

Functional Food Development using *Ulva fasciata* and Its Acute & Chronic Toxicity on *Drosophila melanogaster*

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Abstract

The aim of this investigation is to develop functional food recipes that combines millets and edible seaweed (*Ulva fasciata*), to help maintain blood sugar levels in diabetes mellitus patients. The *U. fasciata* sample was procured from Kanniyakumari, Tamil Nadu, and botanically authenticated at CSMCRI, Rameswaram. The *U. fasciata* thallus was subjected for *Drosophila melanogaster* toxicity studies, to assess the effect on mortality, fecundity, negative geotaxis and feeding behaviors for both acute and chronic durations. It was concluded that the procured *U. fasciata* sample is non-toxic and it improves the physiological functioning of the flies such as neural, muscular, digestive function and feeding potential of the flies. Further, the seaweed was infused into three unique functional food recipes i.e., Ragi Soup, Sorghum Ice Cream and Foxtail Millet Dosa (each infused with 1% dry *U. fasciata*). The functional foods were developed based on trial-and-error method, coupled with sensory panel analysis (semi-trained) to improve the palatability and acceptability. Proximate analysis was performed for all the three food products and fresh *U. fasciata* thallus, to understand their nutritional composition. The developed products contain a good balanced source of nutrients and rich source of minerals, that could help improve the metabolic process of diabetic patients. The optimized functional foods were lyophilized to convert these products into ready-to-eat commercial recipes. To the best of knowledge, this is the first report on developing functional foods that combines millets and edible seaweeds for their anti-diabetic applications.

Introduction

Diabetes Mellitus (DM) is a chronic condition characterized by high blood glucose levels that can lead to various complications if left untreated. Rapid increase in the occurrence of DM in India, has earned the title 'Diabetic Capital of the World' resulting from sedentary life style and drastically changing food habits. Increased commercialization and marketing of processed foods, caused the public to lose interest in traditional foods that are healthy and medicinal in nature. The increasing prevalence of diabetes has prompted the exploration of alternative therapeutic approaches, including the use of traditional food sources such as millets and seaweeds. *Ulva fasciata*, a species of green seaweed, has garnered significant attention owing to its antidiabetic properties. Millets on the other hand, are a group of small-seeded grasses that have traditionally been used as food and fodder in many parts of the world. They have recently gained spotlight as healthier alternatives to refined grains, particularly for individuals with diabetes.

Ulva fasciata commonly known as 'Ribbon Sea Lettuce', is a species of green macroalgae that belongs to the Ulvaceae family. *Ulva fasciata* is a thin, flat, and sheet-like seaweed that can grow up to 50–80 cm in length and width. The thallus of *Ulva fasciata* is green in color and has a ruffled, perforated margin and a small disc-shaped holdfast that attaches it to rocks, shells, or other surfaces. This seaweed is widely distributed in coastal regions around the world, thriving in intertidal and subtidal zones where it forms lush green mats, which are vital components of marine ecosystems (Aguilar-Rosas et al. 2005). The ecological importance of *Ulva fasciata* lies in its role as a primary producer in coastal ecosystems (marine), providing food and habitat for a variety of marine organisms. *Ulva fasciata* is also of interest for its applications in food, cosmetics and pharmaceutical industries. This seaweed is edible and has been consumed as a food in some regions of Asia. Additionally, it has been investigated for its potential use in bioremediation, biofuel and antibiotic production. This seaweed not only plays a crucial ecological role but also has significant economic and high nutritional value (Rajapakse and Kim 2011; Xu et al. 2023; Cotas et al. 2024).

Ulva fasciata is rich in bioactive compounds, such as Ulvans, which have been shown to exhibit various biological activities, such as antioxidant, antibacterial, antifungal, antidiabetic and several other pharmaceutical applications (Mohapatra et al. 2016; El-Beltagi et al. 2022; Animish and Jayasri 2023; Li et al. 2023). Studies have demonstrated that *Ulva fasciata* extract can; (i) significantly reduce blood glucose levels, (ii) improve insulin sensitivity, and (iii) alleviate the symptoms associated with diabetes in mice animal models (Ramu Ganesan 2013; Lucy Mohapatra et al. 2016). *Ulva fasciata* extract has also been found to have a positive impact on (i) enzymes of carbohydrate metabolism, (ii) inhibiting key enzymes lipid and carbohydrate metabolism and (iii) reduces the risk of developing insulin resistance and related complications (Kim et al. 2008; Lee et al. 2010; Ramu Ganesan 2013; Kim and Park 2024). In addition, seaweed has been used in many traditional medicines of Asia to treat a variety of ailments, including hyperglycemia. The popularity of the East-Asian diet has led to increased interest in seaweed as a food source, partly due to its potential health benefits and partly due to its nutritious value (Lee et al. 2010).

Millets such as Ragi / Finger millet (*Eleusine coracana* L.), Foxtail millet (*Setaria italica* L.) and Sorghum millet (*Sorghum bicolor* L.) have been extensively studied for their potential in managing diabetes (Ahamed V.P. et al. 2024). These grains are rich in fiber, protein, and various micronutrients, making them a suitable choice for individuals with diabetes. In particular, these millets have

been reported to have a low Glycemic Index (GI), meaning they do not cause a rapid spike in blood glucose levels after consumption. Ragi, is known as a good source of calcium and has been traditionally used in the treatment of diabetes in some cultures. The sorghum millet contains magnesium, Vitamin-E, phenolic compounds and tannins that reduces the risk of diabetes. Alternating the consumption of rice or processed wheat grains, with consumption of Ragi, Foxtail millet and Sorghum millet is a common practice among diabetes mellitus patients, which is also recommended by physicians to manage the blood sugar levels.

Functional Food recipes that could help in management of blood sugar levels in diabetic patients. Functional foods are everyday food products that have been enhanced with additional ingredients to provide specific health benefits beyond basic nutrition. These foods are designed to improve health or reduce the risk of disease when consumed as part of a regular diet. To the best of knowledge, there are no known studies that combines Millets and *Ulva fasciata* (edible seaweeds) to provide a nutritious and palatable. The novelty of the current study lies in development of unique functional food recipes that combines low-GI Millets and antidiabetic *Ulva fasciata* that results in delectable functional foods, which could provide palatable and flavor rich food alternatives for diabetes mellitus patients. The combination of Millets and *Ulva fasciata* could potentially provide a comprehensive approach for managing diabetes mellitus. The antidiabetic properties of *Ulva fasciata*, coupled with the nutritional benefits of millets, may help to reduce blood glucose levels, improve insulin sensitivity, and prevent the development of diabetes-related complications. However, further research is needed to fully understand the mechanisms beneath the antidiabetic effects of these foods and to explore their potential as therapeutic agents in clinical application for diabetes management.

Functional foods are recipes that provide health benefits by combining nutritious ingredients that are rich in bioactive molecules. The objective of the current study is to develop Millet based functional foods that are infused with medicinal properties of *Ulva fasciata*. The study also aims to develop series of 'Vegan' recipes, that are suitable for all major diet categories (i.e., Vegan, Vegetarian, Non-Vegetarian, Eggetarian). The study aims to develop recipes that are chemical-free in nature (i.e., No added sugars, No added preservatives, No added emulsifiers, No added additives). The study focuses on developing diabetic-friendly functional foods, by incorporating only food ingredients that are low-GI and diabetes mellitus compatible.

Materials and Methods

Sample Collection & Herbarium Preparation

Fresh samples of edible marine green seaweeds (*Ulva fasciata*) were collected from the Kanyakumari area, Southeast coast of India (8° 6' 24.4368" N 77° 33' 32.7492" E) in Nov 2023. Fresh algae were washed with deionized water twice followed by 70% Ethanol wash and air-dried. Large portion of the biomass was dried in tray dryer (ISOTECH, Tray Drier: ITDCT-150 RE SPL) at 50°C for 24hr and were ground into powder using mixer grinder. Small portion of the fresh algae were stored at -40°C for future analysis. To prepare the herbarium, clean fresh samples of the collected seaweeds were pressed between blotting papers with application of weights. The papers were changed on a daily basis, until the specimen is completely dry. Care was taken not to break / crack the thallus and complete morphology i.e., margin and appearance, of the algal thallus was retained to the best of possibility. The dried algal sample was glued on to an herbarium sheet and was submitted to Central Salt & Marine Chemicals Research Institute (CSMCRI), Marine Algal Research Station (MARS), Madapam, Rameswaram for identification and authentication of the genus and species name of the specimen.

