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Chia Seed Mucilage Versus Guar Gum: Effects on Microstructural, Textural, and Antioxidative Properties of Set-Type Yoghurts

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HIGHLIGHTS

- The effects of chia seed mucilage (CSM) and guar gum (GG) on yoghurt were compared.
- CSM ensured higher antioxidative activity to yoghurts in comparison with GG.
- CSM concentrations up to 2% improved firmness of yoghurts better.
- 2% GG and 3% CSM provided a better microstructure to yoghurts.

Abstract: Texture is an important parameter which influences on the quality and acceptability of yoghurts. The utilize of stabilizers in yoghurt manufacturing has become a prevalent application to improve the textural properties of yoghurts. In this context, guar gum obtained from *Cyamopsis tetragonolobus* is generally used as a natural stabilizer for its thickening and gelling properties. Accordingly, this study evaluated the use of chia seed mucilage as an alternative to guar gum to improve the textural properties of yoghurt. This study focused on the effect of using chia seed mucilage (CSM) and guar gum (GG) at 1, 2, and 3% concentrations on the textural and microstructural characteristics of yoghurts. The results of fortifications with CSM and GG on the physicochemical, sensory, and antioxidative properties of yoghurts were also evaluated. Compared to GG, CSM provided higher antioxidant activity which improved with increasing concentrations of CSM. An enhancement was observed in textural properties of yoghurts containing CSM and GG, but CSM concentrations up to 2% gave better effect on firmness. Besides, the microstructure of yoghurt was enhanced depending on the increase in CSM and GG amounts. No negative effect was determined on the sensory properties of the samples by CSM and GG additions. The results showed that CSM can be used in set-type yoghurt production as an alternative stabilizer by improving firmness and consistency and reducing syneresis. Furthermore, its use is suitable for industrial yoghurt production with regards to sensorial properties.

Keywords: antioxidant activity; firmness; microstructure; stabilizer; yoghurt.

INTRODUCTION

Salvia hispanica L. is a plant species known as 'chia' and is a member of the Lamiaceae family. Chia seeds have had an essential place in basic food product lists of several Central American regions, such as Southern Mexico and Northern Guatemala since pre-Columbian times [1]. The roasted and ground chia seeds in these areas are still consumed as gruel or as a refreshing beverage when soaked in water or fruit juice [2]. Chia seeds have a high oil content, ranging from 25 to 35%, and contain a high proportion of polyunsaturated fatty acids especially, linolenic acid (i.e. accounts for 60% of the oil content) [3]. The chia seed is also comprised of 15-25% protein, 26-41% carbohydrates, 18-30% dietary fiber, and 4-5% ash [4]. A total dietary fiber content of 18-30% is remarkably high, when considering the dietary fiber content of other related food products such as almonds (12.2%), peanuts (8.5%), soybeans (9.6%), and quinoa (7%) [5]. Chia seeds also, contain high levels of antioxidative ingredients, vitamins (mainly B vitamins), and minerals such as calcium, magnesium, and phosphorus [6]. The existence of cinnamic acid, chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol in chia seeds has also been reported [2].

Chia seeds are surrounded by a layer which contains mucilage that is composed of a tetrasaccharide with 4-O-methyl-a-D-glucoronopyranosyl. This mucilage has properties similar to several other popular hydrocolloids now being used [4]. When chia seeds are soaked in water, the mucilage separates and increases the viscosity of the aqueous mixture [2, 7]. Muñoz, Cobos [8] reported on the high water-holding capacity of CSM. CSM exhibited an ability to absorb water at 27 times its own weight, making this capacity characteristically more so than that of oat and wheat. In the late 1990's, Grigelmo-Miguel, Gorinstein [9] noted that as dietary fiber content increased, so did the water holding capacity.

Most studies to improve the textural properties of yoghurt have focused on guar gum [10-12]. These studies have been conducted on the use of guar gum as a natural stabilizer considering its gelling and thickening properties in yoghurt production. Guar gum is produced from seeds of *Cyamopsis tetragonolobus* plant belonging to family Leguminosae and its major polysaccharide is galactomannan [11]. Galactomannan is water soluble and provides the formation of viscous solutions [13]. The effect of guar gum on viscosity can alter depending on the molecular weight of the galactomannan. Because of these, guar gum is used as a food additive due to its emulsifying, water-binding, thickening, and stabilizing properties [14]. Soukoulis, Panagiotidis [15] reported that neutral gums such as guar gum enhanced texture of yoghurts, and so inhibited the syneresis.

Yoghurt is a fermented dairy product that provides many health benefits, high nutritional value, and digestibility. Syneresis is an important defect in yoghurt products, and oftentimes restricts the storage duration and consumer preference. To suppress syneresis and improve sensorial scores, numerous attempts were made in yoghurt processing such as the addition of gelatin [16], inulin and polydextrose [17], date fiber [18] and, *Elaeagnus angustifolia* L. flour [19]. Though there is data available on the high water holding capacity of chia seed and its potential use for improvement in textural parameters, little information is known regarding the performance of CSM as gelling or thickening agents in yoghurt production.

Considering all the aforementioned, the use of CSM in yoghurt production as an alternative to guar gum was evaluated in this study. The main objectives of this study were to monitor the incorporation of CSM, as an alternative ingredient to guar gum, on the textural parameters, syneresis values, microstructure, physicochemical properties, and sensory acceptability of yoghurts; as well as, the enhancing effect of CSM on antioxidative activity and phenolic content of experimental yoghurts during 28 days of shelf-life as compared with guar gum, which is a commercial hydrocolloid gelling additive.

MATERIAL AND METHODS

Materials

Raw cow milk (3.5% fat, 8.2% NFSM) was obtained from the Selcuk University Dairy Farm; medium-heat skim milk powder (34.5% protein, 55% lactose, 3.5% moisture, and 7.2% ash) was supplied from ENKA Dairy Product (Konya, Turkey); guar gum (food grade, Alfasol) was supplied from Molar Chemistry, Turkey; black variety of chia was purchased from a commercial market in Turkey; and the freeze-dried yoghurt starter culture YF-L901, consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, was supplied by Chr. Hansen-Peyma (Istanbul, Turkey).

Mucilage extraction process from chia seeds

The CSM was obtained according to the method of Campos, Dias Ruivo [20]. Distilled water was used in a seed:water ratio of 1:14 for extraction and thereafter, the mixture was subjected to a 70 °C water bath for 3 h (Memmert, WB22, Germany). The mixture was dried overnight at 50 °C in a drying oven and the dried mucilage was separated from the seeds by passing through a sieve (stainless steel) with a 0.5 mm fine mesh.

