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Sustainable Innovations in Oat-Based Yogurts: Modulating Quality and Sensory Properties with Chia Seeds and Honey

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Abstract: This study investigates the impact of adding varying concentrations (1%, 3%, 5%, 7%, and 9%) of chia seeds on the physicochemical and antioxidant properties of oat-based yogurt fortified with 2% honey. The research analyzed changes in pH, titratable acidity, water-holding capacity (WHC), dry matter content, total phenolic content (TPC), and antioxidant activity over a 7-day storage period. The pH values ranged from 4.33 ± 0.01 to 4.57 ± 0.01, with no significant impact observed due to chia seed addition. Titratable acidity increased most rapidly in the 9% chia seed sample, particularly between days 5 and 7. WHC significantly improved with higher chia seed concentrations, with the 9% chia sample reaching 99.9 ± 0.07% compared with 69.9 ± 0.12% in the control. Dry matter content showed a similar trend, with the highest increase observed in the 9% chia sample. TPC and antioxidant activity were positively correlated with chia seed concentration and storage time, with the 9% chia sample exhibiting the highest values on day 7. Sensory evaluation revealed that the 3% chia seed concentration was most preferred by panelists for its balanced texture and flavor. Principal Component Analysis (PCA) highlighted the clustering of higher chia concentrations with improved functional properties. This study presents novel insights into the impact of varying concentrations of chia seeds on the physicochemical properties and antioxidant potential of oat-based yogurt, specifically fortified with honey, contributing to the development of functional plant-based dairy alternatives.

Keywords: Oat-based yogurt; sustainable innovations; chia seeds; honey; quality; principal component analysis (PCA)

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1. Introduction

In the past few decades, the demand for milk alternatives has increased, resulting in a parallel increase in alternative milk products. The increased preference for these products is driven by various factors and consumer demands, including health-related challenges such as lactose intolerance or milk allergy, consumers' concerns about cows' milk hormones and cholesterol, ethical disputes regarding the use of animals, environmental issues, and changes in lifestyle and also marketing that highlight health-promoting properties of these products [1]. Plant-based milk does not contain lactose and the milk proteins that lead to allergies, yet it has a high nutritional value, making it suitable for people with dietary restrictions. The most popular substitutes for dairy and dairy products are oat milk, soy milk, coconut milk, almond milk, and many others. The use of plant-based milk is increasing, and the industry is expanding to produce beverages with favorable features, including beverages that alleviate aging, prevent diseases, or improve nutrition, which can meet different people's needs [2]. Cereal-based beverages have a huge potential either to fulfill this expectation or to act as potential vehicles for functional compounds such as antioxidants, dietary fiber, minerals, prebiotics, and vitamins [3]. In addition to the good nutritional value, the functionality, and the fact that it is a compatible substitute for dairy products, it can be noted that the production of this product involves a fairly easy process. The production process itself is not very complex, but it requires commitment and constant monitoring of changes to obtain a quality product that is safe to consume. Because of the low concentrations of calcium in oats, calcium is often added to the production of oat milk along with other minerals and vitamins, which makes the final product more functional and more appealing to consumers [2]. Plant-based milk alternatives are becoming more and more popular each day, especially with younger generations. These types of products are not so uncommon; they are slowly starting to become a stable alternative to dairy products. While previous studies have focused on the benefits of chia seeds and oat-based products individually [4,5] or in different food applications [6], this study uniquely investigates the synergistic effects of varying chia seed concentrations in oat-based yogurt fortified with honey. This approach aims to deepen the understanding of how these ingredients can enhance the nutritional profile and overall quality of plant-based dairy alternatives. Chia seeds were selected for their rich content of omega-3 fatty acids, fiber, and antioxidants, which contribute to the nutritional enhancement and texture improvement of plant-based yogurts. Comprising 15–25% protein, 30–33% fats, 26–41% carbohydrates, and 18–30% dietary fiber, chia seeds also contain 4–5% ash, along with essential minerals, vitamins, and a dry matter content of 90–93% [4]. Additionally, oat-based yogurt was chosen for its growing popularity and health benefits, such as the presence of beta-glucans that support cholesterol reduction. In summary, this study aims to assess the quality of oat-based yogurt produced in a laboratory setting, providing insights into the formulation process and the effects of chia seeds and honey on various quality parameters.

2. Materials and Methods

2.1. Oat Plant-Based Yogurt Preparation

As a starting material for this study, oat-based milk was prepared in a laboratory environment by the method of Raikos et al. [7]. Oats and water were bought from a local supermarket in North Macedonia and then blended to a smooth consistency using a laboratory blender (MRC, model 800 S, speed 22,000 rpm). Subsequently, the blended mixture was strained through a milk-straining bag to discard the oat remains from the milk. The next step was pasteurizing the freshly prepared oat milk using a small-scale commercial batch pasteurizer for pasteurization (Weck Inc., Luray, VA, USA) for 30 min at a temperature of 90 ± 1.0 °C with constant stirring to avoid lump formation at the oat-based milk. After the pasteurization, the milk was cooled down to a temperature of 45 ± 1.0 °C before adding thermophile culture FD-DVS Selection™ Mild 1 (*Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus*) and probiotic culture nu-trish® LGG® DA (Chr.Hansen, Hoersholm, Denmark). Six samples were obtained for this study, including a control sample that contained 2% honey but no chia seeds. The other samples consisted of oat-based yogurt with 2% honey and varying concentrations of chia seeds as a gelling agent. Chia seeds were added to achieve the desired gel structure, with different concentrations (1%, 3%, 5%, 7%, and 9%) measured in pre-weighed sterile containers. This was followed by an incubation period in an Elecrem Y 140 (Elecrem SAS, Paris, France) incubator that maintained a constant temperature of 43 ± 1.0 °C. Incubation continued until the control samples reached a pH of 4.6 ± 0.1, at which they were moved to cool in a fridge at a temperature of 4.0 ± 1.0 °C. The analyses were performed in triplicates on the first, third, fifth and seventh day of production of oat-based yogurt.

2.2. pH Measurement

The pH of the samples was measured using a pH-meter Testo 206-pH (Testo SE & Co. KGaA, Lenzkirch, Germany), previously calibrated with standard buffers.

2.3. Titratable Acidity Measurement

The titratable acidity was measured by the method previously described by Montemurro et al. [8]. For this analysis, it was required to weigh 10 g of oat-based yogurt, which was then diluted with 90 mL water and the titration was performed with 0.1 mol/L NaOH. Along with the titration, the pH of the yogurt was measured until achieving a value of 8.3 pH for additional accuracy.

2.4. Water-Holding Capacity Measurement (WHC)

The water-holding capacity of the oat plant-based yogurts was assessed using the method outlined by Silva and O'Mahony [9]. Each sample (20 ± 0.01 g) was weighed and centrifuged, and the separated water was measured. *WHC* percentage was then calculated using a formula (Equation (1)).

$$WHC\ (\%) = \left(1 - \frac{a}{b}\right) \times 100 \tag{1}$$

where a is the weight of the supernatant expressed in grams, and b is the weight of the sample expressed in grams.

