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PLANT PECTIN SUBSTANCES.
METHODS OF ISOLATING PECTIN SUBSTANCES

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Information is given on methods of isolating plant pectin substances from various raw-material sources, and the conditions for obtaining high-quality pectin are discussed.

Pectin substances (PcSs) are high-molecular-mass biopolymers, and in soluble or insoluble (protopectin) form are present in practically all plants. Pectins are polyuronides and, thanks to the specificity of their properties, are widely used in many fields of medicine, the food industry, and technology [1-6]. Interest in pectin and its derivatives, which arose as early as the beginning of this century, remains stable even today, as is shown by patents and scientific and technical information.

In world practice, until the present time the traditional raw material for the production of pectin has been wastes of the fruit juice industry — citrus-fruit and apple residues. Citrus residues are favorably distinguished by a high content of pectin with a good jelling capacity [7]. In apple residues the level of pectin amounts to more than 15% of the weight of the raw material. In addition to the sources of pectin that have been mentioned, fairly detailed studies have been made of sugarbeet pulp [8], sunflower heads [9], the valves of cotton bolls [10], the bark of coniferous trees [11], the pulp of medicinal plants [12], etc. Of practical interest for the republics of the CIS are apple residues and sugarbeet pulp — wastes from production of large processing sectors.

On the territory of the former Union three enterprises are operating on the production of apple pectin and there are also small establishments for the production of sugarbeet pectin with a design capacity ensuring less than 10% of the country's requirements. In the Republic of Uzbekistan, a pilot plant for the production of apple pectin with a capacity of 90 tonnes per year working by a fundamentally new scheme has been in operation since 1991.

Investigations in the field of the chemistry of the PcSs have been carried out in the following main directions: the study of methods for isolating pectins and for their qualitative and quantitative characterization, and also the determination of their structure and and finding ways of modifying it with the aim of broadening their range of use. The main information on the chemistry of the PcSs is contained in a series of monographs and periodical publications [4, 5, 13]. Analysis of the literature as a whole shows that there is no single opinion on the chemical structure of protopectin [14], which complicates the task of technologists and the choice of universal methods for the hydrolysis of protopectin.

At the beginning of the investigation of the structure of protopectin, the opinion was expressed that it consisted mainly of a compound of pectin with cellulose [15]. Other investigators consider that the protopectin module is a linkage of the polychains of pectin through calcium ions with the inclusion of residues of molecules of sugar, cellulose, etc. [16, 17]. The structure of protopectin and the PcSs cannot be regarded as having been definitively established even for a concrete material, since their properties vary within wide limits according to the methods of isolation and a number of other factors [18-20]. The quality of the pectin and its yield from the raw material depend mainly on the degree and conditions of hydrolysis of the protopectin,

In the paper that we present, general and specific methods of isolating pectin from the above-mentioned types of raw material with the aim of choosing the optimum conditions are considered, which is of scientific and practical interest. The isolation of pectin in the general form consists of the following stages: the preliminary treatment of the raw material with the aim of eiminating low-molecular-mass carboydrates and pigments; hydrolysis of the protopectin; extraction of the pectin; precipitation of the pectin and its purification; drying and grinding the dried pectin.

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PRELIMINARY TREATMENT OF THE RAW MATERIAL

Low-molecular-mass carbohydrates are eliminated from the raw material, as a rule, by water at low temperatures (not more than 15°C) with the aim of reducing the loss of the water-soluble fraction of pectin [21]. To achieve a comparatively high purity of the raw material with the minimum loss of pectin, the ballast compounds are extracted by organic solvents [20-24] or hydrolysates are subjected to enzymatic treatment. However, raw material containing a fairly large amount of water-soluble auxiliary compounds requires the use of pure or acidified hot water, although under these conditions the losses of pectin are considerable.

Analysis of the literature shows that, on the elimination of nonpectin compounds from the raw material, losses of the desired product are possible, which has an adverse effect on the subsequent stages of the isolation of pectin. The limited volume of literature on the intensification of the elimination of undesirable impurities by their extraction from the raw material with water can also be explained by the possible losses of pectin. Regardless of the method and degree of elimination of ballast substances from the raw material, this stage is connected with economic losses, and this is reflected on the cost of the desired product.

