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PROTEIN ENRICHMENT OF YOGHURT USING SEAWEED EXTRACTS

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ABSTRACT

Seaweed is considered a superfood and is extensively used as a nutritious ingredient in the global food industry. In Sri Lanka, yoghurt containing seaweed extracts is not produced commercially; hence, an initiative was taken through this research. The present study was conducted to develop a novel variety of gelatin-free yoghurt enriched with protein using seaweed extracts, thereby aspiring to widen the consumer audience. This study investigated the nutritional composition of Arthrospira platensis (spirulina) and Gracilaria edulis (red algae). Spirulina was chosen as the nutritional enhancer due to its high protein content, while red algae was used as the gelling agent. Development of the enriched yoghurt supplemented with prepared seaweed extracts was carried out on the basis of the best ratio of extraction, with the highest protein content and best gelling property. The protein content of sixteen different combinations of seaweed yoghurt containing spirulina (0.0%, 0.1%, 0.2% and 0.3%) and red algae (0.0%, 1.0%, 2.0% and 3.0%) was compared against six commercially available yoghurt using the Lowry protein assay. Statistical analysis revealed using spirulina resulted in the production of significantly enriched yoghurt with higher protein content, while red algae proved to be a suitable substitute for gelatin. The highest protein content (0.192g) was observed in the 12th combination incorporating 0.3% spirulina and 2.0% red algae. Microbiological analysis confirmed seaweed extracts do not inhibit probiotic growth. Compared to commercially available yoghurt, seaweed yoghurt is certified to contain sufficient amount of protein and viable probiotics to notably enhance human health.

Keywords: Yoghurt, Spirulina, Red algae, Protein content, Lowry protein assay

INTRODUCTION

Food has been one of the primary necessities of human life. Dairy products in particular provide nutrition and strength due to the macromolecules incorporated within. Amongst the nutritionally complex and biologically active fermented dairy products is yoghurt, whose industrial production was started by Isaac Carasso in 1919. Its assets, costs and values are linked to its biological nature (Aryana and Olson, 2017). Being a probiotic carrier due to the presence of lactic acid bacteria (LAB) Lactobacillus bulgaricus and Streptococcus thermophilus it improves lactose intolerance, enhances immunity and helps maintain body weight. Its acidity provides protection against pathogens and intestinal infections, leading to its rise in consumer demand in the global food market (German, 2014; Weerathilake et.al., 2014). Gelatin is used during yoghurt production to maintain its stability and shape, provide the smooth mouthfeel, creamy texture and shiny appearance. However, its animal sources (calves and pigs) raise concern for those with dietary restrictions like vegetarians and religious consumers (Siriwardhana, 2018). Yoghurt, like any other dairy product, has

to go through end-product testing which is vital part of food manufacturing control strategy. For products in which microbes can survive and grow, routine microbiological analysis is done, which is a biological, molecular or chemical method to detect or enumerate microbes (Zwietering et al., 2016; Sultana et al., 2014; Tebbutt, 2007).

Proteins are crucial to good health. As a macronutrient they are utilized by the body to build and repair muscles and bones, thereby contributing to proper growth and development in children, teens and pregnant women (Bertholf, 2014). They play a key role in hormone synthesis and regulation, coordinate overall physiological functions and can also be used as an energy source to fuel cells. Proteins act as a buffer system, allowing the body to maintain proper pH values of fluids, like blood. Compared to other macronutrients, they are known to be more satiating and help curb hunger. Collectively, these functions make protein one of the most important nutrients for overall good health (Smeuninx et al., 2020). The nutritional value of proteins is measured by the quantity of essential amino acids it contains. The recommended dietary allowance (RDA) of proteins for adults is generally 0.8g.kg−1.day−1. Nonetheless, the actual need varies depending on age, weight, gender and health (Burd et al., 2019). A vast majority of people worldwide, especially young children, are deprived of adequate protein intake due to food insecurity. As a dietary approach to promote wellbeing and minimize adverse health and environmental effects of excess animal protein consumption, incorporation of sustainably sourced seaweed proteins is deemed a promising strategy (Lonnie et al., 2018).

