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RESEARCH OF SORPTION PROPERTIES OF APPLE PECTIN AND ITS INFLUENCE ON THE LEVEL pH STOMACH IN CONDITIONS IN VITRO

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Abstract

Pectin contains about 70% galacturonic acid. The exact chemical structure of pectin has not yet been definitively established, but three main pectin polysaccharides from the plant wall have been isolated and identified. These are homogalacturonans, which contain about 65%, rhamnogalacturonans - 20-35% and substituted galacturonans.

In order to determine the possible mechanism of action of apple pectin, the pH was determined in mixtures that mimic the contents of the stomach, small and large intestine with the addition of hydrochloric acid and alcohol. To determine the pH in the stomach used 0.1 N solution HCl, of a small bowel - the buffer hydrocarbonate with pH 7,5, a large intestine - the buffer hydrocarbonate with pH 8,5.

In vitro models simulating the environment of different parts of the gastrointestinal tract have shown that apple pectin, in contrast to activated carbon and silicon dioxide, exhibits buffering properties, and when alcohol is administered causes a shift in pH to the acidic side. As a result of determining the zero charge point of apple pectin, it can be stated that at pH above 3.69 it adsorbs cations of metals or organic substances, which explains the ability to bind ethanol.

Keywords: *apple pectin, sorption properties.*

Introduction

Pectin contains about 70% galacturonic acid [1]. The exact chemical structure of pectin has not yet been definitively established, but three main pectin polysaccharides from the plant wall have been isolated and identified. These are homogalacturonans, which contain about 65%, rhamnogalacturonans - 20-35% and substituted galacturonans [2, 3, 4].

In the human body, pectin, as a dietary fiber, is not enzymatically digested in the small intestine, but is broken down by the microbiota of the colon. [5, 6, 7]. Forming gels with water, it retains the gelling properties in the gastrointestinal tract, which slows down digestion. In the process of hydrolysis of pectin there is a gradual cleavage of methoxyl groups (demethoxylation) [19]. Completely demethoxylated pectin is called "pectic acid". Between pectin and pectic acid there are a number of intermediate decomposition products of varying degrees of demethoxylation, which are present in the natural mixture of pectin substances. It is clear that pectin has an acidic reaction – pH = 3,3-3,7 [7], which is stored when mixed with distilled water, which also has a weakly acidic reaction [8, 9].

Given the acid reaction of pectin, it was interesting to determine the probability of its interaction with alcohol and the possible neutralization of the latter, as one of the mechanisms of action in alcohol intoxication.

Materials and Methods

In order to determine the possible mechanism of action of apple pectin, the pH was determined in mixtures that mimic the contents of the stomach, small and large intestine with the addition of hydrochloric acid and alcohol. To determine the pH in the stomach used 0.1 N solution HCl [10], of a small bowel - the buffer hydrocarbonate with pH 7,5, a large intestine - the buffer hydrocarbonate with pH 8,5 [11].

Measurements were performed at a temperature of 200C and 350 C using a laboratory ionomer И-160-M.

The ratio of solutions and dosage of apple pectin, activated charcoal and silicon dioxide in all samples was the same.

To clarify the possible mechanism of action of apple pectin, the physicochemical properties of apple pectin were determined.

In order to determine the point of zero charge of nuclear power plants, measurements were performed in the laboratory of the Department of Chemistry of Vasyl Stefanyk Precarpathian National University.

Statistical processing of the data obtained as a result of the experiments was performed using a software environment for statistical calculations R [12], which is distributed under a free license, and «Excel for Windows» using the add-on «Analysis package» (Microsoft Office 2016, with a license key MFM9H-PNXDT-FKJRM-R8Q7Q-3PFHM). The obtained quantitative data corresponded to the normal and abnormal type of distribution (Shapiro-Wilk test), and therefore the interval M ± m was chosen for the description, and the Student's parametric test was used to check the reliability of the data of the studied groups with the control. The data were presented as M± m, where M – arithmetic mean, m – standard error of the arithmetic mean. Since there were 5 comparison groups in the study, the reliability of the difference between the obtained data in these groups was assessed using ANOVA analysis (functions aov() package stats with R) , namely post hoc test (fuction TukeyHSD [12] in package stats with R). Probability $p \leq 0{,}05$ considered sufficient to conclude on the statistical significance of the difference in the data obtained.

