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INTERACTION OF PECTIN SUBSTANCES WITH COPPER, MERCURY, ZINC, AND CADMIUM SALTS

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The interaction of the sage, mint, apple, and ginseng pectins, isolated from tissue culture wastes and purified with copper, mercury, zinc, and cadmium salts, has been studied by the amperometric method with two metal indicator electrodes. The optimum conditions of titration have been determined: pH 3.5-5.0; concentration of pectin substances $5 \cdot 10^{-5}$-$1 \cdot 10^{-3}$ g/ml of solution. It has been established by graphical and mathematical methods that the interaction is accompanied by the formation of compounds with a ratio of the carboxy groups of pectins to the metal cation of two. The IR spectra of sage pectin and of copper and mercury pectinates are given.

The wastes from various enterprises of the foodstuff and pharmaceutical industries contain pectin substances (PSs) which are natural ion-exchangers capable of replacing the hydrogen of the carboxy groups by cations of multivalent metals. This property is used in the prophylaxis of poisoning by heavy-metal salts [1-7]. The inclusion of sugar beet pectin in the food ration of people coming into contact in their work with lead and other poisonous metals is obligatory. The use in various ways of radioactive isotopes in the national economy has expanded the limits of the protective action of pectin substances still more widely [9, 10].

Up to the present, there has been no single opinion on the mechanism of the action of pectins and the nature of the compounds formed with metal cations. This is due to the fact that, depending on the raw material source and the method of isolation, the PSs have different chemical structures. Some authors consider that the binding of cations by polygalacturonic acids takes place through the carboxy and hydroxy groups [4]. According to investigations by G. B. Aimukhamedova et al., Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} form with pectins compounds in which, in addition to the carboxy groups, the hydroxy groups of the macromolecules participate [10].

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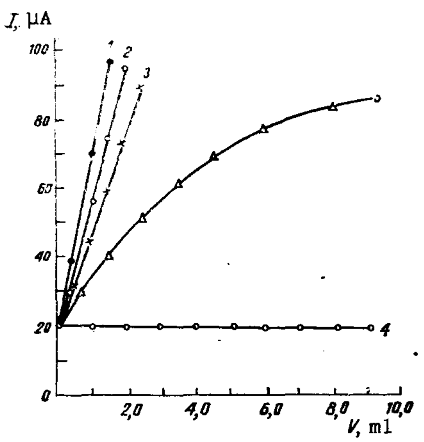

Fig. 1

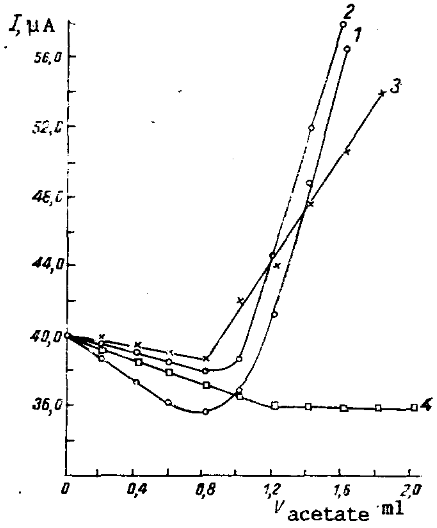

Fig. 2

Fig. 1. Dependence of the current strength on the volume of copper (1), cadmium (2), zinc (3), and mercury (4) acetates and of sage pectin (5); C_{PS} = 0.05%, $C_{metal\ acetate}$ = 0.2%.

Fig. 2. Curves of the amperometric titration (with two indicator electrodes) of sage pectin with copper (1), cadmium (2), zinc (3), and mercury (4) acetates. Volume of the pectin solution 10 ml. 1) $C_{PS} = 1.88 \cdot 10^{-3}$ M, $C_{Cu^{2+}} = 9.72 \cdot 10^{-3}$ M; 2) $C_{PS} = 1.68 \cdot 10^{-3}$ M, $C_{Cd^{2+}} = 7.60 \cdot 10^{-3}$ M; 3) $C_{PS} = 1.90 \cdot 10^{-3}$ M, $C_{Zn^{2+}} = 1.14 \cdot 10^{-2}$ M; 4) $C_{PS} = 2 \cdot 10 \cdot 10^{-3}$ M, $C_{Hg^{2+}} = 9.0 \cdot 10^{-3}$ M. (The concentrations of pectin substances are given in terms of carboxy groups).