Production of set-type yoghurt

Dry matter of milk was standardized to 16% by adding medium-heat skim milk powder and it was then divided into 7 experimental groups including control without any additives. CSM and GG were added separately at 1, 2, and 3% concentrations to each experimental groups except control sample. Each treatment was pasteurized at 90 °C for 10 min and then rapidly cooled to 44 °C for inoculation of the starter culture. The yoghurt mixes were inoculated with 2% starter culture and they were then put into 100 mL sterile plastic containers. All treatments were incubated at 44 °C until the pH reached 4.5, and later cooled and stored at 4 °C. Yoghurt samples containing CSM and GG with concentrations ranging from 1 to 3% were identified as CMY1, CMY2, CMY3, GGY1, GGY2, and GGY3, respectively.

Physicochemical analysis

The pH, which pH-meter (WTW-315i, Germany) was used for its measurement, and titratable acidity of yoghurt samples were determined according to AOAC [21] method. Syneresis was analyzed according to the method of Isanga and Zhang [22]. L*, a*, b* values for color analysis of yoghurts were quantified by using the chroma meter CR-400 (Konica Minolta, Inc., Osaka, Japan) [23]. Protein and fat content of samples were measured by the Kjeldahl and Gerber methods, respectively [24, 25]. Titration acidity, pH and syneresis analyses were monitored throughout 28 days of storage, while other physicochemical analyses were performed on day 14 of storage. All performed analyses were duplicated.

Texture analysis

Texture profiles of experimental yoghurts were determined by using the TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, England) equipped with a 500 N compression load cell and operating at 1 mm/s head speed. The probe was a 25-mm acrylic cylinder, moved speed of 5 mm/s and test speed of 1 mm/s through 10 mm within the sample. The results were given as firmness (g), consistency (g sec), cohesiveness (g), and viscosity index (g sec). Triplicate texture analysis measurements were performed for each sample over the 28 days of cold storage.

Confocal laser scanning microscopy (CLSM) analysis

The inverted confocal laser scanning microscope (A1/A1R, Nikon, Japan) with Nikon Plan Fluor, and a PA:0.30 objective lens was used for the confocal laser scanning (CLSM) analysis. This analysis was performed in the dark and an Ar laser line (488 nm) was used as a light source to excite the fluorescent dye Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA), which is a stain protein. The dye solution was prepared by dissolving 0.2 g of Rhodamine B in 100 mL of distilled water at 20±1 °C. A minimum of four images were obtained for each treatment on day 14 of storage.

Determination of antioxidant activity and total phenolic content

The antioxidant activity of extracted samples according to the method of Öztürk, Aydın [19], was determined by the DPPH scavenging method described by Shetty, Curtis [26] and the ABTS scavenging method conducted by Re, Pellegrini [27]. Total phenolic content of experimental samples was analyzed according to the method of Tseng and Zhao [28]. All trials were duplicated and performed on day 14 of storage.

Sensory analysis

Sensory acceptability of yoghurt samples was assessed by seven trained panelists on the 14th day of storage. A seven-point hedonic scale was used for appraising of color and appearance, odor, body and texture, acidity, taste, and overall preference (i.e. 1-7, Bad-Excellent) [29].

Statistical analysis

The results were analyzed by using one-way ANOVA in Minitab software version 17 (State College, USA). Mean values were compared by the Tukey test at P<0.05, and statistically significant differences among them were indicated by different letters.

RESULTS AND DISCUSSION

Evaluation of physicochemical characteristics of yoghurt samples

It has been determined that adding 3% of GG causes textural problem in yoghurt production. Therefore, yoghurt samples with 3% GG were not analyzed. In parallel with our observation, Brennan and Tudorica [30] reported that the GG should not be used in yoghurt formulations higher concentrations than 2%.

Depending on CSM and GG addition, fermentation time of yoghurts decreased by about 1 hour as compared to plain yoghurt. The most effective reduction was monitored in yoghurts containing 2% CSM. Generally, the fermentation of GG supplemented yoghurts was completed approximately 15 minutes later than that of yoghurts with CSM. The pH results of yoghurts stored for 28 days are presented in Figure 1. On day 1, the pH of yoghurts enriched with CSM ranged from 4.30 to 4.40, which is similar to the pH of plain yoghurt. However, the pH values of yoghurt fortified with guar gum were higher than the other samples. This finding is in contrast with the result of Koksoy and Kilic [31] who reported that guar gum did not affect the pH of ayran, a widely consumed yoghurt-based drink in Turkey. In this study, the addition of guar gum caused higher pH values in yoghurt samples. In all samples, pH decreased significantly (p<0.05) at the end of the storage period as compared to the initial day. Similar results were reported for yoghurts fortified with green lentils [32] and pineapple peel powder [33].

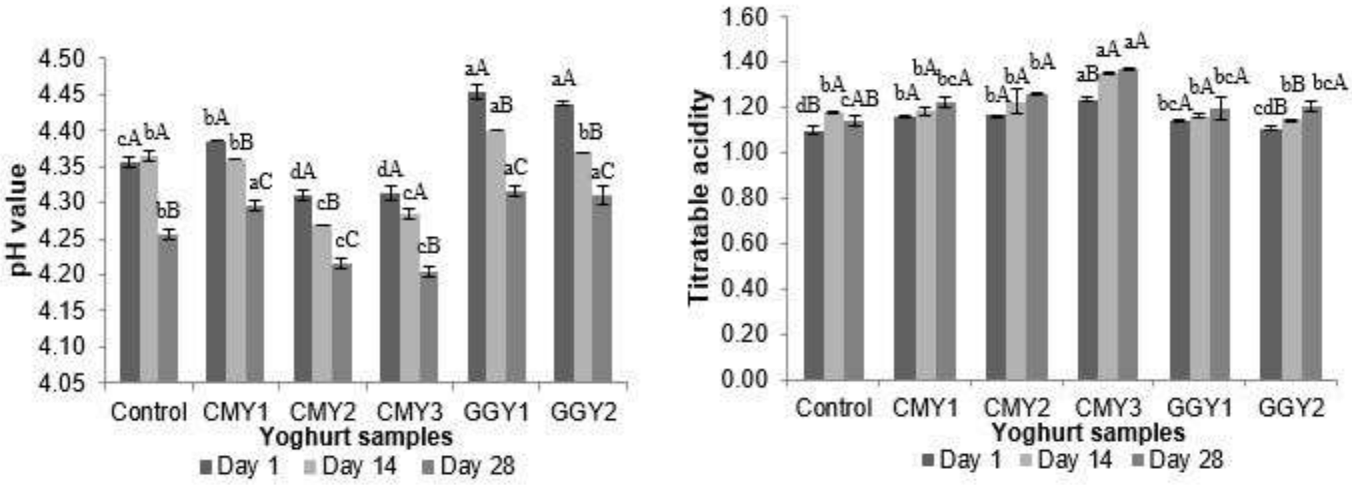

Figure 1. pH and titratable acidity values of experimental samples during storage. CMY1: yoghurt with 1% chia seed mucilage, CMY2: yoghurt with 2% chia seed mucilage, CMY3: yoghurt with 3% chia seed mucilage, GGY1: yoghurt with 1% guar gum, GGY2: yoghurt with 2% guar gum. The error bars represent standard deviation of means (n=2). Lower-case letters present the differences between the samples in the same storage time and upper-case letters show differences between the storage times of samples (P < 0.05).

