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Effect of Storage Period on Physicochemical, Textural, Microbial and Sensory Characteristics of Stirred Soy Yogurt

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ABSTRACT: Development of different soy milk-based products has growing market during the last decade. This research was conducted with the aim of producing stirred soy yogurt and physicochemical, textural, microbial and sensory characteristics during 28-day storage period (days 1, 3, 7, 14, 21 and 28) were studied. Until 14th day, pH and acidity of sample showed a significant decrease and an increase trend ($P<0.05$), respectively, but then pH had an upward trend and acidity had a downward trend ($P<0.05$). Syneresis of the samples had a downward trend from day 1 to 14 and then increased to 28 days, but the viscosity showed a continuously increasing trend ($P<0.05$). Mean comparison of textural parameters showed a significant increase ($P<0.05$) in the first day with other days (28 days of storage), but no significant difference was observed between the other days ($P>0.05$). There was no clear trend regarding the brightness of samples, and factors of a* and b* had no significant difference during storage. Microbial counts of samples decreased from the1 to 28th day. The sensory evaluation of stirred Soy yogurt showed that the overall acceptability of the product decreased during storage, most of which was related to the taste and flavor of the product. According to the results, this research provides good information about the production of stirred soy yogurt as a new product by adding skim milk powder, milk protein concentrate, and corn starch.

***Keywords*:** Physicochemical, Sensory evaluation, Stirred soy yogurt, Storage period, Texture

INTRODUCTION

Considering the lack of food, there are many efforts to find a substitute for protein resources, especially in developing and engaged in malnutrition countries. Soybeans contain a variety of phytoestrogens (Isoflavones) that its amount is about 50 times more than the group in its family such as lentils and beans. According to the role of Isoflavones in maintaining human health and also other nutritional values of soy in preventing many diseases, the soy milk consumption in most countries of South East Asia, America and Europe is very high, while its consumption in Iran is very low (Park et al., 2005). The relatively low price, lack of cholesterol and lactose, presence of essential and unsaturated fats, as well as, reducing the blood fat and cholesterol and preventing the atherosclerosis are some significant benefits and characteristic of soy food products (Osundahunsi et al., 2007; Sengupta et al., 2013). Therefore, many efforts are made to use and produce the delicious and acceptable food products from them.

Variety of products can be highly effective in increasing the consumption and considering the extraction of 5 to 8 kg of milk per kg of soya bean, its production can be fully explained economically (Tamime et al., 1999). Soy milk is a stable emulsion of oil, water, and protein that is made by soaking dry soybean and milling them. Soy milk is made by soaking soybeans for 6 to 16 hours and then crushing them and mixing them with water in various ratios (1 to 7, 1 to 8 and 1 to 10) (Shetty et al., 2006). The amount of soy milk protein (3.6 % wt) is almost similar to cow milk (3.3 % wt) but its amino acid profile is different with the amino acid profile of cow milk protein. Also, soy milk has 1.9 % wt of fat (3.9 % wt in cow milk), 2.8 % wt of carbohydrate (4.7 % wt in cow milk), 0.4 % wt of ash (0.7 % wt in cow milk) and 91.3 % wt of water (87.4 % wt in cow milk) (Smith, 1972; Tamime et al., 1999). Soy milk consumption has grown at an annual growth rate of 21 % per year during the past decade (Min et al., 2005). Soy milk has many oligosaccharides, which mostly are sucrose and other sugars such as Raffinose and Stachyose. Raffinose and Stachyose are known as compounds that cause abdominal bloating, which can be reduced by lactic fermentation. Soy milk consumption is limited due to the strong bean flavor; Although lactic acid bacteria helps to fix the problem of bean flavor and leads to increase the acceptability of the final product (Clark et al., 1994; Granata et al., 1996). In this regard, soy milk based yogurts (namely sogurt) that are obtained from the inoculation of conventional yogurt starter bacteria to soy milk can be appropriate (Pyo et al., 2008). Although, several researches were published on formulation of soy yogurt fortified with fruit pulp and flavor (Drake et al., 2001; Kumar et al., 2003).

