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Evaluation of shelf stability of antioxidant rich seabuckthorn fruit yoghurt

*Selvamuthukumaran, M. and Farhath, K.

BN Division, DFRL, Mysore, India

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Abstract

Antioxidant rich fruit yoghurt was developed utilizing seabuckthorn fruit syrup using yoghurt culture. The resultant product had higher content of fat, protein, carbohydrate and antioxidants (vitamin C, vitamin E, carotenoids, phenols, anthocyanins) when compared to a commercial yoghurt. The shelf stability studies of yoghurt were assessed at room temperature, 15°C and 4°C. The product stored at room temperature exhibited off flavor and acidic taste within a day of storage, while the product stored at 4°C and 15° C exhibited significant increase in acidity and syneresis during 9^{nth} and 6th day of storage period respectively. The total solids, pH and viscosity significantly decreased on 18^{nth}, 9^{nth} and 6th day of storage at 4°C and for 15˚C it was on 9^{nth}, 6th and 3rd day of storage respectively. No remarkable change was noted for fat, protein, carbohydrate and antioxidants during storage. The sensory quality of product drastically reduced after 18 days at 4°C and 9 days at 15°C. The count of both yoghurt cultures viz. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* significantly decreased during the entire storage period. The yeast and mould count was absent in fresh samples, but were detected on 21st day at 4˚C and 6th day at 15˚C. Likewise, the coliform count was initially absent but was detected on 15th and 6th day when stored at 4 and 15˚C temperature respectively. The product was found to be acceptable up to a period of 12 days at 4˚C and 3 days at 15˚C without posing any threat to the consumers.

Introduction

Fermented food product like fruit yoghurt is being manufactured in various countries and they possess significant nutritional as well as therapeutic value (Rasic *et al.*, 1971). These products occupy a prominent position in diet, mainly due to presence of high quality protein, easily digestible fat and an abundance of bio-available calcium and phosphorus. Plain yoghurt has been available in the world market since long, but currently there is a good demand for fruit yoghurt (Gallaghar *et al*., 1974).

Seabuckthorn (*Hippophae rhamnoides*), is a deciduous shrub with yellow or orange fruits (Li and Schroeder, 1996). Such shrub is being domesticated in several countries like China, Russia, Germany, Finland, Romania, France, Nepal, Pakistan and India. The fruit is reported to have considerable medicinal value (Li and Wang, 1998), which aids in treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers and also cardiovascular diseases. Seabuckthorn berries are among the most nutritious and vitamin-rich fruit owing to presence of natural antioxidants viz., vitamin C, E, carotenoids, anthocyanins and phenols (Chauhan *et al.*, 2001). Therefore supplementation of yoghurt with seabuckthorn fruit can enhance its nutritional quality and provide therapeutic value too.

Shelf life plays a crucial role in marketing and sales of perishable or semi-perishable food products. Factors like temperature and time play a significant role in deciding the stability of food product. The aim of the current study was to develop an antioxidant rich 'functional' fruit yoghurt utilizing seabuckthorn fruit pulp and evaluate its shelf stability.

Materials and Methods

Raw materials

Pure freeze dried culture of *S. thermophilus* (NCDC 075) and *L. bulgaricus* (NCDC 008) were procured from National Dairy Research Institute, Karnal, India. These cultures were maintained in reconstituted sterilized skim milk (10% TS). Standardized cow milk (fat 4.5% & SNF 8.5%), skimmed milk powder, fresh pineapple and grape yoghurt samples of Nandini brand was procured locally from Mysore Cooperative Dairy, Karnataka, India. Gelatin (Food grade) was purchased from local market. Seabuckthorn (*Hippophae rhamnoides* L.var. rhamnoides) berries were brought from Field Research Laboratory, Leh, India.

Development of seabuckthorn fruit yoghurt (SFY)

Seabuckthorn fruit yoghurt was prepared using the method of De (1960). The standardized cow milk

*Corresponding author.
Email: *selkuma@hotmail.com*
Tel: 0821 2473290; Fax: 0821 2473468

was blended with SMP (12 g/100 g of milk) and gelatin (0.5 g/100 g of yoghurt base mix, inclusive of SMP), pasteurized at 85˚C for 30 min and cooled to 37˚C. The fruit syrup (TSS of 70˚ Brix) was prepared from seabuckthorn berries by crushing them in a laboratory type blender (Sahyog Enterprise, India) for pulp extraction, straining, adjusting the pulp pH to 5.4 from an initial pulp pH of 2.90 (with food grade 2% sodium bicarbonate). The fruit pulp (50 g) was blended with required quantity of sugar syrup (50 g sugar dissolved in 12.5 ml water for 100 g fruit syrup and finally boiling the content to attain 70˚ Brix). The prepared fruit syrup was added at the rate of 15% to milk and mixed thoroughly with high speed laboratory type stirrer (Sahyog Enterprise, India). The blended mix was inoculated with 2% (v/v, S.thermophilus and L.bulgaricus, 1:1 ratio) of 24 h old culture and incubated at 37 ± 2˚C until pH of 4.4 was obtained. Fruit yoghurt was cooled to 2-4˚C in refrigerator and utilized for further studies.

