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Yogurt Enriched with Chia Seeds: Physicochemical, Microbiological, and Sensory Changes during Storage

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Abstract: This study investigates the physicochemical, microbiological, and sensory properties of yogurt fortified with honey and chia seeds at concentrations of 5% and 10% (*w*/*w*) over a 21-day storage period at 4 ± 1 °C. The addition of chia seeds resulted in initial pH increases, with 5% and 10% chia seeds having pH values of 4.22 ± 0.02 and 4.31 ± 0.01, respectively, compared to 4.20 ± 0.01 for the control yogurt. During storage, the pH decreased by 0.17 units for the control yogurt and by 0.08 and 0.13 units for 5% and 10% chia seeds, respectively. The titratable acidity (TA) increased significantly, by 9.1% for the control yogurt and by 22.9% and 29.6% for the 5% and 10% yogurt, respectively. The protein content increased from 3.22 ± 0.03 g/100 g in the control sample to 3.92 ± 0.08 g/100 g and 4.59 ± 0.00 g/100 g for 5% and 10% chia seeds, respectively, without any significant changes during storage. The number of lactic acid bacteria (LAB) was highest in the control sample (9.005 ± 0.007 log CFU/g) and lowest in the 10% yogurt with chia seeds (8.495 ± 0.007 log CFU/g), with all samples remaining free of harmful microorganisms. The sensory evaluation showed that yogurt with 5% chia seeds received the highest overall acceptability scores, while yogurt with 10% chia seeds had poorer scores for taste and texture. Overall, fortification of yogurt with 5% chia seeds improves its nutritional value and sensory properties without compromising product safety or microbiological quality.

Keywords: yogurt; chia seed; functional foods; physicochemical properties; microbiological properties

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1. Introduction

Chia seeds (*Salvia hispanica* L.), native to Mexico and belonging to the Lamiaceae family, are traditionally used in various forms, e.g., as chia oil, gruel, flour, or consumed whole [1]. With a nutritional profile that includes fiber (18–30%), ω-3 and ω-6 fatty acids, protein (23%), carbohydrates (41%), fats (30%), and ash (4%), these seeds have gained a lot of attention in recent years. Their reputation for providing numerous health benefits, such as blood pressure-lowering and antioxidant effects, contributes to their growing popularity among health-conscious consumers [2–4].

The growing consumer preference for health-promoting foods has fueled the expansion of the functional foods market. These foods, fortified with bioactive compounds such as probiotics and prebiotics, offer health benefits beyond just nutrition. Yogurt, a traditional fermented dairy product known for its probiotic content, is a prime candidate for such fortification [5–7]. The addition of chia seeds to yogurt represents a new approach to enhancing its nutritional and functional properties, in line with the current trend towards functional foods.

Although yogurt naturally contains bioactive compounds produced during fermentation (such as probiotics and health-promoting metabolites), it is often not primarily recognized for these compounds compared to other fortified or enriched foods. However, the fortification of yogurt with various nutrients is becoming increasingly important in dairy science. As dairy products are highly perishable, careful handling during processing, storage, and distribution is essential. The addition of chia seeds could increase the nutritional value and functional properties of yogurt. Chia seeds not only provide important nutrients but can also influence the texture, flavor, and overall quality of yogurt—crucial factors for successful incorporation.

The fermentation of milk by lactic acid bacteria, such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, results in different types of yogurt [8]. Lactic acid bacteria (LAB), including *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Bifidobacterium*, are of central importance for the technology of fermented milk products. These bacteria produce bioactive compounds with nutritional and therapeutic value, such as antioxidants and antidiabetic agents [9]. Yogurt, known for its health benefits, can be further enriched with bioactive compounds and nutrients that alter its matrix and protein profile during gastrointestinal digestion [10]. As a result, yogurt is increasingly being fortified with various plant or animal ingredients to compensate for nutritional deficiencies [11]. Recent studies have investigated the addition of seeds to yogurt to increase its nutritional value, such as yogurt fortified with 1% poppy seeds [12] and controlled yogurt with black cumin seed extract (*Nigella sativa* L.) [13]. Chia seeds have also been studied; Eker and Karakaya [14] investigated the effects of 2% chia seeds on the nutritional properties of yogurt, and Shori [9] developed yogurt with coriander and cumin seeds. The sensory properties of yogurt enriched with various seeds are often compromised, leading to the addition of honey in the production process [15].

The present study aims to evaluate the changes in the physicochemical, microbiological, and sensory properties of yogurt enriched with chia seeds at concentrations of 5% and 10% over a 21-day storage period at 4 ± 1 °C. By analyzing these changes, this study aims to determine the potential of chia seed-enriched yogurt as a functional food with improved nutritional and sensory properties.

2. Materials and Methods

2.1. Material

The chemical composition of bovine milk used for yogurt production was as follows: fat content of 3.69 ± 0.06%, lactose 4.40 ± 0.09%, non-fat solids (SNF) 12.66 ± 0.23%, proteins 3.28 ± 0.03%, and acidity 6.00 ± 1.00 °SH. The bovine milk was analyzed using a Lactoscan MCCWV1 (Sofia, Bulgaria).

A freeze-dried symbiotic starter culture (LBB BY 144–12) was employed for yogurt production. This starter culture, designed for direct inoculation, includes *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. This combination is known for providing medium acidity, medium viscosity, and a strong flavor to the yogurt.

The chia seeds (*Salvia hispanica* L.) were sourced from a local organic shop in Bulgaria. The nutritional composition of the chia seeds was as follows: fats 31.00 ± 0.50%, carbohydrates 44.00 ± 0.60%, and protein 16.00 ± 0.40%.

Organic herbal honey (0.38 ± 0.02% proteins, 75.10 ± 0.50% carbohydrates, 0% fats, and 24.62 ± 0.30% moisture) from Kalofer, Bulgaria, was used to enhance the sensory properties of the yogurt. The honey was used in its raw form without additional processing. The honey was used in its raw form without any additional processing.

The raw materials were tested for their microbiological quality in the form of samples, but no presence of microorganisms was detected even after 72 h of incubation.

2.2. Methods

2.2.1. Production of Yogurt with Different Amounts of Chia Seeds

Bovine milk was pasteurized at 84 ± 1 °C for 40 min. This temperature and duration were chosen based on standard pasteurization protocols to ensure microbial safety. Pasteurized milk (10 L) was produced in a milk pasteurizer (GEA, Düsseldorf, Germany) at LB Bulgaricum LTD, Production Base in Sofia, Bulgaria.

After pasteurization, the milk was cooled to 43 °C. Organic herbal honey was added at a concentration of 5% (*w*/*v*) and mixed thoroughly until dissolved.

