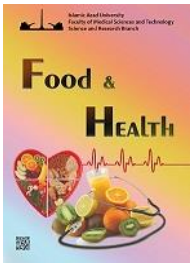

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Production and characterization of dairy dessert enrichment with *Sargassum angustifolium* algae

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ABSTRACT

Milk desserts are semi-solid complexes with a proteinaceous structure that are very popular among Iranian consumers. A milk dessert was prepared with whole milk, sugar, corn starch, gelatin, and *Sargassum angustifolium* powder (SAP). The effect of SAP (0, 0.4%, and 0.8%) on the physicochemical, microbial, and sensory properties of the milk dessert during 30 days of storage was evaluated. The results showed that acidity (0.18-0.21), dry matter (24.57%-24.80%), Brix (22.39-22.63), ash (0.86-0.89), fat (3.52%-3.80%), and protein content (4.05%-4.29%) of the milk dessert increased in the presence of SAP. The results showed that the stability and viscosity of samples containing SAP were higher than samples without this macroalgae. As the level of SAP increased, the color values, total viable bacterial count, and fungi population of the milk dessert samples changed significantly ($p<0.05$). Sensory property evaluation showed that the samples treated with 0.4% SAP had a proper general acceptance score on the 30th day. Finally, the T1-treated sample with 0.4% SAP was chosen as the best formulation for enriched milk dessert production.

1. Introduction

A dairy dessert is a product in which milk is the main ingredient of the formulation. A milk dessert contains at least 50% fresh cow's milk or reconstituted milk, which is prepared with additives such as flavorings, sweeteners, thickeners, and stabilizers after a thermal process such as pasteurization. The purpose of producing dairy desserts is to diversify the household food basket, develop dairy products, and produce an enjoyable product. The most important feature of desserts is the high energy they receive and the pleasant feeling created in the consumer due to the type of ingredients. Dairy desserts are used either with the main meal or as a snack during the day (1). Recent studies indicate a positive relationship between the prevalence of cardiovascular diseases, obesity, diabetes, high blood pressure, cancer, and nutritional factors. Concerns about communication between health and nutritional diet have led to the development of functional foods. These kinds of foods provide health benefits beyond basic nutrition and have demonstrated physiological benefits like reducing the risk of disease. In addition to nutritional factors such as omega fatty acids, fiber, minerals, and vitamins, functional foods contain bioactive compounds with health-promoting effects (2). Macroalgae mostly contain primary metabolites like lipids, proteins, and carbohydrates. In addition, agar, alginate, fucoidan, ulvan, laminarin, pectin, and carrageenan are examples of secondary metabolites. Macroalgae are rich sources of biologically active compounds with antimicrobial, antitumor, anti-inflammatory, and antioxidant properties. Because of the health benefits of macroalgae, more consideration is being given to applying them in food industries (3). A kind of brown macroalgae *Sargassum angustifolium* is reported to be found on the south subtropical coast of Iran. *S. angustifolium* was mainly composed of carbohydrates such as insoluble fibers, proteins, vitamins, minerals like calcium, iron, zinc, and pigments such as carotenoids (4). Several studies have shown that edible algae may be satisfactorily used in various food products such as

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drinks, bakery products, desserts, yogurt, fermented milk, cheese, etc. Tohamy et al. (5), due to the importance of the nutritional value of algae, evaluated the nutritional and functional properties of spreadable processed cheese supplemented with *Spirulina platensis*. Similarly, Agustini et al. (6) reported a positive effect of *S. platensis* on the chemical, rheological, and sensory properties of ice cream and soft cheese. Islami Meshkenani et al. (7) presented a significant increase in protein and iron content of buttermilk produced via fortification with *S. platensis*. Toliaty et al. (8) completed a study in which S. platensis and stevioside were applied in dairy dessert production. In enriched dairy products, *S. angustifolium* may reveal a positive effect on physicochemical properties by improving the rheological and sensory properties. However, when new ingredients are used in an available milk dessert formulation, evaluating its effect on dessert characteristics is necessary. The present study evaluated the production of enriched milk dessert containing *Sargassum angustifolium* powder (SAP), to determine their physicochemical, microbial, and organoleptic characteristics.