Seaweed, a sub-category of algae, is considered a superfood and is extensively used as a nutritious ingredient in the global food industry (Wells et al., 2017). Nowadays, they are being marketed as "functional foods" or "nutraceuticals" due to the presence of highly bioactive secondary metabolites, providing chemical and pharmacological novelty (Ganesan et al., 2019; Bleakley and Hayes, 2017). The presence of polyphenols, polysaccharides and sterols confer health benefits to the consumer, including antioxidant, anti-inflammatory and antidiabetic properties (Brown et al., 2014). Studies reveal regular seaweed consumption lowers dietary and lifestyle-related diseases (Seghiri et al., 2019; Shannon and Abu-Ghannam, 2019). The term 'phyconomy' embraces large-scale, sustainable seaweed farming in coastal waters for economic benefit (Hurtado et al., 2019). Not only are they cultivated for food, but also used as additives, fertilizers and hydrocolloids (thickening agents) owing to their biochemical composition (Gamal, 2010). However, seasonal changes greatly affect their biochemical composition, as stated by Aroyehun and his colleagues (2019).

Arthrospira platensis (spirulina) is a multicellular cyanobacterium, deriving its name from the helical shaped filaments. Having up to 70% protein content (Table 1), it represents an important staple diet in Asia and is used to address food insecurity and malnutrition (Fujisawa et al., 2010). It modulates immune functions and exhibits anticancer, anti-inflammatory, antidiabetic and anti-aging properties, while the lack of a cellulose wall eases digestion (Hoseini et al., 2013; Karkos et al., 2011). Although relatively easy to cultivate due to less competition for arable land, water and extra resources compared to other terrestrial plants, it flourishes only under extremely alkaline conditions (Wang et al., 2013). Regarded as a nutrient-rich dietary supplement without significant side-effects, it is even used by the National Aeronautics and Space Administration (NASA) for astronauts. Spirulina is listed by the United States

Food and Drug Administration (FDA) under the category Generally Recognized as Safe (GRAS) (Banakar et al., 2020; Mohammadi et al., 2013).

Table 1: Nutritional profile of spirulina (Gutierrez-Salmean et al., 2015).

Nutritional profile of Spirulina *Powder (composition by 100 g)*

Macronutrients		***Vitamins***	
Calories	373	Vitamin A (as β-carotene)[a]	352,000 IU
Total fat (g)	4.3	Vitamin K	1090 mcg
Saturated fat	1.95	Thiamine HCL (Vitamin B1)	0.5 mg
Polyunsaturated fat	1.93	Riboflavin (Vitamin B2)	4.53 mg
Monounsaturated fat	0.26	Niacin (Vitamine B3)	14.9 mg
Cholesterol	< 0.1	Vitamin B6 (Pyridox. HCL)	0.96 mg
Total carbohydrate (g)	17.8	Vitamin B12	162 mcg
Dietary fiber	7.7		
Sugars	1.3	***Minerals***	
Lactose	< 0.1	Calcium	468 mg
Protein B	63	Iron	87.4 mg
Essential amino acids (mg)		Phosphorus	961 mg
Histidine	1000	Iodine	142 mcg
Isoleucine	3500	Magnesium	319 mg
Leucine	5380	Zinc	1.45 mg
Lysine	2960	Selenium	25.5 mcg
Methionine	1170	Cooper	0.47 mg
Phenylalanine	2750	Manganese	3.26 mg
Threonine	2860	Chromium	<400 mcg
Tryptophan	1090	Potassium	1,660 mg
Valine	3940	Sodium	641 mg
Non-essential amino acids (mg)			
Alanine	4590	***Phytonutrients***	
Arginine	4310	Phycocyanin (mean)[b]	17.2%
Aspartic acid	5990	Chlorophyll (mean)[b]	1.2%
Cystine	590	Superoxide dismutase (SOD)	531,000 IU
Glutamic acid	9130	Gamma linolenic acid (GLA)	1080 mg
Glycine	3130	Total carotenoids (mean)[b]	504 mg
Proline	2380	β-carotene (mean)[b]	211 mg
Serine	2760	Zeaxanthin	101 mg
Tyrosine	2500		

Gracilaria edulis (Rhodophyta or red algae) is an edible marine algae containing high amounts of polysaccharides, polyphenols and lipids (Table 2). Hence, it is used as a predominant raw material in the production of phycocolloids (agar, agarose and carrageenan) (Torres et al., 2019). Although traditionally used as food, it is extracted for multiple purposes including medicinal, pharmaceutical, biotechnological and cosmeceutical applications (Kasanah et al., 2015; Mohammadi et al., 2013). Red algae sources many biologically active secondary metabolites and serves as a lead in the synthesis of novel natural medicines (Gamal, 2010). It plays a major role in cancer treatment, while possessing antioxidant and antibacterial activities as well (Iyer and Murugan, 2012).

