

**IMPACT OF WHOLE CHIA SEEDS (*SALVIA HISPANICA*) ADDITION ON THE  
PROPERTIES AND STABILITY OF THE SET-TYPE YOGURT**

KARIMA TAZRART<sup>1\*</sup>, LINDA OULD-SAAD<sup>1</sup>, LEILA SMAIL-BENAZZOUZ<sup>2</sup>, DJAMEL  
EDINE KATI<sup>1</sup>

<sup>1</sup>Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000  
Bejaia, Algeria

<sup>2</sup>Laboratoire de Biomathématiques, Biochimie, Biophysique et Scientométrie, Faculté des Sciences de la Nature et  
de la Vie, Université de Bejaia, 06000 Bejaia, Algeria.

\*Corresponding author: karima.tazrart@univ-bejaia.dz

Received on 18 march 2023

Revised on 8 June 2023

**Abstract**

Natural plain yogurts enriched with 1, 2, and 3% of whole chia seeds (Y1, Y2, and Y3 respectively) were produced, in addition to a control yogurt (CY) without supplementation. The physicochemical parameters monitoring was conducted every week for a period of 4 weeks of cold storage (4°C) at the days 1, 7, 14, 21 and 28 (mentioned as D1, D7, D14, D21 and D28 respectively). The results showed that the pH values and titratable acidity didn't fluctuate significantly between samples ( $P>0.05$ ). The syneresis highest degree was observed for CY, which indicates a better capacity of retaining water for fortified yogurts. The total polyphenol contents increased significantly with increasing enrichment rates and ranged from  $0.13\pm 0.006$  to  $0.21\pm 0.02$  mg EAG/g for CY and Y3 respectively on D1. The antioxidant activity, estimated by DPPH and ABTS tests was significantly higher in enriched yogurts (from  $24.1\pm 1.66$  to  $49.4\pm 2.11$ % of DPPH inhibition on D14 for CY and Y3 respectively). The viability of lactobacilli and streptococci was significantly enhanced in yogurts containing chia seeds compared to the control ( $P<0.05$ ). The sensory analysis revealed that the chia seeds addition increased the intensity of smell, aroma, sweetness and consistency of yogurts and decreased their acidity. Y2 and Y3 samples were the most appreciated by the panelists.

**Keywords:** chia seeds, natural plain yogurt, physicochemical properties, antioxidant activity, LAB counts, sensory analysis

**Introduction**

Yogurt is a well-known fermented dairy food, usually made from cow's milk with or without the addition of certain natural milk derivatives, and whose gel structure

results from the coagulation of milk proteins by lactic acid produced by *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* bacteria (Varga, 2006). The production of yogurt and its consumption are constantly growing due to its sensorial, nutritional and therapeutic properties. In fact, yogurt is nutritionally rich in assimilable protein, milk fat, calcium, folate, and niacin. It is also a very good source of phosphorus, potassium, magnesium, iodine, zinc, vitamin B<sub>2</sub>, B<sub>5</sub>, B<sub>6</sub> and B<sub>12</sub> (Arab *et al.*, 2020). Furthermore, yogurt is considered a probiotic carrier; which is defined as “living microorganisms which, when administered in adequate amounts, confer health benefits to the host” (Sánchez *et al.*, 2017). The particularity of yogurt is due to its ability to be combined with various toppings, which gives the finished product wide taste properties (Nadtochii *et al.*, 2020).

Chia (*Salvia hispanica L.*) is an oilseed, considered as the powerhouse of omega-3 fatty acids, containing premium proteins, high amounts of dietary fiber, vitamins, minerals and is a vital source of bioactive compounds (Chaudhary *et al.*, 2021). It represents an excellent source of antioxidants due to the availability of chlorogenic acid, myrcetin and kaempferol, essential fatty acids and other polyphenols (Alamri and Bayomy, 2020). The latter have been associated with different medicinal influences, in particular positive effects on hypertension, cardiovascular diseases, antidiabetic, anti-inflammatory, antimicrobial and immunostimulant activities (Valdivia-López and Tecante, 2015).

Chia seeds are currently used for food fortification in bakery products, dairy products, etc. (Mburu *et al.*, 2021). Therefore, the addition of these seeds, especially in natural yogurt, could improve its nutritional, nutraceutical and sensory properties (taste, color and texture), with a reduced sugar intake. Indeed, chia supplements have been incorporated into yogurt formulations in various forms: whole chia seeds, chia seed extracts, chia seed mucilage and chia seed flour (Pop *et al.*, 2015; Kwon *et al.*, 2019; Atik *et al.*, 2020; Nadtochii *et al.*, 2020) and generally induced higher nutritive value, raised the antioxidant activity and promoted the LAB survival in the enriched samples. On the other hand, most of the studies reported a negative impact of the chia supplementation on the sensorial acceptability of yogurts (Kowaleski *et al.*, 2020; Ribes *et al.*, 2021).

In our study, we investigated other incorporation levels of chia seeds (1, 2 and 3%) and two factors of variability in yogurts properties have been taken into consideration: the enrichment level and the storage period. The main objectives of this work were to follow the evolution of the physicochemical parameters during the period of storage (4 weeks), to assess the bioactive features and the viability of lactic acid bacteria (LAB) and to evaluate the sensory acceptability of chia seed-supplemented yogurts.

## Materials and methods

### Materials

Whole chia seeds, originated from Brazil, were purchased from a local market in Bejaia (Algeria). The milk used for yogurt preparation is a UHT whole milk

(Soummam, Bejaia) and the yogurt starter cultures were provided by Food Mania Company in a lyophilized form (Algiers, Algeria) and the BB<sub>12</sub> comes as a frozen liquid from CHR Hansen (France).

#### **Yogurt preparation**

For natural plain yogurt preparation, 1L of whole milk was heated to 45°C, then, inoculated with LAB starter, consisting in a mixture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii ssp. bulgaricus* and BB<sub>12</sub> (*Bifidobacterium animalis ssp. lactis*). On the other hand, different amounts of whole chia seeds (1, 2 and 3%) were already weighed in ramekins. After agitation for 15 min, volumes of 10 mL of the milk and starter mixture were introduced into the prepared ramekins and carefully stirred. A control yogurt was prepared by introducing the same volume of the mixture in empty ramekins, which were then covered and incubated at 40°C in a yogurt maker (Moulinex DJC241, France) for 15 hours. The day after, the fermented yogurts were preserved at 4°C.

