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# Development and nutritional characterization of functional muesli enriched with *Hippophae rhamnoides* (sea buckthorn) dried berries

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**Abstract**
This present study framed to formulate and evaluation of a functional breakfast muesli incorporating *Hippophae rhamnoides* (Sea Buckthorn) dried berries, a nutrient-dense and bioactive-rich ingredient. Three formulations (T1, T2 and T3) were prepared by varying the proportion of dried Sea Buckthorn berries (100 g, 80 g and 60 g), while other components such as oats, almonds, cashews, pumpkin seeds, honey and milk were made constant. Sensory evaluation identified T2 as the most preferred formulation, achieving an overall acceptability score of 8.2 ± 0.45 on a 9-point hedonic scale. Nutritional analysis of T2 revealed it to be rich in protein (9.1 g/100 g), dietary fiber (6.8 g/100 g) and essential minerals including potassium (415.8 mg/100 g), magnesium (110.2 mg/100 g) and calcium (81.7 mg/100 g). Vitamin analysis showed the presence of substantial levels of vitamin A (98.9 mg/100 g), vitamin C (16.4 mg/100 g) and vitamin D (1.25 mg/100 g). Antioxidant activity by DPPH, FRAP and ABTS assays further established the functional potential of the product. Microbial analysis revealed the product's safety and stability. Aerobic plate count and yeast/mold count below 10 CFU/g and absence of pathogenic bacteria. The incorporation of Sea Buckthorn berries significantly enhanced the nutritional, sensory and functional profile of muesli, offering a promising value-added product for health-conscious consumers.

**Keywords:** Sea buckthorn, *Hippophae rhamnoides*, functional foods, muesli, antioxidant activity, sensory evaluation, vitamin analysis, plant-based nutrition, bioactive compounds, food safety

## 1. Introduction

The global shift towards preventative healthcare and wellness has fueled the demand for functional foods foods that provide health benefits beyond basic nutrition (Martirosyan & Singh, 2015) [11]. Among these, ready-to-eat breakfast cereals particularly muesli have gained popularity for their convenience and health-promoting properties. Traditionally composed of rolled oats, dried fruits, nuts and seeds. Muesli is a source of complex carbohydrates, fiber and plant-derived proteins (Anderson *et al*., 2009) [1]. However, the nutritional quality and bioactive profile of conventional muesli can be significantly enhanced through the inclusion of underutilized, nutrient-dense ingredients.

*Hippophae rhamnoides* L., commonly known as Sea Buckthorn, is a deciduous shrub native to cold-temperate regions of Europe and Asia. The berries of this plant are a rich source of essential nutrients and bioactives including ascorbic acid, carotenoids, tocopherols, flavonoids, folate, phytosterols and polyunsaturated fatty acids such as linoleic and α-linolenic acid (Bal *et al*., 2011; Suryakumar & Gupta, 2011) [5, 15]. Additionally, Sea Buckthorn is renowned for its high antioxidant capacity, attributed to its polyphenolic content, which imparts protective effects against oxidative stress and inflammation (Geetha *et al*., 2002) [9]. Historically, it has been employed in Tibetan and Chinese medicine for the treatment of gastrointestinal, dermatological, and cardiovascular ailments (Yang & Kallio, 2002; Beveridge *et al*., 1999) [16, 7].

Despite its promising nutritional profile, the commercial application of Sea Buckthorn in food products remains limited due to its acidic taste and high perishability. Drying the berries is a practical solution to improve shelf life while preserving most of their nutritional and sensory attributes (Arimboor *et al*., 2006) [4]. Incorporating dried Sea Buckthorn into muesli formulations has the potential to elevate both the health benefits and market value of the product by introducing naturally derived vitamins and antioxidants. earlier studies have examined the use of fruit-based ingredients in cereal products; however, there is limited research focusing on the incorporation of Sea Buckthorn berries into breakfast cereals. This study attempts to bridge that gap by exploring the development of a functional muesli product fortified with dried Sea Buckthorn berries and evaluating its nutritional, antioxidant, microbial and sensory characteristics. The primary aim of this study is to formulate and evaluate three variants of muesli incorporating varying proportions of *Hippophae rhamnoides* dried berries and to assess their nutritional composition, vitamin and mineral content, antioxidant activity, microbial safety and consumer acceptability. The aim is to develop a healthy breakfast product of an underutilized indigenous fruit.

## 2. Materials and Methods

### 2.1 Ingredient procurement

All ingredients used in the development of the muesli formulations were sourced from verified and hygienic outlets. Dried *Hippophae rhamnoides* (Sea Buckthorn) berries were procured from regional herbal suppliers and certified organic vendors through online platforms, ensuring quality and traceability. Rolled oats, almonds, cashews, pumpkin seeds, honey and pasteurized full-fat milk were purchased from local supermarkets.