Figure 1 shows titratable acidity of yoghurt samples stored for 28 days. On the first day of storage, titratable acidity values were found to be between 1.10 and 1.24% lactic acid, with the highest titratable acidity observed in CMY3. This may be due to the positive effect of CSM on the yoghurt bacteria. Indeed, the counts of viable lactic acid bacteria were higher in CMY3 compared to the control sample, but not statistically remarkable (data not shown). This result is supported by the findings of Pop, Vlaic [34] who determined that the addition of 1.4% chia seed to yoghurt increased the number of lactic acid bacteria in comparison with the control sample. Besides, Kwon, Bae [35] reported that the addition of 0.1% chia seed water extract and chia seed ethanol extract into yoghurts significantly increased the number of lactic acid bacteria. Compared to the initial day, a statistical increase in titratable acidity values of CMY3 and GGY2 was observed at the end of the storage period (p<0.05). This increment conforms with the study of Demirci, Aktaş [36] who used rice bran to the yoghurt formulation. It is believed that the high acidity of CMY3 in comparison with control, GGY1, and GGY2, may originate from the beneficial effect of 3% CSM on starter cultures.

Table 1 shows the physicochemical characteristics of experimental yoghurt samples. The protein content was found to vary between 4.82 and 5.66% amongst all treatments. Guar gum addition caused a reduction in protein content of yoghurt samples. Yoghurts enriched with CSM had the highest protein ratio resulting from the large protein content of CSM [5]. The fat contents of experimental samples ranged from 2.00 to 2.90%, while, the highest fat content was observed in CMY3. However, the addition of 1 and 2% CSM did not affect the fat content of samples. Though chia seed have high fat content consisting primarily of alpha-linolenic acid [6], CSM have much lower amounts of fat in comparison, which explains the slight increment in fat content of CMY3 [37].

The L* values of experimental samples were not affected by the addition of CSM or guar gum ($p>0.05$). The addition of 3% CSM increased the a* value (red (+)-green (-)), whereas there was no variation between CMY1, CMY2, and the control sample. 1% and 2% guar gum addition caused a slight decline in redness value of yoghurt samples. Felisberto, Wahanik [38] reported that the use of CSM advanced the a* value of cakes which consistent with our study. Samples fortified with 1 and 2% CSM had statistically similar b* values (yellow (+)-blue (-)) as GGY1 and GGY2 yoghurts, whereas the use of 3% CSM in formulation diminished the yellowness value of yoghurt. In the same way, Felisberto, Wahanik [38] reported that b* value decreased in cakes by increasing the CSM ratio used in formulation.

Table 1. Physicochemical characteristics and sensory scores of yoghurt samples on day 14 of storage.

		Control	CMY1	CMY2	CMY3	GGY1	GGY2
Physicochemical characteristics							
Protein (%)		5.35 ± 0.02^{ab}	5.66 ± 0.16^{a}	5.54 ± 0.03^{a}	5.65 ± 0.01^{a}	4.82 ± 0.03^{c}	5.07 ± 0.19^{b}
Fat (%)		2.20 ± 0.00^{b}	2.00 ± 0.00^{b}	2.00 ± 0.00^{b}	2.90 ± 0.14^{a}	2.00 ± 0.00^{b}	2.20 ± 0.28^{b}
	L*	89.37 ± 0.67^{ns}	89.62 ± 0.38^{ns}	88.91 ± 0.52^{ns}	88.89 ± 0.43^{ns}	89.75 ± 0.14^{ns}	89.08 ± 0.36^{ns}
Color	a*	-4.19 ± 0.06^{ab}	-4.26 ± 0.06^{b}	-4.17 ± 0.15^{ab}	-4.07 ± 0.08^{a}	-4.29 ± 0.05^{b}	-4.34 ± 0.04^{b}
	b*	8.57 ± 0.23^{a}	8.32 ± 0.49^{ab}	8.11 ± 0.14^{ab}	7.86 ± 0.22^{b}	8.10 ± 0.20^{ab}	8.20 ± 0.06^{ab}
Sensory scores							
Color and appearance		5.86 ± 1.07^{ns}	6.29 ± 0.76^{ns}	5.71 ± 0.76^{ns}	5.29 ± 0.76^{ns}	5.86 ± 0.90^{ns}	5.29 ± 0.95^{ns}
Odor		5.43 ± 1.27^{ns}	5.71 ± 0.76^{ns}	5.14 ± 0.69^{ns}	5.57 ± 0.79^{ns}	5.71 ± 0.76^{ns}	5.43 ± 1.13^{ns}
Body and texture		5.43 ± 0.98^{ns}	5.57 ± 1.27^{ns}	5.14 ± 0.38^{ns}	4.43 ± 1.27^{ns}	5.00 ± 1.00^{ns}	4.00 ± 1.29^{ns}
Acidity		5.00 ± 0.82^{ns}	5.29 ± 0.95^{ns}	5.00 ± 1.15^{ns}	5.14 ± 0.69^{ns}	5.29 ± 1.38^{ns}	5.00 ± 0.58^{ns}
Taste		4.57 ± 1.40^{ns}	5.71 ± 0.49^{ns}	4.71 ± 1.11^{ns}	4.00 ± 1.29^{ns}	5.43 ± 1.13^{ns}	4.71 ± 1.11^{ns}
Overall preference		4.71 ± 1.60^{ns}	5.43 ± 0.79^{ns}	4.71 ± 0.95^{ns}	4.29 ± 1.38^{ns}	5.71 ± 0.95^{ns}	5.00 ± 1.00^{ns}

CMY1: yoghurt with 1% chia seed mucilage, CMY2: yoghurt with 2% chia seed mucilage, CMY3: yoghurt with 3% chia seed mucilage, GGY1: yoghurt with 1% guar gum, GGY2: yoghurt with 2% guar gum.
Results are expressed as the mean ± standard deviation (n = 2).
Different letters in the same row are significantly different ($P < 0.05$). ns: not statistically significant.