2.5. Dry Matter Measurement

Approximately 2.0 ± 0.1 g of each oat-based yogurt sample was measured using a moisture analyzer (MJ33, Mettler Toledo, Greifensee, Switzerland) [10].

2.6. Extraction Procedure for Determination of Total Polyphenols Content (TPC) and Antioxidant Activity

The samples were prepared as described by Nakov et al. [11]. Briefly, 10 g of oat-based yogurt was weighed and mixed with 30 mL of methanol in a ratio of 80:20 (80 mL methanol: 20 mL deionized water) and shaken for 30 min. Following this, the samples underwent centrifugation at 8000× g rpm at 4 °C for 30 min. The supernatant was used for evaluation of total polyphenols concentration (TPC) and antioxidant activity.

2.7. Total Polyphenols Determination

This analysis was performed as described by Nakov et al. [11]. A volume of 300 µL of supernatant was transferred into a tube and mixed with 1.5 mL of 1:10 Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA). After 5 min, 1.5 mL 7% sodium carbonate was added, the mixture was homogenized, and allowed to stand for 90 min at room temperature (22 ± 2 °C). Absorbance was measured at 760 nm with a spectrophotometer (UV-VIS Spectrophotometer-UV1800, Shimadzu, Kyoto, Japan). Results were expressed as milligrams of gallic acid equivalent (GAE) per 1 (liter) of yogurt.

2.8. Antioxidant Activity Determination

Exactly 200 µL of the sample was mixed with 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (•DPPH) in methanol (0.1 mmol·L^{-1}). The mixture was homogenized and allowed to stand for 30 min at room temperature (22 ± 2.0 °C). Absorbance was measured at 517 nm with a spectrophotometer (UV-VIS Spectrophotometer-UV1800, Shimadzu, Kyoto, Japan) as previously described by Nakov et al. [11], where methanol was used as a control. Antioxidant activity was determined as a percentage of free radical inhibition using equation (Equation (2)):

$$\%\text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \tag{2}$$

where A_0 is the absorption of the DPPH solution, and A_1 is the absorption of the sample.

2.9. Diacetyl

The diacetyl of the samples was measured according to the method described by Carić et al. [12]. Briefly, 1.0 ± 0.01 g of oat-based yogurt was weighed in test tubes to which 2.0 mL distilled water, 1.0 mL 10% sodium tungstate and 1.0 mL 0.33 mol/L sulfuric acid were added. After mixing on a vortex, the solution was filtrated, and 400 µL of the filtrate was placed in a clean test tube. The filtrate was treated with 4.6 mL distilled water, 1.0 mL of 0.5% creatine in water, and 1.0 mL of 5% α-naphthol in 2.5 mol/L aqueous sodium hydroxide. All the samples were mixed on a vortex, closed, and allowed to stand at 23 ± 1.0 °C for 90 min. Absorbance was measured at 575 nm using an ultraviolet-visible spectroscopy (UV-VIS Spectrophotometer-UV1800, Shimadzu, Kyoto, Japan).

2.10. Color Measurement

The color of the oat plant-based yogurts was determined by the CIE Lab system, with the determination of the *L** (brightness), *a** (+red/−green) and *b** (+yellow/−blue) parameters according to the CIE1976 color system, previously described by Nakov et al. [13].

2.11. Microbiological Analysis

For microbial counts, 10 g of each oat plant-based yogurt was stored at refrigerator temperature and homogenized in 90 mL of a diluent solution (0.85% sodium chloride and 0.10% tryptone), and serial 10-fold dilutions were prepared. Enumeration of Enterobacteria was performed on Violet Red Bile Glucose (VRBG) Agar (Condalab, CAT 1092.00) according to ISO standards [14]. Yeasts and molds were enumerated according to the ISO method [15]. Colony-forming units (CFU) were counted on Petri dishes expressed as log CFU/mL of oat plant-based yogurt.

2.12. Sensory Evaluation

Oat-based yogurts were evaluated by a nontrained sensory panel consisting of 20 semi-trained panelists, including 14 females (aged 22–38 years) and 6 males (aged 22–37 years). Approximately 20 g of every sample cooled at a temperature of 4.0 ± 1.0 °C was placed in previously marked cups in a randomized order. Attributes such as flavor, mouthfeel, appearance, texture, and overall acceptance were rated on a scale ranging from 1 to 10, where 1 represented "extremely dislike" and 10 represented "extremely like". After gathering all the results, an average value for every parameter was calculated. The sensory evaluation of oat plant-based yogurts was carried out in accordance with International Standard Organization (ISO) 8586:2012 [16] on food information, with informed consent secured from all participants. All participants signed an informed consent form and reported no known food allergies.

2.13. Determination of Appropriate Amount of Chia Seed by Principal Component Analysis (PCA)

Table 1 shows the signs used and their meaning. A total of 20 characteristics were taken into account, which included the physicochemical, color, and sensory characteristics of the product, known as a method for selecting informative features.

An approach known as regression feature selection based on neighboring component analysis (FSRNCA) was used to identify suitable features [17]. This method is suitable for distance-based models, making it particularly useful for tasks involving regression analysis. By using FSRNCA, the selection of informative features becomes more efficient and facilitates improved predictive modeling. It is a method for reducing the volume of data in the selected feature vector. A check was made on the influence of the amount of chia on the main characteristics of yogurt. Table 2 describes the relationships between the samples with the additive and the characteristics of the resulting oat-based yogurt. The data were processed using the principal component analysis (PCA) method and are presented graphically. Also, the PCA method was used in constructing a regression model applied to determine the appropriate amount of chia in oat milk yogurt.

Table 1. Features and their meaning.

Feature	Meaning	Feature	Meaning
F1	pH	F11	*L**
F2	DM%	F12	*a**
F3	DPPH Abs	F13	*b**
F4	DPPH AOA	F14	SH, mL NaOH
F5	WHC g sample	F15	flavor
F6	WHC model	F16	month-feel
F7	Polyphenols Abs	F17	appearance
F8	TPC	F18	texture
F9	Diacetyl Abs	F19	overall acceptance
F10	Diacetyl model	F20	Shelf life

Table 2. Feature-additive table.

Sample / Feature	S1	...	Sn
F1	S1F1	...	SnF1
...	...	...	...
Fm	S1Fm	...	SnFm

A regression model more commonly used in food analysis was applied [18]. This model has the following general appearance (Equation (3)):

$$z = b_0 + b_1x + b_2y + b_3x^2 + b_4xy + b_5y^2 \quad (3)$$

where z is the dependent variable and x and y are the independent variables; the coefficients of the model are b. The evaluation of the model is performed depending on the coefficient of determination (R^2), the coefficients of the model, their standard error (SE), t-statistic (tStat), p-value, and Fisher's criterion (F).

For determining the appropriate amount of additive a linear programming algorithm implemented by the linprog function is used. In linear programming, one solves a problem related to finding a vector x such that the linear function $f^T x$, with linear constraints (Equation (4)):

$$\min_x f^T x \quad (4)$$

One of the conditions must be fulfilled (Equation (5)):

$$Ax \leq b \qquad A_{eq}x = b_{eq} \qquad l \leq x \leq u \quad (5)$$

An interior-point-legacy algorithm was used. The algorithm reaches a suitable solution by traversing the interior of the data region. The Matlab software system, Matlab 2017a (The Mathworks Inc., Natick, MA, USA) was used in the processing of the obtained data. All data were processed at a significance level of $\alpha = 0.05$.

