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COMPARATIVE ANALYSIS OF *ULVA LACTUCA* AND *GRACILARIA CORTICATA* FOR FORMULATION OF BAKERY PRODUCTS

KAKOLI BANERJEE[1]*, ARCHANA SNEHASINI TURUK[1] AND BALU ALAGR VENMATHI MARAN MARAN[2]*

[1]*Department of Biodiversity & Conservation of Natural Resources, Central University of Odisha, Sunabeda, Koraput, 763004 India.* [2]*Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.*

**Corresponding author: banerjee.kakoli@yahoo.com, bavmaran@ums.edu.my*
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Abstract: Value was added to breads, cakes and cookies BY incorporating green seaweed (*Ulva lactuca*) and red seaweed (*Gracilaria corticata*) powder. Six lots of each were prepared using the same formulation of 2 % seaweed powder with conventional ingredients) and were evaluated for phytochemical, antioxidants, vitamins and essential amino acids (EAAs). Results indicated that phytochemical content ranged from 0 mg TAE/g dw [total tannin content in Conventional Cake (CCa)] to 4.12 mg GAE/g dw [total phenol content in *Gracilaria corticata* Cookies (GcCo)]; antioxidants ranged from 0 [astaxanthin in Conventional Bread (CBr), CCa, Conventional Cookies (CCo)] to 460±0.5 µg/g dw (CCo), 0 (carotenoid in CBr, CCa, CCo) to 398.8±0.4 µg Trolox/g dw (ABTS in GcCo); vitamins ranged from 0 (V-B_5, V-B_{12} and V-C in CBr, CCa, CCo) to 23.66±0.4 µg/g [(V-E in *Ulva lactuca* Cookies (UlCo)] and total EAAs ranged from 0.193 (CCo) to 5.54 g/100g [*Gracilaria corticata* Bread (GcBr)]. Duncan's post hoc analysis proved *G. corticata* products are better than *U. lactuca* and that the post-monsoon period was the best time to collect the seaweed . A cost-benefit analysis showed a slightly higher price for the value-added seaweed products compared with conventional products. Hence, the study recommends value-added bakery products as a sustainable seaweed culture backed market .

Keywords: Bread, cake, cookies, *Gracilaria corticata*, *Ulva lactuca.*

Introduction

Seaweeds or marine macroalgae are a well-known food crop as they are a rich source of health-promoting compounds which can cure many human diseases (Lordan *et al*., 2011). Dias *et al*. (2012) stated antiviral drugs are synthesised either from naturally derived (specialized metabolites) plants or from organic chemicals. Currently, "blue organisms" (marine life) is used in the extraction of antioxidants Vitamin C - Ascorbic acid, Vitamin E - α-tocopherol for the development of drugs to cure human diseases (Malve *et al*., 2016). Historical records utilising seaweeds as medicinal supplements date back to before 300 B.C. These include treatments for cancer, dermatitis, hypothyreosis, involuntary urination, kidney problem, parasitic infections as well as gastrointestinal and glandular disorders (Youngmin *et al.*, 2012). Studies related to the clinical bioactivity of algal extracts (*Gracilaria corticata*, *Sargassum muticum*, *Ulva clathrata, U. compressa*, *U. flexuosa*, *U. intestinalis*, *U. lactuca, U. linza*) have also proved the antioxidant capacity of seaweed, especially for dealing with the oxidation of free radicals (Farasat *et al*., 2014; Pinteus *et al.*, 2017; Sundaramurthy & Shantaram, 2017).

Phytochemicals, vitamins and amino acids are among the most favourable seaweed elements as a food source and nutraceuticals that are important for health. Antioxidants are synthesised by plants which inhibits oxidation in living organisms. Dietary seaweed, including brown, red and green seaweed are consumed by almost 75 % of the population in East and Southeast Asian countries as they have been found to contain diverse bioactive compounds that have various health benefits including anti-

hypertensive, antioxidant and anti-inflammatory effects. As such seaweed helps improve the immune response to viral infections.

Oxidative stress which is a combination of the development of free radicals like antioxidants and Reactive Oxygen Species (ROS) are an essential element in the pathogenesis of chronic diseases in human beings (Fedoreyev *et al.*, 2018). Viral infections are magnified by oxidative processes that help with the replication of infected cells and inhibit proper cell function (Gullberg *et al*., 2015). Antioxidant compounds help the immune system (lymphocytes) via apoptosis, releasing pro-inflammatory chemokines [(interleukin-1, interleukin-6 and Tumour Necrosis Factor apha (TNF apha)] which inhibits viral replication.

Vitamin E is another antioxidant that helps with to prevent superoxide production that interacts strongly with the antioxidant systems (Milito *et al*., 2020). Hence, the intake of exogenous antioxidants can help prevent such cellular oxidative stress. The present paper has tried to compare the nutritional components of value-added bakery products infused with green (*Ulva lactuca*) and red (*Gracilaria corticata*) seaweed for commercial use.

Materials and Methods

Seaweed Sampling

Edible seaweeds (*Ulva lactuca* and *Gracilaria corticata*) was collected seasonally by hand from the Tenneti Park area situated at latitude 17º44'50.207''N and longitude 83º20'59.2434'' E on the Visakhapatnam coast of Andhra Pradesh from August 2019 to July 2020 (Figure 1). The seaweed samples were identified using standard taxonomic keys (Rao & Sreeramulu, 1964). The fresh seaweed was collected and washed with distilled water to remove sand, sea salt, settled mollusc shells and epiphytes and oven dried (Labotech, Lab instrument, B.D. Instrumentation, India) at 40°C for 24 hours. It was then powdered with the help of a mixer grinder (Bajaj-750 classic mixture grinder) and stored in a sterilised plastic poly pack at ambient conditions for use in additional experiments.