Syneresis

Effects of CSM addition and guar gum on syneresis, an undesirable feature in yoghurt quality, are shown in Table 2. CMY2, CMY3, and GGY2 had the lowest syneresis values (11.22±0.22, 11.70±0.30, and 11.46±0.06%, respectively). The use of CSM gave the samples lower syneresis compared to plain yoghurt at the end of the 28 days of cold storage. Syneresis decreased with the increasing of mucilage concentration from 1% to 2%, but no statistical difference in syneresis values were observed between CMY2 and CMY3. GGY2 however, had statistically higher syneresis values whereas, 1% GG supplementation generated no statistical difference in comparison to plain yoghurt. In all CSM levels of addition, the positive effect of CSM fortification on decreasing yoghurt syneresis may be caused by the higher proportions of protein and fiber contents present in this mucilaginous fraction. Likewise, Baú, Garcia [39] mentioned that soy fiber reduced whey separation from kefir. Indeed, Dickinson [40] reported that hydrocolloids rich in protein are good stabilizers as a result of their numerous hydrophobic groups that decrease surface tension in interfaces. Alfredo, Gabriel [3] also stated that fiber content is an important factor to consider when increasing the gel forming and water retention capacity of a matrix. This is in accordance with explanations given by Coorey, Tjoe [37], who reported that chia seed gel had a better water retention capacity than commercially used guar gum (10.7 times lower) and gelatin (37 times lower).

In the present study, at the end of the evaluated storage, syneresis values of all yoghurt samples containing both CSM and guar gum were statistically lower than plain yoghurt, with the exception of GGY1. The presence of 1% CSM in yoghurt had significantly lower syneresis values in contrast to that of 1% guar gum at the end of the storage. Syneresis values remained slightly low in yoghurt samples containing 2%

CSM in comparison to those with the guar gum at the same addition rate; however, this difference was not statistically significant. In a previous study, Timilsena, Adhikari [41] found that, regarding guar gum and CSM, chia seed gum demonstrated high water retention capacity comparable to that of guar gum. In this regard, their findings coincided with our results about the behaviors of CSM and guar gum for yoghurt syneresis. Moreover, Kwon, Bae [35] revealed that 0.1% chia seed aqueous extract and ethanol extract improved water holding capacity in yoghurts due to the high reactivity of the polyphenols they contain, such as chlorogenic and caffeic acid. Overall however, the samples containing 2% CSM had remarkably lower syneresis in comparison to all other treatments (i.e. samples containing 1% and 3% CSM). This is not at all surprising, given that Srisuvor, Chinprahast [17] revealed similar results, when their study demonstrated that the most desirable whey separation in yoghurts was produced with 2% inulin and polydextrose as compared to those with 1% and 3% addition. Timilsena, Adhikari [41] reported that CSM had limited solubility at concentrations above 2%, like most polysaccharides of plant origin, which parallels with our findings.

Table 2. Syneresis of experimental yoghurt samples during storage.

		Day 1	Day 14	Day 28
Syneresis (%)	Control	22.06 ± 0.06^{bA}	21.08 ± 0.08^{bB}	15.08 ± 0.02^{aC}
	CMY1	22.42 ± 0.02^{bA}	21.84 ± 0.16^{aB}	14.20 ± 0.20^{bC}
	CMY2	22.40 ± 0.20^{bA}	16.34 ± 0.18^{cB}	11.22 ± 0.22^{cC}
	CMY3	19.12 ± 0.12^{dA}	15.78 ± 0.02^{dB}	11.70 ± 0.30^{cC}
	GGY1	23.02 ± 0.06^{aA}	16.46 ± 0.16^{cB}	15.16 ± 0.16^{aC}
	GGY2	20.72 ± 0.28^{cA}	15.48 ± 0.16^{dAB}	11.46 ± 0.06^{cC}

CMY1: yoghurt with 1% chia seed mucilage, CMY2: yoghurt with 2% chia seed mucilage, CMY3: yoghurt with 3% chia seed mucilage, GGY1: yoghurt with 1% guar gum, GGY2: yoghurt with 2% guar gum.
Results are expressed as the mean ± standard deviation (n = 2).
Different letters in the same row and column are significantly different ($P < 0.05$).
Lower-case letters present the differences between the samples in the same storage time and upper-case letters show differences between the storage times of samples.

Textural properties

The variations in the textural characteristics of yoghurt are shown in Table 3. With the exception of plain yoghurt, an increment in firmness progressively occurred throughout the assessed storage period in all yoghurt formulations. This finding may be due to the gradual decrease in syneresis amounts within this period. Over the evaluated storage period, it was found that the greater firmness values were obtained from yoghurt samples with 1% and 2% CSM (422.38±2.59 and 404.22±4.86 g, respectively) compared to other yoghurt samples. Overall, the presence of CSM in yoghurt, up to an appropriate concentration (2%), resulted in increased firmness in comparison to plain and guar gum supplemented yoghurts; most probably due to its high protein, fiber content, and fat content. Concerning this matter, it was reported that higher protein content generated a higher percentage of cross-linkage of the gel structure reflecting a stronger and firmer formation [42]. Additionally, Ranadheera, Evans [43] indicated that high dietary fiber content in yoghurt had been associated with lower syneresis; while fat globules in the protein network were indicated to play a critical role in increasing water retention which is relevant to a constant gel matrix [44]. Hence, it is a normal circumstance that chia rich in protein, fat, and dietary fiber could avoid whey expulsion from a stable gel network, thus improving firmness and viscosity in yoghurt. However, our results displayed that the addition of CSM greatly advanced firmness as opposed to the findings reported by Basiri, Haidary [45] where the addition of flax seed mucilage to yoghurt showed reduced firmness.

With the exception of plain yoghurt, firmness and consistency values consistently augmented over the course of the storage period in parallel with the findings of Serra, Trujillo [46], and Sahan, Yasar [47]. As expected, increments in the firmness and consistency values of fortified yoghurts with CSM correlated with decline in the whey separation over the evaluated storage. Vianna, Canto [48] have recently reported a similar trend in yoghurt between spontaneous syneresis and firmness values during storage. The most effective consistency result was obtained from GGY2 samples, followed by yoghurts enriched with CSM. Contrary to other findings, the addition of CSM at 2% or 3% ratios had no greater effect in regards to firmness than ones with 2% GG. On the other hand, cohesiveness values remained higher at the end of the storage period in yoghurts enriched with 2% CSM compared to other treatments; however, some previous studies observed contrasting results where the cohesiveness values of inulin, quince seed mucilage and flax seed mucilage added yoghurt samples were similar to plain yoghurts during and at the end of the storage [45, 49]. In spite of this, it is clear that CSM enrichment had desired outcomes as compared to plain yoghurt.

