Bacteriostatic action of biopectin

Acción bacteriostática de la biopectina

Butova Svetlana Nikolaevna[1], Tarasova Veronika Vladimirovna[2], Shhegoleva Irina Dmitrievna[3], Musika Maxim Yurevich[4], Krasnova Yuliya Valerevna[5]

[1]Doctor of biological sciences, professor, head of the department «Biotechnology and technology of bioorganic synthesis products», Moscow State University of Food Production, 125080, Moscow, Volokolamskoe avenue, 11, tel.: +7(499)750-01-11 add. 72-01, 65-67; Email:vbutov@bk.ru, https://orcid.org/0000-0003-1398-1998

[2]Candidate of technical sciences, docent, docent of the department «Biotechnology and technology of bioorganic synthesis products», Institute of Engineering Physics, 142210, Moscow region, Serpukhov, Bol`shoj Udarny`j lane, 1a, tel.: +7(499)400-05-75; Email:sod@bk.ru, https://orcid.org/0000-0002-5722-9872

[3]Candidate of technical sciences, docent of the department «Biotechnology and technology of bioorganic synthesis products»; Email: shegoleva.mgupp@yandex.ru, https://orcid.org/0000-0003-2835-345X

[4]Senior lecturer of the department «Biotechnology and technology of bioorganic synthesis products»; Email: muzyka@mgupp.ru, https://orcid.org/0000-0003-2271-7638

[5]Chief technologist; Email: krasnova@iifmail.ru, https://orcid.org/0000-0003-2977-5486

Abstract

In recent times, a rapid development of industry and adverse environmental conditions have led to a deterioration in the health of the population. Thus, in order to limit the adverse effect of harmful environmental factors and eating disorders, the healthcare policies should be focused on using curative and preventive nutrition as a part of strategic approach to the well-being of the nation. The use of pectin-containing substances and additives is, in the opinion of experts, a promising trend in the modern food industry. Pectic substances, according to WHO, are absolutely safe and biocompatible with the tissues of human body. Health-promoting properties of pectic substances are now actively used in the treatment of various gastrointestinal, cardiovascular diseases and metabolic disorders. Today, one of the promising trends in biochemistry is enzymatic extraction of pectin from plant materials. This article is devoted to the study of sensory, biochemical, physicochemical and microbiological indicators of pectin obtained by the fermentation of black currant, citrus and apple pomace, zucchini, carrots and pumpkin, as well as to the study of the bacteriostatic activity of 'biopectin'. According to the results of all studies, it was found that all biopectins demonstrated bacteriostatic properties. The greatest bacteriostatic activity was shown by biopectin from black currant. The bacteriostatic activity of biopectin makes it possible to use it in food products with curative and preventive properties.

Key words: biopectin, bacteriostatic activity, microbiological indicators, microbial pathogens.

Resumen

En los últimos tiempos, un rápido desarrollo de la industria y las condiciones ambientales adversas han llevado a un deterioro en la salud de la población. Por lo tanto, para limitar el efecto adverso de los factores ambientales nocivos y los trastornos alimentarios, las políticas de atención médica deben centrarse en el uso de la nutrición curativa y preventiva como parte del enfoque estratégico para el bienestar de la nación. El uso de sustancias y aditivos que contienen pectina es, en opinión de los expertos, una tendencia prometedora en la industria alimentaria moderna. Las sustancias pécticas, según la OMS, son absolutamente seguras y biocompatibles con los tejidos del cuerpo humano. Las propiedades promotoras de la salud de las sustancias pécticas ahora se usan activamente en el tratamiento de diversas enfermedades gastrointestinales, cardiovasculares y trastornos metabólicos. Hoy, una de las tendencias prometedoras en bioquímica es la extracción enzimática de pectina a partir de materiales vegetales. Este artículo está dedicado al estudio de los indicadores sensoriales, bioquímicos, fisicoquímicos y microbiológicos de la pectina obtenidos por la fermentación de grosella negra, cítricos y orujo de manzana, calabacín, zanahorias y calabaza, así como al estudio de la actividad bacteriostática de la 'biopectina'. Según los resultados de todos los estudios, se encontró que todas las biopectinas demostraron propiedades bacteriostáticas. La biopectina de grosella negra mostró la mayor actividad bacteriostática. La actividad bacteriostática de la biopectina permite su uso en productos alimenticios con propiedades curativas y preventivas.