Acute & Chronic Toxicity Analysis on *Drosophila melanogaster*

Drosophila melanogaster was used as model organism to test for acute and chronic toxicity of the procured *Ulva fasciata* samples. The study groups were mainly divided in to Parent generation and F1 generation flies. The Parent generation was considered to be 'acute toxicity' group, which consisted of 30 flies i.e., 15 male and 15 female flies, that were exposed to the intervention for 7 days period and then were subjected for further analysis. The F1 generation was considered as 'chronic toxicity' group, which consisted of the flies that were eclosed newly in the experiment after the removal of parent flies from the container. The F1 generation flies were considered as 'chronic toxicity' group, since these flies spent their entire life cycle i.e., egg, larva, pupa and adult fly stages, all in the food (media) that has the intervention.

The *D.melanogaster* flies were maintained in Semolina media, with a composition of 100g of Semolina, 100g of Jaggary, 10g of Agar-agar, 10g of Yeast and 7.4ml of Propionic Acid for every 1 Liter of the media. The cultures were maintained in an incubator at

25°C. All the experiments were carried in an air conditioned room with a temperature similar to the incubator.

To investigate the toxicity effects of the test sample, the flies were segregated into three groups with each group consisting of 30 flies (15 male & 15 female flies), i.e., i) Control group (fed with semolina media); ii) 1% Seaweed group (fed with 1% *U.fasciata* dry powder mixed in semolina media) & iii) 2% Seaweed group (fed with 2% *U.fasciata* dry powder mixed in semolina media). The Parent flies were allowed to feed on the treatment condition for 7 days and were removed from the media and subjected for toxicity assays. While the newly hatched larvae of the F1 generation were allowed to continue feeding on the media till they eclose into a new adult fly. Once the F1 generation flies eclose from their pupa stage, they were transferred to a new container, consisting of the same media composition as designated based on the group that they belong. Newly eclosed flies of the F1 generation are allowed to continue feeding on the media for 7 days, post which they were subjected for assays. The toxicity effects on the *D. melanogaster* was observed in regards to mortality, fecundity, morphology, negative geotaxis assay and feeding assay after the intervention period of acute and toxicity groups.

The intervention period for the Parent flies is 7 days of feeding on the specified media, while the intervention period for the F1 generation flies is 7 days of continued feeding from the day of their eclosure. Post this intervention period, the flies are subjected for the toxicity assays. Parent flies are exposed to the intervention only for 7 days at their adult fly stage, while the F1 generation spend their entire life cycle in the intervention.

Mortality of the flies were monitored on a daily basis, to identify whether any of the Parent flies or F1 generation flies die during the observation period. The number of flies that die during the observation period is noted and plotted in a graph. Fecundity of the flies were monitored after the Parent fly's incubation period is complete. The number of newly eclosed flies in the container, after removal of the Parent flies is counted for each group and was statistically compared. Decrease in number of eclosed flies, denotes reduction in the reproduction potential and indicates toxic effects of the intervention.

Negative geotaxis assay also known as climbing assay is performed to quantify the locomotion ability of the flies. After the completion of the intervention period, the flies are transferred to an empty container and were subjected for the assay. The flies were gently tapped till all the flies reach to the bottom of the container and a timer was started to identify the total number of flies that cross a 6 cm height distance from the bottom of the container within 6 seconds time period. The number of flies that cross the marked 6 cm distance within 6 seconds time period was noted and the pass percentage of the flies were calculated based on total number of flies in the experiment each time. The pass percentage was normalized so as to consider the pass percentage rate of the Control group as 100% and the test groups were compared for their increase or decrease in the pass percentage.

Feeding assay is performed to quantify the ability of the flies to consume food. After completion of the intervention period, the flies are transferred to a new container, consisting of 'Feeding assay media'. The feeding assay media is the same semolina media substituted with 1mg/g of methylene blue dye (MB). The flies were allowed to feed on the feeding assay media for 24 hours, post which, the flies are anesthetized, washed with distilled water and homogenized in ethanol, to extract the MB dye present in their digestive tract and internal organs. The amount of MB present in the flies were quantified using UV spectrophotometry. The absorbance of the extract was measured at 664nm. A total of 5 flies were subjected for feeding assay from each group at a time, in triplicates. The amount of dye present in the Control group flies is normalized to 100% percentage, so as to compare and quantify the amount of dye present in the test groups.

Development of Ragi Soup infused with *Ulva fasciata*

Material Required

Finger Millet (*Eleusine coracana*) was purchased from a local supermarket in Bengaluru, Karnataka, India and edible seaweed (*Ulva fasciata*) was procured from the Kanniyakumari, Tamil Nadu, India. Other ingredients such as vegetables and spices (carrots, capsicum, peas, sweet corn, spring onion, garlic, cinnamon, salt, and pepper) were procured from local market in Bengaluru, Karnataka, India.

Preparation of Ragi Milk

Raw millets were placed in a bowl and immersed in water and left for soaking. Soaking facilitates softening of the millet for easier blending. Subsequently, the soaked grains were drained and rinsed thoroughly with distilled water. In a blender, soaked Ragi millets were ground with water in various ratio for different time duration until smooth consistency was achieved. The ground mixture was

allowed to settle and strained through 0.6mm sieve to separate the liquid from the millet pulp. The filtrate is collected and stored in refrigerator until further use.

Preparation of Vegetable Homogenate

All the vegetables that were used for soup as ingredient i.e., Carrots, Capsicum, Sweetcorn, and Peas, were ground into a smooth paste using blender. Each trial had varying ratios / proportions of the ingredients. The vegetable homogenate was incorporated in to the soup, so as to obtain a uniform taste and texture for the final product. Also to facilitate the lyophilisation and conversion of the product into a ready-to-eat recipe. The lyophilized powder of the final product would not contain any variations due to vegetable chunks, hence making the nutritional value uniform throughout the batch of production.

Preparation of Ragi Soup Infused with *Ulva fasciata*

The soup recipe was optimized for every 100 ml of Ragi milk. Add Ragi milk and suitable amount of water in a cooking pan and boil the mixture. Add the vegetable homogenate and the varying proportions of ingredients for each trials i.e., salt, pepper, cinnamon, spring onion, peas, sautéed garlic. The mixture is allowed to cook for 15mins. Based on the final volume of the soup (calculated and estimated prior) the amount of seaweed powder (*U.fasciata* dry powder) would be calculated and added appropriately (i.e., 1g of dry seaweed for every 100ml of the final product).

Development of Sorghum Ice Cream

Material Required

Sorghum millet (*Sorghum bicolor* L.), Guava (*Psidium guajava*), monk fruit sugar, sweeteners, and cocoa powder were procured from local market at Bengaluru, Karnataka, India. Seaweed (*Ulva fasciata*) was procured from the Kanniyakumari, Tamil Nadu, India. The purchased ingredients were stored in refrigerator until further use.

Preparation of Condensed Sorghum Milk

The optimal process for preparation of Condensed Sorghum Milk was optimized based on trial-and-error method. Sorghum millet was weighed and soaked overnight at room temperature in water at varying ratio. The soaked millet was washed thoroughly and excess water was drained. The soaked millet was then boiled for 5 min with varying ratio of water. The mixture was finely ground in a mixer by pulverizing at maximum speed for 10 min. The coarsely grounded mixture was filtered using a strainer with 0.6mm sieve to obtain the milk. The obtained Sorghum milk was boiled with constant stirring, until the desired consistency of Condensed Sorghum Milk was attained.

Preparation of Sorghum Ice Cream Infused with *Ulva fasciata*

For every 100g of the prepared Condensed Sorghum Milk, varying proportions of the ingredients i.e., Cocoa Powder, Maple Syrup, Monk Fruit Sugar and *Ulva fasciata* were added and mixed uniformly using a blender. This mixture was whipped with a mixer and chilled in the freezer before serving. The proportion of each ingredient varied for each trial, however, the p

Development of Foxtail Dosa

Material Required

Foxtail millet (*Setaria italica* L.), fenugreek, urad dhal and salt were procured from local market at Bengaluru, Karnataka, India. Seaweed (*Ulva fasciata*) was procured from the Kanniyakumari, Tamil Nadu, India. The purchased ingredients were stored in refrigerator until further use.