The nutritional value of soy yogurt is dependent on the milk by which the yogurt is produced and considering that soy milk is obtained from mixing water with crushed soybeans or soy flour with different ratios, so the amount of its nutrients is indirectly related to the water added to crushed soybeans or soy flour (Tamime et al., 1999). Soy yogurt is a good replacement for conventional yogurt, especially in remote geographical areas where transfer of milk and yogurt and other products is difficult and keeping the animals is not provided. However, milk and soy yogurt have high value in Nigeria and South Africa countries (Park et al., 2005).

Often the texture defects in yogurt are caused by the changes in apparent viscosity and syneresis which lead to the rejection of the final product. These changes may be caused by variation in the milk composition, as well as the change in the process and conditions of storage and maintenance. Hydrocolloids (thickeners) and dairy additives that are added to the milk are for fixing the defects and providing a firm texture and decreasing the syneresis (Ares et al., 2007; El-Sayed et al., 2002). Hydrocolloids in stirred yogurt are widely used and the main purpose of adding them is creating and maintaining the desirable characteristics such as good texture, viscosity, consistency and suitable appearance and preventing the syneresis in the final product (Jimoh et al., 2007; Keogh et al., 1998). Therefore, the research was conducted with the aim of producing the stirred soy yogurt that ordinary corn starch and milk protein concentrate were used to produce and improve its texture. In order to study the quality and other characteristics of the produced product, the physicochemical, textural, microbial and sensory characteristics of stirred soy yogurt in a 28-day storage period (days 1, 3, 7, 14, 21 and 28) were studied.

Materials and Methods

Raw materials

Soy milk was provided by Isfahan soy milk Company (Isfahan city, Iran). Skim milk powder and milk protein concentrate (MPC) were bought from Isfahan Pegah Company (Isfahan, Iran) and Germany Meggle Company, respectively. Ordinary corn starch was prepared from Tehran Glucosan Company (Tehran, Iran). The yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (TY947 and TY367) was purchased from the representation of Christian Hansen Company of Denmark. The culture mediums of violet red bile agar and Dichloran Glycerol Agar (DG18) were purchased from the representation of Germany Merck Company. Other chemicals had a high purity and for the chemical analysis.

Preparation of stirred soy yogurt

At first, based on a weight percentage of initial soy milk (average 8 % of dry matter), 2 % of skim milk powder, 1 % of milk protein concentrate (MPC), and 1% of ordinary corn starch were added to it and thoroughly mixed. Then, it was pasteurized at a temperature of 85 °C for 15 minutes and got cold to 42-43 °C. The rennet bacteria, including *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were added to the soy milk mixture with the ratio of 0.7 and 0.5 % wt, respectively, and were poured in polyethylene containers. The containers were put in the incubator at 44 ° C for 4-6 hours and then the samples were removed from the incubator and put in the ice and water bath to temperature drop of containers approximately 5 to 7 °C. Then, the samples were fully stirred in their respective container to obtain a stirred soy yogurt with uniform texture. Stirred soy yogurt was put in the refrigerator at the temperature of 4 ° C. After 24 hours, all the tests of first day were conducted.

pH and acidity
pH of stirred soy yogurt samples was carried out by inserting the pH meter probe (Metrohm, model 827, Switzerland) in the samples. Acidity was also performed by the titration method. Therefore, a certain amount of yogurt was weighed and 0.5 mL of Phenolphthalein reagent was added to it. Then, the titration continued by a 0.1 Molar sodium hydroxide solution to reach the stable pink color. The amount of acidity was calculated based on the percentage of lactic acid (Tamjidi et al., 2012).

Syneresis
The sensitivity of samples to syneresis was conducted by centrifuging 20 g of each sample per 350 g for 5 minutes (Sigma, 2K15 model, Germany). The serum separated on the Falcon surface was separated by pipette and reported as the percentage of syneresis (V/W %) (Panesar et al., 2012).

$$\text{Syneresis percentage} = \frac{\text{volume of upper phase}}{\text{Sample weight}} \times 100$$

Apparent viscosity
The apparent viscosity of the stirred soy yogurt samples was conducted by Brookfield Viscometer (Brookfield Viscometer, DVII, USA) at 5 ° C. Measurements was performed by the spindle, No 4, and at rotational speed of 3 RPM. The results were reported as centipoises after 50 seconds of rotation in the samples (Izadi et al., 2014).