Palabras clave: biopectina, actividad bacteriostática, indicadores microbiológicos, patógenos microbianos.

Introduction

The current chemicalization of agriculture and rapid industrial development lead to the entrance of a large number of xenobiotics to the environment[8].

Curative and preventive nutrition occupies an important place in the scope of medical and biological measures against the effects of harmful environmental factors on human body[7].

The nutritional status of individuals and the population is the main indicator of the well-being and development of a country. The most important discoveries in all food science in the 20th century were based on the establishment of a link between nutritional habits and chronic cardiovascular, gastrointestinal diseases, allergies, etc.

The consumption of excessive amounts of animal fats, increased consumption of easily digestible carbohydrates, lack of complete proteins and dietary fiber can lead to eating disorders and even to a significant mortality of the population.

According to nutritionists, the human need for nutrients cannot be satisfied only by traditional food and diets.

On the other hand, the problem of food allergy and food intolerance, which have developed into a medical and social problem, is of particular importance: 30% of the world's population suffers from allergic diseases.

One of the most promising trends in modern medicine are health and sanitary measures that prevent diseases and retard their development. These measures include the use of biologically active substances (BAS) such as alkaloids, glycosides, polysaccharides, essential oils, organic acids, antibiotics, coumarins, quinones, flavonoids, etc., contained in certain plants.

According to numerous authoritative studies, pectic substances, pectin-containing additives and products hold an important position in the development of functional foods with health-promoting properties.

Literature Review

Analysis of data on dietary habitsof industrialized countries population showed a high intake of fats and sugars[3]. Poor nutrition leads to obesity, metabolic disorders, cardiovascular and gastrointestinal diseases, which is due to insufficient consumption of products containing dietary fiber, and in particular pectic substances[16].

The tissues of all higher plants contain two main forms of pectic substances - protopectin and pectin (hydropectin). Propectin is a strong combination of pectin with cellulose; its cleavage is an additional source of pectin production[12].

Pectic substances are natural anionic polysaccharides obtained from the cell walls of higher plants. Pectin (E440) is a group of high molecular heteropolysaccharides that makes up the primary cell walls and intercellular formations of higher plants[6]. Pectic substances are recognized by WHO as absolutely safe.s

Analysis of clinical material proves that patients receiving pectic substances showed a noticeable improvement in their health[13].

According to the recommendations of the Ministry of Health of the Russian Federation, the daily intake of pectic substances must be 3-4 g, and for people living in environmentally damaged regions, the norm should be 15-16 g per day[19].

To date, the numerous studies are underway to identify pectic substances, their structure, properties and methods of production. The pectin is of great importance in various sectors of the food industry: oil and fat production, bakery, confectionery, dairy industries, the production of beverages and food additives, - as well as in pharmaceutical industry and cosmetics production[9,12].

In the pharmaceutical industry, pectin is used in slow-release drugs and in biologically active food supplements that help lower cholesterol, stimulate the intestinal motility, eliminate toxic substances, reduce the risk of cancer and stimulate the growth of intestinal microflora.

In medicine, new pectin-based biomaterials have been developed to prevent adhesions after surgical interventions[9].

In the cosmetic industry, pectic substances act as water-holding and lift-restructuring agents, and also as anti-inflammatories.

Beneficial physiological effects and technological properties of pectic substances are widely used in food industry, as shown in Table. 1 [11,12,14,15,20].