2. Materials and methods

2.1. Materials

The *Sargassum angustifolium* powder (SAP) was obtained from the Algal Bank-Algal Bioresource Development Company in Shiraz, Iran. Food-grade corn starch, gelatin, and vanilla were prepared from the Golha Company. Sodium hydroxide, sulfuric acid, isoamyl alcohol, boric acid, plate count agar (PCA), Baird-Parker agar (BPA), lauryl sulfate tryptose (LST) broth, eosin methylene blue agar (EMB), and yeast extract glucose chloramphenicol agar (YGC) were provided by the Merck Company (Darmstadt, Germany).

2.2. Preparation of milk dessert

The base formulation for the milk dessert consists of 190 mL of whole milk (3%), 20 g of sugar, 5 g of corn starch, 4 g of gelatin, 0.2 g of vanilla, and different concentrations of SAP (0, 0.4%, and 0.8%). Pasteurized whole milk (3% fat) was mixed with sugar, gelatin, vanilla, and different concentrations of SAP and was stirred well at 85°C with a magnetic stirrer (IKA, Staufen, Germany) at 250 rpm for 10 minutes. Subsequently, the dispersion was heated to 90°C for 20 minutes and cooled down to 40 °C. The desserts were placed in 200 ml plastic containers with lids previously sanitized with 0.5% sodium hypochlorite and stored under refrigeration (4 °C ± 1 °C) for up to 30 days (9).

2.3. Physico-chemical analysis

The pH, dry matter, and ash content of samples were measured using a digital pH meter (Taiwa, AZ 86502), oven (Memmert, Germany), and furnace (Tehran Godazeh Saz, Iran), respectively (8). The titratable acidity was evaluated by titrating samples with 0.1 N NaOH. The Gerber and the Macro Kjeldahl method measured samples' fat and protein content. Syneresis was quantified with 10 g of sample by centrifugation as the percentage of supernatant liquid after centrifugation of the gel for 20 min at 2790 g. The apparent viscosity of milk desserts was assessed using a viscometer (DV II + LV, Brookfield, Middleboro, MA, USA), equipped with an LV4 spindle. The samples were poured into the measuring vessel and measurements were carried out at fourteen angular speeds of the viscometer spindle ranging from 1.5 to 100.0 rpm (9). A Hunterlab (Colorflex EZ, USA) was applied to measure the whiteness (L*), red/greenness (a*), and yellow/blueness (b*) values of the milk dessert enriched with SAP (10).

2.4. Microbial analysis

For the microbiological analysis, 10 g of the samples were homogenized into a sterile glass with 90 ml of sterilized saline solution (0.95% w/v) to obtain the initial dilution (1/10). By applying this dilution, a number of decimal dilutions were prepared using the same diluent. All plates were placed in an incubator for 48 h at 37°C but for mold and yeast evaluation 5 days at 25 °C were used. The results were expressed as Log 10 CFU/g (11).

2.5. Sensory analysis

A consumer panel of nine panelists (four women and five men aged between 20-30) performed the sensory analysis using a five-point hedonic scale ranging from one (dislike extremely) to five (like extremely). The sensory parameters included color, taste, flavor, texture, and general acceptability. These parameters were analyzed on the thirtieth day of storage. Twenty grams of dessert samples were prepared in numbered plates and released to panelists at a temperature of four ± one degree Celsius before having a meal. The panelists used water after each test to wash their mouths (12)

2.6. Statistical analysis

Experiments were performed in triplicate, and significant differences between means were analyzed using one-way ANOVA and Duncan post hoc tests (SPSS version 22). Differences were considered significant at $p<0.05$. Nonparametric data were analyzed by applying Kruskal-Wallis tests.