Results and Discussion

Our research showed the following (Table 1). Mixing apple pectin with distilled water shows the pH level of apple pectin and sets a value of 3.7 by 200 C and decreases to 3.4 by 350 C, consistent with the literature [13, 14]. The combination of malic pectin with hydrochloric acid causes a clear increase in acidity to pH = 2.0 at both temperatures.

Mixing of apple pectin with alcohol showed an increase in pH to 4.3, and when introduced into this mixture of distilled water, a decrease in pH = 3.6 was observed by 200C and 3,2 by 350C, which can be explained by changes in the physicochemical properties of apple pectin and an increase in active dissolved galacturonic acid residues.

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Addition to the mixture of hydrochloric acid and alcohol of the comparison agent of activated carbon changed the reaction to 4.2, and when introduced into the mixture of silicon dioxide - to pH = 4.4 and these values did not change with increasing temperature of the test samples.

The mixture of malic pectin with hydrochloric acid and alcohol caused a significant shift of pH to the acidic side: changed the alkaline reaction of alcohol from 5.5 to 2.17 (200C) and 2.0 (350C), that is, more than twice as much as the reference drugs. It is possible that the neutralization reaction reduces the concentration, absorption and subsequent toxic effects of alcohol.

Since the bulk of ingested alcohol is absorbed in the small intestine, we were interested to know about the effect of apple pectin on the pH in the small and large intestine. In order to simulate the environment of the small intestine used bicarbonate buffer with a pH of 7.5.

It is noted that when a mixture of apple pectin with water and hydrochloric acid to the "small intestine" with pH of 7.5 to 200C there was a significant shift of the reaction to the alkaline side, although the reaction was still weakly acidic and remained so with the introduction of alcohol (Table 2). The combination of an aqueous apple pectin solution with a buffer with pH = 7.5 also gave an acidic reaction - 6.8 at room temperature and the addition of alcohol did not change this position. At the same time, the increase in the temperature of the mixture to 350C caused a significant shift of pH to the acidic side, both with the addition of HC1 and without it: 4.26 and 4.69, respectively.

It should be noted that in mixtures of bicarbonate buffer with water and activated carbon and silicon dioxide, the pH did not change at 200 C, and with the addition of alcohol to them slightly increased - up to 7.6. At the same time, the increase of the temperature of the studied mixtures to 350C caused a sharp shift of the pH in the mixtures with activated carbon to the alkaline side - to 9.85 and 9.94 with the introduction of alcohol. In mixtures with silicon dioxide, a slight decrease in pH was noted, which can be explained by the weakly acidic reaction of this reference drug.

The result of measuring the activity of hydrogen ions by mixing a buffer solution, an aqueous solution of apple pectin, 0.1 N HCl solution and 40% ethanol solution was interesting for us. It is in this mixture that we obtained the most acidic reaction at both temperatures (Table 2).

Presumably, the sharp shift of the pH to the acidic side in the studied samples with apple pectin at a temperature of 350 C is explained by an increase in solubility and fluidity, a decrease in viscosity and an increase in the number of free anionic groups of galacturonic acid.

In our opinion, the results of the study of pH changes during the reproduction of environmental conditions in the lumen of the colon were also interesting (Table 3).

Aqueous solution of apple pectin when mixed with water and bicarbonate buffer, simulating the environment of the colon with pH = 8.5, caused a sharp shift of the reaction to the acidic side at both temperatures (Table 3).

