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Development and characterization of k-carrageenan (*Kappaphycus alvarezii)* incorporated bun

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Functional foods with elevated levels of fibre content are of high demand because of its several health benefits. A study was conducted for the development of fibre enriched bakery products. As a part of this study fibre enriched bun was developed using k-carrageenan (*Kappaphycus alvarezii)* as the source of fibre. The bun was prepared with the incorporation of various concentrations (2-8%) of κ-carrageenan powder. Comparative analyses of the physical, chemical, textural, structural and sensorial characteristics of bun were conducted. The highest concentration of k-carrageenan that was sensorily acceptable for incorporation in bun was 6 per cent, beyond which sensory parameters like taste and texture showed unacceptability. Radical scavenging activity assays revealed an improved activity with increased concentration of k-carrageenan.The shelf-life analysis of the sample was done after packing in low density polythene (LDPE) pouches. The present study has demonstratedthat k-carrageenan can be used as a competent constituent for the fortification of bun to utilize the health benefits of marine fibre.

Key Words : k-carrageenan, Bun, Fibre, Antioxidant activity, SEM, Texture profile analysis

Abbreviations : ABTS- (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), DPPH -2,2-diphenyl-1-picrylhydrazyl; di(phenyl) - (2,4,6-trinitrophenyl) iminoazanium), UV - Ultra violet, Vis- Visible

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INTRODUCTION

Now-a-days functional foods are a popular area of research in processed food industry. Professional and international health organizations defined the functional foods as a food given an additional function by incorporating new ingredients that provide health benefits (Feili *et al.*, 2013). Generally, dietary fibres in food are not enzymatically degradable in the human digestive tract. Presence of soluble dietary fibre in the food reduces the absorption of carbohydrates into the bloodstream (Yangilar, 2013). This indirectly helps to prevent rapid increase in blood sugar levels which result in the development of type 2 diabetes. Other important roles of dietary fibre in the human diet include reduction of cholesterol levels in the body, as well as regulating weight gain (Kurek and Wyrwisz, 2015). Increased consumption of fibre content significantly reduces obesity (Slavin, 2005) and reduces the risk of colon cancer (Buttriss and Stokes,

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2008). Bun and bread are one of the popular baked products and are consumed by a major population of people all over the world. White bread, bun and similar baked products do not help to attain the desired fibre requirement of the human body. So, the incorporation of fibre into these baked products will help to overcome these drawbacks and produce fibre enriched products.

Seaweed is an excellent source of bioactive compounds such as carotenoids and dietary fibre. k-carrageenan is a natural polysaccharide obtained from the edible red seaweed *Kappaphycus alvarezii.* It is commonly known as *Eucheumacottonii* (Munoz *et al.*, 2004). The classification of carrageenan into various types such as λ, κ, ι, ε and μ is based on the concentration of sulphate groups present in it. Sulphated polysaccharides obtained from marine algae have diverse biological activities including immunomodulatory, anticoagulant, antithrombotic, antiviral and antitumor effects. k-carrageenan exhibit cholesterol-lowering effect (Necas and Bartosikova, 2013) and is a rich source of minerals. They can also act as an excellent source for free radical scavengers (Souzan *et al.,* 2012). Recent research shows that carrageenan has anti proliferative activity in cancer cell lines as well as inhibitory activity on tumour growth. Earlier studies showed that k-carrageenan has excellent activity against the colon cancer and it can be used for cancer therapy (Raman and Doble, 2015).

The present study includes the development of fibre enriched bun by the incorporation of k-carrageenan and evaluation of its nutritional and physico-chemical characteristics.

METHODOLOGY

Materials:

Refined wheat flour, fat, milk powder, salt, sugar and instant yeast were procured from local market and κ-carrageenan was obtained from Aquarev Industries, Gujarat. All the chemicals used were of analytical grade. 250 gauges Low Density Polyethylene (LDPE) pouches were used as the packaging material.

Preparation of bun:

Bun was prepared using refined wheat flour as the major ingredient and k-carrageenan powder at various concentrations like 2 per cent, 4 per cent, 6 per cent and 8 per cent was used to replace refined wheat flour. Formulation of the ingredients are summarized in Table A. The ingredients were mixed by using a hand mixer to obtain smooth and elastic dough. Thereafter the dough was divided and kept in a bun mould for proofing for 90 minutes at room temperature. After proofing the dough was baked in an Oven maintained at 180^0 C for 18min. Buns were cooled at room temperature and packed in LDPE pouches. The buns prepared using above mentioned concentrations of k-carrageenan was subjected to different analytical methods to determine the best suitable formulation having sensory acceptability and improved nutritional value.

