Original Research Article

Utilization of Encapsulated Seabuckthorn and Spirulina for the Development of Functional Bread

ABSTRACT

Bread is one of the staple foods and largely consumed throughout the globe. Seabuckthorn (*Hippophae rhamnoides L.*) is rich in nutrients and bioactive compounds possess numerous health benefits. Spirulina also known as 'Food of the future' is source of good quality protein, fiber and other nutrients. The aim of the study was to develop bread from encapsulated seabuckthorn and spirulina powder and to evaluate the proximate composition of bread. The result showed that the colour L*, a* and b* value of bread increases with increases encapsulated seabuckthorn at 2.5, 5, 7.5, 10, 12.5 and 15%. The loaf weight and volume also increase while the specific volume and crust to crumb ratio decreases with addition of encapsulated seabuckthorn (2.5, 5, 7.5, 10, 12.5 and 15%) and spirulina powder (3%) in the bread. The addition of encapsulated seabuckthorn (2.5, 5, 7.5, 10, 12.5 and 15%) and spirulina powder (3%) in the bread increased the crude protein content, ash, crude fat, crude fiber, carbohydrate whereas the moisture, water activity and energy decreased. The study has shown that the functional bread can be developed from encapsulated seabuckthorn and spirulina powder which would enhance the nutrition and prevent malnutrition.

Keywords: Functional bread, Seabuckthorn, Spirulina, Protein, Proximate composition

1. INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides L.*) is an ecologically and economically important thorny shrub, which is widely distributed in various regions of Asia, Europe and North America (Perino-Issartier *et al.,* 2011). Ladakh remains the major site for natural seabuckthorn resource with over 70% of the total area in the country followed by Uttrakhand, Himachal Pradesh and North east region. Seabuckthorn berries locally known as, Tses-ta-lulu, are among the most nutritious of all fruits with 11,500 ha under pure Seabuckthorn vegetation and around 30,000 ha in the mixed forest cultivation/cover with Willow (Salix), Poplar (Populus) etc. in transHimalayan Ladakh region (Tamchos and Kaul, 2019). Sea buckthorn has been valued as a medicinal and edible plant that is rich in nutrients and bioactive compounds since ancient time and its development and use are of great significance (Lyu *et al.,* 2021). The seabuckthorn formulations are often prescribed by local *Amchi* for treatment of health issues like indigestion, throat infection, gynecological problem, ulcer, gastritis, bronchitis, acidity, diarrhea, hypertension, blood disorder, fever, tumor, gallstone, cough, cold, food poisoning etc. (Stobdan *et al.,* 2013). Despite being socio-economically highly valuable and major site of seabuckthorn resources, it is used to a limited extent by the locals and its potential for sustainable utilizationis still not realized to the fullest.

Spirulina is a multicellular, filamentous, free-floating cyanobacterium or photosynthetic blue green known to be the richest source of nutrient like protein, fibers, vitamins and minerals. It can be potentially used as a functional food and supplements that are safe to consume in the right amount as humans foods (Liestianty *et al.*, 2019) and thus considered as "Food of the future" and an ideal food for astronauts (Joshi *et al.,* 2014). United Nation has hailed Spirulina as the possible 'food of the future' in its World Conference held during 1974 (Pundir, 2023) and it is considered as generally recognized as safe (GRAS) for consumption without toxicological effects and approved by FDA (USA) (Ahmed *et al.* 2023). *Spirulina platensis* is commonly produced and cultivated in Asian countries particularly in India, Japan and China (Moorhead *et al.,* 2011). Spirulina content highly valuable bioactive compounds provide countless health benefits including antioxidants, anti-inflammatory, anticancer, antifungal, antibacterial, antiviral, anti-allergic, hypoglycemic and antidiabetic properties (Saraswathi and Kavitha, 2023). It also lowers the cholesterol and boost the immune system (Mohan *et al.*2014). It can be used to treat children suffering from malnutrition. It is an excellent source of good quality protein (60-70%) with all essential amino acids in perfect balance and also provide high concentration of minerals and B-complex vitamins specially vitamin B12 which is usually found in animal tissues (Saharan and Jood, 2017). Spirulina also prevent and slows down the progression of neurodegenerative diseases like Alzheimer's and Parkinson's (Trotta *et al*. 2022).