Table 2: Nutritional profile of red algae (Pal et al., 2015).

Nutrient	Amount present	Nutrient	Amount present
Ash	38.91 g/100 g	Calcium	295.50 mg/100 g
Crude protein	9.58 g/100 g	Copper	0.20 mg/100 g
Crude fibre	10.40 g/100 g	Zinc	1.00 mg/100 g
Crude lipid	2.00 g/100 g	Iron	67.35 mg/100 g
Saturated fatty acid	48.92% of total fatty acids	Manganese	4.16 mg/100 g
Total amino acids	889.78 mg/g of protein	Nickel	0.92 mg/100 g
Moisture	88.88%	Cobalt	0.24 mg/100g
Vitamin C	28.50 mg/100 g	Sulphate	106.20 mg/100 g
Carbohydrate	45.92%	Chlorine	1170.00 mg/100g
Potassium	8633.00 mg/100 g	Lead	1.11 mg/100 g
Magnesium	549.50 mg/100 g	Cadmium	0.14 mg/100 g
Phosphorus	278.50 mg/100 g	Sodium	158.50 mg/100 g

In Sri Lanka, gelatin-free yoghurt enriched with protein incorporating the combination of spirulina and red algae extracts is not produced commercially. Hence, an initiative was taken through this research, which aspires to widen the consumer audience through the production of a novel variety of gelatin-free yoghurt enriched with protein using seaweed extracts. During the present study, spirulina was introduced as the nutritional enhancer to improve the protein content of yoghurt, while red algae was used as the gelling agent, substituting gelatin.

Experimental Procedure

Study design

The present study was conducted during the period from June 2020 to September 2020 within the laboratory premises of the Department of Biotechnology, Faculty of Science, BMS. The methodology was adapted from procedures described by Godlewska et al., 2016 and Janairo et al., 2011 with some minor modifications.

Sample collection

Spirulina powder (100g) was purchased from 'Spirulina Sri Lanka' in Neboda, Kalutara. Fresh red algae, which is one of the most commonly available seaweed varieties in Sri Lanka, was collected from the coastal belt of Galle in the southwest. It was transported to the laboratory premises in a cellophane bag with marine water to maintain the conditions of its natural habitat.

Preparation of red algae extraction

The red algae was separated from marine water and washed well under running water for 30 minutes until the macroscopic epiphytes, tiny pebbles, sand particles and other extraneous matter were cleared off. Using distilled water, it was re-washed three times with continuous agitation, whilst each round lasted 10 minutes. The wet weight was measured (65.05g) and the dry weight was calculated as 10% (6.505g). Using a Bunsen burner, the red algae was boiled for 45 minutes in distilled water with a volume 45 times its dry weight. The clear liquid extract was filtered through a muslin cloth and refrigerated at 4-5°C (Figure 1).

Figure 1: *Clear red algae extract.*

Preparation of seaweed yoghurt

The yoghurt cups were washed and ultraviolet (UV) treated for 15 minutes. Sixteen different combinations of spirulina extract (0.0%, 0.1%, 0.2% and 0.3%) and red algae extract (0.0%, 1.0%, 2.0% and 3.0%) were weighed out and added to each cup accordingly (Table 3), to investigate the best ratio of seaweed extraction, giving rise to the highest protein content and best gelling property. Duplicate samples were generated per combination, producing a total of thirty-two sample cups. Yoghurt samples without seaweed extracts were used as the control.

