A STUDY OF THE COMPLEXING AND GELLING ABILITIES OF PECTIC SUBSTANCES

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Abstract: Cranberries, lingonberries, blueberries, and food systems based on these berries with different solvents and sugars have been studied. A physicochemical analysis of cranberries, lingonberries, and blueberries has been conducted. Crystalline pectin from cranberries, lingonberries, and blueberries has been isolated to determine the degree of etherification. The gelling ability of the pectic substances has been studied. The effect of different solvents and sugars on the rheological properties of food systems containing pectin has been examined. A comparative estimation of the gelling ability of the pectic substances not properties, and blueberries, and blueberries and in chitosan and alginate gelling agents has been conducted. The viscous properties have been found to increase in the series: cranberry pectin < lingonberry pectin < sodium alginate < chitosan. The complexing ability of pectins with respect to copper and iron ions has been studied and compared to that of casein. Casein exhibits a lower complexing ability with respect to iron ions than pectin. It has been found that the complexing properties of pectin vary with its concentration: the more dilute the solution, the higher the complexing ability of pectin.

Key words: pectic substances, gelling ability, blueberries, cranberries, lingonberries, rheological properties, complexing ability

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INTRODUCTION

The food industry is being rapidly developed. At the same time, the competition between manufacturers is escalating. The manufacturers strive to anticipate the customer needs by paying ever-increasing attention to the quality of products and introducing advanced technologies. The perception and attitude of consumers to food products have a huge impact on the sales of the products. These requirements have led to the formulation of a new generation of foodstuffs that favorably affect the human body [1, 2].

The most important curative measure aimed at reducing the adverse effects of physical, chemical, and biological factors of the environment on human health is the use of pectin preparations approved by the Ministry of Health of the Russian Federation in the healthful and dietary meals of all groups of the population.

Pectins are widely used in all sectors of the food industry. Pectin is a purified hydrocarbon obtained by extraction from plant raw materials. Pectins are used as gelling, stabilizing, thickening, water-retaining, and clarifying agents, as substances facilitating filtering, and as a means for encapsulation. For example, in the dairy industry, pectins are actively used in the manufacture of yogurts, cheese, ice cream, milk-fruit desserts, and fermented and acidified dairy products; pectins are used as an emulsifier for the manufacture of mayonnaise and liquid margarines in the fat-and-oil industry. Pectins are successfully used for the production of marmalade, jelly fillings, whipped confectionery products, such as marshmallows and pastille, and candy pastes.

In the European system of codification of food additives, pectin has the number E440.

According to the current nomenclature, pectic substances include protopectin, pectin, pectinic acid and pectinates, pectic acid and pectates [3].

• Protopectin is water-insoluble natural pectin with a complex structure, which has not yet been precisely specified. It is believed that its composition includes all the above complexes.

• Pectin, or soluble pectin—water-soluble polygalacturonic acids methoxylated to varying degrees—is formed from protopectin under the action of acids, alkalis, or protopectinase enzyme.

• Pectinic acid is macromolecular polygalacturonic acid with carboxyl groups partially etherified with methanol. Salts of pectinic acid are referred to as pectinates.

• Pectic acid results from complete demethoxylation of pectinic acid. The solubility of pectic acid is lower than that of pectinic acid. Salts of pectic acid are referred to as pectates.

The main functional feature of pectin as a gelling agent is the ability to form gels in aqueous solutions in the presence of a certain amount of sugar and acid or calcium ions. In addition, pectin can absorb and rid the body of biogenic toxins, anabolic steroids, xenobiotics, metabolites, and biologically harmful substances capable of accumulating in the body [4-6].

Thus, we should put emphasis on two main properties of pectin: complexing and gelling abilities, which are used depending on the sphere of application.

Any plant raw material with a high content of pectin can be used for the production of pectic substances. Four basic types of feedstock are processed: apple pomace, sugar beet pulp, sunflower heads, and citrus peels. These types of raw materials are not basic for the regions of Siberia and the Far East; therefore, the problem of the complete use of local fruit and berry resources is of particular importance. The present-day technologies for isolating pectin from plant raw materials involve the use of significant amounts of acids and ethanol. Data on the enzymatic hydrolysis of pectin-containing feedstocks are hardly available. The application of ultrafiltration and sorption on ion-exchangers in the technology for purification of extracts of beet pectin and some other types of pectins has been reported [3, 7].

Recently, studies on the isolation, purification, and examination of the properties of pectins derived from nonconventional plant raw materials have been developed. It is known that valuable sources of pectic substances are fruits and berries, including cranberries, lingonberries, and blueberries. These berries are abundant in the Siberian region because of their easy maintenance, high frost resistance, early ripening, special gustatory qualities, and a wide range of medicinal properties.