Now a days people are demanding new food products with functional properties and bread is one of the staple foods and largely consumed throughout the globe (Popa *et al.,* 2022). Most of the baked products are made from refined flours which lack important nutrients (proteins, fiber, etc) and the most consumed on daily basis is white bread made from refined flour (Akbas and Kilmanoglu, 2022). Bread being consumed widely represents a good product for addition of various highly nutritious ingredients for consumer health benefits (Villasante *et al.,* 2022). Development of bread from encapsulated seabuckthorn and spirulina powder will enrich the nutritional value, widen the food applications and provide the health benefits to consumers to combat protein energy malnutrition and the nutrient deficiencies. The present study was conducted to investigate the use of encapsulated seabuckthorn and spirulina powder for the development of functional bread and to evaluate the proximate composition of bread.

2. MATERIAL AND METHODS

Carotenoids from seabuckthorn was extracted by green extraction method using olive oil and microwave assisted extraction (MAE). 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 order to improve the nutritional quality of bread, wheat flour was supplemented with the encapsulated seabuckthorn 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 (1.5 g), sugar (2.5 g), salt (2 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 in warm water with dissolve sugar for 5 min. 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 ℃ for a period of 30 minutes. During the process of fermentation knock backing was done to reduce the alcohol production and retain CO_2 and increase in gas retention capacity. The temperature rise due to knock back. The dough was kept for intermediate proofing at a temperature of 35℃ 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℃ 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 ℃ for 30 minutes. Bread was depanned after baking and cooled down to room temperature to facilitate slicing and to prevent the condensation of moisture in wrapper. 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)	Encapsulated seabuckthorn (g)	Spirulina powder (g)
$\mathbf{T_1}$	100.00	00.00	00.00
$\mathbf{T_2}$	94.50	02.50	03.00
$\mathbf{T_3}$	92.00	05.00	03.00
$\mathbf{T_4}$	89.50	07.50	03.00
$\mathbf{T_5}$	87.00	10.00	03.00
$\mathbf{T_6}$	84.50	12.50	03.00
$\mathbf{T_7}$	82.00	15.00	03.00

2.1 Colour (L*, a*, b*): Colour measurement of the sample was done by using Hunter Lab colour analyzer (Hunter Lab Color Flex Reston VA, USA-45/0) according to the method of Grabowski *et al.* (2006).

2.2 Loaf Weight: The loaf weight was measured directly by using weighing balance.

2.3 Loaf volume: The loaf was cooled for 3 hrs and loaf volume was measured using the rapeseed displacement method. The loaf was put in a container, filled with rapeseed and the volume of seed displaced by loaf was measured as loaf volume.

2.4 Specific volume: The specific volume of bread was calculated according to (AACC, 2000) method by dividing volume (ml)by weight (g)

Loaf specific volume(ml/g) = Loaf volume(ml)/ Loaf weight(g)

2.5 Crumb to crust ratio: It was be determined by separating crust and crumb using sharp blade and weighing each component (Barett *et al.*, 2005)

2.6 Proximate composition: Moisture content, Crude fat, Crude protein, Crude fiber, total ash and Total carbohydrates were determined according to the method described in AOAC (2012).

2.7 Total Energy(kcal): The total energy value of the bread formulation was calculated according to (Ak *et al.* 2016) using the formula as shown in the following equation:

Total energy (kcal/100 g) = [(% available carbohydrates×4) + (% protein×4) + (% lipid×9)]

2.8 Statistical analysis: The data obtained were expressed as the mean value of three replications and were statistically analyzed by Duncan test ($p < 0.05$) using IBM SPSS Statistics 23.