#### **pH, acidity and syneresis rate of plain yogurts**

The yogurts' pH values were determined using a pH-meter (BANTE instruments, Changhai, China). Titratable acidity (g lactic acid/100 g) was determined by titrimetric methods. Syneresis degree, expressed as proportion of free whey, was measured according to the method described by Purwandari *et al.* (2007). 1.5 g of yogurt were weighed into hemolysis tubes and then centrifuged at 5000 rpm for 20 min (Nuve 200, Ankara, Turkiye) at room temperature. The supernatants were finally recovered and weighed. Syneresis rate is expressed as free whey percentage, and calculated as follows:

$$\text{Syneresis (\%)} = \frac{\text{Weight of the supernatant (g)}}{\text{Weight of yogurt sample (g)}} \times 100 \quad (1)$$

#### **Extraction and quantification of phenolic compounds**

Phenolic compounds were extracted from 1.5 g of yogurt in 30 ml of an ethanol/water mixture (80:20, v/v) as described by Hosseinian and Mazza (2009). For phenolics quantification, the method explained by Cardozo *et al.* (2010) was followed. The results were expressed in equivalent mg of gallic acid per 1 g of sample (mg GAE/g), referring to a calibration curve prepared with a concentration gradient of gallic acid.

#### **Antioxidant activity estimation**

##### *Determination of DPPH radical scavenging activity*

DPPH radical scavenging activity (DPPH RSA) was estimated following the method detailed by Brand-Williams *et al.* (1995). 1 ml of DPPH solution was introduced into a test tube, and then 600 µl of ethanolic extract were added. Test tubes were vigorously shaken and the mixture was incubated in the dark for 30 min at room temperature. The absorbances were finally measured using a spectrophotometer at

517 nm (Secomam 30100, Alès, France). A mixture of 1 ml of DPPH solution and 600  $\mu$ L of extraction solvent was used as a control sample and the results were obtained using the hereafter equation:

$$\text{DPPH RSA (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

#### *Evaluation of antioxidant activity by the ABTS test*

The percentage of ABTS<sup>+</sup> radical inhibition was evaluated by the method described by Re *et al.* (1999). The ABTS solution at 7 mM was obtained by dissolving 38.4 mg of ABTS, 6.62 mg of potassium persulphate and 10 mL of distilled water. The mixture was then agitated for 5 min and incubated overnight at room temperature and away from light. To obtain an optical density of  $0.7 \pm 0.02$  at 734 nm, the solution was diluted with distilled water. For ABTS<sup>+</sup> inhibition test, 200  $\mu$ L of the extract to be assayed were added to 1800  $\mu$ L of the ABTS solution, and after 6 min of incubation in the dark, the absorbance was measured at 734 nm. A control was prepared by replacing the extract with the extraction solvent. The results were calculated following the formula:

$$\text{ABTS}^+ \text{ inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (3)$$

#### **Enumeration of LAB**

For the selective enumeration of *S. thermophilus*, we used the M17 culture medium, while the MRS medium (agar of Man, Rogosa, Sharpe) was chosen for *L. bulgaricus*. Petri dishes were incubated under anaerobic conditions at 44°C for 48 h and 44°C for 72 h, respectively. Colonies were counted only in dishes containing between 30 and 300 colonies. The results were expressed as log colony forming units per milliliter (log cfu/mL) according to the formula: CFU= number of colonies/plate factor x dilution factor.

#### **Sensory analysis**

In order to evaluate the sensorial characteristics of yogurts enriched with chia seeds, a sensory analysis was performed by ten expert judges (aged from 28 to 53 years old) at the Sensory Analysis Laboratory of the University of Bejaia (Algeria). The attributes evaluated were: smell, aroma, sweetness, acidity, consistency and texture on a 5-point scale. The experts were also asked to give a preference score for each sample on a scale of 1 to 9. Codes were assigned to the different yogurts (Y100, Y101, Y102 and Y103 which correspond respectively to the control yogurt and the yogurts enriched with 1, 2 and 3% of chia seeds). The judges were provided with sufficient quantities of the coded samples to taste them as many times as they wished and they were asked to rinse their mouths after each sample tasting.

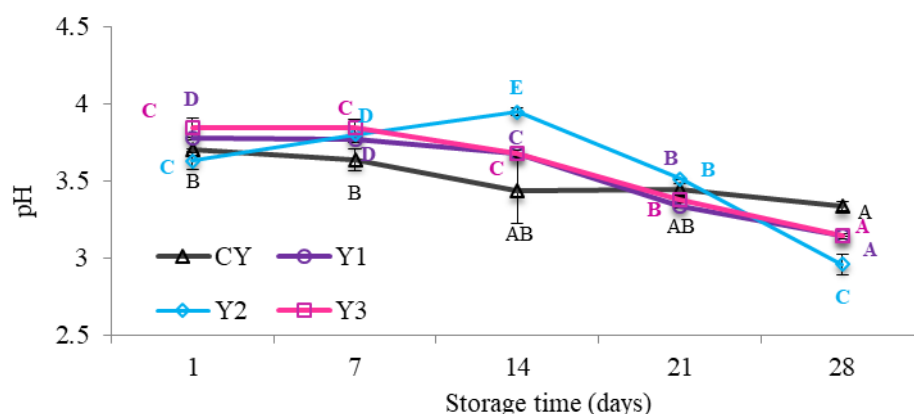
### Statistical analysis

All measurements were carried out in triplicate and weekly, for a period of four weeks (days 1, 7, 14, 21, and 28). Multiple sample comparison of the means (ANOVA) and Fisher's least significant differences (LSD) were applied to establish statistical significant differences between samples. Statistical analyses were carried out by the software XL-STAT 7.0 Addinsoft 2014 (Paris, France) and differences were considered significant at  $P < 0.05$ .

## Results and discussion

### Evolution of samples pH

pH is determined by the density of ionized hydrogen ions in a sample. This chemical index is very important and can indicate the completion of the fermentation process (Tizghadam *et al.*, 2021). It is considered an important quality attribute for dairy products during storage (Nadtochii *et al.*, 2020). Our results revealed that the addition of chia seeds did not have a considerable impact on the pH of the produced yogurts (Figure 1). In fact, it can be observed that on the seventh day of incubation, there was a slight rise in pH when passing from the control yogurt to the yogurt enriched with chia seeds ( $3.63 \pm 0.07$  for CY and  $3.77 \pm 0.01$ ;  $3.8 \pm 0$  and  $3.84 \pm 0.05$  for Y1, Y2 and Y3 respectively). On days 1, 14, 21 and 28 of incubation, pH fluctuations were generally independent of the enrichment rate.