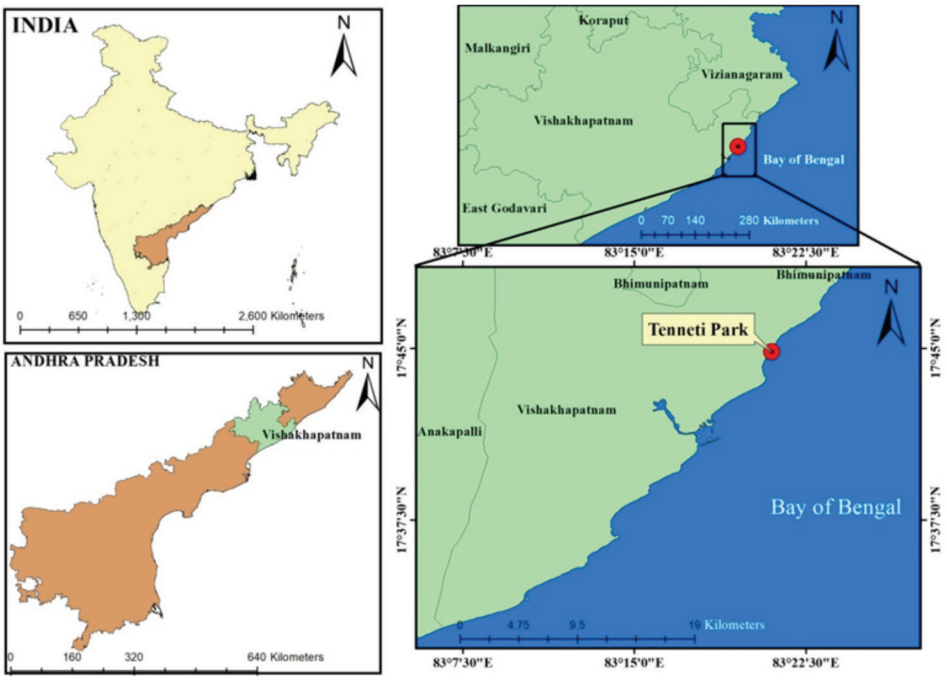

Figure 1: Map of sampling site

Preparation of Bakery Products

Before mixing or preparation of the dough or batter for the products, the raw materials were obtained from the different registered International Organisation for Standardisation (ISO) and the Food Safety and Standards Authority of India (FSSAI) companies. It was autoclaved and kept in a desiccator for complete dehydration to prevent any microbial action to extend the shelf life of the prepared products. Whole wheat bread was prepared following a standard protocol (Adeniji, 2012). Branded and trademarked ingredients were used: for all weighed ingredients including whole wheat flour (Aashirvaad), salt (Tata), sugar (Trust), yeast (Gloripan), commercial water] were mixed thoroughly, and the dough was left to ferment for an hour. Then it was microwaved at 220°C for 20 minutes. The cake batter was prepared by using the procedure outlined by Seth & Kochhar (2018). All branded weighed ingredients including white flour (Ahaar), sugar (Trust), baking powder (Weikfield), baking soda (Weikfield), milk (Omfed), sunflower oil (Fortune), vanilla essence (Symega) was mixed and baked for 20 minutes at 180°C. The dough of whole wheat cookies was made using the method used by Ceserani *et al*., 2008. Al weighed ingredients including whole wheat flour (Aashirvaad), sugar (Trust), baking powder (Weikfield), milk (Omfed), butter (Amul) were mixed together thoroughly and after the cookies were shaped, they were baked at 160°C for 20 minutes.

For the purpose of this experiment, breads, cakes and cookies were prepared with 20 g/1kg of finely powdered *U*. *lactuca* and *G*. *orticate* as ingredients. After the products were baked, the product was kept in a desiccator to completely remove any moisture, packed in plastic poly packs and kept in a refrigerator for use in further analysis. It was compared with conventional breads, cakes and cookies to check their composition and economic viability (Table 1).

The Conventional Bread, *Ulva lactuca* Bread and *Gracilaria corticata* Bread will be expressed in this report as (CBr, UlBr and GcBr), Conventional Cake, *Ulva lactuca* Cake and *Gracilaria corticata* Cake are expressed as (CCa, UlCa and GcCa) and Conventional Cookies, *Ulva lactuca* Cookies and *Gracilaria corticata* Cookies are expressed as (CCo, UlCo and GcCo).

Quality Control Checks

For the purpose of quality control checks the physical aspects of the products including weight was measured using an electronic digital kitchen weighing scale, the specific gravity was measured against AACC (1983) standards, the volume of bread and cake was measured using the AACC (2000b), the volume of the cookies was measured using the method outlined by Jemziya & Mahendra (2017), the specific volume was measured using AACC (2000a), , the diameter, height and thickness was measured using a Vernier calliper 125 mm, while weight loss was calculated with Rodriguez-Garcia (2012) method In terms of textural hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness: products were tested using methods outlined in AACC (2000a) Finally the colour properties [the colour of breads (crusts and crumbs), cakes and cookies] were determined as per the standard methodology using a : Colorimeter.

Sensory Evaluation

The sensory analysis (appearance, colour, aroma, taste, softness, crispiness and overall acceptability) of bread, cake and cookies was also tested via a survey with 50 respondents and the values were expressed in a hedonic scale from 1 to 5 (1-disliked very much=1.00-1.80, 2-disliked moderately=1.90-2.60, 3-neither liked nor disliked=2.70-3.40, 4-liked moderately=3.50-4.20 and 5-liked very much=4.30-5.00) (Emmanuel-Ikpeme, 2012).

Analysis of Seaweeds and Bakery Products

Phytochemical Screening

The powdered seaweed and their baked product samples were placed in test tubes and soaked with four different solvents namely methanol,

acetone, petroleum ether and chloroform at 80% concentrations and kept for 24 hours. The mixture was centrifuged, and the supernatant extracts were analysed for qualitative phytochemical screening of alkaloids, glycosides, flavonoids, steroids, saponins, tannins, terpenoids, phenol, and anthraquinone (Yadav & Agarwal, 2011). For quantitative estimations the total flavonoid, total phenol, total alkaloid and total tannin count was tested using the following protocols.

Total flavonoid count was estimated using the $AlCl_3$ colorimetric method devised by Chang *et al*., 2002. The dried 0.5 g powdered sample was soaked in 10 mL of ethanol (80 %), homogenised and extracted for 30 minutes at 4000 rpm. The supernatant (0.5 mL) was treated with 10 % $AlCl_3$ (0.1 mL), CH_3CO_2K (0.1 mL) and 2.5 mL of distilled water. It was then mixed thoroughly and incubated for 30 minutes under ambient conditions. The reading was measured at 415 nm absorbance with a spectrophotometer and expressed as mg quercetin equivalence per gramme of dry weight (mg QE/g dw) using quercetin as standard.

The total phenol concentration was logged as per the procedure outlined by Sadasivam & Manickam (2007). 0.1 g dried sample was treated with 80% ethanol (5 mL) and rested for 12 hours. The sample was then powdered with mortar and pestle and centrifuged for 20 minutes at 10,000 rpm. About 0.1 mL of supernatant was collected and transferred to a 25 mL Erlenmeyer flask and the volume was adjusted with distilled water. 0.1 mL of the resulting solution was extracted, treated with an FC reagent (0.5 mL) and incubated for half an hour. The absorbance was measured at 650 nm . The result was expressed as mg gallic acid equivalence per gramme of dry weight. (mg GAE/g dw).

The total alkaloid content was evaluated using the procedure given in Harborne & Harborne (1973). A gramme of the powdered sample was soaked in 40 mL CH_3COOH (10 %) in CH_3OH and rested for 4 hours. Then, the filtered extract was kept in a water bath till it became 1/4th of the actual volume. Drop wise undiluted NaOH was added to the evaporated extract till the precipitation was complete. The mixture was kept for between 2 and 3 hours and the precipitation was collected on filter paper. The remaining mixture was soaked in 1 % NH_4^+ solution and rested for 30 minutes. After which the solution was oven-dried, The oven-dried residue was weighed to calculate the product/s alkaloid content and expressed in alkaloid mg/g.