Textural properties
The texture characteristics of stirred soy yogurt during storage were conducted by doing the back extrusion test by a Texture Analyzer (Brookfield, LFRA 4500, USA). The diameter of the probe used, the amount of penetration, and its speed was 38.1 mm, 20 mm and 1 mm per second, respectively. The metal cylinder with a diameter of 45 mm and height of 95 mm was used to test that approximately 100 g of samples was poured into the metal cylinder for conducting the test. Textural parameters (hardness, apparent modulus, adhesiveness and the work done for hardness) were calculated by using software of device (TexturePro Lite software). All measurements were performed at 5 °C (Tamjidi et al., 2012).

Colorimetric test
Measuring the color parameters of samples was conducted by the method of photographing with a digital camera (LUMIX Panasonic, TZ5 model, Japan) in the box with controlled light conditions. Color parameters (L*, a*, b*) were determined in Photoshop software (Version 8.0, San Jose, CA) and was standardized by special cards (RAL K7 classic, Germany) (Izadi et al., 2014).

Microbial counting
For counting Coliforms, the molds and yeasts of stirred soy yogurt during the storage period, the culture media of violet red bile agar and Dichloran Glycerol Agar (DG18) were used, respectively. Microorganisms count was done according to standard methods (Jimoh et al., 2007).

Sensory evaluation
Sensory evaluation samples of stirred soy yogurt during the storage period was conducted by using the Hedonic method and considering the parameters such as texture, taste, mouth feel and overall acceptance. The test was performed on a rating scale of 1 to 7 (the larger number represents the desirability) for each parameter and by 20 half-trained panelists (Yang et al., 2010).

Statistical analysis
All experiments were carried out in a completely randomized block design with at least two replications. In order to analyze the data, the method of ANOVA with SAS 9.0 software was used. All mean comparisons were conducted by the test of LSD (least significant difference) at the level of 5 %.

Results and Discussion

pH and acidity of stirred soy yogurt
The body, texture, physical stability, flavor and aroma of the stirred soy yogurt were related to pH (Granata et al., 1996). As shown in Figure 1, during the storage period up to 14 days, pH of the samples showed a significant decreasing trend at the level of 5 % but then pH had an insignificant upward trend, and its difference was

significant (P< 0.05). The acidity of stirred soy yogurt during storage up to 14 days showed a significant increase in the level of 5 %, but then the acidity had a significant downward trend. Izadi *et al.* (2014) reported a decrease and an increase trend (P<0.05) in pH and acidity of yogurt during 28 days of storage (Izadi et al., 2014). Decreasing pH and increasing acidity in the yogurt during storage are due to the metabolic activity of yogurt starters that produce lactic acid by fermenting lactose. In fact, considering the trends observed for pH and acidity of stirred soy yogurt, the trend of decreasing pH can be attributed to both beneficial and harmful microorganism activity. Microorganisms can lead to decrease pH by sugar consumption and production of organic acids. By finishing the sugar resources, the microorganisms consume the proteins in an environment and this will increase pH.