Table 3: Combinations of spirulina and red algae extracts

Spirulina % (w/w) / Red algae % (v/w)	0.0	0.1	0.2	0.3
0.0	0.0,0.0 (1)	0.1, 0.0 (2)	0.2, 0.0 (3)	0.3, 0.0 (4)
1.0	0.0, 1.0 (5)	0.1, 1.0 (6)	0.2, 1.0 (7)	0.3, 1.0 (8)
2.0	0.0, 2.0 (9)	0.1, 2.0 (10)	0.2, 2.0 (11)	0.3, 2.0 (12)
3.0	0.0, 3.0 (13)	0.1, 3.0 (14)	0.2, 3.0 (15)	0.3, 3.0 (16)

500mL of whole milk was boiled until 15% (75mL) of it condensed. During boiling, 10% sugar (50g) and a few drops of vanilla essence were added and gently stirred continuously with a whisk to prevent the formation of a cream layer. The milk was boiled until 85°C and left to cool until 60°C before adding 8mL to each cup. It was further allowed to cool down to 40°C, following which 2mL starter culture mixture was added consisting of 1.5% (0.15g) starter culture, bringing the total volume of yoghurt per cup to 10mL. They were incubated at 37°C for 16 hours. Finally, the samples were refrigerated at 4-5°C to prevent overgrowth of probiotics.

Visual observation

The gelling property (rheological behavior) of the yoghurt samples were visually observed to deduce the candidacy of red algae as a substitute for gelatin.

Preparation of reagents

Biuret reagent: 250mL was prepared, with 20g of sodium hydroxide (NaOH) pellets, 0.375g of copper sulphate (CuSO4) and 1.5g of potassium sodium tartrate (KNaC4H4O6·4H2O).

Folin-Ciocalteu reagent (FCR): 40mL was prepared after diluting the reagent x10 to get a suitable working concentration.

Biochemical analysis

The protein content of seaweed yoghurt (SY) samples was compared against six

commercial yoghurt (CY) samples. This was done employing the Lowry protein assay, a biochemical examination. The samples were taken from the refrigerator, left to thaw and stirred till liquified. Serial dilution was performed; an aliquot of 100µL was taken in a test tube and topped up to 1000µL with 900µL of distilled water (10-1 dilution). The process was repeated until 10-3 dilution was reached, to which 2667µL of biuret reagent was carefully added and incubated at 37°C for 15 minutes, turning purple in the presence of proteins. Following this, 333µL of diluted FCR was added and incubated at 37°C for 30 minutes for the development of the characteristic molybdenum blue color (Figure 2), which is directly proportional to the protein concentration.

Figure 2: *Development of the characteristic colors after the addition of reagents.*

The spectrophotometer was blanked at 750nm using distilled water. 3mL of each solution was transferred to a cuvette without disturbing the pellet and the absorbance was taken. All readings were repeated in triplicates for data validation and error assessment. The average values were calculated, and the protein content was estimated using the bovine serum albumin (BSA) standard curve.

Statistical analysis

The quantitative data were entered into a Microsoft Excel spreadsheet (version 2019). One-way analysis of variance (ANOVA) test was performed to compare the mean absorbance values and test for statistical differences between the quality of SY and CY. Statistical significance was assessed at 5% level of significance ($p \leq 0.05$).

Microbiological analysis

The effect of seaweed on the growth of probiotics was investigated employing the streak plate method. In a conical flask, 11.2g of nutrient agar powder was added to 400mL of distilled water, thoroughly mixed and autoclaved at 121°C for 30 minutes. Inside the laminar flow hood, 15mL of the agar solution was poured into petri plates and left to solidify. Quadrant streaking was performed amidst two Bunsen burners for the control yoghurt, best combination of SY and all the CY. The plates were incubated at 37°C for up to 48 hours.

RESULTS AND DISCUSSION

Supplementation of fermented dairy products like yoghurt with seaweed is a promising strategy in boosting their nutritional value and stimulating their functionality. Spirulina being a historical Asian cuisine was selected as the nutritional enhancer due to its high protein content and secondary metabolites that exhibit multiple characteristics and benefits to the host (Varga et al., 2002). Red algae was chosen as a substitute for gelatin due to its rheological nature and gelling property. It forms a rigid gel in water at room temperature even at low concentrations and is heat tolerant, making it a highly valued raw material in the food industry (Kasanah et al., 2015). Applications of seaweeds in the food industry were touched down upon by Kumar and his colleagues (2020) in their study, including its use as a texture

improver in dairy products like yoghurt, cheese and cream.

