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Studies on physico-chemical and microbiological attributes for development of soy blended herbal frozen yoghurt

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Abstract
Yoghurt is popular cultured dairy product in most countries. It is healthy and delicious food due to its high nutritive and therapeutic value. Yogurt also provides amount of growth factors and minerals which includes vitamin B_1, vitamin B_2 and vitamin B_{12} along with Ca, P, Mg, folic acid and niacin. Soy milk provides all essential amino acids in the amount needed for human health. Cinnamon as a supplement has effects on health and disease is loaded with powerful antioxidants such as poly phenols. The present investigation was conducted for development of acceptable soy blended herbal frozen yoghurt prepared from admixture of soy and buffalo milk in the ratio of 50:50, 60:40 and 40:60. To this preparation cinnamon was added as herbal additive at two different level of 0.05 and 0.08 percent and orange pulp was added to each preparation at 10 and 15 percent level. The data was collected and statistically analysed it was found that the among all the treatments T_7 (60:40:0.05:15) scored higher in overall acceptability and was considered as optimized product. The physico-chemical analysis revealed that the optimized product had fat (4.82%), protein (5.29%), carbohydrate (15.71%), ash (1.03%), T.S. (26.85%), moisture (73.14%) and acidity (0.74) and microbiological analysis revealed that the optimized product had SPC**(**$\times 10^3$cfu/ml**)** 15.47 and coliform had nil.

Keywords: herbal frozen yoghurt, soy milk, cinnamon, fat, protein, carbohydrate, acidity

Introduction
Yoghurt is a most popular fermented milk product worldwide. It provides nutrients in significant amounts, in relation to its energy and fat content, making it a nutrient-dense food. Yoghurt is similar to dahi in its physical appearance and it possesses excellent nutritional and therapeutic properties. Yoghurt has many health benefits beyond the basic nutrition it provides, such as improved lactose tolerance, a possible role in body weight and fat loss, and a variety of health attributes associated with probiotic bacteria (Mckinley, 2005) [19]. Yoghurt is belonging to fermented milks products and has increased its world popularity and consumption (Tamime and Robinson, 1999) [28]. Yoghurt is a main source of protein, fats, carbohydrates and mineral for children (Bibiana *et al*., 2014) [14]. Because production of yoghurt with omega-3 fatty acids may be an alternative for the increasing health-conscious of consumers. Yoghurt can be ideal to lactose intolerant as they can tolerate yoghurt better than the other dairy products. Lactose present in yoghurt is easily digested than lactose in other milk products. This is because of beta galactosidase system present in lactic acid bacteria in yoghurt (Martini *et al.* 1991) [18]. Lactic acid gives refreshing taste to the yoghurt and the by-products impart characteristic aroma (Rasic and Kurman, 1978) [23]. Frozen yoghurt popularity has increased and continues to grow; making it one of the most frequently consumed frozen desserts around the world. As the popularity of yoghurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers (Allgeyer *et al*., 2010) [1]. Frozen yogurt contains a considerably lower amount of fat in comparison with ice cream (Marshall, 2003) [17] and it is well known that excessive intake of fat is connected of higher risk of obesity and cardiovascular diseases (Devereux *et al*., 2003) [3]. Frozen yoghurt is a dessert that combines the texture of ice cream with nutritive and healthy properties of yoghurt (Rezaei *et al*., 2011) [24] and also combines with the flavors and textures of ice-cream and sherbet. Frozen yogurt is perceived as a healthier alternative to ice cream (Isik *et al*., 2011; Milani and Koocheki, 2011) [9, 21].

Buffalo milk is one of the richest milk from a compositional point of view, it contains higher levels of fat, solid not fat, total solid, total protein, lactose and ash (Dubey and Gupta, 1988) [4] and buffalo milk is responsible for high energy and nutritive value. Buffalo milk contains higher proportion of triglycerides, casein and whey protein than cow milk (Sindhu, 1998) [25]. Similarly, the proportions of butyric acid containing triglycerides are also higher in buffalo milk as compared to cow milk (Ramamurthy, 1974). Lactose is a disaccharide present in milk and milk products consisting of glucose and galactose (Ganzle *et al*., 2008) [5]. Milk proteins are well known for their high nutritional quality as well as bioavailability. The biological value provides a measurement of efficient the body utilizes protein consumed in the diet. A food with a high biological value is correlates to a high supply of the essential amino acids (Hoffman and Falvo, 2004) [7].