Table 1 - The use of pectic substances in the food industry

Sector of food industry	Technological properties of pectin
Confectionery	Gelatinizing agent
Baking industry	Gelatinizing agent Improving agent Water-retaining agent Emulsifier
Beverage industry	Stabilizer Thickening agent
Canning industry	Jelling agent
Dairy industry	Stabilizer Additive prolonging the shelf life of food
Fat and oil production	Emulsifier Structure-forming additive Stabilizer
Beer brewing	Clarifying agent

Table 1 clearly shows that pectic substances have various technological functions and are used in almost all sectors of the food industry.

Pectic substances have numerous favorable physiological effects: complex-forming activity, based on the interaction of pectin molecules with metal ions (including heavy and radioactive metals); enhancing the cholesterol excretion from the body; reduction of allergic effects, regulation of metabolism and normalization of gastrointestinal tract functions; antitumor, antimetastatic, antimicrobial activities and others[2,5]. In addition, these hydrocolloids are excellent intestinal sorbents, due to their protective and prophylactic properties actively used in the treatment of various gastrointestinal, metabolic and cardiovascular diseases[10]. The target of pectin physiological action depends primarily on its composition, which is determined by the type of plant material from which they are produced. The most common plants containing pectin are apples, citrus pomace, sugar beet[1].

But it is worth noting that the assortment of pectin containing additives and products is very limited. This is primarily due to the acute shortage of pectin in the Russian food industry.

The pectin deficiency, in turn, is due to the fact that the pectin production capacity is not sufficient in Russia to meet the needs of food enterprises. The modern technologies of pectin extraction, including hydrolysis and use of corrosive acids, purification, concentration and drying of pectin extracts, are quite energy-intensive and insufficiently profitable[5].

About 95% of all pectin commercialized in Russia is imported. The world's leading producers of pectin are Herbstreith & Fox (Germany), Cargill (France), CP Kelco (Denmark), Danisco (Czech Republic), Andre Pectin (China). The German company Herbstreith and Fox (41%) was leader in the supply of pectin to Russian market in 2018[18].

Rationale

As noted above, the development of production technologies, studies on the structure and properties of pectic substances are promising and relevant areas of science.

One of the worthwhile methods of pectin production is enzymatic hydrolysis of waste plant materials. Pectin, produced with the help of enzymes, is called biopectin. The research of the pharmacological properties of biopectin is quite relevant today. In this regard, our work seeks to investigate the bacteriostatic ability of biopectin towards various microorganisms. The following tasks were set:

1. Analysis of scientific and technical literature on this topic.
2. Definition of organoleptic, physicochemical and biochemical indices of biopectins.
3. Definition of sanitary and microbiological indicators of biopectins.
4. Study of the biopectins effect on microorganisms causing pyoinflammatory diseases.

It was expected that biopectins would show bacteriostatic ability towards various microorganisms, which would allow to create therapeutic and prophylactic products enhancing the barrier functions of the body.

Materials
Biopectins obtained by the enzymatic method from apple, citrus, blackcurrant, carrot, squash and pumpkin were used in the experiments.

Equipment
Analytical balance Acculab ALC-210d4, counterbalance MASS VK-600, water bath Armed DK420, benchtop centrifuge EBA-20, electric cabinet drying 2B-151, muffle furnace PM-8, electric stove “Lazur”, Chizhhov’s modernized digital device PChMTs, paper and glass filters, pH-meter with glass electrode LP-3, photoelectric colorimeter KFK-2-UHL 4.2., ultrathermostat LT-111a, glass capillary viscometer VPG-1 with capillary diameter of 0.8 mm, thermostat air-dry TCO-1 / 80 SPU.