The milk was inoculated with the freeze-dried starter culture (LBB BY 144–12) at a concentration of 40 mg/L. Homogenization was carried out at 1200 rpm for 60 s to ensure an even distribution of the starter culture.

The chia seeds were added to sterile plastic cups at concentrations of 5 g/100 g and 10 g/100 g. The inoculated milk was then distributed among these cups, mixed thoroughly, and incubated at a constant temperature of 43 ± 1 °C (Memmert GmbH, Schwabach, Germany) until the yogurt reached a pH of 4.6 ± 0.1 (approximately 240 min). All samples were produced in three batches (control without chia seeds, 5 g/100 g chia seeds, and 10 g/100 g chia seeds).

After fermentation, the yogurt was cooled to 4 °C and stored in the refrigerator for 24 h. The analysis was carried out on days 0, 7, 14, and 21.

Figure 1 shows a flow chart for the preparation of the yogurt with different amounts of chia seeds.

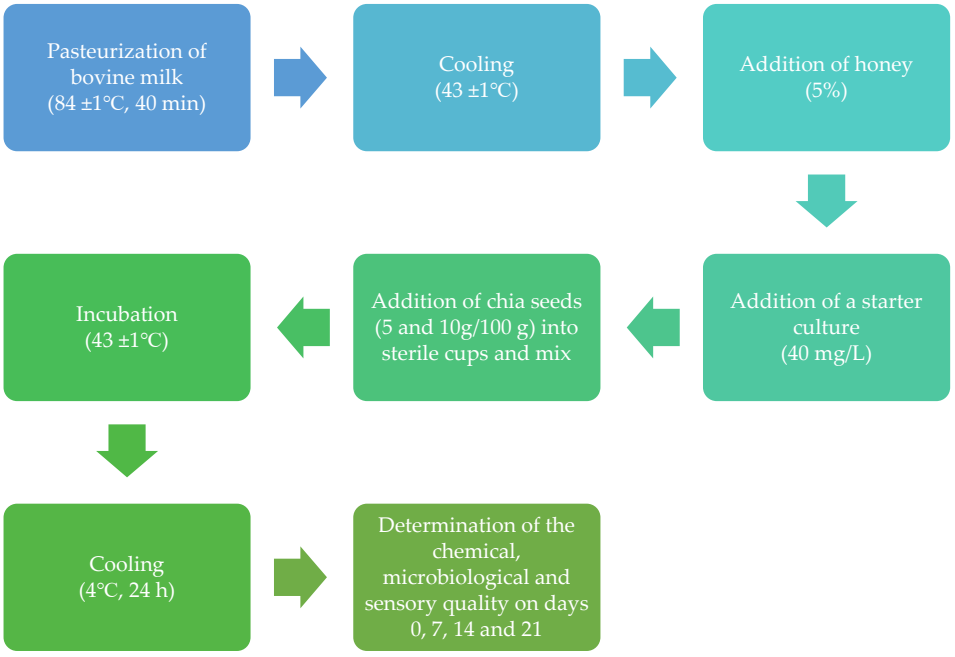


Figure 1. Flowchart for the preparation of yogurt with different amounts of chia seeds.

2.2.2. Microbiological Analysis

For the microbiological analysis, 10 g of yogurt sample was mixed with 90 mL of buffered peptone water (oxoid) in a sterile stomacher bag and homogenized in a blender.

The microbiological analyses followed the ISO standards: enumeration of *Enterobacteriaceae* (ISO 21528-2) [16], yeasts and molds (ISO 6611) [17], coagulase-positive staphylococci (ISO 6888-1) [18], *Salmonella* spp. (ISO 6579-1/A1) [19], *Listeria monocytogenes* and *Listeria* spp. (ISO 11290-1) [20], and *Lactobacillus* spp. and *Streptococcus* spp. (ISO 7889) [21]. Decimal dilutions and plate counting methods were used, with results expressed as CFU/mL. Bacterial counts were performed in triplicate.

The change in the number of *Lactobacillus* spp. and *Streptococcus* spp. during the storage of bovine yogurt was determined according to the method of BDS ISO 7889:2005 [22], with triplicate samples analyzed at each time point.

2.2.3. Changes in Chemical Properties during Storage

The changes in the chemical properties of enriched bovine yogurts with different proportions of chia seeds were determined using different methods over a storage period of 21 days. The proteins in the yogurt were determined according to BDS EN ISO 8968–1:2014 [23]. The pH value of the yogurt was determined using a pH meter (Testo SE & Co. KGaA, Lenzkirch, Germany). The titratable acidity of the yogurt was measured as Soxhlet–Henkel degree (°SH) according to BDS 1111: 1980 [24] and calculated using the following equation:

$$^{\circ}\mathrm{SH} = \mathrm{a}\cdot 2\cdot \mathrm{F}, \quad (1)$$

where *a* is the volume (mL) of 0.1 M sodium hydroxide used for the titration and *F* is the factor of 0.1 M sodium hydroxide.

All chemical analyses were performed in triplicate to ensure accuracy and reproducibility.

2.2.4. Sensory Analysis

The sensory analysis of the bovine yogurt was performed according to the European Union guidelines for ethics and food-related research, and informed consent was obtained from all participants [25]. The sensory evaluation aimed to assess the influence of chia seed concentration on the sensory properties of the yogurt during the storage period.

The sensory analysis was performed by a group of twenty subjects. All subjects had given informed consent prior to their participation and met the inclusion criteria. These included no health impairments of sensory perception (such as anosmia or color blindness) and a typical preference for dairy products.

The sensory evaluations were conducted in a special sensory analysis room equipped with individual tasting booths to minimize external influences and ensure a controlled environment. The room had neutral temperature and lighting conditions to avoid bias in the sensory evaluation.

The panelists were given a brief introduction to familiarize them with the objectives of this study and the yogurt samples to be evaluated. Each yogurt sample was presented in identically coded cups to avoid any bias. Samples were served at room temperature, and participants were instructed to rinse their mouths with water between tastings to cleanse the palate.

Sensory attributes assessed included aroma, taste, texture, aftertaste, and appearance. Each attribute was rated using a 9-point hedonic scale, with scores ranging from 1 to 9: extremely dislike (1), very strongly dislike (2), moderately dislike (3), slightly dislike (4), neither dislike nor like (5), slightly like (6), moderately like (7), very like (8), and extremely like (9). The overall sensory score for each yogurt sample was calculated by averaging the scores of the attributes evaluated. This overall score was used as one of the response variables to optimize the yogurt recipe and evaluate the acceptability of the different chia seed concentrations.