Soybeans are known to have highly beneficial health effects and worldwide consumption. Soybean contains all the three nutrients as a carbohydrate, protein and fat required for good nutrition, fiber, vitamins and minerals. It content high poly unsaturated fatty acid. Soybean has more than twice the amount of minerals, mainly calcium, iron, zinc and phosphorus than any other legume (Itapu, 2003 and Venter, 2004) [10, 29]. Soy products relatively high in soy protein and which utilize the majority of the bean are soymilk and tofu (Liu, 1997) [16]. Soy protein contain high concentration of isoflavones that exerts protective properties against breast, prostate, colon and lung cancers (Kirupa et. al., 2011). Soy milk is a beverage made from soybeans, which is rich in protein, vitamins, and minerals and free from lactose and cholesterol. Soy milk provides all essential amino acids in the amount needed for human health. It is a rich source of nutrients, soybean has a number of phytochemicals, which offer various health benefits. The consumption of soy foods has been linked to the prevention and treatment of chronic diseases, lowering cancer mortality rates, and reducing the risk of heart disease due to the cholesterol lowering effect of soy proteins (Sirtori *et al*., 1993 [26]; Messina *et al*., 1994 [20]; Kennedy, 1995) [13]. The final composition of soymilk is about 8-10% total solid, 3.6% protein, 2% fat, 2.9% carbohydrates and 0.5% ash, and it depends on processing conditions and soybean variety used (Liu, 1997) [16].

Cinnamon is a common spice used by different cultures around the world for several centuries. The genus *cinnamomum* is commercially known as cinnamon and is considered as one of the oldest spices in the world and cinnamon oil is widely used in the food processing, flavouring, cosmetic, confectionaries and pharmaceutical industries (Jayatilaka *et al*., 1995) [11]. Cinnamon is available in two forms - the cinnamon stick or ground cinnamon powder. The spice is actually obtained from the inner bark of the cinnamon tree. Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products (Huang *et al*., 2007) [8]. Cinnamon is used in traditional medicine and It has been tested on a variety of clinical conditions, such as bronchitis or diabetes (Castro, 2018).

The word "orange" is derived from Sanskrit term Narang. Orange is an evergreen flowering tree. Orange tree was cultivated in Southern China and Northern India. Oranges are the nutritional fruit, with all of its types bundled within a powerhouse of nutrients and that is beneficial for so many of our vital functions. Orange in colour is just like their pulpy flesh. Orange is rich source of vitamin C, flavonoids, phenolic compounds and pectin. The main flavonoid found in citrus species are narirutin, hesperidin, naringin and eriocarpin (Guanieri *et al*., 2007 and Kamran *et al*. 2009) [6, 12]. Citrus fruits can be used for the production of valuable products viz. biogas, ethanol or volatile flavouring compound or used for extraction, separation and purification of bioactive molecules with great interest in the development of healthy products. Orange peel is medicinally used against fungi (Strange *et al*. 1993) [27].

Materials and Methods

The experiment "Studies on physico-chemical and microbiological attributes of Development of Soy blended Herbal Frozen Yoghurt" was carried out in research lab, Warner College of Dairy Technology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad-211007, U.P. (India).

Procurement and collection of ingredients

Buffalo Milk was purchased near village of Allahabad. Soy milk was made in Research lab WCDT, SHUATS, Allahabad. Cinnamon was purchased from local market of Allahabad. Orange was purchased from local market of Allahabad. Sugar was purchased from local market of Allahabad. Stabilizer was purchased from local market of Allahabad. Yoghurt culture (NCDC-144) was purchased from National Dairy Research Institute, Karnal (Haryana).

Admixing soy & buffalo milk

Buffalo milk was collected from near village of Allahabad. Which was standardized at 4.5% fat and 9.0 % SNF. It was then mixed with prepared soy milk in following way.

T1-50 Parts of buffalo milk (BM): 50 parts of soy milk(SM).
T2-60 Parts of buffalo milk (BM): 40 parts of soy milk(SM).
T3-40 Parts of buffalo milk (BM): 60 parts of soy milk(SM).

Addition of cinnamon & Orange pulp

Cinnamon and Orange pulp was added after addition of stabilizer.