Proximate analysis:

Moisture, carbohydrate, protein, fat, ash and total fibre content were estimated according to the standard method by AOAC (2005). The analyses were done in triplicates.

Texture profile analysis:

Textural properties of buns with different concentrations of k-carrageenan and that of the control bun were measured by using Texture Analyser Shimadzu (EZ-LX HS), equipped with 10mm diameter cylindrical

Table A: Formulation of ingredients used for the preparation control (without the incorporation of carrageenan) and various concentrations (2-8%) of carrageenan incorporated bun

Ingredients	Control	2%	4%	6%	8%
Refined wheat flour (g)	100	98	96	94	92
Carrageenan (g)	0	2	4	6	8
Sugar (g)	30	30	30	30	30
Salt (g)	1	1	1	1	1
Skim milk powder (g)	2	2	2	2	2
Calcium propionate (g)	0.5	0.5	0.5	0.5	0.5
Instant yeast (g)	3	3	3	3	3
Fat (g)	3	3	3	3	3
Water (ml)	50	50	50	50	50

metal probe. Bun was kept horizontally on the plate and compressed to 60 per cent of its height with a test speed of 10mm/sec using 100N load cell. Texture profile analysis (TPA) measured the parameters like hardness, hardness, cohesiveness, springiness, gumminess and chewiness (Feili *et al.*, 2013). All the values were recorded in triplicates.

Colour analysis:

The crust and crumb colour of the bun samples were measured by using Hunter LabEZ colorimeter which is based on (CIE) L*a*b* scale. The instrument was calibrated in the beginning (Feili *et al.*, 2013) and then the crust and crumb colour of the product were measured.

Sensory evaluation:

Sensory evaluation for the freshly baked bun was carried out by semi-trained panellist members (n=10) with the help of a score card. Panellists were asked to evaluate different attributes of the bun such as appearance, colour, taste, texture and overall acceptability on the basis of 9-point hedonic scale (Wichchukit and O'Mahony, 2015). A score of above 4 was considered as a margin of acceptance.

Antioxidant activity assays:

DPPH radical scavenging activity assay:

Methanolic extracts of bun samples was incubated with DPPH (2,2-diphenyl-1-picrylhydrazyl) reagentand the absorbance was read at 515nm using UV–Vis Spectrophotometer (Mishra *et al.*, 2012) andascorbic acid was used as the standard. Activity was expressed as percentage of radical scavenging activity which is equivalent to that of µg/ml of ascorbic acid.

ABTS radical scavenging activity assay:

Methanolic extracts of different bun samples were mixed with ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) reagent and stored for 30 min in dark condition at room temperature. The absorbance was read at 734nm using UV-Vis spectrophotometer ((Re *et al.,* 1999). Butylatedhydroxylanisol (BHA) was used as the standard. Activity was expressed as percentage of radical scavenging activity which is equivalent to that of µg/ml of BHA.

Scanning electron microscopic analysis:

The structural morphology of the bun samples was observed using scanning electron microscopy (SEM). Bun crumbs were cut into cubes of side 1cmand freeze dried for 6h (Turkut *et al.*, 2016). Prior to the examination samples were sputter coated with gold-palladium to make it electrically conductive (Turabi *et al.*, 2010). Micrographic images of the crumb parts were taken at the 30X magnification. Images were analysed and the difference in pore size was measured using image J software.

Physical properties of the flour and storage studies of bun:

Based on the results of physico-chemical characterization and sensory analysis, the most suitable formulation for preparation of bun was selected and it was taken up for further studies.The samples were observed for changes that occurred during the period of storage. Texture and colour of the sample was analysed and was also taken up for sensory evaluation every day during the period of storage. The changes in the colour, texture and sensory scores were recorded and the data was statistically analysed using one-way ANOVA at significance $p<0.05$. The bun flour formulation containing the selected concentration of k-carrageenan was physically analysed to determine the bulk density, packed density, water holding capacity, oil holding capacity and swelling capacity (Raman *et al.,* 2019). Storage studies were carried out for the selected formulation of k-carrageenan incorporated bun. The product was packed in LDPE pouches and stored at ambient temperature (28 ± 2°C) to studythe storage stability.

Statistical analysis:

All the analyses were conducted in triplicates and the results are expressed as mean ± standard deviation. Statistical analysis of the data was done using SPSS 18.0 software for windows (SPSS Inc., Chicago,USA). Comparisons of the significant one-way ANOVA were performed by Duncan's multiple range comparison tests at a level of $p<0.05$ to designate the significant differences among samples.