Chemical analysis

The pH scan 2 (Elico Instruments Private Limited, India) was used to measure the final pH of yoghurt samples at room temperature (23±4˚C). The product was subjected to chemical analyses viz., total solids, fat and acidity (ISI, 1960); protein (Sathe, 1999); ash, fiber (AOAC, 1984) and carbohydrate (calculated based on difference). Viscosity was determined (at 4 and 15˚C) using a RV Brookfield Viscometer (Brookfield Engineering Laboratories, Massachusetts, USA). Vitamin C was determined through titration method using 2,6-dichlorophenol-indophenol (Ranganna, 1986); vitamin E was estimated spectrophotometrically at 536 nm using ferric chloride reagent (UV-Visible Spectrophotometer 1601, Shimadzu, Columbia, MD, USA) (Desai, 1984). Total carotenoids (Arya *et al*., 1979); anthocyanins (Clydesdale and Francis, 1976) and phenols (Shikanga *et al*., 2010) were determined spectrophotometrically at 450, 535 and 765 nm, respectively. Antioxidant activity was estimated spectrophotometrically by (1,1-Diphenyl-2-picrylhydrazyl) DPPH method (Singh *et al*., 2008). Syneresis of sample was determined according to the procedure of Rodarte *et al.* (1993).

Microbial analysis

The microbial load in the fruit yoghurt samples were analyzed initially and during storage up to 21 days, at an interval of 3 days. IDF (1988) method was used for enumeration of *S. thermophilus* and *L. bulgaricus*. The coliform, yeasts and mould counts were enumerated by serial dilution as per the method of Harrigan and Mccance (1976).

Sensory evaluation of yoghurt

A panel comprising of 15 trained judges, aged 30-55 years evaluated the fruit yoghurt samples for their sensory quality. The coded (3 digit) samples were presented one at a time in random order to the judges. The judges evaluated the product for colour, taste, aroma, body and texture and overall acceptability. A 9-point hedonic scale was used for scoring the product (Swaminathan, 1995).

Statistical analysis

All experiments were replicated three times. Data were subjected to analysis of variance (ANOVA) using Microsoft Excel. The paired comparisons of means were performed using Duncan's test (Steel and Torrie, 1980).

Results and Discussion

Physico-chemical characteristics of seabuckthorn fruit and pulp

The physico-chemical characteristics of seabuckthorn fruit and pulp are shown in Table 1. The colour of the seabuckthorn fruit was orange-yellow, oval shaped, 5-7 mm in size and had a sour taste. The fruit had good nutritional profile in terms of carbohydrate, fat, crude fibre, protein and minerals. Katiyar *et al.* (1990) also reported similar proximate results while analyzing seabuckthorn fruit berries from Himalayan region of India. The chemical characteristics of seabuckthorn fruit pulp revealed that the pulp had less amount of total sugar (6.45%), had low pH (2.90) and high acidity (1.84%) when compared to pulp obtained from grape fruit berry viz. 13.97%, 3.75 and 1.28% with respect to total sugar, pH and acidity (Selvamuthukumaran and Farhath, 2014). It also contained appreciable amount of antioxidants viz., vitamin C, vitamin E, carotenoids, anthocyanins and phenols which exerts beneficial antioxidant activity. Among the antioxidants present, the concentration of total phenols, vitamin C and vitamin E was higher than the rest. Chauhan *et al.* (2001) were also reported similar results of high antioxidants viz. vitamin C, phenols and carotenoids while analyzing pulp of seabuckthorn fruit berry.

