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Development and characterization of marine fibre enriched biscuits

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A biscuit is a flour-based baked food product which is accepted by all age groups. Carrageenan is a natural polysaccharide obtained from red seaweeds andis a rich source of fibre. The diets rich in fibre have a positive effect on health, thus, carrageenan was incorporated into biscuits to enhance the fibre content. Different percentages (2 to 6%) of carrageenan were incorporated in biscuits among which 6 per cent was found to be the best. Fibre rich biscuits were subjected to physiochemical analysis. The antioxidant activity and total polyphenol contents were estimated. Snap test to determine the crispiness and textural characterization of biscuit was performed and Scanning Electron Microscopy (SEM) was done to compare the morphological changes in the products due to addition of carrageenan. The stability of the product at room temperature was analysed by monitoring the textural and colour changes and development of rancidity in the product. The storage studies have revealed a shelf-life of 63 days for the product at room temperature when packed in polypropylene trays.

Key Words : Biscuits, Carrageenan, Fibre, Antioxidant, Total polyphenols, SEM, Shelf-life

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INTRODUCTION

Functional food development is currently one of the fastest-growing food product development areas worldwide. Bakery products are widely consumed worldwide, especially bread, bun and biscuits. Biscuits is one of the most common items in bakery, consumed by almost every level of society. It is mainly due to its ready to consume nature, nutritional consistency and availability in various varieties and reasonable prices. Most of the bakery products are used as a medium for the inclusion of different nutritionally rich ingredients, due to its wide consumption and acceptability. Fortification or value addition in bakery products will make it available to the different levels of population and gives wide range of acceptability. Diets high in fibre, such as cereals, nuts, fruits and vegetables, have a positive health effect as their intake is related to a reduction in the occurrence of many diseases (Dhingra *et al.*, 2012).

Marine algae are mainly used for the industrial manufacture of food and non-food additives. Like every manufacturing industry, the manufacture of algae additives creates many by-products and waste, which are typically discarded. As these by-products contain valuable bioactives, fine biochemical and biomolecules, processing

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of these by-products is not justified from an economic, social and environmental point of view. Bioactive compounds from seaweed waste are reported to have several biological activities, including antimicrobial activity, antioxidant activity and lipid peroxidation inhibition, anti-proliferative activity, anti-diabetic effect and anti-inflammatory substances (Tiwari and Troy, 2015). The seaweeds thus, have the potential to be used as a means of countering food safety concerns and reducing the burden of non-communicable diseases that arise from modern lifestyle.

Kappaphycus alvarezii, economically important red tropical seaweed is of high demand due to its cell wall polysaccharide, which is the most important source of kappa carrageenan (Kumar *et al*., 2008). *K. alvarezii* based diet is proved to be cardioprotective and can effectively decrease body weight gain. The seaweed also exhibited strong antioxidant effects and can play an important role in oxidative suppression. The combination of high antioxidant activities and high nutritional content, especially dietary fibre, makes the seaweeds potentially functional food for the prevention of diet induced cardiovascular disease (Matanjun *et al*., 2010). According to Kumar *et al.,* 2014 M *K. Alvarezii* has a reasonable water-holding and oil-holding capacity, which makes it suitable for multiple food applications such as water-holding or as a texture enhancer. It could also be used to boost the viscous quality of the food formulations.

The present study was, therefore, conducted with the intention of using carrageenan powder to prepare fibre rich biscuits and to study its characteristics and shelf stability at room temperature (28±2°C) when packed in Polyvinyl chloride cups Trays PVC lid.

METHODOLOGY

Materials:

Elite Maida flour was used for the formulation of biscuit. Carrageenan (food-grade) was procured from Aquarev Industries (Una, Gujarat, India). The other minor ingredients like sunflower oil, sugar, salt, skimmilk powder, emulsifier (lecithin), sodium bicarbonate, ammonium bicarbonate, invert syrup and vanilla essence were purchased from the local market (Cochin, Kerala, India). The analytical grade chemicals used for the analysis were purchased from HiMedia® and Sigma-Aldrich® (Cochin, Kerala, India).

Preparation of flour blends:

The flour mixtures used for the preparation of biscuits were refined maida flour along with carrageenan powder at defined levels. The different levels of substitution in each sample were as follows: A-0 per cent, B-2 per cent, C-4 per cent and D-6 per cent carrageenan. The four different ratios of flour mixture formulations were used in order to determine the optimum acceptability of carrageenan that can be incorporated in biscuits.