The total tannin count was done following the procedure outlined by Sadasivam & Manickam (2007)where 0.5 g of dry powdered sample was heated to 100 °C with 75 mL of distilled water for 30 minutes. Then, 0.5 mL of the filtered extract was treated with 8 mL of distilled water, Later, 0.5 mL of FC reagent and 1 mL of 10 % $NaCO_3$ was added to the test sample and the sample was rested for half an hour. A reading of 760 nm for the tannin count was then taken. The tannin content was expressed as mg tannic acid equivalence per gramme of dry weight (mg TAE/g dw) using tannic acid as the comparative standard.

Analysis of Antioxidants

Carotenoid was determined according to the procedure used by Kirk & Allen (1965). 0.5 g dried powdered sample was homogenised with 10 mL of 80 % acetone and centrifuged for 15 minutes at 3000 rpm. The supernatant was collected in a 25 mL Erlenmeyer flask. The extraction process was repeated and adjusted the volume with fresh acetone (80 %). The absorbance was recorded at 480, 645 and 663 nm.

$$\text{Carotenoid } (\mu g/g) = \text{Absorbance}_{480} + (0.114 \times \text{Absorbance}_{663}) - (0.638 \times \text{Absorbance}_{645})$$

Astaxanthin was evaluated according to the method of Schuep and Schierle (1995). 0.025 g dried sample was ground with 5 mL DMSO and kept in the water bath at 45 °C for 15 min and cooled at ambient condition. Now, the sample was centrifuged for 3 minutes between 3800-4200 rpm and the supernatant was transferred to a 25 mL Erlenmeyer flask. The centrifuge process was repeated till the sample became colourless. The Erlenmeyer flask was adjusted

with fresh acetone and absorbance was noted at between 471and 477 nm.

The total antioxidant capacity of the extract was evaluated by the phosphor-molybdenum process prescribed by Prieto *et al.* (1999). A dried 0.05 g sample was homogenised with 70% methanol (10 mL). Then, the filtered extract (0.3 mL) was treated with 3 mL of phosphomolybdenum reagent and kept in the water bath for 90 minutes at 90°C. The sample was cooled before the absorbance at 695 nm was recorded. The antioxidant activity percentage was calculated as mentioned below:

Antioxidant Activity (%) = $(A_{sample}-A_{blank}/A_{ascorbic\ acid}-A_{blank}) \times 100$

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was evaluated as stated by Blois (1958). 1 g sample was dissolved in 10 mL methanol, homogenised and left for between 48 and 72 hours. A volume of 1 mL of 0.1 Mm/1DPPH was treated with 3 mL of different levels (ranging from 10 to1000 µg/mL) of sample extract. The solution was mixed properly and rested in a dark place for 30 minutes. The dropping in DPPH free radical absorbance was observed at 517 nm. The mixture with only DPPH solution was taken as control. Below equation was used to calculate the DPPH percentage.

DPPH assay (%) = $(A_{control}-A_{test}/A_{control}) \times 100$

Where $A_{Control}$ = Absorbance of control; A_{Test} = Absorbance of samples

The ABTS (2,2'-azino-bis (3-ethylbenzothia zoline-6-sulfonic acid) assay was analysed by the procedure by Re *et al*. (1999) where 1 g powdered sample was dissolved in methanol (10 mL), homogenised and kept for between 48and 72 hours. 1 mL of sample extracts of different concentrations ranging from 100 to 1000 µg/mL were allowed to react with ABTS solution (2.5 mL). The results were measured at 734 nm. ABTS scavenging activity was expressed as µg Trolox/g dw and the equation below was used to calculate the ABTS as a percentage.

ABTS assay (%) = $(A_{Control}-A_{Test}/A_{Control}) \times 100$

Where $A_{Control}$: Absorbance of ABTS radical in the blank; A_{Test}: Absorbance of ABTS radical with sample solution as standard.

Analysis of Water-Soluble Vitamins

The amount of water-soluble vitamins B_2, B_5, B_7 and B_{12} in the edible seaweed and the bakery products under review was determined by the procedure outlined by Lalitha & Dhandapani (2018).

Vitamin B_2 (Riboflavin): To assess for riboflavins, 1.5 mg of the powdered specimen was placed in a 100 mL Erlenmeyer flask and its volume was regulated with distilled water. 20 mL of filtered solution was transferred into a 25 mL Erlenmeyer flask and again regulated with distilled water. After that, 5 mL was taken from the sample and standard and placed in test tubes and 2 mL of [(HCl (1M), CH_3COOH, H_2O_2, $KMnO_4$ (15% w/v), phosphate buffer (pH 6.8)] was treated. The absorbance was measured at 444 nm with riboflavin in standard opposite to blank.

Vitamin B_5 (Pantothenic acid)*:*The presence of this vitamin was evaluated using 5 mg of the sample , which was put into a 100 mL Erlenmeyer flask and the volume of the sample was regulated with distilled water. A filtered (10 mL) solution was put into an Erlenmeyer flask () and again reworked with distilled water. After that, a 5 mL sample and the standard solution was placed in a 50 mL Erlenmeyer flask and treated with 2 mL HCl (1 M) and boiled for 5 hours at 690°C. The solution was cooled and 2 mL H_3NO reagent (7.5% in 0.1M NaOH), 5 mL NaOH (1M) was added to it, Then it was left for 5 minutes. The pH level was maintained at 2.7±0.1 with HCl (1M) and the volume was adjusted with distilled water. Then, a 5 mL of sample and standard (pantothenic acid) solution was divided into test tubes and 1 mL of 1% $FeCl_3$ solution was added discarding the airlocks. Absorbance was read at 500 nm.

Vitamin B_7 (Biotin)*:* To evaluate for biotin 500 µg samples with 10 mL of DMSO were taken into an Erlenmeyer flask (100 mL). The solution

was heated for 5 min at 600 to 700°C. Then, the volume was adjusted with distilled water and the result was recorded at 294 nm for the sample as well as in standard.

Vitamin B_{12} (Cyanocobalamin): 1 µg sample with 10 mL of distilled water was taken into an Erlenmeyer flask (25 mL). It was treated with 1.25 g Na_2HPO_4, 1.1 g $C_6H_8O_7$ and 1.0 g $Na_2S_2O_5$. The volume was adjusted with distilled water. Then, the solution-filled Erlenmeyer flask was placed in an autoclave at 121°C for 10 minutes. The solution was then filtered and reading of530 nm of standard as well as a sample against blank.

Vitamin C (Ascorbic acid): The test for Vitamin C was evaluated according to the procedure used by Omaye *et al.* (1962), where 1 g of powdered sample was mixed with 10% TCA (4 mL) and centrifuged for 20 minutes at 3500 rpm. The supernatant (0.5 mL) was treated with 0.1 mL of a DTC reagent in a test tube and stood for 3 hours. Then, 0.75 mL of icy cold 65% H_2SO_4 was added to the solution, and it was again left to rest for 30 minutes. Optical density was documented at 520 nm and the result was expressed as µg ascorbic acid per g on dry weight (µg AA/g dw).