Nutritional properties of SFY

The nutritional profile of SFY samples in comparison with commercially available fruit (pineapple) yoghurt is shown in Table 2. The result shows that the experimental fruit yoghurt had higher content of fat, protein, carbohydrate, and

Table 1. Physico-chemical characteristics of seabuckthorn fruit pulp**

Fruit composition	Content	Pulp parameters	Content
Moisture (g/100 g)	53.05 ± 0.03	Total soluble solids (°Brix)	12.00 ± 0.28
		Acidity*** (%)	1.84 ± 0.03
Protein (g/100 g)	2.21 ± 0.37	Total sugars (%)	6.45 ± 0.59
Crude fiber (g/100 g)	4.71 ± 0.25	Reducing sugars (%)	0.85 ± 0.40
Total carbohydrate* (g/100 g)	29.08 ± 0.46	pH	2.90 ± 0.01
Total fat (g/100 g)	9.20 ± 0.22	Vitamin C (mg/100 g)	504.00 ± 0.38
Ash (g/100 g)	1.75±0.05		
Sodium (mg/100 g)	67.00 ± 0.47	Vitamin E (mg/100 g)	190.54 ± 0.86
Potassium (mg/100 g)	625.00 ± 0.86	Total carotenoids (mg/100 g)	6.85 ± 0.74
Calcium (mg/100 g)	667.00 ± 0.39	Total anthocyanins (mg/100 g)	1.48 ± 0.59
Iron (mg/100 g)	17.00 ± 0.54	Total phenols (mg/100 g)	560.00 ± 0.91
Phosphorus (mg/100 g)	72.00 ± 0.77	Antioxidant activity (%)	89.23 ± 0.49

*Total carbohydrate by difference
**Values are mean ± standard deviation of triplicate analysis
***Acidity expressed as % in terms of gm citric acid/100 g

Table 2. Comparison of nutritional quality of Seabuckthorn fruit yoghurt with commercial fruit yoghurts

Parameters	Seabuckthorn yoghurt	Pineapple yoghurt	Grape yoghurt
Total solids (%)	28.9 ± 0.22 a	26.7 ± 0.34 b	27.5 ± 0.21 ab
Acidity (%)	0.86 ± 0.02a	0.88 ± 0.05 a	0.87 ± 0.06 a
Fat (%)	5.4 ± 0.82 a	5.1 ± 0.74 a	5.2 ± 0.69 a
Protein (%)	7.9 ± 0.40 a	7.1 ± 0.53 a	7.2 ± 0.66 a
Carbohydrates (%)	14.5 ± 0.51 a	13.3 ± 0.49 a	14.1 ± 0.59 a
Ash (%)	0.86 ± 0.03 a	0.80 ± 0.06 a	0.82 ± 0.04 a
Vitamin C (mg/100 g)	20.8 ± 0.62 a	0.70 ± 0.21 b	0.61 ± 0.32 b
Vitamin E (mg/100 g)	12.0 ± 1.84 a	ND	3.7 ± 0.96 b
Total carotenoids (mg/100 g)	1.4 ± 0.62 a	0.06 ± 0.02 b	0.04 ± 0.01 b
Total anthocyanins (mg/100 g)	0.05 ± 0.01 a	0 b	0 b
Total phenols (mg/100 g)	24.4 ± 1.87 a	2.2 ± 0.90 b	2.6 ± 0.71 b

Mean values in the same row bearing a common superscript do not differ significantly ($p > 0.05$)
ND: Not detected
Acidity expressed as % in terms of gm citric acid/100 g

antioxidants (vitamin C, vitamin E, carotenoids, phenols, anthocyanins) when compared to commercial fruit yoghurt sample. This may be due to presence of comparatively more amounts of fat, protein, carbohydrate and antioxidant compounds in seabuckthorn fruit. The vitamin E could not be detected in commercial fruit yoghurt sample. Therefore this type of fruit yoghurt can be recommended as a nutritive antioxidant rich fermented food product. Similar antioxidant rich nutritive fruit yoghurt was prepared incorporating fruit juice of acai i.e. *Euterpe oleracea* (Coisson *et al.*, 2005).

Storage stability of fruit yoghurt

The seabuckthorn fruit yoghurt was contained in sanitized 100 ml polyethylene cups and stored at 4°C (refrigerator), 15°C (incubator) and 23 ± 4°C (room temperature) in order to evaluate its shelf stability.

Physicochemical properties of fruit yoghurt

Room temperature storage

The fruit yoghurt stored at room temperature (23 ± 4ºC) exhibited marked deteriorative changes within a day through development of off-flavor and acidic taste, crumbly appearance with lack of smoothness and glossiness (especially due to syneresis), accumulation of gas-holes. Shukla *et al.* (1988) also observed similar result for mango fruit yoghurt stored at room temperature.

Storage at low temperatures

Chemical and nutritional qualities of yoghurt

The physico-chemical properties viz., total solids, acidity, pH, fat and protein content of stored seabuckthorn fruit yoghurt samples at 4 and 15˚C are furnished in Table 3. The result shows that the total solids (TS) of fruit yoghurt decreased to 28.4 and 27.7% at 21 days of storage, when stored at 4 and 15ºC respectively, from an initial value of 28.9% TS. The significant decrease ($p < 0.05$) in total solids could be due to syneresis i.e. oozing out of whey which contains whey proteins, lactose and minerals. Yeom *et al.* (2004) also reported a decreasing trend in TS of strawberry flavored yoghurt during stored at 4 and 22ºC for up to 90 days.

