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Kappaphycus alvarezii Seaweed Powder Incorporated Dairy Ice Cream as a Potential Functional Food

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Abstract

The demand for seaweed-based functional food products is growing significantly due to changes of lifestyles such as increased health awareness and interest on plant-based diets. This study aimed to evaluate the quality parameters of *K. alvarezii* seaweed powder (SWP) incorporated ice cream developed as a novel functional food product with nutritional and therapeutical benefits. Fresh and cleaned seaweeds were oven-dried at 40°C for 48 h and ground to make a fine powder. The radical scavenging activity, total phenolic content and flavonoid content were analysed for the dehydrated seaweed powder. Seaweed powder incorporated ice cream was developed with addition of seaweed powder (0, 0.2, 0.5, 1% w/w) with 100%, 9.8%, 99.5%, and 99% of ice cream mix, respectively. Total phenolic content, flavonoid content and radical scavenging activity of dehydrated seaweed powder were 10.42 mg GAE g^{-1}, 15.51 mg rutin g^{-1}, 21.43%, respectively. A significant difference was observed for titratable acidity among four ice cream samples ($p<0.05$) after the first week of storage. The highest melting rates were exhibited in control ice cream samples. The 0.5% SWP incorporated ice cream was distinguished as the most acceptable ice cream. The 1% SWP incorporated ice cream sample was detected as the sample with the highest hardness. The highest DPPH radical scavenging activity was recorded in 0.2% SWP incorporated sample. Thus, dried powder of *K. alvarezii* can be effectively incorporated to ice cream to enhance its antioxidant content and sensory qualities.

Keywords: Bioactive compounds, Functional foods, Ice cream, *Kappaphycus alvarezii*, Seaweeds

1. Introduction

A wide range of bioactive compounds and other nutritional compounds of red seaweeds have led the development of various kinds of functional foods around the world. Recent studies have found that red seaweeds possess considerable levels of bioactive compounds that deliver their effects such as antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, and antitumor properties. Thus, this provides several benefits including improvement of human health and prevention of diseases [1,2]. Fresh seaweed can be used for direct consumption, dried seaweed or seaweed extracts are used as raw material in food products [3]. *K. alvarezii* is an edible red seaweed (marine algae) which is widely used in food industry as a gelling, thickening, stabilizing agent and texture enhancer [4]. *K. alvarezii* is the source of carrageenan. Numerous scientific studies reported the incorporation of *K. alvarezii* powder in food products such as steam buns [5], crackers [6], buns [7], bread rolls [8], and pastas [9]. *K. alvarezii* is an underutilized resource in Sri Lanka and a promising ingredient for the food industry that can be cultivated in sustainable manner. Ice cream is a nutritious, wholesome frozen dairy dessert which has a smooth texture and cool sensation property and can be consumed in frozen condition by any aged group of people. The composition of ice cream consists of milk fat, milk solid non fat (MSNF), stabilizers, emulsifiers, flavourings, and sugars or sweeteners. The ice cream composition differs from country to country as well among their localities in different markets [10, 11]. Functional foods offer health benefits beyond basic nutrition, helping to prevent chronic diseases such as cancer and heart disease, and improving overall human health. [12, 13]. The addition of seaweed powder into ice cream is scarce due to a lack of awareness among Sri Lankans. Furthermore, there are limited research conducted globally on the addition of *K. alvarezii* as a whole powder in dairy products and the evaluation of their quality attributes. Moreover, there are few scientific research conducted on the evaluation on the quality of seaweed powder incorporated ice cream. Ice cream incorporated with seaweeds like *Kappaphycus alvarezii* can be considered a functional food. Hence, this study aimed at developing and evaluating the quality and functional properties of *K. alvarezii* seaweed powder incorporated ice cream and develop value-added seaweed related dairy products.

2. Materials and Methods

2.1 Collection of seaweed sample

Seaweed samples (*K. alvarezii*) were obtained from a seaweed cultivation farm in Valaipadu, Kilinochchi, Sri Lanka. (9.3378°N 80.0528°E) in March 2022. The collected seaweed samples were washed with fresh water and cleaned thoroughly to remove epiphytes, foreign biota, sand, salt, and other surface contaminants. Samples were stored at -20°C for further analysis.

