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## CHAPTER 9

# Justification of the technology for the use of *Phyllophora* (Zernov field) carrageenan as a regulator of the consistency of food products

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### Abstract

The results of theoretical and experimental studies and progressive solutions regarding the use of the phytocolloid – carrageenan *Phyllophora* (Zernov field) extracted from the Black Sea red algae *Phyllophora Brodyas* food consistency regulator is presented. The technological aspects of the use of carrageenan from the Black Sea red algae *Phyllophora Brody* are substantiated. The study shows that the use of this drug ("PZF" carrageenan) is appropriate for expanding the range of consistency regulators of food industry products. It is found that "PZF" carrageenan extracted from the Black Sea red algae *Phyllophora Brody* has a 3,6-anhydrogalactose content of 21.3 %; the mass fraction of sulfoether groups (in terms of  $\text{SO}_4$ ) is 24.2 %. The concentration dependence of the viscosity of the carrageenan solutions "PZF" is studied. With an increase in the concentration of "PZF" carrageenan to 2 %, the flow index of the solution decreases sharply, which indicates an increase in the structuredness of the system. The dependence of the viscosity of the "PZF" carrageenan solutions on the shear rate gradient in the interval 3–1312  $\text{s}^{-1}$  is established. In the studied range of shear rates, the viscosity of solutions obeys the power law and is described by the Ostwald-Weyl equation. It is found that the reversible destruction of the structure occurs under the action of shear. The degree of thixotropic reduction of the "PZF" carrageenan solution is 87.9 %. The influence of temperature and pH on the rheological properties of "PZF" carrageenan solutions is studied. It is found that at temperatures up to 45 °C, carrageenan macromolecules exist in a spiral conformation, and at higher temperatures they undergo a thermo-reversible transition into a coil conformation. This transition causes a decrease in viscosity and gelation of the solution. It is found that "PZF" carrageenan solutions retain their abnormally viscous properties in a wide pH range. When the pH of the

solution changes from 1 to 11, no signs of a conformational transition of macromolecules of "PZF" carrageenan are detected. In the process of storage, the viscosity of "PZF" carrageenan solutions first increased, and then decreased, regardless of the pH value. A solution with pH=4 has high stability during storage. An acidic environment prevents the development of microorganisms; however, in this case it is not strong enough to cause significant hydrolysis of the polysaccharide. The obtained data on the chemical and physico-mechanical properties of "PZF" carrageenan solutions make it possible to predict the properties of viscous solutions and gels for structured food products.

### **Keywords**

Carrageenan, phytocolloid, consistency regulator, extraction, rheological properties, physical and chemical properties, food technology.

## **9.1 Justification of using carrageenan feasibility as regulators of food products consistency**

### **9.1.1 Analysis of modern theoretical information on the gel-forming ability of carrageenan**

Carrageenan is an anionic sulfated polysaccharide consisting of alternating long linear chains of (1→3)-β-d-galactose and (1→4)-3,6-anhydro-α-d-galactose (3,6-AG) or (1→4)-α-d-galactose with ether sulfates (15–40 %) [1]. These natural polymers are divided into six main forms based on their source, solubility, and sulfate content: kappa, iota, lambda, mu, nu, and theta. Thanks to the numerous works of Riess and co-authors [2], several "saturated", i.e., idealized structures of carrageenan were established. This made it possible to divide carrageenans into so-called "types", which differ in the content of 3,6-anhydro-galactose, the location and number of sulfate groups. Currently, more than 16 carrageenan structures are known, but the main ones are the so-called gelling types, which include kappa- and iota-carrageenans, and non-gelling lambda-carrageenans. These types of carrageenans are of commercial value [3–5].

The practical use of carrageenan is largely determined by its physicochemical properties, which are dissimilar for different types of carrageenans. The gelling properties of carrageenans depend on their chemical structure, the nature of the cation, the temperature of the solution, and the concentration of the polymer. The gelling properties are higher, the fewer the residues of sulfuric acid in the polysaccharide and the higher the content of 3,6-dihydrogalactose in it. Increasing the content

of the latter from 28 to 35 % leads to a significant increase in gelling properties, which can be achieved by special alkaline treatment [6]. Due to the presence of a highly charged sulfate group in the polymer molecule, carrageenans are in solution in the form of stable  $K^+$ ,  $Na^+$  or  $Ca^{++}$  salts. The nature of the cation determines the gelling properties of the polysaccharide. Natural extracts have various gelling properties. Kappa carrageenan does not gel in the  $Na^+$  form, but the addition of  $K^+$ ,  $Ca^{++}$  or  $NH_4$  in the case of kappa and  $Ca^{++}$  in the case of iota promotes the formation of stable transparent gels.

The viscosity of aqueous solutions of carrageenans depends on their type, temperature and pH of the solution, the presence or absence of ions, the concentration and molecular weight of the polymer. Similar to other polysaccharides that have a charge along the entire chain of the macromolecule, the viscosity of solutions increases with increasing concentration and molecular weight of carrageenan and decreases with increasing temperature and ionic strength of the solution. Most commercial samples of carrageenan form solutions with a viscosity of 25 to 500 mPa and a core area of 25 to 100 mPa. At the same time, native lambda-carrageenan can produce solutions with a viscosity of up to 20,000 mPa [5].

Kappa, iota and lambda carrageenans are rationally used as food consistency regulators due to their viscoelastic and gelling properties.

**Fig. 9.1** shows the chemical structures of gelling carrageenans, which are the most commercially used. Iota-carrageenan (iota) is a nearly homopolymer of G4S-DA2S disaccharide units, containing a very low amount (about 5 mol %) of G4S-DA disaccharide units. Kappa-carrageenan (Kappa) is slightly more heterogeneous, up to 10 mol % of G4S-DA2S disaccharide units break blocks of G4S-DA disaccharide units [6]. A third gelling carrageenan, commercially known as kappa-2 or weak kappa (Kappa 2), has gradually received an industrial boost. Kappa 2 replaces Kappa and Iota blends in niche applications where intermediate gelling properties are required between the hard and brittle gels formed by Kappa and the softer but deformable gels formed by Iota [1]. Kappa 2 is a random block copolymer made of G4S-DA sequences (representing 45–80 mol % of the hybrid chain) [7, 8] and G4S-DA2S [9, 10]. Depending on the seaweed and the extraction method used for isolation polysaccharide, the G4S-DA and G4S-DA2S blocks can be separated by more sulfated disaccharide units, such as G4S-D6S (mu-carrageenan) and G4S-D2S,6S (nu-carrageenan) [11].