TABLE 1. Concentration (C) and Volume (V) of the Reactants and the COOH:Me^{2+} from the Results of Amperometric Titration (n = 4, P = 0.95)

$Me(CH_3COO)_2$	C_{PS} M	$C_{Me^{2+}}$, M	V_{PS}, ml	$V_{Me^{2+}}$, ml	COOH:Me^{2+}
$Cu(CH_3COO)_2$	$1,88 \cdot 10^{-3}$	$9,72 \cdot 10^{-3}$	10,0	1,00	1,93±0,03
			15,0	1,52	1,91±0,03
$Hg(CH_3COO)_2$	$2,10 \cdot 10^{-3}$	$9,01 \cdot 10^{-3}$	10,0	1,20	1,94±0,03
			15,0	1,80	1,94±0,04
$Zn(CH_3COO)_2$	$1,90 \cdot 10^{-3}$	$1,14 \cdot 10^{-2}$	10,0	0,82	2,03±0,04
			15,0	1,23	2,03±0,03
$Cd(CH_3COO)_2$	$1,68 \cdot 10^{-3}$	$7,60 \cdot 10^{-3}$	10,0	1,02	2,17±0,05
			15,0	1,54	2,15±0,04

In this paper we give the results of an investigation of the interaction of the number of PSs (sage, apple, mint, ginseng) with copper, mercury, zinc, and cadmium salts, by the amperometric method with two indicator electrodes. The amounts of free (K_f) and methoxylated (K_e) carboxy groups and the degree of esterification (λ) of the pectin substances are given below:

Source of PSs	M, y. e.	K_f, %	K_c, %	λ
Sage	28500	9.15±0.09	6.21±0.09	40.43
Zarya mint	28900	8.41±0.16	5.77±0.07	40.69
Apples	25100	9.60±0.08	6.54±0.09	40.52
Ginseng	29000	4.62±0.09	5.81±0.11	55.71

TABLE 2. Positions and Assignments of the Bands (cm^{-1}) in the IR Spectra of Sage Pectin and of Copper and Mercury Pectinates

Sage pectin	Copper pectinate	Mercury pectinate	Nature of the vibration*
3350	3350	3350	$\nu(OH)$
2900	2900	2890	$\nu(CH)_r$
1740	1745	1745	$\nu(C=O)$
1640	—	—	$\delta(H_2O)$
—	1610	1595	$\nu_{as}(COO^-)$
1440	1440	1440	$\delta_{as}(CH_3)_E$
—	1410	1400	$\nu_s(COO^-)$
1370	1380	1380	$\delta_s(CH_3)_E$
1330	1320	1320	$\delta(CH)_r$
1225	1220	1220	$\nu(C{-}O{-}C)_E$, $\delta(OH)$, $\delta(CH)_r$
1140	1140	1140	$\nu(C{-}O{-}C)$
1095	1080	1080	$\nu(C{-}C)$ $(C{-}O)_r$ ν, $\delta(C{-}OH)_a$
1045	1045	1045	$\nu(C{-}C)$ $(C{-}O)_r$
1020	1020	1010	$\nu(C{-}C)$ $(C{-}O)_r$
955	955	950	$\gamma(OH)_a$
880	885	880	
835	835	835	$\gamma(OH)_a$
765	770	770	
635	635	635	

*E – ester groups; r – groups of pyranose rings; a – alcohol groups.

The relative error of the detrmination at a confidence level of 0.95 and a number of determinations of 5 does not exceed ±2.0%.

We first studied the behavior of solutions of metal acetates and PSs at the corresponding electrodes. As can be seen from Fig. 1, the current strength was directly proportional to the concentration of Cu^{2+}, Cd^{2+}, and Zn^{2+} salts (curves 1-3). For mercury cations (curve 4) no change in current was observed. For the solutions of PSs, a more complex dependence of the current strength and the concentration was obtained (curve 5), which is explained by the simultaneous occurrence of two processes, due to the capacity for the hydrogen cations formed on the dissociation of the carboxy groups for being reduced at the cathode [11], in addition, to the surface-active properties of the PSs, leading to absorption on the metal electrodes and to the blockage of the surface of the latter [12].

Figure 2 shows the course of the titration curves of sage pectin with the metal acetates investigated. As can be seen from Fig. 2, in the case of the cations Cu^{2+}, Cd^{2+}, and Zn^{2+}, both substances participated in the electrode reaction. With an excess of pectin in the solution the change in the current strength was only slight; however, when an excess of the cation was added to the solution the current rose sharply, since these cations are more electroactive. The curves of the titration of a pectin solution with mercury acetate have a different form. After the end-point of titration, the current strength assumed a constant value, since the Hg^{2+} ions do not participate in an electrochemical reaction.

To choose the optimum conditions of titration we studied the influence of various factors on the current strength. As the experiment showed, the most complete interaction took place in the pH interval of 3.5-5.0 for PS concentrations of $5 \cdot 10^{-5}$-$1 \cdot 10^{-3}$ g/ml. The course of the titration curve depended substantially on the nature of the anion of the metal salt. When the pectins were titrated with the chloride, nitrate, or sulfate of the corresponding metal cation, no equivalence point was shown. A quantitative interaction took place only in the case where the acetates were used, since the acetate ions bound the hydrogen cations liberated as the result of the reaction:

$$\mathrm{PS{-}COOH} + 1/2\,\mathrm{Me}^{2+} = \mathrm{PS{-}COOMe}\ 1/2 + \mathrm{H}^+,$$

leading to a shift of the equilibrium to the right. The binding of the hydrogen cations excluded the appearance of the interfering currents due to their reduction at the cathode.