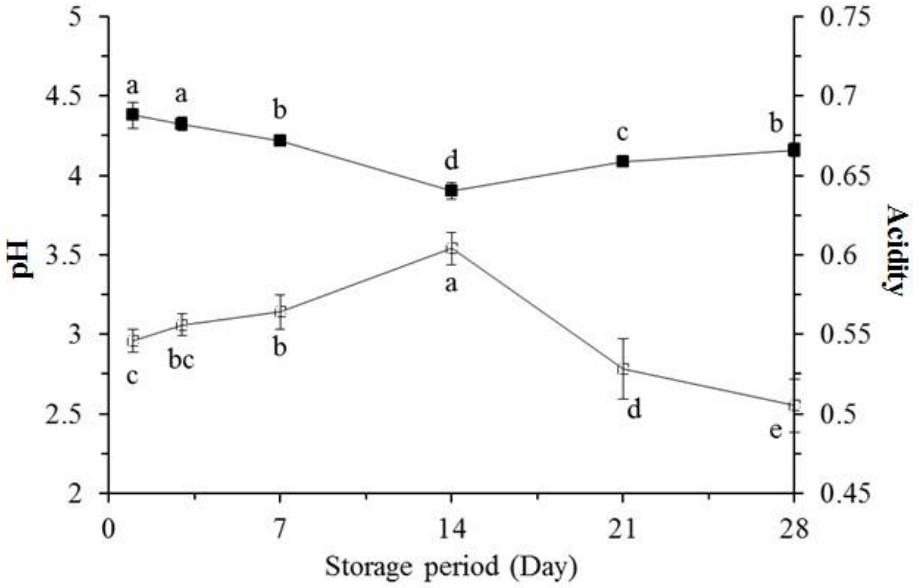

Figure 1. Effect of storage time on pH (■) and acidity (□) soy yogurt stirred

pH and acidity of stirred soy yogurt changed in the range of 3.90-4.38 and 0.55-0.60. Although, previously the optimum pH of yogurt milk is reported 4.2-4.3 (Granata et al., 1996). Relatively high pH value and low acidity are caused by the low amount of lactose in initial soy milk, although it should be considered that skim milk powder and milk protein concentrate were also used in the formulation of stirred soy yogurt. These two compounds have some lactose and the lactose content can be effective in improving the taste and producing lactic acid by starter bacteria of yogurt. Yang and Lee (2010) reported pH and titratable acidity for soy yogurt in the range of 3.90-3.94 % and 0.75- 0.80 % (Yang et al., 2010). Lee et al. (1990) also showed that *Lactobacillus bulgaricus* and *Streptococcus thermophilus* produce more acid (1.67 %) and lower pH (3.9) in cow milk yogurt than acid (1-1.19 %) and pH (4-4.2) in soy yogurt (Lee et al., 1990).

Syneresis

Syneresis is considered as a very important physical test for yogurt quality and is related to the instability of the yogurt gel network and the impossibility of trapping the serum phase in its gel network (Izadi et al., 2014). Syneresis in yogurt occurs due to compression of three-dimensional structure of the protein network that results in decreasing the protein binding power and exiting the water from yogurt. Adding the corn starch has a significant effect on reducing the syneresis of stirred soy yogurt because the hydrocolloids are able to establish the stronger bonds with free water molecules due to high molecular weight.

As shown in Figure 2, comparing the amount of syneresis of samples from the first day to the 14th day had a downward trend and after the 14th day to 28th day showed an increase. It can be stated that considering the hydrolysis and digestion of proteins in the product by microorganisms, the syneresis increases by increasing the storage time, because the proteins making a desirable texture lose their property and their bond with water is disintegrated. In general, the different results have been reported about yogurt syneresis during the storage period. For example, Tamjidi *et al.* (2012) observed that the separation of yogurt serum during storage had a decreasing trend from the first to fourteenth say, but at day 21 increased, and its difference was not significant at day 14 (P<0.05) (Tamjidi et al., 2012). The difference of results in the syneresis process can be due to the different

methods used, the different capabilities of yogurt starter strains in producing the secreted exopolysaccharides and differences in milk composition used.

