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Physico-chemical and biological properties of pumpkin pectin

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Abstract

This study presents the findings of pectin's isolation and purification process derived from the Michurinskaya pumpkin variety. The researchers employed infrared spectroscopy and acid-base titration techniques to determine the presence of free carboxyl groups and the degree of esterification. Additionally, the physicochemical properties of the resulting polysaccharide, including kinematic viscosity and molecular weight, were investigated using viscometry. Furthermore, the fungicidal activity of the resulting polysaccharide was assessed. The acidic polysaccharide, pectin, was extracted from the Michurinskaya pumpkin cultivar using the salt extraction method, yielding 29 %. The overall concentration of OM e- and OAc groups (36.3%) and free carboxyl groups (6.48%) was measured using acid-base titration techniques. Infrared (IR) spectroscopy data validated the obtained results. The spectra revealed signals inside the 1319-1408 cm^{-1} region, corresponding to OMe groups. Free carboxyl groups were also present in the 1730-1760 cm^{-1} range. The molecular weight of pectin measured using the viscometry method was 2.5 kDa. The findings of the antifungal activity investigation indicated that the isolated pectin derived from pumpkins demonstrates inhibitory properties against the growth of *Penicillium* sp.

Keywords

pectin; acid-base titration; viscometry; IR spectroscopy; antifungal activity

Introduction

Pectins are known to be effective biosorbents capable of cleaning the body of radionuclides and various metabolites, including glucose and cholesterol, toxicants and other low-molecular-weight biologically active substances (BAS). In addition to such characteristics, this class of biopolymers is characterized by the demonstration of antiulcer, wound healing, immunomodulatory, antioxidant and antimicrobial activity (1-3).

Another emerging area is the use of pectins in the pharmaceutical industry, formulating new dosage forms characterized by low toxicity compared to the original drug and higher solubility. This increases the therapeutic effect and the gradual release of active components and therefore, a longer-lasting effect (3, 4).

Important factors determining the properties of pectins are the nature of a plant and the method of its extraction from plant materials. Differences in extraction methods and conditions can produce fractions with different molecular weights, °C of esterification and branching of the macromolecule (5). The most widely used method is the treatment of plant cells with aqueous solutions of mineral (sulfuric, hydrochloric, nitric) and organic acids (citric, oxalic, lactic), salts (ammonium oxalate) and sometimes bases (NaOH).

However, these methods are environmentally unsafe (6). Other techniques, such as enzymatic actions or microwave treatment, increase the yield of the product but can lead to de-esterification and partial hydrolysis of high molecular weight pectins to produce shorter polysaccharide fragments (7, 8).

Among the commonly used plants for producing pectin, the following can be distinguished: apple and citrus pomace, beet pulp, sunflower baskets, etc. Pumpkin is a promising source of pectin with characteristics such as a high degree of esterification and macromolecule branching. In works (9, 10), the authors reported the antidiabetic, immunomodulatory, antitussive and antioxidant activities of pectin fractions obtained from different types of pumpkin. Extraction methods affect pumpkin pectin's physicochemical properties and biological activity and make it possible to obtain polysaccharides with various functional properties.

A study reveals that pumpkins are a highly beneficial and cost-effective source of natural pectin (11). Pumpkin pectin forms a gel at a significantly lower concentration than citrus pectin. It is reported that the structure of pumpkin pectin with a high degree of branching of carbohydrate chains contains a more significant number of neutral sugars (pentoses: arabinose, xylose; 6-deoxyhexoses - rhamnose and hexoses - galactose) and esters of acetic acid (12).

These structural characteristics may play a role in enhancing gelation (7). The authors proposed that pectin obtained from pumpkin has two distinct domains. The first domain is a homogalacturonan, formed by the monosaccharide galacturonic acid residues (D-GalpA) and can undergo acetylation or methylation (Figure 1a). The second domain is rhamnogalacturonan (RG-I), which consists of free and methyl-esterified sections of GalpA residues, as well as branches in the form of side chains formed by β-1,4-D-galactose and α-arabinofuranose (Araf), (Figure 1b). In addition to glycosidic components, these polysaccharides encompass other non-glycosidic molecules, including polyphenolic compounds and proteins (Figure 1c).