Table 3. Textural characteristics of experimental yoghurt samples.

	Control	CMY1	CMY2	CMY3	GGY1	GGY2
Firmness (g)						
Day 1	378.47±7.88^{aA}	365.13±5.38^{bC}	351.26±2.05^{cC}	307.66±2.76^{eC}	299.46±0.61^{eC}	334.02±2.59^{dC}
Day 14	362.30±3.53^{bB}	392.80±2.84^{aB}	388.97±1.11^{aB}	339.23±4.32^{cB}	321.00±5.33^{dB}	392.80±3.89^{aB}
Day 28	374.41±3.38^{cAB}	422.38±2.59^{aA}	404.22±4.86^{bA}	370.12±3.44^{cA}	345.06±2.21^{dA}	403.68±3.36^{bA}
Consistency (g sec)						
Day 1	9073.59±27.43^{aA}	8984.08±27.93^{bC}	9041.15±34.39^{abC}	7969.21±3.37^{dC}	7374.29±5.53^{eC}	8315.26±5.22^{cC}
Day 14	8549.83±3.06^{eC}	9386.23±1.69^{cB}	9794.40±4.87^{bB}	8730.63±0.87^{dB}	7897.71±7.18^{fB}	9944.24±0.79^{aB}
Day 28	8795.44±1.44^{fB}	10164.39±3.72^{cA}	10196.04±5.17^{bA}	9287.65±6.35^{dA}	8818.60±4.93^{eA}	10424.90±3.93^{aA}
Cohesiveness (g)						
Day 1	146.74±3.77^{dC}	218.47±2.49^{aB}	210.27±1.39^{bB}	204.52±0.87^{bC}	205.59±0.92^{bB}	196.78±2.27^{cB}
Day14	289.35±0.37^{aA}	249.81±3.04^{bA}	146.97±5.31^{eC}	239.69±1.18^{cdA}	237.55±3.20^{dA}	247.97±3.86^{bcA}
Day28	188.66±0.91^{cB}	160.38±2.29^{dC}	255.94±1.45^{aA}	208.05±1.05^{bB}	150.04±1.86^{eC}	163.68±1.40^{dC}
Index of viscosity (g sec)						
Day 1	442.56±6.09^{cB}	531.30±3.20^{aB}	481.52±4.45^{bB}	479.51±1.36^{bB}	369.42±3.38^{dB}	482.69±0.39^{bB}
Day14	431.61±3.89^{eB}	632.08±2.78^{aA}	282.83±2.38^{fC}	442.61±3.21^{dC}	472.33±1.43^{cA}	574.91±3.39^{bA}
Day28	518.54±4.41^{bA}	200.68±0.84^{eC}	506.06±2.35^{cA}	554.03±1.31^{aA}	353.86±3.24^{dC}	353.77±2.95^{dC}

CMY1: yoghurt with 1% chia seed mucilage, CMY2: yoghurt with 2% chia seed mucilage, CMY3: yoghurt with 3% chia seed mucilage, GGY1: yoghurt with 1% guar gum, GGY2: yoghurt with 2% guar gum.
Results are expressed as the mean ± standard deviation (n = 3).
Different letters in the same row and column are significantly different ($P < 0.05$).
Lower-case letters present the differences between the samples in the same storage time and upper-case letters show differences between the storage times of samples.

Regarding viscosity, no similar trend was observed with respect to the other textural parameters. The viscosity of yoghurts fortified with CSM increased in accordance with the increase of added mucilage, but plain yoghurt remained higher compared to CMY2 and CMY1 at the end of the storage. Samples with 3% CSM and the control group had the highest viscosity values (554.03±1.31 and 518.54±4.41 g sec, respectively) amongst all the treatments after 28 days of storage. Regardless of the incremental amount of guar gum, there were no significant variations in viscosity values of yoghurts with guar gum after 28 days of shelf-life. All the formulations containing CSM and guar gum under varying concentrations, experienced several fluctuations throughout the entire storage period in contrast to previous findings of study conducted by Basiri, Haidary [45] who reported a regular decrease in flax seed added yoghurts during all days of evaluation (0, 14, and 21 days). CMY3 had the greatest viscosity values than that of all assessed yoghurt formulations at the end, which is somewhat comparable with the report by Isanga and Zhang [50] who stated that the higher levels of fat might contribute to higher viscosity of yoghurts.

Microstructural characteristics

The three-dimensional protein network and microstructural images of yoghurt samples are presented in Figure 2. The large pores containing serum between casein particles are apparent in the CLSM images for control, CMY1, and CMY2. These samples contained a heterogeneous protein network with large serum pores; however, such structures were not observed in CMY3, GGY1, and GGY2. The addition of 2% guar gum and 3% CSM led to larger protein cluster formations and smaller serum pores compared to the microstructure of the plain yoghurt. The formation of smaller serum pores may be a result of the lower whey separation in CMY3 and GGY2 as compared with other yoghurt samples (Table 2). The reduction in the amount and size of serum pores enhances the density of the protein matrix, and thereby ensures the formation of larger protein clusters [51].