The initial acidity (lactic acid) of seabuckthorn fruit yoghurt was 0.86% which rose to 1.15 and 1.44% at 21 days, when stored at 4 and 15ºC respectively. The increase in acidity of fruit yoghurt is a normal phenomenon (Ozturk and Oner, 1999). The increase in acidity during storage at both temperatures was significant ($P < 0.05$). The pH of the fruit yoghurt was 4.42 when fresh, which decreased significantly ($p < 0.05$) to 4.08 and 3.74 at 21 days, when stored at 4 and 15˚C respectively. Such observation is in agreement with the results of Ozturk and Oner (1999), who noticed an increase in acidity and decrease in pH of grape flavoured fruit yoghurt during storage at 4ºC.

The fat content of the fruit yoghurt was 5.40% when fresh which slightly decreased to 5.15 and 5.05% at 21st day, when stored at 4 and 15˚C respectively. The protein content of fruit yoghurt was 7.90% when fresh, which decreased to 7.34 and 7.20% at 21st day when stored at 4 and 15˚C respectively. Such decrease in the fat and protein content of the stored product was non significant. The total carbohydrate content of fresh fruit yoghurt was 14.53% (Table 4), which decreased to 14.26 and 14.05% at 21st day of storage at 4 and 15˚C respectively; the change in carbohydrate content was non-significant. Formisano *et al.* (1974) reported similar result with regard to change in fat, protein and carbohydrate content of yoghurt during its storage at 4ºC.

The vitamin C content of fresh fruit yoghurt was 20.8 mg/100 g (Table 4), which remained so up to 21 days of refrigerated (4˚C) storage. The fruit yoghurt stored at 15˚C exhibited a marginal loss of about 3% i.e. its content was 20.1 mg/100 g at 21st day of storage; such change was found to be non significant. Similar trend was also observed for vitamin E; the

Table 3. Physicochemical characteristics of seabuckthorn fruit yoghurt viz., total solids, acidity, pH, fat and protein as affected by storage

Storage period (days)	Total solids (%)		Acidity (%)		pH		Fat (%)		Protein (%)	
	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C
0	28.9 ± 0.2 a	28.9 ± 0.2 a	0.86± 0.02 a	0.86 ± 0.02 a	4.42 ± 0.00 a	4.42 ± 0.00 a	5.40 ± 0.82 a	5.40 ± 0.82 a	7.90 ± 0.40 a	7.90 ± 0.40 a
3	28.9 ± 0.2 a	28.8 ± 0.4 a	0.90 ± 0.01 a	0.94 ± 0.03 a	4.37 ± 0.02 a	4.32 ± 0.04 a	5.36 ± 0.56 a	5.35 ± 0.45 a	7.82 ± 0.57 a	7.80 ± 0.76 a
6	28.8 ± 0.4 a	28.6 ± 0.4 a	0.94 ± 0.03 a	.02 ± 0.05 ba	4.32 ±0.04 a	4.22 ± 0.01 ba	5.32 ± 0.43 a	5.30 ± 0.56 a	7.74 ± 0.49 a	7.70 ± 0.58 a
9	28.7 ± 0.3 a	28.4 ± 0.2 ba	0.98 ± 0.04 ba	1.10 ± 0.07 ba	4.27 ± 0.03 ba	4.12 ± 0.06 ba	5.29 ± 0.77 a	5.25 ± 0.63 a	7.66 ± 0.45 a	7.60 ± 0.43 a
12	28.6 ± 0.4 a	28.3 ± 0.3 ba	1.02 ± 0.05 ba	1.19 ± 0.09 bc	4.22 ± 0.01 ba	4.03 ± 0.05 bca	5.25 ± 0.63 a	5.20 ± 0.69 a	7.58 ± 0.48 a	7.50 ± 0.45 a
15	28.6 ± 0.4 a	28.1 ± 0.2 ba	1.06 ± 0.06 ba	1.27 ± 0.08 bc	4.17 ± 0.05 ba	3.93 ± 0.07 bc	5.22 ± 0.66 a	5.15 ± 0.58 a	7.50 ± 0.45 a	7.40 ± 0.70 a
18	28.5 ± 0.3 ba	27.9 ± 0.3 bc	1.10 ± 0.07 cba	1.35 ± 0.06 bcd	4.12 ± 0.06 bca	3.83 ± 0.08 bcd	5.18 ± 0.49 a	5.10 ± 0.57 a	7.42 ± 0.85 a	7.30 ± 0.53 a
21	28.4 ± 0.2 ba	27.7 ± 0.2 bc	1.15 ± 0.08 cba	1.44 ± 0.04 bcd	4.08 ± 0.07 bca	3.74 ± 0.03 bcd	5.15 ± 0.58 a	5.05 ± 0.40 a	7.34 ± 0.43 a	7.20 ± 0.49 a