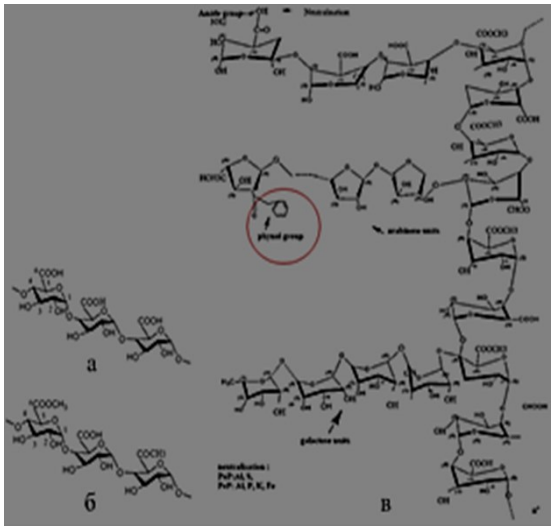

Fig. 1. Chemical structure of pumpkin pectin: A - homogalacturonan, B - esterified homogalacturonan, C - rhamnogalacturonan

This research aims to systematically isolate and characterize pectin from the Michurinskaya pumpkin variety, known for its superior gelling properties compared to other varieties. The study will employ infrared (IR) spectroscopy and acid-base titration to determine the degree of esterification and quantify free carboxyl groups. Additionally, the research will measure the pectin's kinematic viscosity and molecular weight and assess its antifungal activity.

Materials and Methods

Experiment Part

The study's objective was an agricultural product - lar The Michurinskaya variety of pumpkin, scientifically known as *Cucurbita*, belongs to the Pumpkin family. This particular variety was cultivated in 2022 at the scientific and educational centre of the Tambov State Technical University - Michurinsk State Agrarian University, specifically at the "Ecotechnologies named after Yu. G. Skripnikov" Ecotechnologies Centre.

The existing literature does not provide any information regarding the chemical composition of pectin in this particular pumpkin variety. However, experimental evidence has demonstrated a notable gelling capacity compared to other pumpkin kinds.

Materials used in the current study are distilled water (GOST 6709-72), 96% ethyl alcohol (Ekos-1, Russia), analytical grade ammonium oxalate 1-water (Reakhim, Russia), chemical grade concentrated hydrochloric acid (HCl), analytical grade bromothymol blue (C27H28Br2O5S), 0.4% analytical grade red cresol (C21H18O5S) and 0.4% analytical grade red phenol (C19H14O5S1).

The tools and apparatus used in the experiment were an analytical balance Homogenizer Gosmeter VL-210. The experimental equipment utilised in this study includes the LUMME LU-2601, MM-5 magnetic stirrer with heating, TsLN-2 tabletop centrifuge, IRAffinity-1 IR-Fourier spectrometer and UT-4612 thermostat.

Protocol for Pectin polysaccharide extraction: A pumpkin sample weighing 63.32 g was introduced into a 1.5 L glass flask, followed by 500 ml of an aqueous solution containing ammonium oxalate at a concentration of 0.7%. The receptacle was placed within a thermostat set at 60 ° Celsius for 4 hours, with intermittent agitation occurring every 30 minutes. The mixture was subsequently cooled to ambient temperature and subjected to filtration through a bulky viscose filter employing a Buchner funnel to exclude plant cells. The resulting mixture was then stored in a refrigerator at 4°C temperature. The extracts were concentrated to ⁰ volume using a rotary evaporator under vacuum (pressure of 9 mm. of mercury) at 60 °C. The polysaccharide was precipitated by adding a threefold amount of ethyl alcohol to the concentrated solution. A precipitate, specifically pectin glycan, was observed in this instance. The polysaccharides were separated from the ethanol extracts using sedimentation, followed by filtration of pectin using a synthetic fabric filter. The polysaccharide

obtained was dried at ambient temperature until a constant weight was achieved.

