A Systematic Review on Carrageenan: Structure, Safety, and Effects on Food

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Abstract:

Carrageenan is a high molecular weight sulfate polyglycan used to improve the texture of commercial food products. Carrageenan is a natural carbohydrate (polysaccharide) obtained from edible red seaweeds. The name Carrageenan is derived from the Chondrus crispus species of seaweed known as Carrageen Moss or Irish moss in England, and Carraigin in Ireland. Carraigin has been used in Ireland since 400 AD as a gelatin and as a home remedy to cure coughs and colds. It grows along the coasts of North America and Europe. Carrageenans are used in a variety of commercial applications as gelling, thickening, and stabilising agents, especially in food products and sauces. Aside from these functions, carrageenans are used in experimental medicine, pharmaceutical formulations, cosmetics, and industrial applications.

Keywords: Carrageenan, chemical and structures, application

Introduction:

Carrageenan is a generic name for a family of gel-forming and viscosifying polysaccharides, which are obtained by extraction from, was dedicated to this village **(Khalid Mahmood Zia 2017)** Carrageenans have been used as safe food additives for a number of decades and are identified in the system of E-codes in the European Union as E407 (Weiner 2014). They are naturally occurring anionic linear sulphated polysaccharides extracted from red seaweeds of the genus *Rhodophyceae* (**Myra L. Weiner 2017**). The principal constituent of these seaweeds are co-polysaccharides with a linear chain created by β-D-galactose and 3,6-anhydro-α-D-galactose with a varying number of sulphate groups (**Liang Li 2014**). The food industry uses 70 to 80% of total global production of carrageenans, estimated to be around 45 000 tons a year, of which around 45% is used in dairy products and 30% in meat and meat products. Only three dominant types are used in the food industry, i.e. κ-, λ- and ι- carrageenan. Commercial carrageenan is generally a mixture of carrageenans, with κ carrageenan (gelling) predominating over λ-carrageenan (non-gelling) at a ratio of around 3:2 (**Vanessa Leiria Campo 2009**). Carrageenans are widely used due to their excellent physical and functional properties such as thickening, gelling and stabilising qualities. Carrageenans are used to improve the texture of cheeses, puddings and desserts, and as binding agents and stabilisers in the meat industry, for instance in hams and smoked meats (**Mohammad Alnaief, 2018**). Certain species of red seaweeds (figure 1). Carrageenan is derived from a number of seaweeds of the class Rhodophyceae. This particular type of seaweed is common in the Atlantic Ocean near Britain, Europe and North America. When used in food products, carrageenan has the EU additive E-number E407 or E407a. E407a has a slightly different composition; moreover, it contains a considerable amount of cellulose. Carrageenan has no nutritional value and is used in food preparation for its gelling, thickening, and emulsifying properties (**Mihkel Saluri 2017**) and in pharmaceutical applications (**Yurij A. Antonov 2018**) and experimental medicine this substance is often used for the testing of anti-inflammatory agents.

For several hundred years, carrageenan has been used as a thickening and stabilizing agent in food in Europe and the Far East. In Europe the use of carrageenan started more than 600 years ago in Ireland. In the village of Carraghen (Pereira, L., 2016) on the south Irish coast, flans were made by cooking the so-called Irish moss (red seaweed species Chondrus crispus) in milk. The name carrageenin, the old name for carrageenan, was first used in 1862 for the extract from C. crispus.

Carrageenan is a sulfated polygalactan with 15 to 40% of ester-sulfate content and an average relative molecular mass well above 100 kDa. It is formed by alternate units of d-galactose and 3,6-anhydro-galactose (3,6-AG) joined by α-1,3 and β-1,4-glycosidic linkage. Carrageenan is classified into various types such as λ, κ, ι, ε, μ, all containing 22 to 35% sulphate groups. This classification was made

based on its solubility in potassium chloride. The primary differences which influence the properties of carrageenan type are the number and position of ester sulfate groups as well as the content of 3.6-AG. These names do not reflect definitive chemical structures but only general differences in the composition and degree of sulfation at specific locations in the polymer. Higher levels of ester sulfate mean lower solubility temperature and lower gel strength. Kappa type carrageenan has an ester sulfate content of about 25 to 30% and a 3,6-AG content of about 28 to 35%. Iota type carrageenan has an ester sulfate content of about 28 to 30% and a 3,6-AG content of about 25 to 30%. Lambda type carrageenan has an ester sulfate content of about 32 to 39% and no content of 3,6-AG (Gholamreza Kavoosi 2018).