Physical properties of flour:

The physical properties of the flour mixture formulations used for the preparation of biscuits were

Table A: Formula of ingredients used for the preparation of different concentration of carrageenan incorporated (A-0%, B-2%, C-4%, D-6%) biscuit preparation

Composition	Biscuit (100 g)			
		Fibre-rich biscuit		
	A	B	C	D
Flour (g)	50.9	48.9	46.9	44.9
Oil (g)	17.8	17.8	17.8	17.8
Sugar (g)	15.3	15.3	15.3	15.3
Salt (g)	0.3	0.3	0.3	0.3
Skim milk powder (g)	1.0	1.0	1.0	1.0
Emulsifier (g)	0.3	0.3	0.3	0.3
Sodium bicarbonate (g)	0.3	0.3	0.3	0.3
Ammonium bicarbonate (g)	0.5	0.5	0.5	0.5
Invert syrup (g)	0.5	0.5	0.5	0.5
Essence (g)	0.1	0.1	0.1	0.1
Water (g)	13.2	13.2	13.2	13.2
Carrageenan (g)	0	2	4	6

analysed. The properties like bulk density, packed density, water holding capacity, swelling capacity and oil holding capacity was measured as per the method given by (Onipe *et al.,* 2017 and AbdElmoneim and Bernhardt, 2010).

Preparation of biscuits:

The ingredients used for the preparation of biscuits are given in Table A. All in one method was adopted for the preparation, where all the ingredients were mixed together to form the dough. The dough was leavened for 30 minutes at then baked in a rotary type baking oven (Furnace Tech) at 150°C for 20 min. Biscuits of the four different formulations were made in the same manner using the ingredients as mentioned in Plate A.

Powdered sugar, oil, lecithin, skimmed milk powder and invert sugar mixed well to a smooth

↓

Add flavour and salt

↓

Carrageenan mixed with with flour and added

↓

Ingredients mixed well

↓

Kneaded thoroughly and kept for proofing (30 minutes)

↓

Baked at 150°C for 20 minutes

Plate A : Flow diagram for the preparation of marine fibre enriched biscuit

Chemical analysis of biscuits:

Proximate analysis:

The proximate composition of the biscuit samples were determined as per AOAC (2005) method. Moisture content was measured by oven drying (Oxylab) the samples at 102°C until constant weight was obtained. The total protein content was determined by the Kjeldahl method (M/s Pelican Equipments) using a nitrogen to protein conversion factor of 5.8. Fat content was determined using the Soxhlet apparatus (M/s Pelican Equipments). Ashingwas done at 550°C for 6 hours using a muffle furnace (Nabertherm). The total fibre was determined using fibra Plus Fes 02 E (M/spelican equipments). The total carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash percentages from 100 per cent.

Anti-oxidant activity and total polyphenol content

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was carried out as defined by Shimamura *et al*. (2014). Ethanol extracts of the biscuit samples were mixed with DPPH reagent and incubated in dark. The absorbance was measured at 517 nm using UV Spectrophotometer. The antioxidant activity was expressed as percentage of radical scavenging activity which is equivalent to that of µg/ml of ascorbic acid. The total phenolic content of the biscuit extracts was determined using Folin-Ciocalteau method and were expressed as milligram ofGallic acid equivalent per 100-gram dry basis (db) (mg GAE/100g db). (Singleton and Rossi,1965).

Hardness:

Hardness of the biscuit samples were measured using Shimadzu Texture Analyzer (EZ LX HS). Trapezium Software was used to determine the Hardness. The Texture analyzer was equipped witha load cell of 100N. Cylindrical probe of 35 mm diameter was used at a test speed of 50 mm/s and trigger force: 0.1N. The samples were compressed to an extent of 48 per cent. The values are given as an average of three measurements.

Sensory evaluation:

The sensory evaluation of biscuit was carried out by a 10 member trained panel. The sensory quality of biscuits was evaluated using a 9-point Hedonic scale. The Hedonic scale is widely used to calculate the acceptability/preference of a food substance. The descriptors of the sensory attributes for Hedonic rating vary from “like extremely” to “dislike extremely” The attributes evaluated were colour, appearance, texture, flavour and overall acceptability (Ranganna, 2000).