OBSERVATIONS AND ASSESSMENT

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

Proximate:

Proximate composition of the bun with different concentrations of k-carrageenan are mentioned in Table 1. Incorporation of k-carrageenan into bun in different concentrations showed significant changes in the ash and fibre content. It showed an increasing trend with an increased concentration of k-carrageenan. The moisture content of the product also showed an increase from 25 per cent to 34 per cent. The hydrocolloids like k-carrageenan is capable to retain the moisture content even after baking and it remains in the crumb of the bun (Guarda *et al.*, 2004). Whereas, no significant changes were observed in the protein level and fat content in the experimental bun samples. The ash fraction of k-carrageenan is mainly made up of macro-minerals, such as potassium, sodium, calcium and magnesium (Hurtado-Ponce, 1995). Previous studies on white bread incorporated with k-carrageenan showed a significant increase in the mineral level (Raman *et al.*, 2019).

Texture:

Textural properties like hardness 1, hardness 2, cohesiveness, springiness, gumminess and chewiness of bun incorporated with different concentrations of k-carrageenan are determined and areshowed in Table 1. Results showed significant changes in hardness,

Table 2: Physio-chemical properties of control bun and bun incorporated with carrageenan at different concentrations (2-8%)

	Control	2%	4%	6%	8%
Chemical parameters					
Moisture	25.40± 0.12[a]	28.20±0.10[b]	30.30±0.34[c]	32.40±0.17[d]	34.80±0.20[e]
Carbohydrate	64.30±0.12[e]	61.84±0.35[d]	59.39±0.37[c]	56.81±0.17[b]	54.45±0.12[a]
Protein	7.38±0.18[a]	6.93±0.37[a]	7.01±0.18[a]	6.96±0.35[ab]	7.06±0.07[b]
Fat	2.06±0.09[a]	2.43±0.08[c]	2.40±0.09[bc]	2.21±0.18[ab]	2.07±0.05[a]
Ash	1.35±0.05[a]	1.35±0.04[a]	1.61±0.04[b]	2.32±0.09[c]	2.34±0.01[d]
Fibre	0.41±0.07[a]	0.76±0.04[b]	1.93±0.16[c]	2.46±0.18[d]	2.90±0.12[e]
Textural parameters					
Hardness 1 (N)	17.97±0.88[a]	17.19±0.20[a]	20.98±0.79[b]	21.09±1.65[b]	22.87±0.28[b]
Hardness 2 (N)	15.38±0.85[ab]	14.02±0.25[a]	16.80±0.38[bc]	17.43±1.43[c]	20.01±0.28[d]
Springiness (mm)	0.18±0.01[a]	0.21±0.02[b]	0.21±0.00[b]	0.23±0.00[b]	0.54±0.01[c]
Gumminess (Kgf)	3.58±0.15[c]	2.58±0.37[a]	3.11±0.03[b]	3.63±0.04[c]	5.64±0.34[d]
Chewiness (Kgf mm)	0.41±0.09[a]	0.56±0.15[ab]	0.68±0.02[bc]	0.87±0.01[c]	3.24±0.13[d]
Cohesiveness	0.23±0.01[c]	0.19±0.00[b]	0.18±0.01[b]	0.15±0.00[a]	0.15±0.01[a]
Crust colour					
L*	62.60±0.34[a]	66.39±0.16[b]	68.51±0.29[c]	69.63±0.49[d]	69.63±0.35[d]
a*	17.33±0.29[c]	14.23±0.22[b]	14.48±0.20[b]	12.49±0.25[a]	12.19±0.15[a]
b*	40.59±0.45[a]	38.36±0.29[b]	38.31±0.34[c]	35.60±0.28[c]	33.61±0.37[d]
Crumb colour					
L*	83.24±0.20[c]	83.20±0.30[c]	82.79±0.09[c]	82.24±0.36[b]	81.24±0.23[a]
a*	2.00±0.02[a]	2.01±0.10[a]	2.15±0.06[ab]	2.19±0.11[b]	2.23±0.07[b]
b*	20.24±0.13[a]	20.38±0.45[a]	20.56±0.18[a]	21.44±0.25[b]	21.65±0.38[b]
Sensory parameters					
Appearance	8.80±0.42[b]	8.80±0.42[b]	8.70±0.48[ab]	8.80±0.48[ab]	8.30±0.48[a]
Colour	8.90±0.31[a]	8.90±0.31[a]	8.70±0.48[a]	8.60±0.51[a]	8.50±0.52[a]
Texture	8.70±0.48[b]	8.70±0.48[b]	8.10±0.31[a]	8.10±0.31[a]	7.70±0.48[a]
Taste	8.70±0.48[b]	8.70±0.48[b]	8.30±0.48[b]	8.30±0.48[b]	7.20±0.42[a]
Overall acceptance	8.70±0.48[b]	8.70±0.48[b]	8.50±0.52[b]	8.50±0.52[b]	7.40±0.51[a]