Our investigations have shown the economic favorability of the maximum extraction of the compounds accompanying raw pectin in the raw material and low-molecular-mass pectin substances with the subsequent utilization of the extract by various methods, depending on its composition. For example, the performance of aqueous extraction from residues of fruit and vegetable raw material at an elevated temperature and an intensification of the process by a change in pressure from high to low, which promotes the maximum passage of low-molecular-mass carbohydrates, mineral substances, vitamins, and the water-soluble fraction of pectin into the extract and, after some purification, the use of the extract as a basis for the preparation of nonalcoholic beverages.

Both hydrophobic and hydrophilic compounds are extracted fairly effectively from the bark of coniferous trees by aqueous alcoholic solution under certain conditions, and, from the extract, by fractionation using physicochemical methods are isolated such valuable products as lipids, tanning substances, total phenolic compounds, etc. [27-30].

The pectin extract obtained from preliminarily purified raw material contains about 55-60% by weight of the pectin of the substances subjected to extraction and can be concentrated to 10% of the pectin in the concentrate, which appreciably lowers the consumption of alcohol for precipitation or permits direct drying in spray-driers.

HYDROLYSIS OF PROTOPECTIN AND THE EXTRACTION OF PECTIN

The most important stage in the production of pectin is the hydrolysis of protopectin and the extraction of the pectin. The hydrolysis of protopectin is performed under severe conditions, then the mass is diluted with water and extraction is carried out under relatively mild conditions [31]. In practice, as a rule, these processes are combined, i.e., one and the same solution is used.

Existing methods for the hydrolysis and extraction of protopectin can be combined into four main groups: acid, alkaline, salt, and nontraditional. Known methods for the hydrolysis of protopectin are oriented mainly to breaking down the bond of the pectin with the other biopolymers of the raw material while affecting the minimum number of intramolecular bonds [32]

EXTRACTION OF PECTIN BY SOLUTIONS OF ACIDS

Existing and newly developed methods for the industrial production of pectin from various raw materials mainly provide for the hydrolysis of the protopectin by solutions of acids [33]. Highly esterified pectin is extracted fairly effectively from raw material by weakly concentrated solutions of acids, which is explained by the weak chemical bond of the protopectin [34]. For example, pectin from lemon peel is extracted in considerable amount by water at an elevated temperature [35]. In this case, hydrolysis takes place under the action of the residual amounts of citric acid in the raw material.

In order to accelerate the hydrolysis of protopectin and the extraction of pectin, as a rule, 0.2-2.0% solutions of inorganic acids (HCl, H_2SO_3, H_2SO_4, HNO_3) and some low-molecular-mass organic acids (acetic, oxalic, citric, tartaric, etc.) are used [4]. In the production of a pectin concentrate (concentrated pectin extract) it is mainly solutions of H_2SO_3 that are used [36], since, in the concentration of the extract in vacuum evaporators, this acid passes almost completely into the distillate. The use of an H_2SO_3 solution excludes the stage of neutralizing the extract or eliminating the acid by the ion-exchange, or another, method.

In the choice of the type of acid and its concentration, and also the time of extraction, the main criterion is the completeness of the hydrolysis of the protopectin with minimum action on the chemical structure of the pectin in order to prevent a lowering of its jelling capacity.

A number of methods of extraction have been proposed for obtaining pectin from apple residues. In the opinion of some authors [37, 38], the optimum conditions for the extraction of pectin from apple residues are pH 3, temperature 95-98°C, and time 2 h. A somewhat different opinion is maintained by others [39], who consider the optimum pH of the medium to be ~2 (0.2% solution of HCl), the temperature 90°C, and the time of extraction 1 h. According to a Japanese patent [40], pectin is extracted at pH ~ 1 and a temperature of 55°C for 20 h.

The technology adopted by the Bendery pectin factory (Moldova) provides for the hydrolysis of the protopectin and the extraction of pectin with a 0.2-0.3% solution of HNO_3 at ~60°C for 20-24 h. The technological regime used in this factory, together with the hydrolysis of the protopectin, leads to a fall in the degree of esterification to 60-65%, i.e., it ensures the production of a pectin of medium thickening capacity.