During algal extraction, marine residues were cleared off as they could interfere with the biochemical analysis. Lime or vinegar could have been used in the washing process to further mask the foul odor, although that may alter the pH (Bleakley and Hayes, 2017; Chronakis and Madsen, 2011).

Since the yoghurt cups and lids were not heat resistant, they were disinfected with high doses of UV radiation rather than using thermal sterilization. Industrial cups are sprayed with hydrogen peroxide (H_2O_2) while an optimal temperature is reached and dried with hot sterile air (Sansebastiano et al., 2007). Barrier laminated materials are desirable in order to sustain a longer shelf life. Conventionally, polylactic acid (PLA) and polystyrene (PS) cups are used to contain yoghurt to ensure effective stackability, oxygen barrier and water vapor barrier (Aggarwal and Langowski, 2020).

During yoghurt production, concealing the color of spirulina was a challenge, while vanilla extract was added to improve the taste and mask the odor (Garcia et al., 2017; Wells et al., 2017). A study by Dubey and Kumari (2011) demonstrated negative sensory effects on yoghurt at high concentrations of spirulina. Pasteurization destroys the majority of vegetative pathogenic and spoilage bacteria in milk. LAB (probiotics) thrive best between 37°C to 50°C, below which they become inactive and above which they die following a logarithmic pattern (Weerathilake et.al., 2014). They exhibit a symbiotic relationship, helping each other grow until they reach a stable equilibrium. S. thermophilus grows best in a neutral, high oxygen environment, producing lactic acid and minor amounts of formic acid. This creates conditions which kick-start the metabolism of L. bulgaricus, which in turn produces amino acids to stimulate the outgrowth of S. thermophilus. Together they convert the lactose naturally present in milk into lactic acid, forming yoghurt (Chen et al., 2017). The probiotics were introduced into the milk through starter cultures, although not directly. Due to its thickness, the starter culture was mixed with an aliquot of milk after it cooled down to 40°C to form a starter culture mixture. This eases the process of pipetting and transference of minute quantities. Following the fermentation period, the yoghurt samples were refrigerated to inhibit the probiotics and bring them to a dormant state as extensive fermentation period produces acidic yoghurt due to the accumulation of lactic acid (Aryana and Olson, 2017; Han, 2016).

Upon visual observation following incubation for 16 hours, all the yoghurt samples incorporated with red algae extracts had solidified, concluding that all percentages of red algae used were within a suitable range to exhibit gelling properties. However, samples not incorporated with red algae had also solidified (2nd, 3rd and 4th), which can be attributed to the presence of spirulina, inferring that it also possesses gelling property (Seghiri et al., 2019). The colors ranged from cream to intense green depending on the percentage of spirulina used (Figure 3). There was no uniformity in their color and appearance as some spirulina extracts were deposited on the underside of the cups, perhaps due to improper homogenization, the type and amount of spirulina used. Fresh liquid extract is a better alternative which would additionally contain higher protein content. On the downside, the shelf life would be compromised (O'Sullivan, 2016).

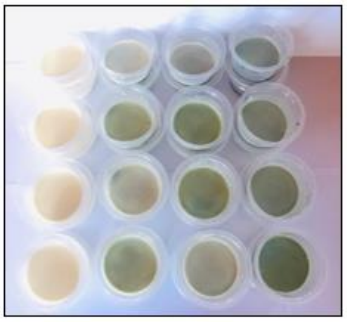


Figure 3: *The color range of SY from cream to intense green depending on the percentage of spirulina used.*

In order to quantify the protein content Lowry protein assay was used, which is based on two reactions. Firstly, in the biuret reaction cupric ions (Cu2+) generated from copper sulphate are chelated and stabilized by sodium potassium tartrate. Under alkaline conditions maintained by sodium hydroxide, they form a purple-colored complex with nitrogen atoms in the peptide bonds, eventually being reduced to cuprous ions (Cu+). Secondly, the Cu+ ions bound to the radical groups of tyrosine, tryptophan and cysteine react with phosphomolybdic-phosphotungstic acid in FCR (oxidizing reagent) to produce an unstable product that eventually gets reduced to the characteristic molybdenum blue color (Janairo et al., 2011). The samples were allowed to stand for a period of time to ensure complete binding of Cu2+ ions to the peptide bonds; the number of peptide bonds directly correspond to the number of amino acids. The Lowry protein assay is highly sensitive to pH changes and low concentrations of proteins in the range of 0.01–1.0mg/mL (Waterborg, 2009).

