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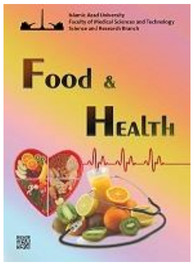

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Enrichment of strawberry frozen yogurt by chia (*Salvia hispanica* L.) seeds

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ABSTRACT

Frozen yogurt is considered as a useful and popular dairy product all over the world. The research was conducted to advance the formulation of strawberry frozen yogurt by chia seed at different concentrations (0 to 3 %). The aim of present study is to improve nutritional quality and health benefits by frozen yogurt enriched with chia seeds. Initially, fatty acid profile in chia seeds was evaluated, then tests such as physicochemical (pH, acidity, fat, and protein content), colorimetric (L^*, a^* and b^*), antioxidant, microbial counts, viscosity, melting rate, and overrun as well as sensory were analyzed on frozen yogurts during 21 days. Finally, the fatty acid profile in frozen yogurts was investigated on optimal and control samples. The results illustrated that 83.73±0.3 of chia seed oil included polyunsaturated fatty acids. The results of physicochemical assays for frozen yogurt represented that treated samples had lower pH and higher acidity, fat, and protein compared to control. Chia improved textural (overrun, viscosity, melting rate) and antioxidant features of frozen yogurts as well as L^* decreased. Enrichment had no significant effects on lactic acid bacteria counts. chia addition showed no significant influence on flavor and generally lacked in odor even in the highest amount. According to all results, the sample with 2 % chia was the most appropriate and improved polyunsaturated fatty acids, especially omega-3. Chia addition can be highlighted as a functional composition.

1. Introduction

Frozen yogurt is regarded as a refreshing and nutritive dessert that combines flavor with the texture of ice cream and yogurt (1). This elaboration results in a beneficial commodity with a fresh flavor and shelf-life stability significantly longer than yogurt (2). It could be considered as a healthy substitute to ice cream for humans suffering from obesity, cardiovascular illnesses, and lactose intolerance because of its less fat volume (3). Frozen yogurt and ice cream can be considered as foams comprising of air cells enclosed through a partially frozen emulsion (4). Functional products are fortified with active components of recognized biological activity that, when consumed in suitable quantitative and qualitative capacities, provide health profits as well as those supplied through prominent nutrients (5). Strawberries are used for their antioxidant activity because of functional combinations, regarded as bioactive molecules (6). Chia (*Salvia hispanica* L.) is native to an area from western Mexico expanding to northern Guatemala and has been used for almost 5,500 years, which includes more fiber, crude protein, antioxidants contents, and α-linolenic fatty acid (omega-3). It demonstrates distinct functional health impacts due to its cardioprotective, anti-inflammatory, and antioxidant features (7). So far, this seed and mucilage were applied by several researchers to enrich yogurt with nutritious properties as well as obtained satisfactory results (8, 9). Although, no research has investigated the influences of chia seed on antioxidant and physicochemical attributes of frozen yogurt. One of the most well-known dairy products is ice cream all over the world. However, it has long been identified as an energetic food and could not be appropriate for particular sections of the population. The purpose of the present study is to investigate physicochemical, textural, and fatty acids of strawberry frozen yogurt with the addition of chia seeds to evaluate consumer acceptance by sensory characteristics.

2. Materials and methods

2.1. Chemicals and reagents

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Fresh bovine milk was attained from the Agricultural Research Farm of Tehran University, Karaj-Iran, and commercial yogurt starter culture (YC-X11; CHR-Hansen, Horsholm, Denmark) was purchased. Chia (*Salvia hispanica* L.) seeds were purchased from a household in Tehran, Iran. The grains were completely powdered using an electric grinder, and then powder particles were filtered through a 200-micron mesh for uniformity. ∝-monoglyceride as an emulsifier (E471) (Sigma-Aldrich, London, UK), vanillin gained from Kalleh Dairy Co., Amol, Iran, and strawberry flavor (TR 12879) were attained from Aromsa (Kocaeli, Turkey). Chemicals and solvents were analytical grade and obtained from Merck Company (Darmstadt, Germany).

2.2. Proximate composition of chia seeds

These attributes of chia seeds were performed and nitrogen using a Kjeltec Digestion System (method 954.01), fat (method 920.39) as well as crude fiber (method 962.09) were measured (10).