Preparation of Foxtail Dosa Batter infused with *Ulva fasciata*

The foxtail dosa batter was prepared based on trial-and-error methods. Batter was optimized for every 300g of Foxtail millet. Varying proportions of fenugreek seeds and urad dhal were added to foxtail millet and the mixture was soaked in water overnight. After soaking, the water was drained and the grains were ground finely in to a uniform consistency. To this varying proportions of salt was added. For every 100g of the final batter (volume optimized in advance), 1g of dry seaweed (*Ulva fasciata*) was added. This

mixture was allowed to ferment overnight at room temperature. Post fermentation, the batter was cast into dosa on a hot pan and served while hot.

Sensory Evaluation

All the trial-and-error attempts for development for Functional Foods were tested with sensory evaluation. Sensory evaluation was done using a 9-point hedonic scale, for different aspects of the foods such as appearance, texture, mouth feel, aroma, taste and overall acceptance. The sensory panel consisted of semi-trained panel members. For each of the trials that were performed, a total of 30 members were involved in sensory evaluation. The feedbacks of the sensory panel members were incorporated in following trials to improve the taste, appearance and overall acceptance of the product. For the final optimized product, a total of 90 members were involved in the sensory evaluation. The final score for the optimized products were plotted based on mean calculation of the 90 sensory evaluation scores provided by the panel members.

Lyophilization

All the three finalized functional foods were subjected for lyophilisation process, to convert the final products into ready-to-eat foods, with increased shelf life. The lyophilisation was done using BioLab, Bench Top Freeze Dryer instrument, set to -40°C for 48 hours. The lyophilized products were reconstituted by adding lukewarm to hot water, to obtain the functional food, ready for consumption.

Proximate Analysis (Nutritional composition)

Proximate analysis of the final products was executed to understand the nutritional composition of the optimized products, with various nutritional aspects such as moisture content, crude protein, crude fat, total ash, crude fibre, total carbohydrates and energy value for every 100g of the optimized final products. Additionally, inorganic components of the foods were also estimated to understand the mineral composition of the optimized foods. The proximate analysis was executed for the fresh *Ulva fasciata* and for the optimized final functional foods, to understand the nutrients inherited from the seaweed to the functional foods.

Determination of Moisture Content (IS: 16072)

The moisture content of the test samples was determined using the Indian Standard (IS: 16072) method. The test samples were weighed to 2-5gms grams of each sample and transferring it to a pre-weighed crucible or petri dish. The samples were dried in an hot air oven at 105°C (220°F). The initial weight of the sample along with the crucible was measured and the weight of the crucible along with test sample was measured for every 1 hour, while in the hot air oven. The observations were recorded, until there is no change in the weight for 3 observations. The final weight was recorded after the samples had cooled in a desiccator or under ambient conditions. The initial weight of each sample was recorded before drying, and the final weight was recorded after drying. The moisture content was calculated as a percentage by subtracting the final weight from the initial weight and dividing by the initial weight, then multiplying by 100. The moisture content was computed using the formula:

$$\text{Moisture Content (\%)} = (\text{Initial weight} - \text{Final weight} / \text{Weight of sample taken}) \times 100$$

This method provides an accurate and reliable measure of moisture content in seaweed samples.

Determination of Crude Protein (IS: 7219)

The crude protein content of the test samples was determined using the Indian Standard (IS: 7219) method. The test samples were prepared by weighing 2–3 grams and transferring it to a digestion flask. The method involves digestion of the samples in sulfuric acid, followed by distillation and titration of the ammonia liberated. The digestion apparatus was set to 400°C (752°F), and the samples were digested for 2 ~ 3 hours, until a clear solution was achieved. The initial weight of each sample was recorded before digestion, and the final weight was recorded after digestion. The distillation apparatus was set to 100°C (212°F), and the ammonia liberated was collected in a boric acid solution. The boric acid solution was titrated with a standard hydrochloric acid solution until the endpoint was reached. The volume of titrant used for each sample was recorded, and the nitrogen content was calculated using the formula:

$$\text{Nitrogen Content (\%)} = [(V - V_{\text{blank}}) \times N \times 1.4] / (\text{Weight of Sample in grams})$$

where V represents the volume of titrant used for the sample (mL); V blank is the volume of titrant used for the blank (mL); N is the normality of the titrant. The nitrogen content was then converted to crude protein content by multiplying by a factor of 6.25, reflecting the average crude protein content of the sample.

Determination of Crude Fat (IS: 7874)

The crude fat content of the test samples was determined using the Indian Standard (IS: 7874) method. The test samples were prepared by weighing 2.5 grams of each sample and drying them as described under IS: 7874. The dried samples were then extracted with petroleum ether or hexane, food grade, in a Soxhlet apparatus. The extraction period was 16 hours at condensation rate of 2 to 3 drops per second. The extract was dried on a steam-bath for 30 minutes. The extract was then cooled in a desiccator, and weighed. This process was repeated at 30-minute intervals until the difference between two successive weighing was less than one milligram or no difference was observed. The crude fat content was calculated as a percentage by mass using the formula:

$$\text{Crude fat (on moisture-free basis)} = 100 \times (M1 - Ma) / m$$

where M1 is the mass in grams of the extraction flask with dried extract

Ma is the mass in grams of the extraction flask

m is the mass in grams of the dried sample taken for the test

Determination of Total Ash (IS: 14433)

The total ash content of the test samples was determined using the Indian Standard (IS: 14433) method. The test samples were prepared by weighing 2 g of each sample and drying them as described under IS: 14433 (1997). The dried samples were then ignited in a muffle furnace at 600°C for 2 hours to produce the total ash. After ignition, the crucible was removed from the furnace and cooled in a desiccator to room temperature. The weight of the crucible and ash was recorded. The total ash content was calculated as a percentage by mass using the formula:

$$\text{Total Ash (\%)} = [(\text{Weight of crucible containing ash}) - \text{Weight of empty crucible} / (\text{Weight of initial sample})] \times 100$$

Determination of Crude Fiber (IS:10226)

The crude fiber content of the test samples was determined using the Indian Standard (IS:10226) method. The test samples were prepared by weighing 2 g of sample and drying them as described under IS:10226 (1982). The dried samples were then ground into a fine powder and defatted by boiling with a standard volumetric solution of sulphuric acid. The insoluble residue was separated and washed with distilled water to remove any impurities. The insoluble residue was then boiled with a standard volumetric solution of sodium hydroxide to break down any remaining insoluble matter. The insoluble residue was separated, washed with distilled water, and dried to a constant weight. The dried residue was then incinerated to determine the loss in mass, which is used to calculate the crude fiber content. The crude fiber content was calculated as a percentage by mass using the formula:

$$\text{Crude Fiber (\%)} = [(\text{Weight of insoluble residue} - \text{Loss in mass upon incineration}) / \text{Weight of sample}] \times 100.$$

Determination of Total Carbohydrate (IS:1656)

The carbohydrate content in test samples was determined using the IS:1656 method, a standard protocol for food product analysis. 5 g of dried and finely ground sample was subjected to hydrolysis with 200 mL of 1.25% sulfuric acid, boiling under reflux for 2.5 hours to convert carbohydrate polymers into simple sugars. The hydrolysed mixture was then neutralized to pH 7 using 1M sodium hydroxide, filtered, and clarified. The resulting filtrate was analysed by the phenol-sulfuric acid method, where a 2 mL aliquot of the filtrate was reacted with 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid. After a 10-minute colour development period, the absorbance was measured at 490 nm using a UV-Vis spectrophotometer. Carbohydrate content was quantified by comparing the absorbance to a standard glucose calibration curve, and results were expressed as grams of carbohydrate per 100 grams of dry test sample.

Determination of Energy (Calculated)

The energy content of test samples was determined using the macronutrient composition-based calculation method. Nutrient analysis was conducted to ascertain the quantities of carbohydrates, proteins, and fats present in the test samples. The caloric contributions of these macronutrients were then calculated using the Atwater general factors, where carbohydrates and proteins each contribute 4 kcal/g, and fats contribute 9 kcal/g. In cases where fiber content was significant, the caloric value for fiber was adjusted to 2 kcal/g. The energy from each macronutrient was summed to obtain the total energy content per 100 grams of the sample. This method allowed for a comprehensive assessment of the caloric value, accounting for the specific nutrient profile of the test samples under investigation.