The technique described in reference (13) was used to determine the degree of esterification and the quantity of free carboxyl groups in the native pectin. The methodology employed in this study involves the titrimetric analysis of both unbound and esterified carboxyl groups of polygalacturonic acid inside isolated pectin. Distilled water was used to dissolve the indigenous pectin. To enhance the solubility of pectin, it is possible to wet it with ethyl alcohol. A fraction of the overall volume was extracted and subjected to a temperature of 40°C. The container was securely sealed and agitated until the pectin achieved full dissolution. A 0.1 N NaOH solution titrated the material with a mixed Hinton's indicator.

A total of six drops of the indicator were introduced into the solution and subjected to titration until the formation of a deep pink hue colour appeared, which persisted for a minute. Considering the volume of the NaOH solution used, 50 ml of the same solution was added. The flask was sealed and remained undisturbed for 3 hours to facilitate the saponification of the esterified carboxyl groups. Next, a 50 ml hydrochloric acid (HCl) was introduced into the solution and subsequently subjected to titration using a NaOH solution. The percentage of esterification of DE in pectin was determined by employing the following formula:

$$DE = \frac{V_2}{V_1 + V_2}\ 100\%$$

Let V1 represent the volume of 0.1 N NaOH solution used in the initial titration, measured in millilitres and V2 denote the volume of 0.1 N NaOH solution employed in the subsequent titration, measured in millilitres.

The determination of the percentage of free carboxyl groups (Kc) was calculated using the prescribed formula:

$$K_c = \frac{V_1\ 0{,}45}{p}$$

V1 represents the volume of a 0.1 N NaOH solution utilised for the initial titration, measured in millilitres. p denotes the weight of pectin, measured in grams.

A Ubbelohde viscometer with a capillary diameter of 0.54 mm was employed for viscosity measurement. The viscometer's capillary and upper ball were filled, ensuring they reached the corresponding mark. Flow time was measured, which refers to the duration it takes to decrease the tube's solution level between two markers. Three measurements were conducted. The kinematic viscosity and molecular weight were determined using the methods provided in reference (14).

An IRAffinity-1 Fourier Transform Infrared (FTIR) spectrometer was used to record the IR spectrum of the material within the wavenumber range of 4000-400 cm^{-1}. The IR spectra were acquired as thin films by drying the materials directly on a silicon single crystal in an oven at a temperature of 50°C. The silicon plate with the deposited sample was subsequently inserted into the apparatus and the resultant spectrum was documented.

The fungal culture *Penicillium* sp was used to test the antifungal action of pumpkin pectin in the biological laboratory of the Department of Toxicology and Mycology of the Lipetsk Regional Veterinary Laboratory, Lipetsk.

The nutrient medium can be prepared by combining 20-30 g of dry Capek agar powder with 1 litre of tap water, followed by a soaking period of 2 hours at room temperature. To ascertain the quantity of water absorbed by the agar, the water was drained and its volume was measured after that. Subsequently, the agar underwent two to three washes using distilled water. The residual constituents of the medium were measured and subsequently dissolved in an equivalent volume of distilled water as the agar that was removed during the soaking process. The resultant solution was supplemented with washed agar-agar, followed by boiling the medium in an autoclave with continuous steam for 1 hour. The resultant medium underwent filtration, followed by its transfer into flasks and subsequent sterilisation in an autoclave operating at a pressure of 0.05 MPa for 20 minutes. The pH of the nutritive medium fell within the range of 6.0-6.8.

The cultivation of microorganisms was carried out by placing a sample of a thin section of a pre-grown fungal culture measuring 2x2 mm in the center of a Petri dish with agar gel and keeping it in a thermostat at 37 °C for 3-4 days. As inhibitors, we used 3% aqueous solutions of pumpkin and apple pectins (isolated according to the same scheme) in a 10 µl volume applied to a Petri dish on the surface of freshly grown colonies of a fungal culture. The inhibitory effect was recorded after 3-7 days.