2.2.1. Fatty acid (FA) determination in chia oil

Chia oil was dissolved in potassium hydroxide and water (85°C, 45 min). 5 mL H_2O and hexane were exposed to liquid-liquid partitioning 2 times, and portions were blended. These portions and unsaponifiable components were washed with 10 % alcohol (pH=7), ultimately dried using a rotary evaporator. From the aqueous layer, saponified FAs were obtained triplicates by hexane. This layer was dried and dissolved in 2 mL boron trifluoride methanol. The obtained mechanism was incubated to attain FA methylation (60°C, 45 min). Fatty acid methyl esters (FAMEs) were extracted by hexane from the cooled combination. FAMEs investigation was performed using gas chromatography (7820A, Agilent, Santa Clara, CA, USA), coupled with an FID and Trace TR-FAME capillary column. Analysis was planned, followed by: temperatures for FID and injector were maintained at 250°C, but for the oven was kept at 80°C. It was elevated to 215°C at 15 min, ultimately up to 215 °C at 20 min. As carrier gas, nitrogen was applied with a split ratio of 1:20 (11).

2.3. Frozen yogurt

Milk (3.1 % fat) was preheated at 65°C for homogenization, then was pasteurized at 85 °C for 15 min and cooled to 45°C. It was inoculated with a 2 % DVS (Direct vat set) yogurt starter (CHN 22, Christian Hansen®, Denmark), and incubated by a commercial freeze-dried lactic acid bacteria (LAB) at 45°C for 4 h (to final pH 4.8). During ice cream production, milk and emulsifier (E471) were heated at 45°C before incorporating 14 % sugar, chia seed powder (0, 1, 2, and 3 %), 6.4 % cream (30 % fat). The mixture was pasteurized (72°C, 10 min) and cooled to 10 °C. Then, 3 % (w/w) strawberry flavor was added as a color compound. Yogurt and ice cream were produced in previous steps, stirred (a ratio of 3:2), and maintained at 4 °C for 10 h (9). Then, the aged blend was frozen for over 25 min by a home ice cream maker (Gaggia mod 60468, Gaggio Montano, I) with constant stirring. Samples (n=16) were filled into an 80 mL container with numbering and stored at 18°C in the shelf life of 0, 7, 14, and 21 days (Table 1).

Table 1. Summary and description of strawberry frozen yogurts in the present study.

SFY_0	Strawberry frozen yogurt on day 0*
SFY_7	Strawberry frozen yogurt on day 7
SFY_{14}	Strawberry frozen yogurt on day 14
SFY_{21}	Strawberry frozen yogurt on day 21
$SFYCI_0$	Strawberry frozen yogurt with 1 % of chia seed on day 0
$SFYC^I_7$	Strawberry frozen yogurt with 1 % of chia seed on day 7
$SFYC^I_{14}$	Strawberry frozen yogurt with 1 % of chia seed on day 14
$SFYC^I_{21}$	Strawberry frozen yogurt with 1 % of chia seed on day 21
$SFYC^{II}_0$	Strawberry frozen yogurt with 2 % of chia seed on day 0
$SFYC^{II}_7$	Strawberry frozen yogurt with 2 % of chia seed on day 7
$SFYC^{II}_{14}$	Strawberry frozen yogurt with 2 % of chia seed on day 14
$SFYC^{II}_{21}$	Strawberry frozen yogurt with 2 % of chia seed on day 21
$SFYC^{III}_0$	Strawberry frozen yogurt with 3 % of chia seed on day 0
$SFYC^{III}_7$	Strawberry frozen yogurt with 3 % of chia seed on day 7
$SFYC^{III}_{14}$	Strawberry frozen yogurt with 3 % of chia seed on day 14
$SFYC^{III}_{21}$	Strawberry frozen yogurt with 3 % of chia seed on day 21

*The day of production

2.3.1.Physicochemical evaluation of frozen yogurt

pH and acidity were determined by pH-meter (Metrohm) and titration respectively. Total protein and fat levels were measured using Kjeldahl and Gerber methods for samples (10). The colorimetric features ($L*$, $a*$ and $b*$) for samples were determined by a Hunterlab colorimeter (D25 DP9000, Hunter Associates Laboratory, Inc., USA), (12).

2.3.2.DPPH radical scavenging assay

The potential of an antioxidant to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was investigated by the spectrophotometric method (Thermo Scientific, Madison, WI, USA) at λ=517 nm (6). The absorbance reduction was a consequence of the component's radical scavenging potential as represented in Equation 1:

$$\text{(Eq.1)} \quad DPPH\ scavenging\ rate(\%) = (A_0 - A_1)\ /A_0 \times 100\%$$

where, the absorption value of blank and sample was A_0 and A_1 respectively.

2.3.3.Rheological properties

Apparent viscosity of mixtures was investigated after 20 h aging through a Brookfield rheometer (Model DV-111, spindle DIN-85) at 4°C by a step-wise program with 0.1 rpm steps from 0.3 to 3 rpm, taking readings each 8 s. A consistency coefficient from the Power Law model was attained for each sample (4).