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

springiness, gumminess, chewiness and cohesiveness with the addition of k-carrageenan. A positive correlation can be observed between fibre and moisture content with textural properties.Substitution of k-carrageenan changed the viscoelastic properties of the dough which resulted in the changes in the textural properties. A gradual increase in hardness can be observed from 4 per cent addition of k-carrageenan onwards, which can be related to the increase in the fibre content. Increase in fibre content breaks the gluten network formation which leads to a change in the textural properties of the final product (Salas-Mellado and Chang, 2003). One-way statistical analysis (ANOVA) revealed significant ($p<0.05$) difference among the formulations for hardness 1, hardness 2, chewiness, springiness and adhesiveness of the bun.

Increase in the hardness and the decrease in cohesiveness of the fibre incorporated bun can be correlated with the earlier studies on the fibre enrichment in wheat bread (Frutos *et al.*, 2008). Cohesiveness is the extent to which a material can be deformed before it breaks (Sullivan *et al.,* 2010), addition of k-carrageenan reduced cohesiveness from 0.23 to 0.15. Significant differences were observed in other textural properties such as springiness, gumminess and chewiness with the addition of k-carrageenan. Springiness and Chewiness of the bun also showed a linear correlation with the increase in fibre content of the bun.

Colour:

The L*, a*, b* values depicting the crust and crumb colour of the bun samples are given in Table 1. The results of the analysis demonstrate that the augmented levels of carrageenan causes a significant increase in crust colour in L* value from 62 to 69 and a decrease in a* and b* values from 17 to 12 and 40 to 33, respectively. That indicates that incorporation of k-carrageenan increased the lightness and reduced the yellowness of the crust colour of the bun meantime there is no significant difference in the crumb colour, only slight reduction in L* value from 83 to 81 was observed, whereas the a* and b* values remained the same. Maillard reaction, which may lead to the browning of crust is retarded with increased fibre content of the product (Gomez *et al.*, 2003 and Feili *et al.*, 2013).

Sensory evaluation:

Sensory score data aredisplayed in Table 1 indicates that no significant difference was observed in the score of colour and appearance of bun samples with different concentrations of carrageenan. The scores of taste, texture and overall acceptability of bun samples containing 8 per cent carrageenan showed significant difference from that of the bun with lower concentrations of carrageenan. 6 per cent carrageenan incorporated bun got better acceptability compared to 8 per cent. With the addition of 8 per cent carrageenan the texture and taste properties showed a significant change which was not appreciated by the sensory panel. The nutritional quality of the bun was enhanced through the addition of carrageenan and the sensory evaluation results depicts a maximum acceptability for bun with 6 per cent carrageenan.

Antioxidant activity of carrageenan incorporated bun:

The free radical scavenging activity of k-carrageenan incorporated bun samples were determined using DPPH and ABTS assays. The results of the analysis demonstrated that the incorporation of carrageenan significantly enhanced the radical scavenging activity in bun samples. In DPPH assay the samples with 6 per cent and 8 per cent of k-carrageenan showed maximum activity with percentage inhibition (PI) values 19 per cent and 33 per cent, respectively which is equivalent to that of 10-15µg/ml ascorbic acid (Fig.1a). Meantime, the samples with less than 6 per cent of k-carrageenan showed negligible levels of activity.

The results obtained from the ABTS radical scavenging activity assay of bun samples with incorporated k-carrageenan is showed in Fig.1b, exhibiting animproved activity. The rate of activity is directly proportional with the concentration of k-carrageenan in bun. The samples with 8 per cent of k-carrageenan showed highest activity (59%) which is equivalent to that of 20 µg/ml of BHA. The enhanced levels of antioxidant activity in food helps to reduce the oxidative stress in tissues (De Souza *et al.*, 2007). Sulphated polysaccharides from marine algae were previously established to possess moderate levels of radical scavenging activity (Souzan *et al*., 2012). k-carrageenan is included in the class of sulphated polysaccharide composed of D-galactose polymerized through β-1,3 glycosidic linkages. As well as their degraded fragments were established to possess antioxidant potential may be contributed by free phosphate,