Results

To isolate pectin, we used pumpkin pulp powder of the Michurinskaya variety, dried to constant weight and crushed.

The method for isolating pectin polysaccharide from pumpkin is shown in Fig. 2.

Previous research demonstrated that ammonium oxalate is the most efficient extractant for isolating pectins from garden plants (15). Therefore, it was employed in this study. Furthermore, an initial evaluation was conducted to determine the influence of several extractants (water, citric acid and ammonium oxalate) on producing the desired pectin from pumpkin. Additionally, the viability of employing an ammonium oxalate solution was verified.

The experiment used a diluted aqueous ammonium oxalate solution to heat dry pumpkin pulp powder. The selection of salt concentration is based on experimental findings indicating the inadequate efficacy of solutions with lower concentrations (below 0.7%). Additionally, employing more significant concentrations of ammonium oxalate solution leads to an elevation in the pH of the medium. Furthermore, using a greater concentration of ammonium oxalate necessitates an extra step of purifying pectins from salt, which involves dialysis.

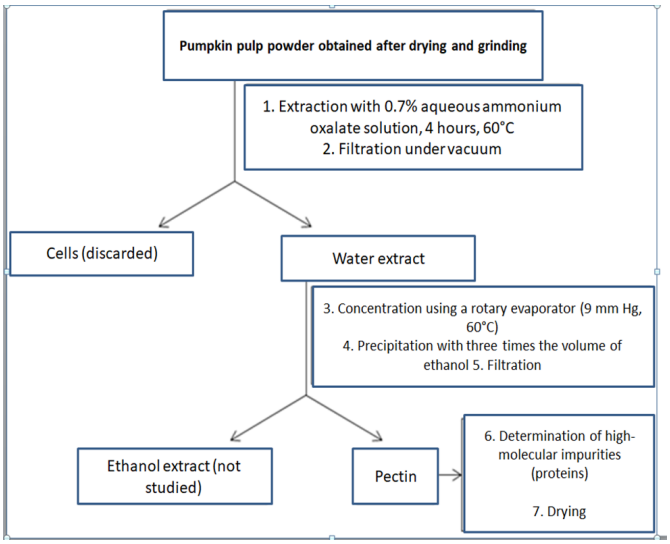

Fig. 2. Scheme for isolating pectin from plant materials

A thermostat was utilised to conduct the extraction procedure, maintaining a temperature of 60°C for 4 hours. The selection of the designated temperature yields enhanced efficacy in extracting polysaccharides while minimising the risk of biopolymer degradation.

The extracts obtained, which consisted of a combination of bio-active compounds with both high and low molecular weight, were subjected to evaporation until they reached one-fourth of their initial volume. This was done to increase pectin yield by using alcohol precipitation. The concentration process was conducted using a rotary evaporator in a vacuum environment to prevent the degradation of bioactive compounds. It is widely acknowledged that the solubility of polysaccharides in an aqueous environment is significantly more excellent than in an alcoholic environment. Consequently, introducing excessive alcohol leads to the precipitation of the polysaccharide. To enhance the organisation of the pectin material and increase the production of the desired polysaccharide, the flasks were refrigerated at a temperature of 4°C for two days. The pectin obtained isolated from the ethanol extract was subjected to air drying. During pumpkin pectin isolation, it is important to consider the potential existence of impurities derived from other biologically active biopolymers, such as proteins and nucleic acids, which cannot be eliminated. The employed extractant, ammonium oxalate, does not entail the simultaneous extraction of nucleic acids, which are typically isolated using acidic solutions.

A negative xanthoprotein response test indicates the lack of protein contamination. The pectin polysaccharide obtained from pumpkin had a yield of 29%. Pumpkin pectin extraction generally results in lower yields compared to other methods. An instance of the extraction of *Cucurbita* pumpkin pectin maximum using cavitation resulted in a yield of 10% (16). The acid extraction of pectin from C. *maxima*, *C. moschata* and *C. pepo* var. *styriaca* resulted in yields of 5% (7), 8-20% (17) and 5-7% (18), respectively. The pectin extraction from *C. maxima* and *C. pepo* var. *styriaca* using microwave technology resulted in yields of 11% (7) and 3.1-7.4% (18), respectively. The enzymatic treatment of *Cucurbita mixta* resulted in a pectin yield of 10% (8).