Mean values in the same column bearing a common superscript do not differ significantly ($p > 0.05$)
Values are Mean ± SD of triplicate analysis

Table 4. Physicochemical characteristics of seabuckthorn fruit yoghurt viz., carbohydrates and antioxidants as affected by storage

Storage period (days)	Carbohydrates (%)		Vitamin C (mg /100 g)		Vitamin E (mg / 100 g)		Total carotenoids (mg /100 g)		Total anthocyanins (mg / 100 g)	
	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C
0	14.53 ± 0.51 a	14.53 ± 0.51 a	20.8 ± 0.6 a	20.8 ± 0.6 a	12.0 ± 1.8 a	12.0 ± 1.8 a	1.46 ± 0.62 a	1.46 ± 0.62 a	0.05 ± 0.01 a	0.05 ± 0.01 a
3	14.49 ± 0.68 a	14.46 ± 0.49 a	20.8 ± 0.6 a	20.8 ± 0.6 a	12.0 ± 1.8 a	12.0 ± 1.8 a	1.46 ± 0.62 a	1.46 ± 0.62 a	0.05 ± 0.01 a	0.05 ± 0.01 a
6	14.46 ± 0.49 a	14.39 ± 0.50 a	20.8 ± 0.6 a	20.8 ± 0.6 a	12.0 ± 1.8 a	12.0 ± 1.8 a	1.46 ± 0.62 a	1.46 ± 0.62 a	0.05 ± 0.01 a	0.05 ± 0.01 a
9	14.42 ± 0.61 a	14.33 ± 0.55 a	20.8 ± 0.6 a	20.8 ± 0.6 a	12.0 ± 1.8 a	12.0 ± 1.8 a	1.46 ± 0.62 a	1.46 ± 0.62 a	0.05 ± 0.01 a	0.05 ± 0.01 a
12	14.38 ± 0.60 a	14.26 ± 0.41 a	20.8 ± 0.6 a	20.6 ± 0.4 a	12.0 ± 1.8 a	12.0 ± 1.8 a	1.46 ± 0.62 a	1.44 ± 0.54 a	0.05 ± 0.01 a	0.04 ± 0.02 a
15	14.34 ± 0.79 a	14.19 ± 0.63 a	20.8 ± 0.6 a	20.4 ± 0.5 a	12.0 ± 1.8 a	11.8 ± 1.9 a	1.46 ± 0.62 a	1.43 ± 0.51 a	0.05 ± 0.01 a	0.04 ± 0.02 a
18	14.30 ± 0.58 a	14.12 ± 0.67 a	20.8 ± 0.6 a	20.3 ± 0.2 a	12.0 ± 1.8 a	11.7 ± 2.0 a	1.46 ± 0.62 a	1.43 ± 0.51 a	0.05 ± 0.01 a	0.04 ± 0.02 a
21	14.26 ± 0.41 a	14.05 ± 0.82 a	20.8 ± 0.6 a	20.1 ± 0.3 a	12.0 ± 1.8 a	11.7 ± 2.0 a	1.46 ± 0.62 a	1.41 ± 0.47 a	0.05 ± 0.01 a	0.04 ± 0.02 a

Mean values in the same column bearing a common superscript do not differ significantly ($p > 0.05$)
Values are Mean ± SD of triplicate analysis

initial value of 12.0 mg/100 g remained unchanged during storage at 4˚C, but exhibited 2% loss at 21st day (i.e. 11.7 mg of vitamin E/100 g) when stored at 15°C; the effect being non-significant (Table 4). The total carotenoids content of fresh fruit yoghurt was 1.46 mg /100 g (Table 4), which remained unchanged during storage at 4˚C, but exhibited a loss of 3% (content was 1.41 mg/100 g) at 21st day of storage. The observed change in carotenoid content was found to be non-significant. Frederiksen *et al.* (2003) also reported slight reduction in the carotenoid content of plain yoghurt during 35 days of storage at 4°C.

The non-significant change in anthocyanin and total phenol content of fruit yoghurt was similar to that of carotenoids. The anthocyanin and total phenol content of fresh sample was 0.05 mg/100 g (Table 4) and 24.4 mg/100 g (Table 5) respectively, which reduced by 2.5% and 2.8% at 21st day when stored at 15˚C. Storage of fruit yoghurt at 4°C did not lead to any change in anthocyanin or total phenol content.