In his comprehensive review of the gelation of carrageenans, Piculell gathered compelling evidence for network formation at the superhelical level for kappa gels, giving hard gels in contrast to soft Iota gels, where the network can occur in a helix. Fewer studies have attempted to systematically compare the network

structure of carrageenan with the corresponding elastic properties of the gel in order to identify structure-elasticity relationships. The purpose of the article [5] is to review the recent progress made in defining such relationships. The aim is to show the lack of rationalization of structural and elastic data by theories developed to describe the special elasticity of filamentous networks. The latter have been used with some success to elucidate the relationship between structure and rheological properties in various biopolymers and polymer gels that have structural and elastic similarities to carrageenan gels. L. Hilliou showed that reports on nonlinear rheological properties of carrageenan gels are still critically lacking in the literature, as emphasized by Van de Velde [8]. The significance of nonlinear elastic properties for the identification of structural features in these networks is emphasized. Preliminary nonlinear rheological data for Kappa, Iota, and Kappa 2 gels are presented. These results demonstrate concentration scaling in the strain-hardening behavior of Iota and Kappa 2 gels. The strain-hardening is analyzed using theories to extract elastically relevant structural features for comparison with structural information.

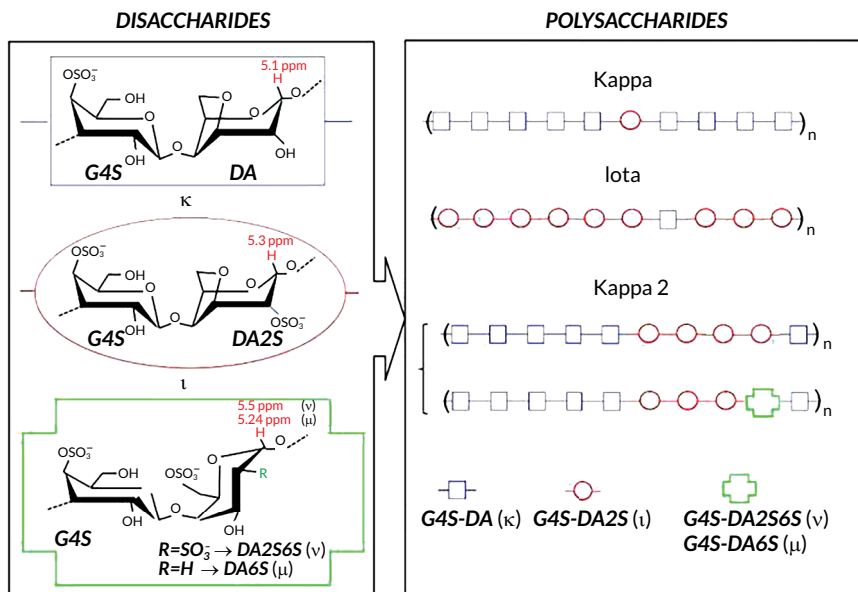


Fig. 9.1 Chemical structures of industrially suitable gel-forming carrageenans

Although many studies used commercial samples as received, most of the results presented here were obtained after performing a purification step to obtain a polyelectrolyte with one type of counterion. This is of primary importance for Kappa, which is known to exhibit specific gel properties depending on the type of salt used to form the gels [12]. In contrast, it was found that Iota does not show such sensitivity to cations unless a significant amount of G4S-DA units remain present as impurities in Iota [13].

Overall, the picture that emerges from the data reviewed by L. Hilliou is that the large differences in the elasticity of the Iota and Kappa gels are difficult to reconcile with their rather similar structures, which essentially consist of semi-flexible filaments with a length  $L$  of the order of 100 nm and a thickness  $d$  of the order of 10 nm, located in a dense network with cell size  $\varepsilon$  of the order of nanometers or tens of nanometers. Structural heterogeneity, where different filament types coexist with different filament aggregates, has long been reported in carrageenan gels. Structural heterogeneity on length scales larger than 100 nm and with correlations in the micron was recently revealed by confocal scanning laser microscopy in Kappa gels, showing significant turbidity in contrast to the uniform and clear Iota gels.

The reviewed here literature on the relationship between gel structure and elastic properties provides ample evidence for the filamentous nature of responsible for the elasticity of carrageenan gels networks. This structure explains the power-law dependence of the linear modulus of elasticity of the gel on the concentration of carrageenan and the strain hardening behavior of Iota gels. Although it is not well documented in the literature, strain failure has been established in a study [14] and rationalized by theoretical models. The latter explains the quadratic concentration scaling of strain hardening, which arises from the rod-like shape of the filaments (with fractal dimensions of 1.7 rank), which gives the network more enthalpic elasticity than entropy. The picture for kappa-gels is much less clear, since difficulties in rheological testing of these materials may explain the scatter of power-law values reported in the reviewed literature to describe the concentration dependence of  $G_0$ . Due to its longer persistence, Kappa self-assembles into straighter and more connected strands compared to Iota. However, a review of the literature shows that both systems exhibit nearly identical chain lengths (about 100 nm) and radii (about 1 nm). It is also reported that the cell sizes in the network are almost the same. Thus, the higher elasticity of kappa gels is attributed to the greater stiffness of the kappa filaments (or filaments), as suggested by the authors who found that kappa forms straighter filaments [15]. Several recent theoretical developments in the elasticity of filamentous networks, briefly

reviewed here, suggest that kappa-gels should exhibit strain hardening in this case. However, strain rarefaction followed by abrupt gel failure is consistently reported in the limited sample of literature reviewed here. This study confirms this high deformation behavior. Based on the results of the literature review, it can be concluded that reoptic measurements will help determine structural changes in the behavior of large deformations. Nonlinear elasticity should be studied using additional methods that include strain time [16], without limiting the study to dynamic vibration tests. In particular, such time must be taken into account when studying the thixotropy of carrageenan gels, which is a matter of industrial interest. Thus, the specific extraction of carrageenans from selected seaweeds seems preferable to the conversion of commercial samples into a single cationic form, since such processes are known to degrade the polysaccharide [17]. It is expedient to study the physicochemical properties of carrageenan extracted from red seaweed, which can be obtained in industrial quantities from undeveloped natural sources.