Determination of Potassium (PL/SOP/CM/139/00)

The potassium content in test samples was determined using the PL/SOP/CM/139/00 method, a standardized procedure for mineral analysis. Approximately 1 gram of test sample was digested in a microwave digestion system with 10 mL of concentrated nitric acid and 2 mL of hydrogen peroxide to ensure complete breakdown of the organic matrix. The digested solution was then cooled, filtered through Whatman No. 42 filter paper, and diluted to a final volume of 50 mL with deionized water. Potassium quantification was performed using Flame Photometry, with the instrument calibrated against standard potassium solutions of known concentrations. The emission intensity at the potassium-specific wavelength (466nm) was measured and compared to the calibration curve to determine the potassium concentration, expressed as milligrams of potassium per 100 grams of test sample.

Determination of Magnesium (PL/SOP/CM/182/00)

The magnesium content in test samples was determined using the PL/SOP/CM/182/00 method, a standardized procedure for mineral analysis. Test samples were weighed to 0.5 grams, and subjected to microwave digestion using a mixture of 5 mL concentrated nitric acid and 2 mL hydrogen peroxide in a sealed digestion vessel. After complete digestion, the solution was cooled, filtered through Whatman No.42 filter paper, and diluted to a final volume of 50 mL with deionized water. Magnesium quantification was performed using Atomic Absorption Spectroscopy (AAS), with the instrument calibrated using standard magnesium solutions of known concentrations. The absorbance was measured at the magnesium-specific wavelength (285nm), and the concentration was determined by comparing the absorbance with the calibration curve. Results were expressed as milligrams of magnesium per 100 grams of dry seaweed sample.

Determination of Sodium (PL/SOP/CM/139/00)

The sodium content in test samples was determined using the PL/SOP/CM/139/00 method, a standardized procedure for mineral analysis. One gram of test sample was digested using a microwave digestion system with 10 mL of concentrated nitric acid and 2 mL of hydrogen peroxide in a sealed digestion vessel to ensure complete decomposition of the organic matrix. The digested solution was then cooled, filtered through Whatman No.42 filter paper, and diluted to a final volume of 50 mL with deionized water. Sodium quantification was performed using Flame Photometry, with the instrument calibrated using sodium standard solutions of known concentrations. The emission intensity at the sodium-specific wavelength (589nm) was measured and compared to the calibration curve to determine the sodium concentration, which was expressed as milligrams of sodium per 100 grams of test sample.

Determination of Calcium (IS:13433)

The calcium content in test samples was determined using the IS:13433 method, a standardized procedure for mineral analysis. Test sample approximately 1 gram each, were accurately weighed and transferred into a 250 mL beaker. The samples were then treated with 20 mL of 1:1 hydrochloric acid (HCl) and heated gently to dissolve the calcium. The solution was filtered to remove any insoluble material, and the filtrate was collected in a 100 mL volumetric flask, made up to volume with distilled water. Calcium quantification was performed using Atomic Absorption Spectroscopy (AAS). The AAS instrument was calibrated using calcium standard solutions of known concentrations. The absorbance of the sample solutions was measured at the calcium-specific wavelength (422.7nm). The calcium concentration in the samples was determined by comparing the absorbance readings with the calibration curve obtained from the standard solutions. The results were expressed as milligrams of calcium per 100 grams of test sample.

Determination of Iron (PL/SOP/CM /264/00)

The iron content in seaweed samples was determined using the PL/SOP/CM/264/00 method, a standardized procedure for mineral analysis. Approximately 1 gram of test sample was digested using a microwave digestion system. The sample was mixed with 10 mL of concentrated nitric acid and 2 mL of hydrogen peroxide in a sealed digestion vessel to ensure complete breakdown of the organic matrix. After digestion, the solution was cooled to room temperature, filtered through Whatman No. 42 filter paper, and diluted to a final volume of 50 mL with deionized water. Iron quantification was performed using Atomic Absorption Spectroscopy (AAS). The AAS instrument was calibrated with iron standard solutions of known concentrations. The absorbance of the sample solutions was measured at the iron-specific wavelength (248.3nm). The concentration of iron in the samples was determined by comparing the absorbance readings with the calibration curve obtained from the standard solutions. The results were expressed as milligrams of iron per 100 grams of test sample.

Results

Seaweed Sample Collection and Authentication

Fresh samples of wild edible green seaweed were collected from the seafloor, with help of local fisher man, from the Kanniyakumari district of Tamil Nadu state. Kanniyakumari is the meeting point of Bay of Bengal, Indian Ocean, and Arabian Sea. The point of sample collection was at 8° 6' 24.4368" N 77° 33' 32.7492" E, Kanniyakumari, Tamil Nadu, India. The collected samples were transported in a clean container under refrigeration to the laboratory for further analysis. The obtained sample was washed with distilled water to remove silica, shells and other unwanted debris from within the thallus of the green seaweed. The cleaned thallus of the collected edible green seaweed is shown in Fig. 1A.

The freshly collected green algae was pressed into a herbarium sheet. The prepared herbarium was submitted to Central Salt & Marine Chemicals Research Institute (CSMCRI), Marine Algal Research Station (MARS), Madapam, Rameswaram. It was identified and authenticated as *Ulva fasciata* by Dr.V.Veeragurunathan, Principal Scientist, MARS Mandapam, CSMCRI. The authenticated herbarium is shown in Fig. 1B.

The procured seaweed samples were dried in tray drier at 50°C for 48 hours to remove moisture and the dried sample was ground to fine powder for further processing. The moisture content of the collected same was 70.54%. Upon drying using tray drier, every 1kg of the fresh sample yielded 295g of dry powder. The dried powder was further utilized for *Drosophila melanogaster* toxicity analysis and Functional Food development.

***Drosophila melanogaster* Toxicity Analysis**

Acute & Chronic

To observe for any physiological and toxic side effects of the procured *Ulva fasciata*, an in-vivo acute and chronic toxicity studies were performed on *Drosophila melanogaster* flies. The flies were segregated into three groups with each group consisting of 30 flies, i.e., i) Control group (fed with semolina media); ii) 1% Seaweed group (fed with 1% *U.fasciata* dry powder mixed in semolina media) & iii) 2% Seaweed group (fed with 2% *U.fasciata* dry powder mixed in semolina media). The effect of seaweed diet was observed both in parent flies (inoculated 30 flies) and F1 generation flies (newly eclosed flies). The observations made for the parent flies are considered to be acute toxicological effects, as the adult flies were exposed to the intervention only for a duration of 7 days. The observations made for the F1 generation flies are considered to be chronic toxicological effects, as the flies were exposed to the intervention, throughout their life span, i.e., from hatching to eclosure (the intervention was terminated on 7th day after eclosure).

There was no mortality observed in both parent and F1 generation flies, as all the 30 parent flies and all the newly eclosed flies were alive throughout the experiment's observation period. The fecundity of the flies were also unaffected, as the seaweed fed groups and control group demonstrated similar number of eclosed flies, without any significant difference. Hence based on the mortality and fecundity observations, it was concluded that, feeding 1% and 2% seaweed (dry *U.fasciata*) in diet, does not cause any alternations in the reproduction capabilities of the flies and also does not cause any toxic effects that leads to death of the flies. These observations confirm no severe acute or chronic toxicity.

Negative geotaxis assay also known as climbing assay, was performed to observe any locomotor deficits in the group fed with seaweed as compared to the healthy control group. The parent flies were allowed to feed on the seaweed infused media for 7 days, post which the negative geotaxis assay was performed. The newly eclosed flies were allowed to continue feeding on the seaweed infused media for 7 days from the day of eclosure, post-which they were subjected for negative geotaxis assay. The results of the negative geotaxis assay are graphically represented in the Fig. 2A. Compared to the control group, the seaweed groups demonstrated significant increase in the negative geotaxis. In parent flies the 1% seaweed group demonstrated 122% pass percentage, while 2% seaweed group demonstrated 140% pass percentage, that are significantly higher than the control group which is normalized to 100%. Similarly, the F1 generation flies also demonstrated increased negative geotaxis in the seaweed fed groups compared to the control group. The 1% seaweed group demonstrated 125% pass percentage, while the 2% seaweed group demonstrated 147% pass percentage, when compared to the control group. Observations of the negative geotaxis assay suggests that, feeding of *U.fasciata* (1% and 2%) significantly increases the locomotor ability of the flies, that could possibly be due to improved neurological function, metabolism and muscle function.

