

Effect of chia (*Salvia hispanica* L.) seed mucilage powder on some physicochemical and rheological properties of ayran drinks

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Abstract

In this study, chia seed mucilage powder (MP) was added into ayran drinks at different ratios (0.05, 0.10 and 0.15 %, w/v), and its effect on physicochemical, rheological, microbiological and sensory properties of ayran drinks was determined during 10 days of storage at 4 ± 1 °C. MP addition did not influence their major chemical composition significantly ($p>0.05$). Increasing MP ratio in drinks decreased the colour L^* values of drinks while increasing their colour a^* and b^* values. Addition of 0.05, 0.10 and 0.15 % MP reduced serum separation values of drinks by 25, 67 and 83 %, respectively. The apparent viscosity value of control samples was 103.20 cP and increased to 134.25, 185.35 and 223.38 cP in ayran drinks with 0.05, 0.10 and 0.15% MP, respectively. The obtained results indicated that chia seed MP up to 0.05% can be used in ayran drink production to reduce serum separation and improve viscosity without any adverse effect on sensory liking scores.

Key words: chia seed mucilage; yoghurt drink; serum separation; viscosity

Introduction

Salvia hispanica L., known as chia, is an annual plant belonging to the *Lamiaceae* family that grows in arid or semi-arid climates (Sandoval-Oliveros and Paredes-Lopez, 2013). The seeds of chia plant, originating from Mexico and northern Guatemala (Coelho and Salas-Mellado, 2014), can be consumed directly as an energy source and can be used in the production of many foods (de Souza Ferreira et al., 2015). Seeds contain 25-40 % oil (Coelho and Salas-Mellado, 2014) while 60 % of the total oil is α -linoleic acid (n-3) and 20 % is linoleic acid (n-6). Pulp remaining after oil extraction contains a significant amount of fibre (33.9 g/100 g) and protein (17 g/100 g) (de Souza Ferreira et al., 2015).

Capitani et al. (2013) reported that chia seeds may contain 50-60 g/kg of mucilage as a part of soluble dietary fibre in their structure, which can be easily extracted and absorb up to 27 times its weight in water and undergo hydration, thus having the potential to be used as a thickener functional ingredient in a variety of foods. They also stated that the basic structure of mucilage is in question and consists of polysaccharide units, that is, a tetrasaccharide containing 4-O-methyl- α -D-glucuronopyranosyl residues in the main chain in the form of β -D-xylopyranosyl branches.

Acidified milk drinks are popular worldwide and have many health benefits and high nutritional value. Ayran (or yoghurt) drink is an acidified milk drink produced by diluting and homogenizing yoghurt with water or the fermentation of standardized milk with starter cultures (Thi et al., 2009; Colakoglu and Gursoy, 2011). Plain yoghurt drinks such as *doogh*, *lassi*, *chaas* and *ayran* are popular in Central Asia, Anatolia, Balkans and the Middle East while yoghurt drinks with fruits and sweeteners are mostly preferred in Europe and the US (Colakoglu and Gursoy, 2011).

Ayran drink, like other acidified milk drinks, is a drink that is prone to textural deterioration during storage due to its low pH. The main structural defects in ayran are its low viscosity and serum separation during storage (Özer, 2006; Thi et al., 2009). Serum separation may increase up to 30 % in ayran drinks sold in retail stores in Turkey (Koksoy and Kilic, 2004). Serum separation that is directly visible through the container of ayran drinks negatively affects consumer preference. In the literature, there are studies on the use of different stabilizers such as high methoxyl pectin, carrageenan, carboxymethyl cellulose and gelatin to eliminate the structural problems like serum separation and low viscosity in ayran and similar acidified milk drinks (Tromp et al., 2004; Guo et al., 2021).

Although there are data available on the high water holding capacity of chia seed mucilage and its potential use for the improvement of textural or rheological properties of foods, limited information is available regarding the use of chia seed mucilage as a gelling or thickening agent in the production of fermented dairy products like yoghurt (Atik et al., 2020). To the best of our knowledge, there is no study in which chia seed mucilage

has been used to prevent or reduce structural defects in ayran production. In this study, aqueous extract of chia seed mucilage was freeze-dried, and mucilage powder was added to ayran drinks. Later, the effect of mucilage powder addition on the physicochemical, microbiological, sensorial and rheological properties (viscosity and serum separation) of drinks was determined.