### 9.1.2 Use of carrageenan in the food industry

Natural food additives, which are able to adjust the beneficial properties and chemical composition of food products, are of particular value for the creation of full-fledged food products for mass and medical and preventive purposes. In the developed countries of the world, there is a constant increase in the production of carrageenan. In the early 1990s, its annual production reached 15.5 thousand tons, and now it is 30 thousand tons [18]. The main companies that produce carrageenan are concentrated in Europe and the USA, its production is developing very actively in the Philippines, Chile and China. The annual sale of carrageenan in the world is more than 200 million USD. The increase in the production of this polysaccharide-hydrocolloid is due to the increase in demand for food products containing carrageenan.

Currently, the food industry uses 80 % of all manufactured carrageenan, and the average consumption of this product is 250 mg/day per person [4]. The use of carrageenan in food products is based on the general recognition of its "safety". In the USA and EU countries, carrageenan is considered safe and useful, approved for use as a food additive. In Japan, it is considered a "natural product" and is not subject to the rules governing the use of food additives.

Leading international health organizations such as the World Health Organization (WHO), the US Food and Drug Administration, the European Union, Health

Canada and other independent international organizations allow the use of carrageenan in food products because it is absolutely safe for human consumption. This is confirmed by comprehensive research by scientists conducted in July 2014 – Carrageenan in Infant Formula [19, 20].

After a comprehensive scientific review of carrageenans by scientists in July 2014, the World Health Organization (WHO) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that carrageenan is safe for use in formula milk, including infant formula children with special diseases. Moreover, carrageenan is an important component of such infant formulas, as it is a source of essential nutrients available to the child's body.

Currently, more than 150 different carrageenan-containing products are produced in the world. The main branches of the food industry that use carrageenan are dairy, confectionery and meat.

Different fractions of carrageenan are widely used in the food industry for gelation, thickening and stabilization of emulsions in systems, on milk and water. They are often used to balance and improve the properties of other gels, as they have the ability to form complexes with other hydrocolloids.

Carrageenan is superior to agar and alginate in cases where high viscosity and accompanying thickening, emulsification and suspension are required. Thus, at low concentrations (0.01–0.03 %), it suspends cocoa particles and prevents the separation of fats during the preparation of pasteurized chocolate milk, soy protein drinks with chocolate additives. Kappa-carrageenan within a narrow range of viscosity, from 7 to 10 mPa, exhibits special properties – it prevents the ability to thicken milk, even at high concentrations, so it can be used as an additive to milk and ice cream. It stabilizes fat in ice cream, condensed milk, baby formula, vegetable oil for salads.

Lambda-carrageenan does not gel by itself, but provides thickening and stabilization of cold milk and is used for the preparation of cold chocolate milk, for thickening and stabilization of quick-dissolving powdered milk puddings, fruit drinks, as well as drinks based on natural plant raw materials, syrups, cheese spreads. Dairy products containing carrageenan do not require homogenization during preparation, the presence of lambda-carrageenan in them gives them a mousse structure.

The quality and range of confectionery products are largely determined by the availability and quality of the used gelling agents – emulsifiers and stabilizers. Carrageenans have a relatively high melting point, and this property is used for the preparation at room temperature of specific fruit confections, very plastic milk puddings of the "zhuangdong – soft ice" type. The aqueous gelling characteristics

of carrageenan are particularly useful in diet foods such as jellies and low-sugar syrups [5].

In the production of creamy desserts, carrageenan significantly affects the rheological properties of the final product. Each manufacturer can choose the appropriate concentration of carrageenan for their technological system in order to produce a product that appeals to the consumer, with maximum shelf life and resistance to the processes of retrogradation and syneresis.

Modified carrageenans have an obvious protective property against fat oxidation and therefore can be used as ideal antioxidants. In the form of a coagulate, carrageenan can be used as a fixative in canned meat, fruit gels, and jellies. Moreover, the polysaccharide added to the mixture with water, to the defatted minced meat, gives the meat its original taste. At the end of 1990, the McDonald's company began trial marketing of low-fat hamburgers called LeenDelux, the fat content of which was reduced by 91 %. Carrageenan gives cutlets juiciness and keeps their shape. At the same time, the taste of the cutlets does not deteriorate.

Specific examples of the use of carrageenan in food products testify to the excessive variety of areas of use of this polysaccharide.

The amount of carrageenan in food products usually does not exceed 2 % and in this concentration it cannot affect digestion. If this amount is exceeded, the process of assimilation of food slows down and there is a feeling of satiety, which in no way affects the further absorption of food in the intestines, so it is possible to hope that carrageenan will be used to prepare products that "increase the feeling of satiety".

Equally important is the fact that carrageenan has a wide range of different biological activities. Pharmacological studies have shown that it has immunostimulating and immunosuppressive effects; it has anticoagulant activity (primarily antithrombotic effect); is an enterosorbent and can be used to remove heavy metals from the body; inhibits lung metastases; used to treat atopic ulcers. Thus, carrageenan is a unique basis for the creation of new therapeutic and preventive products and drugs. Carrageenan is certified for use in EU countries. It is in such products that the food industry "feels" an acute shortage.

## **9.2 Research materials and methods**

### **9.2.1 Block diagram of research**

Theoretical and experimental studies were carried out according to the block diagram shown in **Fig. 9.2**.



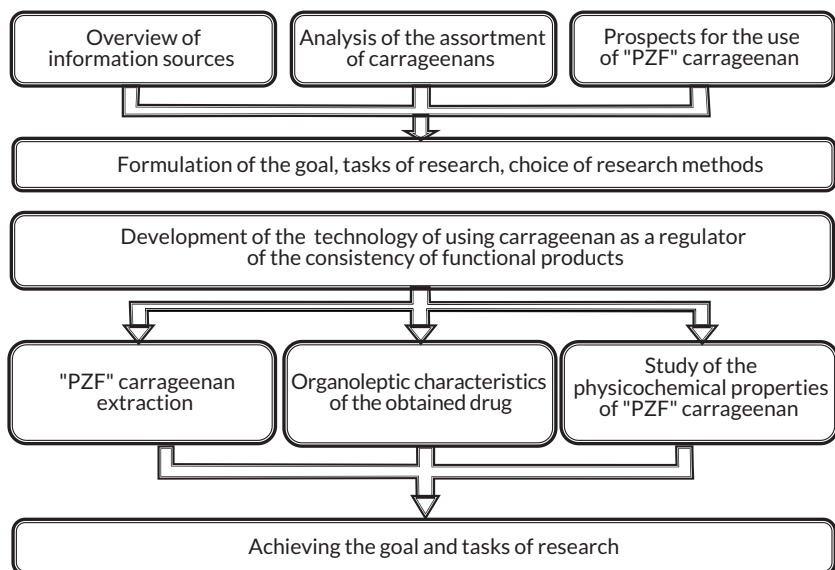


Fig. 9.2 Block diagram of conducting theoretical and experimental research

## 9.2.2 Object of study

Characteristics of "PZF" carrageenan: moisture – 11 %, mass fraction of ash – 25.5 %. The content of 3,6-anhydrogalactose is 21.3 %, the mass fraction of sulfoester groups (in terms of  $\text{SO}_4$ ) is 24.2 %.

