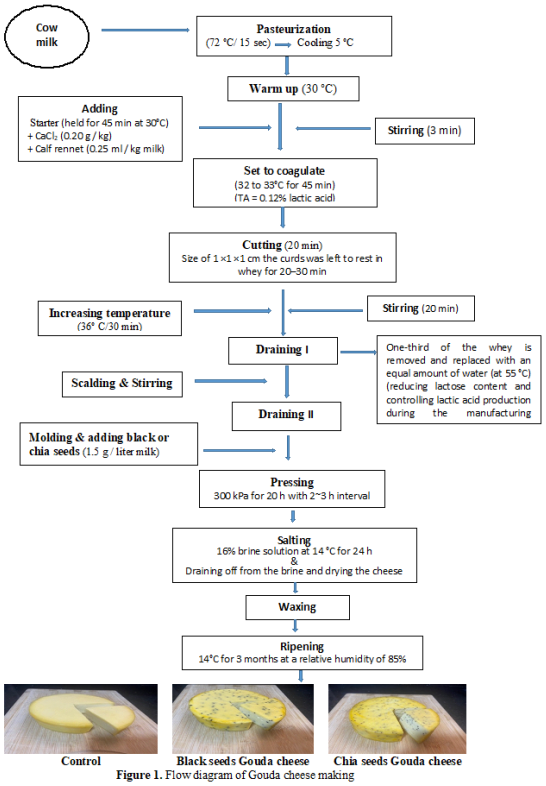

Figure 1. Flow diagram of Gouda cheese making

2.2.2. Chemical composition

The moisture, protein, ash, and titratable acidity contents were determined using the AOAC methods [33]. The Jenway 3510 digital pH meter, a laboratory pH meter with a plastic electrode, was used to measure the pH of the cheese. Salt was determined by titration with $AgNO_3$ using ferric ammonium sulfate, as an indicator, according to the IDF Standard [34]. The Soxhlet method was used to determine the fat content in the cheese samples [35].

2.2.3. Ripening indices

Water-soluble nitrogen (SN) was determined using the method described by Ling [36]. Total volatile fatty acids (TVFA) were calculated using the Kosikowski method [37]. The soluble tryptophan and tyrosine contents of cheese samples were estimated according to Vakaleris and Price [38].

2.2.4. The determination of total phenolic content

Each cheese sample (5 g) was combined with methanol solvent (5 ml) and stirred for 5 min to prepare the extract. The extract let stand at 4°C /2 h and subsequently centrifuged at 8602 × g for 30 min at 4°C. The cheese extracts were ultimately filtered using Whatman No. 42 paper [39]. The total phenolic content (TP) in the crude extract was analyzed using the Folin Ciocalteu reagent test [40]. Each extract was given a proper amount (1 ml) in a 25 ml volumetric flask that had 9 ml of distilled water in it. One milliliter of Folin Ciocalteu phenol reagent was added to the mixture and mixed well. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. Then dilute the solution to 25 milliliters by adding distilled water and mixing well. After 90 minutes of incubation at ambient temperature, the absorbance at 750 nm was measured using a Unicum UV 300 spectrophotometer against the prepared reagent as a blank. Total phenolic content of the sample was reported in milligrams of gallic acid equivalents (GAE) per gram of dry weight.

2.2.5. Determination of antioxidant activity

The antioxidant activity (AA) of cheese samples were determined by two methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and ferric reducing antioxidant power (FRAP). The DPPH radical scavenging was measured according to the method expressed by Baraca *et al*. [41]. The DPPH• (0.1 mM) in methyl alcohol was prepared, and 1 ml of this solution was added to 2 ml of crud extract at concentrations ranging from 1-7 mg/ml. The solution was stirred vigorously and then left to stand at ambient temperature for 30 min in the absence of light. The control sample consisted of all the reaction reagents with the exception of the extract. Subsequently, the absorbance was evaluated at a wavelength of 515 nm. The capacity to scavenge the DPPH• radical was determined through the calculation of the following equation:

DPPH• scavenging activity (%) = (Ac-As/Ac) × 100

Ac stands for the absorption of the control reaction and As for the absorption in the presence of the seed extracts. The results were expressed as IC_{50}, the concentration (mg/ml) of the extract that scavenges 50% of the DPPH radicals.

To evaluate the AA of samples by FRAP, briefly, 30 µl of the sample was mixed with 90 µl of distilled water and 900 µl of a working solution containing 25 ml of 300 mM acetate buffer, 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ solution, and 2.5 ml of TPTZ solution in HCl (40 mM), and kept for 30 min at 37°C. After that, the absorbance of the solution was recorded at 595 nm. FRAP values of samples were obtained through the standard curve (absorbance of different concentration of ferrous sulphate solution at different concentrations ranging 1-10 mmol/L). The results are expressed as mmol $FeSO_4$/g sample [42].

2.2.6. Organic acid analysis

Using a modified procedure outlined by Califano and Bevilacqua [43], organic acids were extracted and assessed with HPLC using an Inert Sustain. A quantity of five grams of cheese that has been grated was added into a centrifuge tube with a volume of 120 ml, along with 20 ml of sulfuric acid (0.013 N). Samples were centrifuged at 6,000 x g for 10 min after shaking for 30 min. The supernatant was then subjected to filtration using a nylon membrane with a pore size of 0.45 µm. An Eclipse AQ-C18 HP column (4.6 mm x 150 mm i.d.,3 µm) was used for the separation. The mobile phase used was 0.05 N sulfuric acid (H_2SO_4). The following was the sequential programming of the mobile phase in a linear gradient for flow rate: 0-4.5 min (0.8 ml/min); 4.5-4.71 min (1 ml/min); 4.71-8.8 min (1.2 ml/min); 8.8-9 min (1.3 ml/min); 9-23 min (1.3 ml/min); 23-25 min (0.8 ml/min). At 210 nm, the diode array detector (DAD) was observed. For each sample, a volume of 5 µL was injected into the high-performance liquid chromatography (HPLC) system. The column was kept at a consistent 55 °C temperature.

2.2.7. Microbiological changes

Twenty-five grams of each cheese sample was mixed thoroughly in 225 milliliters of 0.1% peptone water using a stomacher (FR/BagMixer; Interscience, St. Nom, France) for a duration of three minutes. The cell suspensions were examined for the presence of specific microbial categories through a screening process using serial decimal dilutions. De Man-Rogosa-Sharpe MRS agar medium [44] was used for selective enumeration in order to determine total LAB counts, while M17 medium [45] was used for total lactococci counts. After 48 h of anaerobic incubation at 37°C (lactobacilli) or 30°C (lactococci), colonies were counted. The IDF [46] method was used to count the yeasts and molds. The total lipolytic or proteolytic bacterial count was performed according to Luk [47]. Coliform was counted using violet red bile agar (VRBA) at pH 7.0–7.2; 37˚C for 48 h as described in Hitchins [48].