Feeding assay was performed to understand the appetite, hunger and feeding capacity of the flies. After 7 days of intervention, 10 flies from each group were subjected for the feeding assay i.e., 10 flies were transferred to a vial containing feeding assay media for 24 hours (semolina media infused with 1mg/g of Methylene Blue (MB) dye). Post 24 hours incubation in the feeding assay media, the flies were anesthetized using diethyl ether, further were washed in distilled water and crushed in ethanol. The amount of MB dye present in the supernatant was measured using UV spectrophotometry and were quantified. The intensity of the MB dye is a direct representation of the amount and frequency of feeding habit of the flies. The parent flies demonstrated a strong increase in feeding capacity i.e., the 1% seaweed group demonstrated 30% higher food consumption, while 2% seaweed group demonstrated 60% higher food consumption, in comparison to the control group. Similarly, the F1 generation flies also demonstrated strong increase in the feeding amount i.e., the 1% seaweed group demonstrated 22% higher food consumption, while 2% seaweed group demonstrated 27% higher food consumption, in comparison to the control group. Compared to the parent generation, the F1 generation demonstrated a decreased amount of food consumption, probably due to metabolic adaptation of the F1 generation flies, which could be due to longer exposure of the intervention. The increased food consumption could possibly be due to increased metabolic rate, increased muscle activity, or increased locomotive functions. However, feeding assay strongly suggests that, the flies that were fed with seaweed are consuming and metabolizing food at faster rate, compared to the control group flies.

A strong statistical correlation was observed between the negative geotaxis assay and feeding assay results. The parent flies demonstrated a strong 0.997 correlation coefficient between negative geotaxis and feeding assay, while the F1 generation demonstrated a strong 0.951 correlation coefficient. This strong correlation suggests that the increased food consumption observed in the feeding assay is directly proportional to the increased locomotion demonstrated by the flies in the negative geotaxis assay. The increased movement of the flies, causes higher metabolism and higher energy expense that creates a demand for higher consumption of food to compensate the spent energy. Hence, the negative geotaxis assay and feeding assay strongly suggests, feeding 1% and 2% seaweed (dry *U.fasciata*) in diet, significantly increases the muscle function, neurological function and gastrointestinal (digestion & metabolism) function of the flies.

The individual flies of the F1 generation were subjected for morphological investigation under stereomicroscope, to identify any possible morphological anomalies in the flies due to long term exposure (chronic). The representative image of male and female flies from each group are shown in Fig. 3. The morphology of the seaweed fed flies were unaffected (both male and female), as they did not show any deviation or anomalies, as compared to the control group. The morphology of female control fly compared to the female fly of 1% seaweed group and female fly of 2% seaweed group are shown in Fig. 3A, Fig. 3B and Fig. 3C respectively. The morphology of male control fly, compared to male fly of 1% seaweed group and male fly of 2% seaweed group are shown in Fig. 3D, Fig. 3E and Fig. 3F respectively.

The *Drosophila melanogaster* toxicity studies (acute & chronic) strongly suggests that, *U.fasciata* does not cause any detrimental effects, such as mortality, infertility, morphological anomalies, loss of pigmentation, impaired locomotion, impaired digestion & metabolism. Thus confirming, consumption of *U.fasciata* (1% and 2% in food) is safe and does not cause any undesirable side effects.

Functional Food Development

Current investigation aimed at developing a range of functional foods that delivers the medicinal properties of *U.fasciata* in a palatable form that can be introduced in regular day-to-day diet. The primary goal is to deliver the antidiabetic properties of *U.fasciata* to regulate blood sugar levels. Three different types of foods were developed, that provides options and varieties for the public to choose from, based on convenience, comfort and life style. Soup is a starter for a good meal and aids in increasing appetite and digestion properties. Dosa is considered as one of the main courses that provides satiation (fullness) and acts as the main element in providing majority of protein and carbohydrate portion in a meal. Desserts (ice cream) is usually a finishing component of the meal, that provides sweet and delicious end. Desserts are known to trigger digestion and provide a satisfaction to a good meal. The current investigation was aimed to develop these three different components of a meal, that are mainly based on millets and infused with *U.fasciata*. The soup is based on Ragi (Finger Millet), Dosa is based on Foxtail Millet and Ice Cream is based on Sorghum (Jowar).

The study focused on utilization of diabetic friendly (low-GI) ingredients for all the three food variants, that would provide options for diabetes mellitus patients to enjoy a good wholesome meal. All ingredients used in the product development are known to have a GI index of less than 50 and that are recommended for the diabetic patients. These developed products are infused with 1% of dry seaweed powder (*U.fasciata*), keeping in mind not to exceed the Recommended Dietary Allowance (RDA) of edible marine seaweeds. All the developed products were lyophilized to increase the shelf life and also to convert these products into ready-to-eat recipe for consumers convenience. The photographic representation of all the 3 developed functional foods along with their lyophilized powder are shown in Fig. 4.

All the three products developed in the current study are completely 'Vegan' in nature and are organic without any preservatives or chemical additives. These properties make these products ideal for all group of population (vegan, vegetarian, eggetarian, non-vegetarian, etc.) irrespective of their dietary habit. All the ingredients used in the products are low-GI components, that makes these products suitable for health enthusiasts, who prefer eating organic and healthy over processed and preserved foods. Lyophilization was opted as the mode for preservation, since lyophilization is a physical method and chemical-free method of preservation.

Ragi Soup (Finger Millet)

Photographic representation of the final product 'Ragi Soup' is shown in Fig. 4A. Ragi Soup infused with *U.fasciata* dry powder was successfully developed, following 7 trials. To prepare the Ragi Soup, 'Ragi Milk' was used as the primary base, that consisted of aqueous extract of Ragi seeds. Use of Ragi powder directly for preparation of soup, did not provide a desirable texture (resulted in highly viscous and pasty product), hence 'Ragi Milk' serves as a primary base to obtain a texture that is considered desirable for a soup/stew. The process for extraction of 'Ragi Milk' was optimized based on 5 trials that are tabulated in Table 1. Among the 5 trials, the 5th trial yielded the best quality and quantity of 'Ragi Milk' with desirable texture. The optimized procedure for 'Ragi Milk' extraction was; soaking 1:2 ratio of Ragi:Water for 8 hours, followed by 15 mins of grinding in blender and filtering through a sieve to obtain the 'Ragi Milk' with uniform and smooth texture. This was used as base for preparation of Ragi Soup.

Ragi Soup was prepared based on multiple trials, with various permutation combinations of the ingredients. Among the major 7 trials, the 7th trial yielded the most desirable flavor and texture, based on sensory analysis. The combinations of ingredients for all the 7 trials are tabulated in Table 2. All trials of soup development were immediately followed by sensory panel evaluation, which consisted of semi-trained panel members, for improvement and optimization of the product based on the panel's feedback. The 7th trial of Ragi Soup was identified as most palatable and acceptable by the sensory panel. The results of the sensory panel for the 7th trial of Ragi Soup infused with *U.fasciata* is graphically represented in Fig. 5. The final recipe for the Ragi Soup, that was considered most palatable and acceptable is; 100ml of Ragi Milk, 200ml of water, 7g of carrot, 7g of capsicum, 7g of peas, 7g of spring onion, 14g of sweet corn, 1g of garlic, 1g of salt, 1g of pepper, 1g of cinnamon and 1g of dry *U.fasciata* powder.

The developed Ragi Soup was lyophilized to obtain a ready-to-eat instant soup powder. Every 100ml of the soup yielded 10g of lyophilized powder. Reconstitution of 10g of lyophilized powder, with 90 ~ 100ml hot water resulted in ready-to-eat soup that could be flavored with additional salt or pepper according to consumers preference. Ready-to-eat instant soup powder provides greater advantage in terms of increased shelf life and consumer convenience in consumption and preparation time.

Sorghum Ice Cream (Jowar)

Photographic representation of the developed Sorghum Ice Cream infused with *U.fasciata* is shown in Fig. 4B. The base of the ice cream was provided by 'Condensed Sorghum Milk', that yielded the texture desirable for ice cream / dessert. The trials performed

for optimization of Condensed Sorghum Milk is tabulated in Table 3. Among 9 different trials that were performed, trial 9 was identified as the optimized process for preparation of Condensed Sorghum Milk. The optimized process for preparation of Condensed Sorghum Milk, was identified as; soaking 1:1 ratio of Sorghum:Water for 8 hours followed by boiling for 5mins and blending for 15mins; this mixture was filtered through sieve and the filtrate was boiled again for 5mins to obtain the desired consistency and texture of Condensed Sorghum Milk.