2.14. Statistical Analysis

Statistical analysis was performed using a 2-way analysis of variance (ANOVA) followed by the Fisher (LSD) test. The statistical analysis was performed using XLSTAT software version 2019.2.2 (Addinsoft, New York, NY, USA). The level of significance was set at $p \leq 0.05$.

3. Results and Discussion

3.1. pH and Titratable Acidity

The pH of the oat-based yogurts varied from 4.33 ± 0.01 to 4.57 ± 0.01 during the storage days of the samples (Figure 1). On the first day after production, the highest difference was noticed between the control and sample with 9% chia seeds ($p < 0.05$). Additionally, the samples without honey and chia (data not presented on the graph) showed a noticeably higher pH compared with the control samples (with honey). On the first day, the pH of the non-honey samples was 4.77, followed by 4.68 on the third day, 4.65 on the fifth day, and 4.64 on the seventh day. The impact of honey on the pH of yogurts has been described by Coksun et al. [19], where the samples containing pine honey had lower pH levels compared with the samples without honey. According to Coksun et al. [19], these variations in the pH levels are due to the presence of glucose and fructose in the honey, which contributes to additional fermentation of the yogurt itself. Tazrart et al. [20] reported that the addition of chia seeds did not significantly affect the pH of full-fat yogurts. However, Attalla and El-Hussieny [21] observed a significant decrease in pH values in yogurt mousse fortified with 1%, 2%, and 3% chia seeds compared with the control sample. These findings align with the results of this study, further confirming that chia seed fortification can influence the acidity of certain dairy products. On the other hand, pH reduction was noticed during the storage days in almost all of the samples ($p < 0.05$). Similar findings were reported by Safaa [4], where a slight decrease in pH occurred over time in yogurt fortified with chia flour. These results further demonstrate that chia fortification may contribute to gradual acidification during storage.

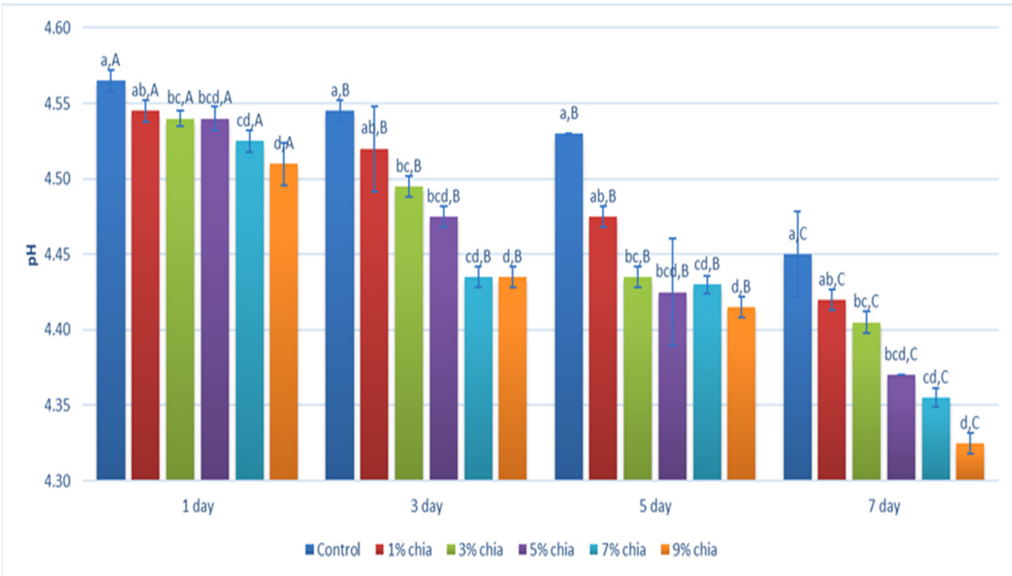


Figure 1. Changes in pH values over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

Regarding the titratable acidity (Figure 2), the sample with 9% chia seeds had the fastest increase in SH°, particularly noticeable between days 5 and 7, displaying a significant increase in SH°. In contrast, the control sample without chia seeds and honey (data not presented on the graph) exhibited the lowest SH° values, showing a gradual increase throughout the storage period. It started at 155 mL·kg^{-1} on the first day, rising to 160 mL·kg^{-1} on both the third and fifth days, and reaching 180 mL·kg^{-1} by the seventh day. Similarly, according to research by Ammar et al. [22], the acidity of the yogurt with added honey was higher compared with the samples without honey. Coksun et al. [19] also found that the samples with added 2% honey had the highest increase in titratable acidity. Atik et al. [23] conducted a comparable study where they added chia seed mucilage to cow's milk yogurt, and they discovered that the acidity of the yogurts with added chia seed mucilage was higher compared with the yogurts without chia seed mucilage. This led to the conclusion that the bacteria in the yogurt are more active in the presence of chia seed mucilage.

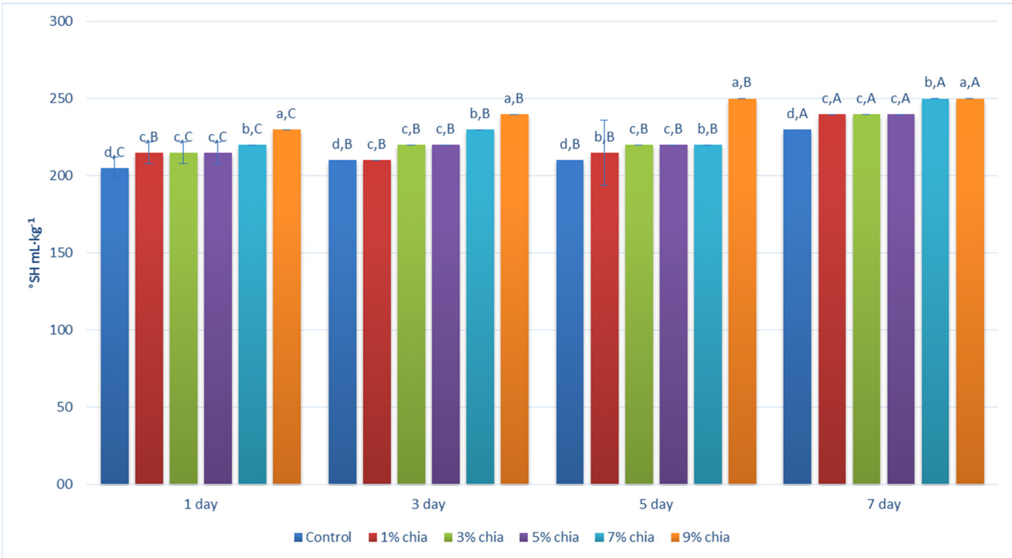


Figure 2. Titratable acidity over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