## 9.2.3 Materials, reagents and equipment

### 9.2.3.1 Products and reagents

*Product: Acetic acid.* Acetic acid is an organic compound, a monobasic carboxylic acid of the composition  $\text{CH}_3\text{COOH}$ . It is produced in accordance with the national standard DSTU 2450:2006 "Vinegars from food raw materials. General technical conditions". Food supplement E260.

*Product: Baking soda.* Chemical name: sodium bicarbonate. Chemical formula:  $\text{NaHCO}_3$ . Baking soda is a crystalline powder-like substance of fine grinding, white

in color, odorless. A distinctive feature of baking soda is its mild alkaline properties, which do not have a negative effect on plant and animal tissues. GOST 2156. Food supplement E500.

*Product: Technical resorcinol.* Chemical name: resorcinol. Chemical formula:  $C_6H_6O_2$ . White, slightly yellowish scales with a specific smell. Water-soluble, soluble in alcohol, diethyl ether. Hardly soluble in benzene, carbon disulfide, chloroform. The chemical properties are the same as those of phenols. Resorcinol requires careful handling, as its vapors and dust are irritating to the respiratory tract and eyes. CAS: 108-46-3.

*Product: Rectified ethyl alcohol.* Chemical name: ethanol. Chemical formula:  $C_2H_5OH$ . Colorless liquid product, clear characteristic odor and fiery taste obtained by distillation of the must from the alcoholic fermentation of the molasses, subsequently rectified. The alcoholic strength achieved during distillation must not be less than 96 % Vol. DSTU 4221:2003.

*Product: Granulated sugar.* Chemical name: Sucrose. Chemical formula:  $C_{12}H_{22}O_{11}$ . It is sucrose in the form of separate white or white with yellowish crystals with sizes ranging from 0.2 to 2.5 mm. The mass fraction of sucrose is 99.55–99.75 % (in terms of dry matter). Mass fraction of moisture – 0.14–0.15 %. In addition to direct use by the population as food, granulated sugar is widely used in the food industry for the production of canned milk, baby food, in the biopharmaceutical industry, etc. DSTU 2316.

*Product: Potassium hydroxide.* Chemical name: Potassium hydroxide. Chemical formula: KOH. Colorless, very hygroscopic crystals, but less hygroscopic than sodium hydroxide. Aqueous solutions of KOH are highly alkaline. It has many industrial and niche applications, most of which utilize its caustic nature and its reactivity toward acids. TU 20.13.25-025-5227004-2015.

*Product: Potassium chloride.* Chemical name: Potassium chloride. Chemical formula: KCl. Potassium chloride is a metal halide salt composed of potassium and chlorine. It is odorless and has a white or colorless vitreous crystal appearance. The solid dissolves readily in water, and its solutions have a salt-like taste. TU 2184-042-00209527-97.

*Product: Hydrochloric acid.* Chemical name: Hydrochloric acid. Chemical formula: HCl. Hydrochloric acid is the aqueous solution of hydrogen chloride gas and the main component of gastric acid, an acid produced naturally in the human stomach to help digest food. Hydrochloric acid is produced synthetically for a variety of industrial and commercial applications. DSTU 3118.

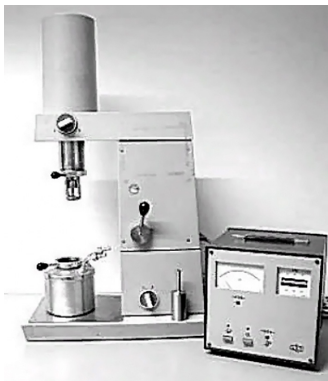
*Product: Isopropyl alcohol.* Chemical name: Isopropyl alcohol. Chemical formula:  $C_3H_8O$ . Colorless transparent liquid with a bitter taste and a sharp smell of alcohol

and acetone. Isopropanol is used as a disinfectant to kill bacteria, viruses and fungi, as well as to clean surfaces and treat skin before injections. Contained in household cleaners and hand sanitizers. DSTU 9805:84.

*Product: Phenolphthalein.* Chemical name: Phenolphthalein. Chemical formula:  $C_{20}H_{14}O_4$ . Triphenylmethane dye, an acid-base indicator that changes color from colorless to red-violet, "crimson". At  $pH > 12$  the indicator becomes discolored again. TU 6-09-5360-88.

*Equipment and chemical utensils:*

- Rheotest-2 rotary viscometer (Germany) (Fig. 9.3);
- pH meter pH-121 (Fig. 9.4);
- KFK-2MP photoelectrocolorimeter (Fig. 9.5);
- technical scales;
- laboratory ionometer;
- electric plates;
- chopper of vegetable raw materials;
- containers;
- precision refractometer;
- water bath;
- stopwatch;
- viscometers;
- measuring flasks, conical flasks, chemical glasses, graduated pipettes;
- burettes, watering cans;
- tripods with rings;
- filter paper.



**Fig. 9.3** Rheotest-2 rotary viscometer (Germany)



Fig. 9.4 pH meter pH-121



Fig. 9.5 KFK-2MP photoelectrocolorimeter

## 9.2.4 Research methods

### 9.2.4.1 Determination of the content of 3,6-anhydrogalactose in "PZF" carrageenan according to the modified Yafe method

2 ml of the test solution (sample) was added to one test tube with a polished reflux condenser, and 2 ml of the standard solution (control) to the second. Then 2 ml of a 0.1 % solution of resorcinol in 96 % ethyl alcohol and 6 ml of a 30 % solution of hydrochloric acid were added to both test tubes. The contents of the test tubes were mixed and heated in a water bath for 8 min at a temperature of 80. After heating, the solutions were cooled and colorimetric at a wavelength of 490 nm [21].

The extinction value of the solution containing the condensation product of oxymethylfurfural formed from 3,6-anhydrogalactose with resorcinol was determined.

The mass concentration of 3,6-anhydrogalactose in the test sample ( $\mu\text{g/ml}$ ) was calculated according to the formula:

$$C = \frac{Q \cdot E_1}{E_0},$$

where  $E_1$  and  $E_0$  – extinction of the tested and standard solutions, respectively;  $Q$  – coefficient that is the ratio of the mass concentration in the standard sample to the volume of the sample.