3. Results and discussion

3.1. Physicochemical Analysis

Table 1 shows the increasing values in the acidity and decreasing values in the pH of different dessert samples during cold storage. The control sample had an initial pH value of 6.48 and reached a level of 6.24 on the 30th day of storage. For T2 samples, the initial value of acidity was 0.185 and had a final value of 0.27 on the 30th day of storage. The pH values and acidity range of dessert samples were 6.05-6.48 and 0.18-0.27, respectively. The Iranian National Standardization Organization (INSO) has set a range for milk dessert pH that

is consistent with recent results (13). However, Toliaty et al. (8) reported a higher range of 7.31-7.46 for the pH values of enriched dairy desserts. A similar pH value was also reported by Aguilar-Raymundo and Vélez-Ruiz (9), with values of 6.35–7.12, for commercial vanilla custard. The pH values of milk dessert had significantly decreased ($p<0.05$) in the presence of SAP, but the acidity increased insignificantly ($p>0.05$). Also, a significant drop in the pH value was observed during storage ($p<0.05$). Acidity is used as an important indicator to determine the quality of dairy products and shows the presence of organic acids. It seems, therefore, that the presence of nutrients such as indigestible fibers in SAP could stimulate LAB's growth during storage (14). In line with our research, Islami Meshkenani et al. (7) reported a decreasing trend in pH value during the storage of probiotic buttermilk containing *Spirulina platensis*. Contrary to our studies, Kaur and Goswami (15) reported that dairy dessert's pH increased significantly with stevia concentration. The dry matter, Brix, and ash content of different dessert samples during cold storage are presented in Table 1. The results revealed that in the presence of SAP, these parameters of dessert samples changed significantly compared to the control samples ($p<0.05$). According to Table 1, the lowest amount of dry matter, Brix, and ash was related to the control sample on the first day, and the highest amount was observed in the sample enriched with 0.8% SAP on the 30th day. The dry matter, Brix, and ash amount of all dessert samples increased over the 30-day storage period. However, this was not significant ($p>0.05$). It was observed that samples with a higher amount of dry matter and Brix revealed greater titratable acidity. Functional compounds such as protein and carbohydrates are abundant in SAP. So, with the increasing concentration of SAP to 0.4% and 0.8%, the dry matter, Brix, ash, and titratable acidity of treated dessert samples increased significantly (9, 14). Samples with a higher content of SAP had a higher amount of dry matter, Brix, ash, and titratable acidity. Similarly, Bchir et al. (16) showed that the addition of fresh and dried *Spirulina platensis* increased the solid contents of yogurt. This finding is also similar to the report of Tarrega et al. (17), wherein the Brix of commercial custards was within the range of 23.5–28.3; that formulation consists of milk, cream, adipate, gelatin, cross-linked starch, and milk powder. Also, Atallah et al. (18) reported ash values 1.01 for low-fat yogurt enriched with *Spirulina platensis*.

Table 1. Physico-chemical characteristics of milk dessert samples with different concentrations of *Sargassum angustifolium* powder (SAP) during storage.