Shelf- life studies:

The shelf-stability of carrageenan incorporated biscuits packed in PVC cups at room temperature (28±2°C) was studied. The changes that occurred in the biscuits were monitored for a period of 9 weeks by evaluating the physical and chemical changes in the product. The samples were taken up for analysis at regular intervals of one week. The physical changes like hardness of the sample was measured using texture analyser and Colour changes that occurred was determined using colorimeter. Peroxide value and free fatty acid value was determined to monitor the development of rancidity in the product. The product was also evaluated by the sensory panel members every week to determine its acceptability.

Peroxide value and free fatty acid value :

Peroxide value and free fatty acid value of the products was analyzed according to the method described by AOAC (2000). Suitable quantity of the sample was blended with anhydrous sodium sulphate, mixed with chloroform and filtered to prepare chloroform extracts. From this chloroform extract 20 ml was taken in a beaker and evaporated off in a water bath to determine the fat content. Another 20 ml of the extract was transferred into another conical flask and evaporated off. 10 ml of neutral alcohol was added to it and titrated against 0.01 N NaOH using phenolphthalein as indicator of endpoint to determine the free fatty acid value. Peroxide value of the samples were determined using another 20 ml of the chloroform extract, which was mixed with 30 ml of glacial acetic acid and a pinch of potassium iodide. The conical flask was kept in dark for 30 minutes and then 50 ml of distilled water was added. Titration against 0.1 N sodium thiosulphate was performed using starch solution as indicator.

Colour analysis:

The measurement of the upper surface colour was carried out with the use of colour flex EZ (Hunterlab, USA) in terms of L *(brightness), a * (redness) and b * (yellowness).The instrument was standardized and then the colour of the samples were measured. The scale for a* corresponds green (negative) to red (positive) and scale for b* varies from yellow (positive) to blue (negative). Colour analyses were carried out in triplicates and the average of three measurements are reported.

SEM analysis:

The scanning electron microscope is one of the most versatile tools accessible for the study and interpretation of microstructure morphology and chemical composition characterizations. Morphological modifications of control and 6 per cent carrageenan incorporated biscuit structures were evaluated by SEM analysis. The samples were cubed and freeze-dried. The dried samples were mounted on stubs and gold-coated in an auto frame sputter coated to make it electrically conductive. Samples were examined in a JEOL scanning electron microscope (JEOL Ltd, Model JSM-6490V, Tokyo, Japan). Micrographic images of the biscuits were taken at 30X magnification.

Sorption isotherm studies:

Static gravimetric method was used to determine the Equilibrium moisture content of biscuits at room temperature (28±2°C) under different conditions of water activity (Jowitt *et al*., 1983). Saturated salt solutions corresponding to a range of water activities from 0.11 to 0.96 was filled in desiccators to achieve the required relative humidity condition. The corresponding relative humidity was checked using a hygrometer. The samples (approximately 2g) in triplicates were weighed and placed in each desiccator and closed tightly to maintain the condition and placed in ambient temperature. The samples were weighed every 24 hours during the entire equilibration period (AOAC, 1990).

Statistical analysis:

All the experiments were conducted in triplicates and the results are expressed as mean ± standard deviation. One-way analysis of variance test was performed using SPSS Windows version 16 (SPSS Inc., Chicago, IL, USA) and the least significant differences were calculated by Duncan's multiple range comparison tests and the significance at $p < 0.05$ was determined.

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Physical properties of flour:

The physical properties of flour used for preparing the different biscuit samples were analysed and is given in Table 1. The bulk density of a sample gives a reflection of its heaviness. The bulk density obtained from this study showed that 6 per cent carrageenan flour has significantly ($p < 0.05$) higher density (0.4559 g/cm^3), while the lowest value (0.0.4194 g/cm^3) of bulk density was recorded for control flour. Packed density of the samples showed no significant difference ($p \leq 0.05$). The WHC and OHC of the flour with carrageenan was significantly ($p<0.05$) higher than that of the plain flour. This indicates that the carrageenan added flours have higher affinity for water. WHC and OHC of 6 per cent carrageenan-flour mix were higher, compared to other samples. The swelling capacity determines the extent to which the volume of flour increases when soaked in water. In this study incorporation of carrageenan has led to a significant ($p < 0.05$) increase in swelling capacity. The ability of flour to link with water and oil is determined by the water absorption capacity and oil absorption capacity, respectively (Adebiyi *et al.*, 2016). Previous studies have reported an increase in the water absorption capacity and significant effect on the farinograph parameters of dough as a result of seaweed incorporation. A significant impact on the volume, crumb colour and textural properties like stickiness and firmness of the final product is also reported (Mamat *et al.*, 2014). There are also reports on the effectiveness and versatility of Hydrocolloids in wheat bread making. It is a safe additive which increases the water absorption capacity of wheat flour (Ferrero, 2017).