#### 9.2.4.2 Determination of the content of sulfoester groups in "PZF" carrageenan

Determination of the content of sulfoester groups in "PZF" carrageenan was carried out gravimetrically in the form of  $\text{BaSO}_4$ .

#### 9.2.4.3 Assessment of organoleptic characteristics

The main requirements for carrageenans are listed in **Table 9.1**.

**Table 9.1** Requirements for the release form of carrageenan

Name of the indicator	Characteristic and norm
Appearance	Finely dispersed powder
Color	From light cream to beige
The smell of carrageenan and gel with a mass fraction of dry carrageenan 0.85 %	Neutral
The taste of the gel with a mass fraction of dry carrageenan 0.85 %	No extraneous aftertaste
The presence of extraneous impurities	Is not allowed

#### 9.2.4.4 Determination of carrageenan solubility

Refractive indices were measured on an IRF-23 refractometer at a temperature of 25 °C. 1 g of the drug was added to 100 g of water and the resulting mixture was

heated in a water bath at a rate of 0.5 °C/min. Every 10 minutes, the value of the refractive index of the mixture was determined. The dissolution was considered complete if the value of the refractive index of the composition acquired a constant value.

#### 9.2.4.5 Study of rheological characteristics

The rotary viscometer Rheotest-2 (Germany) was used to determine the viscosity of Newtonian liquids and conduct rheological studies of non-Newtonian systems [21]. Measuring node – coaxial cylinders.

The range of changing shear rate gradient is from 3 to 1312 s<sup>-1</sup>.

The temperature of the measuring unit was kept with an accuracy of 0.1 °C.

The dependence of viscosity on the shear rate was calculated using the computer program "Techiya".

#### 9.2.4.6 Determination of thixotropic reduction degree of the solutions viscosity

The value of the degree of thixotropic reduction of viscosity of "PZF" carrageenan solutions was calculated by the formula:

$$S = \frac{1}{n} \sum_i^n \frac{\eta_{1i}}{\eta_{0i}} \cdot 100 \%,$$

where  $S$  – the degree of thixotropic restoration of viscosity, %;  $n$  – the number of fixed values of the shear rate gradient;  $\eta_{0i}$  – the viscosity at the  $i$ -th shear rate, which is measured in the mode increasing shear rate gradient, Pa·s;  $\eta_{1i}$  – the viscosity at the  $i$ -th shear rate, which is measured in the mode descending gradient and shear rate, Pa·s.

#### 9.2.4.7 Determination of the pH indicator

One of the main physico-chemical indicators determining the course of the technological process is pH. Hydrogen indicator pH is a value that shows the degree of activity of hydrogen ions (H<sup>+</sup>) in a solution, that is, the degree of acidity or alkalinity of this solution. This characteristic determines the structure formation in the finished product, as well as its storage terms.

The pH of the solutions was determined according to the standard procedure.

To determine the pH, 100 g of a 1 % suspension of the carrageenan preparation in distilled water was prepared. The suspension is dispersed for 15 min at room temperature and the pH is determined on a pH meter pre-calibrated with standard buffers.

### **9.3 Research results and discussion**

#### **9.3.1 Determination of the "PZF" carrageenan characteristics, which determine the possibility of its use as a regulator of food products consistency**

##### **9.3.1.1 Determination of the 3.6-anhydrogalactose content in "PZF" carrageenan**

Carrageenans (sulfated red seaweed galactans) are a unique class of polysaccharides. The basis of the molecules of most galactans is a carbohydrate chain built from alternating residues of (1→3)-β-d-galactopyranose and (1→4)-α-galactopyranose. These 4-linked residues can be partially or completely in the form of a 3.6-anhydro derivative and belong to the D-series.

It is known that a necessary condition for the manifestation of gelling properties is a high content of 3.6-anhydrogalactose residues and a high level of regularity of the structure of polysaccharides.

Compared to other natural polysaccharides, the use of chemical methods in galactans from red algae has a number of features associated with the presence of 3.6-anhydrogalactose and sulfate groups.

An important component of galactans, 3.6-anhydrogalactose is the only 3.6-anhydrohexose found in nature, and it is not found anywhere except in red algae. This monosaccharide is highly prone to degradation in an acidic environment, so that under the conditions of acid hydrolysis of glycosidic bonds, which is used to determine the monosaccharide composition of polysaccharides, 3.6 anhydrogalactose is completely destroyed. Such easy destruction allows selective determination of 3.6-anhydrogalactose in the presence of other sugars by color reaction with resorcinol.

In this research, the determination of the content of 3.6-anhydrogalactose in the studied "PZF" carrageenan was carried out according to the colorimetric resorcinol method of Jaffe (color reaction with resorcinol) with a minor modification proposed by the researchers [21]. The resorcinol reagent was prepared 3 hours before the colorimetric study. Briefly, resorcinol reagent was prepared from 9 ml of resorcinol

solution (1.5 mg/ml), 1 ml of acetaldehyde solution (0.04 vol/vol %) and 100 ml of concentrated hydrochloric acid. Next, a 0.03 ml aliquot of the sample solution (1 mg/ml) was added to a centrifuge tube followed by the addition of 0.2 ml of distilled water. After placing in an ice bath for 5 minutes, 1 ml of resorcinol reagent was added, homogeneously mixed in an ice bath, and then placed at room temperature for 2 minutes. The mixture was incubated for 10 minutes at 80 °C, followed by cooling for 5 minutes in an ice bath. The absorbance of 3,6-anhydrogalactose was measured at 555 nm, and the concentration of 3,6-anhydrogalactose was calculated using a calibration curve with galactose standards. All samples were analyzed in triplicate.

According to the research results, the content of 3,6-anhydrogalactose in "PZF" carrageenan is 21.3%. This is a high content of this component in the general structure of carrageenan. A high content of this monomer can affect the physical and chemical properties of carrageenan. The high content of 3,6-anhydrogalactose in "PZF" carrageenan characterizes it as a drug potentially prone to gel formation and gives grounds for its potential use in functional food products.

### 9.3.2 Study of rheological properties of "PZF" carrageenan

Much attention is paid to the rheological properties of food products, because they determine the quality of condensed or emulsion products and their storage stability [22–24].

When performing the work, a study of the rheological properties of "PZF" carrageenan gels of various concentrations was carried out.