3. RESULT AND DISCUSSION

3.1 Physical properties of bread

The data in Table 2. depicts the Physical characteristics of bread. Regarding colour characteristics, the values of L* (lightness), a* (redness) and b(yellowness) varied between samples. The lightness of the samples containing encapsulated seabuckthorn and spirulina powder were lower compared to control sample, which is due to the addition of the seabuckthorn and spirulina powder which changed the colour of the bread to a darker tone. In contrast, the redness and yellowness of the samples presented higher values for 5% SB and 9% SB (Popa *et al.,* 2022). Similar result were also reported by Gani *et al.* (2021) and Lee and Kim (2020) in saffron and seabuckthorn bioactive encapsulated cookeis and seabuckthorn berry powder added white pan bread, respectively. Obtained results for the physical properties of bread found to be in close agreement with the result of Gökmen *et al.* (2011) who reported increased bread weight and decreased bread volume of breads incorporated with the particles of nano-capsulated flax seed oil. Similarly, Gangakhedkar, (2021) reported increase in loaf weight, decrease in loaf volume and specific volume on incorporation of bitter gourd powder in bread and Nazir, (2018) reported a decrease in crust to crumb ratio from 0.271 to 0.233 in bread supplemented with stabilized rice bran. Our result is also supported by Puramwar, (2013).

Table 2: Physical composition of encapsulated seabuckthorn and spirulina incorporated bread

Treatments	L*	a*	b*	Loaf weight (g)	Loaf Volume (ml)	Specific loaf volume (ml/g)	Crust to crumb ratio
T1 (WF:SE:SP::	75.40^{de}	1.70^{a}	20.01^{ab}	144.00^{a}	548.64^{g}	3.81^{g}	0.270^{e}

100:0:0)							
T2(WF:SE:SP::94.50: 02.50: 03.00)	74.40^{a}	1.72^{a}	19.77^{a}	145.20^{b}	514.08^{f}	3.54^{f}	0.265^{de}
T3(WF:SE:SP:: 92.00: 05.00: 03.00)	74.63^{ab}	1.75^{ab}	20.02^{ab}	146.40^{c}	458.23^{e}	3.13^{e}	0.254^{cd}
T4(WF:SE:SP:: 89.50: 07.50: 03.00)	74.86^{bc}	1.85^{abc}	20.28^{bc}	148.10^{d}	429.49^{d}	2.90^{d}	0.249^{bc}
T5(WF:SE:SP:: 87.00: 10.00:03.00)	75.07^{cd}	1.94^{abc}	20.54^{cd}	150.0^{e}	343.50^{c}	2.29^{c}	0.247^{bc}
T6(WF:SE:SP:: 84.50:12.50:03.00)	75.29^{de}	2.04^{bc}	20.80^{de}	151.80^{f}	333.96^{b}	2.20^{b}	0.239^{ab}
T7(WF:SE:SP:: 82.00:15.00:03.00)	75.49^{e}	2.15^{c}	21.05^{e}	153.70^{g}	322.77^{a}	2.10^{a}	0.233^{a}

WF=Wheat flour SE= Encapsulated seabuckthorn SP= Spirulina powder

The values are given as mean value of three replications. The values followed by different superscripts (a–f) within the same column are significantly different ($p < 0.05$) from each other. Similar letters (superscripts) indicate values that are not significantly different ($p < 0.05$) from each other.

3.2 Proximate composition of bread

With the incorporation of seabuckthorn encapsulates and spirulina powder, there was decrease in the moisture content in all treatments. The highest mean moisture content of 33.86 per cent was recorded in treatment T1 control bread and lowest of 28.21 per cent in treatment T7 (82:15:3:: WF:SE:SP) (Table 3). Similar findings have been reported by Stanciu *et al.* (2023) in bread enriched using organic sea buckthorn pomace and Nilova and Malyutenkova (2021) in bakery products obtained by adding marc powder obtained from blueberry, cloudberry, rowan, and sea buckthorn. Furthermore, Sturza *et al.* (2016) explained that the addition of seabuckthorn flour led to a decrease in the amount of wet gluten in the pastry products, which resulted in a reduction in moisture in the final product.