Biuret reagent should be handled with care and stored in a dark, cool and well-ventilated place as it is light sensitive, leading to dissociation of the complex by photochemical reactions. It must be discarded if any black precipitation is formed. FCR needs to be diluted prior to use as the concentrated version would hinder transmittance during spectrophotometric reading (Janairo et al., 2011; Bertholf, 2014).

Protein concentration was calculated by measuring the absorbance, which is inversely proportional to the transmittance, based on the linear relationship of the Beer-Lambert law. The equation is given as: $A = \varepsilon lc$, where A is the absorbance, ε is the molar absorption coefficient, l is the path length of the light and c is the concentration of a given solution (Dean, 2014; Waterborg, 2009). The average concentration for each sample was calculated based on triplicate readings and the protein content was estimated using the BSA standard curve. Serial dilution was performed initially to dilute the yoghurt samples up to 10-3 and decrease the turbidity, which would otherwise obstruct transmittance when measuring absorbance values (Parnis and Oldham, 2013).

The average protein content of each combination was plotted and compared. As illustrated in Figure 4, the 12th combination (0.3% spirulina and 2.0% red algae) exhibiting apple green color (Table 4) had the highest absorbance value, hence the highest protein content (0.192g), followed by the 14th combination (0.152g). Although the 10th (0.109g) and 13th (0.116g) combinations differ slightly in their bar heights, the error bars overlap; hence, it cannot be concluded that one is better than the other. The 16th combination was expected to have the highest absorbance and protein content due to having the highest percentage of seaweeds (0.3% spirulina and 3.0% red algae), although it did not (0.062g). This could have presumably been due to colloidal or precipitate formation, the pellet being disturbed and transferred to the cuvette, and the sensitivity of the assay as it mainly detects aromatic amino acids. However, besides aromatic amino acids, a wide range of other compounds (drugs,

sucrose, lipids, monosaccharides, hexamines and reducing sugars) interfere with the FCR. In complex food matrices containing several interfering compounds, this leads to inaccurate estimation of protein content (Maehre et. al., 2018).

The 3rd combination (0.2% spirulina and 0.0% red algae) showed the least absorbance value and thus, least protein content (0.014g), followed by the 1st (0.024g) and 4th (0.023g) combinations, both of whose error bars overlap. In accordance with the combination values, the 1st combination (control) showed a low absorbance value as expected (due to already present milk proteins), but not the least. It was noted that red algae also contributed toward increased protein content as combinations without spirulina (5th, 9th and 13th) also showed significant absorbances.

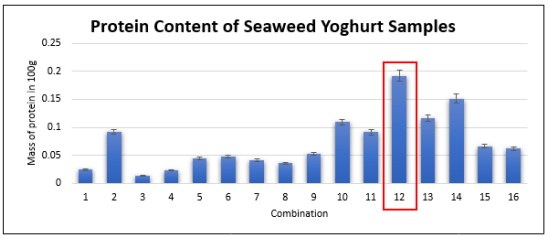


Figure 4: The protein content of SY samples in 100g.

Table 4: The best combination of SY (12th) based on protein content, exhibiting apple green color.

Combination	Appearance	Color
12a	Solid	
12b	Solid	

On comparison, a significant difference was noted between the absorbance values and protein content of SY and CY samples. The protein content of the best SY (12th combination) incorporating 0.3% spirulina and 2.0% red algae was significantly higher than all the CY (Figure 5), proving that spirulina is an excellent source of protein.

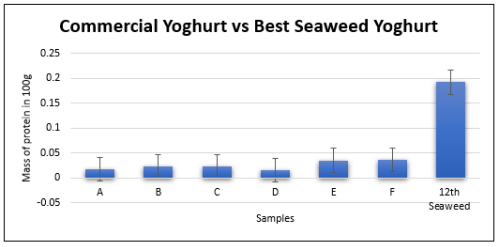


Figure 5: Comparison of six CY against the best SY.

Based on the ANOVA test results, the P-value is 0.00, which is less than the significance level ($p \leq 0.05$), while the F-value is 25.66 and the F-crit is 4.35 (Table 5). This statistically proves that there is a significant difference between the calculated protein values of both SY and CY, with SY possessing a higher protein content due to supplementation with spirulina.