The chemical composition, properties, and total content of pectic substances, the ratio between protopectin and soluble pectin, and their acetyl component in the above feedstocks suggest that pectin of cranberries, lingonberries, and blueberries can be regarded as a promising structure-forming agent.

The gelling ability depends on the molecular weight of pectin, the degree of etherification of its molecule, the content of functional groups, the sugar concentration in the solution, the amount of ballast substances accompanying this pectin, the ambient temperature, and pH of the medium [3].

In selecting technological conditions for the manufacture of food products with a given structure, it is important to maximally preserve the gelling properties of pectic substances during the entire process, including packaging, transportation, and storage of the product. Therefore, it is necessary to study the impact of various factors on the gelling ability of pectic substances derived from various plant materials.

The aim of this study was to examine the gelling ability of pectic substances of cranberries, lingonberries, and blueberries and determine the complexing ability of pectins with respect to copper(II) and iron(III) ions.

In accordance with the stated objective, the following problems were solved:

- Physicochemical analysis of the original feed-stock;

 Isolation of pectic substances from cranberries, lingonberries, and blueberries growing in the Kemerovo and Tomsk regions;

- Examination of the complexing ability of pectic substances of cranberries, lingonberries, and blueberries;

- Analysis of the effect of various solvents and sugars on the rheological properties of food systems containing pectic substances;

- Comparison of the gelling ability of pectic substances contained in cranberries, lingonberries, and blueberries and various gelling agents;

- Examination of the complexing ability of pectic substances with respect to copper(II) and iron(III) ions and comparison of the complexing ability of pectic substances and casein.

MATERIALS AND METHODS

Samples of frozen lingonberries, blueberries, and cranberries growing in the Kemerovo and Tomsk regions and powdered pectin (citrus pectin SS 200, Denmark) were studied.

The physicochemical analysis of the berries was as follows: (1) determination of the content of dry matter and moisture (GOST (State Standard) 28561-90); (2) identification of soluble and insoluble solids (GOST (State Standard) 29031-90); (3) determination of the total acidity (GOST (State Standard) 255555.0-82); (4) identification of nitrates (GOST (State Standard) 29270-95); (5) identification of pectic substances in the form of water-soluble pectins and protopectin (GOST (State Standard) 29059-91); and (6) determination of the degree of etherification of pectins (GOST (State Standard) 29059-91).

Absorbance was measured using a KFK-3 photoelectric colorimeter at a layer thickness of 1 cm.

Rheological experimental data were recorded using a Reotest-2 rotary viscometer.

EXAMINATION OF THE COMPLEXING ABILITY OF PECTIC SUBSTANCES

Heavy metals entering the body can cause a number of metabolic disorders, mostly in redox processes. The formation of metal bicomplexes with various cell components can lead to membrane damage and inhibition of the activity of various enzymes.

Copper is one of the heavy metals that can contaminate food products. The prevention of possible consequences of the penetration of copper into the human body is based on the binding of copper in the complexation with pectin.

In recent years, considerable attention has been paid to the determination of the structure of pectic substances owing to their valuable technical properties and high physiological activity. They have a wide biological action spectrum: many pectins have immunomodulatory effects and can rid the body of heavy metals, biogenic toxins, anabolic steroids, xenobiotics, metabolites, and biologically harmful substances capable of accumulating in the body: cholesterol, lipids, bile acids, and urea [8, 9].

A promising field of application of pectins can be the use of their modified oligomers as a template for the manufacture of extended-release drugs. There are data on studies of the antitumor activity of pectic substances that are products of fermentolysis of deetherified pumpkin pectin; their protective effect is significantly higher than the activity of homogalacturonic oligosaccharides derived from citrus pectin [3]. A variety of new physicochemical, complexing, and physiological properties can be imparted to pectins through chemical modification: etherification, amidation, and acylation [10, 11].

The complexing ability of pectin is based on its ability to form insoluble complex compounds with heavy metals and radionuclides (Fig. 1). It is this property that defines pectin as a prophylactic agent in contaminated and polluted areas, as recommended by the World Health Organization (WHO). The prophylactic daily dose of pectin is 4-5 g; under conditions of radioactive contamination, the daily dose is 15-16 g.



Fig. 1. Formation of a complex compound of pectin with copper ions.

The complexing properties of pectic substances depend on the content of free carboxyl groups, i.e., the degree of etherification of the carboxyl groups with methanol. The degree of etherification determines the linear charge density of the macromolecule, and consequently, the strength and mode of binding of the cations [12, 13].