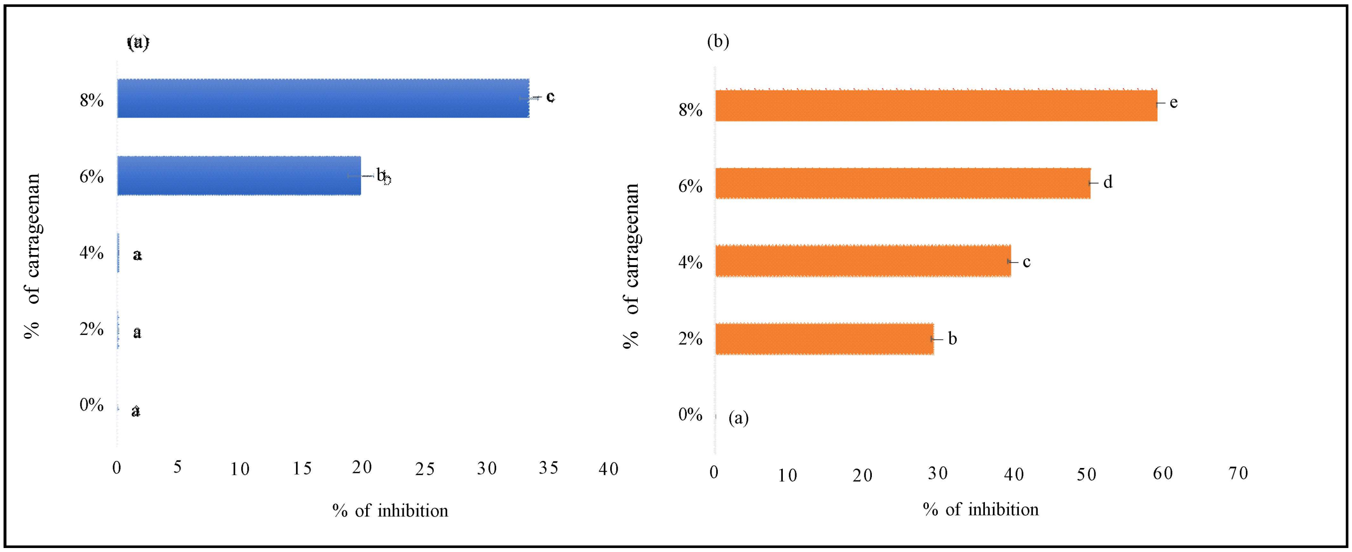

Fig. 1 : Antioxidant activity of k-carrageenan incorporated bun by (a) DPPH and (b) ABTS assay.*Mean value ± standard deviation, one-way ANOVA, Duncan's multiple comparison test values that have different superscripts letters after significantly ($p<0.05$) with each other

hydroxyl and amino side chain groups (Sun *et al.*, 2009).

Micro-structural analysis of bun by SEM analysis:

SEM analysis displays the micro-structural characteristics of the bun samples with different concentrations of k-carrageenan. SEM micrographs (30X) of experimental samples are shown in Fig. 2 (A) represents control, (B) 2%, (C) 4%, (D) 6%, (E) 8% of carrageenan.

The analysis of the pore size area demonstrated an increase in the pore area with an increased concentration of carrageenan. The results can be displayed as (A) 172.97, (B) 240.58, (C) 426.38 (D) 464.97 and (E) 432.83 mm^2 with the pore numbers ranging from 25 to 40 in the given area. Previous study on the incorporation k-carrageenan into the cake batter also showed an increased apparent viscosity and emulsion stability, which improved the volume and porosityof the product (Turabi *et al.,* 2008). Apparent viscosity might help in the entrapment of air into the bun dough and gave higher pore area (Turabi *et al.*, 2010). The k-carrageenan content may interfere in the formation of gluten network which is reflected in the increased pore area and reduced numbers of poresin bun (Sun *et al.*, 2015).

Physical properties of flour and shelf-life analysis of bun:

The results of the analysis illustrate a significant enhancement in the fibre content of bun with increase in concentration of k-carrageenan thereby increasing the nutritional value. Higher levels of antioxidant activity were also observed in samples incorporated with higher concentrations of k-carrageenan. But the sensory scores show that the better acceptable concentration of k-carrageenan that can be incorporated in bun is 6 per cent. The physical properties of the flour used for the development of 6 per cent carrageenan incorporated bun was compared with that of the control bun and the results are given in Table 2. The bulk density and packed density of the formulations used for the preparation of control and 6 per cent k-carrageenan incorporated bun remained the same. The water holding capacity increased whereas oil holding capacity decreased for k-carrageenan incorporated flour. This can be attributed to the presence of more hydrophilic constituents in k-carrageenan (Hodge and Osman 1976). An increase was observed in the swelling capacity of k-carrageenan incorporated flour which shows the increase in volume and this can also be considered as a criteria of better quality product (Achinewhu *et al.*, 1998).