2.2 Preparation of dehydrated seaweed powder

The seaweed samples were cut into small pieces (1 cm) to reduce the particle size [14]. *K. alvarezii* samples were blanched at 88°C [15] for 1 s in a hot water bath to inactivate the enzyme activity and reduce the microbial load. The samples were drained to remove surface water. Samples were oven-dried at 40°C for 48 h [16] using a drying oven (DHG-9146A, Heating Drying Oven, China) to reduce the microbial activity and slow down the chemical reactions which can be negatively affect to the final food product. The oven dried seaweed samples were grounded using a grinder (XPRO DOU, Preethi Kitchen Appliances, Kanchipuram, India) and sieved to make fine powder (2 mm). Dehydrated *K. alvarezii* powder was stored in cold storage at 4°C in air-tight containers.

Fig. 1. Dehydrated *K. alvarezii* seaweed powder **s**ample

2.3 Chemical Analysis of dehydrated seaweed powder

The moisture content of the dehydrated seaweed powder was analysed using moisture analyzer (AGS120, AXIS Spolka Zoo, Gdansk, Poland) immediately after the dehydration process. This provides a baseline measurement and confirms that the drying process was effective in reducing moisture to the desired level.

2.3.1 Preparation of methanol extracts

Methanol extraction of SWP was performed with slight modifications [16]. Dehydrated seaweed powder sample 0.5 g) was measured into a 50 mL polypropylene centrifuge tube and 10 mL of 50% methanol was added. The mixture was vortexed (F202A0270, VELP SCIENTIFICA, Italy) for 20 s and then sonicated (25 kHz 150 W) using a probe ultrasonicator (Soner 203H, Rocker Scientific Co. Ltd, New Taipei City, Taiwan) for 30 min at 55°C. After that, tubes were shaken for 4 h using an orbital shaker (OS-20, Boeckel+Co (GmbH+ Co) KG, Hamburg, Germany) at 6987.5 RCF

Extracts were centrifuged (Sorvall ST 40R, Thermo Fisher Scientific, Langenselbold, Germany) at 2500 rpm for 10 min at 24°C, and the supernatant was collected and transferred into separate centrifuge tubes. Ten milliliters of 50% methanol were added to the remaining solid residue, and centrifuged again to collect the supernatant. Then, the extracts were evaporated at 45°C and 100 mbar using a rotary evaporator (Rotavapor, R-100, Buchi, Labortechnik AG, Flawil, Switzerland). The extracts were stored at -20°C prior to analysis.

2.3.2 Radical scavenging activity (RSA) of dehydrated seaweed powder

The DPPH assay method was followed with slight modifications to determine radical scavenging activity of dehydrated seaweed powder [16]. Accordingly, 100 µL of previously prepared seaweed methanol extract was added into a test tube. One millilitre of 0.1 mM 2,2-diphenyl-1-picrylhdrazyl hydrate (DPPH) in methanol was added. The mixture was vortexed (F202A0270, VELP SCIENTIFICA, Italy) for 1 min before placing at ambient temperature in the dark for 30 min. The absorbance was measured for each sample at 515 nm wavelength by UV/Visible spectrophotometer (Evolution 201, Thermo Fisher Scientific, Shanghai, China). Pure methanol (100 µL) was used as the negative control.

2.3.3 Total phenolic content (TPC)

The Folin-Ciocalteu method was adapted with slight modifications to determine the total phenolic content of SWP [16]. Briefly, 100 µL of seaweed extracts (or gallic acid standard for calibration) were added into test tubes. Then, 1 mL of 10% Folin- Ciocalteu reagent in distilled water and 0.8 mL of 7.5% sodium carbonate were added to each test tube. Tubes were thoroughly mixed by vortexing and allowed to stand for 1 h at room temperature. Calibrations were carried out using gallic acid standards with concentrations ranging from 0.25 to 1.75 mg/mL. Standards and blanks were prepared using the same method as for the sample. Absorbance was measured at 765 nm wavelength using UV/Visible spectrophotometer (Evolution 201, Thermo Fisher Scientific, Shanghai, China). The total phenolic content of the samples was expressed as mg gallic acid equivalent per gram (mg GAE/g).

2.3.4 Total Flavonoid content (TFC)

Total flavonoid content of dehydrated seaweed powder was determined according to the aluminium chloride method [16]. First, 100 µL of seaweed extract or standard (Rutin) was added into a test tube followed by 500 µL of distilled water and 37.5 µL of 5% sodium nitrite. After 6 min, 75 µL of 10% aluminium chloride was added to the tube followed by 250 µL of 1 M sodium hydroxide. Samples and standards were made up to 1.5 mL with distilled water. Calibrations were carried out using Rutin standards series (concentration

range 0.25-1.25 mg/mL). Standards and blanks were prepared using the same method as for the samples. Absorbance was measured at 510 nm wavelength.