Materials and methods

Materials

Ala Çiftçi (Yayla Agro Food Industry and Transport Inc., Ankara, Turkey) brand chia seeds (produced in 2017 in Argentina) were purchased from a national market chain. Analytical grade chemicals were used in chemical analyses.

Extraction of chia seed mucilage

Mucilage extraction from chia seed was conducted by the method of Campos et al. (2016) with slight changes. Chia seeds were mixed with distilled water at a ratio of 1:20 (w/w), and seeds were hydrated at 70 ± 1 °C for 2 hours by a magnetic stirrer (WiseStir, Daihan Scientific Co., Ltd., Gang-Won-Do, Korea). This hydrated seed suspension was lyophilized in a freeze-drier (BW-10B, Vacuum Freezing Dryer, Bluewave, Shanghai, China) until dry. Then, freeze-dried mucilage was sieved (U-1967, 35 mesh, Loyka, Istanbul, Turkey) and this mucilage powder was used in further studies.

Addition of mucilage to ayran drinks

Ayran drinks were produced from yoghurt in the Department of Food Engineering at Burdur Mehmet Akif Ersoy University (Burdur, Turkey). Yoghurt was supplied from a local dairy producer (Bursüt Dairy Products Manufacturing and Sales, Burdur, Turkey) at the same day of the ayran production. Yoghurt was diluted with distilled water, and this mixture (stock drink) was homogenized by a mixer (AR1069 Crust Mix Stand Mixer, Arzum Electrical Home Appliances Industry and Trade Inc., Istanbul, Turkey) for ayran drink production. Table salt (NaCl) (0.75 %, w/v) was added to the stock drink. Stock ayran sample (2 L) was equally divided into four groups. Chia seed mucilage powder was added to ayran drink samples (except for the control group) at different ratios (0.05, 0.10 and 0.15 %, w/v), then samples were mixed at room temperature for 15 minutes at 750 rpm by a magnetic stirrer (WiseStir, Daihan Scientific Co., Ltd., Gang-Won-Do, Korea). Samples were stored at 4 ± 1 °C for 10 days and analysed on the 1st, 5th and 10th days of storage.

Chemical and physicochemical analyses

The pH, acidity (Oysun, 2001), dry matter (ISO/IDF, 2010), fat (Gerber method) (Oysun, 2001) and total nitrogen values (Dumas method) of ayran drinks were determined. The protein contents of samples were calculated by multiplying the total nitrogen value by a factor of 6.38.

Colour measurements

The colour L^* (lightness, 0=black and 100=white), a^* (redness-greenness) and b^* (yellowness-blueness) values of samples were expressed in a scale of CIELAB (Commission International de L'Eclairage) with a colorimeter (Model CR-400, Konica Minolta, Tokyo, Japan). Colour measurements were taken using specular reflection included with the D65 illuminator, 10° observer angle, and 8 mm aperture. Measurements at approximately 5 ± 1 °C were repeated 4 times at an interval of 3 seconds, and averages were recorded as colour values. Measurements were carried out by placing each ayran sample (~20 mL) in an optical glass cell (34 mm diameter) provided by the manufacturer (Gursoy et al., 2016).

Serum separation

Ayran samples were transferred into 25 mL cylinders and stored at 4 ± 1 °C for 10 days. On the 1st, 5th and 10th days of storage, the serum phases on the upper part of cylinders were determined by volume, and results were expressed as % serum separation (Koksoy and Kilic, 2003).