The accepted sample (6% k-carrageenan incorporated bun) was packed in LDPE pouches and were taken up for shelf-life analysis. This was compared with the control (without the incorporation of k-carrageenan) bun. The results of shelf-life studies are given in Table 3. Control and 6 per cent carrageenan

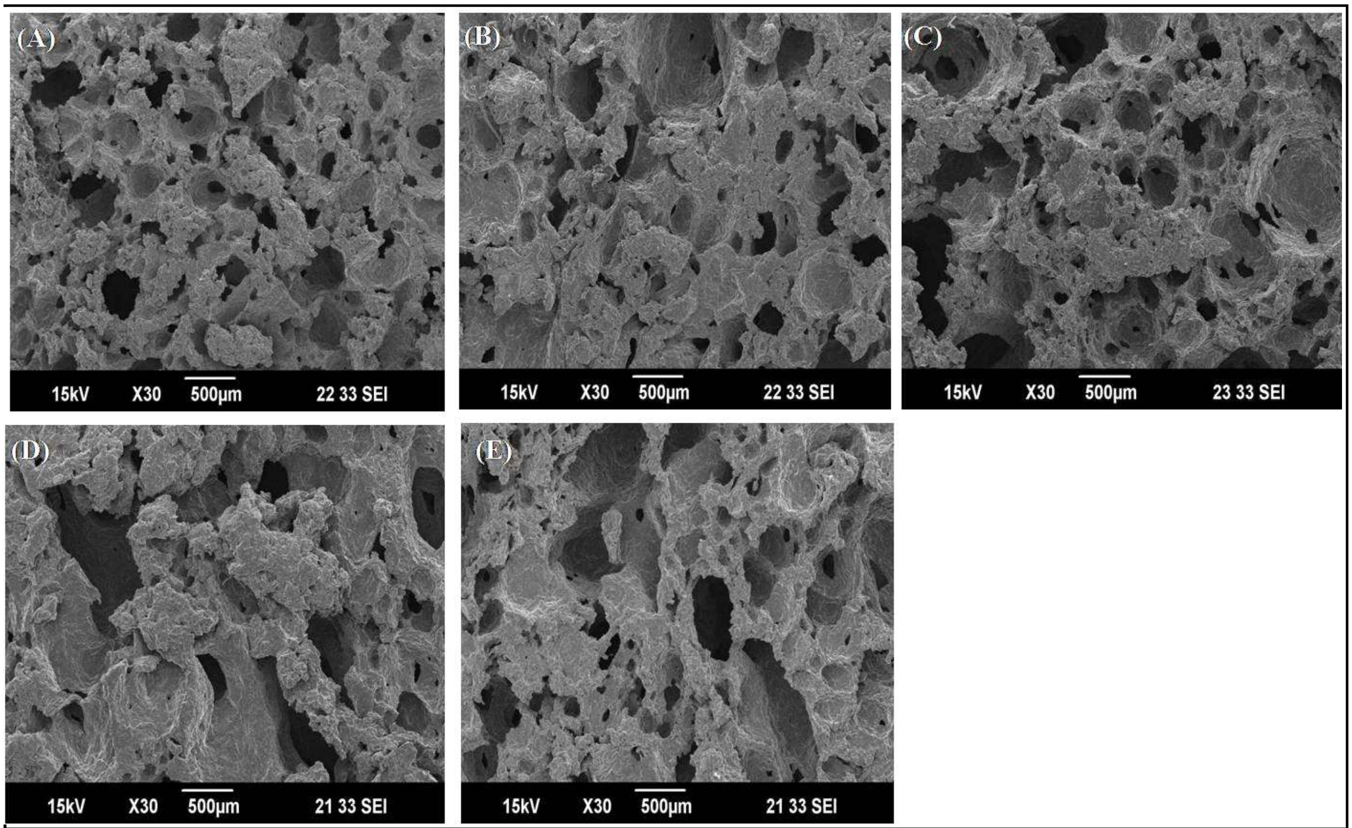

Fig. 2 : Scanning electron microscopy (SEM) micrographs of bun (A) control bun, (B) 2% carrageenan, (C) 4% carrageenan, (D) 6% carrageenan and (E) 8% carrageenan

incorporated samples had a shelf-life of 7 days and both these samples showed similar changes during the entire period of storage. Sensory score shows that buns were in the acceptable range. An increase in the lightness and yellowness was observed and in the case of texture hardness was found to increase (Gupta *et al.*, 2009). On the 8th day of storage mould growth was observed in both the samples. Some studies have shown that dietary fibre could play an important role in improving the shelf-life considering quality issues including increasing the shelf-life of foods because of certain characteristics like water-binding, anti-sticking, rising and thickening capacities (Sabanis *et al.*, 2009). In this study no significant improvement in shelf-life was noticed during the storage period.