2.2.8. Texture profile analysis

A Universal Testing Machine (TMS-Pro) fitted with a (250 Ibf) load cell and linked to a PC running Texture Pro TM texture analysis software (program, DEV TPA withhold) was employed to examine the texture profile of cheese samples. Uniaxial compression of the cheese samples was performed using a flat rod probe (49.95 mm in diameter). Samples of fresh cheese were obtained, and after being ripened for 30, 60, and 90 days, the cheese was sliced into 2 x 2 x 2 cm cubes and measured at 20 °C). Two successive cycles (bite-sized portions) of compression-decomposition were applied to each sample. Using the TMS - Pro texture analyzer and computer interface, data were collected on a computer, and texture profile characteristics were computed. The characteristics of the texture profile was calculated using the method outlined by Bourne [49]. The compressive force (g),

which represents the hardness, is measured at the highest compressive value during the initial bite. Cohesiveness is the ratio of the positive force area during the second compression to that during the first compression (A2/A1). Springiness is defined as the rate at which a deformed sample must return to its original state after the force that caused the deformation has been removed.

2.2.9. Sensory properties of Gouda cheese treatments

Sensory properties of cheese were performed according to Lim [50]. An experience panelist composed of 20 members aged from 30 to 55 yr of Dairy Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt, was assembled. Panel members were selected based on their interest in the sensory properties of Gouda cheese and were trained by testing commercial Gouda cheese. Cubes of cheese samples (approximately 50 g at 20°C) were submitted in a group to the 20 test panelists. They rated the cheese samples on a 9-point hedonic scale for flavor, consistency, color and appearance and overall acceptability using a sensory evaluation sheet.

2.2.10. Statistical analysis

Each experiment was performed three times and the mean values of the results analyzed by SAS software (Version 2004, SAS Institute, Cary, North Carolina, USA). The values were compared using the ANOVA test. The results were expressed as mean ± standard error and the differences between means were tested for significance using Duncan's post hoc analysis at $p<0.05$.

3. Results and Discussion

3.1. Chemical composition analysis

The average chemical analysis of Gouda cheese as impacted by the addition of black seeds (BS) or chia seeds (CS) is summarized in Table 2. At fresh time, the moisture content varied between 44.14±0.22 and 45.54±0.29%. Gouda cheese with CS showed the highest moisture values ($p<0.05$) compared to other treatments. This might be attributed to the functional characteristics of CS, as they have the ability to form gel, thus increasing the water holding capacity, which results in less curd syneresis as well as increasing the moisture content of the cheese. However, gradual significant decrease in the moisture content of all treatments corresponded to a prolonged ripening period. The fat/DM contents of Gouda cheese ranged from 50.20±0.10-50.52±0.46%; a gradual increase was observed to be associated with adding seeds, whether BS or CS ($p<0.05$). This increase could be regard to the chemical composition of the starting materials. While the fat contents were expressed on a dry basis, the differences seemed to be due to the ripening period and were not significant ($p>0.05$). According to Walstra *et al.* [51], the fat/DM content of Edam and Gouda cheese varied between 40 and 50%. Remarkably, the moisture and fat/DM contents of all Gouda cheese treatments met the Egyptian legal standard (EOSQC 2005) and the Codex standard for Gouda cheese, (CXS 266-1966) [52]. Which indicates that Gouda cheese moisture content should not exceed 48% and FDM between 48-55%. Also, met the American and Netherlands standards established for fat in dry matter (FDM) in Gouda cheese, which are 46% and 48%, respectively. As expected, the fat and salt content of cheese increases with increasing ripening time in all treatments due to the decrease in moisture content. The total amount of fat and moisture in the cheese was within the ranges of other studies by Jung *et al*. [4], Hammad [53], Jo *et al*. [54], Ramos *et al*., [55], and Wang *et al*. [56] regarding to the composition of Gouda cheeses. Over all stages of the ripening, the protein content increased ($p<0.05$); this could be related to moisture loss. These results are consistent with the data from Jo *et al*. [54] who suggested that Gouda cheeses with extended ripening periods were probably to be lower in moisture and had higher fat and salt contents than younger cheeses. In addition, BS had higher protein and ash content than C and CS at the end of the ripening period, although CS had the lowest salt content.

3.2. Ripening indices

Table 3 displays the ripening indices values including soluble nitrogen/ total nitrogen, tyrosine, tryptophan, and total volatile fatty acids (TVFAs) as well as titratable acidity (TA%), and the pH value during the ripening period. Coagulating enzymes are primarily responsible for protein degradation due to their capability to hydrolyze αS1-casein and β-casein. Additionally, bacterial proteinases contribute to this increase in the later stages of ripening [57]. At the end of ripening period, the SN/TN ranged from 17.34±0.30 to 18.20±0.10. The results reveal evidently that Gouda cheese with chia seeds led to a slight increase ($p > 0.05$) in the SN/TN, while the values of tyrosine and tryptophan increased significantly ($p<0.05$) and reached 154.88±0.93 to 156.13±0.12 and 54.12±0.31 to 60.12±0.16, respectively. These results could be attributed to the higher moisture retained in cheese, which resulted in an acceleration of proteolysis during cheese ripening. The results in Table 3 demonstrate that the addition of seeds, whether black or chia seeds, remarkably enhanced TVFAs contents ($p<0.05$) compared to the control, although the increase in TVFAs was more noticeable ($p<0.05$) in BS Gouda cheese, where the value reached 30.17±0.31 ml 0.1N NaOH/100g cheese. As the ripening progressed, the values of SN/TN, total nitrogen, tyrosine, tryptophane, and total volatile fatty acids (TVFAs) increased significantly ($P < 0.05$) in all samples. The reasons for these increases include protein hydrolysis, fat degradation, as well as the evolution of soluble nitrogenous and volatile fatty acid molecules. Overall, these findings are consistent with those published by Ardo *et al*. [58]. Titratable acidity ranged from 1.58 to 1.75 % and increased ($p<0.05$) reaching 1.78±0.17 to 1.95±0.04 % after 90 days of ripening, it could notice that there was a general increase in TA% along ripening period (Table 3). Also, the same Table shows the increase in TA% in control was the highest rate among the other samples. This could be due to a further breakdown of lactic acid forming other acids as acetic and propionic as well as amino acids through the protein hydrolysis. The figures tabulated in Table (3) confirmed that opposite to the TA%, the pH value of cheese was gradually lowered as the ripening period of Gouda cheese was progressed. These results are in accordance to those found by Hammad [53].