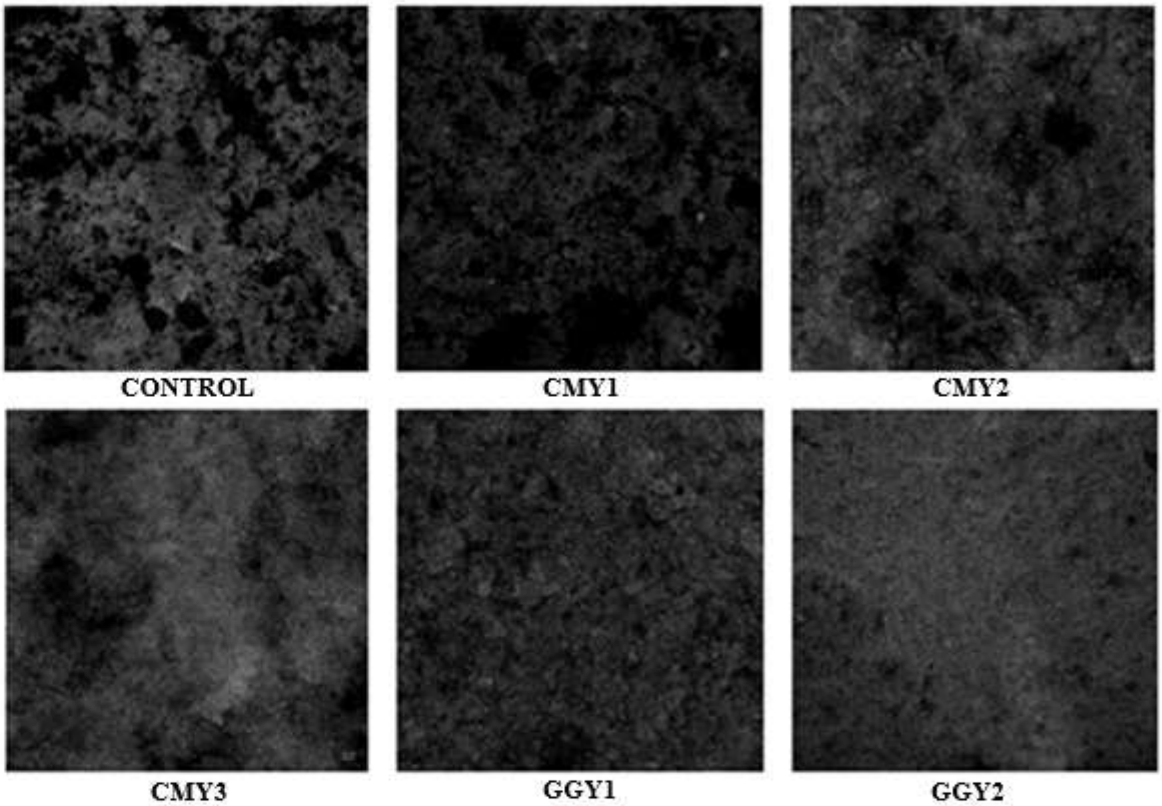

Figure 2. Confocal laser scanning microscope images of experimental yoghurt samples. CMY1: yoghurt with 1% chia seed mucilage, CMY2: yoghurt with 2% chia seed mucilage, CMY3: yoghurt with 3% chia seed mucilage, GGY1: yoghurt with 1% guar gum, GGY2: yoghurt with 2% guar gum. Scale bar: 10 µm. Grey area indicates protein network and dark area shows serum phase.

Remarkable differences were found in the microstructural characteristics of yoghurts enriched with CSM. Depending on its concentration, the addition of CSM affected the serum distribution, serum pore size, and compactness of the protein network. The microstructures of CMY1 and the control sample showed similarities. Nevertheless, as the amount of CSM in formulation increased, the number and size of serum pores decreased, and the density of protein matrix improved. This may have resulted from the water-holding ability of CSM. Indeed, Capitani, Ixtaina [7] observed that CSM had high water absorption capacity. In addition, the denser protein matrix detected in CMY3 rather than in CMY1 and CMY2 is consistent with the results of Krzeminski, Großhable [52] who found that high fat content provided a compact structure for yoghurt gel.

Likewise, the increment in the concentration of guar gum provided small serum pores and great interconnectivity between casein micelles in the yoghurt gel. Zhang, Zhou [53] reported that a rise in concentration of guar gum improves intermolecular chain interactions. Among the experimental samples, the best microstructural features were determined in GGY2, followed by CMY3. The slight microstructural differences between CMY3 and GGY2 were due to the size of the serum pores.

Antioxidant activity and total phenolic content

Chia seeds contain several phenolic compounds which behave like antioxidants such as, caffeic acid, chlorogenic acid, myricetin, quercetin, and kaempferol [54]. Mihaylova and Schalow [55] revealed that high antioxidant properties were obtained by using quercetin in model food systems. The most important phenolic compounds present in chia seeds are caffeic and chlorogenic acids, which protect against free radicals and suppress peroxidation of fats, proteins, and DNA [5]. Considering the data provided above, we anticipated the total phenolic content, determined from the DPPH and ABTS radical scavenging activity, would progressively increase with the addition of CSM. CSM addition to yoghurt resulted in a satisfactory improvement effect on antioxidative activity in comparison to plain yoghurt (Table 4). These results are in agreement with the observations of Alfredo, Gabriel [3] who attributed the high antioxidative capacity to chia fibrous fraction. This aspect was similarly supported by [56], who stated that chia seed and oil presented high antioxidant properties possibly due to the presence of polyphenols, alpha lipoic acid, and other active compounds.

Table 4. Antioxidant activity and total phenolic content of experimental yoghurt samples on day 14 of storage.

	ABTS$^+$ (µM trolox g^{-1})	DPPH (% inhibition)	Total phenolic content (µg GAE g^{-1})
Control	145.88±0.99^e	1.91±0.01^f	7.02±0.04^f
CMY1	162.82±0.93^d	2.15±0.02^d	17.03±0.03^d
CMY2	175.07±0.27^b	2.42±0.01^c	58.80±0.19^b
CMY3	194.47±0.33^a	3.28±0.02^a	72.15±0.22^a
GGY1	163.68±0.79^d	2.06±0.00^e	15.49±0.23^e
GGY2	167.83±0.91^c	2.97±0.02^b	52.44±0.62^c

CMY1: yoghurt with 1% chia seed mucilage, CMY2: yoghurt with 2% chia seed mucilage, CMY3: yoghurt with 3% chia seed mucilage, GGY1: yoghurt with 1% guar gum, GGY2: yoghurt with 2% guar gum.
Results are expressed as the mean ± standard deviation (n = 2).
Different letters in the same column are significantly different ($P < 0.05$).

Sensory acceptability

The results of the sensory acceptability test are given in Table 1. Fortifying yoghurt with CSM and guar gum did not adversely affect all sensory properties. In fact, the scores of all sensory evaluations of enriched yoghurts were statistically similar to those of plain yoghurt. Similarly, Rendón-Villalobos, Ortíz-Sánchez [57] reported that chia addition did not influence sensory properties of corn tortillas.

According to the sensory test, it was observed that sensory properties of experimental samples did not change depending on textural parameters. Likewise, Bedani, Campos [58] mentioned that overall acceptability of yoghurts was not influenced by textural parameters except in terms of firmness. The slight differences between the L*, a*, and b* values of the experimental samples were found to be insignificant with respect to the color scores given by panelists. Taste and overall acceptability scores declined with increasing amount of CSM and guar gum, but these behaviors were not statistically significant. Jandlova, Kumbar [59] asserted that the use of 3% chia flour in yoghurt reduced sensorial acceptability as compared to yoghurt containing 1% chia flour. However, no statistical change was observed in the overall acceptability of yoghurts with the addition of 1 and 3% CSM in this study. Unexpectedly, the panelists showed no special tendency for a type of sample ($p>0.05$). In contrast, Kumar and Mishra [60] reported that stabilizers such as pectin and alginate reduced the flavor and overall acceptability scores of yoghurts. Consequently, the results showed that using CSM as a new stabilizer in yoghurt formulations would not cause a sensory defect.

