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Research Article

Enhanced antioxidant potential of supplemented yogurt with sea buckthorn

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Abstract

Sea buckthorn, *Hippophae rhamnoides*, is a renowned fruit in Chinese traditional medicine. The plant contains a massive number of polyphenols, phytochemicals like vitamin C, flavonoids and carotenoids which act as antioxidants and effective against ailments like cold, cough, inflammation, aging and cancer. Yogurt is an ancient fermented milk product and has been using for nutritional purposes. The current study evaluated the antioxidant potential of sea buckthorn pulp (SP) and shelf life and physicochemical characteristics of supplemented yogurt with sea buckthorn. Yogurt was developed using whole milk supplemented with sea buckthorn by 0, 5, 10, 15 and 20 percent respectively. Physicochemical, phytochemical, microbial and sensory evaluation of yogurt was done at different storage period (0, 7, 14 and 21 days) and obtained data was subjected statistical design. Among 5 treatments T3 showed more acceptability levels in yogurt in terms of physicochemical phytochemical, microbial and sensory evaluation with negligible change in storage period. Negligible changes were shown of ascorbic acid, carotenoids, vitamin E, antioxidant activity and total polyphenolics contents in T3 20.65mg/g, 1.46mg/g, 12.00mg/g, 85.1% and 24.2% respectively. Microbial count of T3 remained < 10 log CFU/ml with negligible change in storage period of 21 days. T3 scored highest on the 9-point hedonic scale during the sensory evaluation as well. This research concluded that sea buckthorn can be supplemented in yogurt for utilization of its antioxidant benefits. in the search for, certification and implementation of safe natural additives. The growing demand for the so-called "clean label" foods is the driving force for many of the conducted researches in the last two decades.

Keywords: Sea buckthorn, polyphenols, antioxidants, fermented, carotenoids

Abbreviations: SP – Sea buckthorn Pulp; DPPH – 2.2-diphenyl-1-picryl-hydrazyl-hydrate
PUFA – polyunsaturated fatty acid, LDL – Low density lipoprotein, MDA – malondialdehyde, ROS – Reactive oxygen species, RNS – Reactive nitrogen species, TBARS – Thiobarbituric acid reactive substances, MetMb – Metmyoglobin, NOMb – Nitrosomyoglobin, CLA – Conjugated linoleic acid, EMF – Extract from male flowers
DDRP – Dried distilled rose petals

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Introduction

Sea buckthorn is an ancient plant with an enormous number of properties, also known as *Hippophae rhamnoides*. It is of major significance because of its medicinal and pharmacological properties. This thorny plant has long, narrow leaves and orange to yellow fruit berries. It is cold impervious and instinctive to Europe and Asia (Christaki 2012). Captivatingly, sea buckthorn fruit was already acknowledged in the primeval times predominantly in traditional Asian medicine (Fu et al. 2014). Numerous little green and brown blooms mature in racemes on the plant. Following the flowering period, sea buckthorn berries mature into delightful and healthy corpulent berries (Amarowicz et al. 2019). For example, phytosterols, which are found in the oil of the sea buckthorn plant and have several health benefits, are found in high concentrations in the plant's oil. Extracts of sea buckthorn oil, pulp chunks, and leaves have been shown to have therapeutic properties (Teleszko et al. 2015). In ancient China, this plant was used to cure coughs, fevers, gynaecological problems, and metabolic and circulatory abnormalities (Russia 2011). Sea buckthorn berries contain an impressive quantity of polyphenols, particularly flavanols. Sea buckthorn has a greater concentration of vitamin C than any other fruit, with an average of 80 milligrams per 100 grams (Olas 2018).

In food science, antioxidants are substances that mitigate the aftereffects of oxidation. Thus, it contributes to the preservation of the quality and extension of the shelf life of food items. An oxidative stress response is reduced by dietary antioxidants and supplementation, which aids in the prevention of many chronic illnesses. The human body's defensive enzymatic system relies on vitamins E and C, which are crucial nutritional antioxidants (Yang et al. 2018). Only a few antioxidants have been proved to have good health benefits that enhance well-being. The favourable effects on the human immune system are determined by the bioavailability of these nutrients, their absorption, and the quantity of these components at certain organ locations (Yang et al. 2018). Potential of antioxidants for protecting cells from oxidative stress responses laid the groundwork for their growth in pharmaceutical and dietary supplement sectors (Godic et al. 2014).