Table 2. Gouda cheese composition as affected by adding black or chia seeds during ripening period at 14°C for 90 days

Component (%w/w)	Ripening period (days)	Treatments		
		C	BS	CS
Moisture	Fresh	44.85± 0.26^{Ba}	44.14±0.22^{Ca}	45.54±0.29^{Aa}
	30	43.25±0.20^{Bb}	42.59±0.39^{Cb}	44.64±0.55^{Ab}
	60	40.93±0.38^{Bc}	40.05±0.17^{Cc}	42.63±0.10^{Ac}
	90	39.02±0.27^{Bd}	37.95±0.31^{Cd}	40.84±0.41^{Ad}
Protein	Fresh	12.98±0.28^{Cd}	13.26±0.20^{Ad}	13.07±0.32^{Bd}
	30	13.73±0.12^{Bc}	14.04±0.16^{Ac}	13.29±0.29^{Cc}
	60	14.99±0.20^{Bb}	15.25±0.14^{Ab}	14.75±0.19^{Cb}
	90	16.06±0.18^{Ba}	16.34±0.17^{Aa}	15.81±0.12^{Ca}
Fat	Fresh	27.69±0.38^{Bd}	28.22±0.48^{Ad}	27.38±0.81^{Cd}
	30	28.76±0.10^{Bc}	29.37±0.21^{Ac}	27.85±0.13^{Cc}
	60	30.75±0.21^{Bb}	32.37±0.58^{Ab}	29.49±0.22^{Cb}
	90	32.41±0.34^{Ba}	33.51±1.26^{Aa}	31.04±0.21^{Ca}
Fat / DM	Fresh	50.20±0.10^{Cd}	50.52±0.46^{Ad}	50.28±0.61^{Bd}
	30	50.68±0.18^{Bc}	51.15±0.39^{Ac}	50.30±0.14^{Cc}
	60	52.05±0.47^{Bb}	52.71±0.42^{Ab}	51.41±0.26^{Cb}
	90	53.15±0.22^{Aa}	54.00±0.60^{Ca}	52.47±0.23^{Ba}
Ash	Fresh	3.29±0.03^{Bb}	4.94±0.16^{Ab}	4.76±0.47^{Ab}
	30	3.50±0.10^{Bb}	4.88±0.10^{Ab}	4.84±0.07^{Ab}
	60	3.85±0.10^{Ba}	5.08±0.36^{Aa}	4.96±0.06^{Aa}
	90	3.92±0.04^{Ba}	5.14±0.21^{Aa}	5.05±0.22^{Aa}
Salt	Fresh	2.28±0.03^{Bd}	2.69±0.10^{Ad}	2.20±0.22^{Cd}
	30	2.91±0.08^{Bc}	3.22±0.09^{Ac}	2.41±0.06^{Cc}
	60	3.08±0.02^{Bb}	3.62±0.21^{Ab}	2.98±0.20^{Cb}
	90	3.46±0.24^{Ba}	3.70±0.13^{Aa}	3.08±0.23^{Ca}
Salt/Moisture	Fresh	5.08±0.16^{Bd}	6.10±0.15^{Ad}	4.83±0.28^{Cd}
	30	6.73±0.08^{Bc}	7.57±0.24^{Ac}	5.39±0.22^{Cc}
	60	7.52±0.09^{Bb}	9.04±0.15^{Ab}	7.22±0.25^{Cb}
	90	8.88±0.21^{Ba}	9.74±0.21^{Aa}	7.29±0.16^{Ca}

There is a significant difference between treatments (means ± SE) for different capital letters in the same row and different lowercase letters in the same column at $p<0.05$ over the ripening period. (C): control Gouda cheese; (BS): black seeds Gouda cheese; (CS): chia seeds Gouda cheese.

Table 3. Ripening indices of Gouda cheese as affected by adding black or chia seeds during ripening period at 14°C for 90 days

Property	Ripening period (days)	Treatments		
		C	BS	CS
SN/TN Ratio (%)	Fresh	8.43±0.25^{Ad}	8.44±0.38^{Ad}	8.46±0.34^{Ad}
	30	11.20±0.55^{Ac}	11.28±0.26^{Ac}	11.80±0.18^{Ac}
	60	14.81±0.14^{Ab}	14.92±0.28^{Ab}	15.65 ±0.22^{Ab}
	90	17.34±0.30^{Aa}	17.75±0.32 ^{Aa}	18.20 ±0.10^{Aa}
Tyrosine (mg/100g)	Fresh	10.92±0.35^{Cd}	11.16±0.33^{Bd}	11.92±0.21^{Ad}
	30	50.73±0.28^{Cc}	51.13±0.22^{Bc}	51.96±0.05^{Ac}
	60	93.76±0.22^{Cb}	94.21±0.27^{Bb}	96.88±0.34^{Ab}
	90	154.88±0.93^{Ca}	155.56±0.32^{Ba}	156.13±0.12^{Aa}
Tryptophan (mg/100g)	Fresh	26.11±0.50^{Cd}	26.20±0.10 ^{Bd}	26.29±0.26^{Ad}
	30	38.22±0.24^{Cc}	38.48±0.20^{Bc}	42.15±0.20^{Ac}
	60	47.16±0.25^{Cb}	47.53±0.25^{Bb}	50.77±0.55^{Ab}
	90	54.12±0.31^{Ca}	54.60±0.23 ^{Ba}	60.12±0.16^{Aa}
Total volatile fatty acids (ml 0.1N NaOH/100g cheese)	Fresh	12.58±0.32^{Cd}	12.81±0.21^{Ad}	12.70±0.10^{Bd}
	30	16.27±0.38 ^{Cc}	18.75±0.20^{Ac}	18.63±0.25^{Bc}
	60	19.53±0.36^{Cb}	21.56±0.12^{Ab}	21.00±0.22^{Bb}
	90	25.43±0.37 ^{Ca}	30.17±0.31^{Aa}	29.30±0.14 ^{Ba}
Acidity (%)	Fresh	1.75±0.10^{Ab}	1.59±0.03 ^{Bb}	1.58±0.17 ^{Bb}
	30	1.87±0.10^{Ab}	1.60±0.20 ^{Bb}	1.62±0.11 ^{Bb}
	60	1.89±0.09^{Ab}	1.67±0.12 ^{Bb}	1.67±0.10^{Bb}
	90	1.95±0.04^{Aa}	1.78±0.05 ^{Ba}	1.89±0.05 ^{Ba}
pH	Fresh	5.53±0.24^{Aa}	5.78±0.02^{Aa}	5.72±0.28 ^{Aa}
	30	5.48±0.46 ^{Aa}	5.67±0.23^{Aa}	5.62±0.20 ^{Aa}
	60	5.38±0.17^{Aab}	5.52±0.18^{Aab}	5.50±0.26^{Aab}
	90	5.25±0.11^{Ab}	5.44±0.17^{Ab}	5.36±0.31^{Ab}

There is a significant difference between treatments (means ± SE) for different capital letters in the same row and different lowercase letters in the same column at $p<0.05$ over the ripening period. (C): control Gouda cheese; (BS): black seeds Gouda cheese; (CS): chia seeds Gouda cheese.

3.3.Antioxidant characteristics of Gouda cheese

3.3.1. Total phenolic content

The effect of BS or CS Gouda cheese on the total phenolic content (TPC) are illustrated in Table 4. The TPC of Gouda cheese treatments represented highly significant differences ($p<0.05$) within the type of cheese and ripening period. CS Gouda cheese had a higher mean TPC value than other cheeses (72.48±0.25 µg GAE/g), followed by BS Gouda cheese (68.86 ±0.10 µg GAE/g) as compared to control (51.38 ±0.19 µg GAE/g). These results concur with Hosseini *et al.* [59] and Kwon *et al.* [60], who found that CS extract significantly raised the TPC of samples when added to yoghurt and Ricotta cheese compared to the control. However, each cheese treatment displayed a similar trend, with high TPC content in fresh samples and decreasing later after 90 days of ripening. This is consistent with Marchiani *et al*. [61] and Giroux *et al*. [62]. It was reported that the total phenolic content of BS and CS (0.87–167.22; 3.42 ±0.06 mg GAE/g sample, respectively) [63,64]. BS and CS Gouda cheese has a higher phenolic content because BS and CS contain high levels of phenolic compounds. Considering that BC and CS could be important sources of phenols and flavonoids [65]. Moreover, there is a hydrophobic association and/or hydrogen bonding between the hydroxyl groups of polyphenolics and the undissociated carboxyl groups in the peptide residues of proteins, which can cause reversible complexation between phenolics and protein residues [66]. Certain simple polyphenols have the ability to cross-link protein particles at high phenolic concentrations in addition to binding to specific protein sites at low phenolic concentrations [67].