At the end of the evaluation period, the data collected from the sensory panel were analyzed to determine the preferences and acceptability of the yogurt samples. Statistical analysis was performed to identify significant differences in sensory characteristics between the samples with different chia seed concentrations.

2.2.5. Statistical Analysis

The results are expressed as mean ± standard deviation. Six replicates (two from every batch) were used for the physicochemical and microbiological parameters. For the sensory analysis, the number of participants was set at 20. To analyze the data, a two-way analysis of variance (2-way ANOVA) was performed to evaluate the effects of the different

chia seed concentrations and storage durations and their interactions on the measured variables. Subsequent comparisons between group means were performed using Tukey's Honestly Significant Difference (HSD) test. The analysis was performed using XLSTAT software, version 2019.2.2 (Addinsoft, New York, NY, USA), with the significance threshold set at $p \leq 0.05$.

3. Results and Discussion

3.1. Change in pH Value and Titratable Acidity during Storage of Yogurt

The pH value of yogurt is a decisive parameter for assessing its quality and safety during storage. Regular monitoring of pH and acidity is essential to ensure product safety and to maintain optimal taste and texture [26]. Our study shows that the addition of chia seeds has a significant effect on the pH value of the yogurt ($p < 0.05$).

On the first day, the pH of the control yogurt (without chia seeds) was 4.20 ± 0.01. In contrast, the chia seed-enriched yogurts had slightly higher initial pH values: 4.22 ± 0.02 for those with 5% chia seeds and 4.31 ± 0.01 for those with 10% chia seeds (Figure 2). This initial increase in pH due to the addition of chia seeds could be due to the buffering capacity of chia seeds, which may attenuate the immediate acidity. Although the increase in pH from 4.20 to 4.22 with the addition of 5% chia seeds is within the measurement error of the pH meter (±0.02) and may not be practically significant, the increase to 4.31 with the yogurt containing 10% chia seeds indicates a remarkable effect.

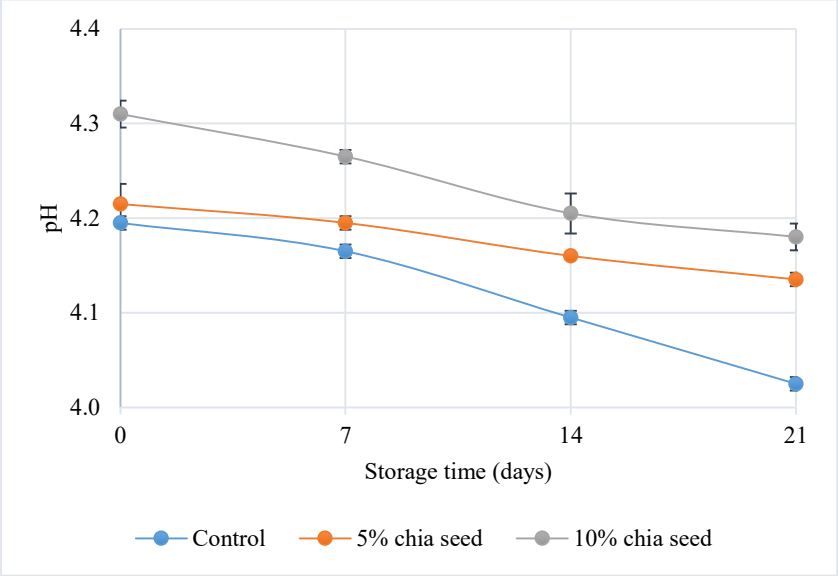


Figure 2. Changes in pH values during storage of bovine yogurt with different percentages of chia seeds.

Chia seeds have a buffering effect, mainly due to their composition, which contains proteins, fibers, and minerals. The proteins in chia seeds can interact with acids and bases through various biochemical reactions, helping to neutralize pH changes. The high fiber content in chia seeds can absorb and bind water, which helps stabilize pH by affecting the solubility and availability of acids and bases. Minerals such as calcium, magnesium, and potassium in chia seeds can also play a role in buffering by reacting with acids and bases to help maintain a stable pH environment. Together, these components contribute to the buffering capacity of chia seeds, which can help stabilize pH fluctuations in the yogurt matrix.

As storage progressed for 21 days, we observed a general decline in pH values in all yogurt samples. In particular, the pH of the control yogurt decreased by 0.17 units, which corresponds to a decrease of 4.05%. For the yogurts with 5% and 10% chia seeds, the pH decreased by 0.08 and 0.13 units, corresponding to a decrease of 1.90% and 3.07%,

respectively. The pH values given are average values. The standard deviations are included to reflect the variability of the measurements.

Statistical analysis of the pH values revealed significant differences between the individual storage days and chia seed concentrations (Table S1). In particular, a two-way ANOVA showed that both storage time and chia seed quantity significantly affected pH ($p < 0.0001$). Post hoc Tukey tests showed that the pH values of yogurts with different chia seed concentrations differed significantly from each other and from the control, especially at different storage intervals.

Our results are consistent with previous studies investigating the effects of chia seeds on yogurt pH. Ribes et al. [27] found a decrease in pH when chia seeds were added as a fat substitute, which is consistent with our results that chia seeds affect the initial pH but do not drastically change the overall trend in pH decrease during storage. Drużyńska et al. [28] observed a similar trend, with pH decreasing from 4.05 to 3.7 within 28 days, suggesting that chia seeds do not significantly affect the rate of pH change compared to plain yogurt.

While chia seeds change the initial pH of yogurt, they do not significantly alter the overall pH dynamics during storage. This suggests that despite their initial effect on pH, chia seeds do not drastically affect the basic acidification process of yogurt during its shelf life.

The following several properties of chia seeds could help inhibit acid formation or slow fermentation:

Mucilage and gel formation: Chia seeds are rich in soluble fiber, which forms a gel when hydrated. This mucilage can have a barrier effect, potentially slowing the movement and availability of substrates for fermentation by lactic acid bacteria (LAB). This could indirectly slow down acid formation [29].

Antimicrobial properties: Chia seeds contain phenolic compounds and other antioxidants that have antimicrobial properties. These compounds may be able to inhibit the growth of certain microorganisms, including those involved in fermentation, thus slowing down acid formation [30].

Water absorption and dilution effect: The high water absorption capacity of chia seeds can lead to a dilution effect in the yogurt matrix. This can reduce the concentration of fermentable sugars available for the LAB, potentially slowing down the fermentation rate and acid production [31].

Titratable acidity (TA) is a key parameter for assessing the quality and fermentation process of yogurt. In our study, the acidity of yogurts enriched with different proportions of chia seeds was analyzed over a storage period of 21 days (see Figure 3).