Proximate composition:

The proximate composition of carrageenan biscuits were quantified and presented in Table 2. The moisture content was observed to range from 0.49 to 1.47 per cent, where the sample with highest percentage of seaweed powder (6%) reported the highest moisture content ($p<0.05$). There were no significant changes in crude fat and protein content of the formulations. Studies have reported presence of high amount of polyunsaturated fatty acids especially omega-3 fatty acids in *K. alverazei* which is beneficial to human health.The amount of dietary fibre significantly increased ($p<0.05$) from 13.61 per cent to 20.57 per cent with the increase in level (2– 6%) of incorporation of seaweed powder. Matanjun *et al.* (2009) confirmed the presence of 25.05 per cent dietary fibre in red algae (*K. alverazei*). Similar trend was observed for ash content. The amount of carbohydrate content in biscuit samples reduced from 61.67 per cent to 52.07 pe cent. The presence of increased dietary fibre content in *K. alvarezii* and its suitability for use in seaweed breads and snack bars as a fibre enhancer is reported in previous studies (Komatsuzaki *et al.*, 2019 and Hajal *et al.*, 2015).

Anti-oxidant activity and polyphenol content:

The free radical scavenging activity of k-carrageenan incorporated biscuit samples were determined using DPPH assay. The results of the analysis demonstrated that the incorporation of carrageenan significantly enhanced the radical scavenging activity. The samples with 6 per cent of k-carrageenan showed maximum activity with percentage inhibition (PI) value of 16 per cent which is equivalent to that of 5-10µg/ml ascorbic acid. The samples with less than 6 per cent of k-carrageenan showed negligible levels of activity. Biscuits incorporated with carrageenan displayed significantly ($p < 0.01$) higher polyphenolic content than control biscuits. The phenolic content was found to increase with

Table 1 : Physical properties of flour-carrageenan mix

Samples	Bulk density (g/ml)	Packed density (g/ml)	Water holding capacity	Oil holding capacity	Swelling capacity (ml/g)
A	$0.4194{\pm}0.00^{a}$	$0.63105{\pm}0.00^{c}$	$1.42635{\pm}0.00^{a}$	$0.8334{\pm}0.00^{a}$	$1.992{\pm}0.00^{a}$
B	$0.4535{\pm}0.00^{b}$	$0.5616{\pm}0.00^{ab}$	$1.51{\pm}0.00^{b}$	$0.9102{\pm}0.00^{b}$	$2.173{\pm}0.00^{b}$
C	$0.4526{\pm}0.00^{b}$	$0.5657{\pm}0.00^{b}$	$1.841{\pm}0.00^{c}$	$1.03185{\pm}0.00^{c}$	$2.3888{\pm}0.00^{c}$
D	$0.4559{\pm}0.00^{b}$	$0.55605{\pm}0.00^{a}$	$2.0328{\pm}0.01^{d}$	$1.0987{\pm}0.00^{d}$	$2.39075{\pm}0.00^{c}$

Results are expressed as : Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other. Substitution level: A- 0%, B-2%, C-4% and D-6%

Table 2 : Compositional analysis of carrageenan incorporated biscuits

Proximate composition	A	B	C	D
Moisture	0.49 ± 0.01^{a}	0.85 ± 0.06^{b}	1.10 ± 0.43^{c}	1.47 ± 0.26^{d}
Ash	0.73 ± 0.02^{a}	1.15 ± 0.006^{b}	1.69 ± 0.07^{c}	2.22 ± 0.17^{d}
Fat	17.50 ± 1.43^{a}	17.48 ± 0.35^{a}	18.47 ± 0.34^{a}	17.37 ± 1.22^{a}
Protein	5.98 ± 0.09^{a}	6.03 ± 0.11^{ab}	6.18 ± 0.21^{bc}	6.27 ± 0.01^{c}
Carbohydrate	$61.67{\pm}3.31^{d}$	58.74 ± 0.67^{c}	54.91 ± 2.02^{b}	$52.07{\pm}1.07^{a}$
Fibre	13.61 ± 1.97^{a}	15.77 ± 0.83^{b}	17.63 ± 1.65^{c}	20.57 ± 0.59^{d}