At a high degree of etherification of pectin (above 90%), the free carboxyl groups that include C6 atoms are significantly spaced apart. In addition, the salts of pectic acid are almost completely dissociated. With a decrease in the degree of etherification, i.e., with an increase in the charge of the macromolecule, the binding between the pectic substances and the cations enhances and the stability constant of the pectates increases. At a degree of etherification of 40%, the conformation undergoes a change, which leads to the aggregation of pectin molecules and the formation of a strong intramolecular chelate bond [3].

The determination of the complexing ability of the analyte with respect to copper is based on the spectrophotometric detection of copper in the form of a copper ammine complex, which has an intense blue color with maximum absorption at 620 nm and is formed after the addition of excess ammonia to a solution comprising copper sulfate according to the reaction

 $CuSO_4 + 4NH_4OH \rightarrow [Cu(NH_3)_4]SO_4 + 4H_2O.$

To specify the maximum absorption, a spectrophotometric absorption curve was plotted.

The concentration of copper in an aqueous pectin so

lution containing Cu^{2+} ions was determined form the absorbance of the solution.

To this end, the absorbance of a set of Cu^{2+} solutions with varying concentration was found. According to the derived experimental data, a calibration graph of absorbance versus Cu^{2+} ion concentration was plotted to find the concentration of copper ions in the pectincontaining solutions. The complexing ability value was found as the difference in the copper concentration in the aqueous solution and the pectin-containing solution. The results of the study are shown in Table 1.

Table 1. Results of examination of the complexing ability of pectin with respect to Cu^{2+} ions

| m (pectin), mg | m _{add} (Cu ²⁺), mg | m _{free} (Cu ²⁺), mg | m _{bound} (Cu ²⁺), mg | Complexing ability mgCu ²⁺ /g of pectin |
|----------------------|--|---|--|---|
| 5 | 400 | 1.2 | 398.8 | 79760 |
| 10 | 400 | 1.2 | 398.8 | 39880 |
| 15 | 400 | 1.2 | 398.8 | 26590 |

A comparative estimation of the complexing ability of pectin and casein has been conducted.

Casein belongs to a group of proteins referred to as phosphoproteins; it comprises a large number of phosphate groups that bind calcium.

Heavy metal ions are bound by casein through a phosphorus caseinate–calcium phosphate complex; this leads to the formation of water-insoluble salts.

To compare the binding ability of pectin with protein, a casein solution was prepared. A weighed portion of casein was dissolved in water with a sodium acetate additive under heating on a water bath. The analysis results are shown in Table 2.

Table 2. Results of examination of the complexing ability of casein with respect to Cu^{2+} ions

| m (casein), mg | m _{add} (Cu ²⁺), mg | m _{free} (Cu ²⁺), mg | m _{bound} (Cu ²⁺), mg | Complexing ability mgCu ²⁺ /g of casein |
|----------------------|--|---|--|---|
| 5 | 400 | 1.9 | 398.1 | 79620 |
| 10 | 400 | 2.1 | 397.9 | 39790 |
| 15 | 400 | 2.4 | 397.6 | 26500 |

To determine the complexing ability of pectin and casein with respect to iron ions, the absorbance of solutions containing FeCl₃ at $\lambda = 395$ nm was found. The analysis results are shown in Tables 3 and 4.

Table 3. Results of examination of the complexing ability of pectin with respect to Fe^{3+} ions

| m (pectin), mg | m _{add} (Fe ³⁺), mg | m _{free} (Fe ³⁺), mg | m _{bound} (Fe ³⁺), mg | Complexing ability mgFe ³⁺ /g |
|----------------------|--|---|--|--|
| 0 | 0 | 0 | 0 | of pectin |
| 5 | 18.0 | 5.0 | 13.0 | 2600 |
| 10 | 18.0 | 9.0 | 9.0 | 900 |
| 15 | 18.0 | 12.4 | 5.6 | 370 |

Table 4. Results of examination of the complexing ability of case in with respect to Fe^{3+} ions

| m (casein), | m _{add} (Fe ³⁺), | m _{free} (Fe ³⁺), | m _{bound} (Fe ³⁺), | Complexing ability mgEa ³⁺ /g |
|----------------|--|---|--|--|
| nig | mg | mg | mg | of casein |
| 5 | 18.0 | 16.0 | 2.0 | 400 |
| 10 | 18.0 | 16.7 | 1.3 | 130 |
| 15 | 18.0 | 16.7 | 1.3 | 90 |

EXAMINATION OF THE GELLING ABILITY OF PECTIC SUBSTANCES

Gelation begins as follows. A hydration shell is formed around the pectin molecules in the solution and prevents the molecules from coming into contact with each other. Since the carboxyl groups of polygalacturonic acids undergo dissociation in the solution, the pectin molecules acquire a charge and mutually repel. The formation of a gel skeleton primarily requires the weakening or elimination of electrostatic repulsion forces. Since the acid contained in the solution is dissociated to a higher degree than polygalacturonic acid, the degree of dissociation of pectin is reduced; that is, the electrostatic charge of its particles decreases.