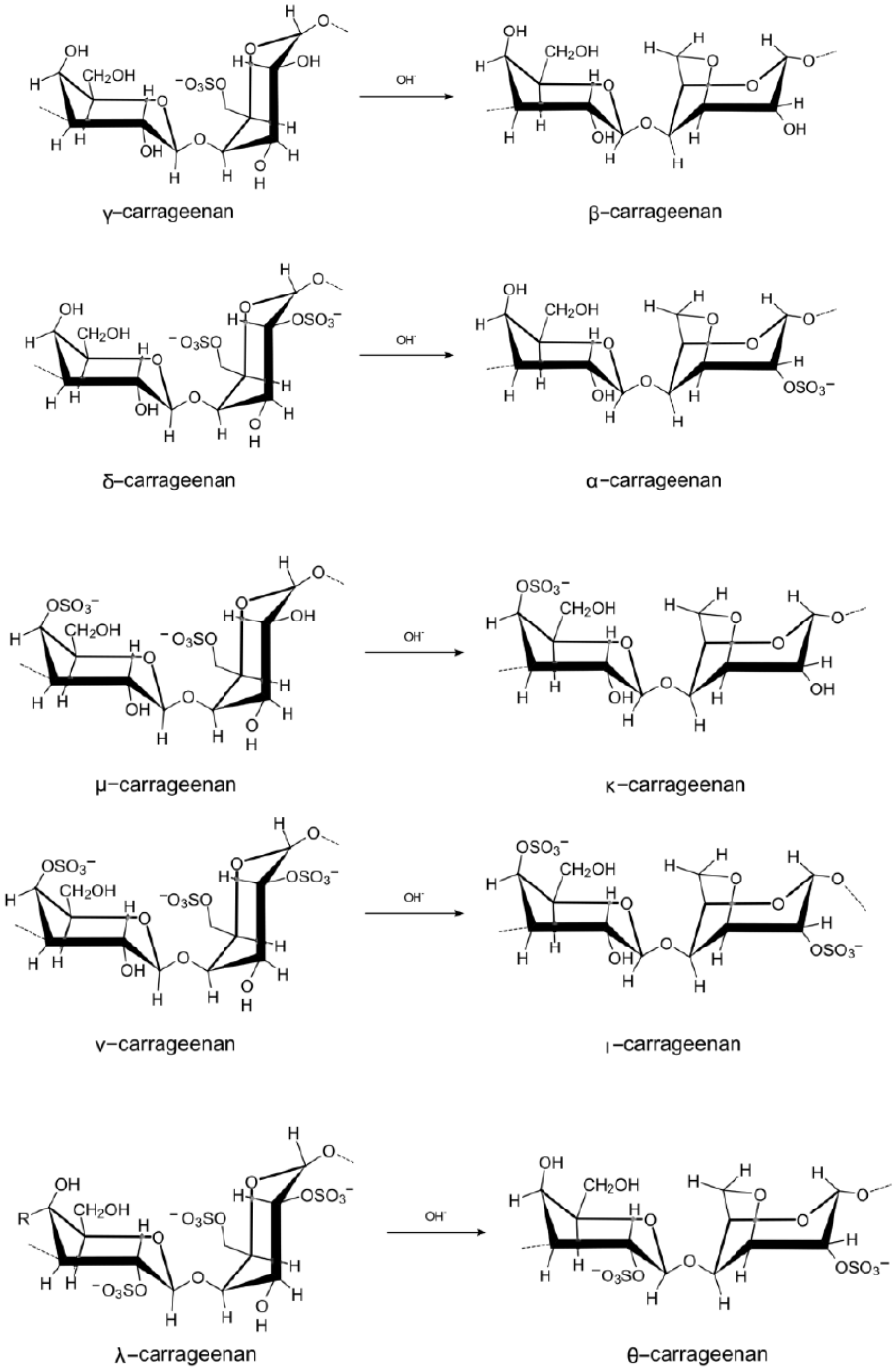

Figure 1. Chemical structure of carrageenans

Properties of Carrageenan:

The chemical reactivity of carrageenans is primarily due to their half-ester sulfate groups which are strongly anionic, being comparable to inorganic sulfate in this respect. The free acid is unstable, and commercial carrageenans are available as stable sodium potassium and calcium salts or, most commonly, as a mixture of these. The associated cations together with the conformation of the sugar units in the polymer chain determine the physical properties of the carrageenans. For example, kappa- and iota-carrageenans form gels in the presence of potassium or calcium ions whereas lambda-carrageenan does not. Reactivity with proteins is exhibited by both gelling and nongelling carrageenans, although regularity of the polymer is an important factor. In most, if not all, cases ion-ion interactions between the sulfate groups of the carrageenan and the charged groups of the protein are involved. Reaction depends on protein/carrageenan net charge ratio, and thus is a function of the isoelectric point of the protein, the pH of the system, and the weight ratio of carrageenan to protein (Mingjin Zhu, 2017).