3.3.2. DPPH radical scavenging activity

Table 4 shows the effects of black or chia seeds on antioxidant activity as determined by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) inhibition assay (AA) of Gouda cheese. The incorporation of CS or BS into Gouda cheese curd increased antioxidant activity either fresh or after 90 days compared to the control (42.58±0.38-37.97±0.14, 30.48±0.32-25.96±0.30, and 21.16±0.24-15.73±0.23%, respectively). During ripening, the antioxidant activity tended to be mildly decreased. The powerful free radical scavenging effect of BS and CS ($p<0.05$) can be illustrated by the increased concentrations of antioxidant flavonoids and phenolic acids [68,69]. Moreover, milk components as casein, whey proteins, selenium, zinc, catalase, glutathione peroxidase, superoxide dismutase, vitamin C, E, and beta-carotene and cheese itself, possess antioxidant activity because of the existence of different bioactive water-soluble peptides due to the proteolysis of milk proteins in the time of the ripening process [54,58,70-73].

The phenolic compounds' oxidation in cheese samples caused a gradual decline in the antioxidant activity of the samples, which agreed with the results of Hosseini *et al*. [59]. Furthermore, the interaction between the hydroxyl groups of phenolic acids, proteins, and polysaccharides could influence the absorption of natural antioxidants within the cheese matrix [74]. Additionally, a decline in antioxidant activity may result from the cheese's moisture content decreasing as the ripening period advances. Corresponding to our results, Ozer *et al*. [75] indicated a decline in the moisture of the feed as a consequence of lower total antioxidant activity values. However, the capability of BS and CS to scavenge free radicals with DPPH was greater than that of the control cheese. CS has been determined to exhibit DPPH free radical scavenging activities of 197.5±7.4 µmol Trolox equivalents (TE)/g and IC_{50} =2.001 mg/ml [62]. Furthermore, it has been shown by Hosseini *et al*. [59] and Beltrán-Orozco *et al*. [76] that the free CS extract had DPPH values of 50.48% and 41.1 µmol TE/g, respectively. There are numerous bioactive and functional phenolic components, as gallic, myricetin, rosmarinic acid, querencetin, cinnamic acid, kaempferol, caffeic acid, alpha-linoleic acid, and caffeic acid. In addition to vitamin C, carotenoids, tocopherols, phytosterols, and flavonoids have been identified as the source of the noticeable AA in chia seeds [26,68,77-81]. In the literature, BS extracts revealed inhibitory activity against butyrylcholinesterase (BChE) (inhibitory concentrations of 50% [IC_{50}] 79.11±6.06 µg/ml); catalase (CAT) with an IC_{50} value of 6.61± 0.27 µg/ml; 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) 7.31-2495.85 µmol/g [82]. The researchers explained the high AA activity of black seed to the existence of significant amounts of phenolic components as carvacrol, 4-terpineol, thymol, thymohydroquinone, etc. [83].

3.3.3. Ferric-reducing antioxidant power assay (FRAP)

The FRAP method is used to evaluate the reducing ability of Fe (III) iron to Fe (II) iron [84]. The beneficial effects of phenolic and antioxidants found in BS and CS have been established in numerous research [68,85]. The literature clarifies that the reducing properties are often linked to the existence of phenolic molecules such as quercetin, kaemferol, caffeic acid and chlorogenic acid in CS [86, 87]. While reductones (i.e., carvacrol, thymol, 4-tertbutylcatechol, etc.) possess the hydrogen-donating ability for BS [88]. BS or CS have an antioxidant effect by breaking the chain of free radicals. The effects of black or chia seeds on the ferric-reducing antioxidant power assay (FRAP) of Gouda cheese are illustrated in Table 4. According to the results of the reducing power, it is noted that the CS and BS treatments have shown higher reducing power ($p<0.05$) than the control. During ripening time FRAP tended to be mildly decreased. However, the FRAP value of Gouda cheese either fresh or after 90 days of ripening with BS or CS exceeded that of the control ($p<0.05$) (8.90±0.16-7.63±0.11, 11.72±0.27-10.11±0.11, and 7.82±0.16-5.96±0.15 mmol $FeSO_4$/g, respectively).

3.4. Organic acid analysis

The organic acids present in cheese are the consequence of normal biochemical processes that cause significant changes during ripening [89,90]. This is the result of many metabolic activities [91, 92]. In particular, lactate and citrate are essential precursors for a number of reactions that lead to the formation of lactic acid, acetic acid, citric acid, formic acid, and pyruvic acid [93]. Most lipases produce butyric acid during lipolysis [94]. Seven organic acids were measured in Gouda cheeses because they have an impact on the synthesis of compounds that give flavor and aroma (Table 5). Rennet enzymes and starter cultures are the primary sources of organic acids

during ripening. Califano and Bevilacqua [43], Jo *et al*. [54], and Ahmed *et al*. [95] reported that there is a direct relationship between temperature and time in the formation of lactic, acetic, citric and pyruvic acids in Gouda cheese. Table 5 shows that the ratio of organic acids increased within the ripening period. These results agreed with the results of the analysis of the content of organic acids in Gouda cheese by Jo *et al.* [54]; Akalin *et al.* [96]; Lues and Bekker [97]; Ong *et al.* [98]; Skeie *et al.* [99] during the time of ripening of pickled white cheese, Cheddar cheese, and probiotic Cheddar cheese, respectively.

Table 4. Antioxidant properties of Gouda cheese as influenced by the addition of black or chia seeds during the ripening at 14°C for 90 days

Antioxidant activity	Ripening period (days)	Treatments		
		C	BS	CS
TPC (µg GAE/g)	Fresh	51.38 ± 0.19^{Ca}	68.86 ± 0.10^{Ba}	72.48 ± 0.25^{Aa}
	30	47.18 ± 0.23^{Cb}	64.12 ± 0.19^{Bb}	66.74 ± 0.26^{Ab}
	60	46.90 ± 0.09^{Cc}	53.62 ± 0.29^{Bc}	63.70 ± 0.30^{Ac}
	90	43.82 ± 0.19^{Cd}	51.94 ± 0.09^{Bd}	58.38 ± 0.47^{Ad}
DPPH radicals scavenging activity (%)	Fresh	21.16 ± 0.24^{Ca}	30.48 ± 0.32^{Ba}	42.58 ± 0.38^{Aa}
	30	19.44 ± 0.21^{Cb}	28.64 ± 0.21^{Bb}	39.27 ± 0.12^{Ab}
	60	16.59 ± 0.18^{Cc}	26.80 ± 0.17^{Bc}	38.10 ± 0.31^{Ac}
	90	15.73 ± 0.23^{Cd}	25.96 ± 0.30^{Bd}	37.97 ± 0.14^{Ad}
FRAP (mmol $FeSO_4$/g)	Fresh	7.82 ± 0.16^{Ca}	8.90 ± 0.16^{Ba}	11.72 ± 0.27^{Aa}
	30	7.10 ± 0.12^{Cb}	8.44 ± 0.16^{Bb}	11.14 ± 0.20^{Ab}
	60	6.21 ± 0.26^{Cc}	8.90 ± 0.21^{Ba}	10.58 ± 0.23^{Ac}
	90	5.96 ± 0.15^{Cd}	7.63 ± 0.11^{Bc}	10.11 ± 0.11^{Ad}