At the same time, under the effect of sugar, the pectin molecules undergo dehydration resulting in the appearance of some "bare" regions with no polarity. The formation of a structural skeleton occurs via the adhesion of individual molecules through their dehydrated regions under the action of intermolecular forces. The skeleton is strengthened owing to hydrogen bonds between the carboxyl and hydroxyl groups of the adjacent chains of the pectin molecules [3]. This interaction between the pectin molecules results in the formation of a cellular structure (Fig. 2).



Fig. 2. Mechanism of gelation of high-etherified pectin [3].

The gelling ability of pectin depends on its molecular weight (degree of polymerization), because an increase in this quantity leads to an increase in the gel strength, on the amount of methyl groups contained in the pectin molecule (degree of methoxylation), the content of free carboxyl groups, and their binding by metals [3, 14, 15].

Depending on the degree of etherification of carboxyl groups, pectins are divided into high- and lowetherified (the degree of etherification is less than 50%), which are obtained from an original feedstock by acidic or alkaline extraction or by enzymatic hydrolysis. Pectins of different nature exhibit significantly different gelling ability. Pectins with a higher quality (highetherified) are prepared from citrus and apple peels; pectins with a lower quality (low-etherified) are produced from sugar beet pulp.

At the first stage, the berries were subjected to a physicochemical analysis in order to determine (1) the content of solids and moisture, (2) the amount of soluble and insoluble solids, (3) the total acidity, (4) the nitrate content, (5) the amount of pectic substances in the form of water-soluble pectins and protopectin, and (6) the degree of etherification of pectins.

Crystallized pectin was isolated from the above berries by acid–ethanol extraction.

The quantitative analysis of pectic substances showed that the weight fraction of water-soluble pectin was 0.78% in blueberries, 0.77% in lingonberries, and 0.465% in cranberries, while the amount of waterinsoluble pectin (protopectin) was 0.52, 0.13, and 0.255%, respectively. The total amount of pectin was 1.3% in blueberries, 0.9% in lingonberries, and 0.69% in cranberries. The resulting pectins had the following degree of etherification: 86.2, 63.4, and 75.9% in blueberries, lingonberries, and cranberries, respectively.

One of the important problems of the technology of food products is to give them desired form and structure. The structure type and mechanical properties of food products determine their texture. The texture is used to estimate the quality of the food product, which can be determined by instrumental measurement of structural and mechanical characteristics. A huge variety of food products prevents from providing any universal recommendations for choosing the method for estimating the rheological characteristics of dispersed food systems in which these properties change during production, storage, packaging, transportation, etc.

The rheological parameters of food systems are among the most important physicochemical characteristics that determine the role of the thickening agent used in food products. Rheological parameters were estimated from the yield point, which was determined on a rotational viscometer, by varying the type of the gelling agent, the degree of fineness of the berries, and the presence of various sugars and solvents.

The rheological tests were conducted for systems with the following ratio of components:

- 0.16 wt % of a thickening agent (sodium alginate; chitosan; blueberry, lingonberry, and cranberry pectin);

- 35.44 wt % of a solvent (milk with a weight fraction of fat of 1.5%, curd whey, and distilled water);

-64.4 wt % of sugar (saccharose, fructose, and sorbite) with allowance for the solid content.

The experimental data were used to plot flow curves in the γ/τ coordinates (strain rate gradient/shear stress) for systems based on water, milk, and whey; the curves are shown in Figs. 3–5.

All the flow curves in the figures deviate from a straight line; hence, the food systems corresponding to these curves are non-Newtonian (quasi-viscous) fluids and belong to structured disperse systems; their viscous properties depend on the composition and velocity gradient.

The rheograms confirm the non-Newtonian flow,

which is characterized by a disproportional decrease in viscosity with increasing shear rate.

Flow curves were plotted for all the systems accord-

ing to the derived values of shear stress and viscosity: logarithmic dependences of apparent viscosity η on shear stress τ (Figs. 6–8).



Fig. 3. Flow curves of the studied systems based on milk.



Fig. 4. Flow curves of the studied systems based on water.

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Fig. 5. Flow curves of the studied systems based on whey.