Conclusion:

k-carrageenan extracted from *K. alverezii* was used for developing the fibre enrichment bun. Different concentrations of k-carrageenan from 2 to 8 per cent were used for the preparation of and the samples were subjected to different analysis, the results illustrate that antioxidant properties and other nutritional characteristics were improved by the addition of k-carrageenan. Textural parameters like hardness and chewiness increased with an increase in the concentration of k-carrageenan and

Table 2 : Physical properties of control and 6% carrageenan incorporated flour

Properties	Control	6% Carrageenan
Bulk density (g/ml)	0.40	0.42
Packed density (g/ml)	0.64	0.64
Water holding capacity	1.06	1.33
Oil holding capacity	1.48	1.14
Swelling capacity (ml/g)	2.59	3.49

Table 4 : Texture, colour and sensory changes during the storage time of control bun and 6% carrageenan incorporated bun

Sample	Storage time	Textural parameters					
		Hardness 1(N)	Hardness 2 (N)	Springiness (mm)	Gumminess (Kg f)	Chewiness (Kgfmm)	Cohessiveness
Control	Day 1	17.9±0.88[a]	15.3±0.85[a]	0.19±0.01[a]	3.59±0.16[a]	0.41±0.09[a]	0.23±0.16[a]
	Day 2	17.9±0.88[a]	15.3±0.85[a]	0.19±0.01[a]	3.59±0.16[a]	0.41±0.09[a]	0.23±0.01[a]
	Day 3	20.3±0.44[a]	17.1±0.87[b]	0.19±0.01[a]	3.47±0.04[a]	0.41±0.09[a]	0.23±0.01[a]
	Day 4	20.3±1.22[b]	17.1±0.87[b]	0.19±0.01[a]	3.47±0.04[a]	0.41±0.09[a]	0.23±0.01[a]
	Day 5	20.3±0.44[b]	18.1±0.38[bc]	0.18±0.01[a]	3.47±0.04[a]	0.41±0.09[a]	0.21±0.01[ab]
	Day 6	20.3±0.44[b]	18.1±0.38[bc]	0.18±0.01[a]	3.47±0.04[a]	0.38±0.03[a]	0.19±0.01[b]
	Day 7	22.6±0.51[c]	19.1±0.87[c]	0.18±0.01[a]	3.47±0.04[a]	0.32±0.01[a]	0.19±0.01[b]
6%	Day 1	21.1±1.65[a]	17.4±1.43[a]	0.24±0.00[c]	3.63±0.05[b]	0.87±0.01[b]	0.15±0.00[c]
	Day 2	21.1±1.65[a]	17.4±1.43[a]	0.24±0.00[c]	3.63±0.05[b]	0.87±0.01[b]	0.15±0.00[c]
	Day 3	27.1±0.36[b]	17.4±1.43[a]	0.20±0.03[bc]	3.55±0.04[a]	0.87±0.01[b]	0.15±0.00[c]
	Day 4	27.1±0.36[b]	17.4±1.43[a]	0.20±0.01[bc]	3.55±0.05[a]	0.87±0.01[b]	0.15±0.00[c]
	Day 5	27.1±0.36[b]	17.4±1.43[a]	0.19±0.01[a]	3.55±0.05[a]	0.87±0.01[b]	0.13±0.00[b]
	Day 6	27.1±0.36[b]	17.4±1.43[a]	0.18±0.01[a]	3.53±0.05[a]	0.87±0.01[b]	0.12±0.00[a]
	Day 7	27.8±0.20[b]	17.4±1.33[a]	0.18±0.01[a]	3.52±0.03[a]	0.69±0.01[a]	0.12±0.00[a]
		Colour analysis					
		Crust colour			Crumb colour		
		L*	a*	b*	L*	a*	b*
Control	Day 1	62.6±0.34[c]	17.3±0.29[ab]	40.6±0.45[ab]	83.2±0.24[b]	2.0±0.02[d]	20.2±0.13[d]
	Day 2	62.4±0.12[bc]	17.2±0.69[ab]	40.9±0.36[bc]	83.2±0.06[b]	1.9±0.02[d]	20.1±0.12[d]
	Day 3	62.2±0.10[bc]	17.1±0.07[bc]	41.7±0.36[c]	82.7±0.56[ab]	1.8±0.13[c]	19.9±0.10[d]
	Day 4	61.9±0.08[b]	17.1±0.22[bc]	41.9±0.60[cd]	82.7±0.36[ab]	1.7±0.09[b]	19.6±0.45[c]
	Day 5	61.8±0.12[b]	16.9±0.81[bc]	42.4±0.80[d]	82.4±0.24[ab]	1.5±0.04[a]	19.5±0.16[c]
	Day 6	60.8±0.06[a]	16.6±0.36[bc]	42.9±0.02[d]	82.3±0.30[ab]	1.4±0.07[a]	18.4±0.20[b]
	Day 7	60.4±0.59[a]	16.4±0.19[bc]	43.1±0.04[e]	82.2±0.85[a]	1.4±0.01[a]	17.2±0.07[a]