On the first day of storage, the control of the yogurt (without chia seeds) had a TA of 35.0 ± 0.28 °SH. In comparison, yogurts with 5% and 10% chia seeds had initial TA values of 34.0 ± 0.57 °SH and 32.4 ± 0.57 °SH, respectively. Over the storage period, all yogurt samples showed a significant increase in TA. In particular, the TA value of the control yogurt, increased by 9.1%, while the increases in the yogurts with 5% and 10% chia seeds were 22.9% and 29.6%, respectively ($p < 0.05$).

The observed increase in TA is consistent with previous studies. Kowaleski et al. [26] reported similar trends for yogurts with chia seeds and strawberries, where TA values increased due to the fermentation process. Wang et al. [32] found that continuous fermentation leads to the continuous production of lactic acid and other organic acids such as butyric acid, citric acid, acetic acid and formic acid, which are responsible for the increase in TA.

Statistical analysis using a two-way ANOVA, (see Table S2), revealed significant effects of both chia seed concentration and storage duration on TA ($p < 0.0001$). The addition of chia seeds resulted in lower initial TA values compared to the control ($p < 0.05$). However, the trend that TA increased during storage was the same for all samples. This suggests that although chia seeds affect the initial TA value, they do not significantly alter the overall trend in acidity increase during storage.

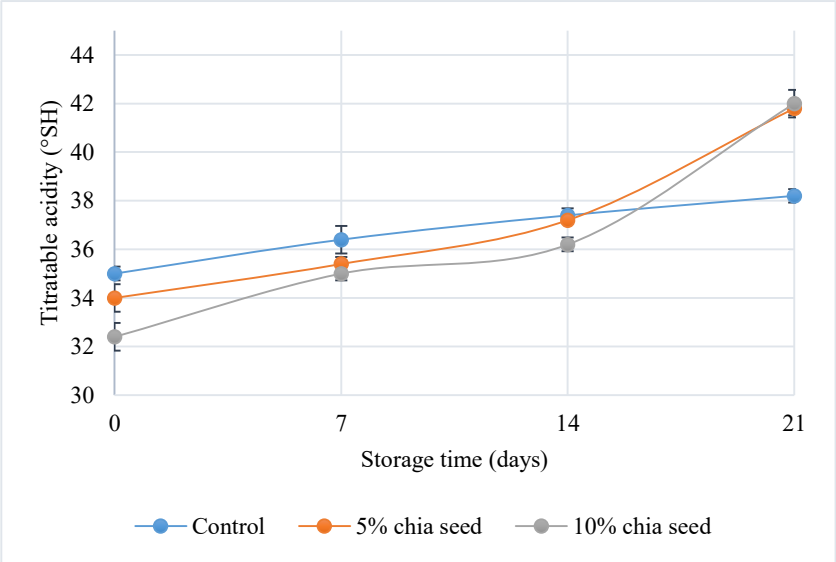


Figure 3. Changes in titratable acidity during storage of bovine yogurt with different percentages of chia seeds.

Significant main effects of storage time (F (3, 96) = 331.3, $p < 0.0001$) and chia seed concentration (F (1, 96) = 6.39, $p = 0.013$) on TA were observed. The interaction between these factors also had a statistically significant effect (F (6, 96) = 29.29, $p < 0.0001$). The increase in TA was more pronounced in the later stages of storage, which reflects the ongoing fermentation process, and is consistent with the results of previous studies [26,27].

The addition of chia seeds affects the initial TA of the yogurt but does not significantly change the general trend in increased acidity over time. The increase in TA during storage is primarily due to the natural fermentation processes in the yogurt.

3.2. Change in Chemical Composition during Storage of Yogurt

Chia seeds form chia seed mucilage (CSM) when they come into contact with water. This mucilage acts as an emulsifier and stabilizer, enhancing the rheological properties and stability of the yogurt while potentially reducing the calorie content of the product [27]. According to Tamime and Deeth [33] and Kim et al. [34], the most important characteristics of dairy products are their functional properties, storage stability, and low calorie content. Chia seeds form chia seed mucilage (CSM) on contact with water, which acts as an emulsifier, stabilizer, or hydrocolloid [35]. This mucilage's improve the rheological properties and stability of yogurt and can also reduce the calorie content [27]. Several mechanisms by which chia seeds can influence the calorie content of yogurt are discussed below as follows:

High fiber content: Chia seeds are rich in fiber, especially soluble fiber, which forms a gel-like substance when hydrated. This gel increases the volume of food without adding calories, as the fiber is not digested and absorbed by the human body. The presence of this gel can create a feeling of fullness and satiety, which can lead to lower calorie intake from other sources [36].

Water absorption: The high water absorption capacity of chia seeds contributes to the formation of the above-mentioned gel. This not only makes the yogurt more voluminous but also means that a smaller amount of yogurt is needed to achieve the same volume or weight, which can reduce the overall calorie content per serving [37].

Nutrient density: Although chia seeds are high in calories due to their fat content, the fats they contain (omega-3 and omega-6 fatty acids) are considered healthier compared to the saturated fats found in many dairy products. When added to yogurt, chia seeds can improve the nutritional profile by adding essential fatty acids and other nutrients without significantly increasing the calorie content compared to traditional fat sources [37].

Displacement effect: When chia seeds are incorporated into yogurt, they can partially displace other high-calorie ingredients. For example, if yogurt is normally consumed with sugary toppings or high-calorie additives, the addition of chia seeds can reduce the need for these additional ingredients and thus lower the overall calorie content of the final product [35].

Despite the mucilage formation, the overall fat content of the yogurt remains unchanged, suggesting that the chia seed mucilage primarily affects the texture and stability of the yogurt rather than its fat composition. This finding underscores the effectiveness of chia seed mucilage as a fat substitute, improving yogurt's texture and stability while maintaining its original fat content.

It is known that yogurt has a protein content comparable to that of raw milk [14]. Protein content is an important quality parameter in yogurt that reflects its nutritional value. In our study, the effect of the addition of chia seeds on the protein content of yogurt during a 21-day storage period was investigated. The results are summarized in Figure 4 and Table S3.