The observed outcomes might be attributed to the characteristics of the primary ingredient (a distinct pumpkin cultivar), the environmental conditions for cultivating melon crops, and the technique employed for isolating the polysaccharides.

There is conflicting information regarding the existence of essential groups in pumpkin pectin. In a previous study (16), 71.9% were reported. However, another study (12) found a lower content of esterifying groups in pectin derived from pumpkin pulp (54.21%) and pectin derived from pumpkin peel (36.36%). Consequently, a titrimetric analysis was conducted to determine the free carboxyl (-COOH) and total content of OAc-and OMe-groups. Titrimetric methods, including acid-base, conductometric, and potentiometric titration, are used to determine DE. These methods remain relevant and are employed in both industrial and scientific-technical facilities. These techniques enable the determination of the overall concentration of O-methyl and O-acetyl groups and the presence of free carboxyl groups. The acid-base titration method was employed in our study.

The titration procedure involved using a titrant composed of NaOH and a mixture of Hinton's indicator, which undergoes a colour change to a deep pink hue close to the equivalence point. Polysaccharide solutions with a uniform concentration of 1% were collected for the study. Each polysaccharide sample underwent three measurements.

The methodology employed in this study was founded upon a two-stage analysis. Fig. 3 illustrates the procedure for doing acid-base titration.

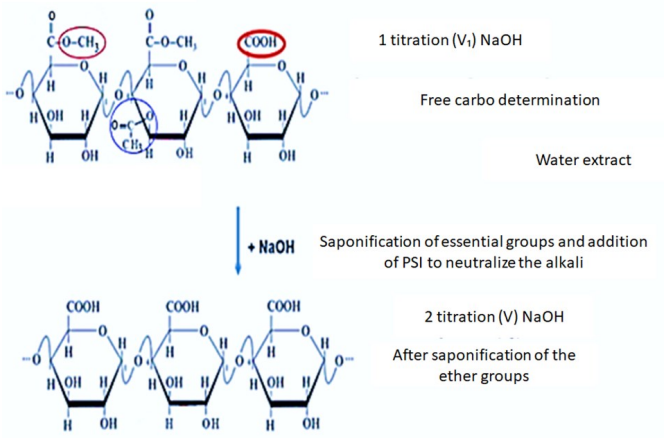

Fig. 3. Scheme for performing acid-base titration

The initial titration phase involves using a NaOH solution to determine the quantity of unbound carboxyl groups. This process entails consuming the necessary volume to neutralise the carboxylic acids. The results are consistent with the titrimetric analysis data, suggesting a relatively modest degree of esterification (DE = 36.3%). Pumpkin pectin exhibits a notable deficiency in ester groups, indicating its elevated gelling capacity attributed to presence of unbound carboxyl groups.

In characterising pectin polysaccharides, the analysis of DE is conducted in conjunction with the measurement of

free carboxyl groups within the macromolecule's composition. To achieve this objective, the data collected during the initial titration phase (titrant volume values V1) are utilised to determine the concentration of carboxyl groups using formula (2), resulting in a value of 6.48%.

Aside from titrimetric approaches, the IR spectroscopic analysis method can also be employed to evaluate the presence of ester and carboxyl groups. In (16), the authors demonstrated a correlation between the degree of esterification and the relative intensity of signals at 1730-1760 cm^{-1} (esterified carboxyl groups) and 1600-1630 cm^{-1} (free carboxyl groups). The band corresponding to carbonyls of ester groups (1731 cm^{-1}) exhibited a positive correlation with the degree of esterification of pumpkin pectin. Conversely, the band associated with free carboxyl groups (1610 cm^{-1}) demonstrated a negative correlation with the degree of esterification of pumpkin pectin. The authors conducted a comparative investigation of the IR spectrum of pumpkin pectin to citrus pectin and apple pectin. The findings revealed that pumpkin pectin exhibited the highest level of esterification, which aligns with the results obtained by the titrimetric approach.