An increasing amount of research suggests that the health advantages of polyphenols in food may be attributable to their antioxidative properties. Yogurt with additional polyphenol-enriched extracts from natural sources can be a practical meal format to satisfy customers' desire in original yoghurt nutrients, starting culture advantages, and added polyphenol extract that provides health benefits (Wang et al. 2020). Antioxidant, anticancer, immunomodulatory, anti-inflammatory, anti-ulcer, anti-microbial, radio-protective, and anti-diabetic qualities are only a few of its many medicinal benefits. Antioxidant-rich phytosterols and sugars are found in abundance in this plant along with a wide range of other nutrients (carotenoids, flavonoids, tocopherol) (Singh et al. 2019).

Currently, this research is conducted to examine the antioxidant potential of sea buckthorn, the production of sea buckthorn-enriched yogurt, and the shelf life and quality acceptability of sea buckthorn supplemented yogurt.

Materials and Methods

The study was carried out at the laboratory of the National Institute of Food Science and Technology at the University of Agriculture, Faisalabad. The milk, yogurt culture, and sea buckthorn fruit were purchased locally in Faisalabad, Pakistan.

Physicochemical analysis of sea buckthorn

Moisture content was examined by using hot air oven and ash was determined by using muffle furnace (AOAC 2016). pH was calculated by using pH electrodes (Caleja et al. 2016), acidity with titration procedure and total soluble solids was determined by refractometer (Soni et al. 2020).

Phytochemical analysis of sea buckthorn

The polyphenolic extraction was carried out according to the gallic acid standard methodology using a spectrophotometer set to 765nm. Antioxidant activity was determined by 2, 2-diphenyl-1-picryl-hydrazyl-hydrate protocol (DPPH) using spectrophotometer at 517nm (Guo et al. 2017). The carotenoids were evaluated using a spectrophotometer set to 470nm, and the ascorbic acid content of sea buckthorn pulp (SP) was determined using the titration method (Selvamuthukumaran and Farhath 2014). And at

562nm, the absorbance of vitamin E was measured (Jadoon et al. 2013).

Preparation of yogurt

Whole milk was pasteurized at 90°C and cooled to 37°C. Later on, it was divided into separate containers. Sea buckthorn was added into the milk according to the treatment plan. The mixed milk was inoculated with 2 percent of yogurt culture and incubated at 43°C. Then yogurt was stored at 4°C for further experiments and storage study (Roy et al. 2015).

Table 1. Treatment plan for preparation of yogurt supplemented with sea buckthorn

Treatment	Sea buckthorn, %
T_0	0
T_1	5
T_2	10
T_3	15
T_4	20

Physicochemical analysis of sea buckthorn fortified yogurt

It was determined by utilizing a hot air oven to analyze the moisture content of the yogurt enriched with sea buckthorn; the ash was assessed using a muffle furnace; and fat, protein, and syneresis were determined using the Gerber technique, Kjeldahl process, and centrifugation protocols respectively (AOAC 2016). Although pH was calculated by using pH electrodes (Caleja et al. 2016), acidity with titration procedure and total soluble solids was determined by refractometer (Soni et al. 2020).

Phytochemical evaluation of sea buckthorn fortified yogurt

The polyphenolic extraction was carried out according to the gallic acid standard methodology using a spectrophotometer set to 765nm. Antioxidant activity was determined by 2, 2-diphenyl-1-picryl-hydrazyl-hydrate protocol (DPPH·) using spectrophotometer at 517 nm (Guo et al. 2017). The carotenoids were evaluated using a spectrophotometer set to 470nm, and the ascorbic acid content of sea buckthorn pulp (SP) was determined using the titration method (Selvamuthukumaran and Farhath 2014). And at 562nm, the absorbance of vitamin E was measured (Jadoon et al. 2013).

Microbial activity of sea buckthorn fortified yogurt

The quantity of bacteria is calculated indirectly using the number of colonies formed by microorganisms' cells during 48 hours at 37°C. The bacteria were grown on nutrient agar by Caleja et al. (2016).