**Figure 1.** Changes in pH values as a function of storage time during yogurt fermentation. CY: control yogurt, Y1: yogurt with 1% chia seeds, Y2: yogurt with 2% chia seeds, Y3: yogurt with 3% chia seeds. Different uppercase letters (A, B, C, D) indicate significant differences among storage times ( $P < 0.05$ ).

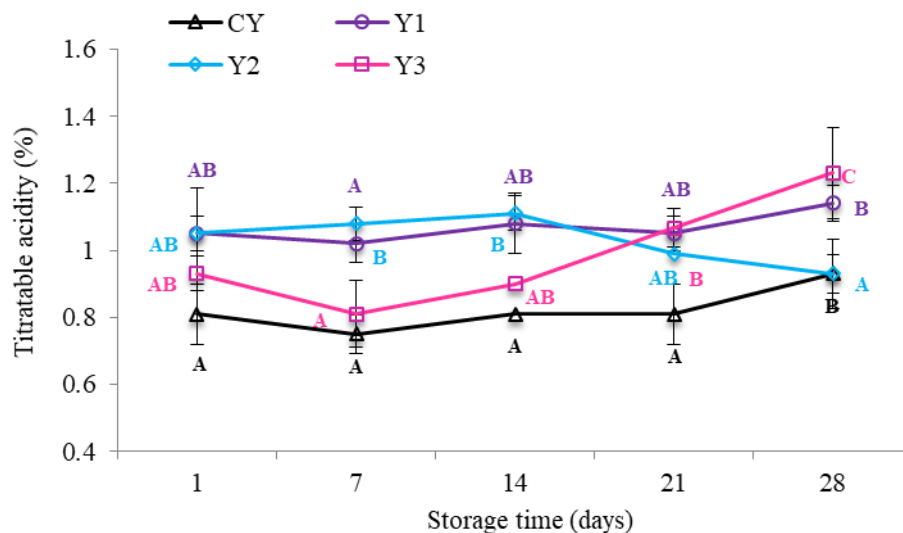
Kwon *et al.* (2019) supplemented yogurt with chia seed extracts and reported lower pH values as compared to the non-enriched sample. This can be explained by the different chia concentration levels used in their study. ANOVA analysis, taking the storage period as a variable, showed that for all samples, the pH tends to decrease gradually as the storage time is extended. The highest pH values are thus observed

on the first day of incubation and the lowest are relative to D<sub>28</sub>. For Y1, the pH drops from  $3.78 \pm 0.01$  on D<sub>1</sub> to  $3.77 \pm 0.01$ ;  $3.68 \pm 0.02$ ;  $3.34 \pm 0.02$  and  $3.14 \pm 0.02$  on D<sub>7</sub>, D<sub>14</sub>, D<sub>21</sub> and D<sub>28</sub> respectively.

Our results are in agreement with those reported by Atik *et al.* (2020) who noticed a significant decrease in pH at the end of the storage period of yogurt enriched with chia seeds mucilage compared to the values noticed on the first day. Similarly, Derewiaka *et al.* (2019) used chia seed oil as an additive to yogurt and found that yogurt enriched with 2% chia seed oil has a lower pH in the first 14 days of storage. They explained the pH changes by the biochemical activity of the still living cells of the lactic acid bacteria. As long as the lactic acid bacteria cells remain biologically active, changes in the chemical composition of the product can be observed.

#### Evolution of samples titratable acidity (TTA)

Acidity measurement is an indicator of bacterial metabolic activity in fermented dairy products (Arab *et al.*, 2020). TTA results (Figure 2) revealed that on days 1, 7 and 14 of incubation, Y1 and Y2 yogurts are significantly ( $P < 0.05$ ) more acid than CY and Y3, while on D<sub>21</sub> TTA was significantly higher in enriched yogurts ( $1.05 \pm 0.05\%$ ,  $0.99 \pm 0\%$  and  $1.06 \pm 0.05\%$  for Y1, Y2 and Y3 respectively, compared to  $0.81 \pm 0.09\%$  for the CY). On D<sub>28</sub>, yogurts Y1 and Y3 were significantly ( $P < 0.05$ ) more acid than CY and Y2. We can therefore state that generally, the incorporation of chia seeds has an impact on the acidity of fortified yogurts regardless of the storage duration.



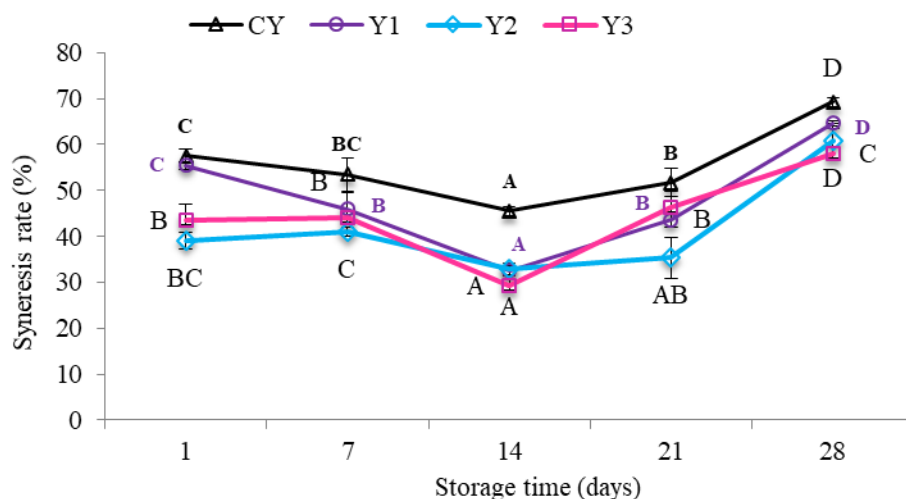
**Figure 2.** Changes in titratable acidity as a function of storage time during yogurt fermentation. CY: control yogurt, Y1: yogurt with 1% chia seeds, Y2: yogurt with 2% chia seeds, Y3: yogurt with 3% chia seeds. Different uppercase letters (A, B, C, D) indicate significant differences among storage times ( $P < 0.05$ ).