Results are expressed as: Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other.Substitution level: A- 0%, B-2%, C-4%, and D-6%

increasing level of carrageenan in biscuit. 2 per cent incorporation of carrageenan in biscuit increased the total phenolic content from 0.25 to 0.292 mg GAE/100g, in the case of 4 per cent carrageenan added biscuit it increased to 0.50mg GAE/100g and to 0.63 mg GAE/100 g db for biscuits having 6 per cent carrageenan. The results are in accordance with the reports showing that seaweed extracts have high total polyphenol content and antioxidant activity (Wang *et al*., 2009). Seaweed-based ingredients are potential natural antioxidants that could be used as active ingredients in functional food products as well as improving oxidative stability of healthy food products for targeted consumers (Corsetto *et al.*, 2020). Kumar *et al*. (2008), has reported the excellent scavenging activity of *K. alvarezii* and its suitability for nutraceuticals and functional food applications.

Hardness:

Hardness is the peak force measured during the first compression cycle. *i.e.*, first bite. The texture of biscuit was measured using the texture analyser which showed that the hardness value decreased with increase in the carrageenan content of the biscuit formulation. The hardness values are given in Table 3. The textural quality of biscuit is highly influenced by the parameters like dough development and quantity of sugar used. The variations in type of raw materials used and the manufacturing conditions will greatly influence the physical properties of biscuits (Alam *et al.*, 2014). Previous studies have reported significant reduction in the textural properties of cooked noodles with increased levels of Seaweed. (Chang, 2011). A decrease in the resistance to snap test during texture analysis is also reported for dietary fibre enriched biscuits (Brennan and Samyue, 2004 and Chakraborty *et al*., 2011).

Sensory evaluation:

The sensory scores for the samples with different concentration of carrageenan are shown in Table 4. According to the data, those biscuits supplemented with 6 per cent carrageenan obtained a higher total score.

Shelf-life studies:

The analysis of different physio-chemical parameters and sensory evaluation showed that 6 per cent of carrageenan is the highest percentage that can be incorporated into biscuits without affecting its characteristics and acceptability. Thus, 6 per cent carrageenan incorporated sample was taken up for shelf life analysis and its storage life was compared with that of the control sample. The samples were packed in PVC cups and stored at ambient temperatures (28±2°C). During the storage period the samples were analysed for physical changes by monitoring the changes in colour and hardness. The development of rancidity in samples was monitored by analysing the peroxide value and free fatty acid value every week during the storage period. The sensory acceptability of the samples was also monitored during the period of storage.

Table 3 :Hardness of carrageenan incorporated biscuits

Samples	Hardness (N)
A	16.06±2.65[a]
B	17.06±1.45[a]
C	21.93±2.17[b]
D	33.3±3.04[c]

Results are expressed as: Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other. Substitution level: A- 0%, B-2%, C-4%, and D-6%

Table 4 : Sensory characteristics of carrageenan incorporated biscuits

Samples	Colour	Appearance	Flavour	Taste	Texture	Overall acceptability
A	8.33±1.15[a]	7±1.00[a]	8±1.00[a]	8±1.00[a]	7±1.00[a]	8±1.00[a]
B	7.33±0.57[a]	6±1.73[a]	6.33±0.57[a]	6.66±0.57[a]	7±1.00[a]	7±1.00[a]
C	7.33±0.57[a]	8±1.73[a]	8.33±1.15[a]	7.33±1.52[a]	7.66±1.52[a]	8±1.00[a]
D	7±1.00[a]	6.66±2.30[a]	7.66±1.52[a]	8.33±0.57[a]	7.33±0.57[a]	8.33±0.57[a]

Results are expressed as: Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other. Substitution level: A- 0%, B-2%, C-4% and D-6%

Peroxide value and free fatty acid value:

The development of rancidity in the product during storage was analysed by measuring the peroxide value and free fatty acid value of the sample and is given in Table 5. A significant increase in FFA and PV was observed during storage of 9 weeks. However, when the shelf-life was over, FFA and peroxide values of the sample remained under permissible level. Hence, it indicates that the biscuits stored upto 9 weeks were good for consumption. Peroxide value levels should not exceed 30 meq peroxide/kg oil in an edible food product (Gotoh and Wada, 2006). It is used as an index for assessing the level of lipid oxidation (Aksu, 2007).

