AATCC Review

08 October 2025: Received
18 December 2025: Revised
12 January 2026: Accepted
13 February 2026: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Evaluation of bioactive components and sensory parameters of encapsulated seabuckthorn and spirulina enriched functional bread

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ABSTRACT

The present study investigates the extraction and encapsulation of carotenoids from seabuckthorn powder and use in functional food applications along with spirulina powder. The obtained functional bread was characterized in terms of bioactive compounds and sensory properties with their storage. The carotenoids content, total phenol content, antioxidant activity, ascorbic acid, calcium and iron increase with the addition of spirulina powder and increase in concentration of seabuckthorn encapsulated carotenoids. The mean carotenoids content (5.07 mg per g), antioxidant activity (39.85 %) and total phenol (7.12 mg GAE per g) were found highest in treatment T_7 (82.00:15.00:03.00::Wheat flour:Encapsualted carotenoids:Spirulina powder). The carotenoids content, total phenol content, antioxidant activity, ascorbic acid, calcium and iron decrease during storage. Based on sensory evaluation of functional bread treatment T_7 (82.00:15.00:03.00::Wheat flour:Encapsualted carotenoids:Spirulina powder) was adjudged to be superior among all treatments for colour, appearance, texture, flavour and overall acceptability scores. The functional bread developed from encapsulated seabuckthorn and spirulina powder being rich in nutritional composition, can provide health benefits to consumers to combat the nutrient deficiencies and protein energy malnutrition.

Keywords: *Seabuckthorn, Carotenoids, Encapsulation, Bioactive components, Functional bread.*

INTRODUCTION

Hippophae rhamnoides L. (*Elaeagnaceae*), a thorny shrub commonly known as seabuckthorn is native to the cold-temperate regions of Europe and Asia (1). In India, seabuckthorn is widely distributed in Ladakh region of J&K followed by Uttrakhand, Himachal Pradesh and North east region. The berries of seabuckthorn are among the most nutritious vitamin and bioactive compound rich fruits found in the plant kingdom and have been referred to as ''Super Healthy Fruits'' (2). Carotenoids stand as valuable bioactive constituents in seabuckthorn which are associated with a plethora of health effects in humans and animals, acting as antioxidants, modulators of the immune systems, provitamin A activity, eye health, anticancer and so on. However, carotenoids are prone to oxidation, to chemical degradation (acidic environment), and have relatively low bioavailability (3) and the direct incorporation of plant extracts in processed food is challenging. The encapsulation technique is the best approach to increase their stability and bioavailability (4).

Spirulina which is known worldwide for its tremendous nutritional contents is a multicellular, filamentous, free-floating cyanobacterium or photosynthetic blue green. Spirulina was originally harvested from Africa and Mexico lakes, dried and consumed as food since long time, but gained more prominence recently after it was used for astronauts on space missions as a dietary supplement. *Spirulina platensis* contains highly valuable bioactive compounds and possess therapeutic potential like antioxidants, anti-inflammatory, anticancer, antifungal, antibacterial, antiviral, anti-allergic, chemo-preventive, hypoglycemic and antidiabetic properties (5). It stimulates the immune system and lower cholesterol (6). United Nation in its World Conference 1974 hailed spirulina as the possible 'Food of the Future' (7) and FDA (USA) approved and considered it as GRAS (Generally Recognized as Safe) without toxicological effects.

In recent years, the global situation on food and nutrition consumption has changed and consumers are becoming more health conscious and demand foods that are rich in micronutrients and bioactive components with potential health benefits. Since most of the baked goods including bread are prepared from refined flours and are lack in most of the vital nutrient like vitamins, minerals, protein and critical amino acids like lysine making them unable to satisfy the consumer demand (8). The growing demand of consumers derived towards development of functional foods that enable the presence of required compounds in the final product. Bread is one of the most advantageous matrices for delivering bioactive chemicals to consumers since it is widely consumed staple food throughout the globe. The functional bread develops from seabuckthorn and spirulina have the potential to enrich the nutritional value, widen the food applications and provide the health advantages for customers to combat the micronutrient deficiencies and protein energy malnutrition. Thus, the purpose of the study was to extract bioactive component from seabuckthorn, encapsulation and utilization along with

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DOI: https://doi.org/10.21276/AATCCReview.2026.14.01.93
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spirulina to produce functional bread and to evaluate the bioactive compounds as well as to determine its preference by consumers.