The highest mean water activity was recorded in treatment T1 control bread and lowest treatment T7 (82:15:3:: WF:SE:SP) (Table 3). With the incorporation of sea buckthorn encapsulates and spirulina powder, there was decrease in the water activity content in all treatments. The water activity index of bread with additives was lower compared to the control Shevchenko *et al.* (2024). Popa *et al.* (2022) reported that water activity values of the tested samples are lower for the samples added with seabuckthorn powder (5% SB 0.946 ± 0.001 and

9%SB 0.945 ± 0.002) compared to Control sample (0.949 ± 0.010). Similar result was also reported by (Lim *et al.*, 2011; Costa *et al.*, 2018; Rajeswari *et al.*, 2018; Nikolau *et al.*, 2022).

The functional bread treatment (T_2,T_3,T_4,T_5,T_6 and T_7) content more crude protein than the control bread treatment (T_1) could be due to the significant quantity of protein in spirulina (Achour *et al.* 2014) and also valuable in malnutrition and immune support (Verma *et al.* 2024). The decrease of protein content in treatments is due to low protein content in seabuckthorn pulp as compared to wheat flour and buckwheat flour (Katoch, 2001). Stanciu *et al.* (2023) reported an increase in protein of functional bread with seabuckthorn pomace powder as compare to control bread. Ewunetu *et al.* (2023) revealed that crude protein, value decrease with increases addition of banana and carrot flour in bread as compare to control. Ursache *et al. (*2018) reported that muffin with microencapsulated powder content protein 10.98% as compare to control muffin which content protein 9.97%. Similarly, Nassar *et al.* (2008) reported in orange pulp biscuits and Peter Ikechukwu *et al.* (2017) in cookies prepared with date palm fruit pulp.

The data in Table 3 depicted that the fat content of bread increased significantly in all the treatments. Katoch, (2001) explained that increase in crude fat content might be due to the good source of fat content in sea buckthorn pulp as compared to wheat and buckwheat flour. Stanciu *et al.* (2023) reported an increase in fat of functional bread with seabuckthorn pomace powder as compare to control bread. Similar results were reported by Ayo and Gidado, (2017) in acha-carrot blend biscuit and Ewunetu *et al.* (2023) in banana and carrot flour bread. Ursache *et al. (*2018) reported that muffin with microencapsulated powder content fat 21.20%, as compare to control muffin which content fat 19.90%. Similar results was also observed by (Olaitan *et al.*, 2017; Azeez *et al*., 2021; El-Hadidy and Dreny, 2020).

The data reported in Table 3 showed that crude fiber increased in all treatments. Stanciu *et al.* (2023) reported an increase in crude fiber of functional bread with seabuckthorn pomace powder as compare to control bread. Almeida *et al.* (2021) observed an increased in crude fibre in sauce content 4% spirulina as compare to control. Similar finding were reported by Ayo and Gidado (2017) in acha-carrot blend biscuit, Rani *et al.* (2020) in orange peel powder cake and Tata *et al.* (2023) in raphia palm fruit pulp powder biscuits. Our results were also support by (Shabeer *et al.*, 2016 ;Ursache *et al.*, 2018; Ewunetu *et al.*, 2023).

The increasing trend in ash content (Table 3) might be due to high minerals in sea buckthorn and spirulina as compared to wheat flour. Similar results have been reported by Asefa *et al.* (2017) cookies prepared with mango pulp and Ayo and Gidado (2017) in acha-carrot blend biscuit. Ewunetu *et al.* (2023) revealed that ash content decrease with increases addition of banana and carrot flour in bread as compare to control. Akbaş and Kılmaoğlu *et al.* (2022) shows that ash content is more in bread enriched with vegetable and fruit extracts than in the control. Ursache *et al. (*2018) reported that muffin with microencapsulated powder content ash 0.82% as compare to control muffin which content ash 0.78%.