Sensory evaluation of sea buckthorn fortified yogurt

Aesthetical evaluation (texture, color, aroma and overall acceptability) was assessed by using 9 points hedonic scale. A panel of ten trained members assessed color, odor, taste, texture and overall acceptance using ranging from extremely like to extremely dislike for each sensory attribute. The samples were served in 20ml of glass cups at different storage period (0, 7, 14, 21 days) in day light (Roy et al. 2015).

Statistical analysis

Each sample was examined in triplicate for each parameter. The obtained data analyzed by two-way ANOVA and Fisher’s LSD model. The findings were analyzed using Statistics 8.1 software (Montgomery 2017).

Results and Discussion

Physicochemical analysis of SP

SP was subjected to the physicochemical analysis (moisture, ash, fiber, pH, acidity and total soluble solids). The results disclosed that the sea buckthorn pulp contains 72.6% moisture, 1.73% Ash, 4.7g fiber, 2.67 pH, 1.80% acidity and 11.3 Brixo Total soluble solids. Buya et al. (2012) conducted the analysis of chemical composition of sea buckthorn fruit comprises of 40.7% of moisture, 1.7% Ash and 9.17g/100mg fiber. Tkacz et al. (2020) assessed the physicochemical properties of sea buckthorn juice at fresh and storage period to evaluate the stability of components.

Table 2. Physicochemical analysis of SP

Physicochemical analysis	Sea buckthorn pulp
Moisture, %	72.6 ± 0.5
Ash, %	1.73 ± 0.02
Fiber, %	4.7 ± 0.03
pH	2.67 ± 0.25
Acidity, %	1.80 ± 0.02
Total soluble solids, Brix°	11.3 ± 0.6

The data labelled as Mean value ± Standard deviations

Phytochemical analysis of SP

Phytochemical evaluation of sea buckthorn revealed that it contains 558.7mg/L GAE total polyphenols and has 89.14% of antioxidant activity, 327.67mg/100g ascorbic acid, 190.32mg/100g vitamin E and 6.8mg/100g carotenoids. Buya et al. (2012) determined the vitamin C and carotenoid content of sea buckthorn fruit and revealed that fruit contain 320mg/100g vitamin C and 7.15mg/100g of total carotenoids. Tkacz et al. (2020) assessed that sea buckthorn is an excellent source of polyphenols and other antioxidant components as it contains 98% bioactive elements in total. Zenkova and Pinchykova (2019) revealed that sea buckthorn berries contain 4.4-13.2mg/100g of carotenoids and 44.7-78.7mg/100g of vitamin C.

Table 3. Phytochemical analysis of SP

Phytochemical analysis	Sea buckthorn pulp
Total polyphenols, mg/L GAE	558.7 ± 1.5
Antioxidant activity, %	89.14 ± 0.1
Ascorbic acid, mg/100g)	327.67 ± 7.5
Vitamin E, mg/100g	190.32 ± 0.3
Total carotenoids, mg/100g	6.83 ± 0.02

The data labelled as Mean value ± Standard deviations

Physicochemical analysis of sea buckthorn fortified yogurt

Moisture content fluctuates in yogurt with 5% of supplementation and in T2 (10%) and T3 (15%) yogurt has negligible changes during storage time. There is significant decrease ($P < 0.05$) in moisture content in control yogurt and T4 (20%) during 21 days of storage. The decreasing trend in moisture over the period of time is due to an increase in protein content of yogurt which increased the total solids by developing complex of protein globules (Barkallah et al. 2017). Ahmed et al. (2020) stated that decrease in moisture of supplemented yogurt during storage is due to the storage conditions and evaporations. Additionally, the absorbance of mineral salts increases the total solids content which lead to the loss of moisture content.

Inorganic matter is useful for determining the mineral content of food goods. Additionally, it maintains the number of microorganisms in food products. The change in ash content of yogurt due to the supplementation of fruit pulp which is rich source of minerals or inorganic matters and ash which represents mineral content of food. Similar outcomes have shown in a study of (Barkallah et al. 2017). They supplemented yogurt with spirulina and had alike results and stated that this change was due to the high mineral (magnesium and calcium) content in the spirulina. Al-Shawi et al. (2020) studied in the fortification of yogurt by adding thyme into it. The results were having similar increasing trend in the ash and stated that this increase in content was due to the collaboration of minerals into the moisture of yogurt. Ahmed et al. (2020) has described that the change in ash is due to the decrease in moisture content during storage period of time. Evaporation during storage cause increase in ash content and due to the absorbance of more mineral contents in yogurt.