The IR spectrum of the pumpkin pectin we isolated contains signals from the functional groups ν(OH)C (3363 cm^{-1}), ν(CH)E and ν(CH)K (2976 cm^{-1}), ν(COO-) (1641 cm^{-1}), ν,δ (C-OH)A (1450 cm^{-1}), δas (CH3)E (1408 cm^{-1}), δs (CH3)E (1381 cm^{-1}), δ(CH3)K (1319 cm^{-1}), ν(C-O-C) (1084 cm^{-1}), ν (C-C) (C-O)K (1045 cm^{-1}), pyranose cycle (877 and 572 cm^{-1}), characteristic of pectin (Fig. 4).

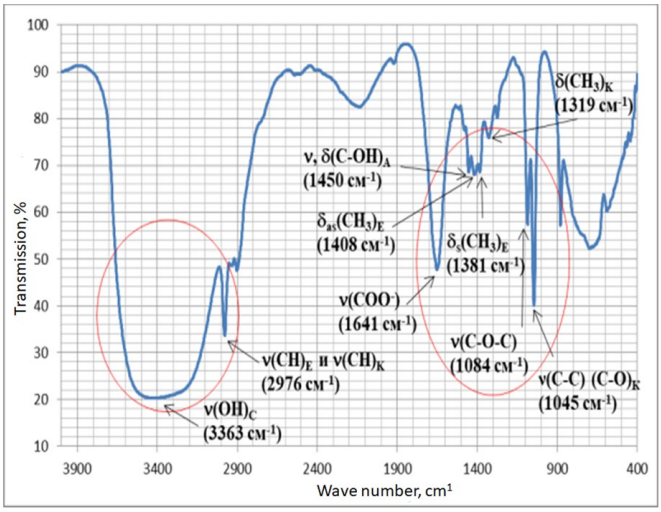

Fig. 4. IR spectrum of pumpkin pectin

The region of 1730-1760 cm^{-1} has a significant signal associated with the stretching vibrations of carbonyls in ester groups. This signal serves as an indication of the existence of esterified carboxyl groups (16). The presence of free carboxyl groups can be inferred from the signal observed in the range of 1600-1630 cm^{-1}, associated with the stretching vibrations of the carboxyanion. Based on the infrared spectrum data presented in Fig. 4, it can be inferred that the prominent peak observed within the range of 1730-1600 cm^{-1} represents a composite signal encompassing both free carboxyl (1641 cm^{-1}) and esterified carboxyl groups (1760-1730 cm^{-1}). This inference is supported by transmission bands within the 1319-1408 cm^{-1} range, confirming the presence of OMe groups. A diminished signal strength suggests a negligible presence of OMe groups.

It is known that the ability of pectin polysaccharides to form gels increases with increasing viscosity and molecular -weight (19). Such characteristics make it possible to assess the quality of pectin and predict the area of its practical applications.

An Ostwald viscometer with a capillary diameter of 0.54 mm was utilised to quantify the viscosity of an aqueous solution containing pumpkin pectin. The viscometric approach is a straightforward and convenient way to determine molecular weight. However, it necessitates understanding the equation's constants that relate viscosity to molecular weight. Among all the known equations that describe the relationship between weight-average molecular weight and internal characteristic viscosity for high-molecular compounds, the Kuhn-Mark equation is the most appropriate for pectins, as stated by Glickman and Orlov. The experimental data demonstrated that the kinematic viscosity of pectin was measured to be 1.43 mm 2/s, while its molecular weight was determined to be 2.5 kDa. Based on the data obtained, it can be inferred that the separated pectin is classified as a glycan with a low molecular weight. Oligogalacturonides possess a low molecular weight, rendering them highly susceptible to absorption within the gastrointestinal system and subsequent renal excretion. This characteristic renders them highly appealing for medical applications.