The monitoring of the evolution of yogurt's acidity during refrigerated storage showed that for CY, Y1 and Y3 samples, the TTA reaches its maximum on D<sub>28</sub>, while Y2 displays the highest value on D<sub>14</sub>. Our results are in agreement with those reported by Atik *et al.* (2020), who fortified yogurt with chia seed mucilage and observed an increase in acidity of fortified yogurts compared to plain yogurt. They deduced that the yogurt bacteria were more active in the presence of chia mucilage. In the same trend, Nadtochii *et al.* (2020) claimed that polysaccharides are a substrate for various starter cultures.

### *Evolution of samples syneresis rate*

Syneresis is a measure of the serum released from the gel when exposed to centrifugal force. It is a quality indicator determining the water retention capacity of the product (Pachekrepapol *et al.*, 2021). The study of the effect of the incorporation of chia seeds on the syneresis of yogurts (Figure 3) showed that, whatever the incubation time of yogurts, CY always records significantly ( $P < 0.05$ ) the highest syneresis rate compared to enriched yogurts and the lowest values are generally noted for Y2; which means that the whey retention for this sample is better and thus indicates a good yogurt quality.

Statistical data relative to the effect of storage on yogurts' syneresis rates showed that regardless of the type of yogurt, the lowest value is always recorded on D<sub>14</sub> of storage and the highest on D<sub>28</sub>. This suggests that after 28 days of storage, the appearance of yogurts deteriorates following the release of a large quantity of whey.

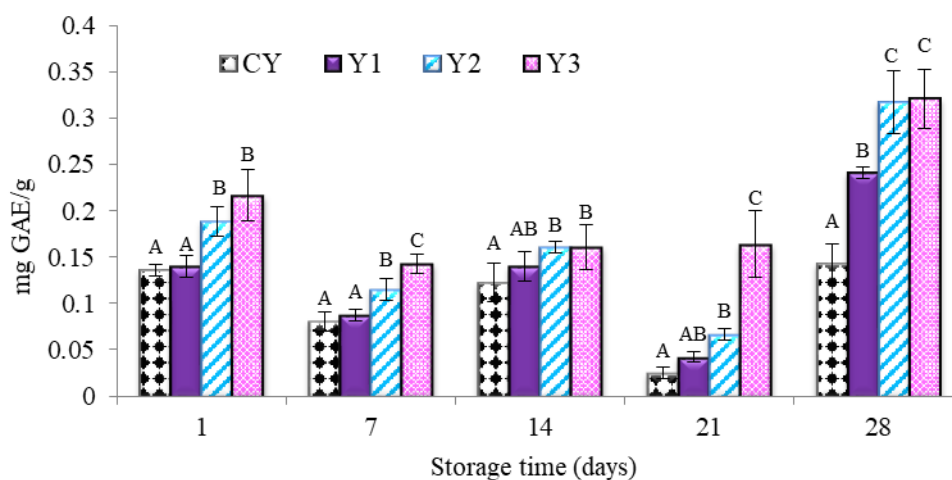


**Figure 3.** Changes in syneresis rate as a function of storage time during the yogurt fermentation. CY: control yogurt, Y1: yogurt with 1% chia seeds, Y2: yogurt with 2% chia seeds, Y3: yogurt with 3% chia seeds. Different uppercase letters (A, B, C, D) indicate significant differences among storage times ( $P < 0.05$ ).

Our results are in agreement with Dal Bello *et al.* (2015) and Atik *et al.* (2020) who enriched their yogurts with chia seed mucilage and  $\omega 3$  polyunsaturated fatty acids respectively. They explained the decrease in yogurt syneresis by the higher proportions of protein and fiber present in the mucilaginous fraction. Ribes *et al.* (2021) in turn explained the decrease in syneresis of chia mucilage-enriched yogurt by the interactions between positively charged casein micelles and negatively charged chia mucilage, which improves the stability of the matrix and reduces the amount of released whey. Syneresis rate has been reported to be proportional to the evolution of acidity throughout the process of storage (Hachana *et al.*, 2017), which explains the increase of yogurt's syneresis rates in the second half of storage period.

#### **Polyphenolic content of obtained yogurts**

Our results, shown in Figure 4, revealed that the content in phenolic compounds of yogurts increases with the increase of the enrichment rate in chia seeds and this, throughout the storage period. On the 7<sup>th</sup> and 28<sup>th</sup> day of storage, the polyphenol content practically doubled when changing from control yogurt to yogurt enriched with 3% chia seeds ( $0.08 \pm 0.01$  to  $0.14 \pm 0.02$  mg/g on D<sub>1</sub> and  $0.14 \pm 0.02$  to  $0.32 \pm 0.03$  mg/g on D<sub>28</sub>). This increase in the level of polyphenols in enriched yogurts is probably linked to the richness of chia seeds in these compounds (Pająk *et al.*, 2019). These were released and solubilized in the yogurt, which induced higher concentrations in the enriched samples.



**Figure 4.** Effect of chia seeds addition on the phenolic content of yogurts. CY: control yogurt, Y1: yogurt with 1% chia seeds, Y2: yogurt with 2% chia seeds, Y3: yogurt with 3% chia seeds. Different uppercase letters (A, B, C, D) indicate significant differences between samples ( $P < 0.05$ ).



Our results are in agreement with those reported by Kwon *et al.* (2019); who enriched their yogurts with chia seed extracts and noticed that their composite samples had significantly higher amounts of phenolic compounds. Regarding the effect of storage on the phenolic compounds level, we observed that whatever the sample analyzed, their content is always significantly higher ( $P < 0.05$ ) on the last day of storage ( $D_{28}$ ). The proteolysis of milk proteins may release amino acids with phenolic side chains such as tyrosine, which could contribute to the increase in phenolic compounds. Furthermore, the microbial metabolism of phenolic compounds in chia yogurt and the production of new phenolic acids during acidification may result in an increase of phenolic groups as the ring structure is broken down (Muniandy *et al.* 2016).