Methods

1. Methods used to evaluate the organoleptic, physicochemical and biochemical parameters of the biopectins

1.1. Organoleptic analysis of biopectins.

1.2. Ash content determination.

1.3. Moisture content determination.

1.4. Determination of the mass fraction of free and methoxylated carboxyl groups.

1.5. Determination of the mass fraction of pectic acid.

1.6. Determination of the mass fraction of ballast substances.

1.7. Determination of hydrogen concentration (Ph) in 1% pectin solution.

1.8. Determination of gelation temperature.

1.9. Determination of β-carotene content.

1.10. Determination of vitamin C content.

2. Determination of sanitary and microbiological indicators of biopectins:

2.1. Determination of the total number of bacteria by counting colonies of mesophilic aerobic and optionally anaerobic microorganisms grown on dense nutrient agar at 30 ° C during 72 hours.

2.2. Detection of yeasts and molds when on Saburo selective agar medium at 24 ° C for 5 days.

3. Plating of microbial suspension with the addition of biopectin on beef-extract agar.

At the first stage, organoleptic, physicochemical and biochemical tests were carried out in order to establish the qualitative characteristics of the biopectins.

Then, the microbiological analysis of biopectins was carried out. For this purpose, the dilution 1:10 in normal saline was prepared, then the fermentation and coli titer were defined. Then the streak inoculation on the Endo medium was made. The test plates with the covers down were placed in a thermostat for 18–24 hours at a temperature of 37°C. The total number of bacteria was determined by counting colonies of mesophilic aerobic and optionally anaerobic microorganisms grown on dense nutrient agar at 30°C for 72 hours. The amount of yeast and molds contained in biopectins was measured by inoculation on Saburo medium at a temperature of 24°C for 5 days.

At the final stage of the work, the effect of biopectins on microorganisms causing pyoinflammatory diseases was investigated. The discharge from a septic wound was put into normal saline and then inoculated on the beef-extract agar. The plates with agar were placed in a thermostat for 20 hours. Then a suspension of early bacterial cultures was prepared. To do this, 5 cm^3 of sodium chloride isotonic solution were added to a test tube with the culture grown on agar, and thoroughly mixed. The number of microbial bodies in 1 cm^3 was counted. Next, the suspension with the number of microbial bodies 107 CFU / cm^3 was prepared. In 9 cm^3 of a 1% solution of biopectin and in 9 cm^3 of a 5% biopectin, 1 cm3 of microbial suspension was introduced and then plated on beef-extract agar immediately after application, and after 2, 4, 24, 48 and 72 hours. The experiment was carried out at the temperature of 37 ° C. During the control experiment, normal saline was used instead of biopectin solution.

Discussion and Results

The results of biochemical, organoleptic and physic-chemical testing are shown in Table 2.

It has been established that citrus and apple biopectins comply with the requirements of GOST 29186[4]. Blackcurrant, carrot, zucchini and pumpkin biopectins have no regulatory and technical requirements, since these plant materials are classified as having no industrial value. These types of biopectins have acceptable physic-chemical parameters. The highest degree of etherification was demonstrated by zucchini pectin, the lowest – by black currant pectin. The physic-chemical parameters of pectin depend on plant materials, extraction conditions and the balance of functional groups.

The microbiological indicators of pectin were monitored for the following groups of microorganisms:

• QMAFAnM and coliform bacteria;

• Opportunistic pathogens, including Escherichia coli;

• Microorganisms causing food spoilage (yeast and mold).

The microbiological parameters of biopectins are within the normal range.

The effect of biopectins on pathogenic microorganisms taken from purulent wounds was also subject of the current study. The results for apple and citrus pectin are shown in Table 4.