3.2. Water-Holding Capacity

Water-holding capacity (WHC) is defined as the ability of gel structures to retain their liquid component, water [24]. The concentration of added chia seeds correlates closely with the WHC of oat plant-based yogurts (Figure 3). As the concentration of chia seeds increases, there is a corresponding increase in the WHC of the yogurts. Particularly noteworthy is the substantial increase in WHC observed when comparing control and oat-based yogurt with 9% chia seed. Additionally, oat plant-based yogurts lacking chia seeds (control) exhibit a WHC of 69.9 ± 0.12%, whereas the sample with 9% chia demonstrates a remarkable WHC of 99.9 ± 0.07%, maintaining consistency from day 1 to day 7. These results align with the findings of Nadtochii et al. [25], who reported similar improvements in water retention when enriching cow's milk yogurt with oats and chia seeds. The improvement in WHC can be attributed to the unique properties of chia seeds, which are rich in dietary fibers, particularly soluble fibers like mucilage. When hydrated, these fibers form a gel-like structure capable of trapping water, enhancing the product's ability to retain moisture. In addition to the soluble fiber content, chia seeds are also rich in proteins and lipids, which contribute to the formation of a more stable gel matrix, further increasing WHC. The hydrophilic nature of chia seeds enables them to absorb and retain significant amounts of water, explaining the notable increase in WHC in samples containing higher concentrations of chia seeds [21,26].

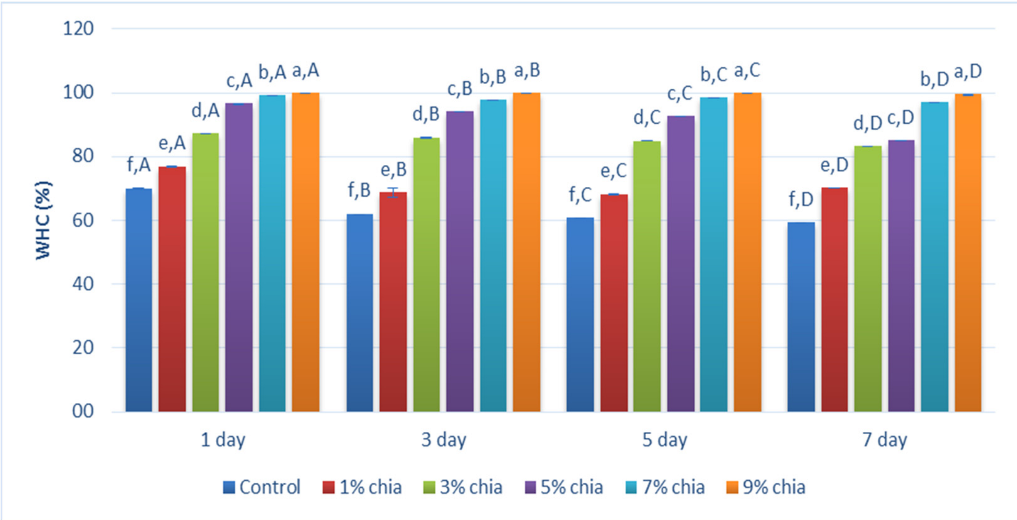


Figure 3. Water-holding capacity (WHC %) over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

3.3. Dry Matter

In parallel with WHC, the concentration of added chia seeds demonstrates a correlation with the dry matter percentage (DM%) in oat plant-based yogurts. Notably, chia seeds influence the DM% of the samples (Figure 4). This is evident in the DM% values observed across different samples: The control sample exhibits a higher DM% on day 1, which progressively increases over time ($p < 0.05$). The sample with 9% chia seeds registers the highest DM%, with a consistent rise observed throughout the storage period until the fifth day ($p < 0.05$). Previous studies [27] have shown that chia seed addition increases dry matter content due to their high fiber and protein content, which retains water and increases the solid fraction.

3.4. Total Polyphenols Content

The results depicted in Figure 5 reveal that the control sample remained stable up to the fifth day ($p > 0.05$), with a slight increase on the seventh day. In contrast, the addition of chia seeds to the samples slightly elevates the phenolic concentration compared with the control sample devoid of chia seeds. Chia seeds are recognized as a rich source of low molecular weight polyphenols, particularly phenolic acids [28]. Beyond phenolic acids, chia seeds also contain various other compounds with antioxidant properties, including lignans, flavonoids, tannins, and tocopherols [29].

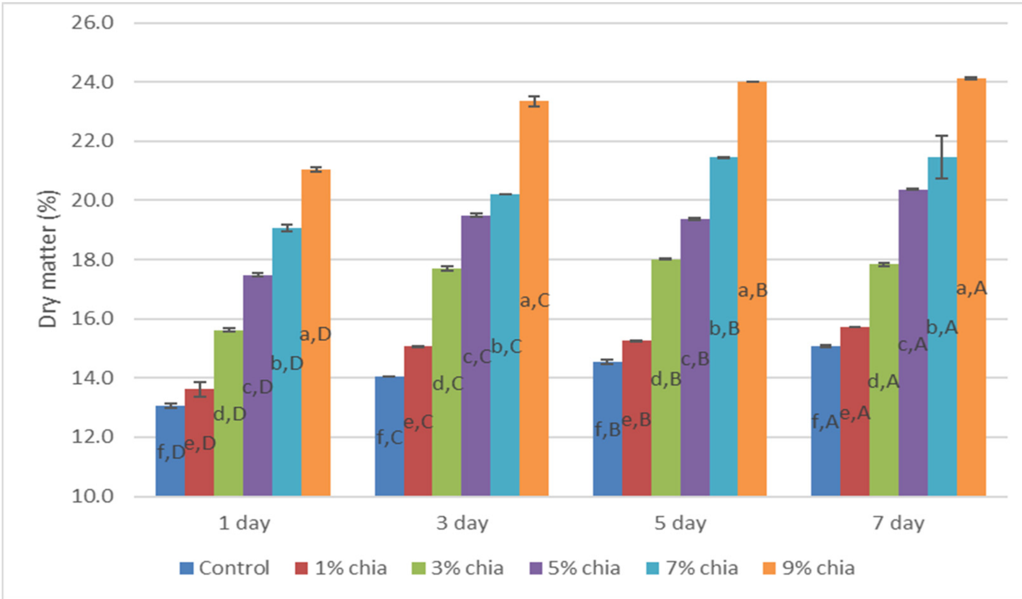


Figure 4. Dry matter (%) over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