At the same time, when the same buffer solution was combined with water and reference drugs, the reaction remained alkaline and hardly changed with the introduction of silicon dioxide and became more alkaline in combinations of bicarbonate with water and activated carbon and alcohol.

Upon introduction to the above aqueous solutions of alcohol, an increase in pH was observed in the sample with apple pectin - up to 4.3 (3.85 in the absence of alcohol) at 200 C and at 350C - up to 4.0.

Therefore, in the studied samples with the comparison drugs, the pH level was equal to or slightly higher than that of the bicarbonate buffer.

To explain the obtained results and to find out the possible mechanisms of action of apple pectin, the point of zero charge of pectin was determined. The measurement was performed in the laboratory of the Department of Chemistry of Vasyl Stefanyk Precarpathian National University.

The point of zero charge, or isoelectric point, is the pH value characteristic of a certain substance, at which the maximum number of ions of this substance in solution acquires zero electric charge, and the substance is the least mobile in the electric field. [2,15,16].

The pH value of the zero charge point (pHpoint of the zero charge) is a very important characteristic of the surface of materials. The pHpoint of the zero charge influence significantly affects the processes of absorption and desorption of ions present in solutions in cationic and anionic forms [15, 16, 17].

If the value pH solution below pHpoint of the zero charge, then the material absorbs mainly anions (see equation 1); if the pH of the solution will exceed pHpoint of the zero charge, then the substance adsorbs mainly metal cations or cations of organic compounds (equation 2).

$$=M{-}OH \ + \ H^+ \xrightarrow{\text{protonation}} =M{-}OH_2^+$$

(pH < pH point of the zero charge) (1)

$$=M{-}OH \ + \ OH^- \xrightarrow{\text{deprotonation}} =M{-}O^- \ + H_2O$$

(pH > pH point of the zero charge) (2)

Thezero point of charge of apple pectin on the pH scale is 3.75. According to a study at pH above 3.69, pectin adsorbs cations of metals or organic substances.

The result confirms the ability of apple pectin to bind (adsorb) alcohol ([C2H5O-]H)+.

According to the results of in vitro studies, it was found that the mixing of apple pectin with alcohol under conditions of simulation of the environment of the gastric lumen and at different temperatures (200C i 350C) there was an increase in pH to 4.3, and when introduced into this mixture of distilled water, a decrease in pH = 3.6 was observed, which can be explained by changes in the physicochemical properties of apple pectin and an increase in active dissolved galacturonic acid residues.

The mixture of malic pectin with hydrochloric acid and alcohol caused a shift of the pH to the acidic side almost twice, compared with activated carbon and silicon dioxide at both temperatures. Presumably, the neutralization reaction occurs precisely due to absorption, which leads to a decrease in concentration, absorption and subsequent toxic effects of alcohol.

When reproducing the environment of the small intestine, the combination of an aqueous solution of apple pectin with a buffer with pH = 7.5 gives a weakly acidic reaction - 6.8 and the addition of alcohol does not change this position at 200C . Increasing the temperature of mixtures to 350C leads to a significant shift in pH acidic side in the presence of apple pectin (1.5-2.2 units) and pH increase in the presence of activated carbon (more than 2.3 units).

An aqueous solution of apple pectin when mixed with bicarbonate buffer with pH = 8.5, which mimicked the environment of the colon, caused a sharp shift in the reaction to the acidic side. At the same time, when the same buffer solution was combined with water and reference drugs, the reaction remained alkaline and hardly changed at both temperatures.

The point zero charge of apple pectin, which determines the sorption capacity, on the pH scale is 3.75. According to the results of the study, it can be stated that at pH above 3.69 apple pectin adsorbs cations of metals or organic substances, which explains the ability of apple pectin to bind (adsorb) alcohol ([C2H5O-]H)+.