The antifungal activity of pectins (pumpkin and apple) was studied using aerobic fungal cultures of the genus *Penicillium*, which causes skin and sinus lesions. The antifungal effect was characterized by recording the inhibition of the growth of fungal cultures when exposed to an aqueous solution of the isolated pectin, Fig. 5.

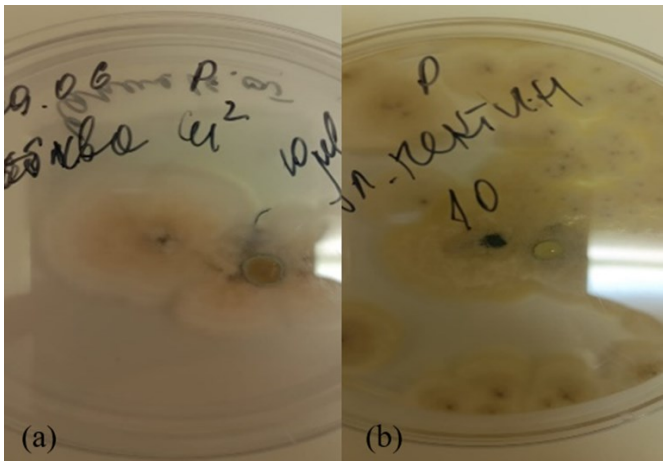

Fig. 5. Inhibition of fungal culture growth by pumpkin (a) and apple (b) pectin

It was found that apple pectin had virtually no effect on the further development of microorganisms. In contrast, pumpkin pectin was noted to have the ability to suppress the growth of colonies throughout the areola of the spread of a drop of solution on the surface of a fungal culture.

For a more detailed characterization of the antifungal properties of the obtained pumpkin pectin, continued microbiological studies are necessary.

Conclusion

As a result of the study, pectin was isolated from the Michurinskaya variety of pumpkin using the salt extraction method, yielding 29%.

The low degree of esterification and low molecular weight of pumpkin pectin from the Michurinskaya variety have been confirmed, setting it apart from pectins derived from other pumpkin types. The acquired data about the extent of esterification and molecular weight present an opportunity to utilise this particular pumpkin cultivar to develop efficient methods for eliminating heavy metal ions, namely lead, mercury and cadmium, from the human body.

Evaluation of the fungicidal activity of the resulting polysaccharide showed that the isolated pumpkin pectin exhibits an inhibitory effect on the growth of *Penicillium* fungi sp., unlike apple pectin. These results suggest the potential for practical use of pumpkin pectin in creating antifungal ointments, gels and patches with prolonged antimicrobial effects.

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Authors' contributions

Jassim designed the experiment, and Mamedov carried out the research. Rodionov supervised the research project. Koltsov, Rybin and Kalmykova reviewed the results and discussed, read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Did you use generative AI to write this manuscript? No.