2.4 Development of dehydrated seaweed powder incorporated ice cream

Dehydrated seaweed powder incorporated ice cream was prepared using 1 L fresh milk (SNF 8.7% and fat 3.5%), 4% milk powder, 8% cream fat, 24% white sugar, 2% liquid glucose, 0.2% gelatine, 0.4% stabilizer, and dehydrated seaweed powder with different levels 0%, 0.2%, 0.5%, 1% (w/w). Ice cream mix was prepared according to the procedure of Deosarka *et al.*, with slight modifications replacing the stabilizer [17, 18]. Each mix was pasteurized at 80°C for 25 sec and cooled down to 70°C. The mixes were homogenized for 150 Pa using a homogenizer and cooled to room temperature. Mixes were stored in the refrigerator at 4°C for 24 h to ensure complete hydration of all ingredients. After aging, mixes were frozen in a batch freezer to achieve the desired overrun. The ice cream was packed into 2 L plastic containers and stored at -18°C.

2.5 Evaluation of chemical and phytochemical properties of seaweed powder incorporated ice cream

2.5.1 pH

Measurements of pH on dehydrated seaweed powder incorporated ice creams were analysed at 0, 7, and 14 days, stored at -18°C using pH meter.
The pH of ice cream (stored at -18°C) at 7 days interval was measured using pH meter (ELE-511, Germany) [19].

2.5.2 Melting rate of seaweed powder incorporated ice cream

Determination of melting rate was carried out with slight modifications [13], after hardening of ice cream at -18°C. Here, 50 g of the ice cream samples were weighed on a wire mesh screen and left to melt into a beaker below the wire mesh at room temperature of 24±2°C until 50% of the sample melted. The time of the first drop and the volume of the melted ice cream (every 5 min) was recorded for each sample. A sigmoidal curve for the kinetics of melting was plotted according to the volume of the melted ice cream versus the time. The slope represented the melting rate (mL/min) of each ice cream accordingly.

2.5.3 Titratable acidity

Titratable acidity of ice cream was determined as described by Fabro and others [20]. Briefly, 20 mL of ice cream sample was added to 40 mL of boiled and cooled distilled water. Five drops of phenolphthalein were added to the mixture. The mixture was titrated with standardized 0.1 M NaOH until the first colour change (to pink) persist for 3 s. One more drop of 0.1 M NaOH was added and final volume of added 0.1 M NaOH was recorded.

2.5.4 Texture Profile

Texture profile of ice cream was analysed in four samples stored at -18°C using the texture analyzer (TexturePro CT V1.8, AMETEK, Brookfield, MA, USA) [21].

2.5.5 DPPH free radical scavenging activity

Determination of DPPH radical scavenging activity was carried out according to Sagdic and Hwang [22, 23]. Ice cream samples were extracted as above procedure to determine phenolic content. Firstly, 0.3 mL of extract was added with 1.2 mL methanol and 1.5 mL of 1 mmol/L DPPH methanol solution. The mixture was kept at room temperature for 30 min and the absorbance were measured at 517 nm.

2.6 Sensory evaluation

Twenty millilitres of each ice cream sample were added to small sized plastic cups. Ice cream samples were organoleptically analysed by 30 untrained panelists focusing on colour, odour, taste, physical appearance, and overall acceptability using 9-point hedonic rating scale. The best seaweed incorporated ice cream sample was chosen according to the results of the sensory evaluation. Each ice cream sample was served at frozen (-18°C) conditions.

2.7 Statistical analysis

All experiments were carried out in triplicate. The statistical analysis was conducted using Complete Randomized Design with one-way ANOVA combined with Tukey's and Dunnet comparison of the mean tests to understand the significant difference between the treatments at 95% significance level. Non-parametric data obtained from sensory evaluation were analyzed using Friedman test. Minitab 19 software and MS Excel were employed for statistical analysis.