Systems with a concentration of 0.07%; 0.08%; 0.1%; 0.15%; 0.2%; 0.3%; 0.4%; 0.5%; 0.6%; 0.7%; 0.8%; 0.9%; 1%; 1.5%; 2% were studied.

There are several approaches to the graphical interpretation of the concentration dependence of the viscosity of polymer solutions. The relationship between the viscosity of solutions of almost any polysaccharide and its concentration is exponential.

The dependence of the viscosity of "PZF" carrageenan solutions on the shear rate gradient in the interval 3–1312 s<sup>-1</sup> was established. As for most high-molecular systems, with an increase in the shear rate, there is an anomalous decrease in viscosity. The obtained dependences are, most likely, sections of the "structural branches" of the complete rheological curves, since there are no signs of a transition to the regimes of flow with the highest or lowest viscosity.

In the studied range of shear rates, the viscosity of solutions obeys the power law and is described by the Ostwald-Weyl equation:



$$\eta = \frac{P}{\dot{\gamma}} = k\dot{\gamma}^{n-1}.$$

The values of the constants  $k$  and  $n$  of the equation for 0.1–2 % of "PZF" carrageenan solutions are given in the **Table 9.2**.

**Table 9.2** The value of the Ostwald-Weyl equation constants

Carrageenan concentration "PZF", %	$k$ , Pa·s	$n$
0.1	$3.7 \cdot 10^{-3}$	0.950
0.3	0.234	0.609
0.5	5.320	0.420
1.0	7.467	0.411
2.0	10.43	0.403

With an increase in the concentration of "PZF" carrageenan to 2 %, the flow index of the solution decreases sharply, which indicates an increase in the structure of the system. Solutions containing more than 2 % "PZF" carrageenan have a fairly developed spatial structure.

Marmalade, jelly candies, fruit jelly desserts have a jelly-like structure. Their technology involves boiling the recipe mixture, processing the marmalade mass, forming, drying. During these technological stages, the mass transitions from the sol state to the gel state. The structure of the gel is characteristic of the finished product. In production, there are situations when the mass prepared for forming, due to various reasons, cannot be formed. Then the transition of the sol structure to the gel structure occurs. There is a need for the reverse process – converting the gel into a sol. In this context, the application of thixotropic properties is important [25].

The process of thixotropic restoration of the viscosity of "PZF" carrageenan solutions, which is represented by the "direct" and "reverse" branches of the viscosity dependence on the shear rate, was studied.

According to the results of experimental data, the degree of thixotropic restoration of viscosity of 1 % "PZF" carrageenan solutions is:

$$S = 87.9 \%$$

When studying thixotropies of gels containing carrageenan, the authors [23] noted that thixotropic recovery is never complete. For cold shear of already formed gels, ripening time before shear was not a significant factor for aqueous gels, but caused

significant differences in the final texture of the model desserts. In our studies of the "PZF" carrageenan solution, the presence of hysteresis was revealed when measuring the effective viscosity in the modes of increasing and decreasing shear rate gradient. This indicates that reversible changes associated with the internal structure occur during the shear impact, but additional research is still required to establish the nature of the structural changes occurring.

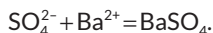
### 9.3.2.1 Determination of the content of sulfoester groups

In addition to 3,6-anhydrogalactose, sulfate groups are an equally important structural element of galactans that determines their properties and chemical behavior. These groups are cleaved in an acidic environment at rates comparable to the rate of hydrolysis of galactoside bonds, so that complete acid hydrolysis of the polysaccharide also results in complete desulfation. In an alkaline environment, including polysaccharide methylation conditions, sulfate groups are usually stable, with one exception: the sulfate at position 6 of the galactose residue bound at position 4 easily undergoes intramolecular substitution with a free hydroxyl at C-3 to form 3,6-anhydrogalactose.

This sequence of reactions (enzymatic sulfation of C-6 followed by enzymatic elimination of sulfuric acid, which leads to the formation of a 3,6-anhydrocycle) is the basis of the biosynthesis of 3,6-anhydrogalactose residues in the galactans of red algae, and in industry, alkaline treatment of algae is often used to increase the content of 3,6-anhydrogalactose and, therefore, to improve the gel-forming properties of polysaccharides released.

Determination of the content of sulfoester groups in "PZF" carrageenan was carried out by the gravimetric method in the form of  $\text{BaSO}_4$ .

The method is based on the precipitation of sulfate ions in the form of a crystalline white precipitate of  $\text{BaSO}_4$  and obtaining the gravimetric form ( $\text{BaSO}_4$ ) by calcinations:



During research, the attention was paid to the process of obtaining the precipitated form. As soon as the sediment settles and the liquid above it becomes clear, check the completeness of the  $\text{BaSO}_4$  precipitation. To do this, 2–3 drops of hot solution of  $\text{BaCl}_2$  are added to the solution above the sediment, being careful not to disturb the sediment. If no turbidity of the solution is observed, the completeness of the precipitation is achieved, otherwise add another 1 ml of hot solution of  $\text{BaCl}_2$ , allow the precipitate to settle and check the completeness of the precipitation again.

Then the beaker is placed, heated for 2–3 hours to ripen the sediment, after which the sediment is filtered and washed.

To check the completeness of sediment washing, a few drops of filtrate are taken on a watch glass and a qualitative reaction is carried out for chloride ions, from which the sediment is washed. Rinsing ends with a negative test result.

After the end of ashing, the sediment in the crucible is roasted in a muffle furnace at a temperature of 800 °C until a constant mass of the crucible with a gravimetric form is obtained.

Based on the results of the analysis, calculate the mass of sulfate ( $\text{SO}_4$ ) in the sample. The proportion is:

1 mol of  $\text{BaSO}_4$  (233.4 g) contains 1 mol of  $\text{SO}_4$  (96.06 g);  
in  $m$  g –  $x$  g.

According to the experiment, the weight of the ashed sediment is 0.588 g.

Thus, the mass fraction of sulfoester groups in "PZF" carrageenan (in terms of  $\text{SO}_4$ ) is 24.2 %.

In accordance with international legislation, carrageenans, as commercial preparations for the food industry, must have a sulfoester group content of at least 20 %. That is, the studied "PZF" carrageenan according to the characteristic – the content of sulfoester groups meets the requirements for commercial carrageenans for the food industry.