Samples	pH		Acidity (%)	
	1st day	30th day	1st day	30th day
Control	6.48 ± 0.01^{Aa*}	6.24 ± 0.03^{Ba}	0.18 ± 0.01^{Ba}	0.21± 0.1^{Ab}
T_1	6.44± 0.01^{Aa}	6.11 ± 0.02^{Bb}	0.183± 0.05^{Ba}	0.25 ± 0.03^{Aa}
T_2	6.40 ± 0.03^{Aa}	6.05 ± 0.06^{Bb}	0.185± 0.04^{Ba}	0.27 ± 0.3^{Aa}
	Dry matter (%)		**° Brix**	
	1st day	30th day	1st day	30th day
Control	24.57± 0.01^{Aab}	24.59 ± 0.04^{Ab}	22.39 ± 0.01^{Aa}	22.39 ± 0.03^{Aa}
T_1	24.65± 0.5^{Aab}	24.68 ± 0.1^{Aab}	22.52± 0.٠6^{Aab}	22.53 ± 0.05^{Aab}
T_2	24.79± 0.2^{Aa}	24.80 ± 0.2^{Aa}	22.63 ± 0.01^{Ab}	22.63 ± 0.06^{Ab}
	Protein (%)		**Fat (%)**	
	1st day	30th day	1st day	30th day
Control	4.05± 0.05^{Ab}	4.07 ± 0.6^{Ab}	3.52 ± 0.01^{Ac}	3.53± 0.07^{Ac}
T_1	4.18± 0.01^{Aab}	4.20 ±0.02^{Aab}	3.68 ± 0.4^{Ab}	3.68 ± 0.02^{Ab}
T_2	4.27± 0.02^{Aa}	4.29 ± 0.2^{Aa}	3.79 ± 0.03^{Aa}	3.80 ± 0.01^{Aa}
	Ash (g/100)			
	1st day	30th day		
Control	0.086 ± 0.004^{Ac}	0.086± 0.002^{Ac}		
T_1	0.088 ± 0.005^{Ab}	0.088± 0.004^{Ab}		
T_2	0.089 ± 0.003^{Aa}	0.089± 0.002^{Aa}		

* Means within each column followed by different letters (a–b) show significant differences ($p<0.05$) between treatments at the same time. Means within each row followed by different letters (A–B) show significant differences ($p<0.05$) at treatment during the storage period. Samples were included (Control (0% SAP), T1 (0.4% SAP), and T2 (0.8% SAP)).

Table 1 revealed a significant increase in different dessert samples' protein and fat content during storage ($p<0.05$). On the first day, the least protein and fat content were observed for the control sample. On the 30th day, the T2 sample with 0.8% SAP exhibited the highest percentage of protein and fat. The results showed that the addition of SAP caused a significant increase in protein and fat content compared to the control sample ($p<0.05$). The presence of fat and protein in SAP has been reported by several researchers (16, 19). This result is related to the incorporation of SAP into dessert samples, which improved its protein and fat content.

3.2. Syneresis percentage and viscosity analysis

A dairy dessert is a three-dimensional protein network in which a continuous liquid fills the entire volume of the system. Considering the negative effect of syneresis on food quality, it is desirable to reduce this phenomenon in dairy desserts (9, 20). The syneresis percentage of different milk dessert samples during cold storage can be observed in Fig. 1. According to the results, the least syneresis was related to the T2 sample (containing 0.8% SAP) on the first day, and the highest amount was observed in the control sample on the 30th day. The results showed that the syneresis percentage of dessert samples had a significant increase during the 30 days of cold storage ($p<0.05$). Although, during cold storage, restructuring of the protein matrix results in water exudation (syneresis), adding

SAP could significantly increase the water retention capacity ($p<0.05$) and produce a highly stable dessert (20). Adding SAP to the dairy dessert strengthens the protein-water bond in the gel network, so the amount of syneresis decreases. Although SAP is a low-calorie food, it is rich in non-digestible essential amino acids, proteins, polysaccharides, vitamins, minerals, and phenolic compounds. It seems that proteins and polysaccharides in SAP interact synergistically with gelling agents to avoid undesirable syneresis (21). Similarly, Alam et al. (22) reported a significant reduction of milk dessert syneresis in the presence of taro starch-guar gum mixture and taro starch-xanthan gum mixture ($p<0.05$). The results were in accordance with the findings of Bierzunska et al. (23), who reported the reducing effect of whey protein on yogurt syneresis. Consistent with our results, Aguilar-Raymundo and Vélez-Ruiz (9) reported a significant increase in the syneresis percentage of dairy dessert that was enriched with chickpea flour during 12 days of storage.