The process for preparation of palatable and desirable Sorghum Ice Cream infused with *U.fasciata* was optimized based on 9 trials. The variations in each of the trials is summarized in Table 4. Each of the trial was followed by sensory panel analysis, to improve the palatability based on feedback of the panel members. The most palatable and desirable recipe was identified as; 100g of Condensed Sorghum Milk in combination with 2.5g of cocoa powder, 5.5g of monk fruit sugar and 1g of dry seaweed powder. This mixture was whipped with a mixer and chilled in the freezer before tasting and serving. The result of the sensory panel analysis for the optimized recipe is graphically shown in Fig. 5. Post preparation of the Condensed Sorghum Milk, the product did not require any heating/cooking. The recipe was mixed, whipped and frozen before serving.

The finalized product was subjected to lyophilization to increase the product shelf life and also to make it convenient for the consumers preparation. Upon lyophilization, every 100g of the Sorghum Ice Cream resulted in 30g of dry powder. Reconstitution of the lyophilized powder with 70 ~ 80ml of hot water, followed by gentle whipping and freezing resulted in obtaining the desirable texture and ready-to-eat Sorghum Ice Cream infused with edible marine seaweed.

Foxtail Millet Dosa

Photographic representation of the developed functional food 'Foxtail Millet Dosa' is shown in Fig. 4C. The investigation was mainly focused on the dosa batter optimization, so as to deliver this product as an instant dosa batter mix, that is commercially popular. A total of 9 trials were performed to produce different variations in the batter and the prepared batter was cooked into a dosa and was tested by the sensory panel. Details of the variations in each trial are tabulated in Table 5. Originally, both Foxtail millet and Sorghum millet both were tested for dosa batter preparation. Based on the sensory panel feedback, the flavor of Sorghum and Seaweed combination was rejected and only Foxtail was further optimized.

The final recipe for preparation of Foxtail Millet Dosa Batter infused with *U.fasciata* dry powder was optimized as; soaking 300g of foxtail millet grains, 5g of fenugreek seeds, 50g of urad dhal for 12 hours, after which the mixture is ground finely in to a uniform texture; following this, 3g of salt and 1g of seaweed were added to the mixture and allowed to ferment for 8 hours at room temperature. After fermentation, the batter was cooked into a dosa and was tested by the sensory panel. The sensory panel feedback for the final optimized product is shown in Fig. 5.

The finalized foxtail millet dosa batter, was then subjected for lyophilization to convert the product into an 'instant dosa batter mix' and to increase the shelf life of the product. Upon lyophilization, every 100g of the dosa batter yielded 40g of dry powder. Reconstitution of the product was possible, by mixing 40g of lyophilized powder with 60 ~ 70ml of lukewarm water, followed by finely mixing the product without any lump formation and allowing to stand for 30mins. The product can then be cooked into dosa of desirable thickness and crispiness.

Sensory Panel Analysis

All the three developed products were tested with semi-trained sensory panel members to understand the acceptability and other aspects of the food. The various trials of the products were evaluated with a minimum of 30 panel members, while the final optimized product was evaluated with a minimum of 90 panel members, so as to obtain a more reliable sensory evaluation. Overall, all three products were scored above 7 for overall acceptance in the 9-point hedonic scale. This shows that the developed products are acceptable in regards to their texture, flavor, appearance, mouthfeel and aroma. However, the aroma of the products exhibited the unique seaweed fragrance, that was disliked by a considerable number of panel members. Overall, majority of the panel members were pleased with the aroma and other aspects of the product, making these developed products suitable and acceptable by the common public. The results of the sensory panel analysis, strongly suggest that, the developed products are palatable and can be accepted by the common public.

Proximate Analysis (Nutritional Composition)

Proximate analysis of all the developed products and the fresh edible marine seaweed was performed to compare the nutritional composition of the products and to understand the contribution of the developed products in the consumers recommended dietary allowance of each component. Results of the proximate analysis are tabulated in Table 6. The fresh *Ulva fasciata* seaweed demonstrated high moisture content (70.5%), which makes this product shelf life very short. Hence, tray drying of the sample was the best method to preserve it for a longer duration and also it would provide a uniform biomass to be infused in the developed products. As a marine plant, *U. fasciata* exhibited a significantly strong inorganic components such as potassium, magnesium, sodium and calcium, however, iron content was not very significant. The high amount of minerals in this product, is evident that the amount consumed on a daily basis to be checked, as it could increase the mineral composition in the consumers. The three developed products inherited the mineral composition of the seaweed and also inherited the key mineral elements present in the millet base that was utilized for product development.

Ragi Soup demonstrated high amounts of sodium content, making it vital for the consumers to limit the intake, if co-morbidities such as blood pressure impairment is present. Sorghum Ice Cream demonstrated a significant level of calcium, that makes the product ideal for early menopausal women and others with bone related ailments. Foxtail Millet Dosa provides a strong level of potassium, magnesium and calcium, making this ideal for elderly women who are susceptible to osteoporosis and other bone related ailments. Overall, the proximate analysis shows a well-balanced proportion of nutrients that are present in the developed products, that gives the consumer a significant proportion of the RDA for each component. This proximate analysis, helps in standardization and label making of the products, for commercial applications.

Table 1
Trials for Ragi (Finger Millet) Milk Extraction process optimization

Process	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Finger Millet Soaking Time	10hrs	12hrs	8hrs	12hrs	8hrs
Finger Millet: Water	1:1	1:2	1:3	1:3	1:2
Blending time	15mins	15mins	15mins	15mins	15mins
Interpretation	Rejected	Rejected	Rejected	Rejected	Selected

Table 2
Trials for optimization of recipe and process for Ragi Soup production

Ingredients	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7
Ragi powder	10g	10g	-	-	-	-	-
Ragi milk	-	-	100ml	100ml	100ml	100ml	100ml
Water	250ml	200ml	200ml	250ml	250ml	200ml	200ml
Carrot	5g	-	3.5g	3.5g	5g	7g	7g
Capsicum	5g	7g	3.5g	3.5g	5g	7g	7g
Peas	5gm	7g	-	3.5g	5g	7g	7g
Sweet corn	10g	-	-	3.5g	5g	14g	14g
Spring onion	-	-	-	-	-	7g	7g
Cabbage	5g	-	3.5g	3.5g	-	-	-
Ginger	1g	0.5g	-	-	1g	-	-
Garlic	1g	-	-	-	-	-	1g
Salt and pepper	1g	1g	0.5g	1g	1g	1g	1g
Cinnamon	-	-	-	-	-	1g	1g
Seaweed	1g	1g	1g	1g	1g	1g	1g
Interpretation	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Selected

Table 3
Trials for Condensed Sorghum Milk Extraction process optimization

Process	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Soaking hours	8hrs	8hrs	8hrs	8hrs	8hrs	8hrs	8hrs	8hrs	8hrs
Sorghum:Water	1:3	1:3	1:3	1:2	1:2	1:2	1:1	1:1	1:1
Boiling	-	5mins	5mins	-	5mins	5mins	-	5mins	5mins
Blending	15mins	15mins	15mins	15mins	15mins	15mins	15mins	15mins	15mins
Boiling	-	-	5mins	-	-	5mins	-	-	5mins
Interpretation	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Selected

Table 4
Trials for optimization of recipe and process for Sorghum Ice Cream production

Ingredients	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Condensed Sorghum Milk	100g	100g	100g	100g	100g	100g	100g	100g	100g
Cocoa Powder	1g	1.5g	2g	2.5g	2.5g	2.5g	2.5g	2.5g	2.5g
Maple Syrup	1.5ml	2ml	3ml	4ml	5ml	-	-	-	-
Monk Fruit Sugar	-	-	-	-	-	3g	4g	5g	5.5g
Seaweed	1g	1g	1g	1g	1g	1g	1g	1g	1g
Interpretation	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Selected

Table 5
Trials for optimization of recipe and process for Foxtail Millet Dosa Batter production

Ingredients	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Sorghum	300	300	300	300	-	-	-	-	-
Foxtail	-	-	-	-	300g	300g	300g	300g	300g
Fenugreek	1g	2g	3g	4g	1g	2g	3g	4g	5g
Urad dhal	50g	50g	50g	50g	50g	50g	50g	50g	50g
Soaking	12 hrs	8 hrs	8 hrs	12 hrs	12 hrs	8 hrs	10 hrs	10 hrs	12 hrs
Salt	3g	3g	3g	3g	3g	3g	3g	3g	3g
Seaweed	4g	3g	2g	1g	5g	4g	3g	2g	1g
Fermentation	16 hrs	16 hrs	8 hrs	8 hrs	16 hrs	8 hrs	8 hrs	8 hrs	8 hrs
Interpretation	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Selected