Physical properties:

Table 6 shows the physical changes, with respect to hardness and colour, that occurred in the product during storage. The changes in hardness of biscuit incorporated with 6 per cent carrageenan were significant during storage for 9 weeks. The hardness decreased from 34.27 to 13.67N which shows the absorption of moisture by the product. The L*, a* and b* values represents the degree of lightness to darkness, redness to greenness, and yellowness to blueness, respectively. All the three components completely describe the colour of an object. From the results, it was observed that, there is significant changes in the colour values during storage. L* value increased significantly ($p < 0.05$) from 69.26 to 71.51. A significant ($p < 0.05$) increase from 6.89 to 11.26 was also observed for a* value. Likewise, b* value raised from 30.27 to 34.82. The changes in colour can be due to the changes in the packaging material that occurs due to

Table 5 : Changes in peroxide value (PV) and free fatty acids (FFA) of 6% carrageenan incorporated biscuit during storage

Storage (weeks)	PV (%)	FFA (% oleic acid)
0	0.71 ± 0.06^{a}	0.25 ± 0.05^{a}
1	1.33 ± 0.04^{b}	0.32 ± 0.006^{b}
2	1.60 ± 0.06^{c}	0.36 ± 0.002^{b}
3	2.31 ± 0.09^{d}	0.44 ± 0.01^{c}
4	3.70 ± 0.10^{e}	0.65 ± 0.005^{d}
5	4.46 ± 0.28^{f}	0.74 ± 0.01^{e}
6	5.44 ± 0.24^{g}	0.93 ± 0.01^{f}
7	6.37 ± 0.22^{h}	1.06 ± 0.05^{g}
8	6.44 ± 0.14^{i}	1.22 ± 0.09^{h}
9	6.92 ± 0.003^{j}	1.25 ± 0.13^{h}

Results are expressed as: Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other.

Table 6 : Physical parameters of 6% carrageenan incorporated biscuits during storage

Storage (weeks)	Hardness	Colour		
		L*	a*	b*
0	34.27 ± 1.85^{e}	69.02 ± 0.56^{a}	6.88 ± 0.45^{a}	29.47 ± 0.19^{a}
1	33.35 ± 3.01^{e}	69.34 ± 0.19^{ab}	6.89 ± 0.03^{a}	29.89 ± 0.01^{b}
2	33.03 ± 4.48^{e}	69.26 ± 0.57^{ab}	7.04 ± 0.02^{a}	30.02 ± 0.11^{bc}
3	28.68 ± 0.34^{d}	69.83 ± 0.15^{bc}	7.35 ± 0.12^{b}	30.03 ± 0.03^{bc}
4	25.88 ± 2.09^{d}	70.16 ± 0.12^{cd}	7.67 ± 0.005^{c}	30.27 ± 0.27^{bc}
5	22.01 ± 1.43^{c}	70.23 ± 0.46^{cd}	8.18 ± 0.18^{d}	30.39 ± 0.18^{c}
6	18.79 ± 1.20^{bc}	70.57 ± 0.12^{d}	8.23 ± 0.15^{d}	30.80 ± 0.55^{d}
7	16.02 ± 0.74^{ab}	70.73 ± 0.21^{d}	9.17 ± 0.05^{e}	31.16 ± 0.17^{d}
8	15.55 ± 0.06^{ab}	71.33 ± 0.26^{e}	9.71 ± 0.01^{f}	33.48 ± 0.01^{e}
9	13.67 ± 1.08^{a}	71.51 ± 0.03^{e}	11.26 ± 0.02^{g}	34.82 ± 0.06^{f}

Results are expressed as: Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other available head space oxygen (Bajaj, 2006). Maillard reactions between sugars and proteins can also lead to colour changes (Lingnert, 1990).