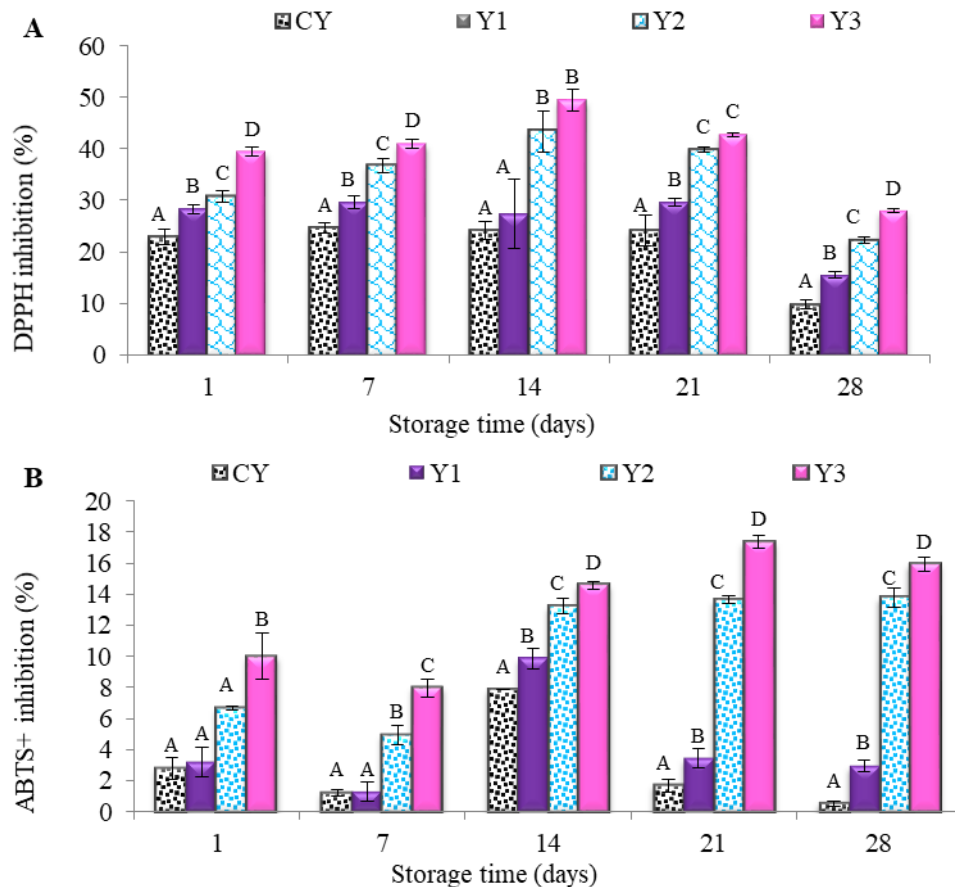
#### ***DPPH radical scavenging activity of obtained yogurts***

The percentage of DPPH radical inhibition increases with the increase in the level of chia seeds in yogurt (Figure 5A). Thus, the highest antioxidant activity is recorded by Y3 and the lowest by the control, regardless of storage time. On days 14 and 21, the percentage of inhibition almost doubled from the control to Y3, while on  $D_{28}$ , the activity of Y3 is three times that of CY ( $27.9 \pm 0.36\%$  against  $9.8 \pm 0.86\%$ ). This can be explained by the fact that fortified yogurts contain a higher proportion of phenolic compounds (Figure 4), provided by chia seeds, and which are known to exert an antioxidant activity (Laczkowski *et al.*, 2018; Pająk *et al.*, 2019). Ahmad *et al.* (2022) reported that yogurt has various antioxidant activities due to amino acids and bioactive peptides produced during fermentation. In addition to this, plant-derived polyphenols greatly enhance its antioxidant properties. To assess the effect of storage time on the antioxidant activity of the produced yogurts, an ANOVA analysis was performed and the results revealed that for all samples, the inhibition percentage on  $D_{28}$  is significantly ( $P < 0.05$ ) the lowest ( $9.8 \pm 0.86$ ;  $15.5 \pm 0.6$ ;  $22.3 \pm 0.63$  and  $27.9 \pm 0.36\%$  for CY, Y1, Y2 and Y3 respectively). For CY and Y1 samples, the highest activity is observed on  $D_7$  ( $24.5 \pm 1.03$  and  $29.6 \pm 1.36\%$  respectively). On the other hand, Y2 and Y3 exhibited maximum antioxidant activity on  $D_{14}$  ( $43.3 \pm 0.54$  and  $49.8 \pm 0.35\%$  respectively). Regarding the decrease in antioxidant activity, observed on the 28th day of storage, our results are in agreement with those of Jiménez-Redondo *et al.* (2022) who enriched their yogurts with cinnamon and explained this reduction after refrigerated storage, to improved protein-phenol interactions during storage.

#### ***Antioxidant activity with the ABTS test of obtained yogurts***

The data of antioxidant activity, as estimated by means of ABTS reduction are illustrated in Figure 5B. The results revealed that on the 1st day of storage, the elevation of the antioxidant activity is insignificant ( $P > 0.05$ ) between the yogurts CY, Y1 and Y2, while Y3 showed significantly higher activity ( $9.98 \pm 1.48\%$ ). After one week of storage, Y2 and Y3 yogurts exhibited significantly higher activity than CY and Y1 ( $4.9 \pm 0.6$  and  $7.9 \pm 0.6\%$  versus  $1.2 \pm 0.17$  and  $1.27 \pm 0.6\%$  respectively). On  $D_1$ ,  $D_{21}$  and  $D_{28}$ , the antioxidant power of yogurts increases progressively and significantly ( $P < 0.05$ ) with the increase in the percentage of chia seeds in the formulation. To follow the evolution of the antioxidant activity during storage, an

analysis of the variance was carried out and the results showed a fluctuation of the activity during the incubation of the yogurts. We can notice that the highest activity is usually induced on days 14 and 21, while the lowest varies between samples.