The total soluble solids (TSS) in food are determined using a refractometer. It shows the sugar content present in food commodities. The increased change in the total soluble solid content is due to the addition of pulp into the yogurt and as concentration increases the TSS content increased. The present study has the similarities with the research Al-Shawi et al. (2020) stated in the research of organoleptic and sensory study of yogurt with thyme extract that the TSS content in yogurt over the storage period was increased. This change was due to the decrease in moisture content and increase in total solids in yogurt which may cause by the evaporation of moisture in the yogurt and balance in the components.

The pH is measured for the acidity or the presence of hydrogen ion in food which give sour taste to the food. There is slight difference in pH of fresh samples but it varied as the storage day increased. Overall, the storage exerted decline effect on pH in all treatments. Outcome of present research was similar to Meenakshi et al. (2018). The more lactic acid the decline in pH and according to this present study has acceptable pH at the end of the study except T3. They stated that lactic acid production decreased the pH of fruit yogurt at different concentration. Other parameters like total soluble solids, total solids, storage temperature and additives also could decrease the pH of yogurt. Dabija et al. (2018) evaluated the physiochemical features of yogurt supplemented with sea buckthorn powder, rosehip powder and extract of grape seed. The research described decreased trend in pH during the storage period of 28 days. They stated that the decline in pH was due to post acidification and increased activity of lactic acid bacteria.

Acidity calculates the hydrogen ion and in term of acidity in food, the flavor of food products is highly depending on acidity. Higher the acidity higher the sour taste of products. The maximum fluctuation in acidity among treatments was calculated in T3 group which has 15% of SP in 21 days of storage with 7 days interval. Amal et al. (2016) stated in their 10 days storage study that lactic acid bacteria production increased the acidity and decreased the pH of fruit yogurt. Similar study of Meenakshi et al. (2018) indicated the addition of fruit pulp increased the acidity of yogurt as the fruit has its own specific acidity level and sourness.

Fat is a critical macronutrient found in food items because it provides energy, promotes cell growth, and makes meals more palatable. The fat content increases as the concentration of pulp increased and so the storage days. Similar results are found in the study of Scibisz et al. (2019) and stated in the research on the different blended and on the top fruit yogurts that during storage fat content could change due to the oxidation process.