Physical characteristics of fruit yoghurt

The viscosity of the fresh experimental fruit yoghurt was 7199.0 cP, which decreased significantly ($P < 0.05$) to 6800.7 and 6463.7 cP at 21st day, when stored at 4 and 15˚C respectively (Table 5). The decrease in viscosity of the fruit yoghurt may be due to reduction in water-binding capacity of proteins, possibly due to further decrease in the pH of product reaching towards isoelectric point of casein (Ozturk and Oner, 1999).

Table 5. Physicochemical characteristics of seabuckthorn fruit yoghurt viz., total phenols, viscosity and syneresis as affected by storage

Storage period (days)	Total phenols (mg /100 g)		Viscosity (cP)		Syneresis (%)	
	4°C	15°C	4°C	15°C	4°C	15°C
0	24.4 ± 1.8 a	24.4 ± 1.8 a	7199.0 ± 3.1 a	7199.0 ± 3.1 a	33.0 ± 0.9 a	33.0 ± 0.9 a
3	24.4 ± 1.8 a	24.4 ± 1.8 a	7142.1 ± 1.9 a	7093.9 ± 2.7 b	34.2 ± 0.4 a	35.6 ± 0.7 b
6	24.4 ± 1.8 a	24.4 ± 1.8 a	7085.2 ± 1.8 ba	6988.9 ± 1.6 c	35.4 ± 0.6 ba	38.3 ± 0.8 c
9	24.4 ± 1.8 a	24.4 ± 1.8 a	7028.3 ± 2.5 ba	6883.9 ± 3.4 d	36.6 ± 0.7 b	40.9 ± 0.5 d
12	24.4 ± 1.8 a	24.2 ± 1.6 a	6971.4 ± 2.0 ba	6778.8 ± 2.3 e	37.8 ± 0.8 bc	43.6 ± 0.6 e
15	24.4 ± 1.8 a	24.0 ± 1.7 a	6914.5 ± 2.2 ba	6673.8 ± 3.9 f	39.0 ± 0.5 bc	46.3 ± 0.4 f
18	24.4 ± 1.8 a	24.0 ± 1.7 a	6857.6 ± 1.7 bca	6568.8 ± 3.6 g	40.2 ± 0.4 bcd	48.9 ± 0.5 g
21	24.4 ± 1.8 a	23.7 ± 1.9 a	6800.7 ± 2.4 bca	6463.7 ± 3.0 h	41.4 ± 0.6 bcd	51.6 ± 0.7 h

Mean values in the same column bearing a common superscript do not differ significantly ($p > 0.05$)
Values are Mean ± SD of triplicate analysis
Viscosity were measured at 4 and 15°C

The syneresis of fresh fruit yoghurt was 33.0%, however on storage for 21 days at 4 and 15˚C the syneresis rose to 41.4 and 51.6% respectively (Table 5). The increase in syneresis of fruit yoghurt may be due to continued increase in the acidity of product during storage. Such change in the syneresis of stored yoghurt was found to be statistically ($P < 0.05$) significant. Ozturk and Oner (1999) has also reported a decrease in viscosity and an increase in syneresis of grape yoghurt during its storage at 4°C.