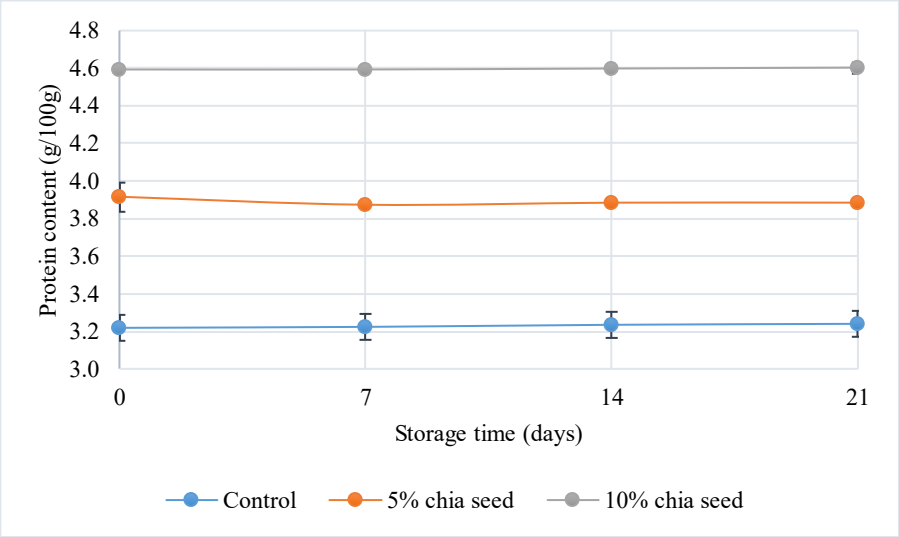


Figure 4. Protein content in fortified bovine yogurts with different proportions of chia seeds during the 21-day storage period.

On the first day of production, the protein content of the control yogurt (without chia seeds) was 3.22 ± 0.03 g/100 g. In contrast, the yogurts with 5% and 10% chia seeds had a higher protein content of 3.92 ± 0.08 g/100 g and 4.59 ± 0.00 g/100 g, respectively. The significant difference in protein content between the control yogurts and the chia seed-enriched yogurts ($p < 0.0001$) emphasizes the contribution of chia seeds, which are known for their high protein content (~19%) [38].

Figure 4 illustrates that the addition of chia seeds significantly increases the protein content in yogurt. This result is in line with previous studies by Ribes et al. [27], who observed a similar increase in protein content when chia seed mucilage was added to yogurt.

Statistical analysis using a two-way ANOVA, as shown in Table S3, confirmed that the addition of chia seeds significantly affected protein content ($F(2, 96) = 5347.4$, $p < 0.0001$). However, there were no significant changes in protein content during the storage period ($F(3, 96) = 0.261$, $p = 0.852$). This stability of protein content during storage suggests that the chia-enriched yogurts effectively maintained their protein content throughout the shelf life.

The interaction between chia seed concentration and storage time showed no significant effect ($F(6, 96) = 0.453$, $p = 0.830$), suggesting that the protein content remains stable regardless of the storage duration. This stability is in line with the Codex Alimentarius

Commission (2010) guidelines [39], which stipulate that yogurt should have a minimum protein content of 2.7%. Our yogurt samples exceeded this standard, confirming that they adequately meet the protein content requirements.

It has been observed that the protein content of yogurt, similar to the fat content, remains stable during storage. This stability is noteworthy as it indicates that the addition of chia seeds, which have a high protein content, did not affect the overall protein content of the yogurt over time. The stability of the protein content is significant, as it indicates that the nutritional value of the yogurt was maintained throughout the storage period.

However, changes in protein content during storage could theoretically be caused by factors such as enzymatic activity or microbial growth, which can affect protein solubility or lead to degradation. In view of these potential influences, it may be useful to investigate not only the total protein content but also the changes in the type of peptides present and the extent of protein hydrolysis over time. This approach would allow a more comprehensive understanding of protein dynamics and interactions within the yogurt matrix.

For example, the study by Khalil et al. [40] showed that while chia seeds increase the initial protein content of yogurt, the stability of protein content could be affected by storage conditions and duration. Analyzing protein hydrolysis or peptide profiles could provide further insight into how chia seeds affect yogurt at the molecular level and potentially reveal more about the interactions between chia proteins and the yogurt matrix during storage.

Chia seeds not only increase the protein content in yogurt but also introduce bioactive peptides with antioxidant properties. These peptides can protect proteins from oxidative damage, thereby enhancing the shelf life and nutritional quality of the yogurt [41]. Additionally, chia seeds contain trypsin inhibitors that may affect protein stability and digestion by inhibiting protease activity, which could otherwise lead to protein degradation during storage [42].

The addition of chia seeds increases the protein content of the yogurt, and this increased level is maintained throughout the storage period. The stability of the protein content in chia-enriched yogurt underlines the robustness of this nutritional enhancement.

3.3. Changes in the Microbial Composition of Yogurt during Storage

The microbiological quality of yogurt enriched with different concentrations of chia seeds was investigated over a period of 21 days at 4 ± 1 °C. The focus was on the microbial composition of the yogurt during storage. The focus was on the number of *lactic acid bacteria* (LAB), which are crucial for the probiotic properties of the yogurt, and on the presence of potentially harmful microorganisms.

Table 1 shows the LAB counts in yogurts with different chia seed concentrations over time. On day 0, the control yogurt (0% chia seeds) had the highest LAB count at 9.005 ± 0.007 log CFU/g. In comparison, the yogurt with 10% chia seeds had the lowest LAB count at 8.495 ± 0.007 log CFU/g. It is noteworthy that yogurt with 5% chia seeds had a higher LAB count (8.795 ± 0.007 log CFU/g) than yogurt with 10% chia seeds. These results suggest that higher concentrations of chia seeds are associated with lower LAB counts.

Table 1. Viability of fortified yogurts with different proportions of chia seeds during the 21-day storage period.

Total LAB (log CFU/g)	Day 0	Day 7	Day 14	Day 21
Control	9.005 ± 0.007	8.980 ± 0.014	8.845 ± 0.007	8.775 ± 0.007
5% chia seeds	8.795 ± 0.007	8.740 ± 0.000	8.620 ± 0.028	8.500 ± 0.141
10% chia seeds	8.495 ± 0.007	8.370 ± 0.014	8.315 ± 0.021	8.150 ± 0.212

Table 1 also illustrates the LAB counts across different storage days. During the 21-day storage period, the LAB count generally decreased for all yogurt samples. For the control yogurt, the LAB count decreased from 9.005 ± 0.007 log CFU/g on day 0 to

8.775 ± 0.007 log CFU/g on day 21. Similarly, the LAB count in yogurt with 5% chia seeds decreased from 8.795 ± 0.007 log CFU/g to 8.500 ± 0.141 log CFU/g, while yogurt with 10% chia seeds showed a decrease from 8.495 ± 0.007 log CFU/g to 8.150 ± 0.212 log CFU/g.

The observed decline in LAB counts over time is consistent with the general trend in probiotic products, where LAB viability tends to decline due to factors such as nutrient deficiency and accumulation of metabolic by-products. The decrease in LAB counts was more pronounced in yogurts with higher chia seed concentrations, especially in the 10% chia seed variant. This trend could indicate that the higher chia seed concentration affects the yogurt matrix or microbial environment in a way that is more detrimental to LAB survival over time.