The hydrolysis of the protopectin and the extraction of the pectin from sugarbeet pulp requires more concentrated solutions of acid. Buzina [41] obtained high-quality pectin with an optimum yield by using a 1.3-2.0% solution of HCl at 70°C for ~2.5 h. To achieve the maximum hydrolysis of the protopectin, some workers use concentrated solutions of hydrochloric acid — up to 10% [42]. A detailed study of the process of extracting the raw material and dry sugarbeet pulp [43] has shown that the optimum conditions for performing the process are pH ~ 0.7 (HCl), temperature 40°C, and extraction time 22 h. There are equally contradictory results on the concentration of the hydrolyzing and extracting agents ensuring the optimum yield and a high-quality pectin from sunflower heads [44-46] and other sources [47, 48].

It is known that in plants part of the pectins may give rise to protopectin in the form of polymers through salt bridges, i.e., bonds between the pectin molecules made through bi- and polyvalent metals. Furthermore, the molecules of pectin liberated in the hydrolysis process which are not linked by an ionic bond, may also form a water-insoluble pectin complex in the presence of ions of bivalent metals, especially calcium:

$$2R-COOH + Ca^{++} \rightarrow (RCOO)_2Ca + 2H^+$$

The fact that the proportion of pectin bound with calcium in the cell walls of the raw material amounts, as a rule, to less than half of the total amount of pectin [49] confirms the possibility of the formation of a complex even during the hydrolysis and extraction of the pectin, which explains the use of fairly strong solutions of acids and the length of the protopectin-hydrolyzing process.

In order to use less concentrated solutions of inorganic acids in the extraction of pectin, detergents are used — oxalic and citric acids, polyphosphates, and aluminium, potassium, and sodium oxalates. The role of detergents in this case consists in the fact that they bind free metal ions and eliminate them from the reaction mixture. The strongest detergent is oxalic acid, since its salts with polyvalent metals are practically insoluble in water:

$$\mathrm{PEC}-\mathrm{Ca}-\mathrm{PEC} + H_2C_2O_4 \rightarrow CaC_2O_4 + 2\,\mathrm{PEC}$$

No less effective has proved to be the method of isolating polyvalent metals from the reaction mixture with the aid of ion-exchange resins. The efficiency of the use of ion-exchange resins of the type of Amberlite IR-120 in the extraction of pectin has been shown [50, 51]. Ion-exchange resins added to the reaction mixture appreciably shorten the time of hydrolysis and raise the yield of pectin.

However, in known accounts of methods of extracting pectin, very little attention has been devoted to the action of ions of polymetals on the hydrolysis of protopectin and the extraction of pectin. The conditions for the hydrolysis of protopectin from the various raw-material sources are dictated to a considerable degree by the amount of ash substances in them. When the latter are eliminated from the raw material during the extraction of the low-molecular-mass compounds with water down to a definite concentration it is possible to create a universal scheme for the hydrolysis of protopectin and the extraction of pectin with the minimum consumption of extracting agent. For example, from apple residues after the elimination of low-molecular-mass compounds for 30 min at pH ~3 at the beginning and ~5 at the end of extraction it was possible to extract 15% of high-quality pectin on the weight of the raw material.

EXTRACTION OF PECTIN BY SOLUTIONS OF SALTS AND ALKALIS

The use of salts as extracting agents is directed mainly to replacing ions of polyvalent metals by monovalent ones, i.e., the transformation of an insoluble pectin complex into a soluble salt. The extractants used are salts of monovalent metals of hydrochloric, nitric, sulfuric, and phosphoric acids, and also polyphosphates and oxalates. Pectin is fairly effectively extracted from apple residues by solutions of the salts NaCl, $(NH_4)_2HPO_4$, NH_4Cl, Na_2SO_4, $Na_6P_4O_{13}$, and $Na_3P_6O_{19}$ [52]. High-quality pectin is obtained from sunflower heads by extraction with a 0.5% solution of $(NH_4)_2C_2O_4$ [53]. Pectin is extracted from the bark of trees of coniferous species by a 1% solution of ammonium oxalate [46]. To obtain pectin from cotton valves and peanut shells a mixture consisting of a 0.5% solution of oxalic acid and a 0.5% solution of ammonium oxalate is used [59, 60].