Table 6. Sensory quality of stored seabuckthorn fruit yoghurt

Storage period (days)	Colour		Aroma		Taste		Body and Texture		Overall acceptability	
	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C
0	8.0 ± 0.2 ^{a}	8.0 ± 0.2 ^{a}	8.0 ± 0.3 ^{a}	8.0 ± 0.3 ^{a}	8.1 ± 0.6 ^{a}	8.1 ± 0.6 ^{a}	8.1 ± 0.3 ^{a}	8.1 ± 0.3 ^{a}	8.0 ± 0.3 ^{a}	8.0 ± 0.3 ^{a}
3	7.7 ± 0.5 ^{a}	7.3 ± 0.5 ^{a}	7.7 ± 0.5 ^{a}	7.3 ± 0.4 ^{a}	7.8 ± 0.3 ^{a}	7.4 ± 0.5 ^{a}	7.8 ± 0.8 ^{a}	7.4 ± 0.5 ^{a}	7.7 ± 0.5 ^{a}	7.3 ± 0.4 ^{a}
6	7.4 ± 0.4 ^{a}	6.5 ± 0.9 ^{ba}	7.5 ± 0.6 ^{a}	6.5 ± 0.5 ^{ba}	7.5 ± 0.4 ^{a}	6.6 ± 0.7 ^{ba}	7.4 ± 0.5 ^{a}	6.6 ± 0.4 ^{ba}	7.4 ± 0.4 ^{a}	6.5 ± 0.5 ^{ba}
9	7.0 ± 0.6 ^{ba}	6.0 ± 0.6 ^{ba}	7.1 ± 0.4 ^{a}	6.0 ± 0.7 ^{ba}	7.2 ± 0.5 ^{a}	6.0 ± 0.6 ^{ba}	7.1 ± 0.3 ^{ba}	6.0 ± 0.6 ^{ba}	7.1 ± 0.8 ^{a}	6.0 ± 0.7 ^{ba}
12	6.7 ± 0.7 ^{ba}	5.7 ± 0.4 ^{ba}	6.8 ± 0.2 ^{ba}	5.6 ± 0.6 ^{ba}	6.8 ± 0.4 ^{ba}	5.6 ± 0.2 ^{bca}	6.8 ± 0.7 ^{ba}	5.6 ± 0.3 ^{bca}	6.7 ± 0.5 ^{ba}	5.6 ± 0.3 ^{bca}
15	6.4 ± 0.5 ^{ba}	NA	6.5 ± 0.5 ^{ba}	NA	6.3 ± 0.7 ^{ba}	NA	6.5 ± 0.4 ^{ba}	NA	6.4 ± 0.7 ^{ba}	NA
18	6.1 ± 0.3 ^{ba}	NA	6.2 ± 0.8 ^{ba}	NA	6.0 ± 0.6 ^{ba}	NA	6.1 ± 0.6 ^{bca}	NA	6.1 ± 0.6 ^{ba}	NA
21	5.8 ± 0.8 ^{ba}	NA	5.6 ± 0.6 ^{bca}	NA	5.7 ± 0.5 ^{bca}	NA	5.6 ± 0.3 ^{bca}	NA	5.6 ± 0.3 ^{bca}	NA

Mean values in the same column bearing a common superscript do not differ significantly ($p > 0.05$)
Mean ± SD; n = 15
NA: Not acceptable; Sensory score are based on 9-point hedonic scale

Table 7. Microbial quality of stored seabuckthorn fruit yoghurt

Storage period (days)	*S.thermophilus* count x 10^8 cfu/g		*L. .bulgaricus* count x 10^8 cfu/g		Yeast & mould count x 10^1 cfu / g		Coliform count x 10^1 cfu / g	
	4° C	15° C	4° C	15° C	4° C	15° C	4° C	15° C
0	5.7 ± 0.3 ^{a}	5.7 ± 0.3 ^{a}	4.8 ± 0.6 ^{a}	4.8 ± 0.6 ^{a}	ND	ND	ND	ND
3	5.5 ± 0.5 ^{a}	5.2 ± 0.8 ^{a}	4.5 ± 0.7 ^{a}	4.4 ± 0.4 ^{a}	ND	ND	ND	ND
6	5.3 ± 0.4 ^{a}	4.8 ± 0.3 ^{ba}	4.3 ± 0.5 ^{a}	4.0 ± 0.5 ^{a}	ND	9.2 ± 0.7 ^{a}	ND	5.2 ± 0.6 ^{a}
9	5.1 ± 0.5 ^{a}	4.5 ± 0.6 ^{ba}	4.1 ± 0.2 ^{a}	3.6 ± 0.7 ^{ba}	ND	19.8 ± 0.9 ^{a}	ND	9.2 ± 0.3 ^{a}
12	4.8 ± 0.3 ^{ba}	4.1 ± 0.7 ^{ba}	3.9 ± 0.3 ^{ba}	3.2 ± 0.6 ^{ba}	ND	60.2 ± 0.6 ^{b}	ND	12.6 ± 0.4 ^{ba}
15	4.7 ± 0.7 ^{ba}	3.9 ± 0.5 ^{bca}	3.7 ± 0.4 ^{ba}	2.8 ± 0.3 ^{ba}	ND	99.1 ± 0.3 ^{c}	2.3 ± 0.6 ^{a}	14.8 ± 0.8 ^{ba}
18	4.6 ± 0.3 ^{ba}	3.7 ± 0.9 ^{bca}	3.5 ± 0.5 ^{ba}	2.4 ± 0.9 ^{bca}	ND	146.7 ± 0.5 ^{d}	5.1 ± 0.5 ^{a}	17.7 ± 0.5 ^{ba}
21	4.5 ± 0.6 ^{ba}	3.6 ± 0.4 ^{bca}	3.3 ± 0.8 ^{ba}	2.1 ± 0.2 ^{bca}	4.6 ± 0.4	188.1 ± 0.4 ^{e}	11.0 ± 0.4 ^{ba}	19.3 ± 0.7 ^{bca}