Preparation of frozen yoghurt

Yoghurt base (cold) was mixed with fruit pulp, stabilizer and emulsifier than it was freezer in an ordinary ice cream freezer outlet (-6ºC). The product was package and hardened (-25ºC) followed by storing (-25ºC).

Physico-chemical analysis

The study on physico-chemical attributes of Development of Soy blended Herbal Frozen Yoghurt of different treatments were analysed for their different analytical methods. Fat percentage of the developed product was estimated by the gravimetric method IS: SP: 18(Part XI)-1981. Protein percentage was estimated by Micro kjeldhal method described in IS: SP 18 (Part XI) 1981. TS content of the developed product was estimated by the gravimetrically method described in IS: SP (Part XI) 1981. Carbohydrate percentage of the product was estimated by calculation. Titratable acidity was estimated as per the procedure laid down in IS: SP 18 (Part XI) (1981). Ash percentage was estimated according to the method described in IS: SP 18 (Part XI) 1981 and moisture percentage was estimated as per procedure laid down in IS: SP 18 (Part XI) 1981.

Microbial analysis

Standard plate count- of the product was estimated as per the

method as given in IS: SP: 18(Part XI)-1981 and Coli form count of the product was estimated as per the method as given in IS: SP: 18(Part XI)-1981.

Statistical analysis

Data was analysed using Analysis of Variance (ANOVA) and Critical difference (C.D) at 5% in WASP software and excel software. The significance were separated at ($p<0.05$).

Treatment combination

A. Three levels of soy milk and buffalo milk-50:50, 60:40 and 40:60.
B. Two levels of orange pulp-10% and 15%.
C. Two level of cinnamon powder - 0.05% and 0.08%.

Treatment	Soy milk	Buffalo milk	cinnamon	Orange
T1	50	50	0.05	10
T2	50	50	0.08	10
T3	50	50	0.05	15
T4	50	50	0.08	15
T5	60	40	0.05	10
T6	60	40	0.08	10
T7	60	40	0.05	15
T8	60	40	0.08	15
T9	40	60	0.05	10
T10	40	60	0.08	10
T11	40	60	0.05	15
T12	40	60	0.08	15