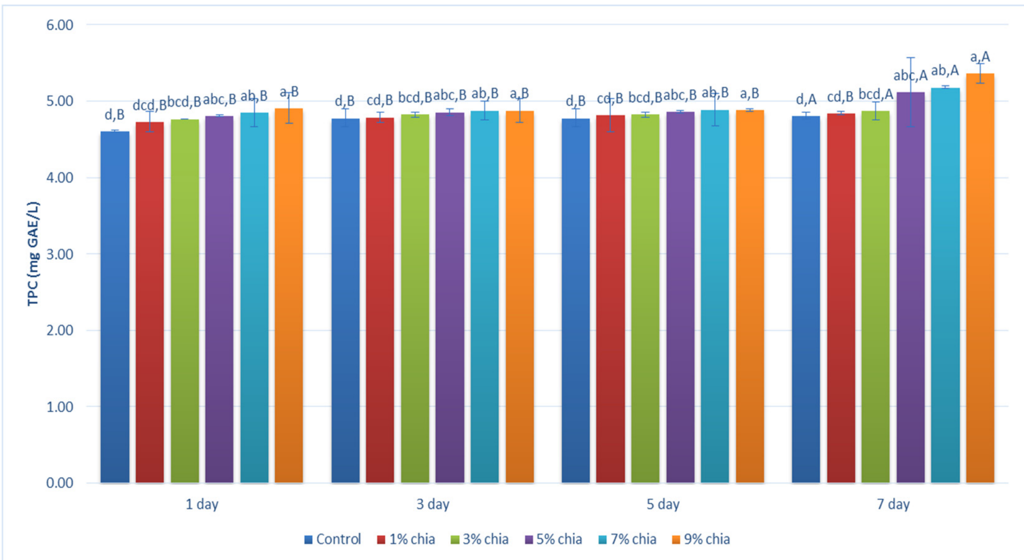


Figure 5. Total phenolic content over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

Research by Kwon et al. [30] supports these findings, demonstrating a significant increase in total phenolic concentration (TPC) in yogurts derived from skim milk with added chia seed extracts, correlating with higher concentrations of chia seed extract. Additionally, the incorporation of honey can significantly impact the total phenolic concentration of oat-based yogurts. Okur et al. [31] found a parallel increase in both the total phenolic concentration and overall antioxidant activity of yogurt produced from cow's milk with escalating concentrations of added honey. Moreover, the fermentation of oats with lactic acid bacteria (LAB) not only enhances their digestibility but also increases the content of soluble dietary fiber and key phytochemicals, such as phenolic compounds. According to Djorgbenoo et al. [32], this fermentation process improves the bioactivity of oat-based nutrients, further contributing to the health benefits of the product. Thus, both chia seeds and honey, in combination with LAB fermentation, are instrumental in elevating the nutritional profile of oat-based yogurts, particularly in terms of phenolic compounds and antioxidant activity.

3.5. Antioxidant Activity

Upon examining Figure 6, the pronounced antioxidant effect of chia seeds becomes evident, with antioxidant activity increasing in correlation with the duration of storage. Notably, all samples exhibit the lowest antioxidant activity on day 1, with a notable surge observed by day 7, where all samples reach peak values. The control sample records the lowest antioxidant activity on the initial day, while the sample with 9% chia seeds demonstrates the highest antioxidant activity on day 7.

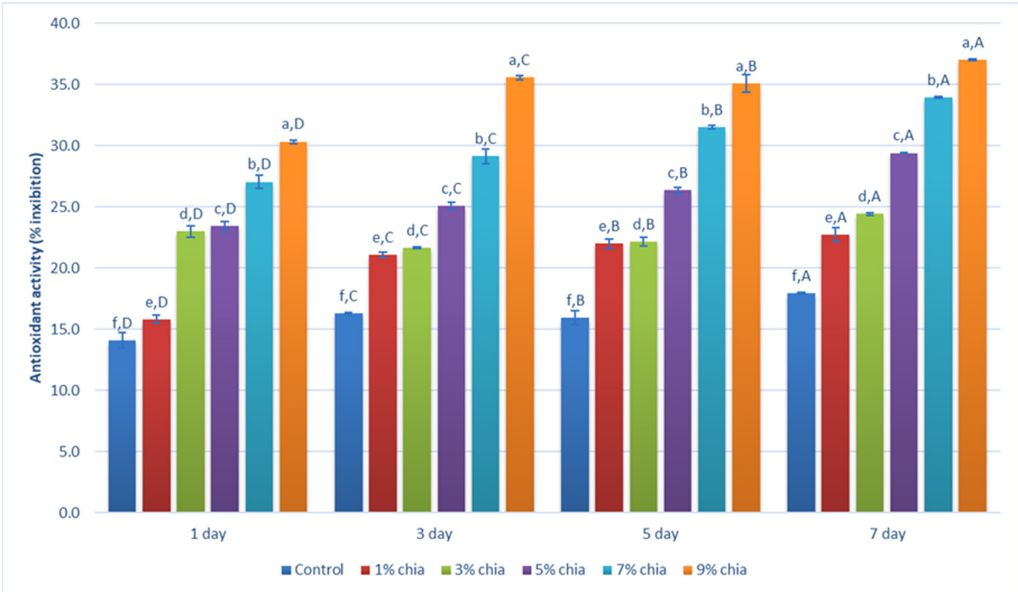


Figure 6. Antioxidant activity over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

Further insights into antioxidant activity are gleaned from the research of Alqahtani et al. [33], who analyzed yogurt produced from goat milk supplemented with oat flour. Their findings underscore the influence of oat flour on the antioxidant activity of yogurt, with increasing concentrations of oat flour yielding a corresponding enhancement in antioxidant activity. Beyond oats, both chia seeds and honey contribute significantly to antioxidant activity, as evidenced by the studies conducted by Menga et al. [34] and Omotayo et al. [35]. These collective findings underscore the multifaceted contributions of various components, including chia seeds, honey, and oats, to the antioxidant profile of oat-based yogurts.

3.6. Diacetyl

The outcomes of the analyses conducted to assess the diacetyl concentration in oat plant-based yogurts are presented in Figure 7. A notable trend emerges upon scrutiny, revealing a substantial rise in diacetyl concentration across all samples from day 1 to day 3. By day 5, a marginal increase is observed in all samples except for the control and sample with 1% chia seeds. Moreover, the final day of analysis highlights a further escalation in diacetyl concentration across all samples, with the sample with 9% chia seeds exhibiting the most significant increase from its initial value. Notably, the control sample registers the lowest diacetyl concentration on day 1, whereas the sample with 9% chia seeds records the highest concentration on day 7. These findings underscore the influence of both chia seeds and storage duration on the diacetyl concentration in oat plant-based yogurts. Ţiţa, A., & Ţiţa, O. [36] documented a similar rise in diacetyl concentration in low-fat stirred yogurt enriched with chia powder during storage. Furthermore, the presence of β-glucan from oats emerges as a pivotal factor in augmenting diacetyl concentration post-fermentation and during storage, a phenomenon corroborated by the studies of Aljewicz et al. [37] and Kütt et al. [38].

