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Yield and quality of carrageenan from *Kappaphycus alvarezii* subjected to different physical and chemical treatments

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ABSTRACT

With a view to findout a suitable method for carrageenan extraction from *Kappaphycus alvarezii*, a detailed investigation was made on quantitative and qualitative estimation of carrageenan subjected to different physical and chemical treatments. The dried material presoaked in KOH and heated for 5 hours at 90^0C and precipitated with propanol gave the maximum yield of 59.4 % and viscosity of 25.25 cps. In $Ca(OH)_2$ treatment, the yield was almost similar to that of KOH treatment but the viscosity was very low (9.45 cps). The gel was found to be brown in colour when treated with NaOH and milky white with $Ca(OH)_2$. KOH gel was thick, translucent with high viscosity. Pretreatments of dried seaweed with NaOH, KOH and $Ca(OH)_2$ followed by pressure-cooking showed relatively higher yield of carrageenan, but the viscosity, clarity and the texture of the gel were found to be poor. Clarity of gel obtained by KOH- methanol treatment was higher when compared to other treatments. The yield of carrageenan was higher when the extracted material was frozen overnight and thawed, but the gel was brown in colour with less rigidity. The present study shows that treatment with KOH gives better yield and quality gel.

Introduction

Carrageenans are commercially important highly sulfated polysaccharides of the galactan group with alternating 1,3 and 1,4 linked galactose residues, which fill spaces between the cellulosic plant structure of red seaweeds (Imeson, 1992), making up between 30 to 80% of this cellulosic material (Whistler and Be Millar, 1997). Carageenans can not be digested by the human body and therefore provide no nutritional benefit, but acts as an additive in the food industry due to its functional properties, which can be used to control moisture, texture and to stabilise foods (Elliason, 1996). Carrageenan is extensively used in the colloid industries like food, pharmaceutical and aquaculture feed (Bautista *et al*., 1990), in emulsion stabilizations, for binding and dispersion, in food particularly in dairy applications to gel, to thicken or as suspending agents (Guisley *et al*., 1980), in medicine (Neushul, 1993) and in meat and poultry (Bixler, 1996). Cajipe (1990) reported that carrageenan processed from *Eucheuma* is used as a medium for the growth of a wide spectrum of microorganisms (bacteria, yeast and other fungi).

Globally there are 24 large processors of carrageenan and less than 10 small factories (Hurtado and Agbayani, 2000). There is no report on commercial scale carrageenan production in India though there is a potential red algal resource through farming. Carrageenan is sold at Rs 1 lakh per tonne (Vijayalakshmi, 2003) and the internal market prices of carrageenan have been reviewed by Krishnamurthy (2005). The price of refined and semi-refined carrageenan extracted from Kappaphycus / *Eucheuma* is in the range of US$ 2,600-3,500 and US$ 1,500-2,000/MT respectively. The present study was designed to investigate the yield and quality of kappa-carrageenan extracted from farmed *Kappaphycus alvarezii*, subjected to different physical (i.e., pressure cooking, freeze and thaw) and chemical (i.e., 0.05N NaOH, KOH and $Ca(OH)_2$) treatment followed by precipitation with organic solvents like methanol, ethanol and propanol and the inorganic salt like potassium chloride (1%).

Material and Methods

Kappaphycus alvarezii (Doty) Doty ex P. Silva cultured in the open sea of Palk Bay near fish farm of CMFRI, Mandapam camp (9°17' N and 79°12'E) was harvested and sun dried. The dried material was transported to CMFRI, Cochin for the extraction of carrageenan. The dried plants were rinsed with freshwater, removed off debris and dried in an oven at 90ºC for 5 h. Known quantity of oven dried seaweed was subjected to different chemical treatments with Potassium hydroxide (KOH), Sodium hydroxide (NaOH) and Calcium hydroxide ($Ca(OH)_2$) by heating in hot water bath for 5 h at 90°C. The suspensions were precipitated with methanol, ethanol, propanol and potassium chloride (KCl) separately and also exposed to physical treatment by pressure-cooking for 15 min followed by freezing and thawing. The viscosities of all the treated samples were measured by using Oswald viscometer. The texture of the gel was noted by visual observation. Clarity of the gel was estimated by taking the transmission of the gel in an UV-visible spectrophotometer (Hitachi model U-2010) with reference to distilled water. All the experiments were carried out in duplicate and the average value is presented in this paper.

