Extraction of pectins from citrus fruits, their qualitative and quantitative analysis for application in the medical and food industries

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Abstract. The paper provides scientific substantiation and experimental confirmation of feasibility, prospects, and simplicity of production of high-quality purified pectin through preliminary multistage extraction of pectin-containing plant raw material, its acid hydrolysis and subsequent precipitation of pectin in the form of calcium salt. One of the most important physical and chemical properties of pectin substances is their ability to sorb and remove toxic substances from the body. These properties make pectin valuable for preventive nutrition, and as a biologically active food supplement.

1. Introduction
Due to environmental deterioration and potential presence of toxic substances, heavy metals and radionuclides in food, creation of food with ingredients that prevent the development of occupational diseases in adverse conditions is of high relevance [1, 2].

Natural polysaccharide pectin exhibits a number of valuable properties due to its chemical structure. Pectin (derived from the Greek word pectos which means to solidify) is water-soluble polysaccharide, free of cellulose, that consists partially or completely of methoxylated polygalacturonic acid residues [3]. Pectin substances are part of all structural elements of the cellular tissue of higher plants in the form of protopectin and pectin in particular. Protopectin is a strong compound with cellulose that splits into pectin [4].

WHO considers vegetable products – pectin substances – as toxicologically safe, and as dietary fibers, these compounds can form complexes with heavy metals, and also remove pesticides and cholesterol from the body. These pectin properties make it widespread in the food and medical industries. It is shown that all pectins, regardless of the nature of their origin, be it fruit and berry or vegetable, are capable of forming complexes and remove toxic substances, biogenic toxins, and antibiotics accumulated in the body. In terms of the degree of esterification (72%), citrus is recognized as the best one, apple is slightly worse, and blueberry pectin has a low degree of esterification [5].

Polygalacturonic acid is the base for the pectin macromolecule (figure 1).
Figure 1. Forms of D-galacturonic acid.

It should be noted that the composition of pectin substances includes neutral monosaccharides, L-arabinose and D-galactose, and fructose, which constitute an insignificant part in the pectin molecule and make it heteropolysaccharide. These monosaccharides are attached to the pectin molecule in the form of side chains, while L-rhamnose is most often found in the pectin backbone.

The chemical composition and structure of pectin is still being studied due to high complexity of this molecule. It is relevant to understand its role in plant growth and development, during fruit ripening and in food processing. The structure of pectins is difficult to identify, since their composition and quality depend on the source of natural origin, accurately chosen and experimentally tested extraction conditions, geographic location and other environmental factors. Pectin can also modify during its isolation from plants, storage, maturity and processing of plant materials [6].

The main mechanism for removing heavy metals from the body in the presence of pectin substances is a chain of biochemical reactions in the human gastrointestinal tract that causes conversion of pectin to polygalacturonic acid, which forms stable complexes with heavy polyvalent metals fecally excreted. This property makes pectin an environmentally friendly and vital food product that is currently in demand for provision of the population.

The ability of pectin to detoxify is determined by the following indicators: the esterification degree, uronid component, binding ability, sorption capacity, etc. To determine the above indicators, an important factor is the source of plant raw materials enriched with pectin, as well as a comparative assessment of methods for production and subsequent purification of pectin substances.

Functional properties of pectins determine their use in the food and medical industries. The main technological property of pectin ingredients is to increase the viscosity or form a gel nanostructure of various strengths [3]. Gelation of low esterified pectins occurs through the formation of hydrogen bonds and as a result of interaction with ions of polyvalent metals, for example, with calcium ions.

Pectin is most widely used in the food industry as a gelling agent for preparation of pastille, jelly, marmalade, marshmallow, and as a gelling agent in production of fruit and berry fillers. Pectin is also added to medicinal bakery products [10]. Pectin is a popular ingredient in food industry and exhibits a range of therapeutic and prophylactic properties. On an industrial scale, pectin substances are produced mainly from pomace (up to 30%) and citrus peel (up to 60%) as by-products of juice production. Commercially pectin is extracted from citrus peel and pomace, but there are some other sources, such as sugar beet, banana peel, mango peel, papaya peel and sunflower heads.

Pectin, a versatile polysaccharide, has been widely used in the food and beverage industry for many years. It is mainly used as a gelling agent, thickener, stabilizer and emulsifier [7]; moreover, pectin is an important source of dietary fiber, which can exhibit therapeutic properties [10]. Apart from the food industry, pectin has application in such vital areas as medicine, where it is used as a carrier for controlled drugs or biologically active release, for example, for nasal, ocular and oral drug delivery [11] and wound healing.
The main types of pectins used in the food industry are citrus and apple pectins, which is due to high degree of esterification naturally inherent in these pectins [11]. Since pectins exhibit cleansing properties, they are successfully used for treatment and prevention of diseases of the gastrointestinal tract, including the liver, gallbladder and pancreas, as well as diabetes mellitus, metabolic disorders, obesity, dysbiosis, hypertension, polyarthritis and other arthroposos. In pharmaceuticals, pectin is actively used in the manufacture of capsules for medicinal formulations.