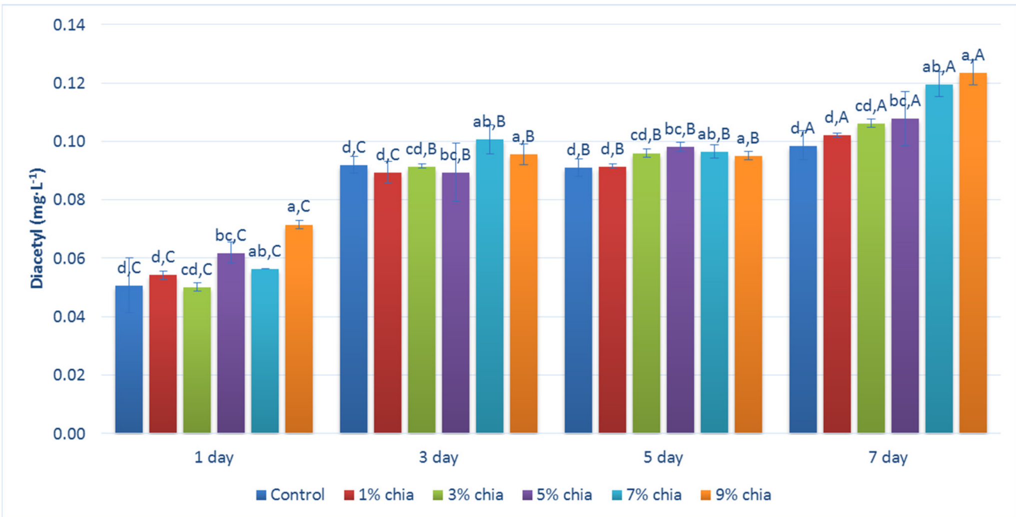


Figure 7. Concentration of diacetyl over the course of storage days of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds). Small letters refer to statistically significant differences ($p < 0.05$) between samples with different quantities of chia seeds; capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

3.7. Color Measurement

Color plays an important role in food products. It often happens that the color and appearance of the product are the decisive factors for whether one product will be preferred and consumed compared with another [39]. Color is the first sensory characteristic that is perceived by consumers, and color itself can alter taste and aroma perceptions [40].

In the results shown in Table 3, it can be observed that there is no significant change between the values from day 1 to day 7 for each oat plant-based yogurt individually. However, when comparing all the samples in one day, there is a noticeable difference in the value for *L**. Starting with the control sample (plant-based oat yogurt with 0% chia seeds), then the plant-based oat yogurts with 1%, 3%, 5%, 7%, and 9% of added chia seeds, there is a gradual decrease in the value for *L**, which means the color is closer to black due to the higher concentration of chia seeds. Ribes et al. [41] produced yogurt from whole and skimmed milk powder, to which they added different concentrations of chia seed mucilage. In their study, although chia seeds were removed with filtration to obtain pure mucilage, yogurts with a higher percentage of added chia seed mucilage had lower *L** values. These results are also confirmed by the research conducted by Pourjavid et al. [42], who added chia seeds and flax seeds to an already prepared yogurt. They observed that chia seeds, due to their dark color, significantly reduced the *L** value.

Table 3. Mean values and standard deviation of color coordinates (*L**, *a**, and *b**) on different percentages of chia seeds in oat plant-based yogurt.

L*	**1 day**	**3 days**	**5 days**	**7 days**
Control	61.90 ± 0.01 ^{a,A}	61.90 ± 0.02 ^{a,A}	61.90 ± 0.02 ^{a,A}	60.94 ± 0.08 ^{a,B}
1% chia	59.75 ± 0.20 ^{b,A}	59.49 ± 0.25 ^{b,A}	59.13 ± 0.55 ^{b,A}	59.13 ± 0.55 ^{b,B}
3% chia	57.15 ± 0.51 ^{c,A}	56.91 ± 0.89 ^{c,A}	56.41 ± 0.52 ^{c,A}	56.35 ± 0.20 ^{c,B}
5% chia	54.15 ± 1.88 ^{d,A}	53.97 ± 0.47 ^{d,A}	53.95 ± 0.06 ^{d,A}	53.12 ± 0.49 ^{d,B}
7% chia	51.90 ± 0.03 ^{e,A}	51.60 ± 0.59 ^{e,A}	51.42 ± 0.13 ^{e,A}	51.17 ± 0.19 ^{e,B}
9% chia	49.90 ± 0.81 ^{f,A}	49.98 ± 0.38 ^{f,A}	49.71 ± 0.04 ^{f,A}	49.08 ± 0.41 ^{f,B}
a*	**1 day**	**3 days**	**5 days**	**7 days**
Control	−1.20 ± 0.07 ^{e,C}	−1.07 ± 0.10 ^{e,B}	−0.94 ± 0.35 ^{e,AB}	−0.77 ± 0.08 ^{e,A}
1% chia	−0.86 ± 0.07 ^{d,C}	−0.75 ± 0.04 ^{d,B}	−0.73 ± 0.02 ^{d,AB}	−0.67 ± 0.04 ^{d,A}
3% chia	−0.55 ± 0.08 ^{c,C}	−0.43 ± 0.04 ^{c,B}	−0.41 ± 0.24 ^{c,AB}	−0.25 ± 0.02 ^{c,A}
5% chia	−0.13 ± 0.07 ^{b,C}	0.03 ± 0.07 ^{b,B}	0.12 ± 0.02 ^{b,AB}	0.16 ± 0.01 ^{b,A}
7% chia	0.10 ± 0.09 ^{b,C}	0.11 ± 0.03 ^{b,B}	0.18 ± 0.05 ^{b,AB}	0.28 ± 0.02 ^{b,A}
9% chia	0.20 ± 0.05 ^{a,C}	0.36 ± 0.39 ^{a,B}	0.44 ± 0.03 ^{a,AB}	0.53 ± 0.01 ^{a,A}
b*	**1 day**	**3 days**	**5 days**	**7 days**
Control	2.79 ± 0.24 ^{e,C}	3.48 ± 0.08 ^{e,B}	3.53 ± 0.16 ^{e,B}	3.60 ± 0.53 ^{e,A}
1% chia	3.05 ± 0.15 ^{de,C}	3.72 ± 0.30 ^{de,B}	3.74 ± 0.52 ^{de,B}	3.79 ± 0.54 ^{de,A}
3% chia	3.46 ± 0.02 ^{cd,C}	3.81 ± 0.03 ^{cd,B}	3.81 ± 0.17 ^{cd,B}	4.02 ± 0.19 ^{cd,A}
5% chia	3.59 ± 0.22 ^{c,C}	3.89 ± 0.10 ^{c,B}	3.96 ± 0.11 ^{c,B}	4.43 ± 0.04 ^{c,A}
7% chia	4.48 ± 0.18 ^{b,C}	4.56 ± 0.01 ^{b,B}	4.58 ± 0.21 ^{b,B}	4.96 ± 0.00 ^{b,A}
9% chia	4.59 ± 0.09 ^{a,C}	4.74 ± 0.14 ^{a,B}	4.82 ± 0.03 ^{a,B}	5.53 ± 0.05 ^{a,A}

Note: Small letters refer to statistically significant differences ($p < 0.05$) between the samples with different quantities of chia seeds; Capital letters refer to statistically significant differences ($p < 0.05$) between storage days.