Fig. 6. Logarithmic dependence of apparent viscosity η on shear stress τ for the studied systems based on milk.



Fig. 7. Logarithmic dependence of apparent viscosity η on shear stress τ for the studied systems based on water.





To describe the flow curves of the studied samples, the Ostwald–de Waele equation was used:

$$\tau = \mathbf{K} \cdot \boldsymbol{\gamma}^{\mathbf{n}}, \qquad \qquad \mathbf{lg} \tau = \mathbf{n} \cdot \mathbf{lg} \boldsymbol{\gamma} + \mathbf{lg} \mathbf{K},$$

where τ is the shear stress, Pa; *K* is the flow consistency index (a measure of viscosity of the fluid); γ is the shear rate gradient, s⁻¹; and *n* is the flow behavior index (characterizes the degree of rheological difference between the studied product and a Newtonian fluid).

To find indices *K* and *n* in the Ostwald–de Waele equation, plots in the $\lg \tau = f(\lg \gamma)$ logarithmic coordinates were constructed. In all the cases, the rheological curves are fairly well approximated by a linear function.

RESULTS AND DISCUSSION

(1) Physicochemical studies of blueberries, lingonberries, and cranberries as a source of pectic substances have been conducted. The biochemical analysis of blueberries, lingonberries, and cranberries has revealed that the weight fraction of moisture is 86.5, 87, and 89.5%, respectively; the weight fraction of solids is 13.5, 13, and 10.5%, respectively; the titratable acidity corresponds to 2, 2.16, and 2.76%; the concentration of nitrate ions does not exceed 0.5 mmol/dm³.

(2) Crystallized pectin in the form of a light brown powder has been isolated from blueberries, lingonberries, and cranberries. Quantitative analysis of the pectic substances has shown that the weight fraction of watersoluble pectin is 0.78% in blueberries, 0.77% in lingonberries, and 0.465% in cranberries; the amount of water-insoluble pectin (protopectin) is 0.52, 0.13, and 0.255%, respectively. The total amount of pectic substances is 1.3% in blueberries, 0.9% in lingonberries, and 0.69% in cranberries. The resulting pectins have the following degree of etherification: 86.2, 63.4, and 75.9% in blueberries, lingonberries, and cranberries, respectively.

(3) The effect of various solvents and sugars on the rheological properties of food systems containing pectic substances has been studied. It has been found that the pectic substances of wild-growing blueberries, lingonberries, and cranberries are fairly effective. Highetherified pectic substances contribute to the strengthening of the structure of the food system because they are stabilized in a gel owing to the combination of hydrophobic interactions and hydrogen bonds.

The use of pectic substances as gelling agents and

various dehydrating agents—saccharose, sorbite, and fructose—has revealed that saccharose is the best dehydrating agent. The addition of saccharose contributes to the formation of hydrogen bonds, which leads to the binding of the solvent, the stabilization of hydrophobic interactions, and, as a consequence, to an increase in the viscosity and the strengthening of the gel structure. Therefore, the use of sugar substitutes in combination with pectin-containing feedstocks significantly decreases the viscosity of the finished product and, hence, its shelf life and quality.

Gels based on fructose and sorbite exhibit a lower apparent viscosity than gels based on saccharose. Therefore, the gel structure can be strengthened by increasing the dose of pectin. In the case of replacing saccharose with other sugars or sugar substitutes, it is necessary to increase their solubility and crystallizability [3, 16].

The action of thickening agents in milk solutions is enhanced compared to solutions based on whey; in turn, the action of these agents in whey-based solutions is more intense than in water solutions. A considerable increase in viscosity occurs owing to the formation of associates of the gelling agent with macromolecular components of the food system. Thus, the efficiency of thickening agents is determined not only by the structural features of their molecules, but also by the composition of the food raw material.

(4) A comparative estimation of the gelling ability of the pectic substances contained in blueberries, lingonberries, and cranberries and in chitosan and sodium alginate gelling agents has been conducted. The viscous properties increase in the series: cranberry pectin < lingonberry pectin < blueberry pectin < sodium alginate < chitosan.

(5) The complexing ability of pectin with respect to copper and iron ions has been studied. A comparative estimation of the binding ability of pectin and casein with respect to metal ions has been conducted. The complexing ability depends on the concentration of both pectin and casein. It has been revealed that casein exhibits a lower complexing ability with respect to iron ions than pectin. It has been found that the complexing properties of pectin vary with its concentration: the more dilute the solution, the higher the complexing ability of pectin. The highest complexing ability of pectin of 80 000 $mgCu^{2+}/g$ of pectin is observed at a pectin concentration of 0.005 mg/cm³.

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