At pH levels below the isoelectric point the protein has a net positive charge and thus can undergo direct electrostatic interaction with the negatively-charged carrageenan. The commercially important reaction of carrageenan with kappa-casein in milk is specific for this protein and unique in that it can occur at pH levels above the isoelectric point of the casein. It appears to be due to a region of positively charged amino acid residues in the kappa-casein molecule which can interact electrostatically with the sulfate groups of the carrageenan even though the net charge of the casein is negative (Mingjin Zhu, 2017).

The functionality of carrageenans in various applications depends largely on their rheological properties. Carrageenans, as linear, water-soluble, polymers, typically form highly viscous aqueous solutions. This is due to their unbranched, linear macromolecular structure and polyelectrolytic nature. The mutual repulsion of the many negatively charged half-ester sulfate groups along the polymer chain causes the molecule to be highly extended, while their hydrophilic nature causes it to be surrounded by a sheath of immobilized water molecules. Both of these factors contribute to resistance to flow.

Viscosity depends on concentration, temperature, the presence of other solutes, and the type of carrageenan and its molecular weight. Viscosity increases nearly exponentially with concentration. This behaviour is typical of linear polymers carrying charged groups and is a result of the increase with concentration of interaction between polymer chains. Salts lower the viscosity of carrageenan solutions by reducing the electrostatic repulsion among the sulfate groups. This behaviour is likewise normal for ionic macromolecules. At low temperature and high enough salt concentration, however, carrageenan solutions may gel, with an increase in apparent viscosity. This is particularly true for the strongly gel-inducing cations, K^+ and Ca^{++}. At high temperatures, however, Ca^{++} lowers viscosity to a greater extent than does Na^+ or K^+.

Viscosity decreases with temperature. Again, the change is exponential. It is reversible provided that heating is done at or near the stability optimum at pH 9, and is not prolonged to the point where significant thermal degradation occurs. Both gelling (kappa-, iota-) and nongelling (lambda-) carrageenans behave in this manner at temperatures above the gelling point of the carrageenan. On cooling, however, the gelling types will abruptly increase in apparent viscosity when the gelling point is reached, provided that the counter-ions (K^+ and Ca^{++}) promotive of gelation are present.

Viscosity increases with molecular weight in accordance with the Mark-Houwink equation:

$[h] = KM^{a}$

Where [h] is intrinsic viscosity, M is an average molecular weight (since carrageenans are polydisperse) and K and a are constants. Intrinsic viscosities correlate well with practical viscosity measurements taken at 1.5% concentration and 75°C.

Commercial carrageenans are available in viscosities ranging from about 5 mPa.s to 800 mPa.s when measured at 1.5% concentration and 75°C. Carrageenan solutions having viscosities less than 100 mPa.s have flow properties very close to Newtonian. At higher viscosities the solutions exhibit shear-thinning behaviour and it becomes necessary to specify the shear rate at which the measurement is

taken. Where non-Newtonian behaviour is expected viscosity measurements should be made at a shear rate comparable to that encountered in the application considered.

Carrageenans specifically tailored for water-thickening applications are usually lambda types or the sodium salt of mixed lambda and kappa. They dissolve in either cold or hot water to form viscous solutions. There high water viscosities are desirable, and the high molecular weight and hydrophilicity of lambda contribute to this. For gelling applications a low viscosity in hot solution is usually desirable for ease in handling, and, fortunately, high gel strength carrageenans (mixed calcium and potassium salts of kappa or iota) fulfill this requirement because of their lesser hydrophilicity and the effect of the calcium ions. Kappa- and iota-carrageenans and furcellaran form gels on cooling of their hot solutions in the presence of certain cations, notably K^+ and Ca^{++}. Heating is required to bring them into solution under these conditions. According to Rees (Jingjing Liu, 2015) carrageenans which form aqueous gels do so because of double helix formation. At temperatures above the melting point of the gel thermal agitation overcomes the tendency to form helices and the polymer exists in solution as random coils. On cooling the polymer chains become interlinked through double helix formation to form "domains" (G. Agoda-Tandjawa, 2017).