References

1. Bush PL. Pectin: Chemical Properties, Uses and Health Benefits . P. L. Bush, Ed. New York: Nova Science Publishers; 2014.
2. Kuzmann E, Garg VK, De Oliveira AC, Klencsár Z, et al. Mössbauer study of the effect of pH on Fe valence in iron-polygalacturonate as a medicine for human anaemia. Radiation Physics and Chemistry. 2015;107:195-8. https://doi.org/10.1016/j.radphyschem.2014.11.001
3. Lara-Espinoza C, Carvajal-Millán E, Balandrán-Quintana R, López-Franco Y, Rascón-Chu A. Pectin and pectin-based composite materials: Beyond food texture. Molecules. 2018;23(4):942. https://doi.org/10.3390/molecules23040942 .
4. Minzanova ST, Mironov VF, Vyshtakalyuk AB, Tsepaeva OV, et al. Complexation of pectin with macro-and microelements. Antianemic activity of Na, Fe and Na, Ca, Fe complexes. Carbohydrate Polymers. 2015;134:524-33. https://doi.org/10.1016/j.carbpol.2015.07.034
5. Košťálová Z, Hromádková Z, Ebringerová A. Structural diversity of pectins isolated from the Styrian oil-pumpkin (*Cucurbita pepo* var. styriaca) fruit. Carbohydrate Polymers. 2013;93(1):163-71. https://doi.org/10.1016/j.carbpol.2012.05.017
6. Ptichkina NM, Markina OA, Rumyantseva GN. Pectin extraction from pumpkin with the aid of microbial enzymes. Food Hydrocolloids. 2008;22(1):192-5. https://doi.org/10.1016/j.foodhyd.2007.04.002
7. Yoo S, Lee B, Lee H, Lee S, et al. Structural characteristics of pumpkin pectin extracted by microwave heating. Journal of Food Science. 2012;77(11):C1169-73. https://doi.org/10.1111/j.1750-3841.2012.02960.x
8. Cui S, Y Chang. Emulsifying and structural properties of pectin enzymatically extracted from pumpkin. Food Science and Technology. 2014;58(2):396-403. https://doi.org/10.1016/j.lwt.2014.04.012
9. Quanhong L, Caili F, Yukui R, Guanghui H, Tongyi C. Effects of protein-bound polysaccharide isolated from pumpkin on insulin in diabetic rats. Plant Foods for Human Nutrition. 2005;60:13-6. https://doi.org/10.1007/s11130-005-2536-x
10. Nagai M, Sato T, Watanabe H, Saito K, Kawata M. Purification and characterization of an extracellular laccase from the edible mushroom *Lentinula edode*s and decolorization of chemically different dyes. Applied Microbiology and Biotechnology. 2002;60:327-35. https://doi.org/10.1007/s00253-002-1109-2
11. Ramachandran P, Dhiman AK, Attri S. Extraction of pectin from ripe pumpkin (*Cucurbita moschata* Duch ex. Poir) using eco-friendly technique. Indian Journal of Ecology. 2017;44(6):685-9.
12. Salima B, Seloua D, Djamel F, Samir M. Structure of pumpkin pectin and its effect on its technological properties. Applied Rheology. 2022;32(1):34-55. https://doi.org/10.1515/arh-2022-0124
13. Klebanova NA. Titrimetric determination of carboxyl groups of fermentation products of high and low-esterified pectins [Electronic resource] / N.A. Klebanova A.V. TarasovaV.A. Sedakova A.V. Klebanov . Problems of sustainable development of regions of the Republic of Belarus and neighboring countries: collection of scientific articles of the X I International Scientific and Practical Internet Conference ; 2022 - Access mode: https://www.elibrary.ru/item.asp?id=49514822.
14. Doynikova AI. Isolation of beet pectin. Preparation of pectin metal complexes with iron ions [Text]: bachelor's final qualifying work / Doynikova Anna Igorevna. - Kazan; 2020;122-:115-122.
15. Shuklova AS. Development of technology for producing pectin from plant raw materials *A rtemisia absintium l., Cerasus vulgaris l., C helidonium Majus l. and Mentha piperita* l . [Text]: master's final qualifying work / Shuklova A.S.- Lipetsk, 2020.
16. Torkova AA, Lisitskaya KV, Filimonov IS, Glazunova OA, et al. Physicochemical and functional properties of Cucurbita maxima pumpkin pectin and commercial citrus and apple pectins: A comparative evaluation. PloS One. 2018;13(9). https://doi.org/10.1371/journal.pone.0204261
17. Yang X, Zhao Y, Lv Y. Chemical composition and antioxidant activity of an acidic polysaccharide extracted from Cucurbita moschata Duchesne ex Poiret. Journal of Agricultural and Food Chemistry. 2007;55(12):4684-90. https://doi.org/10.1021/jf070241r
18. Košťálová Z, Aguedo M, Hromádková Z. Microwave-assisted extraction of pectin from unutilized pumpkin biomass. Chemical Engineering and Processing: Process Intensification. 2016;102:9-15. https://doi.org/10.1016/j.cep.2015.12.009
19. KrasnovaYu. V. Development of biotechnologies of functional food products based on pectin-whey gels [Text]: thesis. Ph.D. tech. Sciences: 05.18.07 / Yulia Valerevna Krasnova -M;2020; 150.