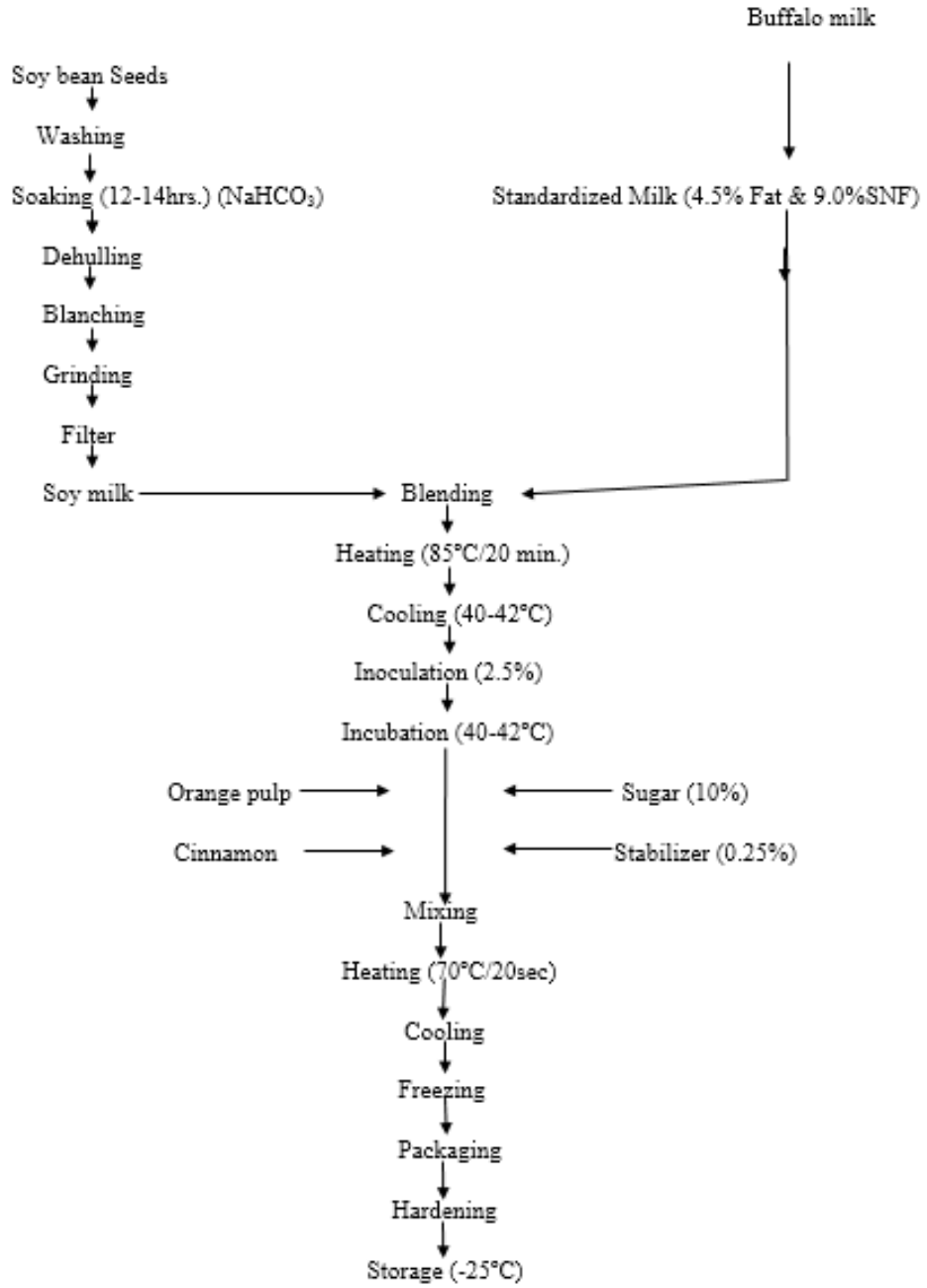

Fig1: Schematic for preparation of development of soy blended herbal frozen yoghurt

Results and Discussion

The data was collected on different aspects as per the methodology have been tabulated and analyzed statistically; the findings are also illustrated diagrammatically. The results obtained from the analysis are presented and discussed on chemical and microbiological analysis of development of soy blended herbal frozen yoghurt.

Table 1: Average data for physico-chemical analysis for development of soy blended herbal frozen yoghurt.

Particulars	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Physico-Chemical Analysis												
Fat (%)	4.51	4.51	4.52	4.52	4.81	4.81	4.82	4.83	4.21	4.21	4.22	4.24
Protein (%)	5.45	5.47	5.50	5.52	5.24	5.26	5.29	5.31	5.65	5.67	5.70	5.90
Carbohydrate (%)	14.98	14.99	15.57	15.59	15.12	15.15	15.71	15.73	14.85	14.88	15.44	15.47
Ash (%)	0.94	0.95	1.00	1.10	0.97	0.99	1.03	1.04	0.94	0.96	1.00	1.20
Total Solid (%)	25.88	25.92	26.59	26.73	26.14	26.21	26.85	26.91	25.65	25.72	26.36	26.81
Moisture (%)	74.10	74.08	73.43	73.27	73.85	73.79	73.14	73.09	74.37	74.28	73.65	73.19
Acidity(%LA)	0.68	0.67	0.76	0.78	0.68	0.67	0.74	0.75	0.72	0.71	0.80	0.81

(Average of three replications)

Fat percentage of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean fat percentage for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 4.51, 4.51, 4.52, 4.52, 4.81, 4.81, 4.82, 4.83, 4.21, 4.21, 4.22 and 4.24 respectively. The tables indicate that the highest mean fat percentage 4.83 was obtained by the sample (T_8) whereas lowest mean fat percentage 4.21 was obtained by the samples (T_9 andT_{10}).

Protein percentage of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean protein percentage for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 5.45, 5.47, 5.50, 5.52, 5.24, 5.26, 5.29, 5.31, 5.46, 5.67, 5.70 and 5.90 respectively. The tables indicate that the highest mean protein percentage 5.90 was obtained by the sample (T_{12}) whereas lowest mean protein percentage 5.24 was obtained by the samples (T_5).

Carbohydrate percentage of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean carbohydrate percentage for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 14.98, 14.99, 15.57, 15.59, 15.12, 15.15, 15.71, 15.73, 14.85, 14.88, 15.44 and 15.47 respectively. The tables indicate that the highest mean carbohydrate percentage 15.73 was obtained by the sample (T_8) whereas lowest mean carbohydrate percentage 15.44 was obtained by the samples (T_{12}).