Rheological analyses

Rheological measurements were carried out using a viscometer (Brookfield, Model DV2T, Brookfield Engineering Laboratories, Middleboro, MA, USA) using a spindle no. 21 in a small sample adapter (13RP) that allows measurement at constant temperature. In the measurements, 8 mL of sample was used and measurements were carried out at a constant temperature of 5 ± 1 °C. The temperature was kept constant using the jacket of the small sample adapter (SC4-45Y) and a cooled circulating water bath (WiseCircu, Daihan Scientific Co., Ltd., Gang-Won-Do, Korea). Flow behaviour index (n) and consistency coefficient (K , Pa.s ^{n}) values were determined by the exponential (power law) model, where Eq. 1 used [δ shear stress (Pa) and $\dot{\gamma}$ shear rate (s⁻¹)] (Steffe, 1996).

$$\delta = K(\dot{\gamma})^n \quad /1/$$

Microbiological analyses

Ayran samples were aseptically sampled on the 1st and 10th days of storage. Samples (10 mL) were diluted in 90 mL

sterile Ringer solution (Merck KGaA, Darmstadt, Germany) and homogenized using the Colworth Stomacher 400 (Seward Laboratory, West Sussex, UK). Appropriate dilutions were prepared and pour-plated. MRS and M17 (Merck KGaA, Darmstadt, Germany) agar mediums were used for enumeration of lactobacilli and streptococci, respectively. MRS agar was incubated anaerobically at 37 ± 1 °C for 48 hours while starter streptococci was determined on M17 agar under aerobic incubation after 48 hours of incubation at 37 ± 1 °C (Colakoglu and Gursoy, 2011).

Sensory analyses

Volunteer sensory panel included staff members or students ($n=20$) of the university, who were regular consumers of ayran drinks. Four different ayran drinks in plastic cups numbered with three-digit random codes were served to these panellists in a statistically balanced order, and panellists were asked to evaluate samples how they liked or disliked on a 7-point hedonic scale (7=like extremely and 1=dislike extremely). Panellists assigned scores to each sample in partitioned booths under daylight. They were instructed to cleanse their palates thoroughly with water between samples. The panel evaluated ayran drinks on the days 1st and 10th of storage.

Statistical analyses

Analysis of variance (ANOVA) of the SAS package software program (The SAS System for Windows 9.0, Cary, NC, USA) was used to determine statistically significant differences among means. Separation of means for significant differences was conducted using the Duncan's multiple-range test at $\alpha=0.05$ level. Data were presented as mean \pm standard deviation.

Results and discussion

Chemical and physicochemical properties

The dry matter, fat and protein contents of ayran samples produced are given in Table 1, and insignificant differences were found among ayran drinks ($p>0.05$). Similar dry matter contents of drink samples ($p>0.05$) might result from the very low addition rate of chia seed mucilage powder. In the Turkish Food Codex, it is indicated that the protein ratio in yoghurt drinks must be at least 2.0 % (Anonymous, 2009). The protein contents of ayran drinks produced in this current study were in good agreement with the value stated in the codex.

The addition of chia seed mucilage to ayran drinks significantly influenced the colour L^* , a^* and b^* values of drinks depending on the ratio (Table 1). Colour L^* value is an important quality parameter in yoghurt drinks and

other fermented milk products, and it is desirable that this value be high for yoghurt drinks (Erkaya et al., 2015). While the addition of 0.05 % mucilage to yoghurt drinks did not influence the colour L^* value of the samples significantly ($p>0.05$), L^* value decreased significantly when mucilage addition ratio was 0.10 or 0.15 % ($p<0.05$) (Table 1). In comparison to control ayran drinks, the incorporation of chia seed mucilage to yoghurt drinks significantly influenced the colour a^* and b^* values of drink samples ($p<0.05$). Overall, as the mucilage ratio was increased in ayran drinks, the colour a^* values of drink samples decreased while colour b^* values increased ($p<0.05$). A negative value of a^* indicates the greenness of ayran drinks. It increased from -2.36 to -2.19 in ayran drinks with 0.15 % chia seed mucilage, and this increase was found statistically significant ($p<0.05$). Similarly, the yellowness of ayran drinks (b^*) steadily increased with an increase in the mucilage content of ayran drinks. The distinctive gray/dark colour of mucilage powder could be the reason of these changes (Campos et al., 2016).