Conclusions. In vitro models simulating the environment of different parts of the gastrointestinal tract have shown that apple pectin, in contrast to activated carbon and silicon dioxide, exhibits buffering properties, and when alcohol is administered causes a shift in pH to the acidic side. As a result of determining the zero charge point of apple pectin, it can be stated that at pH above 3.69 it adsorbs cations of metals or organic substances, which explains the ability to bind ethanol.

References

1. Taran N.G. Adsorbents and ion exchangers in the food industry. M .: Light and food industry, 1983: 248 p.
2. Pectins: properties, preparation, application. [Internet]. Available from: http://newchemistry.ru/letter.phpn?_id=6344.
3. Fedorenko Yu.V. Protective role of pectin and calcium in separate and combined action of lead and fluorine. Experimental and clinical physiology and biochemistry. 2003; 4: 13-18.
4. Mohnen D. Pectin structure and biosynthesis. Current Opinion in Plant Biology. 2008; 11: 266–277. [Internet]. Available from: www.sciencedirect.com.
5. Jensen N.S., Canale-Parola E. Nutritionally limited pectinolytic bacteria from the human intestine. Appl Environ Microbiol. 1985;50:172–173.
6. Jensen N.S., Canale-Parola E. Bacteroides pectinophilus sp. nov. and Bacteroides galacturonicus sp. nov.: two pectinolytic

bacteria from the human intestinal tract. Appl Environ Microbiol. 1986;52:880–887.
7. Lopez-Siles M., Khan T.M., Duncan S.H., Harmsen HJM, Garcia-Gil L.J., Flint H.J. Cultured Representatives of Two Major Phylogroups of Human Colonic Faecalibacterium prausnitzii Can Utilize Pectin, Uronic Acids, and Host-Derived Substrates for Growth. Appl Environ Microbiol. 2012; 78(2): 420–428. doi: 10.1128/AEM.06858-11.
8. Baranova I.I., Zaporozhskaya S.N. Comparative characteristics of reoparameters of gelling agents of different origin. Zaporishs.med.journal. 2008; 4 (49): 81-84.
9. Bugrov O.D., Martyniuk I.M. Dynamics of pH indicators in distilled, double-distilled and pyrogen-free water during storage [Internet]. Available from: http://stattionline.org.ua/agro/109/20603-dinamika-pokaznikiv-ph-u-distilovanij - bidistilovanij-i-apirogennij-vodi-v-procesi-zberigannya.html.
10. Gastric juice. Wikipedia. [Internet]. Available from: https://uk.wikipedia.org/ wiki / gastric juice.
11. Physiological, biochemical and biometric indicators of the norm of experimental animals. Directory. SPB. "LEMA"; 2013: 116.
12. R Core Team. R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. -2018. URL https://www.R- project.org/.
13. Caffall K.H., Mohnen D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydrate Research.[net]. 2009; http://plantometrics.com/files/biblio_attachments/ Caffall,K (2009) Pectin-review.pdf.
14. Dongowski G., Lorenz A., Proll J. The Degree of Methylation Influences the Degradation of Pectin in the Intestinal Tract of Rats and In Vitro. J. Nutr. 2002;132:1935–1944.
15. Makarevich N.A., Bogdanovich N.I. Theoretical foundations of adsorption: a textbook. North (Arctic.) Feder. Univ. M.V. Lomonosov. Arkhangelsk: SAFU; 2015: 362 p.
16. Parfit D., Rochester K. Editors. Adsorption from solutions on solid surfaces. M .: Mir; 1986: 488.
17. Pogorely V.K. Regularities of adsorption of natural bioactive compounds on the surface of nanodispersed silica. Surface. 2009; 1 (16): 322-349.