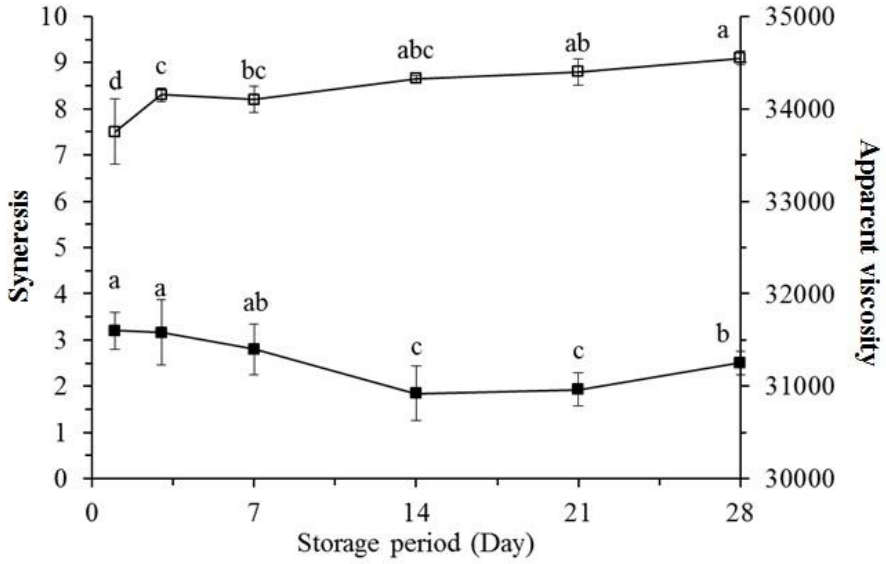

Figure 2. Effect of storage time on the syneresis (■) and apparent viscosity (□) of stirred soy yogurt

Apparent viscosity

According to Figure 2, the viscosity of the samples during 28 days of storage of stirred soy yogurt showed a steadily increasing trend. The highest viscosity was also reported for after 28 days of storage. This increase in viscosity during storage may be due to changes in protein-protein binding in a three-dimensional protein network of yogurt and their rearrangement (Sahan et al., 2008). In addition, the interactions of soy protein and starch can increase the viscosity of the product. The more time passes from the storage, the more interactions of dry matters with each other and also with water increase that finally lead to increase the volume of dry matter molecules and increase the viscosity of samples. Abu-Jdayil and Mohameed (2002) reported an increase in the apparent viscosity of concentrated yogurt during storage that its reason was reported the development of gel structure during storage (Abu-Jdayil and Mohameed, 2002). During storage for 8 days at 4°C, no significant changes in the viscosity of the soy yogurt samples were observed by Lim (Lim, 2013). Celik *et al.* (2006) was reported that the viscosity of the fruit-flavored yogurt (by adding cornelian cherry paste and sugar at different ratios) increase rapidly up to day 7, and continued to increase slowly up to day 14 of storage and afterward decreased slowly (Celik et al., 2006).

Textural characteristics

The texture of a food is feeling and understanding of human from the rheological behavior of the food that is derived from its structural elements (Domagala et al., 2005). Texture is one of the main characteristics that expresses the quality of yogurt and is effective on appearance, mouth feel and overall acceptance (Ares et al., 2007). According to Table 1, mean comparison of textural parameters of stirred soy yogurt in the first day with other days (during 28 days of storage) showed that had a significant increase ($P<0.05$) but no significant difference was observed between other days ($P>0.05$). The increasing trend during storage is due to changes in arrangement and the binding of proteins with each other and corresponds with the increase in viscosity of stirred soy yogurt. Regarding the adhesiveness of stirred soy yogurt samples, it can be stated that given that the adhesiveness force is the force required to overcome the surface adsorbent force among particles, so the more gel structure and protein network of yogurt have hardness, the more adhesiveness force increases. This result is similar to previous study about increasing textural properties of whole and skimmed flavored set-type yogurt during long storage (Salvador et al., 2004). But, Domagala *et al.* (2005) reported that the hardness of yogurts (with the addition of oat-maltodextrin as the fat substitute) increased till the 14th day of storage, and next decreased (21 days) (Domagala et al., 2005).

Table 1. Effect of storage time on textural parameters of stirred soy yogurt

Storage time (Day)	Textural properties			
	Hardness (g)	Apparent modulus (g/sec)	Adhesiveness (g.sec)	Work done to hardness (g.sec)
1	331.50±0.33^{b}	53.61±0.05^{b}	2726.17±2.73^{b}	5623.86±5.62^{b}
3	364.65±7.29^{a}	58.97±1.18^{a}	2998.79±59.98^{a}	6186.25±123.73^{a}
7	371.28±18.56^{a}	60.04±3.00^{a}	3053.31±152.66^{a}	6298.72±314.93^{a}
14	374.59±3.75^{a}	60.58±0.61^{a}	3080.57±30.81^{a}	6354.96±63.55^{a}
21	376.25±37.62^{a}	60.85±6.08^{a}	3094.203±309.42^{a}	6383.08±638.31^{a}
28	377.91±0.38^{a}	61.11±0.06^{a}	3107.83±3.11^{a}	6411.20±6.41^{a}

- The results have been reported as the mean of two replications ± standard deviation.
- Non-common letters in each column indicate the significant differences at the level of P<0.05.