CONCLUSION

Chia seed mucilage is rich in nutrients such as protein, fat, and dietary fibers and serves as a good natural stabilizer source by enhancing textural properties such as firmness, consistency, and reducing syneresis. Compared with guar gum, the addition of chia seed mucilage into yoghurts at a 2% level was the most desirable when taking into consideration firmness and syneresis values over the 28 days of storage. Moreover, chia seed mucilage provided higher antioxidant activity in comparison to guar gum. Hence, chia seed mucilage fortification improved not only textural behaviors of yoghurt samples, but also significantly promoted total phenolic content and antioxidant activities thereby, augmenting the health benefits of yoghurt. Microstructural characteristics in yoghurt samples were positively affected by the enrichment with chia seed mucilage, especially CMY3, which had the greater gel network images, followed by GGY2, as compared to the other treatments. From the sensorial aspects, supplementation of chia seed mucilage and guar gum had no adverse effect on consumer acceptability. Outcomes of this study revealed that chia seed mucilage provided better textural characteristics in yoghurt as well as its higher health promoting effect when compared to guar gum. Consequently, the general evaluation of textural, microstructural, physicochemical, and sensory attributes demonstrated that chia seed mucilage usage can be a satisfactory natural alternative stabilizer for yoghurt manufacturing.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Scheer JF. The magic of chia: revival of an ancient wonder food. California: Frog Books, 2001. 175 p.
2. Reyes-Caudillo E, Tecante A, Valdivia-López MA. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (Salvia hispanica L.) seeds. Food Chem. 2008 Mar; 107(2):656-63.
3. Alfredo V-O, Gabriel R-R, Luis C-G, et al. Physicochemical properties of a fibrous fraction from chia (Salvia hispanica L.). LWT-Food Sci Technol. 2009 Dec; 42(1):168-73.
4. Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, et al. Chemical and functional properties of chia seed (Salvia hispanica L.) gum. Int J Food Sci. 2014 Mar; 2014:1-5.
5. Muñoz LA, Cobos A, Diaz O, et al. Chia seed (Salvia hispanica): an ancient grain and a new functional food. Food Rev Int. 2013 Sep; 29(4):394-408.
6. Ixtaina VY, Nolasco SM,Tomás MC. Physical properties of chia (Salvia hispanica L.) seeds. Ind Crop Prod. 2008 Nov; 28(3):286-93.
7. Capitani MI, Ixtaina VY, Nolasco SM, et al. Microstructure, chemical composition and mucilage exudation of chia (Salvia hispanica L.) nutlets from Argentina. J Sci Food Agric. 2013 Jul; 93(15):3856-62.
8. Muñoz LA, Cobos A, Diaz O, et al. Chia seeds: Microstructure, mucilage extraction and hydration. J Food Eng. 2012 Jan; 108(1):216-24.
9. Grigelmo-Miguel N, Gorinstein S, Martín-Belloso O. Characterisation of peach dietary fibre concentrate as a food ingredient. Food Chem. 1999 May; 65(2):175-81.
10. El-Sayed E, El-Gawad IA, Murad H, et al. Utilization of laboratory-produced xanthan gum in the manufacture of yogurt and soy yogurt. Eur Food Res Technol. 2002 Oct; 215:298-304.
11. Mudgil D, Barak S, Khatkar B. Texture profile analysis of yogurt as influenced by partially hydrolyzed guar gum and process variables. J Food Sci Technol. 2017 Sep; 54(12):3810-7.
12. Toniazzo T, Berbel IF, Cho S, et al. β-carotene-loaded liposome dispersions stabilized with xanthan and guar gums: Physico-chemical stability and feasibility of application in yogurt. LWT-Food Sci Technol. 2014 Dec; 59(2):1265-73.
13. Mudgil D, Barak S, Khatkar BS. Guar gum: processing, properties and food applications—a review. J Food Sci Technol. 2014 Mar; 51(3):409-18.
14. Parija S, Misra M, Mohanty A. Studies of natural gum adhesive extracts: an overview. J Macromol Sci, Part C: Polym Rev. 2001 Feb; 41(3):175-97.
15. Soukoulis C, Panagiotidis P, Koureli R, et al. Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. J Dairy Sci. 2007 Jun; 90(6):2641-54.
16. Kiros E, Seifu E, Bultosa G, et al. Effect of carrot juice and stabilizer on the physicochemical and microbiological properties of yoghurt. LWT-Food Sci Technol. 2016 Jun; 69(2016):191-6.
17. Srisuvor N, Chinprahast N, Prakitchaiwattana C, et al. Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana purée. LWT-Food Sci Technol. 2013 Apr; 51(1):30-6.
18. Hashim I, Khalil A, Afifi H. Quality characteristics and consumer acceptance of yogurt fortified with date fiber. J Dairy Sci. 2009 Nov; 92(11):5403-7.
19. Öztürk Hİ, Aydın S, Sözeri D, et al. Fortification of set-type yoghurts with Elaeagnus angustifolia L. flours: Effects on physicochemical, textural, and microstructural characteristics. LWT-Food Sci Technol. 2018 Apr; 90(2018):620-6.
20. Campos BE, Dias Ruivo T, da Silva Scapim MR, et al. Optimization of the mucilage extraction process from chia seeds and application in ice cream as a stabilizer and emulsifier. LWT-Food Sci Technol. 2016 Jan; 65(2016):874-83.
21. AOAC. Official methods of analysis of AOAC International. 16th edition. Arlington, VA, USA, Association of Analytical Communities, 1995.
22. Isanga J, Zhang G. Production and evaluation of some physicochemical parameters of peanut milk yoghurt. LWT-Food Sci Technol. 2009 Jul; 42(6):1132-8.
23. Aryana KJ. Folic acid fortified fat-free plain set yoghurt. Int J Dairy Technol. 2003 Oct; 56(4):219-22.
24. Helrich K. Official methods of analysis. Food composition; additives; natural contaminants. Arlington, VA: Association of Official Analytical Chemists, 1990.
25. Standard B. Gerber method for the determination of fat in milk and milk products. 1955; BS696 Methods, BSI, London.
26. Shetty K, Curtis OF, Levin RE, et al. Prevention of vitrification associated with in vitro shoot culture of oregano (Origanum vulgare) by Pseudomonas spp. J Plant Physiol. 1995 Apr; 147(3-4):447-51.