Vitamin-E (α-tocopherol): The test for Vitamin E was evaluated as described by Baker *et al.* (1980). 1 g sample was homogenised with ethanol (1.6 mL) and petroleum ether (2 mL) and centrifuged. About 0.2 mL of $C_{10}H_8N_2$ was added to the supernatant (0.5 mL) and stand in the dark for 5 minutes. A reddish colour extract appeared measuring 520 nm. The result was expressed as µg α-tocopherol per g on dry weight (µg α-tocopherol/g dw).

Analysis of Essential Amino Acids

The essential amino acid was estimated using the procedure described by Lumbessy *et al.* (2019) where 1 g of dried specimen was placed in a reaction tube and 6 N HCl (4 mL) was added to it , it was then left to stand for 24 hours at 110 °C to encourage the reflux process. Then, the sample was air-cooled and 6N NaOH was added to it to neutralise and raise its pH level to 7. 10 µL of the filtered extract was mixed with 300 µL of ortho-phthalaldehyde and the solution was rested for 1 minute to allow it to settle for proper extraction. Then, 20 µL of the extract was placed on the HPLC column to isolate the essential amino acids and compare them with 20 types of standard amino acids, the results were then recorded.

Statistical analysis

The seaweed and bakery product values were measured in triplicate and expressed as mean ±SD (standard deviation). Th e seaweed was analysed seasonally with their bakery products. Duncan's multiple test range ($p < 0.05$) was applied in order to find the difference in their quantitative phytochemical, antioxidants, vitamins and essential amino acids compositions using IBM SPSS statistics 21.0.

Results and discussion

Nutraceuticals are often mentioned as a Generally Recognized as Safe (GRAS) food product. According to FAO (2019), seaweed was designated as natural food in 2050.

The present study has utilised *U. lactuca* and *G. corticata* as ingredients for preparing breads, cakes and cookies, the composition for which is given in Table 1. Commercial products have been used as controls whereas the incorporation of 2% of powdered seaweed has been used as the experimental product.

The seaweeds *U. lactuca* and *G. corticata* and their products bread, cake and cookies) were estimated for phytochemicals, antioxidants, vitamins and essential amino acids explained below.

Role of phytochemicals

Phytochemicals from seaweeds were extracted in methanol, acetone, petroleum ether and chloroform where methanol gave a comparatively better result in the qualitative analysis of phytochemicals for the edible seaweeds under review and bakery products made with them.

Table 1: Ingredients used for the preparation of the bakery products with their economics

Ingredients	Price in Rs/kg or Litre	The Formulation of Dough and Cost-Effectiveness of Our Product by Comparing it with Conventional Whole Wheat Bread			The Formulation of Batter and Cost-Effectiveness of Our Product by Comparing it with Conventional Cake			The Formulation of Dough and Cost-Effectiveness of our Product by Comparing it with Conventional Whole Wheat Cookies		
		CBr	UlBr	GcBr	CCa	UlCa	GcCa	CCo	UlCo	GcCo
Whole Wheat flour	35	1 kg (Rs. 35)	1 kg (Rs. 35)	1 kg (Rs. 35)	-	-	-	1 kg (Rs. 35)	1 kg (Rs. 35)	1 kg (Rs. 35)
White flour	30	-	-	-	1 kg (Rs. 30)	1 kg (Rs. 30)	1 kg (Rs. 30)	-	-	-
Salt	20	10 g (Rs. 0.2)	10 g (Rs. 0.2)	10 g (Rs. 0.2)	-	-	-	-	-	-
Powdered sugar	40	20 g (Rs. 0.8)	20 g (Rs. 0.8)	20 g (Rs. 0.8)	800 g (Rs. 32)	800 g (Rs. 32)	800 g (Rs. 32)	500 g (Rs. 20)	500 g (Rs. 20)	500 g (Rs. 20)
Yeast	372	20 g (Rs. 7.5)	20 g (Rs. 7.5)	20 g (Rs. 7.5)	-	-	-	-	-	-
Commercial water	20	500 mL (Rs. 10)	500 mL (Rs. 10)	500 mL (Rs. 10)	-	-	-	-	-	-
Baking powder	300	-	-	-	13 g (Rs. 3.9)	13 g (Rs. 3.9)	13 g (Rs. 3.9)	5 g (Rs. 1.5)	5 g (Rs. 1.5)	5 g (Rs. 1.5)
Baking soda	300	-	-	-	13 g (Rs. 3.9)	13 g (Rs. 3.9)	13 g (Rs. 3.9)	-	-	-
Milk	55	-	-	-	1000 mL (Rs. 55)	1000 mL (Rs. 55)	1000 mL (Rs. 55)	400 mL (Rs. 22)	400 mL (Rs. 22)	400 mL (Rs. 22)
Sunflower oil	185	-	-	-	600 mL (Rs. 111)	600 mL (Rs. 111)	600 mL (Rs. 111)	-	-	-
Vanilla Essence	1750	-	-	-	3 mL (Rs. 5.25)	3 mL (Rs. 1.84)	3 mL (Rs. 1.84)	-	-	-
Butter	480	-	-	-	-	-	-	500 g (Rs. 240)	500 g (Rs. 240)	500 g (Rs. 240)
Seaweed powder	*U. lactuca* powder (Rs. 525) [a] *G. corticata* powder (Rs. 375) [b]	-	20 g (Rs. 10.5)	20 g (Rs. 7.5)	-	20 g (Rs. 10.5)	20 g (Rs. 7.5)	-	20 g (Rs. 10.5)	20 g (Rs. 7.5)
Total price (Rs.)		**53.5**	**64**	**61**	**241.05**	**251.55**	**248.55**	**318.5**	**329**	**326**

CBr: Conventional Bread; UlBr: *Ulva lactuca* Bread; GcBr: *Gracilaria corticata* Bread; CCa: Conventional Cake; UlCa: *Ulva lactuca* Cake; GcCa: *Gracilaria corticata* Cake; CCo: Conventional Cookies; UlCo: *Ulva lactuca* Cookies; GcCo: *Gracilaria corticata* Cookies

[a]https://www.alibaba.com/trade/search?fsb=y&IndexArea=product_ en&CatId=&SearchText=ulva+lactuca+powder&selectedTab=product_en

bhttps://www.alibaba.com/product-detail/Dry-Gracilaria-Seaweed-for-Agar-Agar_1600071594578.html?spm=a2700.details.maylikeexp.2.16686590FuYeAm

A quantitative phytochemical analysis for edible seaweed is given in Table 2 and baked goods with a seaweed component is given in Figure 2. In the quantitative analysis of the seaweed, the total flavonoid (mg QE/g dw) (mean: Ul-0.35±0.05, Gc-0.64±0.04), phenol (mg GAE/g dw) (mean: Ul-1.7±0.4, Gc-2.8±0.4), alkaloid (mg/g dw) (mean: Ul-3.5±0.5, Gc-9.5±0.5) and tannin (mg TAE/g dw) (mean: Ul-0.018±0.005, Gc-0.025±0.005) was analysed seasonally. Phytochemicals was at its lowest in *U. lactuca* over the pre-monsoon and was at its highest in *G. corticata* over the post-monsoon period. The results and data coincided with that of Arulkumar *et al*. (2018) and Prasedya *et al*. (2019). However, Lomartire & Gonçalves (2022) found that the presence of polyphenols and sulphated polysaccharides, alkaloids, flavonoids and tannins in seaweed has manifested superior antiviral activity. Our study has observed higher alkaloid contents (even more than the total flavonoid and phenol levels due to the positive correlation between temperature on phytochemical which reduces the moisture content and increases the nutritious properties of seaweed (Gupta *et al*., 2010 and Elmegeed *et al*., 2014).