MATERIAL AND METHODS

Location of work done

The experiment was carried out in the Division of Food Science and Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, India.

Raw materials

Seabuckthorn was procured from Ladakh and Spirulina was purchased online. Refined flour and other raw materials were purchased from local market of Jammu.

Preparation of functional bread

Carotenoids form seabuckthorn was extracted by green extraction method using olive oil and domestic microwave oven. Dried seabuckthorn powder was weighed and mixed with green solvent olive oil in the ratio of 1:10(w/v) and extracted using domestic microwave with alternate 30s on and 30s off for 10 min. Then it was centrifuged at 3500 rpm for 30 min and the result extracts were encapsulated by using 2% sodium alginate and 5% calcium chloride, forming encapsulated beads. Then the beads were washed three time with distilled water and dried in hot air oven at 35°C for 24hrs. In order to improve the nutritional quality of bread, wheat flour was supplemented with the best-formulated seabuckthorn encapsulates and spirulina powder in the ratio as shown in (Table 1). As per the formulation of bread all the ingredients required for bread preparation was measured yeast (5 g), sugar (10 g), salt (1.5 g), oil (10ml), water, flour and supplements as per the treatment for the development of dough. The flour was sieved and yeast was activated by putting for 5 min in warm water with dissolve sugar. After that all the ingredients were mixed and kneaded for 20 minutes to make a soft dough. After kneading the dough was kept for fermentation at a temperature of 27 °C for a period of 30 minutes. The dough was kept for intermediate proofing at a temperature of 35°C for 30 min. After proofing the dough was divided manually and moulded in a rectangular greased mould pan. The dough was then final proofed at temperature of 35°C for 35 min to give volume to bread. After final proofing the bread was kept in preheated oven and baked at a temperature of 180 °C for 30 minutes. Bread was depanned after baking and cooled down to room temperature to facilitates slicing and to prevent the condensation of moisture in wrapper, Figure 1 and 2. The prepared breads were then packed in LDPE bags and stored for further analysis.

Table 1: Development of bread food from encapsulated seabuckthorn and spirulina powder

Treatments	Wheat flour (g)	Encapsualted seabuckthorn (g)	Spirulina powder (g)
T_1	100.00	00.00	00.00
T_2	94.50	02.50	03.00
T_3	92.00	05.00	03.00
T_4	89.50	07.50	03.00
T_5	87.00	10.00	03.00
T_6	84.50	12.50	03.00
T_7	82.00	15.00	03.00

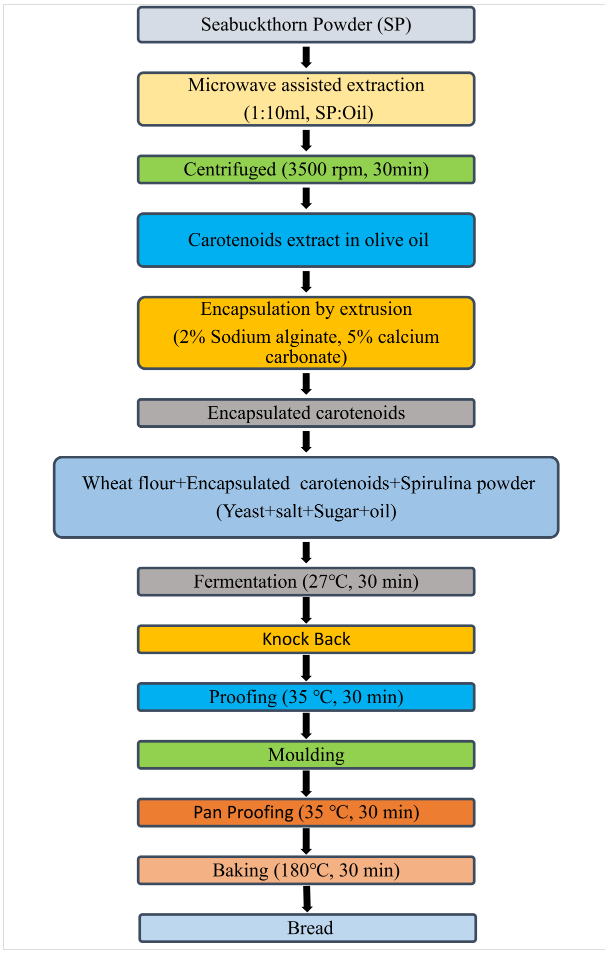

Figure 1: Flowsheet for formulation of seabuckthorn encapsulated carotenoids and spirulina powder enriched functional bread