In the analysis of plant-based oat yogurts with varying concentrations of chia seeds (0%, 1%, 3%, 5%, 7%, and 9%), it was observed that yogurts with 0%, 1%, and 3% chia seed additions exhibited negative values for the parameter *a**. Conversely, yogurts with 5%, 7%, and 9% chia seed additions displayed positive *a** values. This pattern indicates that unaltered plant-based oat yogurt inherently possesses a greenish hue, as denoted by the negative *a** values. Conversely, with the incorporation of higher chia seed concentrations, a transition in the yogurt's coloration occurs from green (negative *a**) to red (positive *a**), signifying a perceptible change in its visual appearance. In their study, Kwon et al. [30]

incorporated a minor concentration of chia seeds into yogurt samples, wherein it was consistently observed that all samples exhibited negative values for parameter *a**. On the other hand, the plant-based oat yogurt with 0% added chia seeds (Control) had the lowest value for *b** (2.79 $\pm$ 0.24), while the sample with 9% added chia seeds had the highest value for *b** (4.59 $\pm$ 0.09). These results indicate that the addition of chia seeds increases the value for *b**, which is confirmed by the research of Pourjavid et al. [42], where all the samples with added chia seeds and flax seeds had a positive value for *b**.

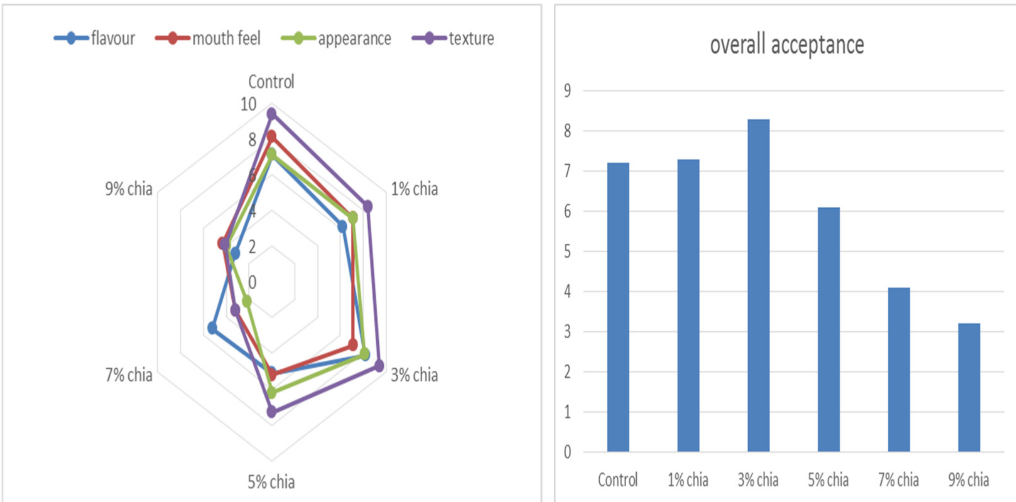
The statistical analysis of the color results for plant-based oat yogurts with different chia seed concentrations revealed that the addition of chia seeds significantly affects the examined parameters ($p < 0.05$). Additionally, the color parameters show significant changes ($p < 0.05$) during various storage periods for these oat yogurts.

3.8. Microbiological Analysis

The microbiological quality of dairy products significantly influences their perishability [39]. Notably, our analysis of microbiological quality yielded satisfactory results throughout the storage period. This was attributed to stringent adherence to hygienic practices during both manufacturing and storage, as evidenced by the absence of *Enterobacteria*, mold, and yeast in the yogurt treatments (data not disclosed). These findings align with those reported by Alqahtani et al. [33].

3.9. Sensory Evaluation

Upon analyzing each sensory parameter—flavor, mouthfeel, appearance, texture, and overall acceptance—Sample 4, containing 3% chia seeds, emerged as the highest-rated by the panelists, achieving an overall acceptance score of 8.3 (Figure 8). Both the control and the sample with 3% chia seeds received the highest texture ratings, scoring 9.4 each, while the control sample excelled in mouthfeel. In contrast, the sample with 9% chia seeds received the lowest scores for flavor and overall acceptance, both at 3.2, while Sample 6 (7% chia seeds) had the lowest appearance (score of 2) and mouthfeel (score of 3). Overall, the sample with 3% chia seeds consistently outperformed other samples across all sensory attributes, while the 7% and 9% chia seed samples showed the lowest scores.



(**a**) (**b**)

Figure 8. (**a**,**b**). Sensory scores of oat-based yogurts with different percentages of chia seeds. Control (plain oat-based yogurt with 2% honey); 1% chia (plain oat-based yogurt with 2% honey and 1% chia seeds); 3% chia (plain oat-based yogurt with 2% honey and 3% chia seeds); 5% chia (plain oat-based yogurt with 2% honey and 5% chia seeds); 7% chia (plain oat-based yogurt with 2% honey and 7% chia seeds); 9% chia (plain oat-based yogurt with 2% honey and 9% chia seeds).

The preference for the 3% chia seed yogurt can be attributed to the optimal balance between enhanced texture from the chia seeds and a smooth, desirable mouthfeel. In higher concentrations, such as 7% or 9%, the thicker, more viscous texture from excessive mucilage formation was perceived as less pleasant. As supported by Kibui et al. [43], in studies of cow's milk yogurt with varying chia concentrations, samples without chia seeds were rated higher in texture, taste, appearance, and overall acceptance compared with those with higher chia concentrations, reinforcing a preference for lower chia levels. At concentrations of 1%, the chia seeds may not have been enough to improve texture, while at 5% or higher, the texture became too thick or gritty, impacting mouthfeel negatively. Higher chia seed concentrations, such as 10%, also led to a pronounced bitterness that affected the aftertaste and reduced consumer preference. Over time, this bitterness intensified in yogurts with higher chia content, likely due to the degradation of chia seed components during storage [44]. Texture also deteriorated more in yogurts with 10% chia seeds, likely due to excessive mucilage, which resulted in an undesirable gel-like consistency [44]. As a result, overall acceptability ratings declined over time, particularly in samples with higher chia concentrations.

A moderate chia seed concentration, such as 3%, provided the best balance between textural improvement and flavor integrity. This concentration not only enhanced the yogurt's sensory appeal but also improved its water-holding capacity (WHC), helping to minimize syneresis and extend shelf life. These findings are consistent with consumer preferences for plant-based products that offer both functional benefits and enjoyable sensory properties. Similar results were observed in studies by Safaa [4] and Attalla and El-Hussieny [21], where the 3% chia seed addition resulted in the highest sensory scores for yogurt and yogurt mousse, supporting its use as an optimal concentration in dairy alternatives.

3.10. Principal Component Analysis

Figure 9 shows the result of the selection of informative features. The most informative feature is the "*a**" component from the Lab color model, followed by the "*b**" component. WHC can be placed in third place in terms of in formativeness. Equal informative weights have dry matter, AOA, and L (Lab), as well as the sensory characteristics of residual flavor, general appearance, and texture.

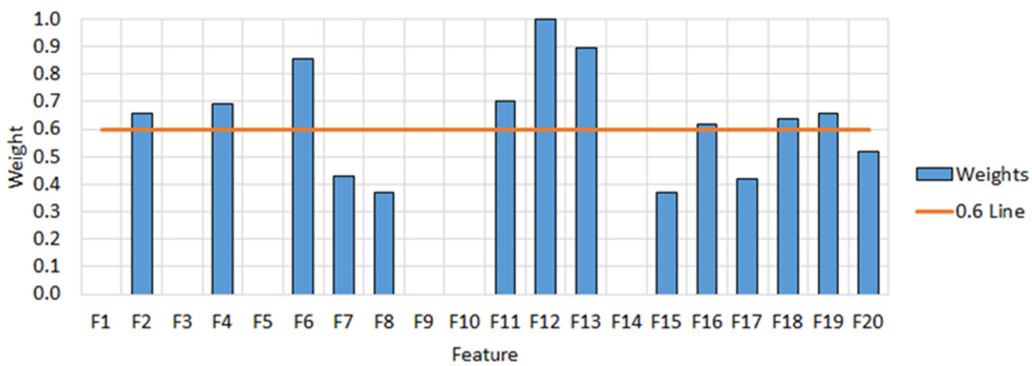


Figure 9. Results from feature selection.