2.3.4.Meltdown rate

This index was measured regarding to Skryplonek *et al.* (13) with several alternations. A scoop of the sample (30 g) was located on a stainless-steel screen with openings (1×1 mm) and then above a beaker. The collected sample weight was registered in a beaker at 20°C after 45 min, and the meltdown rate was calculated according to Equation 2:

(Eq. 2) $$\text{Meltdown rate (\%)} = \frac{\text{wt of melted sample}}{\text{wt of scoop}} \times 100$$

2.3.5.Survival of constitutive lactic acid bacteria (LAB)

The LAB counts were measured using plating suitable dilutions in samples on MRS (de Man, Rogosa and Sharpe) agar plates and at 37°C incubated for 48 h. Cell numbers of frozen yogurts were expressed initially during 21 days (2).

2.3.6.Sensory evaluation

The sensory attributes were evaluated using the descriptive panel and 15 trained panelists, including females and males, which gained more experience. The panel consisted of experts at Agricultural Engineering Research Institute, Karaj, Iran. Following features such as smoothness, appearance, flavor, mouthfeel, texture, and overall acceptance were investigated by assessors. 5 points of a hedonic scale was applied with structure levels from 1 to 5 such as 1 dislike and 5 like extremely. The sensory assay of frozen yogurt samples was replicated on a distinct day but under the same situations as explained above (12).

2.3.7.Overrun condition

This index was performed by comparing the weight of the frozen yogurt mixture and its certain volume in a container. It was measured according to Equation 3: (14).

(Eq. 3)
$$\text{Overrun} = \frac{\text{weight of unit mix} - \text{weight of equal volume of frozen yogurt} \times 100}{\text{weight of equal volume of frozen yogurt}}$$

2.3.8.Fatty acid compound of frozen yogurt

Homogenate samples were obtained using chloroform/ methanol, potassium chloride, butylated hydroxyanisole, and an interior standard. The aqueous layer was attained after collecting the organic phase by chloroform as well as organic extracts were mixed and percolated using pipettes comprising cotton and anhydrous sodium sulfates to eliminate moisture and contaminants. An aliquot of extracted lipid was evaporated through nitrogen gas and samples were transesterified in boron trifluoride, methanol, and hexane on a heating block. FAMEs were evaluated by a Varian 3900 gas chromatograph and Agilent GC system included a 6890N GC and G1540N flame ionization detector (FID), (7).

2.4. Statistical analysis

Initially, normality test was applied and then a factorial test was conducted in an entirely randomized design by 3 replications with average and standard deviation, thus, factors were chia powder (0, 1, 2, and 3 %) and storage time (1, 7, 14 and 21 days). At a level of 0.05, Duncan's multiple range tests were performed. In the present study, overrun is a markedly prominent feature and for stepwise regression method, this function was taken as a dependent variable and others were considered as independent factors to determine effects of the most important feature for overrun and SPSS software number 20 was applied.

3. Results and discussion

3.1. Chia seeds components

Chia seeds represented markedly higher percentages of carbohydrates (41.43±1.14) as compared to fat (25.12± 0.28) and protein (22.08±0.02). Unsaturated fatty acids (UFA) were mainly detected, which 91.37 % monounsaturated fatty acids 7.64 % (MUFA) and polyunsaturated fatty acids 83.73 % (PUFA) were recorded. The highest FAs in chia were ascribed to α-Linolenic acid (63.24 %), followed by linoleic acid (18.30 %) with oleic acid (7.60 %), and were depicted in Table 2.

Table 2. Experimental design of fatty acid profile in chia seeds.

Peak type	Concentration (w/w %)
Myristic acid (C14)	0.91±0.1
Palmitic acid (C16)	5.17±0.3
Stearic acid (C 18)	2.51±0.2
Saturated fatty acids	8.63 ± 0.2
Oleic acid (C:18:1n9c)	7.60±0.2
Monounsaturated fatty acids	7.64 ± 0.4
Linoleic acid (C:18:2n6c, omega-6)	18.30±0.2
α-Linolenic acid (C:18:3n3, omega-3)	63.24±0.1
Arachidic acid (C 20, 4n6c, omega-3)	0.85±0.3
Eicosanoic acid (C20:1n9, omega-3)	0.50±0.1
Polyunsaturated fatty acids	83.73 ± 0.3

In Chile, this seed provided nutrient percentages such as 34.57 carbohydrate, 30.22 fat, and 25.32 protein and these researches published the average 4.59 % carbohydrate, 32.16 % fat, and 18.18 % protein of Brazilian chia (15, 16). In the previous study, 64 % and 80 % FAs such as linolenic and linoleic acids were identified in chia seeds, respectively. α-linolenic acid (39.64 %) was the most abundant followed by linoleic acid (46.21 %) in chia seeds, while saturated fatty acid (SFA) was less than 10 % (11).