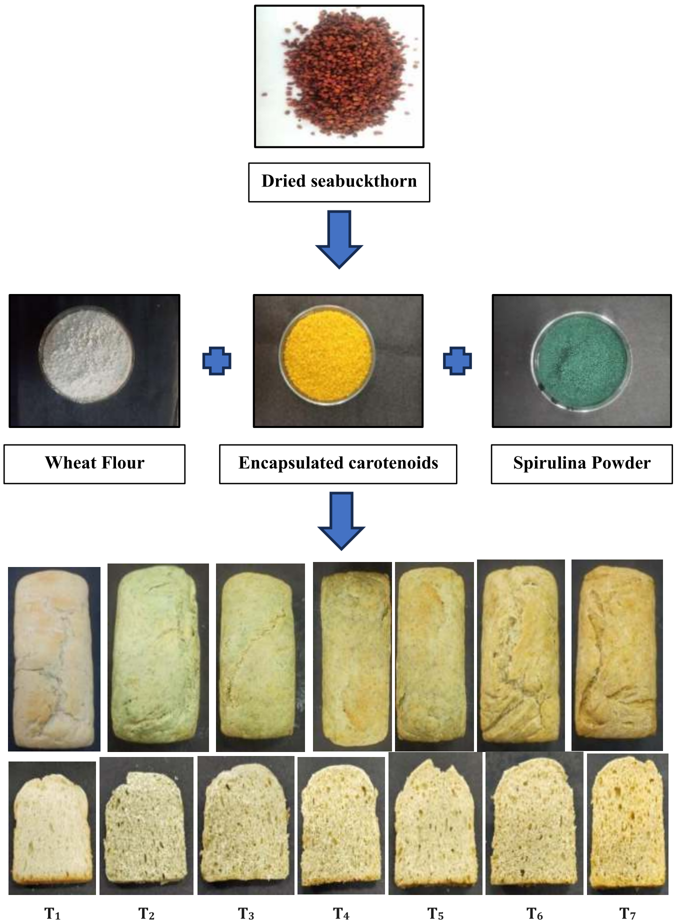

Figure 2: Formulation of Functional bread

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Method of analysis

Total carotenoid content of encapsulated seabuckthorn carotenoid and spirulina enriched functional bread were analyzed by employing the methodology of (9). Total carotenoid content (TCC) of these samples were then calculated by the following equation suggested by a study (10) using the specific coefficient. The total phenolic content of sample was estimated by Folin- Ciocalteau (FC) method (11). The free radical scavenging activity of sample was analyzed by the method described by (12) and the per cent inhibition of DPPH was calculated. Ascorbic acid/Vitamin C of encapsulated seabuckthorn carotenoid and spirulina enriched functional bread was estimated by method as described by (13). Calcium and iron contents of encapsulated seabuckthorn carotenoid and spirulina enriched functional bread were analyzed by following the procedure given by (14). The developed products was evaluate for its sensory parameters like colour, taste, aroma, texture and over all acceptability by panel of 10 judges using the 9 point hedonic rating scale (scores assigned as 9 "like extremely" to 1 "dislike extremely"). The nutritional compositions and sensory qualities data were statistically analyzed by using Factorial Completely Randomized Design (FCRD) methods to see the significant and non-significant differences among them.

RESULTS AND DISCUSSION

The result of total carotenoid content of functional bread is shown in Table 2. It has been found that the mean total carotenoid content increased significantly from 0.24 mg per g in T_1 (100:00:00::WF:SE:SP) to 5.07 mg per g in Treatment T_7 (82.00:15.00:03.00::WF:SE:SP) with increased in encapsulated seabuckthorn and spirulina powder. Roman *et al.* (4) also stated an increase in total carotenoids with the increases in the concentration of seabuckthorn encapsulate microcapsules. Ursache *et al.* (15) stated more total carotenoids in muffins containing encapsulated seabuckthorn extract powder than in the control sample (muffins). Gurung *et al.* (16) also revealed increase in beta carotene content of pumpkin puree fortified biscuits. During storage periods of 21 days, the mean total carotenoid content decreased from 4.16 to 4.07 mg per g which might be due to the oxidative degradation of colour pigment. Thakur, (17) reported a decrease in beta carotenoids in crackers from ripe pumpkin (*Cucurbita moschata*) during three months of storage.