Mean values in the same column bearing a common superscript do not differ significantly ($p > 0.05$)
Values are Mean ± SD of triplicate analysis; ND – Not detected

Sensory evaluation of fresh and stored fruit yoghurt

The fresh fruit yoghurt had an overall acceptability score of 8.0 based on nine-point hedonic scale. A score of 6.1 was taken as the lowest limit implying end of shelf life of product. The fruit yoghurt remained sensorily acceptable up to 18 days at 4˚C and just 9 days when stored at 15˚C (Table 6). The increased acidity of yoghurt samples stored at higher temperature and the consequent development of off-flavour such as alcoholic accompanied by syneresis led to the product being rated unacceptable. The yoghurt sample stored at 15˚C was unacceptable on 12th day itself. Such decrease in the score for colour, aroma, taste, body and texture and overall acceptability of yoghurt with progress of storage was found to be statistically significant ($p < 0.05$). Tarakci and Kucukoner (2004) also observed reduction in the sensory scores of yoghurt samples made using morello cherry during refrigerated storage.

Microbiological evaluation of fresh and stored fruit yoghurt

The microbial population of fresh and stored fruit yoghurt at 4 and 15˚C are provided in Table 7. The freshly prepared fruit yoghurt had 5.7 x 10^8 cfu/g of *S. thermophilus*, which decreased on 21st day to 4.5 x 10^8 and 3.6 x 10^8 cfu/g when stored at 4 and 15˚C respectively. The *L. bulgaricus* count of the yoghurt sample also decreased from initial count of 4.8 x 10^8 cfu/g to 3.3 x 10^8 and 2.1 x 10^8 cfu/g respectively on 21st day in product stored at 4 and 15˚C. Such decrease in the count of *S. thermophilus* and *L. bulgaricus* of the stored product was found to be significant ($P < 0.05$). The decrease in count of *S. thermophilus* and *L. bulgaricus* bacteria may be due to the accumulation of lactic acid in product during storage at above conditions. The decrease in the count of lactic acid bacteria viz., *S. thermophilus* and *L. bulgaricus* during refrigerated storage in strawberry and cherry Swiss style low fat yoghurt and also in mango and grape fruit yoghurts has been reported (Keating and White, 1990; Shukla *et al.*, 1988; Ozturk and Oner, 1999).

The fresh yoghurt was free from any yeast and mould count, however they were detected in yoghurt on 21st day when stored at 4˚C and 6th day when stored at 15˚C. The yeast and mould count increased from 9.2 x 10^1 to 188.1 x 10^1 cfu/g on 21st day when stored at 15˚C; the count for the same was 4.6 x 10^1 cfu/g at on 21st day when stored at 4˚C. This may be at initial stage, yeast and moulds might be present but not enough in number and in later stage, they might have grown to an extent in order to form a colony in agar. The increase in yeast and mould count of the stored product at above temperatures were significant ($p < 0.05$). Tarakci and Kucukoner (2004) also reported an increase in the yeast and mould count of yoghurt samples made using fruit flavors (viz., cornelian and rosehip marmalade, morello cherry, grape molasses) during refrigerated storage. The coliform count also followed similar pattern to yeast and mould count i.e. absent in fresh samples but detectable on 15th and 6th day when stored at 4 and 15˚C respectively. The coliform count of 2.3 x 10^1 cfu/g on 15^{nth} day and 5.2 x 10^1 cfu/g on 6th day increased to 11.0 x 10^1 and 19.3 x 10^1 on 21st day, when stored at 4 and 15˚C, respectively. Con *et al.* (1996), an increase in the coliform count of fruit flavoured yoghurt (viz., sour cherry, orange, strawberry, banana) was also observed during refrigerated storage.

The increase in the coliform count in the stored product at above temperatures were found to be significant (P < 0.05). According to Food Safety and Standard Authority of India (FSSAI, 2012) coliform was not permitted in milk and milk related products, therefore in present case the fruit yoghurt was acceptable up to 12 days at 4˚C and only 3 days at 15˚C.

Conclusion

Antioxidant rich fruit yoghurt can be developed utilizing seabuckthorn fruit pulp, along with other ingredients of yoghurt mix, incorporating beneficial bacteria like *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* The resultant product contained rich amount of fat, protein, carbohydrate and antioxidants (viz., vitamin C, vitamin E, carotenoids, phenols and anthocyanins) when compared to commercially available yoghurt products. The fruit yoghurt can be safely stored up to a period of 12 days at 4°C and 3 days at 15°C, without deterioration in the microbiological qualities. The seabuckthorn fruit yoghurt may provide health benefits ascribed to the presence of carotenoids, vitamins and polyphenols.

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