Colorimetric test

Comparing the colorimetric parameters of stirred soy yogurt is shown in Table 2. The results showed that the factors a* and b* have no significant difference with each other during 28-day storage period (P<0.05). But there was no clear trend regarding the brightness of samples, and the highest brightness was reported after 14 days. Generally, the brightness of stirred soy yogurt is much less than the brightness of conventional stirred yogurt that it may be due to the properties of scattering the light of coagulated casein micelles compared to soy globulin proteins (Lee et al., 1990).

Table 2. Colorimetric parameters (L*, a*, b*) of stirred soy yogurt during storage period

Storage time (day)	Colorimetric parameters		
	L*	a*	b*
1	65.25±0.21^{c}	-3.95±0.50^{a}	16.05±0.78^{a}
3	65.80±0.00^{ab}	-4.15±0.50^{a}	16.25±0.35^{a}
7	65.25±0.07^{c}	-4.00±0.71^{a}	15.95±0.07^{a}
14	65.95±0.07^{a}	-3.95±0.71^{a}	16.35±0.07^{a}
21	65.30±0.28^{bc}	-3.65±0.71^{a}	15.85±0.07^{a}
28	65.30±0.28^{bc}	-3.90±0.14^{a}	16.20±0.28^{a}

- The results have been reported as the mean of two replications ± standard deviation.
- Non-common letters in each column indicate the significant differences at the level of P<0.05.

Microbial analysis

Based on Figure 3, the results showed that the number of Coliforms, molds and yeasts had a continuous decreasing trend from the first to 28 day that the decrease in the microbial population during storage can express the unfavorable conditions for microbial growth. In this regard, Jimoh and Kolapo (2007) reported that two days after the production of soy yogurt, bacteria and yeast count showed an upward trend and after this time to 16th day had a downward trend (Jimoh and Kolapo, 2007).