The significant decrease in LAB numbers with increasing chia seed concentration is consistent with the findings of Kowaleski et al. [26], who indicated that approximately 6% chia seed is optimal for maintaining LAB viability. Higher concentrations of chia seeds could alter the yogurt matrix or create a less favorable environment for LAB.

A detailed statistical analysis, including means, variances, and two-way ANOVA results, can be found in Supplementary Table S4.

The microbiological analyses confirmed that all yogurt samples, regardless of chia seed concentration, met safety standards throughout the storage period. No harmful microorganisms, such as *Enterobacteria*, *yeasts* and *molds*, *coagulase-positive staphylococci*, *Salmonella*, or *Listeria monocytogenes*, were detected in any of the samples after 21 days of storage. This indicates that the yogurt maintained its safety profile and was free from pathogenic contamination, reflecting the effectiveness of the storage conditions and initial heat treatment.

The absence of pathogenic microorganisms and the overall stability of LAB counts despite varying chia seed concentrations emphasize the robustness of the yogurt in maintaining safety and quality standards. The observed decrease in LAB counts over time and at higher chia seed concentrations suggests that while chia seeds improve the nutritional profile of yogurt, their higher concentrations may affect the viability of LAB. This effect underlines the importance of optimizing the concentration of chia seeds to achieve a balance between nutritional value and microbial viability.

While chia seeds contribute positively to the nutritional profile of yogurt, their concentration needs to be carefully controlled to ensure optimal LAB viability and maintain the probiotic benefits of yogurt. The results provide valuable insights into how ingredient concentration and storage time can influence the microbiological quality of fermented dairy products.

3.4. Change in Sensory Analysis during Storage of the Yogurts

Figures 5–9 show the results of the sensory evaluation of yogurt samples with different chia seed concentrations (control, 5% chia seed, and 10% chia seed) on days 0, 7, 14, and 21 of storage. Table S5 contains the detailed results of a two-way ANOVA followed by a Tukey's HSD test for the sensory evaluation of yogurts fortified with different proportions of chia seeds over a storage period of 21 days. Sensory attributes evaluated included color, taste, aftertaste, texture, and overall acceptability.

The ANOVA results show significant effects of chia seed concentration and storage time on the sensory properties.

For color, the addition of chia seeds had a significant effect ($F(2, 11) = 21.527$, $p < 0.0001$), with values decreasing as the concentration of chia seeds increased. Storage time also had a significant effect on color ($F(3, 11) = 4.287$, $p = 0.007$), with a significant decline by day 14 and an even more significant decrease by day 21. This trend is shown visually in Figure 5. Higher concentrations of chia seeds have a negative impact on the visual appeal of the yogurt, which may influence consumer preference and perception of freshness.

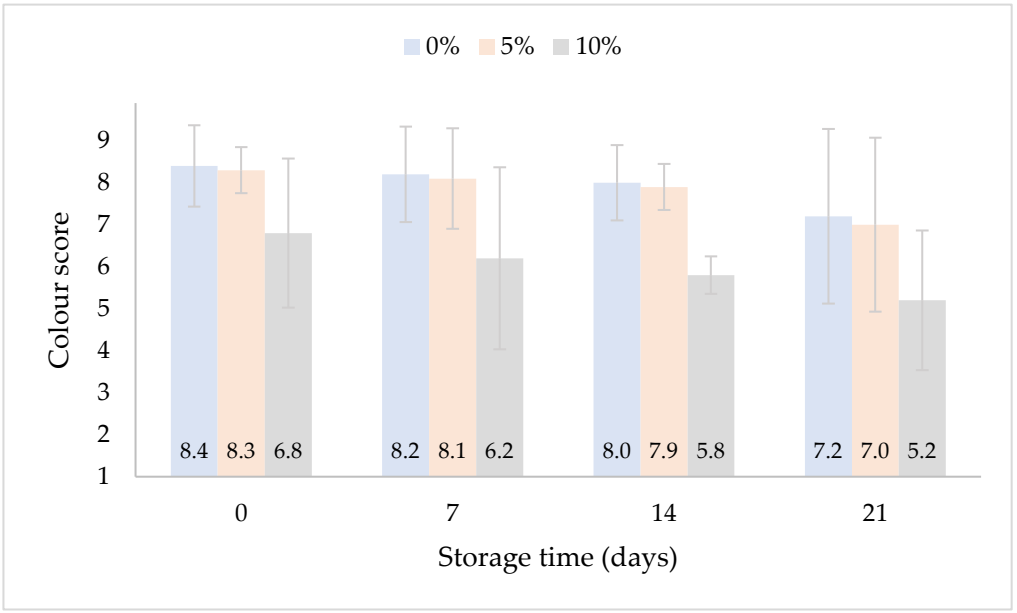


Figure 5. Color scores of fortified yogurts with different proportions of chia seeds during a 21-day storage period.

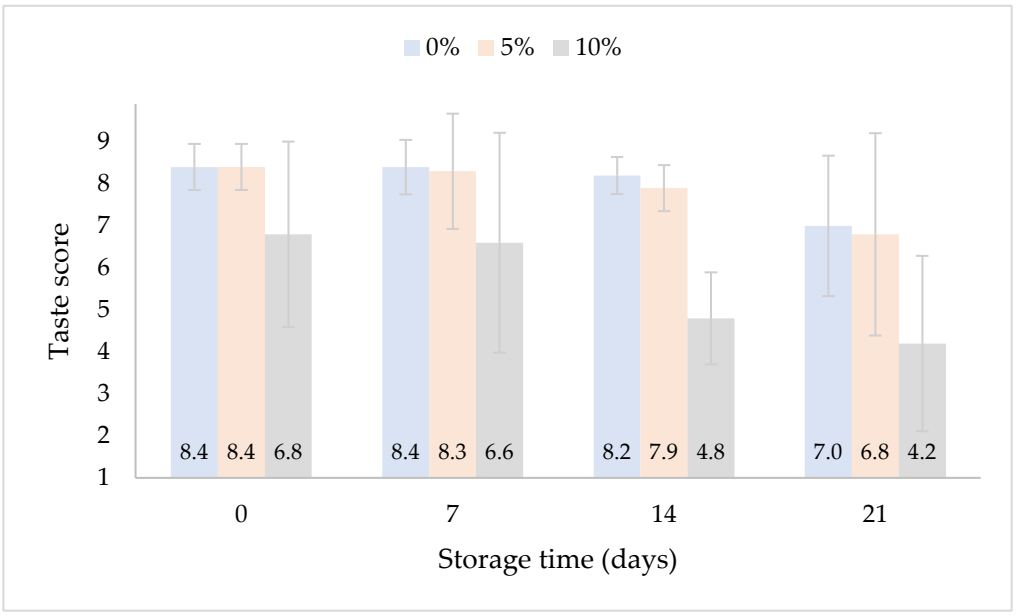


Figure 6. Taste scores of fortified yogurts with different proportions of chia seeds during a 21-day storage period.