### 2.2 Product preparation

Three formulations of muesli were prepared T1, T2, and T3 by varying the quantity of Sea Buckthorn dried berries (100 g, 80 g and 60 g respectively), while keeping other ingredients constant. The composition of each formulation is summarized in Table 1.

**Table 1:** Formulation for the preparation of muesli

| Ingredients | T1 | T2 | T3 |
|---|---|---|---|
| Sea buckthorn dried berries | 100 gm | 80 gm | 60 gm |
| Oats | 80 gm | 60 gm | 40 gm |
| Almonds | 4 gm | 4 gm | 4 gm |
| Cashew | 3 gm | 3 gm | 3 gm |
| Pumpkin seeds | 10 gm | 10 gm | 10 gm |
| Honey | 10 gm | 10 gm | 10 gm |
| Milk | 100 ml | 100 ml | 100 ml |

**Preparation process**

Ingredients were weighed according to formulation and subjected to dry processing. Rolled oats, almonds, and pumpkin seeds were lightly roasted at 120°C for 8-10 minutes to enhance flavor and reduce residual moisture. After cooling, all dry ingredients were mixed thoroughly. Honey was gently warmed and added as a natural binder, and the mixture was blended to ensure uniform distribution. The final mixture was air-dried at room temperature (~25°C) for 1 hour and then packed in food-grade, airtight containers to prevent moisture uptake and microbial contamination. Samples were stored at ambient temperature (25 ± 2°C) until further analysis.

### 2.3 Sensory evaluation

A sensory evaluation was conducted using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) to assess aroma, taste, texture, appearance, and overall acceptability. A panel of 15 semi-trained individuals (aged 21-45) participated in the evaluation under controlled sensory lab conditions. Randomized coding of samples ensured unbiased evaluation. Mean sensory scores were calculated and analyzed using one-way ANOVA to identify the most preferred formulation, with $p<0.05$ considered statistically significant (Meilgaard *et al*., 2007) [12].

### 2.4 Nutritional analysis

Proximate analysis of the optimized muesli sample (T2) was carried out using official AOAC methods (AOAC International, 2005, 2022). Analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Moisture was Determined by oven-drying to constant weight at 105°C (AOAC 925.09). Ash content was Measured by incinerating samples in a muffle furnace at 550°C (AOAC 942.05). Protein Estimated using the Kjeldahl method and calculated as Nitrogen × 6.25 (AOAC 981.10). Fat Extracted with petroleum ether using Soxhlet extraction (AOAC 985.29). Fiber Determined by sequential acid and alkaline digestion (AOAC 985.29). Carbohydrates Calculated by difference method Carbohydrates (%) = 100 - (Moisture + Protein + Fat + Ash + Fiber).

### 2.5 Mineral and vitamin analysis

Mineral content (iron, calcium, magnesium, potassium) was analyzed using standard instrumental techniques. Iron Determined via Atomic Absorption Spectrophotometry (AAS) per AOAC 999.11; Calcium & Magnesium**:** Measured using AAS according to AOAC 984.27; Potassium was by (AOAC 965.09). All measurements were conducted in triplicate and reported in mg/100 g dry weight. Vitamins A, C, and D were quantified using the following methods: Vitamin A**:** by (AOAC 976.15). Vitamin C**:** Measured by titration with 2,6-dichlorophenolindophenol (AOAC 967.21). Vitamin D (AOAC 2012.13). Vitamin contents were expressed in mg per 100 g of the product.

### 2.6 Antioxidant activity

Three different *in vitro* antioxidant assays were performed: DPPH Radical Scavenging Activity**:** Reduction of DPPH radicals at 517 nm; results expressed as % inhibition (Brand-Williams *et al*., 1995) [8]. FRAP (Ferric Reducing Antioxidant Power)**:** Measured by reduction of $Fe^{3+}$ to $Fe^{2+}$ at 593 nm; results in µmol Fe(II)/g (Benzie & Strain, 1996) [6]. ABTS Radical Cation Decolorization Assay: Absorbance measured at 734 nm after ABTS+ generation; expressed as % inhibition (Re *et al*., 1999) [13].

### 2.7 Microbial analysis

Microbial quality was assessed using standard microbiological procedures as per ISO and AOAC guidelines: Total Aerobic Plate Count: Performed on Plate Count Agar; results expressed as CFU/g (AOAC 990.12). Yeast and Mold Count Enumerated using Potato Dextrose Agar (AOAC 997.02). *Enterobacteriaceae* Determined via selective media (Violet Red Bile Glucose Agar); presence/absence recorded. *Staphylococcus aureus*: Detected using Baird-Parker Agar, confirmed via coagulase test (AOAC 2003.07).