Sensory evaluation:

The sensory assessment is shown in Table 7. Perusal of sensory data showed a significant decline in the sensory score of biscuits during storage. The colour score was found to decrease from 8.33 to 5.33, appearance score from 8.66 to 4.66, texture score from 8.66 to 4.33, flavour score from 8.66 to4.66, taste score from 8.00 to 5.33 and overall acceptability from 8.46 to 4.86.

SEM analysis:

The scanning electron microscope (SEM) images of control and 6 per cent carrageenan incorporated biscuits were compared in order to detect morphological differences in the samples and is given in Fig. 1. The micrographs of control biscuit contain homogenously and densely packed starch granules. But a heterogeneous structure was obtained in the case of 6 per cent.

Carrageenan incorporated biscuit. The carrageenan incorporated biscuit had larger pores than the control biscuit which can be due to the fibre incorporated. Fibre holds more water which expands and evaporates during

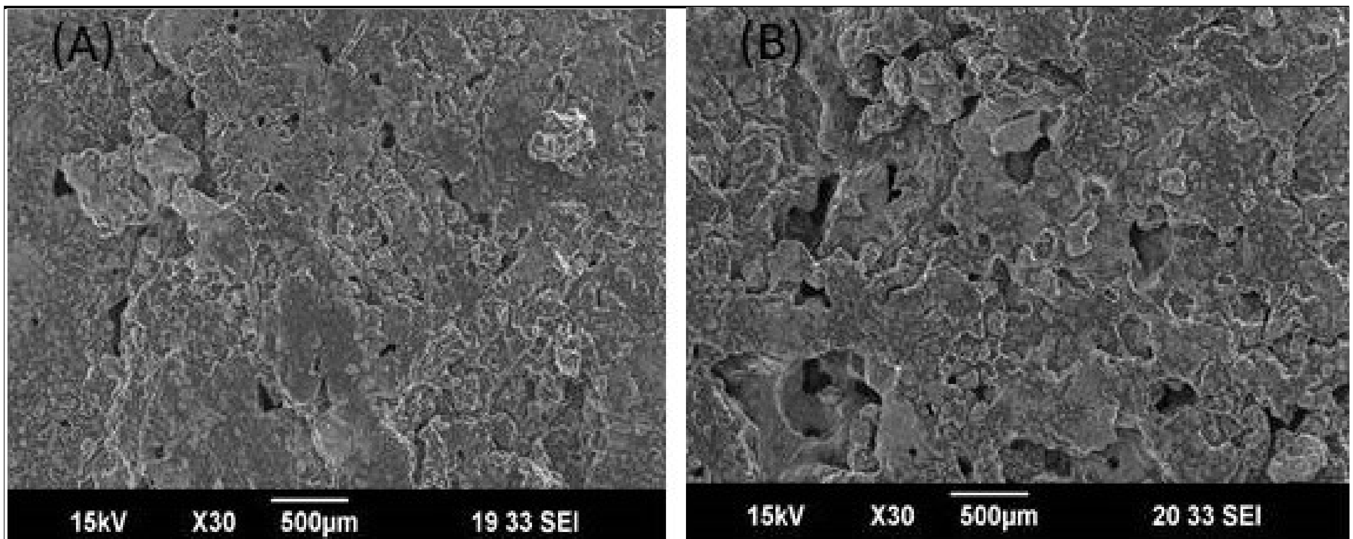

Fig. 1 : SEM micrographs of control (A) and 6 % carrageenan (B) biscuits

Table 7 : Sensory characteristics of 6% carrageenan incorporated biscuitsduring storage