When discussing the obtained results, it should be noted that  $\kappa$ -carrageenan consists of a repeating unit consisting of a disaccharide,  $\beta$ -(1→3)-d-galactose-4-sulfate and  $\alpha$ -(1→4)-3,6-anhydro-d-galactose.  $\iota$ -carrageenan has two sulfate groups in the disaccharide repeating unit;  $\beta$ -(1→3)-d-galactose-4-sulfate and  $\alpha$ -(1→4)-3,6-anhydro-d-galactose-2-sulfate.  $\lambda$ -carrageenan consists of  $\beta$ -(1-3)-d-galactose-2-sulfate and  $\alpha$ -(1→4)-d-galactose-2,6-disulfate, including three sulfate groups. It should be noted that the presented structure is ideal, and real samples contain a number of different types of sequences. Under appropriate conditions,  $\kappa$ -carrageenan and  $\iota$ -carrageenan in aqueous solutions undergo a thermoreversible sol-gel transition, while gelation does not occur in  $\lambda$ -carrageenan with a larger number of electrolyte groups. It is widely recognized that the gelation of carrageenan is based on the formation of a double helical structure. Carrageenan adopts a random helix conformation in the sol state, and low temperature induces the anhydro-galactose sequences to twist in a double helical fashion. Further aggregation also favors the formed double helical moieties. Part of the hydrate sequences functions as a helix break. Eventually, the aggregation of the double helices forms a domain of crosslinks and leads to

an infinite network structure sufficient to complete gelation. Since the repeating units of carrageenan have an electric charge in the sulfate groups, counterions have been found to play a role in gelation.

Thus, the experimentally determined mass fraction of sulfoester groups in "PZF" carrageenan (in terms of  $\text{SO}_4$ ), which is 24.2 %, gives reason to consider "PZF" carrageenan potentially suitable for gelation. Therefore, it is advisable to carry out further research in the direction of studying the rheological properties of the viscous systems that it forms, in conditions that are realistically close to the technologies for making jelly desserts.

### 9.3.3 Study of the temperature influence on the rheological properties of "PZF" carrageenan solutions

In practice, solutions of polysaccharides, which are used to adjust the consistency of food products, are subjected to temperature effects. This determines the theoretical and practical interest of data characterizing the effect of temperature on the rheological properties of "PZF" carrageenan solutions.

Fig. 9.6 shows the dependence of the viscosity of the "PZF" carrageenan solution on temperature.

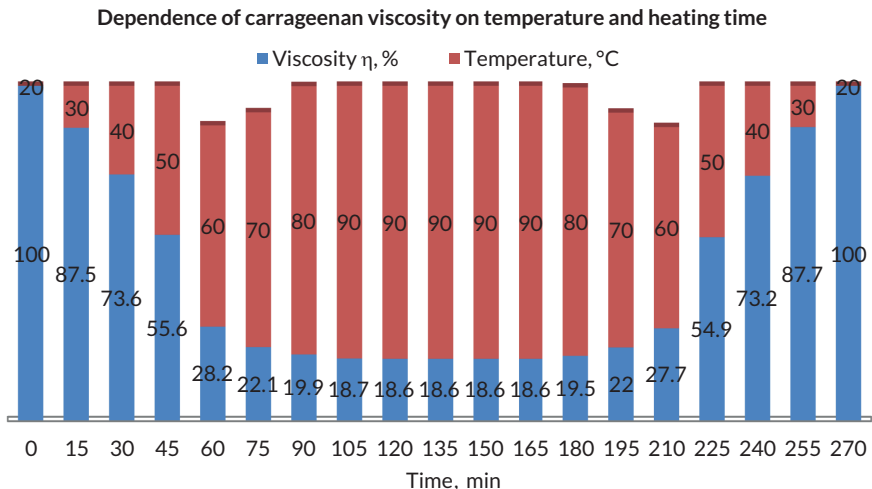


Fig. 9.6 Diagram of the dependence of carrageenan solution viscosity on temperature and heating time

As the temperature rises from 25 °C to 40–45 °C, a relatively small decrease in viscosity is observed. A further increase in temperature above 45 °C leads to a sharp drop in viscosity, at 70–90 °C the decrease in viscosity becomes pronounced. The resulting dependence is practically symmetrical with respect to the inflection point. A somewhat unusual temperature dependence of the viscosity of the "PZF" carrageenan solution was revealed. It can be assumed that the most probable cause is a change in the conformation of the "PZF" carrageenan macromolecules under the influence of temperature. This assumption is due to the fact that in concentrated solutions at normal temperatures, sulfated galactans (including carrageenans) are in a spiral conformation. On the other hand, an increase in the intensity of thermal motion in such solutions inevitably leads to the transition of a high-molecular compound from one conformation to another, and, in the absence of chemical transformations, from a more ordered to a less ordered one.

As a rule, low temperatures and even freezing are used to preserve food products. The thermal stability of food products under conditions of freezing and thawing is a very important factor that determines the quality of the product during storage, so the next stage was to investigate the effect of low temperatures on the rheological and thixotropic properties of carrageenan gels.

Before the experiment, gels from "PZF" carrageenan were pre-frozen at a temperature of -18 °C and after thawing were subjected to research.

It was established that after thawing, the gels formed by "PZF" carrageenan lose their strength the more the concentration of carrageenan is higher. Thus, gels with concentrations of 3 g/l lost 29 %, 5 g/l - 40 % and 8 g/l - 74.3 %.

Questions regarding the possible use of "PZF" carrageenan in food products subject to freezing require further research.

#### **9.3.4 Study of the pH effect on the rheological properties of "PZF" carrageenan solutions**

The formation of product quality is carried out at all stages of the technological process of its production. Many technological indicators that ensure the creation of a high-quality product depend on the active acidity (pH) of the food system.

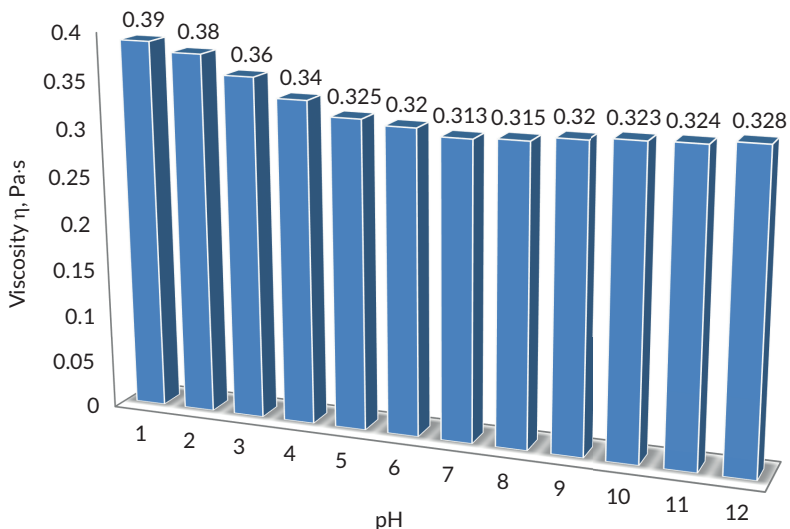
Almost every food product has optimal conditions for the cooking process. It is extremely important that the "PZF" carrageenan solution has the necessary rheological properties under these conditions. One of the criteria for the suitability of a solution of a high-molecular compound as a gelling agent is the pH range within which this drug retains its technological and consumer properties.