Table 4. Physicochemical analysis of sea buckthorn fortified yogurt

Treatments	Storage days	Moisture, %	Ash, %	TSS, Brix°	pH	Acidity, %	Fat, %	Protein, %	Syneresis
T_0	0	80.3^{c}	0.8^{hi}	9.3^{e}	4.55^{a}	0.68^{a}	2.5^{o}	3.2^{e}	32.63^{a}
	7	78.0^{c}	0.8^{efgh}	8.8^{e}	4.5^{a}	0.71^{a}	2.7^{n}	3.3^{e}	32.85^{a}
	14	74.66^{c}	0.9^{efg}	7.6^{e}	3.9^{b}	1.3^{b}	2.7^{n}	3.5^{e}	37.59^{a}
	21	75.0^{c}	1.15^{d}	7.2^{e}	3.3^{c}	1.7^{c}	3.0^{m}	3.9^{d}	30.83^{a}
T_1	0	81.0^{b}	0.76^{i}	12.2^{d}	4.5^{a}	0.70^{a}	3.7^{l}	3.6^{d}	32.06^{a}
	7	81.0^{b}	0.8^{gh}	11.7^{d}	4.5^{a}	0.75^{a}	3.7^{l}	3.9^{d}	31.53^{a}
	14	80.0^{b}	1.3^{e}	10.0^{d}	3.5^{b}	0.80^{b}	4.0^{k}	4.3^{cd}	31.20^{a}
	21	81.0^{b}	1.23^{bc}	10.0^{d}	3.3^{b}	1.0^{b}	4.4^{j}	4.7^{cd}	30.40^{a}
T_2	0	83.1^{a}	0.85^{gh}	14.0^{c}	4.4^{a}	0.78^{a}	4.5^{j}	5.3^{c}	32.9^{a}
	7	82.6^{a}	0.9^{efg}	13.23^{c}	4.2^{a}	0.88^{a}	4.8^{i}	5.6^{c}	32.9^{a}
	14	82.0^{a}	1.13^{b}	12.0^{c}	3.3^{a}	0.98^{a}	5.0^{h}	6.27^{bc}	33.03^{a}
	21	82.0^{a}	1.54^{a}	12.0^{c}	3.2^{b}	1.0^{b}	5.3^{g}	6.5^{c}	33.5^{a}
T_3	0	83.1^{a}	0.87^{fgh}	16.3^{b}	4.4^{a}	0.86^{a}	5.4^{g}	7.5^{bc}	32.9^{a}
	7	82.8^{a}	0.92^{ef}	15.0^{b}	4.3^{ab}	0.94^{ab}	5.4^{f}	7.6^{bc}	34.5^{a}
	14	82.6^{a}	1.16^{cd}	15.0^{b}	3.2^{b}	1.0^{b}	6.0^{e}	7.87^{bc}	38.3^{a}
	21	81.0^{a}	1.55^{a}	14.92^{b}	2.7^{c}	1.5^{c}	6.4^{c}	8.09^{b}	40.3^{a}
T_4	0	83.1^{a}	0.89^{efg}	15.0^{a}	4.4^{ab}	1.1^{ab}	6.2^{d}	8.0^{bc}	34.25^{a}
	7	83.0^{a}	0.89^{efg}	16.0^{a}	4.5^{a}	1.0^{a}	6.8^{b}	8.0^{bc}	35.34^{a}
	14	81.0^{a}	0.96^{e}	16.1^{a}	4.2^{c}	1.0^{c}	6.8^{ab}	8.12^{b}	36.02^{a}
	21	80.0^{a}	1.2^{bc}	16.92^{a}	4.0^{d}	1.3^{d}	6.9^{a}	9.2^{a}	37.00^{a}

T_0 – Control group; T_1 – Yogurt with 5% sea buckthorn; T_2 – Yogurt with 10% sea buckthorn; T_3 – Yogurt with 15% sea buckthorn T_4 – Yogurt with 20% sea buckthorn; Mean carrying the different letter within the column differ significantly ($P<0.05$).

However, there was not significance change ($P > 0.05$) in fruit yogurts as a result of antioxidant components present in fruit. Al-Shawi et al. (2020) documented in the research of organoleptic and sensory study of yogurt with thyme extract that there was an increase change in fat percentage of yogurt as compared to the control yogurt. The fat content generally depends on the milk composition but in case of supplemented yogurt it depends on the oil content of supplementation which increases the fat content of yogurt.

The other most important nutrient of food is protein which affects the taste of food and provides nutrition to the human body as it is the pivotal component in the growth and development. The maximum value determined at the 21st day in T4 with 20% SP supplemented yogurt. Increasing trend has seen after treatments in comparison of control yoghurt, because of the fruit's protein component, this protein level is high. Results of present study has the similarities with Scibisz et al. (2019) where stated in the research on the different blending and on the top fruit yogurts that during storage there was change in the nitrogen complexes in yogurt which led to the minute change in protein content of fruit yogurt. The hydrolysis of yogurt protein, which increased the amount of free amino acids in yogurt, contributed to the change in total protein content of yogurt during storage.

An undesirable build-up of whey protein on the surface of yogurt is known as syneresis which is the most important characteristics of food product. The constant increasing trend can be seen in the T3 and T4 with 15% and 20% of SP. Outcomes of present study is familiar to Wang et al. (2020) study in which they studied the characteristics of yogurt by adding apple pomace in it. According to their research's outcomes, syneresis caused by the re-arrangements in the protein globules leads to the producing masses in fruit yogurt. Jeong et al. (2018) incorporated green tea powder into the yogurt and stated the outcomes that by adding 1% green tea powder did not affect syneresis property of yogurt. By increasing the amount of supplementation by 2-3% increased the syneresis which is due to the decrease in gel matrix of yogurt during storage.