In terms of taste, chia seed concentration had a significant effect ($F(2, 11) = 26.974$, $p < 0.0001$). Yogurts with 10% chia seeds had significantly lower flavor scores than those with 5% chia seeds. In addition, storage time had a significant effect on the flavor score ($F(3, 11) = 8.319$, $p < 0.0001$), with a significant decline observed by day 21, especially for the samples with 10% chia seeds. Figure 6 illustrates this trend. Lower taste scores at higher chia seed concentrations and longer storage time indicate that consumers may find the taste less pleasant due to increasing bitterness.

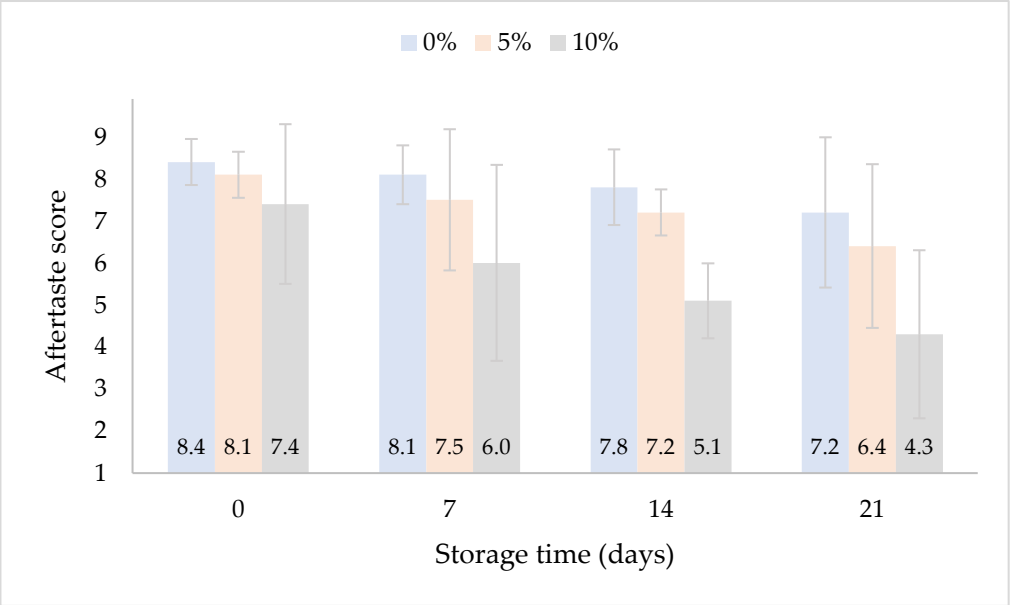


Figure 7. Aftertaste scores of fortified yogurts with different proportions of chia seeds during a 21-day storage period.

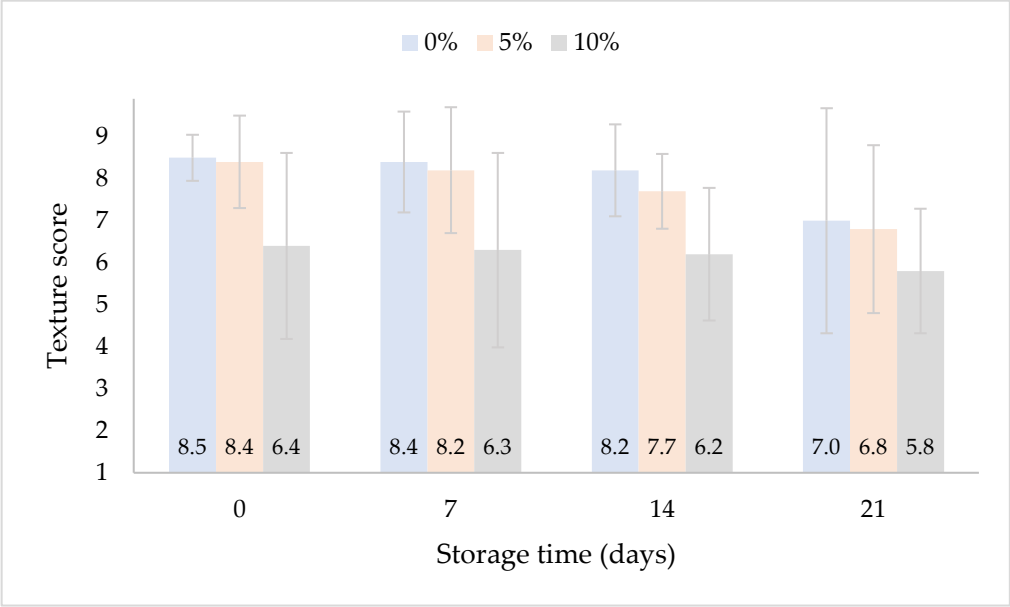


Figure 8. Texture scores of fortified yogurts with different proportions of chia seeds during a 21-day storage period.

Both chia seed concentration (F (2, 11) = 18.366, $p < 0.0001$) and storage time (F (3, 11) = 7.506, $p = 0.0001$) had significant effects on aftertaste. Higher chia seed concentrations resulted in lower aftertaste values, and storage duration further reduced these values. Figure 8 shows the decrease in aftertaste values with higher chia seed concentration and longer storage. According to Laswlees and Heymann [43], aftertaste involves lingering flavors that linger after consumption. The negative effect on aftertaste at higher chia seed concentrations suggests that the lingering bitterness could affect the overall sensory experience, potentially reducing consumer satisfaction.