There is a significant difference between treatments (means ± SE) for different capital letters in the same row and different lowercase letters in the same column at $p<0.05$ over the ripening period. (C): control Gouda cheese; (BS): black seeds Gouda cheese; (CS): chia seeds Gouda cheese.

While the fermentation process is ongoing, lactose is converted into lactic acid, which is considered essential to the quality, processing, and the initial stages of cheese ripening [47,90]. Higher organic acid content was caused by a rapid increase in lactic acid concentration, which varied from 874.34± 0.46 to 967.33±0.11 mg/1000g ($p<0.05$) during the ripening of the cheese. In all cheese treatments, lactic acid concentrations were significantly higher than those of other acids. Lactic acid can also change through a number of additional pathways to produce different organic acids and flavor components [90]. Citric acid exhibited irregular changes as well with a slight tendency to decline towards the end of ripening, reaching 9.56 ±0.29 to 30.32 ±0.34 mg/1000g (Table 5); comparable outcomes were mentioned by Akalin *et al*. [96]. This decline could be explained by citrate metabolism starters such as *Lactococcus lactis* subsp. *cremoris*, which utilize citrate (Cit+) as an energy source. Thus, citrate serves as both a substrate and a product in the Krebs or citric acid cycle; citrate is often co-metabolized with other sugars such as lactose, and the Gouda cheese's texture is impacted by the resulting CO_2 and leads to the "eye" formation in some Gouda cheese [90]. Furthermore, citrate can be used by the starter as a substrate to produce pyruvic acid [100]. Citric acid can be further metabolized into a number of flavor compounds, primarily 2,3-butanedione (diacetyl) and acetoin [89]. Formic acid ranged from 3.69±0.29 to 28.30±0.36 mg/1000g of cheese, then increased in the second month and remained mostly constant thereafter (Table 5). The initial rise in formic acid could be explained by the presence of *Lactococcus lactis* subsp. *Lactis* that encode enzymes involved in the reduction of pyruvate to several end-products other than homolactic acid, including acetic acid and formic acid from lactose [101]. Comparable results were obtained by Califano and Bevilacqua [43] for various cheeses. Short chain fatty acids, including propionic acid and butyric acid, were released due to the lipolytic and proteolytic actions of starters and secondary microflora [89]. Moreover, the breakdown of the milk fat globule membrane during the cheesemaking process can aid in the lipolysis process and the release of fatty acids like butyric acid as well [102]. The low level of butyric acid (ranged from 2.13±0.10 to 9.45±0.33 mg/1000g), which may be due to the fact that cow's milk has a lower fat content than buffalo milk, resulting in cheese containing a lower concentration of butyric acid. At the end of ripening, the amounts of propionic acid and succinic acid were higher than ($p<0.05$) butyric acid. In the case of acetic acid, it was created through multiple reactions by lactic acid bacteria during the early ripening phase [98]. It could also be caused by the breakdown of citric acid. Acetic acid showed irregular changes ($p<0.05$) throughout the ripening period. The highest percentage was recorded at 60 days of ripening period, reaching 30.15± 0.24 mg/1000g.

3.5. Microbiological changes

The total lactobacilli and lactococcus counts, the proteolytic and lipolytic bacterial counts, and the yeasts and molds counts of the Gouda cheese treatments during the 90-day ripening time at 14°C are displayed in Figure 2 (a), (b), (c), (d), and (e). The lowest counts of lactobacilli and lactococci were observed for the control cheese (7.87 and 6.40 log cfu ml^{-1}) in comparison to the BS (7.98±0.12 and 6.60±0.10 log cfu ml^{-1}) and CS (7.93±0.10 and 7.00 ±0.09 log cfu ml^{-1}) treatments (Figure 2 a & b). As ripening progressed, all cheeses showed an overall decline in lactobacillus and lactococcus counts reached (7.26±0.10-5.90±0.09;7.34±0.25-5.97±0.19;7.52±0.24-

6.12±0.11 log cfu ml^{-1}) for C, BS, and CS, respectively. This may be related to unfavorable conditions for its growth in cheese, such as low pH, increased salt content, decreased fermentable carbohydrates, low ripening temperature, and low water activity. These outcomes align with the findings of Wang *et al.* [56]; Ong *et al.* [98]. A progressive rise in the number of lipolytic and proteolytic bacteria (Figure 2 c & d) was observed during the ripening process in all cheese treatments, reaching (6.49±0.13-6.33±0.20; 6.56±0.19-6.36±0.14; and 6.69±0.17-6.65±0.13 log cfu ml^{-1}) for C, BS, and CS, respectively. Similar observations were documented by Pereira *et al*. [57]; Ur-Rehman *et al*. [103]; El-Nagar *et al.* [104].

No yeasts and molds colonies appeared in any cheese treatments until 60 days of ripening (Figure 2 e). However, only a few colonies had been observed after 90 days of ripening (less than 3 log cfu ml^{-1}). The numbers of yeasts and molds were correlated with an increase in lactic acid and other acids, this lowered the pH and created the ideal environment for their growth. These findings are consistent with those of El-Nagar *et al.* [104]. It is noted that yeasts and molds numbers were lower in BS compared to C and CS. This may be due to the antiyeast activity of the BS quinones thymohydroquinone (THQ) and thymoquinone (TQ). This result agrees with Halamova *et al*. [105], who suggested that THQ and TQ are effective antiyeast agents. Additionally, the decline in yeast and fungal number may be due to the fact that Gouda cheese is typically aged with wax rind. The absence of coliform bacteria in all treatments might be attributed to the efficiency of pasteurization and proper hygiene practices throughout the manufacturing and ripening period of cheese treatments.