The pH value of milk and dairy products, known as actual acidity, is generated by other compounds in equilibrium with free and active hydrogen ions (Walstra et al., 1999). In the technology of fermented milk products, the production and maturation steps of these products are practically monitored by determining pH values. Moreover, pH value is also used as an important criterion in explaining the chemical and microbiological changes that occur during ripening in ayran drinks (Colakoglu, 2010). In this current study, changes in the pH and acidity values of ayran drinks during 10 days of storage are given in Table 2. The pH values of ayran drinks decreased significantly at the end of storage ($p<0.05$). In the samples of control drink and drink with 0.05 % mucilage powder, five-day storage did not change pH values significantly ($p>0.05$). Acidity values of the control group and drinks with 0.10 % chia mucilage powder did not change statistically during storage ($p>0.05$). For the drinks with 0.15 % chia mucilage powder, titratable acidity values decreased on the 5th day of storage ($p<0.05$) but remained similar during storage ($p>0.05$).

Serum separation is the formation of serum phase in the upper part of the product as a result of the precipitation of large particles towards the bottom of the container, due to the aggregation and sedimentation of the particles during storage, which is seen in acidic fermented milk products (Ertugay et al., 2012). Serum separation is one of the main quality parameters for yoghurt and yoghurt drinks. Table 2 indicates that as the ratio of mucilage powder in ayran drinks

increased, the serum separation values of drinks decreased. For example, control drinks had a serum separation value of 10 mL at the 5th day of storage while drinks with 0.05, 0.10 and 0.15 % chia mucilage powder had serum separation values of 9, 4 and 2 mL, respectively. The incorporation of 0.15 % chia mucilage to ayran drinks provided a decrease of approximately 83 % in the serum separation value of drinks.

Rheological properties

In the rheological analyses of ayran drinks, coefficients of determination (R^2) ranged from 0.97 to 0.99, and the exponential law (power law) model was found appropriate for ayran drinks. All ayran drinks exhibited a pseudoplastic flow behaviour, which was indicated by a flow behaviour index less than 1 (Table 3). The apparent viscosity values of ayran drinks determined at 70 rpm increased significantly in proportion to the addition ratio of chia seed mucilage ($p<0.05$). While the incorporation of 0.05 and 0.10 % mucilage powder to ayran drinks did not change the consistency coefficient value of control drinks (1562 cP) significantly ($p>0.05$), the addition of 0.15 % mucilage powder increased the consistency coefficient of control sample to 11311 cP ($p<0.05$). Viscosity is one of the most important quality parameters in yoghurt and yoghurt-like fermented milk products, and the viscosity of dairy products is directly related to the physical structure of the product.

In the physical structure of fermented milk products, viscosity is related to the physical interactions of the proteins with each other and the denatured serum proteins to trap the milk serum and fat globules in the protein network (Lucey, 2004; Reiner et al., 2009). The water-binding feature of the mucilage (Capitani et al., 2013) could contribute to an increase in the apparent viscosity values of ayran drinks. The formation of elastically active structural networks among proteins could increase with the presence of chia seed mucilage in the acid gel, and this could also have a positive synergistic effect of gel structuring and rheological properties (Soukoulis et al., 2019). Zeta potential of chia seed mucilage solutions is negative because of the presence of appreciable quantities of uronic acids, and the negativity decreases with a decrease in pH of solution (Timilsena et al., 2015). Electrostatic interactions of milk proteins with charged sites of polysaccharides of mucilage may play a dominant role for improved rheological properties (Smykov, 2020).

Table 1. Dry matter, fat and protein contents (%) and colour values (CIE LAB) of different ayran drinks produced in this study

Sample ^a	Dry matter ^b (%)	Fat (%)	Protein (%)	CIELAB colour values		
				L^*	a^*	b^*
A	8.26±0.08 ^A	2.20±0.00 ^A	2.29±0.18 ^A	85.21±0.04 ^A	-2.36±0.01 ^C	3.85±0.01 ^D
B	8.43±0.08 ^A	2.15±0.07 ^A	2.30±0.11 ^A	85.18±0.00 ^A	-2.27±0.01 ^B	4.08±0.00 ^C
C	8.28±0.02 ^A	2.00±0.00 ^A	2.26±0.09 ^A	84.68±0.03 ^B	-2.20±0.00 ^A	4.34±0.01 ^B
D	8.44±0.08 ^A	2.05±0.07 ^A	2.34±0.11 ^A	83.73±0.04 ^C	-2.19±0.01 ^A	4.67±0.01 ^A

^aControl yoghurt drink (A) and samples with 0.05 % (B), 0.10 % (C) and 0.15 % (w/v) (D) chia seed mucilage powder

^bDifferent superscript letters in the same column indicate statistical differences ($p<0.05$).