The data reported in Table 2 showed that the addition of spirulina powder and encapsulation seabuckthorn enhance total phenol content significantly in functional bread. The maximum mean value of 7.12 mg GAE per g was recorded in Treatment T_7 (82.00:15.00:03.00::WF:SE:SP) and minimum of 3.20 mg GAE per g in T_1 (100:00:00::WF:SE:SP). Saharan and Jood, (18) reported an increase in total phenolic content with increase in spirulina powder in breads (dry matter basis). The results of present study are supported by (19). They observed that polyphenols of bread incorporated with seabuckthorn pomace powder was higher than that of the control. During 21 days of storage, the mean total phenol content significantly decreased which might be due to oxidative degradation of total phenol. Rafiq (20) observed decrease in mean total phenol content of encapsulated peel extract soup stick during 180 days of storage.

The functional bread with highest encapsulated seabuckthorn concentration shows highest antioxidant activity than the control bread (Table 3).

The highest mean antioxidant content of 39.85 per cent was recorded in Treatment T_7 (82.00:15.00:03.00::WF:SE:SP) whereas, lowest of 33.85 per cent in T_1 (100:00:00::WF:SE:SP). Saharan and Jood, (18) reported an enhance in antioxidant activity with the addition of spirulina powder in formulation of breads (dry matter basis). Ursache *et al.* (15) found more antioxidants in muffins containing encapsulated seabuckthorn extract powder than in the control sample (muffins). Roman *et al.* (4) reported an increase in antioxidant activity with increase in the concentration of seabuckthorn microcapsules. The results are also in agreement with Stanciu *et al.* (19) who noticed that antioxidant activity value of bread incorporated with seabuckthorn pomace powder was higher in comparison to the control sample (bread). During 21 days of storage, the antioxidant activity of functional bread significantly decreased which might be due to oxidation. Choskit, (21) found decrease in antioxidant activity of seabuckthorn blended cookies during 90 days of storage.

The ascorbic acid content increased with the incorporation of spirulina powder and encapsulated carotenoids in bread which is due to higher ascorbic acid content in seabuckthorn and spirulina as compared to wheat flour (Table 3). The highest mean ascorbic content of 9.72 mg per 100 g was noted in Treatment T_7 (82.00:15.00:03.00::WF:SE:SP) whereas, lowest of 0.45 mg per 100 g in T_1 (100:00:00::WF:SE:SP). Similar findings were reported by (22) in seabuckthorn cookies and (16) in pumpkin puree fortified biscuits and (21) in seabuckthorn powder incorporated cookies. There was significant decrease in ascorbic acid content from initial mean value of 5.88 mg per 100 g to 5.30 mg per 100 g during 21 days of storage which may be attributed to its oxidation to dehydro-ascorbic acid during storage. Such trend of decrease in ascorbic acid during storage was noticed by (23) in apricot blended crackers.

Table 4 illustrated the calcium and iron content of spirulina powder and encapsulated seabuckthorn carotenoids supplemented functional bread. The highest mean calcium and iron content was found in the functional bread formulated with the highest concentration of encapsulated seabuckthorn carotenoids. Similar results were suggested by (19) in bread incorporated with seabuckthorn pomace powder and (24). During a storage period of 21 days the mean calcium and iron content of functional bread decreases which might be due to their interaction with other components like carbohydrates and proteins (25). Singh, (26) found that after 21 days of storage, the calcium and iron content of pearl millet cake significantly decreased.

Fig. 3. reflected the sensory acceptability of encapsulated seabuckthorn and spirulina incorporated functional bread by the panelists. With the addition of spirulina and increased amount of encapsulated seabuckthorn supplementation there was increased in colour, texture, taste, aroma and overall acceptability. Treatment T_7 (82.00:15.00:03.00::WF:SE:SP) exhibited the highest mean colour, taste, texturr, aroma and overall acceptability score of 7.41, 8.33, 8.38, 7.33 and 7.86, while Treatment T_1 (100:00:00::WF:SE:SP) recorded the lowest score of 6.59, 6.68, 6.86, 6.58 and 6.68, respectively. During storage period of 21 days, the mean colour, tastes, texture, aroma and overall acceptability scores decreased from 7.49 to 6.67, 7.90 to 7.18, 8.29 to 7.34 ,7.64 to 6.56 and 7.86 to 6.94, respectively. Similar trend of sensory scores were also suggested by (27), (28), (18) and (21).