Phytochemical evaluation of sea buckthorn fortified yogurt

Polyphenols are widely recognized for their antioxidant activity and role in inhibiting free radical scavenging in the human body system. The minimum value 4.26 was measured at the 21st day of control yogurt and maximum value was determined at the 0 day of T4. Similar research has revealed similar tendencies. A mixed fluctuations in the polyphenolic content was seen in yogurt with several extracts (rosemary, oregano and dill) (Shori 2020). In this study it was indicated that the polyphenolic content was increased till 14th day from 66.97µg GAE/ml oregano and 61.51µg GAE/ml rosemary to 85.12µg GAE/ml and 80.66µg GAE/ml respectively and decreased during the 21 days of storage time period. She stated that this is due to the hydrolysing action of yogurt microbes on the polyphenolic complex during refrigerated storage. (Arfaoui 2020) added dates into the yogurt and after the evaluation stated that dates increased the polyphenolic content of yogurt but decrease in the content during cold storage is due to the phenolic compound's interaction with the milk protein of yogurt.

Antioxidant activity is measured by the DPPH protocol which prevents oxidation of cellular structures and other molecules present in living cells. The antioxidant activity increased in treatments 5%, 10%, 15% and 20% respectively at 0th day was due to the addition of fruit in yogurt. There is a decline trend in the antioxidant activity of the SP yogurt during the storage period. Findings of present study has similarity with the research of (Yadav et al. 2018). Shori (2020) in her research on the phenolic compounds in yogurt from different extracts documented that antioxidant activity of rosemary and oregano decreased from 86% and 88% to 73% and 58.19% respectively during the 21 days of storage in refrigerator which is due to the change in ferrous ion chelating agents present in the extracts of polyphenols.

Carotenoids, a provitamin A is a fat-soluble vitamin and having antioxidant activities. Control yogurt and T3 with 15% of SP remain same contents of carotenoids from the first day of storage till the 21 days. Other treatments T1, T2 and T3 exhibit decline trend during storage at 14th and 21st day of storage. Similar results can be seen in the study on the spirulina supplemented yogurt. Patel et al. (2019) stated that there was negligible change in carotenoid from 13.28 to 13.10 µg/100g during storage period. This could be due to the nature of carotenoid which is fat soluble and the yogurt contains more fat during storage.

Ascorbic acid or vitamin C is highly active antioxidant and helps in the reduction and prevention of oxidative stress and related diseases. There is no drastic change during the time period. Minor changes can be seen at 14th day of storage in all treatments. The difference between the mean values 23.46 to 1.0 which is because sea buckthorn is a rich source of ascorbic acid. It provides it yellow to orange color and bitter sour taste which cause high in acidity. The decrease in moisture content and increase in total solids also reflects in the decline of water-soluble vitamin Scibisz et al. (2019) stated in the research on the different blended and on the top fruit yogurts that during storage the amount of ascorbic acid had decreased because of the storage conditions as ascorbic acid is vulnerable to the heat treatments and storage conditions.

There is significant difference ($P < 0.05$) of vitamin E content with the treatments. As the percentages increases the content increased. There is no effect of storage on vitamin E, after 14th day there is slight decrease in vitamin E content. Results of present study has resemblances to the research of Estrada Andino (2011) in which the researcher evaluated the storage stability of vitamin E and omega-3 fatty acids. Researcher described that the decline trend of vitamin E content after 14th day in yogurt is due to the many factors like light, heat and oxygen which can cause oxidation process.