The data showed that the total flavonoid content (mg QE/g dw) of bakery products with seaweed ranged between 0.023±0.004 (CBr) and 0.85±0.05 (GcCo), the total phenol content (mg GAE/g dw) ranged between 0.54±0.04 (CCa) and 4.5±0.5 (UlCa), total alkaloid content (mg/g dw) was between 2±0.5 (CCo) to 10.6±0.5 (GcBr) and the total tannin content ranged from 0.001±0.0004 (CBr) to 0.022±0.005 (UlCo) (Figure 2). The upgrade of phytochemicals in value-added products in contrast to conventional products were observed. Similar results were observed by Cox & Abu-Ghannam (2013), who had incorporated *Himanthalia elongata* (brown seaweed) in breadsticks which enhanced its phytochemical content.

In order to define the differences between the phytochemical constituents, Duncan's post hoc analysis was performed on the two different species which showed significant differences in select components ($p<0.05$) excepting *G. corticata* in total flavonoid count in the pre-monsoon period. Significant variations ($p<0.05$) were also observed in total flavonoid, alkaloid, phenol and tannin levels with respect to the products with the seaweed additive excepting UlBr and CCa (in the case of total flavonoids) and total alkaloids (in CCa and UlCo). Since all the reported values were below RDA standards, it can be recommended for inclusion in the daily diet of human beings.

Role of Antioxidants

Antioxidants typically have the capability of inhibiting or scavenging free radicals which helps in inhibiting lipid oxidation (Gupta & Abu-Ghannam, 2011). Augmented production of volatile species in the human body reduces fitness and in a long run reduces an individual's lifespan. Seaweed contains antioxidants which limit oxidative damage from hydrogen peroxide (H_2O_2); superoxide radical anion (O_2^-); hydrogen free radical (HO) and singlet oxygen (O_2) as has been documented by Halliwell and Gutteridge (2007). Although it is a known fact that there are several antioxidant compounds in seaweed their use as dietary products is subpar (Cornish & Garbary, 2010).

Carotenoids and astaxanthin are components found in green and red algae whose ingestion as food helps in improving oxygenation in the body. In the present study, the antioxidant capacities of *U. lactuca* and *G. corticata* were monitored where the values for carotenoid (µg/g dw) ranged between 122±0.5 in *G. corticata* and 1281±0.5 in *U. lactuca* during monsoon with a mean value of 1149±0.3 in *U. lactuca* and 247±0.5 in *G. corticata* (Table 2). The maximum availability of pigment was indicated during the monsoon season because of lower light absorption and greater depth of water (Schubert & Garcia-Mendoza, 2006). In our study, the values for astaxanthin, TAC, DPPH, and ABTS were shown to have similar trends (with the minimum levels seen in pre-monsoon period and the maximum levels seen during the monsoon) as has been stated by Arulkumar *et al.* (2018) and Prasedya *et al*. (2019).

Table 2: The quantitative phytochemicals, antioxidants, vitamins and EAAs in *U. lactuca* and *G. corticata*

Phytochemicals	*Ulva lactuca*	Permissible level of *Ulva* sp.	*Gracilaria corticata*	Permissible level of *Gracilaria* sp.
Total flavonoid content (mg QE/g dw)	0.35±0.05	0.024[a]-55.04[b]	0.64±0.04	0.341[d]-500[f]
Total phenol content (mg GAE/g dw)	1.7±0.4	0.03[1]-8.03[c]	2.8±0.4	2.50[d]-220[f]
Total alkaloid content (mg/g dw)	0.35±0.05	0.079[a]-2.96[d]	0.95±0.05	5.6[f]-9.60[d]
Total tannin content (mg TAE/g dw)	0.018±0.005	0.0075[a]-226.3[e]	0.025±0.005	40-80 %[g]
Antioxidants				
Carotenoid (µg/g dw)	1149±0.3	72[h]-12730[i]	247±0.5	4[p]-5490[q]
Astaxanthin (µg/g dw)	1430±0.5	(102-150)[j]	540±0.5	-
TAC (µg AA/g dw)	717±0.5	554[k]-9200[l]	26±0.5	-
DPPH IC50 (mg/ml dw)	1.99±0.5	0.14[m]-112.6[n]	1.74±0.5	(0.028-0.32)[r]
ABTS (µg Trolox/g dw)	13.48±0.5	(3.29-5.94)[o] mg/ml	263.56±0.5	(50-250)[s] µg/ml
Vitamins (µg/g dw)				
Vitamin B_2	3.97±0.5	0.86[t]-6.2[u]	2.47±0.5	(0.05-2.9)[x]
Vitamin B_5	0.87±0.05	0.3[u]-112[v]	0.37±0.05	-
Vitamin B_7	4.1±0.4	(3.2-2.6)[u]	0.45±0.05	-
Vitamin B_{12}	19.27±0.4	(0.08-0.18)[u]	0.087±0.005	(1.54-21)[w]
Vitamin C	3.4±0.4	2.7[u]-2410[v]	1.63±0.3	(16-149)[y]
Vitamin E	2.9±0.5	0.6[w]-8.6[u]	0.22±0.04	(10.2-1400)[v]
Essential amino acids (g/100g dw)				
Histidine	0.26±0.04	35.5-39.9 %[z]	0.30±0.05	3.43-8.96 mg/100mg[ab]
Isoleucine	0.38±0.04		0.59±0.05	
Leucine	0.63±0.05		0.88±0.04	
Lysine	0.37±0.04		0.57±0.04	
Methionine	0.14±0.04		0.16±0.04	
Phenylalanine	0.56±0.04		0.63±0.04	
Threonine	0.85±0.04		1.01±0.4	
Valine	0.6±0.04		0.89±0.04	
Tryptophan	0.18±0.04		0.29±0.04	
Total EAAs	4		5.28	