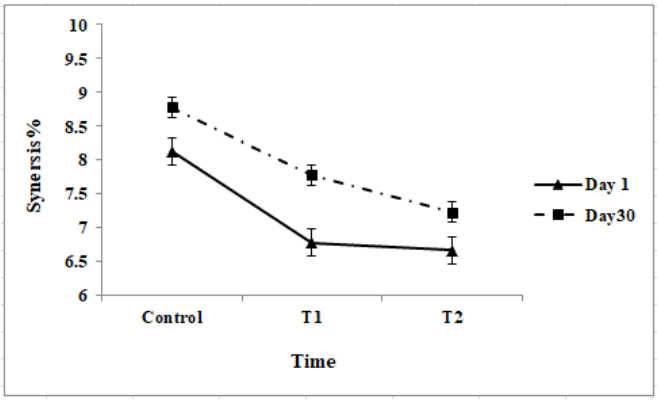

Fig. 1. The syneresis (%) of milk dessert samples containing different concentrations of *Sargassum angustifolium* (SAP) during storage (Control (0% SAP), T1 (1.5% SAP), T2 (3% *S. angustifolium*).

During the production and formulation of new dairy products, viscosity is evaluated as a factor that affects textural and sensory characteristics and ultimately consumer acceptance. Fig. 2 shows the apparent viscosity of different milk dessert samples during cold storage. The results showed that the apparent viscosity of all samples had an insignificant reduction over time of storage ($p>0.05$). Such behavior can be explained by a reduction in the formation of protein-protein crosslinks and protein-water interactions over the 30 days of cold storage (24). However, Bierzunska et al. (23) noted that adding polymerized whey protein and whey protein concentrate to yogurt significantly increased the apparent viscosity during storage. The results revealed that the addition of SAP caused a significant increase in the dessert samples' viscosity compared to the control sample ($p<0.05$). The increase in viscosity can be attributed to the SAP protein structure and the creation of intercellular interactions. Indeed, the existence of fiber and hydrophilic compounds with a hydroxyl group in the structure of SAP significantly increases the product's viscosity. A similar trend has been reported by Atallah et al. (18) for the effect of *Spirulina platensis* on the viscosity and stability of low-fat yogurt. Also, Agustini et al. (25) noted that adding *Spirulina platensis* to yogurt increased viscosity compared to the control samples (26).

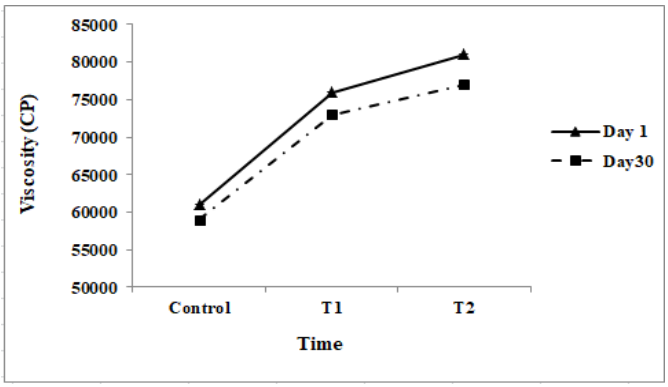

Fig. 2. The viscosity of milk dessert samples containing different concentrations of *Sargassum angustifolium* (SAP) during storage (Control (0% SAP), T1 (1.5% SAP), T2 (3% SAP).

3.3. Color parameters analysis

The color parameters of brightness (L*), red-green (a*), and yellow-blue (b*) of milk dessert samples are shown in Table 2. The results revealed that enriching the milk dessert with SAP significantly decreased the L* index ($p<0.05$). Presumably, the presence of color compounds in SAP caused the reduction in brightness and transparency of milk dessert samples. In addition, the L* index of milk dessert samples decreased insignificantly during 30 days of storage ($p>0.05$). Similarly, Hong et al. in 2020 showed that the L* index in yogurt samples decreased in the presence of paprika extract due to the migration of color compounds from paprika to yogurt (27). These results were consistent with Ardalanian et al.'s (10) report, which noted a reduction in the L* index in buttermilk samples enriched with ginseng extract during storage. Adding SAP causes a significant increase in the a* value, which is a negative index ($p<0.05$). It seems that the increase in the red color of treated samples is due to the red color compounds in SAP. During the storage period, the a* value decreased insignificantly ($p>0.05$), which can be due to the decomposition of red color compounds and the oxidation of pigments in milk dessert. During storage times, the samples enriched with 0.8% SAP (T2) had the highest a* value, and the control samples had the lowest a* value. Table 2 indeed shows that in the presence of SAP, the b* index, which represents the yellow color, increased significantly ($p<0.05$). It seems that SAP contains compounds that effectively increase the yellow color and reduce the blue color. These results are consistent with those of Ardalanian et al. (10), who reported an increase in the b* index in buttermilk samples enriched with ginseng extract. This suggests that certain additives can influence the color parameters of dairy products.