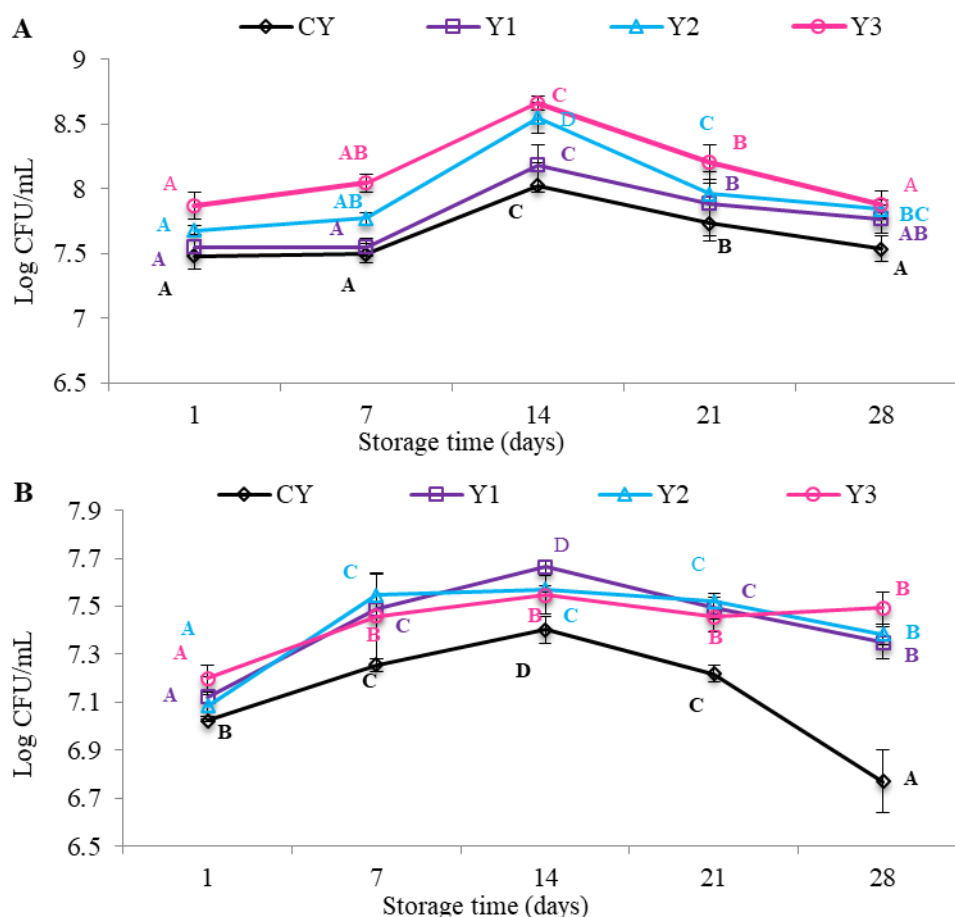


**Figure 5.** Effect of chia seeds addition on DPPH scavenger activity (A) and ABTS<sup>+</sup> radical inhibition (B) of obtained yogurts. CY: control yogurt, Y1: yogurt with 1% chia seeds, Y2: yogurt with 2% chia seeds, Y3: yogurt with 3% chia seeds. Different uppercase letters (A, B, C, D) indicate significant differences between samples ( $P < 0.05$ ).

Our results agree with those reported by Kwon *et al.* (2019) and Atik *et al.* (2020) who fortified yogurt with chia seed extracts and chia seed mucilage respectively, and noted an increase in free radical scavenging abilities with the increase of added products. Atik *et al.* (2020) attributed the high antioxidant capacity of chia seeds to their fiber fraction. They explained that chia seeds and oil exhibited high antioxidant properties, likely due to the presence of polyphenols, alpha-lipoic acid, and other active compounds.

### Bacterial counts

In yogurt, the main strains of LAB contribute to sugar metabolism and extracellular carbohydrates release, generating lactic acid and other flavoring substances, which have an antiseptic effect, modify yogurt acidity and impart distinctive flavors to fermented milk (Ahmad *et al.*, 2022). Our results (Figure 6A) revealed that on days 1, 7, 14, and 21 of incubation, a significant elevation in the number of viable streptococci occurred, starting from the enrichment rate in chia of 2%, while on D<sub>28</sub> the noticeable increase ( $P < 0.05$ ) begins from Y1. Furthermore, from the 7<sup>th</sup> day of incubation, the number of viable lactobacilli (Figure 6B) was significantly higher in enriched yogurts ( $7.48 \pm 0.03$ ,  $7.54 \pm 0.08$  and  $7.45 \pm 0.17$  log cfu/mL for Y1, Y2 and Y3 respectively) compared to CY ( $7.25 \pm 0.02$  log cfu/mL).



**Figure 6.** Changes in viability of *Streptococcus thermophilus* (A) and *Lactobacillus bulgaricus* (B) during yogurt fermentation. CY: control yogurt, Y1: yogurt with 1% chia seeds, Y2: yogurt with 2% chia seeds, Y3: yogurt with 3% chia seeds. Different uppercase letters (A, B, C, D) indicate significant differences among storage times ( $P < 0.05$ ).

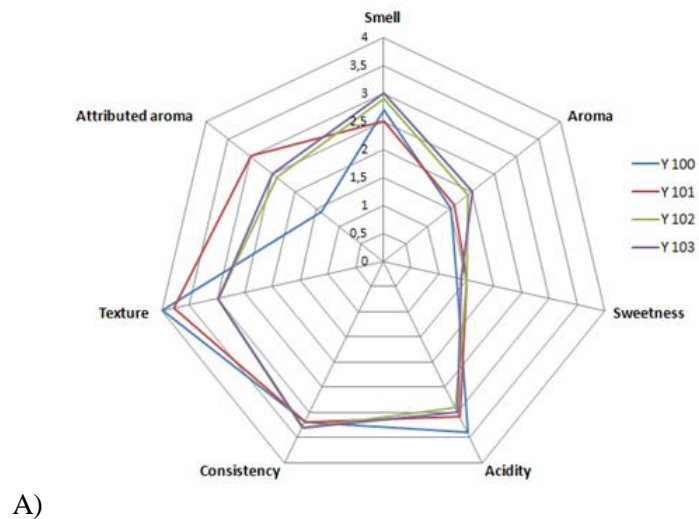
Thus, the addition of chia seeds had a stimulating effect on the growth of yogurt probiotics. Our results are in agreement with those reported by Pop *et al.* (2015) and Kwon *et al.* (2019) who explained the increase in the number of bacteria in yogurts enriched with whole chia seeds at 1.4 % and chia seed extracts respectively, by the high concentrations in antioxidants and polyphenols (especially chlorogenic acid and caffeic acid), provided by the supplements and which have been reported to increase the viability of LAB during yogurt fermentation. The statistical study of the effect of the storage duration on the growth of streptococci showed a gradual increase of their number starting from D<sub>1</sub> ( $7.48 \pm 0.02$ ,  $7.54 \pm 0.05$ ,  $7.68 \pm 0.03$  and  $7.87 \pm 0.1$  log cfu/mL for CY, Y1, Y2 and Y3 respectively), until reaching maximum values (for all the samples) on D<sub>14</sub> ( $8.02 \pm 0.17$ ,  $8.18 \pm 0.15$ ,  $8.54 \pm 0.11$  and  $8.66 \pm 0.05$  log cfu/mL) and then will gradually decrease until the last day of incubation ( $7.53 \pm 0.1$ ,  $7.76 \pm 0.1$ ,  $7.84 \pm 0.07$  and  $7.88 \pm 0.1$  log cfu/mL). The same trend was observed for Lactobacilli since increased acidity leads to inhibition of LAB growth and carbon source also starts to become exhausted with an increase in the bacterial population resulting in a period of attenuation of the bacteria.