Values are expressed as mean ± SD (standard deviation) of triplet experiment

ahttp://dx.doi.org/10.15739/IJAPR.009; bhttps://doi.org/10.1007/s40415-015-0200-8; chttps://www.researchgate.net/publication/259581022_Qualitative_and_quantitative_profiles_of_secondary_metabolites_of_chosen_Chlorophyta_and_Ochrophyta_from_Gulf_of_Mannor; dwww.globalresearchonline.net; ehttps://doi.org/10.1016/j.biteb.2019.100273; fhttps://doi.org/10.12980/jclm.5.2017J7-124; ghttps://actascientific.com/ASMI/pdf/ASMI-02-0159.pdf; hhttps://Doi.org/10.1016/j.sjbs.2015.01.010; ihttps://www.idosi.org/aejaes/jaes3(3)/21.pdf; jhttp://www.idosi.org/ajbas/ajbas1(5-6)09/3.pdf; khttps://www.imedpub.com/articles/evaluation-of-antibacterial-and-antioxidant-activities-of-seaweedsfrom-pondicherry-coast.pdf; lhttps://sci-hub.se/https://doi.org/10.1016/j.jphotobiol.2019.111622; mhttps://doi.org/10.1016/j.sjbs.2015.01.010; nhttps://www.journalcra.com/sites/default/files/issue-pdf/16960.pdf; ohttps://doi.org/10.7508/gjesm.2015.01.005; phttps://doi.org/10.1016/j.sjbs.2015.01.010; qhttps://doi.org/10.7324/JAPS.2012.21118; rhttps://doi.org/ 10.1186/s40538-017-0110-z; shttps://doi.org/10.1016/j.carbpol.2016.09.011; thttps://doi.org/10.21315/tlsr2017.28.2.9; uhttps://www.ijpbs.com/ijpbsadmin/upload/ijpbs_5bf6d98ec62bd.pdf; vfile:///C:/Users/cuo/Downloads/LeonelPereiraChapter2%20(7).pdf; xhttps://doi.org/10.21077/ijf.2016.63.3.60073-11; xhttps://doi.org/10.3390/molecules24122225; yfile:///C:/Users/cuo/Downloads/Dyuthi-T2168%20(2).pdf; zhttps://doi.org/10.21315/tlsr2017.28.2.9; abhttps://www.ijpbs.com/ijpbsadmin/upload/ijpbs_5bf6d98ec62bd.pdf

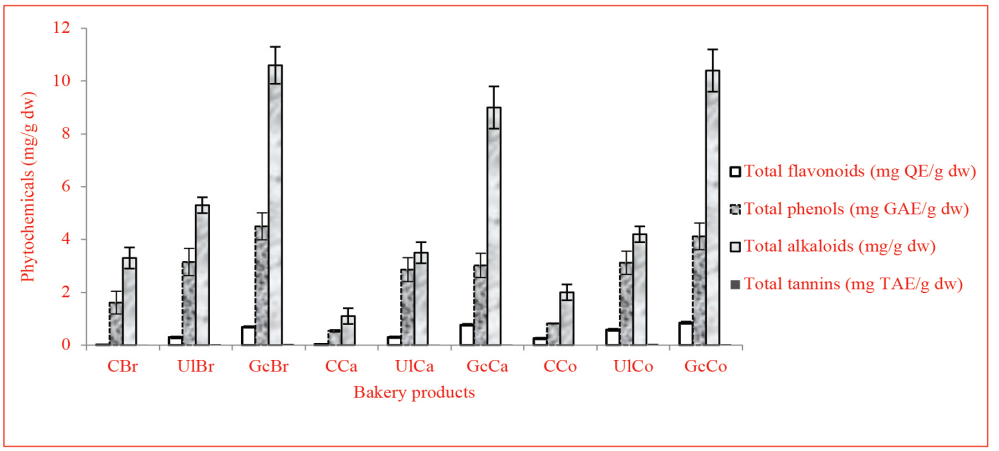

Figure 2: Quantitative phytochemical content of seaweed bakery products

QE: Quercetin equivalent; GAE: Gallic acid equivalent; TAE: Tannic acid equivalent; RDA: Recommended Dietary Allowance RDA: Total flavonoid content: 250-400 (mg d^{-1}) *; Total phenol content: 500-1000 (mg d^{-1})**; Total alkaloid content: not detected; Total tannin content: 1.5-2.5 (g d^{-1})***

*https://doi.org/10.1016/j.biopha.2015.02.028; **https://doi.org/10.17221/166/2013-CJFS; ***https://doi.org/10.1002 jsfa.27403 30116

The carotenoid content (µg/g dw) of bakery products varied from 0 (CBr, CCa, CCo) to 379±0.5 (UlCo), astaxanthin content (µg/g dw) varied from 0 (CBr, CCa, CCo) to 460±0.5 (UlCo), TAC content (µg AA/g dw) varied from 0.10±0.05 (CBr) to 166±0.4 (UlCo), DPPH content (IC50 mg/ml dw) varied from 0.52±0.5 (CBr) to 3.33±0.4 and ABTS content (µg Trolox/g dw) varied from 8.9±0.5 (CBr) to 398.8±0.4 (GcCo) (Figure 3).

The calculated data has showed maximum content of carotenoid, astaxanthin, TAC and DPPH in UlCo whereas

ABTS content was observed to be rich in GcCo in contrast to CBr, CCa and CCo. Our results were supported by Cofrades *et al*. (2008), Cox & AbuGhannam (2013) and Moroney *et al*. (2015). Significant variations were observed at a 5 % level of significance for every antioxidant property between the two seaweeds. Similar observations were noted in the case of the baked goods with those seaweed additives. The total antioxidant capacity (TAC) has shown a comparatively higher value in *U. lactuca* (717±0.5 µg AA/g dw) whereas radical scavenging activity (ABTS) has shown a comparatively higher value in *G. corticata* (263.56±0.5 µg Trolox/g dw) (Table 2). This proves that that baked goods with *U. lactuca* and *G. corticata* may be promoted as an antioxidants for preventing viral infections by inhibiting angiotensin-converting enzymes (ACE and restricting virus ability to enter the cell (Hoffmann *et al*., 2020). Farasat *et al*. (2014) and Saeed *et al*. (2020) have studied five different seaweeds among which *U. lactuca* has recorded the highest content of TAC. Both astaxanthin and carotenoid (compound) are lipophilic scavengers that enhance the antioxidant activity of *U. lactuca* (Heim *et al*., 2002; Mittler, 2002). Additionally, these compounds depended on the switching of aromatic rings and the arrangement of the hydroxyl moieties (Balasundram *et al*., 2006). Accordingly, the higher content of carotenoid and astaxanthin has enhanced the TAC level of the UlBr. However, the presence of polyphenol compounds has increased the ABTS of *G. corticata* (Mittler, 2002). Alike studies were done by Sansone *et al.* (2020). Numerous earlier studies on different algal extracts of DPPH, ORAC, ABTS and TAC have also been studied which have proved their antioxidant capacity (Pinteus *et al*., 2017). Furthermore, the measured results were well within RDA prescribed standard values.