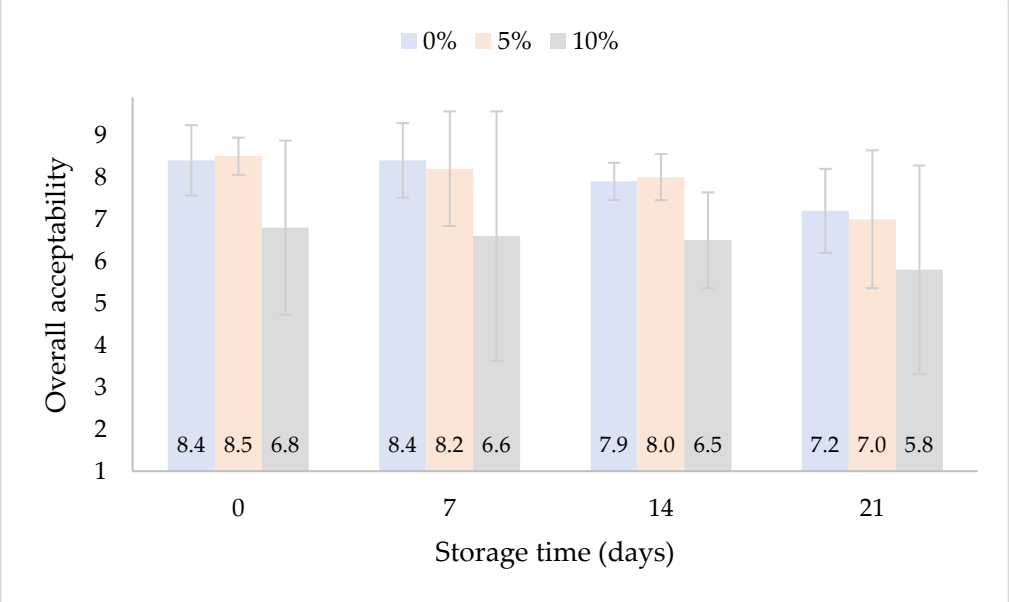


Figure 9. Overall acceptability scores of fortified yogurts with different proportions of chia seeds during a 21-day storage period.

In particular, higher concentrations of chia seeds (10%) led to a more pronounced and less desirable persistent bitterness. This bitterness was perceived by the test subjects as an off-flavor that persisted even after consumption. In contrast, yogurts with a lower chia seed concentration (5%) had a more neutral aftertaste.

The observed bitterness at higher chia seed concentrations had a negative effect on overall preference. Panelists reported that the more intense bitterness reduced the overall acceptability of the yogurt. This shift in preference underscores the importance of balancing chia seed concentration to minimize undesirable aftertaste while maximizing nutritional benefits.

The aftertaste of the yogurt changed over the storage period. With increasing storage time, the bitter taste became more pronounced in yogurts with higher chia seed concentrations. This change is attributed to the degradation of the chia seed components and their interaction with the yogurt matrix, which can lead to increased bitterness and other off-flavors. Chemical processes such as oxidation of lipids and microbial activity could also contribute to these changes in the flavor profile.

Texture scores were significantly affected by chia seed concentration ($F(2, 11) = 13.141$, $p < 0.0001$) and storage time ($F(3, 11) = 2.916$, $p = 0.038$). Yogurts with 10% chia seeds showed a more significant deterioration in texture than those with 5% chia seeds. Figure 8 illustrates this deterioration over time. The deterioration in texture, possibly due to excessive mucilage formation leading to an undesirable gel-like consistency, may affect mouthfeel and overall acceptability.

Overall acceptability was affected by both chia seed concentration ($F(2, 11) = 11.892$, $p < 0.0001$) and storage time ($F(3, 11) = 3.181$, $p = 0.027$). The yogurt with 5% chia seeds maintained a higher overall acceptability rating compared to the yogurt with 10% chia seeds. Figure 9 shows a decline in overall acceptability over time, particularly for yogurts with higher chia seed content. This suggests that a moderate chia seed concentration is optimal to maintain high sensory quality and to balance the nutritional benefits of chia seeds with the sensory attributes preferred by consumers.

The results from Table S5 and the corresponding Figures 5–9 underline the importance of chia seed concentration and storage time for the sensory quality of the yogurt. Yogurt with 5% chia seeds showed better preservation of sensory properties throughout the storage period than yogurt with 10% chia seeds. This suggests that a moderate concentration of

chia seeds is optimal for maintaining sensory quality and provides a balance between nutritional value and consumer preferences.

These results are consistent with previous research recommending a lower concentration of chia seeds to optimize sensory properties. Eker and Karakaya [14] recommend a maximum concentration of 3% chia seeds, while Kowaleski et al. [26] consider 6% to be optimal. Our study suggests that a concentration of 5% chia seeds may be the best compromise for yogurt fortification.

Careful selection of the chia seed concentration is crucial for maintaining the sensory properties of the yogurt throughout its shelf life. The data presented in Figures 6–9 and Table S5 provide a comprehensive overview of how different concentrations and storage times affect sensory quality and serve as a guide for future formulations for improved dairy products.

4. Conclusions

Our study shows that the addition of chia seeds to yogurt significantly affects both the nutritional and sensory properties of the product during the storage period.

The addition of chia seeds, especially at a concentration of 5%, effectively increases the protein content of the yogurt, indicating a nutritional benefit. Although chia seeds affect the initial pH and titratable acidity of yogurt, these effects do not significantly alter the overall pH and acidity trends during storage.

Sensory evaluation shows that yogurts with 5% chia seeds have better sensory properties than those with higher concentrations. A higher chia seed content can have a negative impact on color, taste, aftertaste, and texture, which can affect consumer acceptability. In particular, yogurts with 10% chia seeds showed a significant deterioration in these sensory properties over time.

In addition, higher concentrations of chia seeds are associated with lower lactic acid bacteria (LAB) viability, suggesting that while chia seeds provide nutritional benefits, they may alter the yogurt matrix or microbiological environment in a way that impairs LAB survival. Therefore, careful management of chia seed concentration is critical to balancing nutritional benefits with the preservation of probiotic properties.

Based on our results, a chia seed concentration of 5% is recommended to optimally enhance the nutritional profile of the yogurt while maintaining sensory quality. This concentration provides a beneficial increase in protein content while maintaining product safety, sensory appeal, and microbiological vitality throughout the storage period.

This study underlines the potential of chia seeds as a beneficial ingredient in yogurt. Chia seeds are a valuable addition to yogurt as they offer functional benefits and a proven nutritional content. However, the concentration of chia seeds must be carefully tested to ensure that the final product has high sensory quality and optimal probiotic benefits throughout its shelf life.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10080431/s1. Table S1: Results of a two-way ANOVA with a post hoc Tukey test for pH values of fortified bovine yogurts. Table S2: Results of a two-way ANOVA with a post hoc Tukey test for titratable acidity (°SH) of fortified bovine yogurts. Table S3: Results of a two-way ANOVA with a post hoc Tukey test for protein content (%) in fortified bovine yogurts. Table S4: Results of a two-way ANOVA with a post hoc Tukey test for total lactic acid bacteria count (LAB) in fortified bovine yogurts. Table S5: Results of a two-way ANOVA with a post hoc Tukey test for sensory scores of fortified bovine yogurts.

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