### **Sensory evaluation**

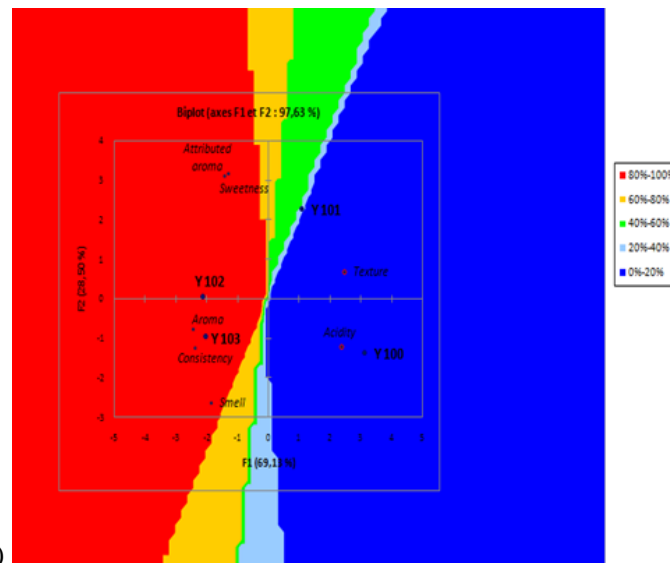
The sensory profiles of the yogurts are presented in Figure 7A. According to the results obtained, the sensory characteristics differed between the four samples as follows: the control (Y100) was characterized by its smoother texture, more acidic taste and less intense aroma compared to enriched yogurts. However, the latter were characterized by a more intense smell, aroma and sweetness, more consistency and granular texture, particularly the samples Y103 and Y102. As a result, the addition of chia seeds affected the sensory characteristics of the yogurts; it increased the intensity of smell, aroma, sweetness and consistency of the samples and decreased their acidity. It might be explained by the richness of chia seeds in aromatic compounds, sugars, fibers and vegetable polymers (Chaudhary *et al.*, 2021).

An External preference mapping (PREFMAP) was created (Figure 7B) on XLSTAT after grouping the panelists according to their preferences by performing Hierarchical Cluster Analysis (HCA), and applying Principal Component Analysis (PCA). This map allows relating preferences to the sensory characteristics of the products expressed by the panelists. This method is important because it is only on this basis that the marketing, research and development teams can adapt the products to the consumers' tastes. According to the results obtained in Figure 8.B, the percentage of satisfaction of the panelists was 100% for the yogurts enriched with chia seeds (Y103 and Y102); they were the most appreciated since they are characterized by their consistency and high intensity of smell, aroma and sweetness, compared to the other yogurts. On the other hand, only 20 to 40% of the panelists appreciated the yogurt Y101 and just 0 to 20% of them appreciated the control. This latter was the least preferred because of its high acidity, as compared to the other products. Consequently, it is worth noting that an improvement in sensory profiles was found in the fortified yogurts and the addition of 2% and 3% of chia seeds was more appreciated by the panelists. In contrast, Kowaleski *et al.* (2020) supplemented yogurts with whole chia seeds at 6, 10 and 14 % and reported a better acceptance for

non-supplemented yogurts. This may be attributed to the differences in the amounts of seeds used in yogurt formulation, as shown in the results reported by Nouri and Basiri (2021), who stated that a negative impact was distinguished by the high chia powder ratio in the formulation.



A)



B)

**Figure 7.** A) Spider graph of the sensory profile of the formulated yogurts. Y100: control yogurt; Y101: yogurt enriched with 1% of chia seeds; Y102: yogurt enriched with 2% of chia seeds; Y103: yogurt enriched with 3% of chia seeds. B) Preference mapping of the formulated yogurts. Y100: control yogurt, it is located in the blue zone corresponding to the judges' satisfaction percentages ranging from 0 % to 20 %; Y101: yogurt enriched with 1% of chia seeds, it is located in the light blue zone corresponding to the judges' satisfaction ranging from 20 % to 40 %; Y102: yogurt enriched with 2% of chia seeds; Y103: yogurt enriched with 3% of chia seeds. Y102 and Y103 are in the red zone, which corresponds to the percentages of satisfied judges ranging from 80% to 100%.

## Conclusions

Our study demonstrated that the chia seeds addition conferred better syneresis rates to enriched yogurts (particularly Y2), and significantly increased their polyphenolic contents and antioxidant activity. The results also revealed that the survival and growth of yogurts' probiotics were stimulated by the presence of *Salvia hispanica* seeds. The supplementation with chia positively affected the sensory attributes of yogurts since Y2 and Y3 samples have been well rated in terms of smell, sweetness and consistency and those same samples received the maximum percentages of satisfaction by the panelists. We can attest that the enrichment of natural plain yogurt with whole chia seeds enhanced the overall quality of yogurts and the supplementation rates of 2 and 3% allowed the best results.

## Acknowledgments

The authors of this manuscript would like to thank the students Faiza Yahiaoui and Kahina Hatri for their precious help in the realization of the experiments.