Table 2. Changes in the pH, acidity and serum separation values of different ayran drinks during storage

Sample ^a	Storage time (day)	pH ^b	Acidity value (% lactic acid)	Serum separation (mL)
A	1	3.87±0.04 ^A	0.58±0.00 ^{B-D}	0.0±0.0
	5	3.86±0.01 ^A	0.60±0.01 ^{BC}	10.0±0.0
	10	3.64±0.02 ^D	0.59±0.03 ^{BC}	12.0±0.0
B	1	3.77±0.06 ^B	0.60±0.01 ^B	0.0±0.0
	5	3.80±0.01 ^B	0.57±0.00 ^{CD}	9.0±0.0
	10	3.55±0.00 ^{EF}	0.58±0.01 ^{B-D}	9.0±0.0
C	1	3.71±0.00 ^C	0.58±0.01 ^{B-D}	0.0±0.0
	5	3.79±0.02 ^B	0.59±0.01 ^{B-D}	4.0±0.0
	10	3.56±0.01 ^{EF}	0.56±0.01 ^D	4.0±0.0
D	1	3.60±0.01 ^{DE}	0.65±0.01 ^A	0.0±0.0
	5	3.80±0.00 ^B	0.57±0.00 ^{CD}	2.0±0.0
	10	3.53±0.00 ^F	0.57±0.00 ^{CD}	2.0±0.0

^aControl yoghurt drink (A) and samples with 0.05 % (B), 0.10 % (C) and 0.15 % (w/v) (D) chia seed mucilage powder

^bDifferent superscript letters in the same column indicate statistical differences ($p < 0.05$).

Table 3. Rheological properties of different ayran drinks on the 5th day of storage.

Sample ^a	Flow behavior index ^b (n)	Consistency coefficient (k, cP)	Apparent viscosity (cP, at 70 rpm)
A	0.35±0.02 ^A	1561.50±149.20 ^B	103.20±2.55 ^D
B	0.52±0.00 ^A	967.00±4.24 ^B	134.25±4.03 ^C
C	0.35±0.05 ^A	2848.50±651.25 ^B	185.35±7.57 ^B
D	0.07±0.11 ^B	11311.25±5020.55 ^A	223.38±11.83 ^A

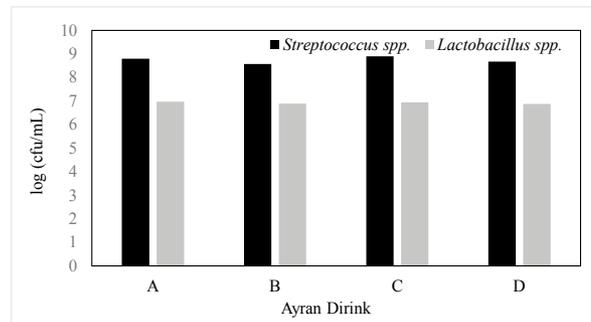
^aControl yoghurt drink (A) and samples with 0.05 % (B), 0.10 % (C) and 0.15 % (w/v) (D) chia seed mucilage powder

^bDifferent superscript letters in the same column indicate statistical differences ($p < 0.05$).

Weak reversible complexes can form between anionic polysaccharides and proteins that carry almost zero total charge or total negative charge while the distribution and type of charges on the surface of casein micelles create a repulsive barrier that contributes to the stability of micelles (Smykov, 2020).

Microbiological properties

The microbiological properties of ayran drinks produced in this study are given in Figure 1. Logarithmic counts of *Streptococcus* spp. and *Lactobacillus* sp. were not influenced by the addition of chia seed mucilage powder into ayran drinks ($p > 0.05$). In the Turkish Food Codex, it was indicated that the counts of total specific microorganism in ayran must be at least 10^6 cfu/g (Anonymous, 2009). The total starter culture values of ayran drinks were consistent with the value stated in the codex at the end of storage period.