The data in Table 3 depicted the carbohydrate content of bread and shows that the highest mean carbohydrate was recorded in treatment T7 and lowest treatment T1 control bread. Ak *et al.* (2016) reported a decrease in carbohydrate from 56.45 in control to 51.09 in bread contain 10% spirulina powder. Urasache *et al.* (2018) reported 46.60±2.71% carbohydrate in control muffins and 49.60±1.54 % in muffins with seabuckthorn microencapsulated powder.

The data in Table 3 showed that the energy content of bread decreased significantly in all the treatments. Roman *et al.* (2022) reported a decrease in energy value of mayonnaise with the increase concentration of seabuckthorn encapsulated powder. Stanciu *et al.* (2023) observed that all samples containing sea buckthorn pomace powder presented lower energetic values compared to the control sample. Ak *et al.* (2016) reported a decrease in total energy from 272.59kcal in control to 256.32 kcal in bread contain 10% spirulina powder. El-Hadidy and Dreny (2020) reported a decrease in caloric value with the increase concentration of doum fruit pwder in toasted bread and Ewunetu *et al.* (2023) revealed that caloric value decrease with increases addition of banana and carrot flour in bread as compare to control.

Table 3: Proximate composition of encapsulated seabuckthorn and spirulina incorporated bread

Treatments	Moisture (%)	Water activity	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Carbohydrate (%)	Energy (kcal)
T1 (WF:SE:SP:: 100:0:0)	33.40^{g}	0.949^{g}	11.40^{a}	1.64^{a}	2.01^{a}	1.64^{a}	51.92^{b}	406.20^{g}
T2 (WF:SE:SP:: 94.50: 02.50: 03.00)	31.80^{f}	0.914^{f}	13.95^{f}	1.71^{b}	2.14^{b}	1.68^{b}	50.86^{a}	396.33^{f}
T3 (WF:SE:SP:: 92.00: 05.00: 03.00)	31.05^{e}	0.897^{e}	13.66^{e}	1.75^{c}	2.18^{b}	1.71^{b}	51.83^{b}	387.42^{e}
T4 (WF:SE:SP:: 89.50: 07.50: 03.00)	30.29^{d}	0.879^{d}	13.36^{d}	1.80^{d}	2.23^{c}	1.75^{c}	52.80^{c}	378.78^{d}
T5 (WF:SE:SP:: 87.00: 10.00:03.00)	29.53^{c}	0.860^{c}	13.06^{d}	1.85^{e}	2.29^{d}	1.78^{c}	53.78^{d}	370.16^{c}
T6 (WF:SE:SP:: 84.50:12.50:03.00)	28.78^{b}	0.842^{b}	12.76^{c}	1.91^{f}	2.35^{e}	1.82^{d}	54.73^{e}	361.53^{b}
T7 (WF:SE:SP:: 82.00:15.00:03.00)	28.03^{a}	0.824^{a}	12.48^{b}	1.98^{g}	2.46^{f}	1.87^{e}	55.64^{f}	352.91^{a}

WF=Wheat flour SE= Encapsulated seabuckthorn SP= Spirulina powder

The values are given as mean value of three replications. The values followed by different superscripts (a–f) within the same column are significantly different ($p < 0.05$) from each other. Similar letters (superscripts) indicate values that are not significantly different ($p < 0.05$) from each other.

4. CONCLUSION

The crude protein, fiber, fat, ash and carbohydrate was found higher in bread containing highest seabuckthorn encapsulates and spirulina powder than the control bread. It was concluded that encapsulated seabuckthorn and spirulina powder can be used for development of functional bread. The functional bread developed from encapsulated seabuckthorn and spirulina powder can enrich the nutritional value, widen the food applications and provide the health benefits to consumers to combat the nutrient deficiencies and protein energy malnutrition.

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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