27. Re R, Pellegrini N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999 May; 26(9-10):1231-37.
28. Tseng A, Zhao Y. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. Food Chem. 2013 May; 138(1):356-65.
29. Larmond E. Laboratory methods for sensory evaluation of food. Canada: Research Branch, Dept. of Agriculture, 1977.
30. Brennan CS, Tudorica CM. Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilisation of barley beta-glucan, guar gum and inulin. Int J Food Sci Technol. 2008 Feb; 43(5):824-33.
31. Koksoy A and Kilic M. Use of hydrocolloids in textural stabilization of a yoghurt drink, ayran. Food Hydrocoll. 2004 Jul; 18(4):593-600.
32. Agil R, Gaget A, Gliwa J, et al. Lentils enhance probiotic growth in yogurt and provide added benefit of antioxidant protection. LWT-Food Sci Technol. 2013 Jan; 50(1):45-9.
33. Sah B, Vasiljevic T, McKechnie S, et al. Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. LWT-Food Sci Technol. 2016 Jan; 65(2016):978-86.
34. Pop C, Vlaic R, Fărcaş A, et al. Influence of pollen, chia seeds and cranberries addition on the physical and probiotics characteristics of yogurt. Bull UASVM Food Sci Technol. 2015; 72(1):141-2.
35. Kwon H, Bae H, Seo H, et al. Chia seed extract enhances physiochemical and antioxidant properties of yogurt. J Dairy Sci. 2019; 102:4870-76.
36. Demirci T, Aktaş K, Sözeri D, et al. Rice bran improve probiotic viability in yoghurt and provide added antioxidative benefits. J Funct Foods. 2017 Sep; 36(2017):396-403.
37. Coorey R, Tjoe A, Jayasena V. Gelling properties of chia seed and flour. J Food Sci. 2014 Apr; 79(5):859-866.
38. Felisberto MHF, Wahanik AL, Gomes-Ruffi CR, et al. Use of chia (Salvia hispanica L.) mucilage gel to reduce fat in pound cakes. LWT-Food Sci Technol. 2015 Oct; 63(2):1049-55.
39. Baú TR, Garcia S, Ida EI. Evaluation of a functional soy product with addition of soy fiber and fermented with probiotic kefir culture. Braz Arch Biol Technol. 2014 Jan; 57(3):402-9.
40. Dickinson E. Hydrocolloids as emulsifiers and emulsion stabilizers. Food Hydrocoll. 2009 Aug; 23(6):1473-82.
41. Timilsena YP, Adhikari R, Kasapis S, et al. Molecular and functional characteristics of purified gum from Australian chia seeds. Carbohydr Polym. 2016 Jan; 136(2016):128-36.
42. Tamime AY. Structure of dairy products. John Wiley & Sons, 2008. 791 p.
43. Ranadheera CS, Evans C, Adams M, et al. Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. Food Chem. 2012 Dec; 135(3):1411-18.
44. Tamime AY and Robinson RK. Tamime and Robinson's yoghurt: science and technology. Elsevier, 2007.
45. Basiri S, Haidary N, Shekarforoush SS, et al. Flaxseed mucilage: A natural stabilizer in stirred yogurt. Carbohydr Polym. 2018 May; 187(2018):59-65.
46. Serra M, Trujillo A, Guamis B, et al. Evaluation of physical properties during storage of set and stirred yogurts made from ultra-high pressure homogenization-treated milk. Food Hydrocoll. 2009 Jan; 23(1):82-91.
47. Sahan N, Yasar K, Hayaloglu A. Physical, chemical and flavour quality of non-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage. Food Hydrocoll. 2008 Oct; 22(7):1291-7.
48. Vianna FS, Canto AC, da Costa-Lima BR, et al. Development of new probiotic yoghurt with a mixture of cow and sheep milk: effects on physicochemical, textural and sensory analysis. Small Rumin Res. 2017 Apr; 149(2017):154-62.
49. Pimentel T, Cruz A, Prudencio S. Influence of long-chain inulin and Lactobacillus paracasei subspecies paracasei on the sensory profile and acceptance of a traditional yogurt. J Dairy Sci. 2013 Oct; 96(10):6233-6241.
50. Isanga K, Zhang M. The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. Int J Dairy Technol. 2009 Jul; 58(3):180-4.
51. Sodini I, Remeuf F, Haddad S, et al. The relative effect of milk base, starter, and process on yogurt texture: a review. Crit Rev Food Sci Nutr. 2004 Aug; 44(2):113-37.
52. Krzeminski A, Großhable K, Hinrichs J. Structural properties of stirred yoghurt as influenced by whey proteins. LWT-Food Sci Technol. 2011 Dec; 44(10):2134-40.
53. Zhang LM, Zhou JF, Hui PS. A comparative study on viscosity behavior of water-soluble chemically modified guar gum derivatives with different functional lateral groups. J Sci Food Agric. 2005 Aug; 85(15):2638-44.
54. Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants. J Am Oil Chem' Soc. 1984 May; 61(5):928-31.

55. Mihaylova D, Schalow S. Antioxidant and stabilization activity of a quercetin-containing flavonoid extract obtained from Bulgarian Sophora japonica L. Braz Arch Biol Technol. 2013 May-Jun;56(3): 431-8.
56. Da Silva Marineli R, Lenquiste SA, Moraes EA, et al. Antioxidant potential of dietary chia seed and oil (Salvia hispanica L.) in diet-induced obese rats. Food Res Int. 2015 Oct; 76(2015):666-74.
57. Rendón-Villalobos R, Ortíz-Sánchez A, Solorza-Feria J, et al. Formulation, physicochemical, nutritional and sensorial evaluation of corn tortillas supplemented with chía seed (Salvia hispanica L.). Czech J Food Sci. 2012; 30(2):118-25.
58. Bedani R, Campos M, Castro IA, et al. Incorporation of soybean by-product okara and inulin in a probiotic soy yoghurt: texture profile and sensory acceptance. J Sci Food Agric. 2014 May; 94(1):119-25.
59. Jandlova M, Kumbar V, Jarosova A, et al. Sensory evaluation of yoghurt with addition of baobab powder, milk thistle flour, cricket flour, chia flour. MendelNet. Brno, Czech Republic 2018 (7-9 Nov),285-9.
60. Kumar P, Mishra H. Mango soy fortified set yoghurt: effect of stabilizer addition on physicochemical, sensory and textural properties. Food Chem. 2004 Oct; 87(4):501-7.