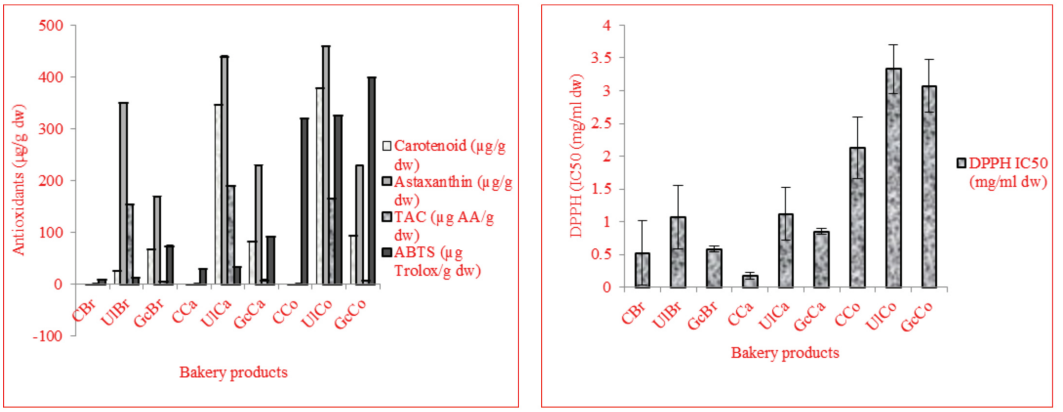

Figure 3: Antioxidant capacity of seaweed baked goods

AA: Ascorbic acid equivalent; Trolox: Trolox equivalent; TAC: Total antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); RDA: Recommended Dietary Allowance

RDA: Carotenoid: 15-180 (mg/d)*; Astaxanthin: 4-40 (mg/d)**; TAC: 29,006 µmol ET/day***; DPPH: not detected; ABTS: not detected

*Longe, J., ed. *The Gale Encyclopedia of Alternative Medicine*, second edition, 2004. Natural Standard Patient Monograph: "Vitamin A (Retinol)" and "Beta-carotene." Office of Dietary Supplements web site: "Vitamin A and Carotenoids." Natural Medicines Comprehensive Database web site: "Beta-carotene"; **https://www.webmd.com/vitamins/ai/ingredientmono-1063/astaxanthin#:~:text=Astaxanthin%20has%20been%20used%20safely,for%20up%20to%2012%20months; ***https://doi.org/10.1111/ijfs.12577

Role of Vitamins

Vitamins are comprised of essential micro-nutrients needed by the human body which cannot be synthesised on their own. Seaweed is a well-known factor of water-soluble necessary vitamins (V-B_2, V-B_5, V-B_7, V-B_{12}, V-C, V-E) and hence can be used as a food supplement to meet the dietary requirements of the human body like antioxidants. Vitamins also help restrict the SARS-CoV-2 virus and promote better immunity (Vázquez-Calvo*et al*., 2017). Seaweed like *Gracilaria* sp. contain comparatively higher levels of V-C wompared with terrestrial vegetables (Hernández-Carmona *et al*., 2009).

In the present study, V-B_2, V-B_5, V-B_7, V-B_{12}, V-C and V-E has showed maximum values during the monsoon season and minimum values during the pre-monsoon season except for V-B_2 (µg/g) that ranged between 2.2±0.5 in *G. corticata* during the post-monsoon period and between 4.6±0.5 for *U. lactuca* during the monsoon period. Lalitha & Dhandapani (2018) has also had similar results w.r.t. vitamins in seaweeds (Table 2).

In the case of the baked goods the vitamins (µg/g) i.e., V-B_2 was highest in UlCa (10.25±0.4) and lowest in CCo (0.72±0.04), V-B_5 in UlCo (2.44±0.4) it was also notably absent in CBr, V-B_7 highest in UlBr (19.15±0.5), lowest in CCa (12±0.5) (), V-B_{12} highest in UlCo (5.7±0.4) and negligible in conventional products (CBr, CCa, CCo).

Similarly, V-C was absent in CBr, CCa, CCo and highest in UlBr (2.85±0.4). V-E was limited in CBr (12.4±0.5) and highest in UlCo (23.66±0.4) (Figure 4). Likewise, result was studied by Senthil *et al*. (2011), whose incorporation of *Kappaphycus alvarezzi* (red seaweed) powder in spices enhanced its vitamin V-B_1, V-B_2 and V-E content.

Significant differences ($p<0.05$) were observed in all the components of vitamins excepting V-B_5 and V-E in *G. corticata* during the monsoon season. Similar differences in the vitamin levels of the baked products in both conventional and experimental products were observed at a 5% significance. However, vitamins were comparatively higher in products with *U. lactuca* and *G. corticata* which proves

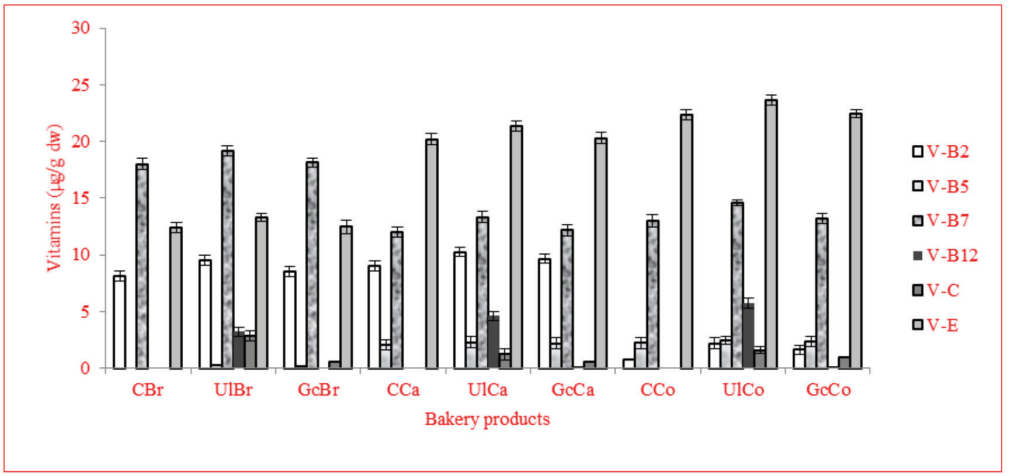

Figure 4: Water-soluble vitamin composition in seaweed bakery products

*Recommended Dietary Allowance (RDA): V-B_2: 1.6 (mg/d); V-B_5: 6.0 (mg/d); V-B_7: 30 (µg/d); V-B_{12}: 6.0 (µg/d); V-C: 75 (mg/d); V-E: 10 (mg/d)

Food Safety and Standards Authority of India (FSSAI): V-B_2: 1.5 (mg/d)*; V-B_5: 5.0 (mg/d)**; V-B_7: 30 (µg/d)***; V-B_{12}: 1.5 (µg/d)**; V-C: 40 (mg/d)**; V-E: 7.5-10 (mg/d)**

*Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation, Bangkok, Thailand, 21-30 September 1998; **Codex (CAC /GL 2-1985-Guidelines on nutrition labelling (applied only for individuals older than 36 months);***ICMR (Nutrient Requirements and RDA for Indians: A report of the expert group of the ICMR, 2010).

their supremacy over the conventional products (Figure 4). The data obtained for vitamins in the prepared products was well within the acceptance range laid out by the FSSAI and the RDA.

Role of Essential Amino Acids

Amino acids are a binding component for proteins and have an important function in nutrition. Seaweed is well known for its functional amino acids like histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan (Uribe *et al*., 2018).