Table 5. Phytochemical evaluation of sea buckthorn fortified yogurt

Treatments	Storage days	Total polyphenols	Antioxidants, %	Carotenoids, mg/100g	Ascorbic acid, mg/100g	Vitamin E, mg/100g
T_0	0	5.1^{h}	78.75^{h}	0.021^{l}	1.0^{e}	0.05^{e}
	7	5.1^{h}	78.07^{h}	0.021^{l}	1.0^{e}	0.05^{e}
	14	4.9^{h}	16.45^{i}	0.21^{k}	1.0^{e}	0.05^{e}
	21	4.26^{i}	70.55^{k}	0.21^{k}	1.0^{e}	0.04^{e}
T_1	0	10.39^{g}	80.31^{g}	0.49^{h}	6.00^{d}	2.80^{d}
	7	10.39^{g}	80.32^{g}	0.49^{h}	5.98^{d}	2.80^{d}
	14	10.33^{g}	78.13^{h}	0.47^{i}	5.8^{d}	2.84^{d}
	21	10.32^{g}	75.1^{i}	0.44^{j}	5.76^{d}	2.67^{d}
T_2	0	19.21^{f}	85.09^{d}	1.0^{e}	14.6^{c}	6.90^{c}
	7	19.21^{f}	85.00^{d}	1.0^{e}	14.6^{c}	6.90^{c}
	14	19.14^{f}	81.4^{f}	0.90^{f}	14.2^{c}	6.63^{c}
	21	18.96^{f}	77.90^{h}	0.88^{g}	14.06^{c}	6.61^{c}
T_3	0	24.4^{c}	89.2^{b}	1.46^{a}	20.68^{b}	12.00^{b}
	7	24.0^{c}	88.09^{b}	1.46^{a}	20.65^{b}	12.00^{b}
	14	24.0^{c}	83.91^{e}	1.46^{a}	20.65^{b}	11.90^{b}
	21	23.86^{d}	78.4^{h}	1.44^{a}	20.64^{b}	11.83^{b}
T_4	0	26.0^{a}	92.7^{a}	1.40^{b}	23.45^{a}	13.3^{a}
	7	26.0^{a}	92.07^{a}	1.40^{b}	23.41^{a}	13.3^{a}
	14	25.48^{b}	88.06^{c}	1.36^{c}	23.1^{a}	12.00^{a}
	21	23.3^{e}	85.00^{d}	1.33^{d}	23.01^{a}	12.6^{a}

To – Control group; T_1 – Yogurt with 5% sea buckthorn; T_2 – Yogurt with 10% sea buckthorn; T_3 – Yogurt with 15% sea buckthorn T_4 – Yogurt with 20% sea buckthorn; Mean carrying the different letter within the column differ significantly ($P<0.05$).

Microbial activity of sea buckthorn fortified yogurt

Maximum values were measured on the 0th and 21st days, respectively of the control group and T4 with 20% of SP. T1, T3 and T4 values 8.1, 8.7 and 9.4 remained same at 0 and 7th day and had slight rise at 14th day. The considerably increase measured in T2 with 10% of SP in yogurt. The results of present study have resembled to the (Meenakshi et al. 2018), the increase in microbial load is mainly due to the increase acidity of yogurt. Developed acidity over the storage period fluctuates the bacterial count. There is slight change in the microbial load during storage time which correlates with lowering in pH but in T4 the pH was 4 at the 21st day which is almost same as in other treatments initial time of storage cause the increase in the microbial load. This result has the similarity to the research of Amal et al. (2016). They also stated that the increase in the microbial count is due to the high polyphenolic contents present in the treatments.

Sensory evaluation of sea buckthorn fortified yogurt

Aesthetic appearance of a dairy product is very important in terms of food product quality. Globally, food products with stable sensory attributes during a particular storage time are preferred. Sensory analysis for the SP supplemented yogurt consisted of various characteristics i.e., taste, aroma, color, texture and overall acceptability. This examination was carried out over the storage period of 0, 7, 14 and 21 days using a 9-hedonic scale.

Color is a significant component in influencing the purchasing behavior of customers and consumers. The non-significant effect of treatment on the color can be seen in the mean values as it causes the decrease in scores from judges. The decrease in values during storage period is due to change in color which caused by the increase in acidity and fat, decrease in pH and moisture. Results of present study have resemblances with the study of Al-Shawi et al. (2020).

Then, taste is regarded as one of the most important components of sensory analysis in order to assure the high quality of a food product. According to the table there is decline trend over the period of time which is due to change in chemical composition. The drastic decrease in scores of T4 is maybe due to the high concentration of SP in yogurt which is acidic and sour in taste. The change in taste is due to the oxidation process during storage and most of the time many minerals exhibit metallic flavors during storage period (Barkallah et al. 2017).

Texture is another important factor of aesthetical attributes. Consumers always attract to textured products. The change in texture compare in control is due to the changed texture of pulp added in yogurt. Findings of present study has the similarities with Dabija et al. (2018).

Aroma is the typical pleasant and distinctive smell that is specific to certain food products. Many consumers reject the product without tasting it merely on the base of its odor. The 10 panellists gave scores to the different concentration of yogurt. The least scores give to the highest treatment at 0, 7, 14 and 21st day of storage. Outcomes of this study has the similarities with Gurkan and Hayaloglu (2017) who studied on the sensory effects of basil in yogurt.