This occurs regardless of the counterions present and does not directly lead to gelation. Only when potassium or other gel-promoting cations are present will the domains aggregate to form a three-dimensional network. An alternative model of carrageenan gelation, based on cation-induced aggregation of single helices has also been proposed Regardless of the mechanism it appears that the occurrence of 1,4-linked 6-sulfated residues in the polymer chain of either kappa- or iota-carrageenan detracts from the strength of their gels. This is ascribed to kinks, produced by these residues, in the chain which inhibit the formation of double helices. Alkali modification of the carrageenan during processing increases the gel strength of the product by removal of these kinks through conversion of 6-sulfated residues to the 3,6-anhydride. Increased hydrophobicity from the added anhydride residues may also contribute to gelation.

Kappa-carrageenan and furcellaran gels are relatively rigid and are subject to syneresis. Incorporation of locust bean galactomannan along with kappa or furcellaran yields a more compliant gel. "Smooth" regions of the mannan chain (i.e., regions carrying no galactose side groups) are believed to bind to the double helices of the kappa to reduce their tendency to aggregate (María D. Torres, 2018). Iota-carrageenan by itself yields compliant gels with very little tendency to undergo syneresis. Here the 2-sulfate groups on the 3,6-anhydride residues act as wedging groups to prevent the tightly-packed aggregation believed responsible for the rigidity of kappa gels. Whereas potassium is more effective than calcium in inducing gelation of kappa the reverse is true for iota-carrageenan.

All carrageenans have the ability to form gels by cooling a solution of the carrageenan in hot milk. Even lambda-carrageenan, which does not gel in water regardless of the cations present, will form a gel at levels of 0.2% or more by weight of the milk. This gelation is ascribed to the formation of carrageenan-casein bonds, as previous described. With kappa- and iota-carrageenan as well as furcellaran there is, in addition to the carrageenan-casein interaction, a water-gelling effect from the cations present in the carrageenan as well as the Ca^{++} and K^+ present in the milk. These cations appear to be required for milk gel formation as milk which has been ion-exchanged to remove Ca^{++} and K^+ does not gel with the sodium salts of lambda, kappa, or iota. On the other hand the strength of milk gels is enhanced by the addition of soluble calcium and potassium salts in a manner quite similar to that in water gels. The presence of fat influences the behaviour of carrageenans in milk. Strongly gelling kappa-carrageenans can be used in high-fat systems but are not well tolerated in low-fat systems wherein they may exert a destabilizing action resulting in whey separation. For the latter, weak milk-gelling kappa-carrageenans with high ester sulfate and moderate to high 3,6-anhydride are more suitable. The reason that strongly-gelling types can be employed in high-fat but not low-fat systems is due in part to the disperse fat phase. This apparently tempers the carrageenan-casein complex, serving to interrupt aggregation to some extent. Interaction may also occur between carrageenan and the phospholipid which is present as a monomolecular layer covering the disperse globules of butterfat in the milk. Since the phospholipid contains basic amino groups with which the ester sulfate groups of the carrageenan can react it is very likely that electrostatic bonds are formed between the carrageenan and

phospholipid. This may account for the extraordinary effectiveness with which very low levels (ca. 50 ppm) of carrageenan stabilize evaporated milk against fat separation.

Carrageenans are susceptible to depolymerization through acid-catalyzed hydrolysis. At high temperatures and low pH this may rapidly lead to complete loss of functionality. They can be used in acid systems, however, if not subjected to prolonged heating. The rate of hydrolysis at a given pH and temperature is markedly lower if the carrageenan is in the gel rather than the sol state. This can be achieved by ensuring that gel-promoting cations are present at sufficient concentration to raise the gel melting temperature above the temperature to which the carrageenan will be subjected. Carrageenan is listed by the U.S. Food and Drug Administration (FDA) as generally Recognized as Safe (GRAS) (G. Agoda-Tandjawa, 2017). Following reports of cecal and colonic ulceration in guinea pigs and rabbits induced by a highly degraded carrageenan provided, ironically, for the symptomatic relief and cure of peptic and duodenal ulcers in man, intensive investigations into the safety of carrageenans were carried out by the FDA and other groups sponsored by the carrageenan industry. By lace 1976 food grade carrageenan, defined as having a water viscosity of no less than 5 mPa.s at 1.5% concentration and 75°C (U.S. Food and Nutrition Board, 1981) had been demonstrated to be safe.