The use of amino acids undoubtedly helps in physiological recovery in the case of malnourished people with low immunity levels and chronic diseases. Hence, the inclusion of amino acids in the daily diet is said to be a first-line nutrition therapy. According to Livesey (1987), a diet with a high biological protein value consisting of essential amino acids (EAAs) (as in the case of seaweeds) is adequate (>150 mg/g protein) to meet amino acid deficiencies in human beings.

Our study also reflected a maximum value of amino acids in monsoon as per the earlier studies by Benjama & Masniyom, (2011). Out of the nine EAAs threonine was the highest in case of in *U. lactuca* and *G. corticata* followed by valine > leucine > phelylalanine > isoleucine > lysine > histidine > tryptophan > methionine respectively (Table 2). In case of the seaweed infused baked products, the EAAs (g/100g) i.e. histidine ranged between 0.016±0.004 (CCo) and 0.40±0.04 (UlBr, GcBr), isoleucine ranged between 0.030±0.004 (CCo) to 0.64±0.03 (GcBr), leucine ranged from 0.029±0.004 (CCo) to 1.22±0.4 (GcBr), lysine ranged between 0.015±0.004 (CCo) and 0.034±0.04 (CBr, GcBr), methionine ranged between 0.019±0.004 (CCo) and 0.28±0.04 (UlBr, GcBr), phenylalanine ranged between 0.027±0.004 (CCo) and 0.98±0.05 (UlBr, GcBr), threonine ranged between 0.020±0.004 (CCo) and 0.79±0.04 (GcBr), meanwhile valine ranged between 0.25±0.004 (CCo) and 0.82±0.05 (GcBr) and tryptophan ranged between 0.012±0.004 (CCo) and 0.23±00.04 (GcBr) (Figure 5).

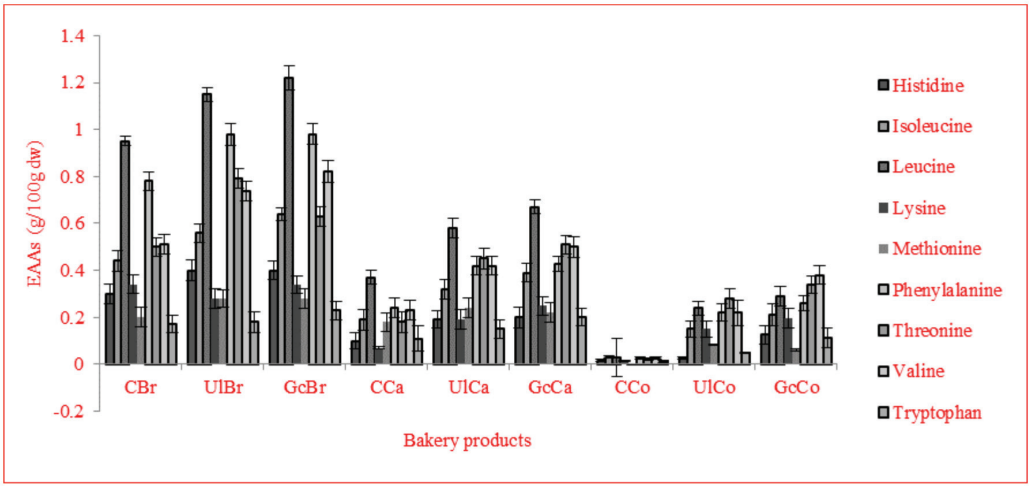

Figure 5: Essential amino acids (EAAs) in seaweed bakery products

*FAO/WHO/UNU g/100g: Histidine: 1.5; Isoleucine: 4.2; Leucine: 4.2; Lysine: 4.2; Methionine: 2.2; Phenylalanine: 2.8; Threonine: 2.8; Valine: 4.2; Tryptophan: 1.4; Total EAAs: 27.5

**FSSAI (mg/kg body wt/day): Histidine: 12; Isoleucine: 23; Leucine: 44; Lysine: 35; Methionine: 18; Phenylalanine: 30; Threonine: 18; Valine: 29; Tryptophan: 4.8; Total EAAs: 213.8

*FAO/WHO/UNU (2002). Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. In a Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (2002: Geneva, Switzerland), WHO technical report t series; no. 935; **Ref: Nutrient requirement and RDA for Indian – A report of the Expert Group of the ICMR (2010).

A similar result was seen by Lopez-Lopez *et al*. (2009), who incorporated *H. elongata* (brown seaweed) in frankfurters which elevated its amino acid profiles. Significant changes ($p<0.05$) were recorded in relation to EAAs in seaweed products. However, UlCa, GcBr and GcCo have shown some similar amino acids to CBr, CCa and CCo (Figure 5). Nevertheless, all the data on EAAs were within FSSAI and RDA limits set by the Government of India.

Eventually, there was no doubt that the incorporation of seaweed powder has enhanced the value-added products. However, the high temperature of microwave oven has been revealed to influence the withholding of and/or caused reduction in select essential components in final seaweed products.

The physical, textural and colour properties of the baked products revealed a positive side effect following the addition of seaweed powder. The results found there was a slight increase in the weight (g) (seaweed products), its specific gravity (UlCa, GcCa), and volume (cm^3) (UlCa, GcCa; The volume increase was almost double in UlCo, GcCo), Even its density (g/cm^3) (UlBr, GcBr, UlCo, GcCo), height (cm) (UlCa, GcCa, UlCo, GcCo), and thickness (cm) (UlCo, GcCo) as well as its weight loss (%) (UlCa, GcCa) was higher in comparison with conventional products. Additionally, the incorporated seaweed powder enhanced the crispiness of the biscuit and softness of breads and cakes, improved the binding properties of the dough and enhanced the appearance of the value-added products. Furthermore, the crispiness was also maintained by keeping the prepared products in the desiccator and removing any excess moisture.

Alternatively, a simple taste test using 50 respondents and applying a hedonic scale and reported a good result of acceptability. Among all baked goods, UlCa had the highest acceptability rating at 64.7% (liked very much) whereas, UlBr recorded the lowest=score at 7.8% (disliked very much).

Conclusions

The phytochemical, antioxidant, vitamin and EAAs analysis of seaweed and seaweed infused baked goods has proven to be a cost-effective nutrient supplement that could improve food security. This paper recommends that the phytochemicals (4.12±0.5 mg/g in GcCo) and total essential amino acids (5.54 g/100g in GcBr) has showed better value in *Gracilaria* products whereas antioxidants (398.8±0.4 µg/g in UlCo) and vitamins (23.66±0.4 µg/g in UlCo) has highlighted enriched results within *Ulva* products. All the nutritional variables studied in this research work are well within the permissible level of FSSAI, and RDA. This has also been supported by the quality and sensory results which proved its economic viability and sustainability. In this study breads, cakes and cookies being the most common economically viable food for the poorest of the poor to increase immune system efficiency. However, the sustainability of the products is based on a back-up nursery of seaweed culture. This will open two economic avenues for the community. This study recommends joint efforts of researchers and industry to bring this new functional food to the market fortified with seaweed.

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