Week	Colour	Appearance	Flavour	Taste	Texture	Overall acceptability
0	8.33±0.57^{c}	8.66±0.57^{f}	8.66±0.57^{f}	8.66±0.57^{c}	8±1.00^{f}	8.46±0.11^{h}
1	8.33±0.57^{c}	8.33±0.57^{f}	8.33±0.57^{ef}	8.33±0.57^{c}	8±0^{ef}	8.4±0.11^{h}
2	8±0^{bc}	8±0e^{f}	7.66±1.15^{def}	7.66±1.15^{bc}	7.66±0.57^{def}	7.8±0.11^{g}
3	8±0^{bc}	7.66±0.57^{def}	7.33±0.57^{cdef}	7.33±0.57^{bc}	7.33±0.57^{cde}	7.53±0.34^{fg}
4	8±0^{bc}	7±1.00^{cde}	7±1.00^{bcd}	7±1.00^{bc}	7.33±0.57^{bcd}	7.2±0.34^{ef}
5	8±1.0^{bc}	6.66±0.57^{bcd}	7±1.00^{bcd}	7±1.00^{bc}	7±1.00^{bc}	7±0.11^{de}
6	7.33±0.57^{bc}	6.33±0.57^{bc}	6.33±0.57^{bcd}	6.33±0.57^{bc}	7±1.00^{bc}	6.66±0.2^{cd}
7	7±1.00^{b}	5.66±1.15^{bc}	6±0^{abc}	6±0^{bc}	6.66±0.57^{bc}	6.33±0.11^{c}
8	5.66±0.57^{a}	5.66±0.57^{bc}	5.66±0.57^{ab}	5.66±0.57^{ab}	6.33±0.57^{b}	5.8±0.11^{b}
9	5.33±0.57^{a}	4.66±0.57^{a}	4.66±1.15^{a}	4.66±1.15^{a}	6.33±0.57^{a}	4.86±0.11^{a}

Results are expressed as Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other.

baking leading to the formation of cavities with different sizes (Arun *et al.*, 2015). The non-homogeneous structure indicates that starch granules are adhering to the protein matrix (Izydorczyk and Dexter, 2008).

Sorption isotherm studies:

Moisture sorption isotherms have a wide range of application in the area of research on foods. It can be used to predict the shelf-life of the product, calculate the drying time, to model the moisture changes that occur during storage (Labuza, 1968 and Al-Muhtaseb *et al.*, 2002). The sorption isotherm of biscuits (Fig. 2) showed sigmoid characteristic which is similar to the results obtained for other sorption studies (Ikhu-Omoregbe, 2006).The moisture sorption isotherm clearly shows that the critical moisture content of control sample is 25.83 per cent and that for 6 per cent carrageenan incorporated sample is 27.66 per cent with respect to 96 per cent relative humidity. The water activity of the both the samples was 0.27. Water activity which is a factor depending upon the storage temperature, relative humidity and moisture content of the product plays an important role in the shelf stability of the product. It can enhance and deteriorate the rate of microbial and physico-chemical deterioration in the product. Lower water activity enhances the shelf stability of the product (Sanni, 1996; Ukhun and Dibie, 1991; Ofuya and Akpoti, 1988 and Chuzel and Zakhia, 1991).

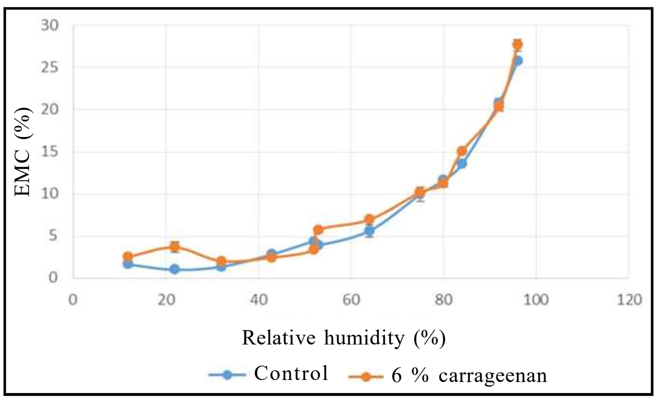

Fig. 2 : Sorption isotherm of control and 6 % carragreenana biscuis

Conclusion:

Seaweeds are reported to have the potential to be used as a means of countering food safety concerns and reducing the burden of non-communicable diseases that arise from modern lifestyle. Kappa carrageenan is extracted from the cell wall polysaccharide of red tropical seaweed *Kappaphycus alvarezii.* In this study Kappa carrageenan was incorporated in biscuits in different concentrations and analysed to find the best concentration that can be incorporated in biscuits. 6 per cent kappa carrageenan incorporated biscuit was found to have higher antioxidant activity with percentage inhibition value of 16 per cent. The total polyphenol content (0.63mg GAE/100g db) was also higher for 6 per cent carrageenan incorporated biscuits. 6 per cent carrageenan incorporated was also having a higher fibre content and was sensorily acceptable. This sample was taken up for further analysis, packed in PVC cups and stored at room temperature to evaluate the storage life. The product was stable at room temperature (28±2°C) for 63 days.

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