The following feature vector is selected (Equation (6)):

$$FV = [F2\ F3\ F6\ F11\ F12\ F13\ F16\ F18\ F19]. \quad (6)$$

Figure 10 shows the results of a principal component analysis (PCA). It can be seen that in the control sample, the texture and the general sensory evaluation are the closest. The addition of 1% chia affects WHC and aftertaste. From 3% to 5% significantly changes the "*b**" component of the Lab color model. This means that the color of the yogurt mainly changes in this range. Amounts from 7% to 9% of chia addition affect dry matter, AOA, and the "*a**" component of the Lab color pattern.

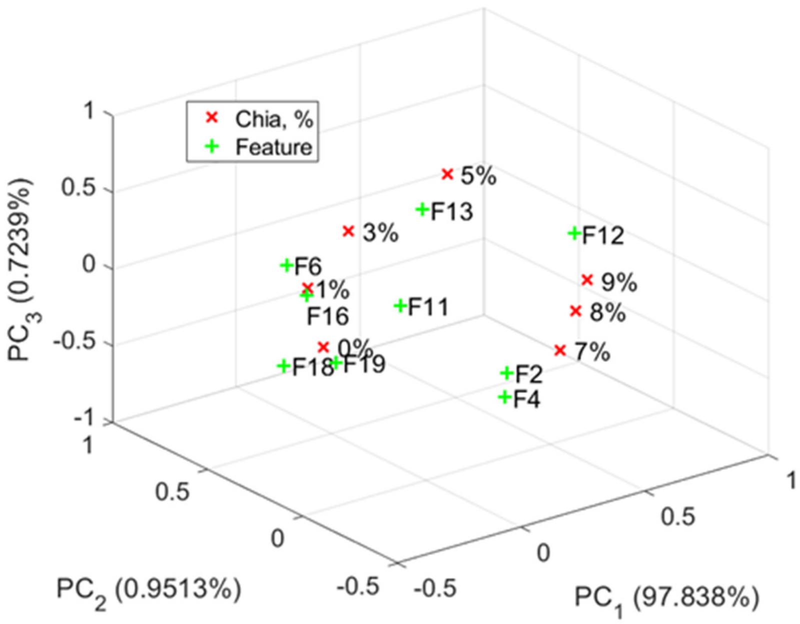


Figure 10. Results from PCA.

The regression analysis results revealed that the coefficient of determination (R2) was around 0.996. This suggests that roughly 99.6% of the changes in the dependent variable were accounted for by the model. The adjusted R2 value was around 0.991, suggesting that the fit of the model was robust and unaffected by the number of predictors. The F-statistic F(4, 16) = 530.44 << Fcr = 3 with a *p*-value below a certain significance level (<<0.05) indicates that the overall model is statistically significant.

Also, the regression analysis shows that the variables PC_1 and PC_2 are statistically significant predictors of the dependent variable "amount of which". The interaction "$PC_1 \times PC_2$" may not be so highly significant, and its effect requires further investigation. The overall model appeared to describe the data reasonably well, as evidenced by the high R-squared and adjusted R-squared values, as well as the standard error of SE = 0.35.

The resulting model has the form (Equation (7)):

$$O = 4.9 + 0.28PC_1 + 0.53PC_2 - 0.007{PC_1}^2 + 0.034PC_1PC_2 \quad (7)$$

Figure 11 shows the results of determining the appropriate amount of chia in oat milk yogurt. When applying a linear programming algorithm, a suitable quantifier of 2.53% was determined. This value is located in the negative levels of PC_1 and close to the positive ones of PC_2. It follows that the data on the second principal component significantly influences the determination of the permissible amount of chia in oat milk yogurt. Additionally, the same results were performed during the sensory evaluation of the oat-based yogurt, where Sample 4 (3% chia seeds) was evaluated with higher scores.

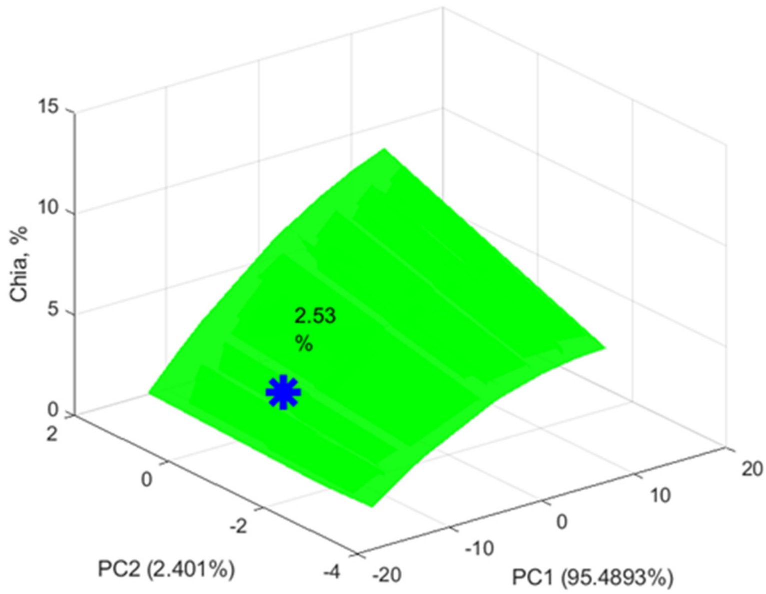


Figure 11. Determination of appropriate amount of chia in oats yogurt.

4. Conclusions

In conclusion, this study offers valuable insights into the quality and characteristics of oat-based yogurt, particularly regarding the effects of chia seeds and honey as additives. The analysis revealed that the incorporation of chia seeds significantly influenced key parameters, including pH, titratable acidity, water-holding capacity (WHC), dry matter percentage (DM%), total polyphenols, antioxidant activity, and diacetyl levels. Chia seeds notably enhanced the physicochemical properties of the yogurt, improving its texture, stability, and antioxidant content, with higher concentrations leading to increased WHC and DM%. Sensory evaluation showed generally favorable ratings for chia seed-enriched samples, although careful attention to the concentration is crucial for maintaining optimal flavor and texture profiles. While honey's impact on pH and titratable acidity was observed, further exploration is needed to clarify its role in the fermentation process within this study's context. Principal component analysis identified key factors influencing yogurt quality, with chia seed concentration and sensory attributes being significant. This research underscores the importance of selecting and integrating additives like chia seeds and honey to enhance product quality and consumer acceptance, while future studies should investigate additional additives and processing techniques to improve the nutritional profile and sensory appeal of plant-based dairy alternatives.

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