Table 1. pH values of water, alcohol 40%, 0.1 N HCl solution and their mixtures with apple pectin and comparison drugs

Substances and their mixtures	pH, 200 C	pH, 350 C
Distilled water	5,8	5,8
Alcohol 40 %	5,5	5,5
0.1 N solution HCl	1,0	1,0
apple pectin + water	3,7	3,4
apple pectin + 0,1 N solution HCl	2,0	2,0
apple pectin + water + alcohol 40 %	3,6	3,2
apple pectin + alcohol 40 %	4,3	4,1
apple pectin + 0,1 N solution HCl + alcohol 40 %	2,17	2,0
charcoal + 0,1 N solution HCl + alcohol 40 %	4,2	4,2
silicon dioxide + 0,1 N solutio HCl + alcohol 40 %	4,4	4,5

Table 2. The effect of apple pectin and comparison drugs on pH under conditions of simulation of the small intestine and the introduction of 40% ethanol solution

Substances and their mixtures	pH, 20° C	pH, 35° C
Buffer solution pH 7,5 + water + apple pectin + 0,1 N solutio HCl	6,64	4,26
Buffer solution pH 7,5 + water + apple pectin	6,8	4,69
Buffer solution pH 7,5 + water + charcoal	7,5	9,85
Buffer solution pH 7,5 + water + silicon dioxide	7,5	6,59
Buffer solution pH 7,5 + water + apple pectin + 0,1 н розчин HCl + alcohol	6,0	4,35
Buffer solution pH 7,5 + water + apple pectin + alcohol	6,8	4,73
Buffer solution pH 7,5 + water + charcoal + alcohol	7,6	9,94
Buffer solution pH 7,5 + water + silicon dioxide + alcohol	7,6	7,03

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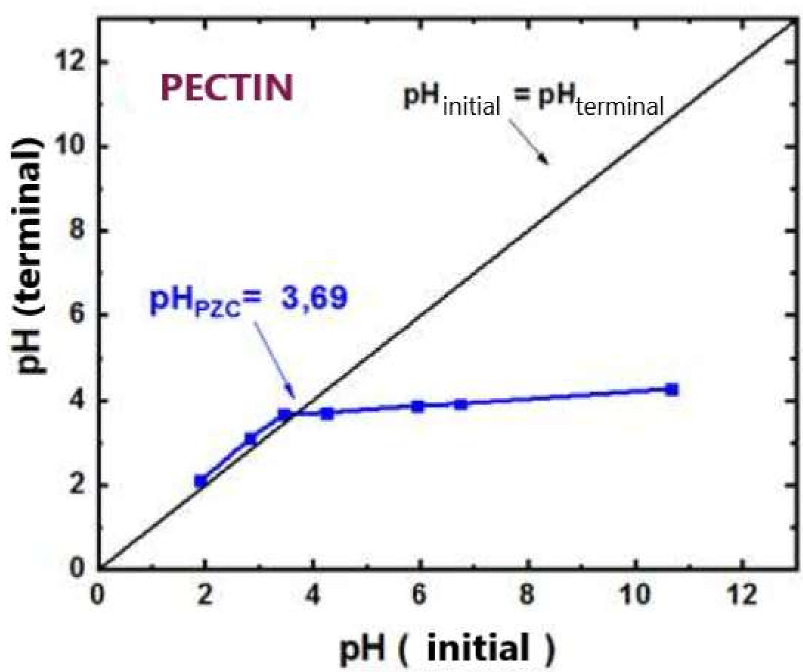
Table 3. The effect of apple pectin and comparison drugs on the pH under the conditions of simulation of the environment of the colon and the introduction of 40% ethanol solution

Substances and their mixtures	pH, 200 C	pH, 350 C
Buffer solution pH 8,5 + water + apple pectin	3,85	3,6
Buffer solution pH 8,5 + water + charcoal	8,4	8,9
Buffer solution pH 8,5 + water + silicon dioxide	8,4	8,2
Buffer solution pH 8,5 + water + apple pectin + ethanol solution	4,3	4,0
Buffer solution pH 8,5 + water + charcoal + ethanol solution	8,5	9,3
Buffer solution pH 8,5 + water + silicon dioxide + ethanol solution	8,5	8,2

Figure 1. The point of zero charge of the pectin surface



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