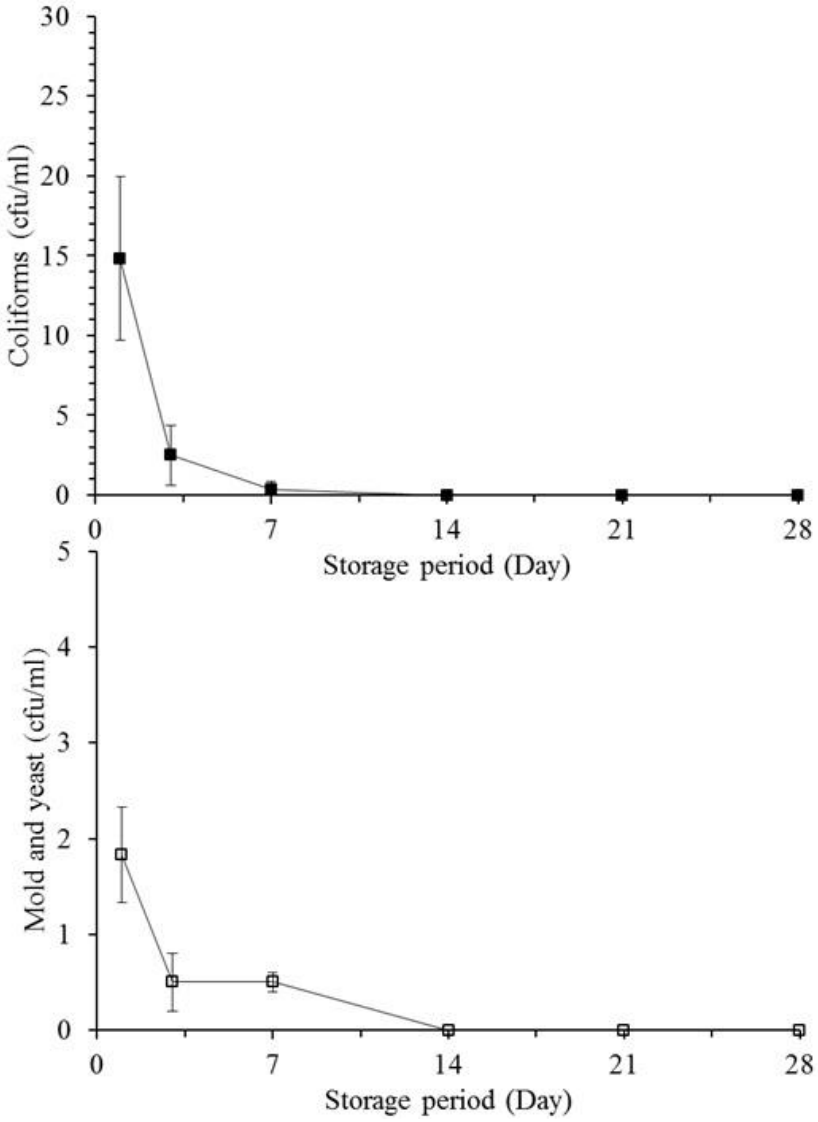

Figure 3. The effect of storage time on the number of Coliforms (■), Molds and yeasts (□) of stirred soy yogurt

Sensory evaluation

Average sensory scores of panelists are shown in Table 3. Panelists did not observe a difference between the texture of samples during the storage period on days 1, 3, 7, 14 and 21 but on 28th day the stirred soy yogurt obtained a lower textural score that was significant compared to previous before days ($P<0.05$). Statistical analysis of taste and flavor of stirred soy yogurt showed that the score of panelists steadily decreased during 28-day storage period and on the first day, stirred soy yogurt has had better taste and flavor and has gotten the highest score. The score of panelists about the mouth feel of the samples showed no clear trend. Totally, based on the scores of panelists, overall acceptance of samples decreased during storage period that probably the production of compounds obtained from the fermentation of soy milk can lead to decrease the average score. A suitable solution to this problem is adding a small amount of sugar or flavoring ingredients to the yogurt. To improve the taste of soy yogurt the fruit extractions with certain tastes and flavors can be used that somewhat leads to decrease the undesirable taste of soy (beany flavor). In a study on qualitative evaluation and acceptance of soy yogurt with different fruit flavors and colors, Osundahunsi *et al*. (2007) reported that strawberry flavor showed the highest score and orange flavor had the lowest score (Osundahunsi et al., 2007). As seen, during 28-day storage period of stirred soy yogurt, a significant difference was observed in the final days ($P<0.05$).

Table 3. Sensory evaluation of stirred soy yogurt during storage period

Storage time (day)	Characteristics of sensory evaluation			
	Texture	Taste	Mouth feel	Overall acceptance
1	4.21^{a}±0.20	6.60^{a}±0.05	5.20^{c}±0.20	5.22^{a}±0.15
3	6.36^{a}±0.10	5.20^{b}±0.10	5.18^{c}±0.08	4.86^{bc}±0.01
7	4.50^{a}±0.30	4.70^{c}±0.20	6.10^{a}±0.05	5.04^{ab}±0.19
14	4.70^{a}±0.05	4.50^{c}±0.30	5.50^{b}±0.30	4.88^{bc}±0.20
21	4.61^{a}±0.00	4.00^{d}±0.05	$5.30b^{c}$±0.10	4.63^{c}±0.01
28	3.14^{b}±0.61	3.71^{d}±0.10	4.35^{d}±0.15	3.67^{d}±0.31

- The average numbers are obtained from the scores of 20 panelists ± standard deviation.
- Non-common small letters in each column indicate a significant difference at the level of P<0.05.

Conclusion

The increased need for protein consumption in developed countries has led to attempts to find an alternative resource of protein by cereal seeds. On the other hand, the prevalence of cardiovascular disease and lactose intolerance, also helped to advance this issue. The study was conducted with the aim of producing stirred soy yogurt. Its physicochemical, textural, microbial and sensory characteristics during storage period (28 days) were studied. Based on the above results, the production of stirred soy yogurt can be more studied as a new product of soy milk, but at first for more success in the production of this product and its marketing, it seems necessary to improve the sensory characteristics of the product.

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