Table 5. Organic acid content (mg/1000g) of Gouda cheese as influenced by the addition of black or chia seeds during ripening period at 14°C for 90 days

Sample	Ripening time (days)	Lactic acid	Butyric Acid	Propionic acid	Acetic acid	Citric acid	Succinic acid	Formic acid
C	Fresh	875.51±1.27^{Cd}	2.13±0.10^{Ad}	13.10±0.13^{Cd}	12.67 ± 0.23^{Bd}	3.65± 0.14^{Cd}	19.68±0.16^{Cd}	5.68±0.20^{Bd}
	30	1099.2 ±0.10^{Cc}	4.28± 0.17^{Ac}	15.28±0.15^{Cc}	16.17±0.22^{Bc}	5.55±0.22^{Cc}	31.56±0.15^{Cc}	10.64±0.2^{Bc}
	60	1204.28 ±0.5^{Cb}	7.63±0.18^{Ab}	33.21±0.26^{Cb}	28.32±0.24^{Ba}	10.98±0.30^{Ca}	37.91± 0.20^{Cb}	23.94±0.29^{Bb}
	90	1775.51±2.76^{Ca}	9.45±0.33^{Aa}	42.56±0.07^{Ca}	24.21±0.46^{Bb}	9.38± 0.27^{Cb}	49.84± 0.38^{Ca}	31.95± 0.06^{Ba}
BS	Fresh	967.33±0.11^{Bd}	1.24±0.13^{Cd}	13.36±0.28^{Bd}	13.50±0.28^{Ad}	17.30± 0.36^{Ad}	44.96 ±0.15^{Bd}	3.69± 0.29^{Bd}
	30	1689.25±0.15^{Bc}	2.25± 0.25^{Cc}	22.96±0.37^{Bc}	17.32±0.44^{Ac}	23.96± 0.41^{Ab}	70.99± 0.96^{Bc}	7.30± 0.17^{Bc}
	60	1752.23±0.97^{Bb}	3.14± 0.16^{Cb}	49.42± 0.53^{Bb}	29.64 ±0.44^{Aa}	25.65 ±0.29^{Aa}	90.36±0.11^{Bb}	28.71± 0.17^{Bb}
	90	1875.13±0.28^{Ba}	4.60± 0.28^{Ca}	60.69±0.13^{Ba}	26.31±0.39^{Ab}	22.62±0.15^{Ac}	92.27±0.31^{Ba}	32.88±0.31^{Aa}
CS	Fresh	874.34± 0.46^{Ad}	1.86±0.11^{Bd}	13.54±0.25^{Ad}	13.69± 0.26^{Ad}	9.56±0.29^{Bd}	52.23±0.57^{Ad}	28.30± 0.36^{Ac}
	30	1873.62±0.47^{Ac}	2.30± 0.16^{Bc}	28.30±0.20^{Ac}	18.10±0.86^{Ac}	12.21± 0.40^{Bc}	104.13±0.74^{Ac}	41.41±0.36^{Ab}
	60	1889.93± 0.61^{Ab}	3.58± 0.27^{Bb}	66.43±0.31^{Ab}	30.15± 0.24^{Aa}	27.32± 0.17^{Bb}	115.43± 0.29^{Ab}	47.31±0.43^{Aa}
	90	1955.34±1.09^{Aa}	4.94± 0.16^{Ba}	71.29± 0.34^{Aa}	27.34± 0.26^{Ab}	30.32 ±0.34^{Ba}	124.88± 0.29^{Aa}	41.41± 0.36^{Ab}

There is a significant difference between treatments (means ± SE) for different capital letters in the same column and different lowercase letters in the same column at $p<0.05$ over the ripening period. (C): control Gouda cheese; (BS): black seeds Gouda cheese; (CS): chia seeds Gouda cheese.

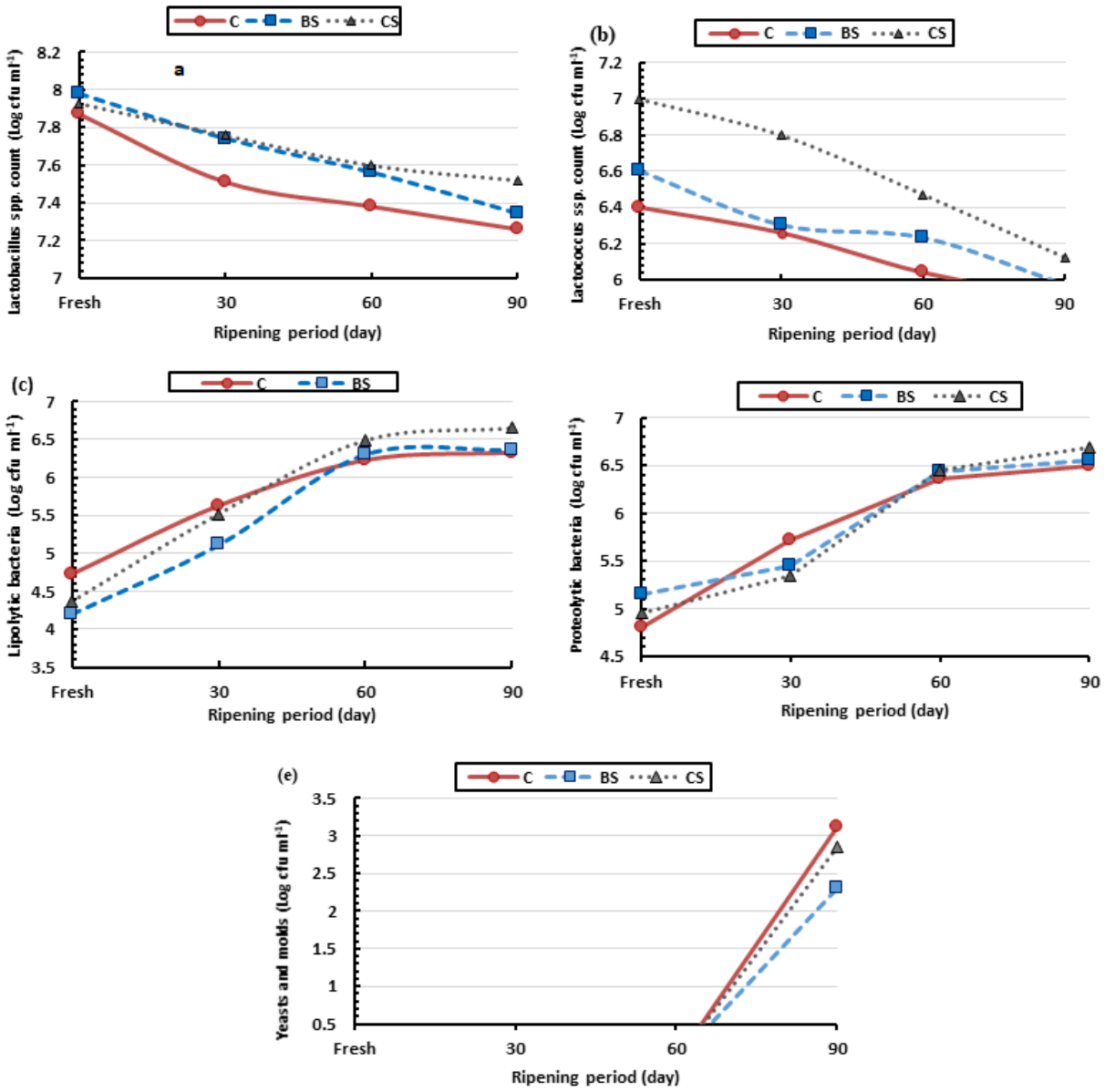

Figure 2. Microbial count in Gouda cheese as influenced by the addition of black or chia seeds during the ripening period at 14ºC for 90 days. C: Control Gouda cheese, BS: Black seeds Gouda cheese, CS: Chia seeds Gouda cheese