3 Results and Discussion

3.1 Bioactive properties of dehydrated seaweed powder

DPPH radical scavenging activity of dehydrated seaweed powder was 21.43±3.65%. A recent study conducted on *K. alvarezii* [24] showed that radical scavenging activity of methanolic extract of *K. alvarezii* powder was 24.12±0.35%. DPPH free radical scavenging activity of the present study was also in agreement with the scavenging activity values on previous studies [25, 26]. DPPH radical scavenging activity is used to determine antioxidant activity by measuring the scavenging capacity of the antioxidant using a stable free radical α, α-diphenyl-β-picrylhydrazyl [27]. The total phenolic content of seaweed powder was determined using Folin-Ciocalteu method. The total phenolic content of

dehydrated *K. alvarezii* powder showed 10.42±0.29 mg GAE/g DW. Recent studies [24] reported that the phenolic content of *K. alvarezii* powder was 12.51±0.56 mg GAE/100 g DW. Such variations of the phenolic content can be caused due to several factors such as location, environmental conditions, type of extraction solvent, type of drying method and chemical composition of each extract. Researchers have predicted higher phenolic contents of the seaweed extract due to high content of hydrophilic and hydrophobic antioxidants [24, 27-29]. The total flavonoid content of *K. alvarezii* powder was determined using Aluminium chloride method. Total flavonoid content of dehydrated *K. alvarezii* powder was 15.51±4.22 mg Rutin/g DW. Furthermore, a previous study [24] had indicated that the flavonoid content of *K. alvarezii* powder (12.51±0.56 mg GAE/100 g DW) was high than the present study. These differences in flavonoid contents in different studies may be due to variables such as the geographical location and environmental conditions of the seaweed grown, type of extraction solvent and type of drying method used [24,26].

Table 1
Bioactive properties and compounds of *K. alvarezii* seaweed powder.

Bioactive properties and compounds	
DPPH radical scavenging activity	21.43±3.65%
Total Phenolic content	10.42± 0.29 mg GAE/g DW
Total Flavonoid content	15.51±4.22 mg Rutin/g DW

Mean values (± standard deviation) of

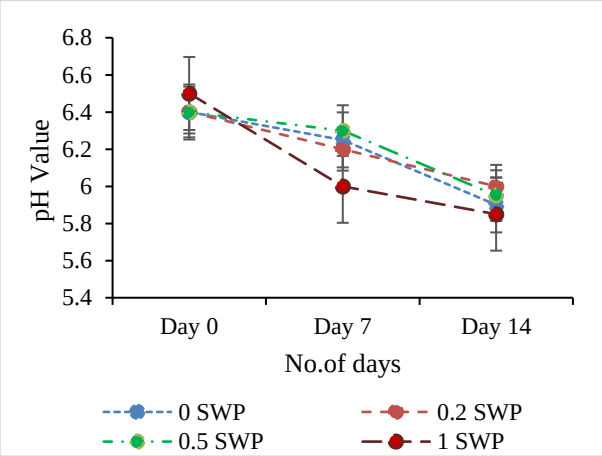

Fig 1. pH values of seaweed powder incorporated ice cream

pH Changes

The pH value of normal ice cream is about 6.3[30],. The composition of the ice cream mix such as TS content – 50.7%, MSNF content – 39.2%, Fat content – 8%) affects on the acidity and pH of the ice cream and the composition was constant for all treatments.During the frozen storage of 14 days, pH of the ice cream samples was reduced. Immediately after the preparation of ice cream, the highest pH was recorded in 1% SWP incorporated ice cream while the lowest in control samples.

Hardness of seaweed powder incorporated ice cream

Hardness is a term used to describe the resistance of the ice cream to deformation when an external force is applied. Overrun, ice crystal size, ice phase volume, and extent of fat destabilization are the main factors that affect to the hardness of the ice cream [31]. The highest hardness values of seaweed incorporated ice cream were recorded in 1% SWP incorporated ice cream sample while the lowest recorded in the control samples. The addition of seaweed powder has no significant effect on ice cream ($p>0.05$). The hardness values of control, 0.2% SWP, 0.5% SWP, and 1% SWP samples were exhibited as 0.71±0.05 kg, 2.88±0.53 kg 2.92±0.83 kg and 3.65±1.01 kg, respectively. The slight increase of ice crystals during freezing may have led to a rise of hardness of the ice cream samples [18]. Higher cohesiveness values improve ice cream stability [32]. There was a significant difference between the cohesiveness of the four treatments ($p<0.05$). The highest cohesiveness was recorded in control samples while the lowest was recorded from the 0.5% SWP incorporated ice cream.

Table 2
Hardness of *K. alvarezii* seaweed powder incorporated ice cream.