## References

- Ahmad, I., Xiong, Z., Hanguo, X., Khalid, N., Khan, R.S. 2022. Formulation and characterization of yogurt prepared with enzymatically hydrolyzed potato powder and whole milk powder. *Journal of Food Science and Technology*, **59**(3), 1087-1096.
- Alamri, E., Bayomy, H. 2020. Therapeutic applications and health promoting properties of chia seeds (*Salvia hispanica*): review. *International Journal of Biosciences*, **16**, 345-353.
- Arab, R., Freidja, M.L., Oomah, B.D., Benali, S., Madani, K., Boulekbache-Makhlouf, L. 2020. Quality parameters, probiotic viability and sensory properties of probiotic stirred sesame yogurt. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology*, **44**(1), 9-25.
- Atik, D.S., Demirci, T., Öztürk, H.İ., Demirci, S., Sert, D., Akın, N. 2020. Chia seed mucilage versus guar gum: Effects on microstructural, textural, and antioxidative properties of set-type yoghurts. *Brazilian Archives of Biology and Technology*, **63**, 1-12.
- Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, **28**(1), 25-30.
- Cardozo, M.L., Ordoñez, R.M., Zampini, I.C., Cuello, A.S., Dibenedetto, G., Isla, M.I. 2010. Evaluation of antioxidant capacity, genotoxicity and polyphenol content of non conventional foods: Prosopis flour. *Food Research International*, **43**(5), 1505-1510.
- Chaudhary, N., Dangi, P., Bishnoi, S. 2021. Chia Seeds—A renewable source as a functional food. In *Handbook of Cereals, Pulses, Roots, and Tubers* (pp. 235-252). CRC Press.
- Dal Bello, B., Torri, L., Piochi, M., Zeppa, G. 2015. Healthy yogurt fortified with n-3 fatty acids from vegetable sources. *Journal of Dairy Science*, **98**(12), 8375-8385.
- Derewiaka, D., Stepnowska, N., Bryś, J., Ziarno, M., Ciecierska, M., Kowalska, J. 2019. Chia seed oil as an additive to yogurt. *Grasas y Aceites*, **70**(2), e302-e302.
- Hachana, Y., Rejeb, R., Chiboub, N., Zneidi, I.A. 2017. Variation factors of yoghurt quality during the manufacturing process. *Journal of New Sciences*, **41**, 2243-2252.
- Hosseinian, F.S., Mazza, G. 2009. Triticale bran and straw: Potential new sources of phenolic acids, proanthocyanidins, and lignans. *Journal of Functional Foods*, **1**(1), 57-64.
- Jiménez-Redondo, N., Vargas, A.E., Teruel-Andreu, C., Lipan, L., Muelas, R., Hernández-García, F., Sendra E., Cano-Lamadrid, M. 2022. Evaluation of cinnammon (*Cinnamomum cassia* and

- Cinnamomum verum*) enriched yoghurt during refrigerated storage. *LWT-Food Science and Technology*, **159**, 113240.
- Kowaleski, J., Quast, L.B., Steffens, J., Lovato, F., dos Santos, L.R., da Silva, S.Z., de Souza D.M., Felicetti, M.A. 2020. Functional yogurt with strawberries and chia seeds. *Food Bioscience*, **37**, 100726.
- Kwon, H.C., Bae, H., Seo, H.G., Han, S.G. 2019. Chia seed extract enhances physiochemical and antioxidant properties of yogurt. *Journal of Dairy Science*, **102**(6), 4870-4876.
- Laczkowski, M.S., Gonçalves, T.R., Gomes, S.T., Março, P.H., Valderrama, P., Matsushita, M. 2018. Application of chemometric methods in the evaluation of antioxidants activity from degreased chia seeds extracts. *LWT*, **95**, 303-307.
- Mburu, M., Paquet-Durand, O., Hitzmann, B., Zettel, V. 2021. Spectroscopic analysis of chia seeds. *Scientific Reports*, **11**(1), 9253.
- Muniandy, P., Shori, A.B., Baba, A.S. 2016. Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packaging and Shelf Life*, **8**, 1-8.
- Nadtochii, L.A., Baranenko, D.A., Lu, W., Safronova, A.V., Lepeshkin, A.I., Ivanova, V.A. 2020. Rheological and physical-chemical properties of yogurt with oat-chia seeds composites. *Agronomy Research*, **18**(3), 1816-1828.
- Nouri, M., Basiri, M. 2021. Enrichment of strawberry frozen yogurt by chia (*Salvia hispanica* L.) seeds. *Food & Health*, **4**(4), 28-34.
- Pachekrepapol, U., Kokhuenkhan, Y., Ongsawat, J. 2021. Formulation of yogurt-like product from coconut milk and evaluation of physicochemical, rheological, and sensory properties. *International Journal of Gastronomy and Food Science*, **25**, 100393.
- Pająk, P., Socha, R., Broniek, J., Królikowska, K., Fortuna, T. 2019. Antioxidant properties, phenolic and mineral composition of germinated chia, golden flax, evening primrose, phacelia and fenugreek. *Food Chemistry*, **275**, 69-76.
- Pop, C., Vlaic, R., Fărcaș, A., Salanță, L., Ghițășan, D., Semeniuc, C., Rotar, A.M. 2015. Influence of pollen, chia seeds and cranberries addition on the physical and probiotics characteristics of yogurt. *Bulletin UASVM Food Science and Technology*, **72**(1), 141-2.
- Purwandari, U., Shah, N.P., Vasiljevic, T. 2007. Effects of exopolysaccharide-producing strains of *Streptococcus thermophilus* on technological and rheological properties of set-type yoghurt. *International Dairy Journal*, **17**(11), 1344-1352.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**(9-10), 1231-1237.
- Ribes, S., Peña, N., Fuentes, A., Talens, P., Barat, J.M. 2021. Chia (*Salvia hispanica* L.) seed mucilage as a fat replacer in yogurts: Effect on their nutritional, technological, and sensory properties. *Journal of Dairy Science*, **104**(3), 2822-2833.
- Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., Margolles, A. 2017. Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, **61**(1), 1600240.
- Tizghadam, P., Roufegari-nejad, L., Asefi, N., Jafarian Asl, P. 2021. Physicochemical characteristics and antioxidant capacity of set yogurt fortified with dill (*Anethum graveolens*) extract. *Journal of Food Measurement and Characterization*, **15**, 3088-3095.
- Valdivia-López, M.Á., Tecante, A. 2015. Chia (*Salvia hispanica*): A review of native Mexican seed and its nutritional and functional properties. *Advances in Food and Nutrition Research*, **75**, 53-75.
- Varga, L. 2006. Effect of acacia (*Robinia pseudo-acacia* L.) honey on the characteristic microflora of yogurt during refrigerated storage. *International Journal of Food Microbiology*, **108**(2), 272-275.