Table 6. Sensory evaluation of sea buckthorn fortified yogurt

Treatments	Storage days	Color	Taste	Texture	Aroma	Overall acceptability	Total plate count
T_0	0	8^a	8^c	8^c	7^c	8^c	8.0^a
	7	8^a	8^b	8^c	7.7^c	8.0^c	8.0^a
	14	6.5^a	5.7^b	5.7^b	5.7^c	6^b	8.3^a
	21	$6d^a$	3.8^a	4.0^a	4.5^b	5.5^b	8^a
T_1	0	8^a	8.2^c	8.2^c	8.2^c	8.2^c	8^a
	7	7.7^a	7.0^b	7.2^c	7.0^c	7.2^c	8.1^a
	14	6^a	5.7^b	5.9^b	5.4^b	6.0^b	8.7^a
	21	$4b^a$	4.0^a	4.8^a	4.0^a	5.4^b	8.8^a
T_2	0	7.8^a	7.8^c	7.8^c	7.8^c	7.8^c	8.2^a
	7	7^a	7.2^b	7.2^c	4.2^b	7.2^c	8.2^a
	14	6.6^a	5.8^b	6.0^b	5.6^c	6.0^b	8.7^a
	21	4.0^a	4.0^a	4.1^a	4.3^a	5.4^a	8.9^a
T_3	0	7.9^a	7.8^c	7.9^c	7.9^c	7.8^c	8.7^b
	7	6.7^a	6.4^b	6.4^b	6.4^c	6.6^c	9.1^a
	14	6.0^a	6.0^b	6.0^b	5.6^b	6.0^c	9.1^a
	21	4.4^a	4.5^a	4.4^a	4.2^a	5.2^b	9.7^a
T_4	0	7.7^a	6.4^c	6.4^c	6.4^c	6.6^c	9.4^{ab}
	7	6^a	5.0^b	5.0^b	5.0^b	5.3^c	9.4^{ab}
	14	6.2^a	4.0^b	4.09^a	$4b^c$	4.5^b	9.4^{ab}
	21	3.6^a	3.3^a	3.3^a	3.3^a	3.8^a	9.7^a

To – Control group; T_1 – Yogurt with 5% sea buckthorn; T_2 – Yogurt with 10% sea buckthorn; T_3 – Yogurt with 15% sea buckthorn T_4 – Yogurt with 20% sea buckthorn; Mean carrying the different letter within the column differ significantly ($P<0.05$).

Acceptance of food is crucial from a marketing standpoint. Appropriateness and acceptability of food products affects consumer's choice. As a result of the fruit's high pulp content, T4 treatment has a non-significant acceptance. Other treatments got much better scores over the other characteristics. Least score was given to the T4. Outcomes of present study has the similarity with the research of Dabija et al. (2018) in which they examined the physicochemical properties of fruit supplemented yogurt with sea buckthorn powder, grape seed extract and rosehip powder separately. Al-Shawi et al. (2020) stated in the research of organoleptic and sensory study of yogurt with plant extract that over the storage period of time there was a significant change in texture and flavor of yogurt due to the change in chemical composition of yogurt.

Conclusions

Antioxidants are compounds that hinder, delay, or completely destroy free radical and oxidant processes. They contribute to the avoidance of oxidative stress and its consequences for human health. Antioxidants serve as a general defensive mechanism in both plant and animal cells and tissues. Sea buckthorn berries include a significant number of polyphenol components, particularly flavanols. Sea buckthorn has more vitamin C than other fruits, around 80 milligrams per 100 grams. Physiochemical, phytochemical determination, microbial analysis and sensory evaluation of raw pulp and yogurt were conducted. Among all treatments in physiochemical determination particularly pH and acidity were acceptable till 15% of supplementation during storage. In the treatment having 20% pulp showed high acidity and pH over the period of time which may lead to the high microbial load. The data indicate that moisture, pH, and syneresis have decreased, while protein, fat, ash, and acidity have increased. Phytochemicals indicate a steady state to the 14th day of storage, with a little drop on 21st days of storage. Panellists' ratings varied throughout the sensory examination. However, Food and Drug Administration (FDA) allows adding 15% of fruit to the yoghurt to get maximum of its potential as this study also favors to the percentage allowed by the FDA.

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