On the industrial scale, the use of solutions of salts for extracting pectin has not found wide use because of the high consumption of extracting agent per unit of pectin obtained. In the extract, the concentration of salt is greater than the concentration of extracted pectin, which complicates the purification of the pectin and the manufacturing wastes, including the effluents.

The use for extracting pectin of solutions of caustic alkalis is also directed to the replacement of polyvalent metals from protopectin by monovalent cations with the formation of soluble pectates. The main methods in this direction are early developments and are used mainly for obtaining pectin from vegetable raw material [54]. The extraction of pectin by solutions of alkalis takes place fairly effectively at a pH of 7-12 [55-58]. The most frequently extractants are solutions of NH_4OH [54, 55].

In the presence of alkali, in addition to the hydrolysis of the protopectin, chemical changes in the structure of the pectin consisting in its de-esterification take place. The pectin substances obtained by the alkali method belong to the low-methoxyl pectins and have a limited sphere of application.

NONTRADITIONAL WAYS OF EXTRACTING PECTIN

Eltinay et al. have shown that a high yield of pectin can be achieved by the enzymatic hydrolysis of protopectin. The use of enzymes eliminates a number of problems connected with equipment constructed from corrosion-resistant types of metals and with the utilization of the effluents; however, in this method prolonged treatment of the raw material (about 20 h) is necessary [62] and the pectin obtained has a low jelling index.

The advantages of samples of pectin obtained by extraction with enzymes include a high degree of esterification, ensuring a good solubility in water and, correspondingly, the possibility of their use in the production of nonalcoholic beverages. This has been confirmed by our investigations in the isolation of pectin substances during solid-phase fermentation [63].

Recently, investigations have been developed in the field of the so-called acid-free cold regime for the hydrolysis and extraction of pectin. The hydrolysis of protopectin is carried out in a hydroacoustic extractor-disintegrator with a cavitation number of 0.4-1.1 [64]. During treatment in the hydroacoustic extractor the raw material is converted into a gel-like mass from which it is possible to separate the extract only by microfiltration. However, our investigations have shown that to achieve a satisfactory yield of pectin the mass being treated must be acidified to pH 4, so that, in fact, the process can hardly be called acid-free hydrolysis.

In order to exclude or reduce to a minimum the use of chemical reagents in the hydrolysis of protopectin, the water used is subjected beforehand to electrochemical activation. Pectin has been extracted by electrochemically activated water at pH $\sim$ 2 and a temperature of 80°C for 1 h, after which it was washed twice with water [65]. However, when an experiment was performed on the pilot-plant scale it was impossible to lower the pH to 2 by the electrochemical activation of pure water. Extraction at higher pH values of the medium does not ensure the complete hydrolysis of the protopectin and the extraction of the pectin. This method has found use for obtaining an extract of enriched low-molecular-mass pectin to be used for the production of beverages.

In our view, mechanochemical methods of isolating pectin have promise but require further investigations. The above-mentioned and other nontraditional methods of extracting pectin from various raw materials have not been tested on the industrial scale, which prevents their being given a comparative evaluation.

The analysis of existing methods shows that to obtain PcSs for use in the food industry it is desirable at this stage to use the acid method of hydrolyzing protopectin and pectin with the maximum decrease in the concentration of extracting agent by intensifying the hydrolysis of the protopectin.

PRECIPITATION, PURIFICATION, AND DRYING OF PECTIN

The pectin extracts — especially those from apple residues — obtained in existing factories by acid hydrolysis contain an average of ~1% of dry matter, including ~0.35% of PcSs.