Carrageenan is extracted from the raw material with water at high temperatures. The liquid extract is purified by centrifugation and/or filtration. The liquid extract may be converted into a powder by simple evaporation of water to yield the so called drum dried carrageenan. Proper release of the dried material from the dryer roll requires addition of a small amount of roll-stripping agents (mono- and diglycerides).

The content of mono- and diglycerides is responsible for the drum dried carrageenans being turbid in watery solutions, and drum dried carrageenan consequently finds little use in water gel applications. Also, drum dried carrageenans contain all soluble salts present in the extract, which may influence the properties - for instance solubility of the carrageenan.

Most of the carrageenan used in foods is isolated from the liquid extract by selective precipitation of the carrageenan with isopropanol. This process gives a more pure and concentrated product.

Application of Carrageenan in Food:

Carrageenans are used to gel, thicken, or suspend; therefore they are used in emulsion stabilization, for syneresis control, and for bodying, binding and dispersion. Major uses are in foods, particularly dairy applications. Tables 1and 2 list dairy and water-based applications respectively (Jingjing Liu,2015).

Carrageenan is unique in its ability at very low concentration (ca. 300 ppm) to suspend cocoa in chocolate milk; no other gum has been found to match it. A very delicate milk gel structure, undetectable on pouring or drinking the milk, is believed to hold the cocoa in suspension. A substantial differential ("spread") between the concentrations at which settling of cocoa occurs and that at which visible gelation is evident is required for practical stabilization. This is achieved by careful selection of weed type and quality.

The use of iota-carrageenan in dessert gel formulations affords gels which have textures very similar to those of gelatin gels. They have an advantage over gelatin gels in that their melting point is higher, so that they find a ready market in tropical climates or where refrigeration is not available. This is offset to some extent by the different mouth-feel, since these gels do not "melt in the mouth", as does gelatin. A further advantage is that iota gels retain their tender structure on aging, whereas gelatin tends to toughen. This is important for ready-to-eat desserts, an item popular in Europe.

Kappa-carrageenan or furcellaran by itself is unsatisfactory for dessert gel applications due to the "short", brittle structure of its gel. This can be ameliorated by the incorporation of locust bean galactomannan into the formulation, and kappa-locust bean or iota- kappa-locust bean blends are also offered for this application. To achieve sparkling-clear gels it is necessary to use a locust bean gum which has been clarified by filtration. The clarified gum is produced for this purpose by several of the major carrageenan manufacturers.

CGNs are extracted from specific seaweeds, including *Gigartina*, *Chondrus*, and *Eucheuma*, and are used by the food industry to improve the texture of food products by acting to thicken, stabilize,

or emulsify dairy products, salad dressings, infant formulas, processed meat, soy milk, and other food products (Jingjing Liu, 2015).

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Industrial vinegar production is a biochemical process which utilises bacteria. Osuga et al. (Jingjing Liu, 2015) described the use of a bubble-mixed reactor and κ-carrageenan gel beads as carriers for the continuous production of acetic acid. An improvement was attempted through the use of an air-lift reactor, using the culture of *Acetobacter* species K1024 isolated from a commercial vinegar broth. More recently, a successful continuous production of vinegar was reported using a bubblemixed tabletop bioreactor with κ-carrageenan-immobilised *Acetobacter suboxydans* cells.

Fermented milk products can be obtained by simultaneous acidification and inoculation of skimmed milk by immobilised mixed cultures. Three different strains of *Lactococcus lactis* and one strain of *Leuconostoc mesenteroides* were separately immobilised in κ-carrageenan/locust bean gum gel (2.75% and 0.25% w/w, respectively) and used in a 2-L stirred reactor (Jingjing Liu, 2015).

Mensour and colleague described the immobilization system of yeast cells (*Saccharomyces* sp.) for beer production using κ-carrageenan beads continuously produced by a static mixer process. Ethanol production from glucose using cells of *Zymomonas mobilis* immobilized in κ-carrageenan was investigated in a fluidised bed fermenter. This research was extended to study the production of ethanol from starch (G. Agoda-Tandjawa, 2017) using the bacteria co-immobilised with an industrial glucoamylase in carrageenan gel beads and used in a glass column fermenter. The carrageenan gel matrix was reported to provide protection of immobilised *Saccharomyces cerevisiae* cells (G. Agoda-Tandjawa, 2017) and continuous ethanol production from pineapple cannery waste was attempted using these yeast cells immobilised in κ-carrageenan (G. Agoda-Tandjawa, 2017).

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