3.2. Changes in physiochemical characteristics of frozen yogurt

The highest and lowest pH was observed in SFY_0 (6.21) and $SFYC^{III}_{21}$ (5.60), respectively. pH treatments decreased markedly by expanding storage time, while $SFYC^{II}$ and $SFYC^{III}$ had the lowest pH in a constant period of time, there was no significant difference for pH in other treatments ($p>0.05$). The highest and lowest acidity was obtained 54.51 and 34.28 D° for $SFYC^{III}_{21}$ and SFY_0, respectively. The results outlined that acidity treatments elevated noticeably by extending shelf life. The results illustrated that $SFYC^{III}$ had the most acidity and because of distinct acidities at end of incubation time, acidity was improved and the dissimilarities in buffering capacities among structure and chia. Metabolic activities of starter bacteria were stimulated and acidity was expanded by increasing chia. Furthermore, microorganism activation was reduced and an increment in acidity was avoided by an increase of sugar level in frozen yogurt (9). The highest fat level was observed at 3.67 % $SFYC^{III}_{21}$ and the lowest 2.80 % SFY_1. Fat content was improved by adding chia and over time from first to end of storage time (Table 3). The trend of acidity changes was in contrast to pH variations and more acidity was represented by more microbial growth in $SFYC^{III}$ among others. The general trend indicates an upward change in acidity. The results of the current study were similar to the role of inulin oligofructose in physicochemical attributes of probiotic frozen yogurt (17). A high proportion of omega-3 UFAs was pointed out and fat was improved by adding chia for frozen yogurt (11). The results revealed the highest protein content for $SFYC^{III}$ and the lowest protein belonged to controls. The results displayed that storage time had no significant effect on protein content (Table 3). The slight increase in protein level for treatment samples was corresponded to protein percentage of chia and bacterial growth level compared to untreated (11). The highest and lowest L^* value was recorded from 79.03 to 80.18 in SFY and 64.29 to 65.20 in $SFYC^{III}$, respectively. Also, the highest a^* value was observed in treated $SFYC^{III}$ (6.20 to 6.71), while the lowest was detected SFY (4.46 to 4.66). The highest value of b^* index was reported in the range of 3.01 to 3.34 and no significant difference was found in mentioned value among other samples (Table 4). The shelf-life extension had no significant effect on color attributes in all samples ($p>0.05$). The addition of chia decreased lightness and straightly related to caramel color, which is created by the caramelization process and Maillard reaction during drying powder. Another reason was that chia addition increased viscosity, so light refraction was reduced as well as whiteness. Enrichment with chia led to expanding red and yellow color of treated samples. In other research, the color of fermented milk was investigated and lightness was reduced by increasing acidity (13). Yogurt color with the addition of starches could be considered as natural and attractive because of brighter color (18). The chia addition varied yogurts color, causing in products with a darker yellow color. Researchers also expressed the same results for dairy products (13).

3.3. Antioxidant properties of frozen yogurt

Statistical analysis revealed that elevating chia, time, as well as chia × time exhibited a significant influence on EC_{50}. Variance analysis and comparison of average EC_{50} demonstrated that $SFYC^{III}$ represented the lowest EC_{50} (12.18 mg/mL) on the day of production, with the highest potential for DPPH, but SFY showed the most EC_{50} (42.80 mg/mL) on the 21st day with the lowest antioxidant activity. Expanded storage time decreased significantly free radical scavenging of

Table 3. The impact of chia seed in the physicochemical function of frozen yogurts over the storage of 21 days (mean ± standard error).