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Table 2: Effect of treatments and storage period on carotenoids content (mg/g) and total phenol content (mg/100g) of functional bread

Treatments	Carotenoids content (mg/g)				Mean (Treatment)	Total phenol content (mg GAE/g)				Mean (Treatment)
	Storage (days)					Storage (days)				
	0	7	14	21		0	7	14	21	
T_1 (100:00:00::WF:SE:SP)	0.30	0.24	0.23	0.19	**0.24**	3.98	3.19	2.97	2.67	**3.20**
T_2 (94.50:02.50:03.00::WF:SE:SP)	4.52	4.45	4.44	4.41	**4.45**	4.79	4.01	3.51	3.31	**3.90**
T_3 (92.00:05.00:03.00::WF:SE:SP)	4.64	4.60	4.58	4.55	**4.59**	5.31	4.61	3.80	3.61	**4.33**
T_4 (89.50:07.50:03.00::WF:SE:SP)	4.74	4.72	4.69	4.66	**4.70**	5.86	5.21	5.05	4.88	**5.25**
T_5 (87.00:10.00:03.00::WF:SE:SP)	4.87	4.83	4.80	4.78	**4.82**	6.41	5.81	5.49	5.33	**5.76**
T_6 (84.50:12.50:03.00::WF:SE:SP)	4.98	4.95	4.91	4.89	**4.93**	6.84	6.34	6.10	5.96	**6.31**
T_7 (82.00:15.00:03.00::WF:SE:SP)	5.11	5.09	5.06	5.05	**5.07**	7.51	7.11	7.01	6.88	**7.12**
Mean (Storage)	**4.16**	**4.12**	**4.10**	**4.07**		**5.81**	**5.18**	**4.84**	**4.66**	

Effects	$C.D_{(p\leq0.05)}$	$C.D_{(p\leq0.05)}$
Treatment (T)	**0.01**	**0.02**
Storage (S)	**0.01**	**0.01**
Treatment x Storage	**0.03**	**0.05**

WF=Wheat flour SE= Seabuckthorn encapsulated carotenoids SP= Spirulina powder

Table 3: Effect of treatments and storage period on antioxidant activity (%) and ascorbic acid content (mg/100g) of functional bread

Treatments	Antioxidant activity (%)				Mean (Treatment)	Ascorbic acid (mg/100g)				Mean (Treatment)
	Storage (days)					Storage (days)				
	0	7	14	21		0	7	14	21	
T_1 (100:00:00::WF:SE:SP)	34.62	34.12	33.74	32.93	**33.85**	0.67	0.53	0.38	0.25	**0.45**
T_2 (94.50:02.50:03.00::WF:SE:SP)	36.63	36.25	35.90	35.15	**35.98**	3.44	3.17	3.02	2.89	**3.13**
T_3 (92.00:05.00:03.00::WF:SE:SP)	37.35	37.02	36.69	35.72	**36.69**	4.77	4.52	4.38	4.17	**4.46**
T_4 (89.50:07.50:03.00::WF:SE:SP)	38.05	37.71	37.40	36.75	**37.47**	6.09	5.83	5.68	5.48	**5.77**
T_5 (87.00:10.00:03.00::WF:SE:SP)	38.76	38.48	38.18	37.68	**38.27**	7.42	7.18	7.06	6.92	**7.14**
T_6 (84.50:12.50:03.00::WF:SE:SP)	39.47	39.23	38.94	38.55	**39.04**	8.75	8.5	8.34	8.07	**8.41**
T_7 (82.00:15.00:03.00::WF:SE:SP)	40.21	40.01	39.73	39.47	**39.85**	10.07	9.8	9.63	9.38	**9.72**
Mean (Storage)	**37.87**	**37.54**	**37.22**	**36.60**		**5.88**	**5.64**	**5.49**	**5.30**	

Effects	$C.D_{(p\leq0.05)}$	$C.D_{(p\leq0.05)}$
Treatment (T)	**0.04**	**0.01**
Storage (S)	**0.03**	**0.01**
Treatment x Storage	**0.08**	**0.02**