Treatment	Hardness (kg)
Control	0.71±0.10[a]
0.2% SWP	2.88±0.53[a]
0.5% SWP	1.92±1.45[a]
1% SWP	3.65±1.75[a]

Titratable acidity of seaweed powder incorporated ice cream

Milk proteins, mineral salts and dissolved gases are responsible for the natural acidity of the ice cream. The activity of lactic acid bacteria causes the developed acidity in commercial level ice cream production [20]. There was a significant difference between the titratable acidity of four ice cream samples ($p<0.05$). The highest acidity was reported in control samples while the lowest in the 0.2% SWP incorporated ice cream sample.

Table 3.

Titratable acidity of *K. alvarezii* seaweed powder incorporated ice cream.

Treatment	Titratable acidity mg of Lactic acid per 100 mL
Control	192.1±5.20[a]
0.2% SWP	162.1±4.50[b]
0.5% SWP	169.5±5.20[b]
1% SWP	187.5±2.60[a]

Mean values (± standard deviation) of seaweed powder incorporated ice creams.

DPPH Radical Scavenging Activity of seaweed powder incorporated ice cream

DPPH free radical scavenging activity of SWP incorporated ice cream was evaluated at day 7. According to the results, the incorporation of seaweed power to ice cream

significantly increased the RSA compared to the control sample. Radical scavenging activity of control, 0.2% SWP, 0.5% SWP, and 1% SWP powder incorporated ice cream samples were 10.75±1.91, 14.67±2.03, 16.12±0.68 and 16.54±3.61%, respectively. The 0.5% and 1% SWP incorporated ice cream samples were significantly higher in DPPH radical scavenging activity compared to other ice cream samples ($p>0.05$), revealing the fact that *K. alvarezii* can be potentially significant source of antioxidants.

Sensory evaluation of seaweed powder incorporated ice cream

The effect of the incorporation of *K. alvarezii* powder on the sensory properties of the ice cream was determined evaluating colour, odour, taste, texture, mouthfeel, physical appearance, and overall acceptability. The results revealed that the acceptability of physical appearance and taste were higher in 0.5% sample than the control sample. Texture of the ice cream with 0.5% SWP scored higher value compared to 0.2%, 1% and control samples. The acceptability of mouthfeel of ice cream samples with 0.2% SWP and 0.5% SWP was significantly different from samples with 1% SWP and control ($p<0.05$). The colour acceptability ranged from 6.5-7.62 where the 0.5% SWP ice cream sample showed the highest acceptability to the colour. According to the results of the sensory evaluation, it can be concluded that, the 0.5% SWP incorporated ice cream sample had a higher overall acceptability compared to other samples ($p<0.05$). From the sensory evaluation, 0.5% seaweed powder incorporated ice cream was chosen as the most acceptable ice cream and it can be designed as the value-added final product.

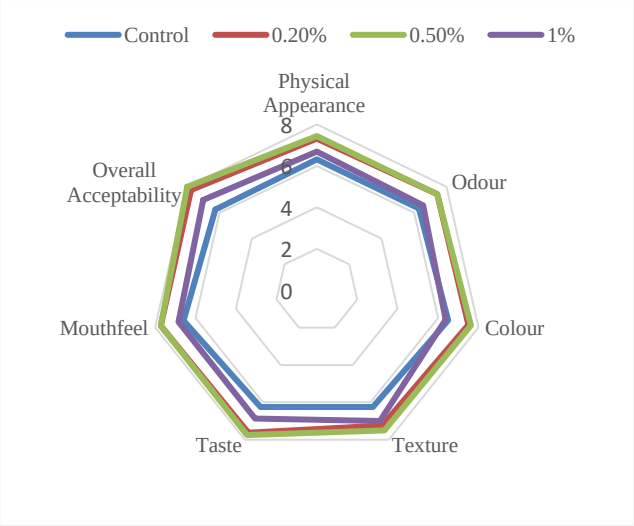

Fig. 2. Sensory evaluation results of seaweed powder incorporated ice cream.

Conclusions

The incorporation of *Kappaphycus alvarezii* seaweed powder significantly enhances the antioxidant properties and acceptability of ice cream, with 0.5% SWP providing the optimal balance of these benefits. While higher concentrations of SWP also improve antioxidant activity, they may lead to diminished textural and sensory qualities. Therefore, incorporating 0.2% to 0.5% SWP into ice cream formulations is recommended to achieve the best overall sensory and functional attributes, potentially positioning the product as a desirable functional food.

Conflicts of Interest

No conflicts of interest to declare.

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