Purification of the pectin is carried out before and after its precipitation. Treatment with enzymes is performed with the aim of freeing the extract from foreign biopolymers [66, 67], while mineral salts are eliminated by dialysis or with the aid of ion-exchange resins [68]. As a rule, the purification of pectin is combined with its concentration by precipitation with organic solvents or by the formation of water-insoluble complexes of pectin with polyvalent metals. Methyl and ethyl alcohols are used as precipitants [69, 70]. Pectin precipitates quantitatively from an acidified alcoholic solution while a number of the low-molecular-mass compounds accompanying the pectin, including the ash substances, pass into the mother solution. The pectin precipitate is treated with aqueous alcohol to eliminate the last traces of ballast compounds and acid and then with pure alcohol to dehydrate it.

In order to eliminate treatment with enzyme, PcSs are precipitated by polyvalent metals, for which aluminum salts are mainly used [71, 72]. On the precipitation of pectin by aluminum salts, the desired product separates out exclusively in the precipitate, while not only low-molecular-mass but also high-molecular-mass compounds such as starch pass into the mother solution. The pectin precipitate is subjected to repeated treatment with acidified aqueous alcohol to eliminate the last traces of aluminum, and this presents certain difficulties from the technological point of view.

After the elimination of the metal ions by an acidified aqueous alcoholic solution the precipitate is dehydrated with pure alcohol in a similar way to that used in the precipitation of pectin by organic solvent. In principle, these methods ensure a fairly high purity of the pectin, but they require, a high consumption of alcohol. To obtain one tonne of pectin requires the use, on average, of about 150 m^3 of alcohol. The expenditure on the regeneration and rectification of alcohol in these volumes amounts to a considerable part of the cost of the pectin.

More favorable, in our view, is the drying of a concentrated extract of pectin in a spray-dryer and, where necessary, the purifiction of the dried pectin by washing with aqueous alcohol.

Stoikov and Kravanov [73] have shown that impurities are more effectively eliminated from pectin precipitated with aluminum salts by treating a dried and ground powder with acidified aqueous alcohol. The spray-dried product has a relatively large surface, which can ensure a high efficacy of the elimination of impurities. Our investigations have shown that low-molecular-mass compounds, including ash substances, are eliminated from a dry pectin concentrate by treatment with acidified aqueous alcohol for 15-20 min. The proportion of ash substances by weight in the purified pectin amounts to about 0.5%, which is considerably lower than in preparations obtained by precipitating the pectin from aqueous solution with alcohol and washing with acidified aqueous alcohol. Moreover, after treatment with alcohol and drying the product does not require grinding.

It is possible to consider the comparatively low consumption of alcohol — 15-20 m^3 (without taking regeneration into account) per one tonne of finished product as an advantage of the scheme of purifying pectins by treating the spray-dried product with aqueous alcohol.

Methods are known for drying aqueous solutions of pectin by spraying [74-76]. Sulc et al. [74] consider it desirable to obtain pectin by precipitating it from concentrated solutions of alcohol, dissolving the precipitate in water, and spray-drying. Other applications [75] give information on the spray-drying of untreated concentrates of pectin. The concentration of an extract, purification of the pectin in ultrafiltration apparatuses, and spray-drying is more favorable from the economic point of view.

The efficiency of the purification of pectin by ultrafiltration has been shown [77-80]. By two filtrations (with dilution by water) through a UAM 200 membrane, an extract of pectin from apple residues (pH about 4-5) can be freed from 90% of the ballast compounds and the concentration of desired product can be raised to 5% in the concentrate. A 92-95% purity of the pectin in the concentrate from an extract of sugarbeet pulp obtained by concentration and purification on ceramic membranes has been achieved. The spray-dried pectin possessed fairly high solubility in water and jelling capacity.

A scheme for obtaining pectin including purification, the concentration of the pectin extract on ultrafiltration apparatuses, and the spray-drying of the concentrate is the most promising in our view.

Thus, an analysis has been made of the isolation, purification, and drying of pectin substances from various plant sources. The value of a treatment of the raw material before the hydrolysis of the protopectin and the possibility of creating more flexible technological schemes of isolating pectins from various sources, and also the role of polyvalent metals in the choice of the optimum conditions for the hydrolysis of protopectin, have been shown. Possible ways of obtaining high-quality pectins by drying a purified extract in a spray-dryer have been discussed.

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