Table 6
Proximate analysis of Functional Foods infused with green edible marine seaweed (*Ulva fasciata*)

Sl.No	Parameter	Units	Fresh Seaweed	Ragi Soup	Sorghum Ice Cream	Foxtail Dosa Batter
1	Energy	KCal/100g	106.7	38	107	152
2	Moisture	%	70.54	89.86	69.9	61.45
3	Crude Protein	g/100g	5.83	2.22	3.6	5.94
4	Crude Fat	g/100g	0.25	N.D	0.36	0.4
5	Total Ash	g/100g	3.1	0.72	3.7	1.08
6	Crude Fiber	g/100g	5.76	1.2	1.95	2.9
7	Carbohydrate	g/100g	20.28	7.2	22.44	31.13
8	Potassium (K)	mg/100g	887	18.6	19.6	340
9	Magnesium (Mg)	mg/100g	453	68	80	110
10	Sodium (Na)	mg/100g	93	136	58	49
11	Calcium (Ca)	mg/100g	410	96	156	180
12	Iron (Fe)	mg/100g	10	N.D	1	2.6

Note: N.D denotes 'Not Detected'

Discussion

The increasing demand and popularity for millets as an alternative to rice and wheat is a clear sign that the public are becoming more conscious about healthy foods and are attempting to manage metabolic disorders by choosing functional foods over processed foods. The drastic increase in diabetes mellitus has dramatically increased the popularity of millets among diabetes patients for managing blood sugar level. Scientific investigations in the past decades have proven that millets such as Ragi, Sorghum, Foxtail, Kodo, Little millets are significantly effective in managing type 2 diabetes mellitus (Oh et al. 2020; Ren et al. 2022; Sawant and Tavhare 2023). Several research investigations have developed functional foods based on millets, for effective management of diabetes mellitus and other metabolic disorders (Bora et al. 2019).

Edible marine seaweeds have proven to be effective source of bioactive molecules that help regulate blood sugar levels in diabetes mellitus. Several studies on utilizing edible marine seaweeds as active ingredients in functional foods have been investigated, to develop a possible diet-based management solution for diabetes mellitus (Mohamed et al. 2012; Murray et al. 2018; Jayakody et al.

2021b). Edible marine seaweeds also serve as a rich source of minerals, that are important for bone regulation, nerve and muscle function, kidney function, glucose metabolism and regulations of several other body metabolism (Singh et al. 2016; Lozano Muñoz and Díaz 2022).

To the best of knowledge, there are no reported Functional Foods that combines the health benefit of millets and green seaweed together. The current investigation is a novel study that combines the antidiabetic properties of millet and green seaweed (*Ulva fasciata*) to deliver functional foods that are delicious, enjoyable and are also medicinally beneficial in management of metabolic disorders such as diabetes mellitus.

Although studies on *Ulva fasciata* in functional food development is limited, the few studies that are reported are following the similar line of work compared to the current study. A study by Jayakody et.al., (2021) has developed a 'Nori' snack using *Ulva fasciata*, in attempts to increase the consumption and popularity of edible green seaweeds in Asia. 'Nori' is a snack made by pressing dried seaweeds in to thin sheets, that can be used in various food recipes, such as seasoning, wrappers for sushi, and other types of Japanese cuisine foods. The authors developed the Nori sheets using dried *U. fasciata* and has performed proximate analysis and sensory analysis for the product. The authors reported high amounts of calcium and iron in the dried snack, however, potassium was not detected in the final product. The results of the current investigation also correlate high amount of calcium and significant amount of iron; however, it contradicts the concentration of potassium. According to the results of the current study, the fresh *U. fasciata* thallus, contains 887mg/100g of potassium (K), whereas, in the Jayakody et.al., (2021) result, potassium was not detected (ND) in the dried Nori sheets. This variation could be due to the different methods employed for mineral quantification i.e., Jayakody et.al., used XRF method to quantify minerals, whereas in the current study, authors have used both AAS and Flame Photometry to quantify the minerals. Hence, this could be the primary cause for variation in the reported levels of potassium (K). Nevertheless, the results of both the study strongly suggests that *Ulva fasciata* is a significant source of minerals, such as calcium, iron, magnesium and potassium (Jayakody et al. 2021a).

A study by Egodavitharana et.al., (2023) has developed edible green seaweed infused crackers (functional food), that has incorporated *Ulva lactuca* and *Ulva fasciata* as two different variants of the product. The authors utilized wheat as the primary base element to form the crackers and have infused the edible marine seaweeds, to enrich the product with minerals and other bioactive molecules (Egodavitharana et al. 2023). Since the functional food is a baked product, in which the ingredients are exposed to high temperature such as > 220°C, there is high probability of the bioactive molecules to be denatured. However, such high temperatures will not affect the concentration and properties of the inorganic elements i.e., minerals. These crackers would still have increased health benefits due to the mineral composition, however the stability of the bioactive molecules in the final product are to be investigated. The authors have also performed proximate analysis and sensory analysis of the products and concluded that the developed products are palatable and acceptable (Egodavitharana et al. 2023). Work flow of this study is very much similar to the current investigation. However, the current study aims to develop functional foods, that delivers both the bioactive molecules and minerals in its native form. The recipes of the functional foods developed in the current study does not include high temperatures, making sure that the bioactive molecules are unaffected and the product can deliver the anti-diabetic properties of the bioactive molecules unhindered.

These literature evidences strongly suggest that, development of functional foods using millets (such as Ragi, Foxtail, Finger millets, etc.) and edible marine seaweeds (such as *Ulva fasciata*) are gaining attention among the researchers and is one of the possible and feasible solution for management of metabolic disorders via dietary supplements. Combination of millets and edible seaweeds is a novel venture for providing a solution to manage diabetes mellitus in the form of Functional Foods.

Conclusion

Millet based functional foods infused with *Ulva fasciata* appears to be a novel nutritional solution for management of diabetes mellites. The current study has developed three functional food recipes (i.e., Ragi Soup, Sorghum Ice Cream and Foxtail Millet Dosa) that combines health benefits of millets with nutraceutical properties of edible green seaweed *Ulva fasciata*. The antidiabetic properties of *Ulva fasciata* and the nutritional benefits of millets make them attractive food choices for individuals with diabetes to help maintain blood sugar levels. The efficacy of these functional foods are yet to be investigated either in animal models and or via clinical study. This study provides a new venture for combining multiple diabetic-friendly food ingredients into a unique recipe that could demonstrate heightened beneficial effects compared to the individual components.