Parameters	Storage time (day)	Samples			
		SFY	**$SFYC^{I}$**	**$SFYC^{II}$**	**$SFYC^{III}$**
pH	0	6.22 ± 0.0125^{a}	6.13 ± 0.0057^{b}	6.05 ± 0.0152^{d}	5.99 ± 0.0057^{e}
	7	6.10 ± 0.0057^{c}	6 ± 0.0050^{e}	5.94 ± 0.006^{f}	5.78 ± 0.011^{g}
	14	5.93 ± 0.001^{f}	5.86 ± 0.010^{g}	5.81 ± 0.011^{h}	5.73 ± 0.011^{j}
	21	5.78 ± 0.0017^{i}	5.73 ± 0.017^{i}	5.68 ± 0.011^{k}	5.60 ± 0.0001^{l}
Acidity	0	34.28 ± 0.258^{l}	38.70 ± 0.116^{k}	40.96 ± 0.153^{j}	42.82 ± 0.445^{h}
	7	38.60 ± 0.145^{k}	41.59 ± 0.021^{i}	44.37 ± 0.276^{f}	46.59 ± 0.133^{e}
	14	43.67 ± 0.528^{g}	46.63 ± 0.233^{c}	49.79 ± 0.438^{d}	50.91 ± 0.063^{c}
	21	49/63 ± 0.14^{d}	50.92 ± 0.318^{e}	51.68 ± 0.333^{b}	54.51 ± 0.234^{a}
Fat	0	2.80 ± 0.029^{h}	2.88 ± 0.012^{gh}	2.93 ± 0.001^{gh}	3.10 ± 0.015^{g}
	7	3.13 ± 0.0960^{fg}	3.24 ± 0.065^{ef}	3.36 ± 1.089^{ef}	3.50 ± 0.046^{cd}
	14	3.47 ± 0.036^{de}	3.51 ± 1.129^{cd}	3.44 ± 1.145^{de}	3.64 ± 0.012^{ab}
	21	3.53 ± 0.076^{bc}	3.56 ± 0.012^{bc}	3.61 ± 0.029^{bc}	3.67 ± 0.164^{a}
Protein	0	3.98 ± 0.46^{d}	4.36 ± 0.34^{c}	4.50 ± 0.08^{b}	4.68 ± 0.09^{a}
	7	3.97 ± 0.36^{d}	4.34 ± 0.65^{c}	4.48 ± 0.25^{b}	4.65 ± 0.45^{a}
	14	3.97 ± 0.12^{d}	4.33 ± 0.12^{c}	4.46 ± 0.65^{b}	4.64 ± 0.15^{a}
	21	3.95 ± 0.29^{d}	4.31 ± 0.42^{c}	4.45 ± 0.21^{b}	4.62 ± 0.06^{a}

Table 4. Effect of chia seed on the color attribute (*L**, *b** and *a**) in frozen yogurts during 21 days of shelf life (mean ± standard error).

Storage time (day)	***L****				***b****				***a****			
	Samples											
	SFY	**$SFYC^{I}$**	**$SFYC^{II}$**	**$SFYC^{III}$**	**SFY**	**$SFYC^{I}$**	**$SFYC^{II}$**	**$SFYC^{III}$**	**SFY**	**$SFYC^{I}$**	**$SFYC^{II}$**	**$SFYC^{III}$**
0	80/04±066^{a}	73.64±0.42^{b}	68.89±0.44^{c}	64.58±0.54^{d}	2.47±0.03^{b}	2.68±0.05^{b}	2.85±0.02^{b}	3.02±0.06^{a}	4.46±0.15^{c}	4.94±0.09^{bc}	5.74±0.24^{ab}	6.29±0.04^{a}
7	79.03±1.05^{a}	73.30±0.97^{b}	68.18±0.57^{c}	64.29±1.03^{d}	2.45±0.07^{b}	2.70±0.05^{b}	2.78±0.03^{b}	3.11±0.13^{a}	4.50±0.13^{bc}	4.81±0.36^{bc}	5.83±0.30^{ab}	6.71±0.23^{a}
14	80.18±0.84^{a}	74.48±1.01^{b}	68.34±0.16^{c}	64.60±0.75^{d}	2.58±0.28^{b}	2.53±0.11^{bc}	2.69±0.32^{b}	3.34±0.44^{a}	4.54±0.12^{bc}	4.78±1.59^{bc}	5.57±0.16^{ab}	6.37±0.09^{a}
21	79.14±0.48^{a}	73.28±0.77^{b}	68.99±0.23^{c}	65.20±0.56^{d}	2.47±0.04^{b}	2.65±0.08^{b}	2.76±0.06^{b}	3.01±0.10^{a}	4.66±0.05^{bc}	4.66±0.33^{bc}	5.59±0.19^{ab}	6.20±0.10^{a}

DPPH in treatments. For control, peptides derived, which are capable of absorbing oxidative compounds due to casein protein and strawberry flavor assisted to the antioxidant effect, thus it was able to partially inhibit DPPH radical. A review illustrated the antioxidant function of dairy products, such as 26.41 mg/mL raw milk, 23.5 µg/mL cheeses, and 32.4 mg/mL yogurt (19). The effect of distinct compounds was observed on antioxidant status of fortified samples compared to control as follows: extract from *Stevia rebaudiana* in yogurt, dark cocoa powder with hazelnut and green tea extract in ice cream, cinnamon bark extract in stirred yogurt, and Brazilian red propolis in yogurt by Helal and Tagliazucchi (20); Santos *et al.* (21), respectively. In the present study, antioxidant activity was improved by chia, as reported by other researchers because of some bioactive compounds such as tocopherol, carotenoids, and glycosides (11).