Ash percentage of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean ash percentage for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 0.94, 0.95, 1.00, 1.10, 0.97, 0.99, 1.03, 1.04, 0.94, 0.96, 1.00 and 1.20 respectively. The tables indicate that the highest mean ash percentage 1.20 was obtained by the sample (T_{12}) whereas lowest mean ash percentage 0.94 was obtained by the samples (T_1).

Total Solid percentage of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean total solid percentage for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 25.88, 25.92, 26.59, 26.73, 26.14, 26.21, 26.85, 26.91, 25.65, 25.72, 26.36 and 26.81 respectively. The tables indicate that the highest mean total solids percentage 26.91 was obtained by the sample (T_8) whereas lowest mean total solids percentage 25.65 was obtained by the samples (T_9).

Moisture percentage of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean moisture percentage for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 74.10, 74.08, 73.43, 73.27, 73.85, 73.79, 73.14, 73.09, 74.37, 74.28, 73.65 and 473.19 respectively. The tables indicate that the highest mean moisture percentage 74.37 was obtained by the sample (T_9) whereas lowest mean moisture percentage 73.14 was obtained by the samples (T_7).

Acidity percentage lactic acid of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean Acidity percentage lactic acid for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12} are 0.68, 0.67, 0.76, 0.78, 0.68, 0.67, 0.74, 0.75, 0.72, 0.71, 0.80 and 0.81 respectively. The tables indicate that the highest mean acidity percentage 0.81 was obtained by the sample (T_{12}) whereas lowest mean acidity percentage 0.67 was obtained by the samples (T_6). Kolapo and Olubamiwa, 2012) [14] observed that slightly lesser value of titratable acidity as % lactic acid of coconut milk containing soy yoghurt.

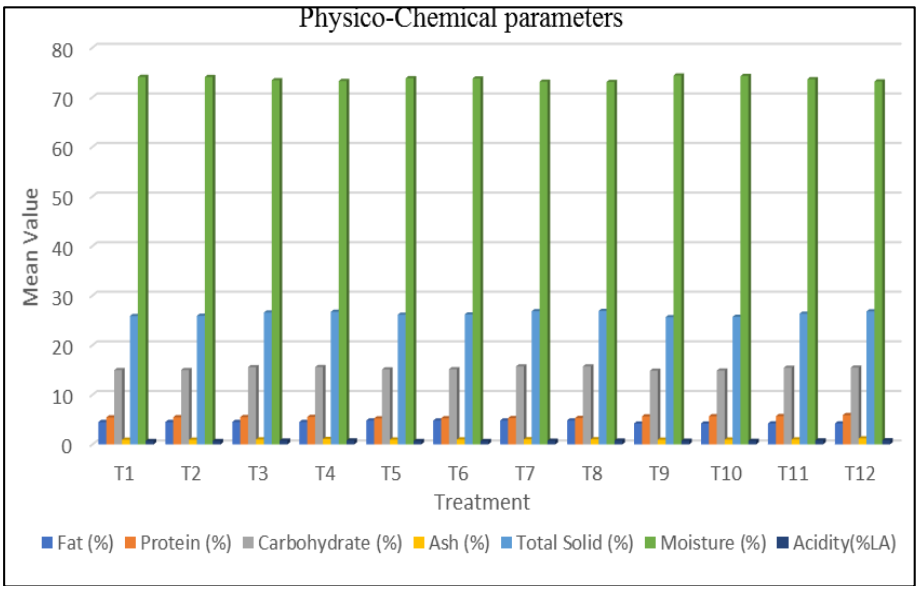

Fig 2: Graphical representation of physico-chemical parameter for development of soy blended herbal frozen yoghurt

Table 2: Average data for microbial analysis for development of soy blended herbal frozen yoghurt.

Particulars	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Microbiological analysis												
SPC (×10^3cfu/ml)	17.71	17.59	16.00	15.85	17.45	17.36	15.47	15.32	18.4	18.00	17.14	15.68
Coli form	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

(Average of three replications)

SPC (x10^3cfu/ml) of herbal frozen yoghurt samples of different treatments was observed through the table 1, the mean Standard plate count for T_1,T_2,T_3,T_4,T_5,T_6,T_7,T_8,T_9,T_{10}, T_{11} andT_{12} are 17.71, 17.59, 16.00, 15.85, 17.45, 17.36, 15.47, 15.32, 18.40, 18.00, 17.14 and 15.68 respectively. The tables indicate that the highest mean SPC (x10^3cfu/ml) 18.40 was obtained by the sample (T_9) whereas lowest mean SPC (x10^3cfu/ml) 15.32 was obtained by the samples (T_8). Coliform count was found to be absent in all the samples of herbal frozen yoghurt of different treatments which indicates that proper hygienic conditions were maintained during the preparation and storage condition of the product.