Table 5: Comparing CY and SY using one-way ANOVA

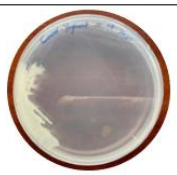
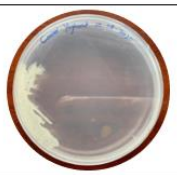
Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Seaweed yoghurt	16	116220.66	7263.7911	24706699		
Commercial yoghurt	6	124096.24	20682.707	48387027		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	785748279.3	1	785748279	25.655595	5.9184E-05	4.351243503
Within Groups	612535622.2	20	30626781			
Total	1398283902	21				

A qualitative microbiological analysis employing the streak plate method was carried out as part of end-product testing to observe the effects of seaweed on the growth of probiotics. Quadrant streaking was carried out in order to better isolate

and identify bacterial colonies after incubation, which provides an optimal growth environment for the bacteria (Zwietering et al., 2016; Tebbutt, 2007). The streaked petri plates were visually observed and cross-sectionally analyzed after both 24 hours and 48 hours (Table 6). The growth of probiotics S. thermophilus and L. bulgaricus in SY was unaffected by seaweed extract type and concentration. Similar results were achieved in a study carried out by O'Sullivan and his colleagues using seaweeds (2016). The works of Guldas and Irkin (2010) concluded that the addition of spirulina in fact promotes the growth of fermenting bacteria. Fadaei and his co-workers (2013) through their research observed a decline in the pH of yoghurt samples, which in turn had a stimulatory effect on the growth of probiotics.

On the other hand, none of the CY presented probiotic growth, most likely due to preservatives, acidification, oxygen barrier packaging and low water vapor transmission rate (Musyoka, 2018; Sultana, 2014; Sansebastiano et al., 2007). However, CY should contain about 106 cfu/mL of viable bacteria. A study by Ranasinghe and Perera (2016) exposed that only 37.5% of marketed brands fulfil this requirement.

Table 6: *Visual observation and cross-sectional analysis of probiotic growth after incubation*

Sample	Probiotic growth (24 hours)	Probiotic growth (48 hours)
Control yoghurt		
SY (12th combination)		

CONCLUSION

To conclude, supplementing yoghurt with spirulina resulted in the production of a novel yoghurt enriched with a higher content of protein compared to CY. All samples with red algae had solidified, proving its candidacy as a substitute for gelatin. Statistical analysis revealed the best ratio of SY was the 12th combination incorporating 0.3% spirulina and 2.0% red algae, while microbiological analysis confirmed the presence of seaweed extracts do not inhibit probiotic growth. SY is thus certified to contain sufficient amount of protein and viable probiotics to notably enhance human health.

Future Work

Due to this being a pilot study, the samples were synthesized on a small scale (10g/sample). The success of this study paves way for large-scale research (100g/sample), involving improvements through sensory evaluation (color, taste, smell, texture and overall appearance). The effects of seaweeds on other nutrient factors were not taken into consideration, which need to be thoroughly studied in the future. This study can be extrapolated to explore the potential of seaweeds as a source of prebiotic biological activity as well. Trials need to be conducted with a random population for unbiased results in order to firmly establish the positive benefits of the novel yoghurt, ultimately leading to branding and development.

ABBREVIATIONS

Abbreviation	Full form
ANOVA	Analysis of variance
BSA	Bovine serum albumin
Cu+	Cuprous ions
Cu2+	Cupric ions
CuSO4	Copper sulphate
CY	Commercial yoghurt
FCR	Folin-Ciocalteu reagent

FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
H2O2	Hydrogen peroxide
KNaC4H4O6·4H2O	Potassium sodium tartrate
L. bulgaricus	Lactobacillus bulgaricus
LAB	Lactic acid bacteria
NaOH	Sodium hydroxide
NASA	National Aeronautics and Space Administration
PLA	Polylactic acid
PS	Polystyrene
RDA	Recommended dietary allowance
S. thermophilus	Streptococcus thermophilus
SY	Seaweed yoghurt
UV	Ultraviolet

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Conflict of Interest

The authors have not declared any conflict of interest.

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