3.4. Viscosity of frozen yogurt

The apparent viscosities were found in the range of 315.15 to 111.17 cP, which were belonged to $SFYC^{III}_{21}$ and SFY_0, respectively. No significant difference was revealed between apparent viscosity with time and 1 or 2 % chia concentrations ($p>0.05$), however, 3 % level developed significantly this factor ($p<0.05$). Hydrocolloids as stabilizers prevent ice crystals growth during shelf life by inhibiting the transfer of water from small to large ice crystals (13). Results outlined that chia could act as a stabilizer. The certain compositions improved viscosity, such as soy protein in strawberry flavored ice cream (22), guar and Arabic gums in yogurt (1), κ-carrageenan in lactose-free frozen yogurt (13), and chia seeds with strawberries in yogurt (7), as similar to chia powder in frozen yogurt for the present research.

3.5. Melting properties of frozen yogurt

The lowest meltdown rate was detected from 46.69 to 48.95 % in $SFYC^{III}$ and the highest levels were observed from 78.44 to 81.56 % during 21 days. Positive correlation coefficients among textural functions were pointed out and more significant was observed between viscosity and meltdown rate ($p<0.05$). The increase in viscosity proves to be corresponded to meltdown rates, in that elevating of chia significantly decrease meltdown rate, again fractionally because of water linking influence in chia which is consistent with former study exhibiting that stabilizer increases the structure of a cohesive matrix and could lead to a gel network such as proteins and carbohydrates in a more stable product. More acidity led to acid coagulation of milk protein whit chia, as a result, viscosity enhanced and meltdown behavior was reduced owing to mentioned factors. It was higher with elevated addition of inulin (4), guar and Arabic gums (1) as well as guar gum and date syrup (9) in frozen yogurt, as declared in the present study, and contrast-enhanced corn starch or κ-carrageenan did not significantly impact melting point regarding to Skryplonek *et al.* (13). The results of viscosity and melting attributes represent that chia could play as a stabilizer because of its capacity for linking water. As a result, water molecules were trapped and incapable to transfer freely among other molecules matrix (4).

3.6. Viability of Lactic Acid Bacteria during Storage

Changes of LAB presented that there was a nearly 1.5 log cycle reduce target bacteria in the control sample on 14th day. This revealed that freezing caused destructive influences on LAB most possibly according to freezing injury and the number of cells lowered to an average of 2 to 2.5 log cycle on 21st day. Our results displayed that reduction of LAB counts in each treated sample appeared close 1 to 1.5 log cycle on 21st day (Table 5). Regarding guidelines and information from the Food and Agriculture Organization of the United Nations (7), yogurts should include $\geq 10^7$ CFU g^{-1} LAB during validity time. The presence of nutrients such as polysaccharides, proteins and fats of chia seeds could be used by LAB and their growth rate improved as indicated in treatment samples. It was obvious that a lowered substrate was distinguished over storage time and the death rate of LAB was statistically higher, as well as the volume of bacteria decreased. Accordingly, the number of LAB lowered over time. Similar to the present study, Pop *et al.* (23) added chia seeds (1.4 %) to yogurt and development were observed in the viability of probiotic bacteria during shelf life. Also, Kwon *et al.* (8) supplemented yogurt with chia extract and detected an increase in LAB counts. In the contrary, Kowaleski *et al.* (7) stated that the addition of chia did not influence LAB survival and *Bifidobacteria*.

3.7. Sensory properties of frozen yogurt

Results revealed that chia addition showed no significant influence on flavor and generally lacked in odor even in the highest amount. The texture scores $SFYC^{I}$ were only similar to SFY. Chia in $SFYC^{II}$ was detected by 70 % of assessors. $SFYC^{III}$ was just ranked better than SFY in terms of body and texture ($p<0.05$). $SFYC^{III}_{21}$ had the highest texture points and no difference was observed between $SFYC^{I}$ and $SFYC^{II}$. The incorporation of chia significantly influenced the appearance functions of frozen yogurt. The color of treated samples was darkened by chia powder and appearance scores were lowered

Table 5. Influence of chia concentration on EC50 and LAB counts for frozen yogurts over a period of 21 days (mean ± standard error).