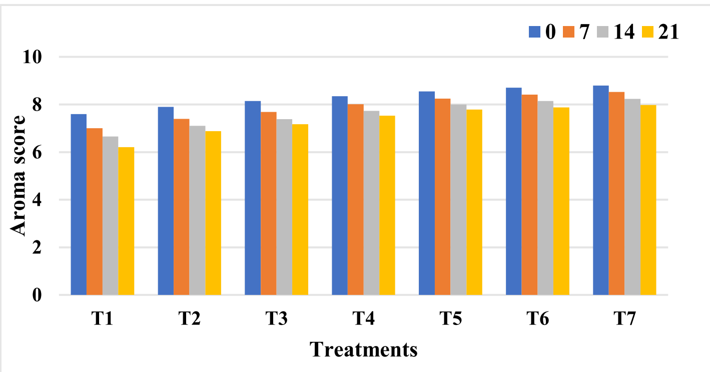
WF=Wheat flour SE= Seabuckthorn encapsulated carotenoids SP= Spirulina powder

Table 4: Effect of treatments and storage period on calcium and iron content (mg/100g) of functional bread

Treatments	Calcium content (mg/100g)				Mean (Treatment)	Iron content (mg/100g)				Mean (Treatment)
	Storage(days)					Storage(days)				
	0	7	14	21		0	7	14	21	
T_1 (100:00:00::WF:SE:SP)	47.10	46.76	46.29	45.97	**46.53**	3.07	2.76	2.26	2.00	**2.52**
T_2 (94.50:02.50:03.00::WF:SE:SP)	50.01	49.70	49.32	49.03	**49.51**	4.18	3.87	3.49	3.27	**3.70**
T_3 (92.00:05.00:03.00::WF:SE:SP)	51.21	50.98	50.33	50.07	**50.64**	4.39	4.16	3.57	3.38	**3.87**
T_4 (89.50:07.50:03.00::WF:SE:SP)	52.71	52.56	52.13	51.93	**52.33**	4.70	4.50	4.07	3.92	**4.29**
T_5 (87.00:10.00:03.00::WF:SE:SP)	54.51	54.36	53.65	53.49	**54.00**	5.00	4.85	4.25	4.10	**4.55**
T_6 (84.50:12.50:03.00::WF:SE:SP)	56.52	56.37	55.88	55.74	**56.12**	5.35	5.19	4.74	4.61	**4.97**
T_7 (82.00:15.00:03.00::WF:SE:SP)	58.82	58.69	58.24	58.11	**58.46**	5.79	5.66	5.26	5.14	**5.46**
Mean (Storage)	**52.98**	**52.77**	**52.26**	**52.04**		**4.64**	**4.42**	**3.94**	**3.77**	

Effects	$C.D_{(p\leq0.05)}$	$C.D_{(p\leq0.05)}$
Treatment (T)	**0.04**	**0.02**
Storage (S)	**0.03**	**0.01**
Treatment x Storage	**0.09**	**0.03**

WF=Wheat flour SE= Seabuckthorn encapsulated carotenoids SP= Spirulina powder



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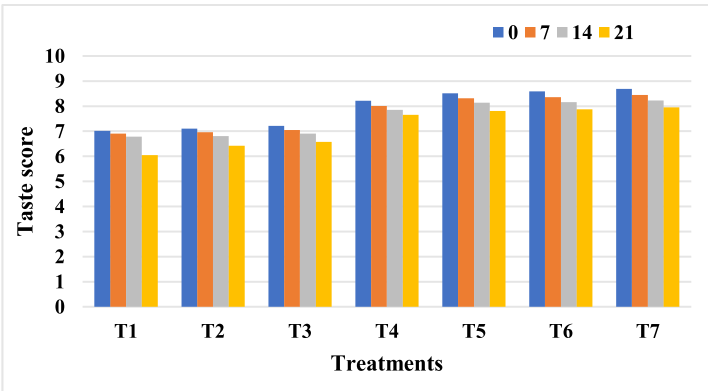

b)