## 3. Results and Discussion

### 3.1 The sensory evaluation of T1, T2 and T3 samples of muesli

The incorporation of *Hippophae rhamnoides* (Sea Buckthorn) dried berries into muesli formulations demonstrated significant improvement in both sensory and nutritional profiles. Among the three formulations (T1, T2 and T3), formulation T2 (80 g sea buckthorn berries) showed as the most preferred by the sensory panel, scoring highest across aroma (8.1 ± 0.53), taste (8.3 ± 0.47), texture (8.0 ± 0.50), appearance (8.2 ± 0.43) and overall acceptability (8.2 ± 0.45) on the 9-point hedonic scale (Table 2). This can be attributed to the optimal balance between the tart flavor of the berries and the natural sweetness of honey and nuts, aligning with previous findings on fruit-enhanced cereal products (Meilgaard *et al.*, 2007) [12].

**Table 2:** Sensory parameters of tested sample (T1, T2, T3)

| Attributes | T1 | T2 | T3 |
|---|---|---|---|
| Aroma | 7.2±0.63 | 8.1±0.53 | 6.8±7.0 |
| Taste | 6.9±0.58 | 8.3±0.47 | 7.0±0.62 |
| Texture | 7.0±0.61 | 8.0±0.50 | 7.2±0.65 |
| Appearance | 7.4±0.55 | 8.2±0.43 | 6.9±0.66 |
| Overall acceptability | 7.1±0.60 | 8.2±0.45 | 7.0±0.58 |

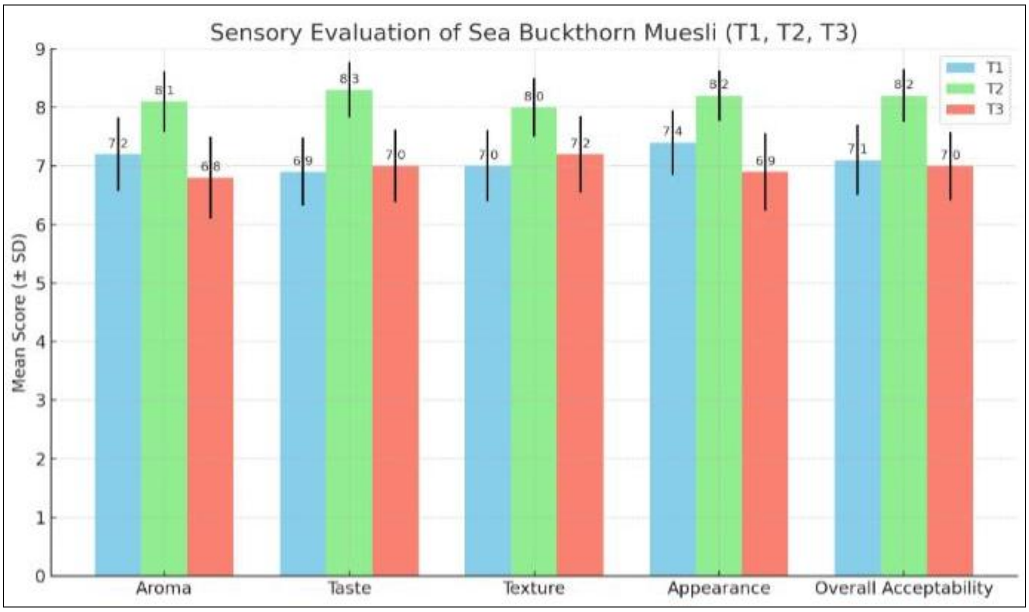


**Fig 1:** Sensory parameters of tested sample (T1, T2, T3)

### 3.2 The Proximate analysis of muesli (T2 Sample)

Proximate analysis of T2 revealed moisture content of 11.4%, indicating a stable product suitable for dry storage with minimal microbial risk (Table 3). Protein content was recorded at 9.1 g/100 g, suggesting the formulation is a moderate source of plant-based protein, mainly contributed by oats, seeds, and nuts. Fat content (12.6 g/100 g) was within the desirable range, predominantly derived from almonds, cashews, and pumpkin seeds, which are known to contain beneficial monounsaturated and polyunsaturated fatty acids (Riediger *et al.*, 2009) [14]. The carbohydrate level (62.4 g/100 g) confirms the product as an energy-dense breakfast option, with a notable fiber content of 6.8 g/100 g, enhancing digestive benefits. Ash content (2.7%) indicates the presence of bioavailable minerals, an observation corroborated by the mineral analysis.