3.6. Texture profile analysis

According to Bourne [49], texture is the structure of food and is recognized as an essential factor for product acceptability that influences consumer perception. Table 6 shows the texture analysis of C, BS, and CS cheese treatments during the ripening period at 14°C for 90 days. Hardness is a mechanical structural characteristic that refers to the amount of force needed to compress the sample. After 30 days of ripening, hardening increased significantly for all cheeses. This can be correlated to moisture decrease, which serves as a plasticizer in the protein matrix and degradation of casein and an increase in interactions between proteins [106]. According to Creamer and Olson [107], the concentration of small peptides and free amino acids produced by casein hydrolysis increased during the ripening process. These compounds are capable of binding free water molecules, strengthening the casein matrix and increasing both hardness values and the resistance of cheese to deformation. However, the hardness was significantly lower (p<0.05) in CS (45.70±0.34 N) than in BS (56.20±0.10 N) or C (50.40±0.11 N) at all ripening times examined. The addition of chia seeds was the major contributor to the decrease in hardness in Gouda cheese, as chia seeds have a high water-retention capacity, which can absorb about twenty-three times their weight in water [108,109].

The work required to overcome the attraction between food and other surfaces is known as adhesiveness. Appropriate adhesiveness is beneficial for taste and flavor release [110]. Gouda cheese treatments represented a gradual increase in adhesiveness values during the ripening period and were recorded as 0.71±0.04, 0.61±0.03 and 0.32±0.03 (mj) for the C, BS and CS, respectively after 90 days of ripening period. It was reported that cheese adhesiveness correlated positively with fat in dry matter (FDM) and salt in moisture (S/M) levels [111]. Fat exists as globules in the protein matrix network of the curd and acts as a plasticizer to prevent the formation of cross-links between the casein chains [112]. Higher fat contents improve the melting point and adhesiveness of cheeses. The ratio of fat and salt in the cheese rises as its moisture level decreases during the period of ripening, which may lead to an increase in adhesiveness values. However, adhesiveness was lower in CS and BS than in C throughout the ripening period.

Cohesiveness is a mechanical structural characteristic that refers to the degree to which a food can be deformed before it breaks and results from the force exerted by internal links in the food. CS exhibited better cohesiveness when compared to C or BS. All treatments showed a decrease in cohesiveness during ripening, which is associated with the reduction in moisture amount [113]. Moreover, cheese cohesiveness was shown to decrease due to the proteolysis process during ripening. In contrast, cohesive forces in cheese and proteolysis have an inverse relationship [114]. Springiness is a mechanical structural property that relates to the speed and degree of recovery from a deformative force. It is noted that the springiness values decreased during the ripening period in treatments BS and CS compared to the control. The reason for the decline in springiness values could be due to the release of calcium ions from mono-calcium and dicalcium para-k-casien molecules and the enzymatic degradation of para-κ-caseinate molecules [115]. Compared to BS and CS treatments, the C treatment displayed more springiness (6.52±0.38, 6.59±0.01, and 6.60±0.10, respectively). Our findings agree with the data provided by Zheng *et al.* [116] they showed that Cheddar-type cheese has a high hardness but a low cohesiveness and springiness due to its low moisture level. The gumminess is the value that represents the amount of energy needed to break down the semi-solid sample into a state that facilitates swallowing. It is expressed as hardness × cohesiveness [117]. The gumminess of the various treatments shows notable variations between the cheeses prepared with chia or black seeds and the control sample. Changes in gumminess were similar to changes in hardness for C, BS, and CS because of the decrease in moisture content during the ripening process, these results are consistent with those of Park *et al*. [118]. CS Gouda cheese had lower gumminess value (5.52±0.08 N) than control and black seeds Gouda cheese (5.88±0.13 and 6.19±0.18 N).

Table 6. Texture profile analysis of Gouda cheese as influenced by the addition of black or chia seeds during ripening period at 14°C for 90 days

Treatment	Ripening period (Days)	Hardness (N)	Adhesiveness (mj)	Cohesiveness (Ratio)	Springiness	Gumminess (N)
Control	Fresh	9.80 ± 0.20^{Bd}	0.17 ± 0.02^{Ba}	0.60 ± 0.10^{ABc}	6.60 ± 0.10^{Aa}	5.88 ± 0.13^{Bd}
	30	22.90 ± 0.39^{Bc}	0.19 ± 0.0^{Bb}	0.67 ± 0.03^{Abb}	6.60 ± 0.21^{Aa}	15.34 ± 0.17^{Bc}
	60	38.90 ± 0.09^{Bb}	0.32 ± 0.02^{Bb}	0.70 ± 0.09^{ABab}	6.59 ± 0.20^{Ab}	27.23 ± 0.27^{Bb}
	90	50.40 ± 0.11^{Ba}	0.71 ± 0.04^{Ba}	0.74 ± 0.04^{Aba}	6.56 ± 0.11^{Ab}	37.30 ± 0.28^{Ba}
BS	Fresh	11.90 ± 0.35^{Ad}	1.40 ± 0.26^{Aa}	0.52 ± 0.11^{Bc}	6.52 ± 0.38^{Ca}	6.19 ± 0.18^{Ad}
	30	26.10 ± 0.27^{Ac}	0.19 ± 0.01^{Ab}	0.64 ± 0.03^{Bb}	5.89 ± 0.09^{Cb}	16.70 ± 0.26^{Ac}
	60	41.20 ± 0.14^{Ab}	0.22 ± 0.03^{Ab}	0.69 ± 0.01^{Bab}	5.46 ± 0.08^{Cc}	28.43 ± 0.38^{Ab}
	90	56.20 ± 0.10^{Aa}	0.61 ± 0.03^{Aa}	0.70 ± 0.02^{Ba}	5.14 ± 0.25^{Cc}	39.34 ± 0.26^{Aa}
CS	Fresh	08.90 ± 0.18^{Cd}	0.19 ± 0.01^{Cb}	0.62 ± 0.05^{Ac}	6.59 ± 0.01^{Ba}	5.52 ± 0.08^{Cd}
	30	21.50 ± 0.26^{Cc}	0.24 ± 0.04^{Cb}	0.69 ± 0.05^{Ab}	6.20 ± 0.10^{Bb}	15.6 ± 0.20^{Cb}
	60	30.80 ± 0.56^{Cb}	0.30 ± 0.10^{Ca}	0.72 ± 0.03^{Aab}	5.89 ± 0.27^{Bc}	14.84 ± 0.06^{Cc}
	90	45.70 ± 0.34^{Ca}	0.32 ± 0.03^{Ca}	0.76 ± 0.04^{Aa}	5.74 ± 0.12^{Bc}	34.73 ± 0.18^{Ca}

There is a significant difference between treatments (means ± SE) for different capital letters in the same row and different lowercase letters in the same column at $p<0.05$ over the ripening period. (C): control Gouda cheese; (BS): black seeds Gouda cheese; (CS): chia seeds Gouda cheese.