Table 2 – The main indicators characterizing biopectins

Indicator	Plant materials used for biopectin production					
	Black currant	Citrus fruits	Apple	Zucchini	Carrot	Pumpkin
Moisture content, %	4,70	5,59	4,90	4,50	5,20	4,70
Ash content, %	1,60	1,70	1,50	1,20	1,60	1,30
pH of 1% solution	3,20	3,10	3,40	4,00	3,50	4,00
Acetyl groups content, %	2,10	1,20	1,30	0,17	1,30	0,62
Carboxyl content, %	17,30	10,00	11,3	2,32	2,98	2,57
Methoxyl content, %	6,70	9,20	8,30	10,05	11,15	10,89
Etherification degree, %	63,00	72,00	68,00	81,20	78,90	80,90
Pectic acid content, %	40,00	45,00	46,00	37,0	40,00	39,00
β-carotene content, mg/100 g	¾	¾	¾	¾	19,88	4,31
Vitamiv C content, mg/100 g	85,00	21,20	10,70	2,80	3,50	¾
Ballast substances content, %	32,00	29,00	29,90	29,50	30,20	30,00
Gelation temperature, °C	60,00	84,00	84,00	79,00	74,00	78,00
Appearance	powder					
Colour	Maroon	Light yellow	cream	Light yellow	Light-orange	Yellow
Taste	no					
Odour	no					

Table 3 – Microbiological indicators of biopectins

Plant material	QMAFAnM CFU/g (not more than)	coliform bacteria		Yeast CFU/g (not more than)	Mold fungi CFU/g (not more than)
		Growth on Kessler medium	Fermentation titer		
Apple	2,1´10[4]	no	>0,1	—	9
Citrus fruits	3,0´10[4]	no	>0,1	—	13
Black currant	8,0´10[4]	no	>0,1	—	11
Carrot	2,6´10[4]	no	>0,1	—	13
Pumpkin	5,0´10[4]	no	>0,1	—	7
Zucchini	1,1´10[4]	No5	>0,1	—	19

Table 4: Apple and citrus biopectin effect on pathogenic microorganisms causing pyoinflammatory diseases

	Apple pectin		Citrus pectin	
		CFU/cm^3		CFU/cm^3
Immediately	Control measurement	2,8×10^6	Control measurement	3,0×10^6
	1% pectin solution	2,8×10^6	1% pectin solution	3,1×10^6
	5% pectin solution	2,7×10^6	5% pectin solution	3,0×10^6
In 2 hours	Control measureent	2,8×10^6	Control measurement	3,3×10^6
	1% pectin solution	2,7×10^6	1% pectin solution	3,0×10^6
	5% pectin solution	1,0×10^6	5% pectin solution	2,2×10^6
In 4 hours	Control measurement	3,0×10^6	Control measurement	3,9×10^6
	1% pectin solution	2,3×10^6	1% pectin solution	2,8×10^6
	5% pectin solution	8,0×10^5	5% pectin solution	9,0×10^5
Через 24 часа	Control measurement	3,6×10^6	Control measurement	4,6×10^6
	1% pectin solution	1,6×10^6	1% pectin solution	1,3×10^6
	5% pectin solution	1,1×10^5	5% pectin solution	3,6×10^5
In 48 hours	Control measurement	4,2×10^6	Control measurement	5,3×10^6
	1% pectin solution	7,0×10^5	1% pectin solution	9,0×10^5
	5% р-р пектина	5,0×10^4	5% р-р пектина	8,4×10^4
In 72 hours	Control measurement	4,8×10^6	Control measurement	6,2×10^6
	1% pectin solution	4,0×10^5	1% pectin solution	7,3×10^5
	5% pectin solution	2,0×10^4	5% pectin solution	2,8×10^4

Table 5: Blackcurrant and carrot biopectin effect on pathogenic microorganisms causing pyoinflammatory diseases pectin solution