Storage time	Antioxidant attributes				LAB counts (log CFU/g)			
	SFY	$SFYC^{I}$	$SFYC^{II}$	$SFYC^{III}$	SFY	$SFYC^{I}$	$SFYC^{II}$	$SFYC^{III}$
0	26.89 ±1.49^{d}	19.78 ± 0.88^{bc}	16.84 ± 1.10^{b}	12.18 ± 0.97^{a}	7.31 ± 0.07^{a}	7.32 ± 0.06^{a}	7.30 ± 0.04^{a}	7.34 ± 0.03^{a}
7	30.79 ± 0.71^{e}	21.88 ± 2.36^{c}	17.93 ± 0.33^{b}	15.53 ± 2.10^{b}	6.23 ± 0.05^{e}	6.69 ± 0.07^{c}	6.80 ± 0.07^{bc}	6.97 ± 0.06^{b}
14	36.76± 1.23^{h}	23.40 ± 1.17^{c}	20.65 ± 1.60^{c}	18.43 ± 2.04^{b}	5.72 ± 0.12^{f}	6.21 ± 0.05^{e}	6.32 ± 0.03^{de}	6.57 ± 0.08^{d}
21	42.80 ± 0.99^{f}	26.53 ± 1.43^{d}	24.09 ± 1.35^{cd}	21.58 ± 2.27^{c}	5.08 ± 0.08^{g}	6.17 ± 0.04^{e}	6.30 ± 0.05^{de}	6.53 ± 0.05^{d}

from 5, which correlates with a higher level of chia powder. Averages of smoothness score for control were significantly ($p<0.05$) higher compared to treated samples. A negative impact was distinguished by higher chia powder ratio on mouth feel scores of frozen yogurts. Generally, the total acceptability scores of each sample were more than 3 on a scale of 1 to 5. Assessors preferred SFYC^{I} more than control. It pointed out that chia developed scores up to a level of 1 %, however further addition declined them. Assessors give scores to SFYC^{II} close to SFY and SFYC^{III} showed the lowest. As in the case of total acceptance, SFYC^{I} was ranked superior to others (Fig. 1). Linear correlation would be suitable to explain the relationship between texture using sensory and instrumental analysis. The presence of chia probably caused a distinct taste compared to control in the mouth, but this fresh taste was not so desirable to panelists. The rest of the experimental frozen yogurts ranked inferior compared to control. The results of some previous researchers were in line with the sensory evaluation of our research (4, 7, 9, 13) and another study had results dissimilar to ours (1).

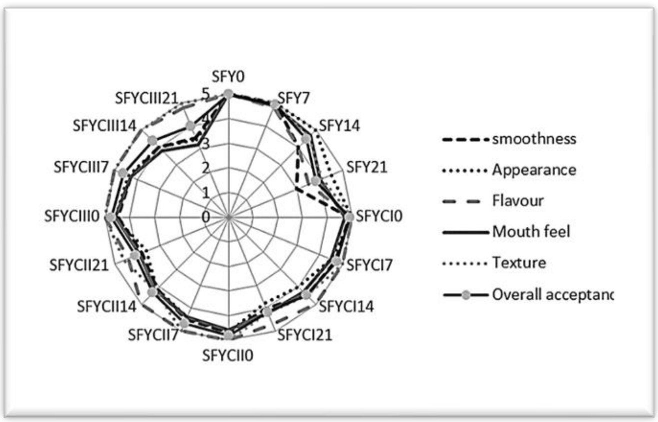

Fig. 1. Effect of chia addition on the sensory properties of strawberry frozen yogurt samples.

3.8. Overrun of frozen yogurt

Table 6 demonstrated an overrun range between 21.31 and 28.95 %, the highest volume belonged to SFYC^{III} from 28.80 to 28.95% on 14th and 21st day, respectively. The amount of this factor was the lowest without significant difference in SFY from 21.31 at production day to 21.54 at 7th day. The same range of overrun was reported in other studies (2, 13, 22). However, chia is comparable to milk-based on functionality, strong foaming stability of this value could be ascribed to the improvement of overrun levels. Protein, hydrocolloids, and fat are prominent for air incorporation (1), which was found in chia seed. According to previous studies, the overrun of frozen yogurt is positively influenced by stabilizers, which avoid air bubbles from collapsing (4, 9), as chia in the present study. In contrast, another researcher stated that overrun was not influenced by κ-carrageenan or corn starch (13). In stepwise regression evaluation, overrun feature was considered as a function variable and another determined attribute was taken as an independent parameter. The variance analysis results demonstrated that the regression relationship was significant at the level of 1 %. The effective characteristics such as protein, viscosity, acidity, L^*, and antioxidant attributes revealed a positive impact on overrun as well as pH and melting rate exhibited a negative impact. The major and independent factors recognized total variance (89%) in decomposition into principal compositions. The initial parameter measured 45 % of changes for variables comprising viscosity, L^*, acidity, antioxidant potential (positive effects), and also pH, a^* and melting value (negative effects). The second parameter was attributed to 35 % changes in variables such as protein and fat (positive effect) and also the viability of LAB and b^* (negative effect), statistical tables were not shown.