**Figure 1.** *Streptococcus* spp. and *Lactobacillus* spp. counts (cfu/mL) of control (A) and ayran drinks with 0.05 % (B), 0.1 % (C) and 0.15 % (w/v) (D) chia seed mucilage powder at the 10th day of storage.

Sensory properties

Changes in the sensory liking scores of ayran drinks during 10 days of storage are presented in Table 4. Results showed that liking scores for each group of ayran drinks were not influenced by the storage period ($p > 0.05$). The highest liking scores were determined in control and ayran drink with 0.05 % chia seed mucilage powder at the beginning of storage. Sensory liking scores of all samples were statistically similar at the end of storage period ($p > 0.05$). Based on the numeric results of sensory liking scores shown in Table 4, it could be concluded that the incorporation of 0.05 % chia mucilage powder to ayran drinks is recommended without any negative effect on the liking scores of ayran drinks.

Mucilage addition may change sensory properties of yoghurt drinks like doogh. Doogh is an acidified milk drink, which is produced by adding salt and water into yoghurt in Iran. Studying effect of quince seed mucilage addition on the sensory properties of doogh samples, Pirsā et al. (2018) reported a positive relationship between the sensory properties and volatile components of doogh drinks.

Table 4. Changes in the sensory liking scores (n=20) of different ayran drinks during 10 days of storage

Sample ^a	Storage time (day)	Liking score ^b
A	1	6.25±0.71 ^A
	10	5.13±1.55 ^{ABC}
B	1	6.13±0.99 ^{AB}
	10	6.13±0.64 ^{AB}
C	1	4.25±2.43 ^C
	10	5.13±1.73 ^{ABC}
D	1	4.00±1.77 ^C
	10	4.75±0.71 ^{BC}

^aControl yoghurt drink (A) and samples with 0.05 % (B), 0.10 % (C) and 0.15 % (w/v) (D) chia seed mucilage powder

^bDifferent superscript letters in the same column indicate statistical differences ($p < 0.05$).

Conclusions

Overall, the obtained results indicated that the incorporation of chia seed mucilage powder significantly increased apparent viscosity values of ayran drinks while decreasing their serum separation values in comparison to control sample ($p < 0.05$). The addition of 0.05, 0.10 and 0.15 % mucilage powder resulted in a 25, 67 and 83 % reduction in serum separation values, respectively. Under the conditions studied, the incorporation of chia seed mucilage powder in ayran production did not alter the proximate composition and microbiological counts (*Streptococcus* spp. and *Lactobacillus* spp.) of ayran drinks. Results of this study showed that the incorporation

of chia seed mucilage powder to ayran production could be a successful alternative for improving the rheological properties (increased viscosity and reduced serum separation) of ayran drink samples and it can be used at a level of 0.05 % without any adverse effect on sensory liking scores for ayran drinks.

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Utjecaj dodatka praha sluzi chia sjemenki (*Salvia hispanica* L.) na neka fizikalno-kemijska i reološka svojstva ayrana

Sažetak

U ovom istraživanju, prah sluzi chia sjemenki (MP) dodan je u ayran napitke u različitim omjerima (0,05, 0,10 i 0,15 %, w/v) te je ispitan njegov učinak na fizikalno-kemijska, reološka, mikrobiološka i senzorska svojstva ayran napitaka tijekom 10 dana skladištenja na 4 ± 1 °C. Dodatak MP nije značajno utjecao na njihov glavni kemijski sastav ($p > 0,05$). Povećanje omjera MP u pićima smanjilo je L^* vrijednosti boje pića dok je povećalo njihove a^* i b^* vrijednosti boje. Dodatak 0,05, 0,10 i 0,15 % MP smanjio je vrijednosti sinereze napitaka za 25, 67 odnosno 83 %. Privedna viskoznost kontrolnih uzoraka bila je 103,20 cP i porasla je na 134,25, 185,35 i 223,38 cP u ayran napicima s 0,05, 0,10 i 0,15 % MP. Rezultati su pokazali da se MP chia sjemenki do 0,05 % može koristiti u proizvodnji ayrana, kako bi se smanjila sinereza i poboljšala viskoznost bez ikakvih negativnih učinaka na senzorska svojstva.

Ključne riječi: sluz *chia* sjemenki; jogurt; odvajanje seruma; viskoznost

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