3.4. Microbiological properties

The microbiological properties of milk desserts enriched with SAP are presented in Table 3. Significant statistical differences were observed in milk dessert samples' total viable

bacterial count and fungi population during storage ($p<0.05$). Also, in the presence of SAP, the total viable bacterial count and fungi population decreased significantly ($p<0.05$). After 30 days, the total viable bacterial count in control dessert samples and treated samples containing 0.8% SAP were 2.83 and 2.78 log CFU/g, respectively. Also, the fungi population decreased from 1.77 log CFU/g in control samples to 1.60 log CFU/g in treated samples containing 0.8% SAP on the 30th day. No contamination with *Escherichia coli* and *Staphylococcus aureus* (Coagulase-positive) was observed in the tested dessert samples. The results were consistent with the microbiological limits for dessert samples determined by the

Table 2. Changes in L*, a*, and b* values of milk dessert enriched with *Sargassum angustifolium* (SAP) during storage.

Samples	L*		a*	
	1st day	30th day	1st day	00th day
Control	89.22 ± 0.02^{Aa*}	89.21 ± 0.01^{Aa}	-4.95 ± 0.01^{Ac}	-4.93± 0.09^{Bc}
T_1	87.10± 0.01 ^{Ab}	87.96 ± 0.02^{Ab}	-4.47± 0.02 ^{Ab}	-4.45 ± 0.03^{Bb}
T_2	86.07 ± 0.02^{Ac}	85.88 ± 0.05 ^{Ac}	-4.11± 0.04 ^{Aa}	-4.08 ± 0.3^{Ba}
	b*			
	1st day	30th day		
Control		20.04± 0.01 ^{Ac}	20.01 ± 0.03^{Ac}	
T_1		24.65± 0.5 ^{Ab}	24.68 ± 0.1^{Ab}	
T_2		24.79± 0.2 ^{Aa}	24.80 ± 0.2^{Aa}	

*Means within each column followed by different letters (a–b) show significant differences ($p<0.05$) between treatments at the same time. Means within each row followed by different letters (A–B) show significant differences ($p<0.05$) at treatment during the storage period. Samples were included (Control (0% SAP), T1 (0.4% SAP), and T2 (p0.8% SAP)).

Table 3. Microbiological properties of milk dessert samples enriched with *Sargassum angustifolium* (SAP) during storage.

Samples	Total viable count (log_{10} CFU)		*Staphylococcus aureus* (*Coagulase-positive*)	
	1st day	30th day	1st day	30th day
Control	2.80 ± 0.22^{Aa*}	2.83 ± 0.31^{Aa}	-	-
T_1	2.76 ± 0.13^{Aab}	2.79 ± 0.35^{ABab}	-	-
T_2	2.74 ± 0.18^{Ab}	2.78 ± 0.18^{Ab}	-	-
	Escherichia coli		Mold/Yeast (log_{10} CFU)	
	1st day	30th day	1st day	30th day
Control	-	-	1.60± 0.19^{Aa}	1.77 ± 0.23^{Ba}
T_1	-	-	1.47 ± 0.31^{Ab}	1.69 ± 0.11^{Bb}
T_2	-	-	1.47 ± 0.31^{Ab}	1.60± 0.19^{Bc}

*Means within each column followed by different letters (a–b) show significant differences ($p<0.05$) between treatments at the same time. Means within each row followed by different letters (A–B) show significant differences ($p<0.05$) at treatment during the storage period. Samples were included (Control (0% SAP), T1 (0.4% SAP), and T2 (p0.8% SAP)).