2. Results and discussion

Pectins are found in all plants, however the main raw materials for pectin production are citrus peels, pomace and beetroot pulp. The above raw materials are used to produce pectin of three types: highly esterified (H-pectin), low esterified (L-pectin) and amidated pectin. They differ in the degree of esterification of carboxyl groups per 100 carboxyl groups of pectinic acid and the degree of its amidation [6].

The process of pectin production has been extensively studied for many years, but it is still relevant [3] and includes the following main stages:

• preparation of pectin-containing plant materials;
• pectin extraction by hydrolysis using mineral and/or organic acids;
• filtration and settling overnight in 95% ethanol;
• washing of the isolated pectin with a 5 ml portion of acetone to remove remaining impurities;
• thorough filtration and drying of the purified pectin in an oven at 35–40 °C;
• calculation of the product yield (%);
• grinding of the dried pectin isolate to obtain a powdery mass;
• drying, grinding and mixing of pectin with sugar to a standard degree of strength.

However, pectin isolation using enzymes greatly facilitates the technological process and is considered most efficient.

To isolate individual polysaccharide fractions, a sequential extraction of air-dried raw materials (after preliminary defatting with chloroform) is performed with water (monosaccharides, water-soluble polysaccharides prevail), then oxalate buffer (pectin substances) and sodium carbonate solution (hemicellulose, proteins). Further precipitation is carried out with acetone, salt solutions, electrolysis and alcohols, and hydrolyzed with acids. The pharmacopoeial method is a gravimetric method based on the precipitation of polysaccharides from aqueous solutions with ethanol and acetone.

Since the reaction with amino acids results in colored products (melanoidins), raw materials for pectin production should contain a minimum amount of reducing sugars. In food production, the concentration of ballast substances in the dry pectin residues should not exceed 30%. In manufacturing of medicinal products and dietary supplements, the concentration of impurities should not exceed the norms specified in the XII edition of State Pharmacopoeia and Manufacturing Specifications [12].

Table 1. The yield of pectin substances depending on the hydrolyzing agent.

| Air-dried citrus peel | Hydrolyzing agent, concentration 0.3 N | Time of hydrolysis, h | Mass of pectin, g | Yield, % (per mass of raw materials = 25 g) |
|-----------------------|---------------------------------------|----------------------|------------------|--------------------------------------------|
| Grapefruit            | hydrochloric acid                     | 2                    | 1.26             | 5.04                                       |
|                       | citric acid                           | 4                    | 1.30             | 5.2                                        |
| Lemon                 | hydrochloric acid                     | 2                    | 1.18             | 4.72                                       |
|                       | citric acid                           | 4                    | 1.19             | 4.76                                       |
| Orange                | hydrochloric acid                     | 2                    | 1.09             | 4.36                                       |
|                       | citric acid                           | 4                    | 1.16             | 4.64                                       |
Table 1 shows that the concentration of the hydrolyzing agent and the time of sample hydrolysis do not have a significant effect on the overall product yield, depending on the type of the raw material.

The most promising method for production of purified pectin is enzymatic catalysis. The method implies exposure of the test material previously purified from pectolytic enzymes to the action of the cellocandin enzyme preparation (a complex of hemicellulases and cellulases), which ultimately leads to the release of low-degraded pectin [2].

Depending on the scope of application of the original product, it is possible to separately determine soluble pectin, protopectin or the amount of pectin substances. In this case, quantitative determination of the studied substances is carried out by the gravimetric method, which is based on the determination of calcium pectate in terms of pectic acid.

It should be noted that thermal stability of pectin ingredients in some food products depends on fruit and berry raw materials, syrup concentration, pH of the extract medium, and other factors. The optimal concentration of calcium ions improves the thermal stability of the confiture filling [11].

Table 1 presents comparative experimental data on the pectin yield depending on different factors.

3. Conclusion
The role of pectin in the medical industry is indisputable. Various domains of the pectin structure reduce the risk of certain types of cancer and cardiovascular diseases, soften the glycemic index and slow down gastric transit the body’s energy consumption is controlled. Colon bacteria ferment pectin to form short-chain fatty acids, which are beneficial to the health of the body. The interaction of pectin and polyphenolic compounds promotes a systemic anti-inflammatory effect. In addition, pectin is an effective means of delivery of exogenous nutraceuticals or drugs using emulsion or hydrogel technology for targeted delivery to the colon [7].

Due to the cleansing properties of pectin substances, they are successfully used for treatment and prevention of diseases of the gastrointestinal tract, including the liver, gallbladder and pancreas, as well as diabetes mellitus, metabolic disorders, obesity, dysbiosis, hypertension, polyarthritis and other arthronosos. Thus, the development of the pectin industry is promising for various areas of the national economy.

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