	Blackcurrant pectin		Carrot pectin	
		CFU/cm^3		CFU/cm^3
Immediately	Control measurement	2,4×10^6	Control measurement	3,0×10^6
	1% pectin solution	2,5×10^6	1% pectin solution	3,1×10^6
	5% pectin solution	2,1×10^6	5% pectin solution	3,0×10^6
In 2 hours	Control measurement	2,8×10^6	Control measurement	4,2×10^6
	1% pectin solution	1,9×10^6	1% pectin solution	3,0×10^6
	5% pectin solution	8,0×10^5	5% pectin solution	2,2×10^6
In 4 hours	Control measurement	3,1×10^6	Control measurement	4,8×10^6
	1% pectin solution	1,1×10^6	1% pectin solution	2,6×10^6
	5% pectin solution	3,8×10^5	5% pectin solution	9,0×10^5
In 24 hours	Control measurement	4,5×10^6	Control measurement	5,4×10^6
	1% pectin solution	9,0×10^5	1% pectin solution	1,3×10^6
	5% pectin solution	7,0×10^4	5% pectin solution	3,6×10^5
In 48 hours	Control measurement	6,0×10^6	Control measurement	5,8×10^6
	1% pectin solution	6,0×10^5	1% pectin solution	9,0×10^5
	5% pectin solution	2,1×10^4	5% pectin solution	8,0×10^4
In 72 hours	Control measurement	6,8×10^6	Control measurement	6,4×10^6
	1% pectin solution	4,0×10^5	1% pectin solution	6,0×10^5
	5% р-р пектина	1,3×10^4	5% pectin solution	1,8×10^4

Exhibits 1 and 2 illustrate the effect of pumpkin and zucchini biopectins on pathogenic microorganisms.

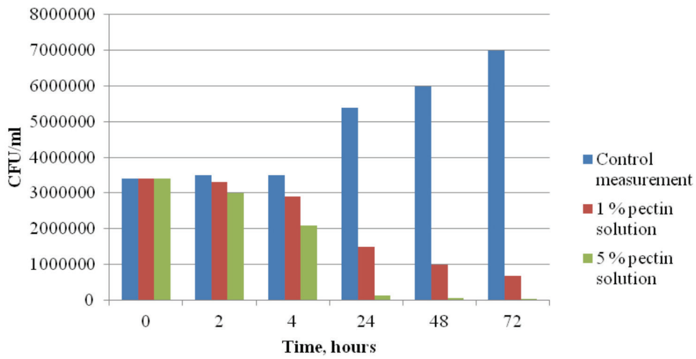

Exhibit 2: Effect of pumpkin pectin on pathogenicmicroorganisms

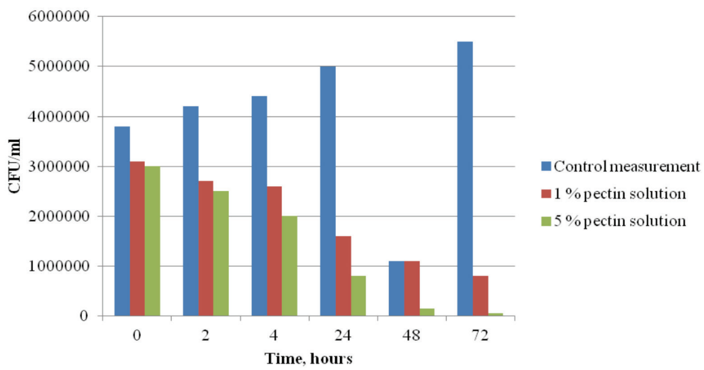

Exhibit 3: Zucchini pectin effect on pathogenic microorganisms

Based on the data, it can be stated that all the biopectins show a bacteriostatic effect on pathogenic microorganisms. They are active 2 hours after the beginning of the experiment. In 72 hours, the pathogenic microflora was significantly suppressed, and the best results were achieved with the use of 5% blackcurrant biopectin solution.

Conclusions

1. The basic quality indicators of tested biopectins meet all the requirements of regulatory documents and technical standards.
2. The biopectins used in the experiments meet the microbiological requirements.
3. Biopectins produced from any kind of plant material demonstrate bacteriostatic activity towards pathogenic microorganisms.
4. The blackcurrant biopectin is characterized by the highest bacteriostatic activity.
5. The pharmacological properties of biopectins, including their bacteriostatic activity, give grounds to their use in the production of therapeutic food.

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