Iranian National Standardization Organization (INSO) (ISIRI 14681, 2012) (13). In agreement with our findings, Secim and Ucar (28) reported an absence of *S. aureus* (Coagulase-positive) contamination in milk dessert samples, but all the tested dessert samples were contaminated with coliforms. Also, Jadhav and Raut (29) observed that about 40% of ice cream samples had *E. coli* contamination.

3.5. Sensory evaluation

The results of the sensory analysis using the chi-square test for smell, color, texture, flavor, and overall acceptability on the 30th day of storage are revealed in Fig. 3. The data analysis revealed that adding SAP to milk dessert samples had no significant effect on the smell parameters ($p>0.05$). However, in the presence of SAP, treated samples revealed significant differences in terms of color, flavor, texture, and general acceptance ($p<0.05$). The sample containing 0.4% SAP received the highest scores for color and flavor parameters, but the T2 sample with 0.8% SAP had the lowest flavor scores. The highest texture score was related to the T1 sample containing 0.4% SAP, while the T2 sample containing 0.8% SAP had the lowest texture score. After 30 days of storage, the T1 sample containing 0.4% SAP revealed the highest overall acceptability score. In line with our research, Choobkar et al. (20) reported that adding cinnamon powder positively affects the general acceptance of pudding products.

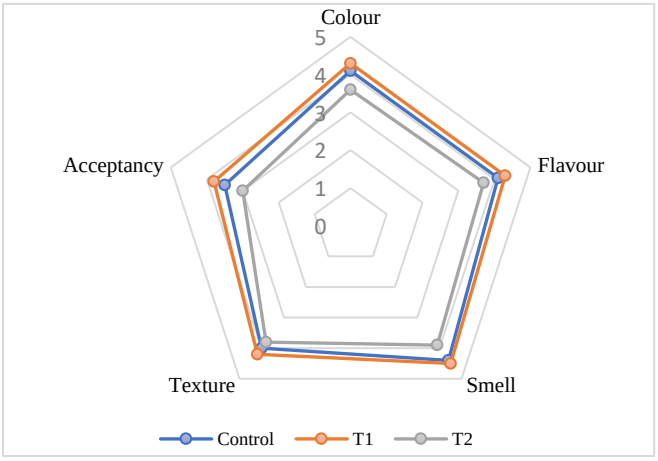

Fig. 3. The Sensory evaluation of milk dessert samples containing different concentrations of *Sargassum angustifolium* during storage (Control (0% *S. angustifolium*), T1 (0.4% *S. angustifolium*), T2 (0.8% *S. angustifolium*).

Also, Jalalvand et al. (30) revealed that the presence of *Spirulina platensis* significantly reduced the sensory evaluation score of buttermilk. These findings highlight the

impact of various additives on the sensory properties of dairy products.

4. Conclusions

In this study, adding *Sargassum angustifolium* powder (SAP) at the levels of 0.4% and 0.8% significantly decreased the total viable bacterial count and fungi population. This revealed the antibacterial potential of SAP in extending the shelf life of milk desserts. The increasing shift of antibacterial activity in the treatment over the 30-day storage period was consistent with the increment in acidity. The sample that presented higher antibacterial activity showed higher levels of acidity. In the presence of SAP, the syneresis percentage was significantly reduced compared to the control sample. The viscosity of the treated dessert samples increased in the presence of SAP. An elevated SAP concentration enhanced the amount of fat, protein, dry matter, and ash in treated samples. The highest satisfaction score for stability and overall acceptance score of the milk dessert sample belonged to the T1 sample with 0.4% SAP at the end of storage time (30 days). On the basis of all data obtained in this study, the T1 sample was observed to be the best treatment with desirable characteristics for the production of enriched milk desserts.

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