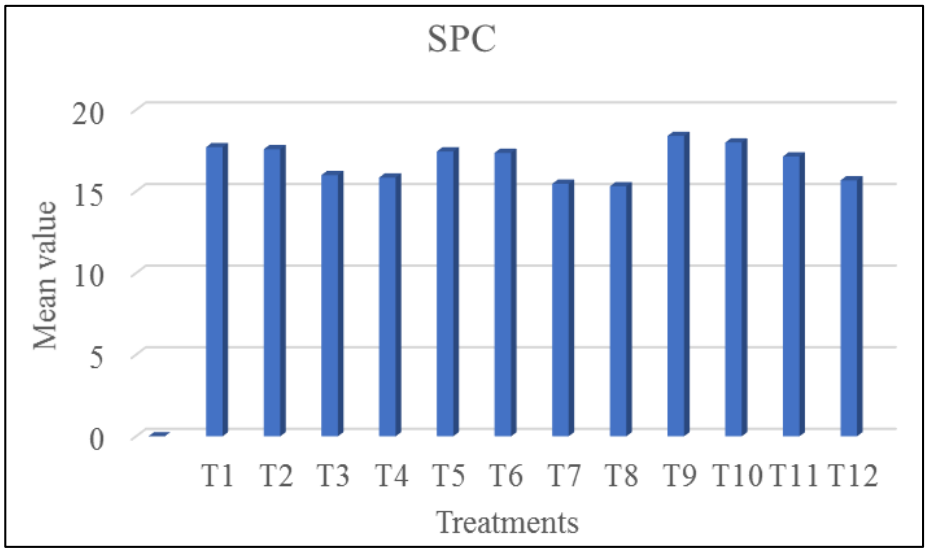

Fig 3: Graphical representation of microbiological parameter for development of soy blended herbal frozen yoghurt

Conclusion

Overall results of the study indicate that the extract of cinnamon and orange pulp can be added satisfactorily into the preparation for development of herbal frozen yoghurt. Cinnamon is packed with a variety of protective antioxidants that reduces free radical damage and show the aging process. Oranges are low in calories and full of nutrients they promote clear, healthy skin and help lower risk for many diseases like asthma, arthritis, diabetic, gallstones, and cholera etc. The herbal frozen yoghurt using different ratios of soy and buffalo milk admixture containing various levels of cinnamon extract and orange pulp was subjected to physico-chemical and microbiological analysis.

The data obtained on various parameters were statistically analyzed and found that among all combinations, highest mean fat percentage 4.83 was obtained by the sample (T_8) whereas lowest mean fat percentage 4.21 was obtained by the samples (T_{10}). Similarly, highest mean protein percentage 5.90 was obtained by the sample (T_{12}) whereas lowest mean protein percentage 5.24 was obtained by the samples (T_5). Highest mean carbohydrate percentage 15.73 was obtained by the sample (T_8) whereas lowest mean carbohydrate percentage 15.44 was obtained by the samples (T_{12}). Likewise, highest mean ash percentage 1.20 was obtained by the sample (T_{12}) whereas lowest mean ash percentage 0.94 was obtained by the samples (T_1). The highest mean total solids percentage 26.91 was obtained by the sample (T_8) whereas lowest mean total solids percentage 25.65 was obtained by the samples (T_9). Similarly, highest mean moisture percentage 74.37 was obtained by the sample (T_9) whereas lowest mean moisture percentage 73.14 was obtained by the samples (T_7) and highest mean acidity percentage 0.81 was obtained by the sample (T_{12}) whereas lowest mean acidity percentage 0.67 was obtained by the samples (T_6). The highest mean SPC(x10^3cfu/ml) 18.40 was obtained by the sample (T_9) whereas lowest mean SPC(x10^3cfu/ml) 15.32 was obtained by the samples (T_8) and coliform count was found to be absent in all the samples of development of herbal frozen yoghurt of different treatments which indicates that proper hygienic conditions were maintained during the preparation.

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