Results and Discussion

The yield, viscosity and clarity of carrageenan of *Kappaphycus alverezii* under different treatments are given in the Fig. 1 to 3 respectively. The carrageenan yield ranged from 2.8% to 72.9%. The maximum yield was obtained with NaOH extraction followed by pressure-cooking (72.9%) but the viscosity of the gel was low (12.04 cps). Carrageenan extracted with KOH followed by methanol precipitation resulted in high yield (62.4%) and quality gel with viscosity 24.21 cps and transmission 70.2%. Carrageenan extracted with KOH showed yield ranging from 51.4 to 65.7 % (Fig.1). The gel was translucent, thick, rigid and the viscosity ranged from 12.94 to 25.25 cps. (Fig.2). The clarity of the gel was also very good showing transmission of 70.2 % (Fig.3) when precipitated with methanol. Carrageenan extracted with KOH followed by pressure-cooking resulted in a brown colour solution with very less transmission (21.7%) and least viscosity (12.94 cps). The brown colouration was more prominent when it was extracted with NaOH followed by pressure-cooking showing viscosity of 12.04 cps and 19.7% transmission, with sediment settling at the bottom of the beaker. The clarity was low when the carrageenan was extracted with $Ca(OH)_2$ followed by pressure-cooking. Milky white precipitation was observed with low clarity showing transmission of 4.4 % and low viscosity (9.19 cps). Highly significant positive correlation was observed between yield and viscosity in KOH ($r^2 = 0.69$; $P<0.05$) and NaOH ($r^2 = 0.99$; $P<0.01$) extraction respectively. Precipitation of carrageenan with ethanol and propanol also resulted in yield ranging from 49.2 to 59.4 %. Viscosity of