To study the effect of acidifying agents, 30 % acetic acid was used, which was added to gels with different concentrations to pH 1–2. The pH was monitored using a pH meter.

The most acceptable alkaline agent for the food industry is calcium bicarbonate (baking soda) – the cheapest reagent that creates milder hydrolysis conditions compared to alkalis.

To study the effect of alkaline agents, a 15 % solution of baking soda was used, which was added to gels of various concentrations to a pH of 10–11. The pH was monitored using a pH meter.

**Fig. 9.7** shows the dependence of the viscosity of "PZF" carrageenan solutions on pH at a shear rate of  $1312 \text{ s}^{-1}$  and a temperature of  $25 \text{ }^{\circ}\text{C}$ .



**Fig. 9.7** Dependence of viscosity of "PZF" carrageenan solutions on pH

As the pH increases from 1 to 10, the viscosity decreases slightly. In a more alkaline environment, the viscosity of the solution drops steeper, probably due to partial ionization of hydroxyl groups of mannose and especially galactose units. In weakly acidic and weakly alkaline environments (pH=5–8.5), the viscosity varies within relatively small limits (no more than  $0.009 \text{ Pa}\cdot\text{s}$ ). With an increase in alkalinity up to pH=9.5–10, the viscosity of the "PZF" carrageenan solution remains almost at the previous level.

As the acidity of the environment increases, the viscosity of "PZF" carrageenan-based solutions increases significantly. An increase in the concentration of hydrogen ions complicates the dissociation of groups that give macromolecules a negative charge (sulfoether groups). A decrease in the dissociation degree reduces the mutual repulsion of polyanions, contributing to the formation of intermolecular bonds and increasing viscosity. "PZF" carrageenan retains abnormally viscous properties in a strongly acidic environment, up to pH=1.

A study of the effect of pH on the stability of viscous solutions of "PZF" carrageenan during storage was of some practical interest.

Experimental data characterizing the dependence of the viscosity of "PZF" carrageenan solutions on the storage time at 25 °C are shown in **Table 9.3**.

**Table 9.3** Viscosity of carrageenan solutions during storage

Storage time, hours	Viscosity, % of the initial value				
	pH=2	pH=4	pH=6	pH=7	pH=10
0	100	100	100	100	100
12	102.1	101.6	100.9	100.9	100.2
24	102.8	103.3	102.1	101.5	101.8
36	103.9	105.2	104.8	104.9	105.6
42	103.6	105.8	105.1	105.3	106.5
48	102.7	105.9	105.5	105.4	106.5
54	102.1	106.2	105.3	104.9	106.2
60	100.8	106.7	105	104.8	104.6
66	99.5	106.7	104.7	104.4	104.4
72	98.3	106.9	104.2	103.9	104.4
78	97.6	106.9	103	102.7	104.1
84	95.4	106.8	102.3	100.3	103.3
90	94.5	105.5	100.1	97.5	102.1
90	92.5	104.7	97.8	96.3	99.6
102	89.9	103.6	97.1	93.5	97.5
108	87.5	103.1	94.7	92.2	96
114	85	101.9	92.7	89.1	94.2
120	82.9	99.7	91.5	86.6	92.5
132	77.4	97.3	83.9	83.1	88.4
144	74.1	95.1	82.3	77.5	86
156	69.7	93.1	78.2	73.3	83
168	65.8	92.3	74.7	67.6	80.2

The viscosity of "PZF" carrageenan solutions 1 hour after preparation is taken as 100 %. In the process of storage, the viscosity of the solutions first increased, and then decreased, regardless of the pH value. The increase in viscosity at the initial stage can possibly be explained by some increase in the concentration of "PZF" carrageenan due to the partial evaporation of water from the viscous solution. The subsequent decrease in viscosity is most likely caused by the hydrolysis of carrageenan.

The fastest, after 1.5–2 days, the viscosity of "PZF" carrageenan solutions begins to decrease at pH=2. Clots with a neutral and alkaline reaction environment fully retain their rheological properties during 3–3.5 days of storage. A week after preparation, the "PZF" carrageenan solution loses up to 30 % of its initial viscosity. A solution with pH=4 has high stability during storage. An acidic environment prevents the development of microorganisms, however, in this case, it is not strong enough to cause noticeable hydrolysis of the polysaccharide.

## Conclusion

1. The technological aspects of the use of carrageenan from the Black Sea red algae "*Phyllophora Brody*" are substantiated. It is shown that the use of such a drug ("PZF" carrageenan) is expedient for expanding the range of consistency regulators of food industry products.

2. The concentration dependence of the viscosity of "PZF" carrageenan solutions is studied. With an increase in the concentration of "PZF" carrageenan to 2 %, the flow index of the solution decreases sharply, which indicates an increase in the structure of the system.

3. The dependence of the viscosity of "PZF" carrageenan solutions on the shear rate gradient in the interval 3–1312 s<sup>-1</sup> is established. In the studied range of shear rates, the viscosity of solutions obeys the power law and is described by the Ostwald-Weyl equation. Reversible destruction of the structure occurs under the action of shear. The degree of thixotropic reduction of "PZF" carrageenan solution is 87.9 %.

4. The effect of temperature on the rheological properties of "PZF" carrageenan solutions is studied. It is assumed that at temperatures up to 45 °C, carrageenan macromolecules exist in a spiral conformation, and at higher temperatures undergo a thermoreversible transition into a coil conformation. This transition causes a decrease in viscosity and gelation of the solution.

5. It is established that "PZF" carrageenan solutions retain their abnormally viscous properties in a wide pH range. When the pH of the solution changes



from 1 to 11, no signs of a conformational transition of "PZF" carrageenan macromolecules are detected.

6. The obtained data on the chemical and physical-mechanical properties of "PZF" carrageenan solutions indicate the expediency of continuing research with the aim of forming viscous solutions for structured food products with the expansion of their functional purpose.

### Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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