3.7. Sensory evaluation of cheese

Cheese quality is mostly determined by sensory perception, which is a complicated process. It is affected by numerous factors, including the amount of aromatic compounds, texture, and appearance [119,120]. Consumer acceptability of Gouda cheeses is mostly influenced by flavor. Nevertheless, there is an expectation of an ideal texture, and deviation from the desired or expected texture has an adverse impact on total satisfaction [121]. The results of the panel scores for the three treatments of Gouda cheese, whether fresh or during ripening period, are represented in Figure 3 (a), (b), (c), and (d). With a total score out of 9 – point hedonic scale for flavor; consistency; color and appearance; overall acceptability. The flavor scores were gradually increased over the ripening period. All cheese treatments had higher flavor scores at the end of the ripening period, which may be due to partial proteolysis and lipolysis of the cheese and the release of compounds that are subsequently broken down into various volatile flavor compounds by intracellular enzymes released by the starter bacteria in the cheese. These findings agree with Wang *et al*. [56];

Collins *et al*. [122]; Semeniuc *et al*. [123]; Su Oh *et al*. [124]. Anyway, the sweet and mild flavor of Gouda cheese is a result of fermentation and ripening, which also contribute to its rich and variable flavor profile [125]. Figure 3 (a) demonstrates that BS gained higher score values for flavor. This may be explained by the presence of thymoquinone which is a type of phytochemical molecules [126]. According to Cakir *et al*. [127], the addition of black seeds and the increase of volatile compounds content (total concentrations of alcohols, aldehydes, ketones, sulfur compounds, esters, terpenes, and other compounds) improved sensory properties of Erzincan Tulum (traditional Turkey cheese) both qualitatively and quantitatively. Regarding consistency, the panel scores during cheese ripening demonstrate that the addition of CS enhanced the consistency of Gouda cheese. It might be explained by the lower hardness levels, as chia seeds have the ability to bind water. A little weakness in the consistency of Gouda with BS was observed other than the control. There are slight differences between the treatments regarding the sensory evaluation values of color and appearance, and there are no significant differences between them ($p > 0.05$). Based on the findings, fresh cheese from all treatments had approximately equal score points for color and appearance, whether fresh or during the ripening period, and no significant differences were found ($p > 0.05$). However, it is noteworthy that the overall acceptability of all cheese types increased significantly after the first month of ripening ($p<0.05$). There is a significant difference ($p<0.5$) in flavor and overall acceptability for the formulated cheeses during the ripening period.

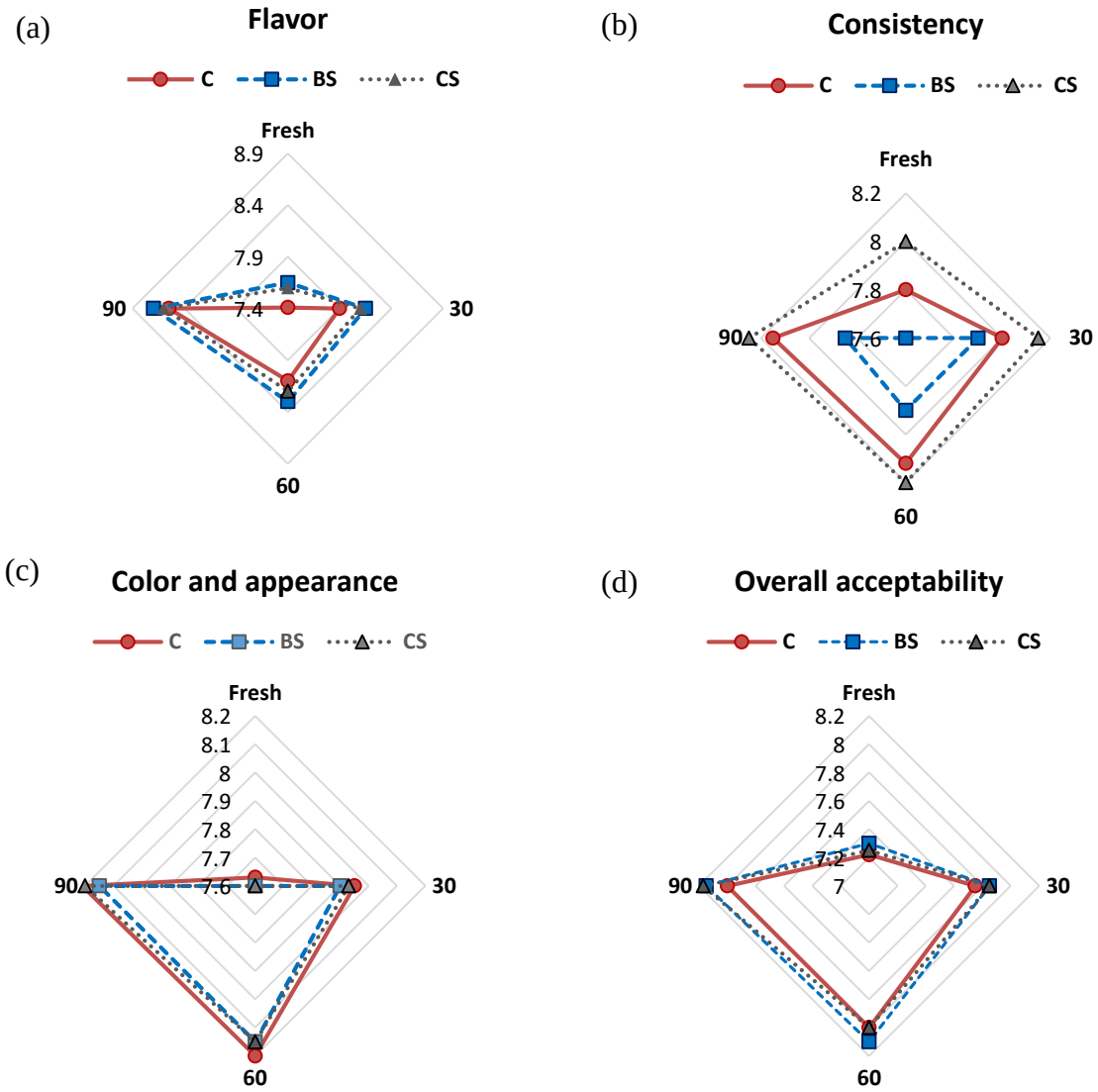

Figure 3. Sensory profile of Gouda cheese as influenced by the addition of black or chia seeds during the ripening period at 14ºC for 90 days. C: Control Gouda cheese, BS: Black seeds Gouda cheese, CS: Chia seeds Gouda cheese

4. Conclusion

This study suggests that black or chia seeds are used to naturally provide phenols that are high in antioxidants when manufacturing Gouda cheese from cow's milk. Incorporation of black or chia seeds into Gouda cheese significantly increased ($p<0.05$) the total phenolic content, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, and ferric reducing antioxidant power assay (FRAP) of the resultant cheese. The effect of the seeds was seen in the higher protein and ash content of the treated cheese compared to the control. Furthermore, there was an improvement in the textural and sensory properties of the produced cheese compared to the control. According to these findings, the addition of black or chia seeds as a rich source of natural antioxidant agents can provide nutritional value and improve all sensory attributes of Gouda cheese, making it healthier and more functional for consumers.

5. Conflicts of interest

The authors declare that they have no conflict of interest.

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