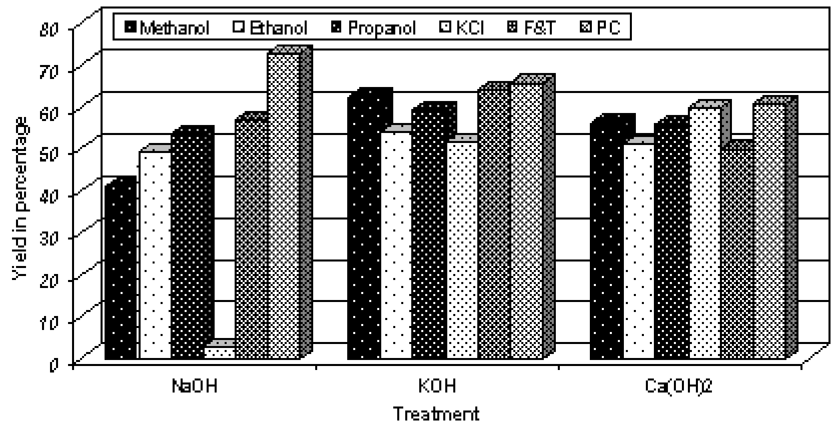

Fig. 1. *Yield of carrageenan under different treatments*

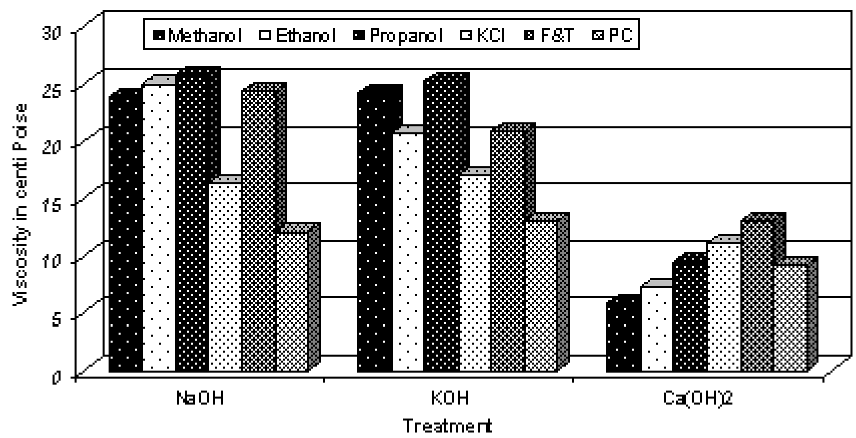

Fig. 2. *Viscosity of carrageenan under different treatments*

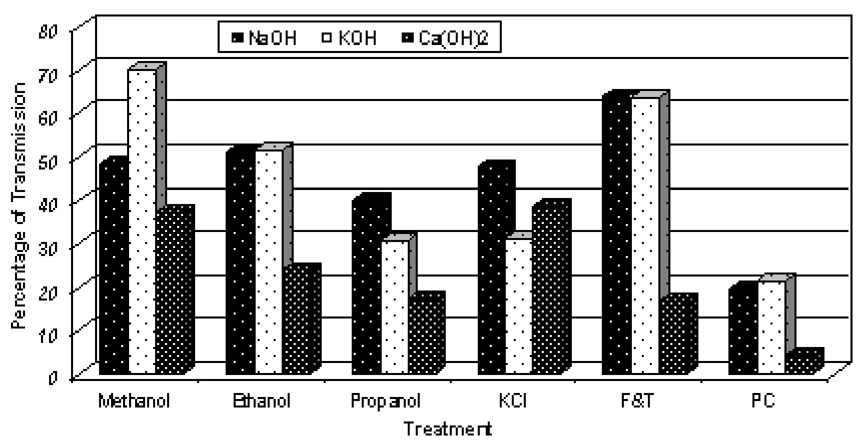

Fig. 3. *Clarity of carrageenan under different treatments*

carrageenan was found to increase with the increase in carbon atom of alcohol. The propanol extracted material was more rigid and translucent than that precipitated with ethanol and methanol. Significant negative correlation ($r^2 = -1.00$, $P<0.01$) was observed when the gel was subjected to freeze-thaw and pressure-cooking.

In the present investigation, variations in carrageenan yield and quality were recorded by different extraction and precipitation methods. The main variables in extraction methods were the base used to break down the seaweed material, the time that the material was in contact with the base and the temperature at which the reaction occurred, which subsequently affected the gelling properties and structure of carrageenan. *Kappa* carrageenan has 34% 3,6 anhydro-D-galactose groups as part of its repeating structure and 25% ester sulfate groups, which will easily disperse when heated (Ross Kealy, 2003). *Kappa* and *iota* carrageenans form thermoreversible gels by arrangement of the disordered chains into double or triple helices, formed by hydrogen bonding, followed by aggregation of the ordered domains to form a firm, three dimensional stable gel (Ross Kealy, 2003). This observation supports our results of high carrageenan yield obtained during pressure-cooking. On the other hand the viscosity was low and negatively correlated with yield explains that carrageenan exposed to high temperature and pressure looses its properties. It might be due to irregularities in the chain as reported by Whistler and Be Miller (1997) and Rao (1999).

Carrageenan extracted with KOH and precipitated by propanol gave high yield and quality gel. It may be due to the fact that *Kappa* carrageenan forms strong, rigid and slightly opaque gels when potassium ions are present. They link to the sulphate groups causing an anti-parallel double helix structure (Ross Kealy, 2003). Similarly better carrageenan yield was observed when extracted with $Ca(OH)_2$ but the viscosity and clarity of the gel were very poor. As explained by Ross Kealy (2003) in the presence of calcium ions, the gel will contract and become brittle. It may be presumed that monovalent cation prefers to bind the sulphate group than divalent cation. Dennis (2003) reported that *Kappaphycus alvarezii*, when heated in an alkaline solution of potassium hydroxide for about two hours had increased gel strength. The hydroxide part of the reagent penetrates the seaweed, reduces the amount of sulphate in the carrageenan and increasing the 3,6-AG, thereby improving the gel strength of the carrageenan. In the present study too, the gel strength improved by boiling the seaweed with 0.05N KOH for 5h. The present investigation shows that better yield and quality of carrageenan can be obtained from *Kappaphycus alverzii* with KOH treatment.

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