3.9. Fatty acid profile in frozen yogurts

According to instrumental test and sensory evaluation results among treatments, SFYC^{II} was selected and the comparison of its FA profile with SFY was illustrated in Table 7. In SFYC^{II} treatment, the main MUFA was oleic acid (18:1n-9). the most level was PUFA with the highest abundant being linolenic acid (18:3n-3) and linoleic acid (18:2n-6), in the other hand, The highest FAs were detected oleic acid 9.98 %, linolenic acid 4.05 %, and linoleic acid 3.41 %, and regarding

Table 6. The effect of chia seed on textural features (viscosity, overrun, and melting rate) in frozen yogurts over 21 days (mean ± standard error).

	Storage time	Samples			
		SFY	**SFYC^{I}**	**SFYC^{II}**	**SFYC^{III}**
Melting rate (%)	0	81.56 ± 1.25^{d}	62.30 ± 1.91^{c}	65.27 ± 1.313^{b}	48.95 ± 1.693^{a}
	7	80.92 ± 2.03^{d}	60.68 ± 1.80^{c}	55.18 ± 1.928^{b}	48.55 ± 1.086^{a}
	14	79.60 ± 1.56^{d}	60.49 ± 0.71^{c}	54.35 ± 1.638^{b}	47.05 ± 1.092^{a}
	21	78.44 ± 1.91^{d}	59.14 ± 1.26^{c}	53.07 ± 1.648^{b}	46.69 ± 1.924^{a}
Viscosity	0	111.17 ± 1.969^{i}	145.94 ± 6.232^{f}	148.82 ± 5.470^{f}	194.47 ± 10.826^{e}
	7	129.32 ± 3.044^{h}	174.46 ± 3.553^{e}	180.34 ± 2.085^{e}	239.99 ± 5.777^{c}
	14	152.25 ± 3.905^{f}	202.74 ± 7.197^{d}	212.73 ± 7.707^{d}	272.22 ± 4.386^{b}
	21	174.62 ± 2.418^{g}	241.97 ± 32.803^{c}	249.24 ± 8.697^{c}	315.15 ± 4.020^{a}
Overrun	0	21.31 ± 0.500^{f}	26.71 ± 0.35^{d}	27.63 ± 0.12^{c}	28.13 ± 0.77^{b}
	7	21.54 ± 1.248^{f}	26.90 ± 0.28^{d}	27.74 ± 0.59^{c}	28.28 ± 0.67^{b}
	14	22.40 ± 2.679^{e}	27.32 ± 0.73^{cd}	28.14 ± 0.55^{b}	28.80 ± 0.54^{a}
	21	22.81 ± 0.512^{e}	27.71 ± 0.35^{c}	28.10 ± 0.14^{b}	28.95 ± 0.17^{a}

10 % by chia addition, in contrast, PUFA content was elevated about 4 % and health attributes were promoted significantly for selected samples compared to controls. In the present study, the achieved results of FA were similar to those observed by Kowaleski *et al*. (7).

Table 7. Experimental evaluation of fatty acid profile in frozen yogurts.

Fatty acid (%)	**Formulation**	
	SFY	**SFYC**
Lauric fatty acid (C12)	4.15 ± 0.2	3.70 ± 0.2
Myristic acid (C14)	15.76 ± 0.1	12.24 ± 0.3
Palmitic acid (C16)	42.47 ± 0.5	38.00 ± 0.4
Stearic acid (C 18)	14.81 ± 0.3	12.90 ± 0.1
Saturated fatty acids	83.79 ± 1.2	72.87 ± 1.1
Oleic acid (C:18:1n9c)	2.70±0.2	9.98 ± 0.3
Monounsaturated fatty acids	10.20 ± 0.4	17.24 ± 1.5
Linoleic acid (C:18:2n6c, omega-6)	2.60 ± 0.2	3.41 ± 0.2
α-Linolenic acid (C:18:3n3, omega-3)	0.42 ± 0.1	4.05 ± 0.3
Arachidic acid (C 20, 4n6c, omega-3)	0.31 ± 0.3	0.50 ± 0.1
Eicosanoic acid (C20:1n9, omega-3)	0.20 ± 0.0	0.32 ± 0.1
Polyunsaturated fatty acids	5.70 ± 1.3	9.88 ± 0.7

4. Conclusions

Chia percentages were successfully applied in strawberry frozen yogurt preparation. This seed could be included in the functional group because it has been indicated to be nutritionally acceptable and components supply health promotions. Samples with chia addition comprised more protein, acidity, fat, and antioxidant features. Obviously, chia had significant impacts on the consistency and texture of frozen yogurts. The results demonstrated that enrichment had no significant influences on LAB counts of frozen yogurt. According to total acceptance scores, SFYC[I] was preferred superior compared to control. The SFYC[II] was chosen and comparable to the control sample based upon FA. More research is required to desirable find out nutritional improvements correlated with fortification obtaining new edible to fulfill market niches.

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