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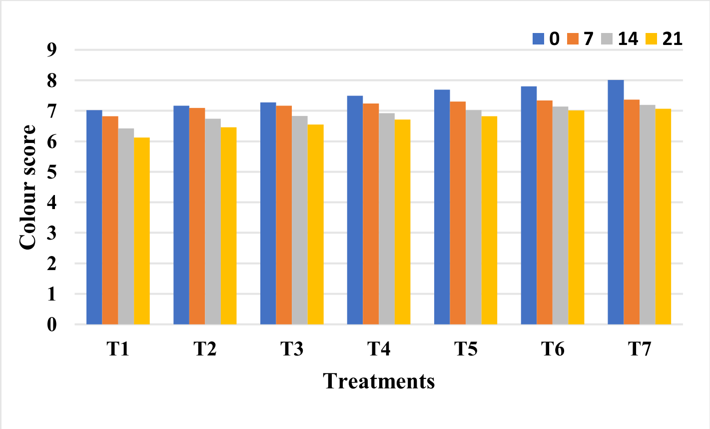

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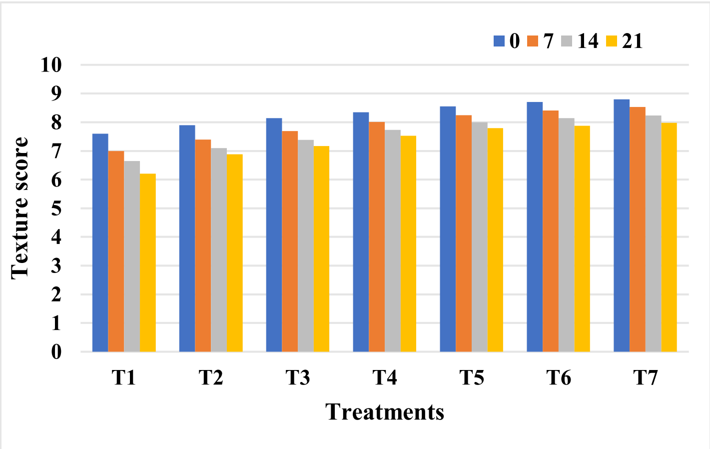

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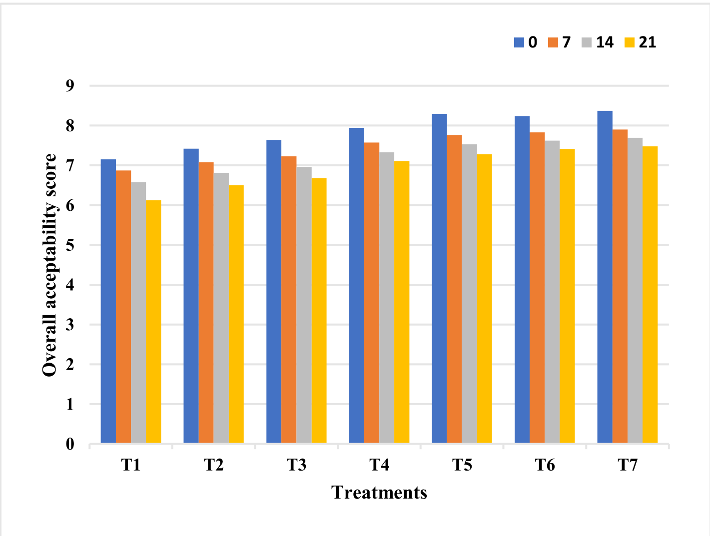

e)

Figure 3: Effect of treatment and storage period on sensory score of functional bread

CONCLUSION

It was concluded that for the development of a consumer acceptable and nutritious functional bread wheat flour, encapsulated carotenoids and spirulina powder can be blended in the ratio of 82:15:3. The incorporation of encapsulated seabuckthorn and spirulina powder enhanced the bioactive components of functional bread. The mean carotenoids content (5.07 mg per g), antioxidant activity (39.85 %) and total phenol (7.12 mg GAE per g) were found highest in treatment T_7 (82.00:15.00:03.00::Wheat flour:Encapsualted carotenoids:Spirulina powder).

The functional bread developed from encapsulated seabuckthorn and spirulina powder being rich in nutritional composition, can provide health benefits and a good source of diet for vulnerable groups to overcome the nutrient deficiencies.

FUTURE SCOPE OF STUDY

Seabuckthorn and spirulina being a rich source of nutrient and potential health benefit can be further explored for development of different value-added products. The extraction of bioactive compounds using oils as solvent can be further study for extraction of different bioactive compounds.

CONFLICT OF INTEREST

There is no conflict of interest

ACKNOWLEDGMENT

The authors wish to express heartfelt gratitude to the Ministry of Tribal Affairs, Government of India for NFST-fellowship and SKUAST-Jammu.

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