Declarations

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Competing Interests

No known competing interest

Availability of Data and Material

Not applicable

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Figures

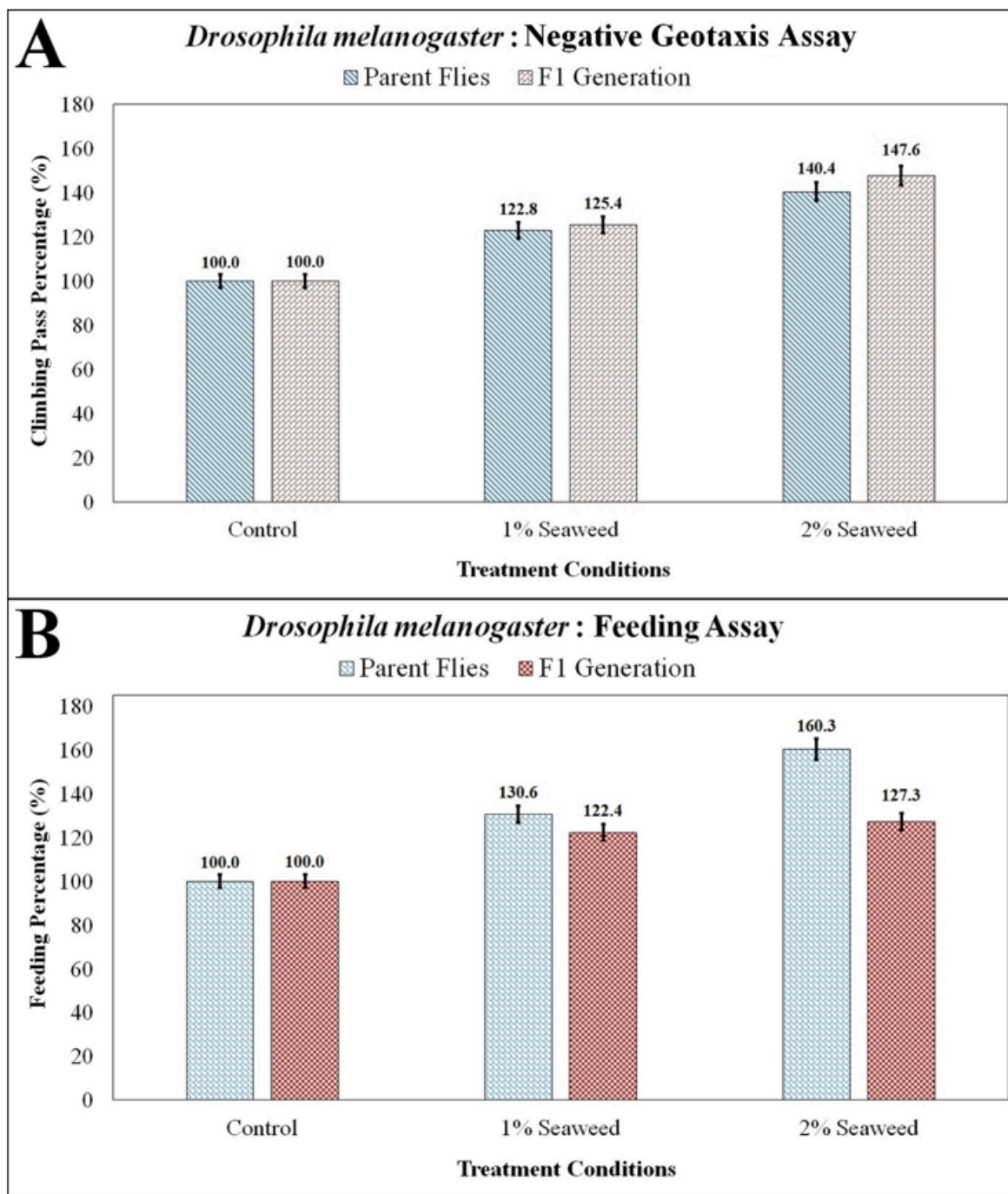


Figure 2

Drosophila melanogaster toxicity studies on dried *Ulva fasciata*; (A) Geotaxis assay; (B) Feeding assay

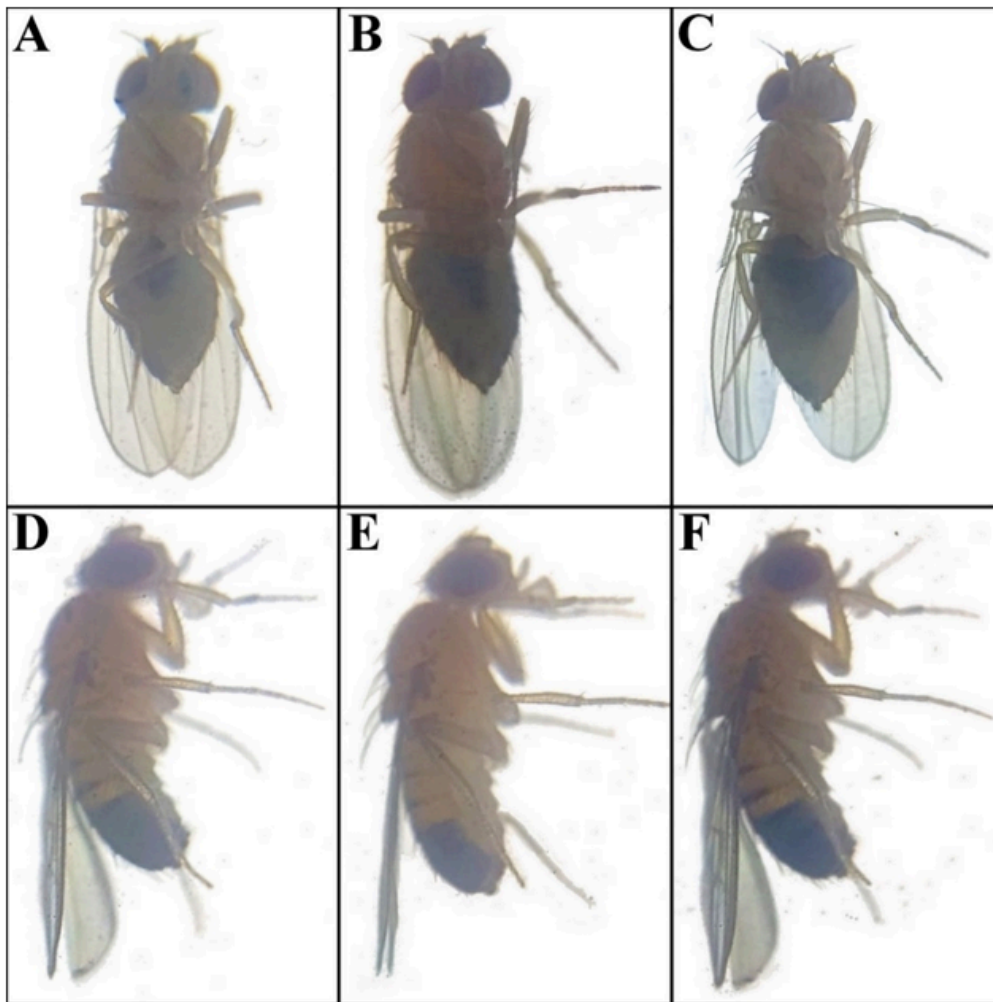


Figure 3

Drosophila melanogaster toxicity studies on dried *Ulva fasciata*. Representative morphology of F1 generation flies subjected to the toxicity studies; (A) Female fly of control group; (B) Female fly of 1% seaweed group; (C) Female fly of 2% seaweed group; (D) Male fly of control group; (E) Male fly of 1% seaweed group; (F) Male fly of 2% seaweed groups.



Figure 4

Final presentation of developed Functional Foods infused with green edible marine seaweed (*Ulva fasciata*); (A) Ragi Soup; (B) Sorghum Ice Cream; (C) Foxtail Millet Dosa.

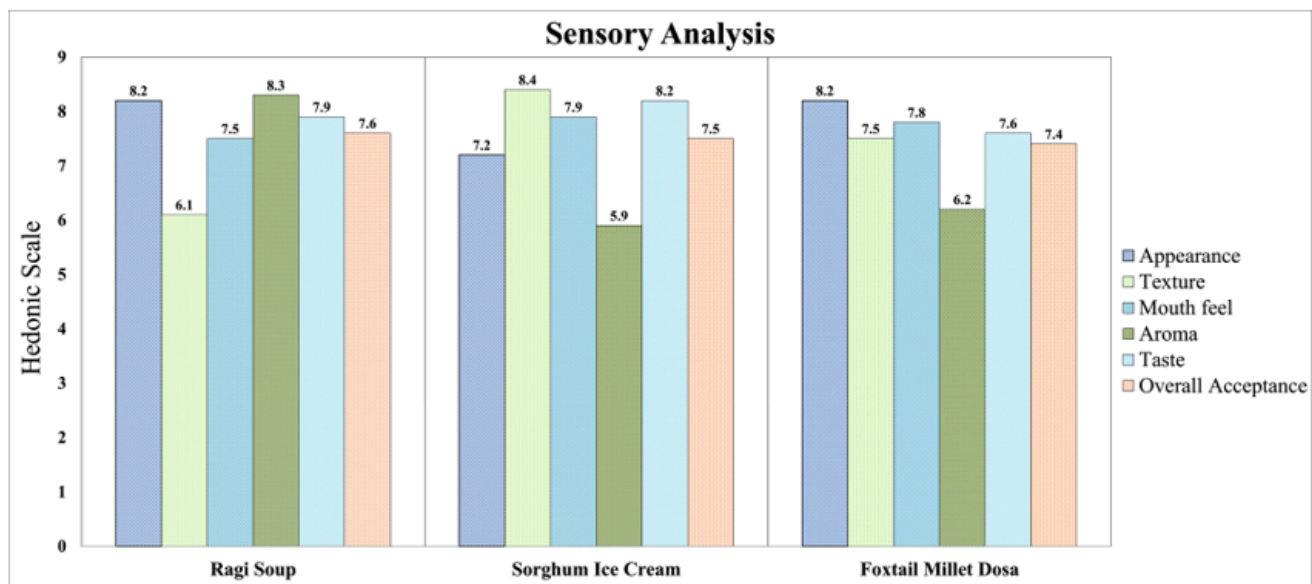


Figure 5

Graphical representation of sensory analysis for developed Functional Foods infused with edible marine seaweed (*Ulva fasciata*)