**Table 3:** Nutritional analysis of muesli

| Test parameters | Results | Units |
|---|---|---|
| Protein | 9.1 | g/100 gm |
| Carbohydrates | 62.4 | g/100 gm |
| Fat | 12.6 | g/100 gm |
| Fiber | 6.8 | g/100 gm |
| Moisture | 11.4 | g/100 gm |
| Ash | 2.7 | g/100 gm |

### 3.3 Mineral and vitamin analysis of muesli (T2 sample)

Mineral profiling revealed high levels of magnesium (110.2 mg/100 g) and potassium (415.8 mg/100 g), essential for neuromuscular function and blood pressure regulation. Calcium (81.7 mg/100 g) and iron (2.3 mg/100 g) were also present in appreciable amounts, supporting bone health and hemoglobin synthesis respectively. These values underscore the micronutrient-dense nature of sea buckthorn berries and their suitability in functional food development (Suryakumar & Gupta, 2011) [5].

Vitamin analysis highlighted significant levels of Vitamin A (98.9 mg/100 g), largely due to the high carotenoid content in sea buckthorn, known for its role in visual health and immune function (Beveridge *et al.*, 1999) [7]. Vitamin C content (16.4 mg/100 g) supports the antioxidant claim and promotes collagen synthesis and iron absorption. The presence of Vitamin D (1.25 mg/100 g) suggests synergistic fortification from milk and seeds, making the formulation valuable for populations at risk of deficiency.

**Table 4:** Mineral and vitamin analysis of muesli

| Test parameters | Results | Units |
|---|---|---|
| Iron | 2.3 | mg/100 gm |
| Calcium | 81.7 | mg/ 100 gm |
| Magnesium | 110.2 | mg/ 100 gm |
| Potassium | 415.8 | mg/ 100 gm |
| Vitamin A | 98.9 | mg / 100 gm |
| Vitamin C | 16.4 | mg /100 gm |
| Vitamin D | 1.25 | mg 100 gm |

### 3.2 Antioxidant activity of muesli (T2 Sample)

Antioxidant activity was notably high, with DPPH radical scavenging at 450.3 ppm, FRAP at 600.2 µmol, and ABTS at 650.5 µmol, confirming the presence of potent phytochemicals and polyphenols (Table 5). These values are consistent with earlier studies affirming the high antioxidant potential of sea buckthorn due to its flavonoid and tocopherol content (Geetha *et al*., 2002; Bal *et al*., 2011) [10, 5]. Such antioxidant capacity contributes to cellular protection and anti-inflammatory effects, enhancing the functional appeal of the product.

**Table 5:** Antioxidant activity of muesli

| Test parameters | Results | Units |
|---|---|---|
| DPPH | 450.3 | PPm |
| FRAP | 600.2 | μmol |
| ABTS | 650.5 | μmol |

### 3.2 Microbial analysis of muesli (T2 sample)

Microbial analysis confirmed the safety of the muesli formulation (Table 6). Total aerobic plate counts and yeast/mold counts were below 10 CFU/g, with no detection of *Staphylococcus aureus* or *Enterobacteriaceae*, reflecting good manufacturing practices and proper storage conditions (AOAC, 2005) [2]. Shelf-life assessment over 90 days showed no significant changes in sensory or microbial quality, affirming the product's stability under ambient conditions.

**Table 6:** Microbial analysis of muesli (T2 Sample)

| Test parameters | Results | Units |
|---|---|---|
| Aerobic plate count | < 10 | CFU/g |
| Yeast and molds | <10 | CFU/g |
| Enterobacteriaceae | Absent | CFU/g |

## Conclusion

The present study successfully demonstrated the formulation and evaluation of a functional breakfast muesli enriched with *Hippophae rhamnoides* (Sea Buckthorn) dried berries, highlighting its potential as a nutrient-dense, antioxidant-rich, and microbiologically safe food product. Among the three formulations, the T2 variant with 80 g of sea buckthorn berries exhibited superior sensory attributes, a balanced macronutrient profile, and enhanced levels of essential micronutrients including calcium, magnesium, iron, and potassium. It also showed significant antioxidant activity, attributed to the bioactive compounds naturally present in sea buckthorn, thereby supporting its functional food status. The absence of pathogenic microbes and stable sensory and microbial quality over storage further reinforced its commercial viability. Overall, the integration of underutilized indigenous fruits such as sea buckthorn into ready-to-eat cereal products not only enhances their nutritional and health-promoting properties but also contributes to dietary diversification and sustainable food innovation. This study provides a scientific foundation for the inclusion of sea buckthorn in mainstream functional food development and encourages further exploration of its bioactive benefits through clinical validation.

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