6%	Day 1	69.6±0.49[d]	12.5±0.25[a]	35.6±0.27[a]	82.2±0.36[a]	2.2±0.11[d]	21.4±0.25[d]
	Day 2	68.6±0.34[c]	12.9±0.01[ab]	35.8±0.08[a]	82.1±0.12[a]	2.1±0.09[d]	21.3±0.21[c]
	Day 3	66.2±0.09[bc]	13.8±0.28[ab]	36.3±0.42[a]	82.0±0.03[a]	1.9±0.01[c]	21.0±0.02[bc]
	Day 4	64.6±0.89[bc]	14.2±1.37[b]	37.4±0.50[b]	81.5±0.15[a]	1.9±0.03[c]	20.9±0.16[abc]
	Day 5	64.9±2.10[b]	15.5±0.45[c]	37.7±0.20[b]	81.4±0.44[b]	1.7±0.03[b]	20.8±0.17[ab]
	Day 6	63.9±1.66[a]	16.0±0.99[c]	37.9±0.78[b]	81.1±0.18[b]	1.7±0.03[ab]	20.7±0.37[ab]
	Day 7	61.2±0.16[a]	16.2±1.11[c]	38.1±0.57[b]	81.0±0.47[b]	1.7±0.04[ab]	20.5±0.23[a]
		Sensory parameters					
		Appearance	Colour	Texture	Taste	Overall acceptance	
Control	Day 1	8.80±0.42[a]	8.90±0.31[a]	8.70±0.48[c]	8.70±0.48[a]	8.70±0.48[b]	
	Day 2	8.80±0.42[a]	8.90±0.31[a]	8.70±0.48[c]	8.70±0.48[a]	8.70±0.48[b]	
	Day 3	8.80±0.42[a]	8.90±0.31[a]	8.50±0.41[bc]	8.70±0.48[a]	8.37±0.48[a]	
	Day 4	8.80±0.42[a]	8.90±0.31[a]	8.30±0.37[b]	8.70±0.48[a]	8.70±0.45[a]	
	Day 5	8.60±0.48[a]	8.90±0.31[a]	8.16±0.35[ab]	8.70±0.48[a]	8.62±0.41[a]	
	Day 6	8.54±0.47[a]	8.90±0.31[a]	8.16±0.35[ab]	8.49±0.51[a]	8.62±0.41[a]	
	Day 7	8.14±0.41[a]	8.70±0.48[a]	7.90±0.50[a]	8.49±0.51[a]	8.40±0.25[a]	
6%	Day 1	8.80±0.48[a]	8.60±0.51[a]	8.10±0.31[b]	8.30±0.48[a]	8.50±0.52[b]	
	Day 2	8.80±0.48[a]	8.60±0.51[a]	8.10±0.31[b]	8.30±0.48[a]	8.50±0.52[b]	
	Day 3	8.80±0.48[a]	8.60±0.51[a]	7.96±0.35[b]	8.30±0.48[a]	8.23±0.25[ab]	
	Day 4	8.80±0.48[a]	8.60±0.51[a]	7.96±0.35[b]	8.30±0.48[a]	8.23±0.25[ab]	
	Day 5	8.54±0.50[a]	8.60±0.51[a]	7.83±0.45[b]	8.30±0.48[a]	8.23±0.25[ab]	
	Day 6	8.54±0.50[a]	8.60±0.51[a]	7.83±0.45[b]	8.23±0.59[a]	8.23±0.25[ab]	
	Day 7	8.47±0.72[a]	8.54±0.48[a]	7.43±0.48[a]	8.23±0.59[a]	7.96±0.41[a]	

*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.

colour analysis showed variation in lightness and yellowness of the crust colour whereas no significant change was observed in the case of crumb colour. SEM images of the bun crumb showed that the incorporation of k-carrageenan has led to an increase in the pore area. Sensory scores suggested that k-carrageenan incorporation above 6 per cent was not appreciated by the sensory panel. Thus, 6 per cent k-carrageenan incorporated bun taken up shelf-life and was compared with that of the control bun. Both control and 6 per cent carrageenan incorporated bun samples showed similar changes during the storage period and had a shelf of 7days after which mould growth was observed. The present-day life style has led to an increased demand for baked goods like bread, bun, etc. whereas these products do not meet the daily nutritional requirements of a human body. This study reveals that the incorporation of k-carrageenan can result in better nutritional qualities with an improved fibre and mineral content and an enhanced antioxidant activity of bun.

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