From the results of titration we calculated the ratio of the amounts of carboxy groups of the pectin and of the metal cations that had reacted from the formula:

$$\mathrm{COOH_{PS}} : \mathrm{Me}^{2+} = \frac{C_{\mathrm{PS}} \cdot V_{\mathrm{PS}}}{C_{\mathrm{Me}^{2+}} \cdot V_{\mathrm{Me}^{2+}}},$$

where C_{PS} and $C_{\mathrm{Me}^{2+}}$ are the concentrations of PS in terms of carboxy groups and of the corresponding cation, M; V_{PS} is the volume of the PS solution taken for titration, ml, and $V_{\mathrm{Me}^{2+}}$ is the volume of the metal acetate solution consumed in titration, ml.

The results obtained are given in Table 1, from which it can be seen that two carboxy groups of sage pectin combined with one metal cation. This means that in the concentration interval studied, over the whole range of ratios only one compound is formed, with the composition $\mathrm{PSCOOMe_{1/2}}$. The type of pectin substance has no influence on the nature of the interaction.

Sage pectin and copper and mercury pectinates were studied by IR spectroscopy. Table 2 gives the positions and assignments of the bands in the spectra.

The absorption at 1440 and 1370-1380 $\mathrm{cm^{-1}}$ shows that some of the carboxy groups were methylated. In a comparison of the IR spectra of the pectinates and the PSs a decrease in the intensity of absorption in the 1740 ± 5 $\mathrm{cm^{-1}}$ region was to be noted, this being connected with the disappearance of unionized carboxyls. In the case of the pectinates, only the C=O stretching vibrations of ester groups appeared. A group of bands in the 1000-1230 $\mathrm{cm^{-1}}$ region, which is characteristic for the vibrations of pyranose rings, was less intense for the pectinates. In the spectra of the copper and mercury pectinates, the ν_s ($\mathrm{COO^-}$) and ν_{as} ($\mathrm{COO^-}$) bands appeared distinctly at 1410 and 1610 $\mathrm{cm^{-1}}$ and at 1400 and 1595 $\mathrm{cm^{-1}}$ respectively, showing a replacement of the hydrogen of the carboxy groups of the PSs by a metal cation.

Thus, the interaction of the pectin substances with copper, zinc, mercury, and cadmium cations is accompanied by the formation of associates with the composition $\mathrm{PS{-}COOMe_{1/2}}$.

EXPERIMENTAL

To prepare the initial solutions, a weighted sample of a PS isolated from the wastes of a tissue culture and purified according to [13] was dissolved in 100 ml of solution with stirring by means of a magnetic stirrer for 2-3 h. The amount of free carboxy groups was determined by the previously proposed method of titrating with 0.01 N carbonate-free NaOH, with the potentiometric recording of the titration end-point [13]. The concentrations of the solutions of metal salts, prepared from "khch" ["chemically pure"] agents were determined accurately by titration.

To investigate the interaction, an ordinary amperometric apparatus with two metal indicator electrodes identical with the metal of the cation of the salt titrated was used, a small potential difference of the order of 0.5 V being applied to the electrodes. The current strength was measured with the aid of a M-95 galvanometer fitted with a sensitivity shunt. After each measurement, the solid indicator electrodes were subjected to mechanical cleaning, followed by washing with distilled water. To plot the curves we used the corrected values of the current strength with allowance for the dilution of the solution [14].

The IR absorption spectra in the 4000-500 $\mathrm{cm^{-1}}$ region were taken on a Specord-75 IR instrument. The samples of PSs and pectinates were obtained in the form of films. The preparation of the films was carried out by M. P. Filippov, who also made the assignment of the bands [15].

SUMMARY

The interaction of a number of pectin substances with copper, mercury, zinc, and cadmium salts has been studied by the method of amperometric titration with two metal indicator electrodes. The optimum conditions of titration have been found. The ratio of the reacting carboxy groups of the pectin to the metal cations has been established by graphical and mathematical methods. The compositions of the pectinates formed have been determined.

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CHANGE IN CHEMICAL COMPOSITION OF TRIACRYLGLYCEROLS OF Rhodotorula gracilis AS A FUNCTION OF SPECIFIC GROWTH RATE

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The position-type composition of yeast triacylglycerols is represented mainly by types that are also characteristic for plant oils. To obtain unsaturated triacylglycerols it is better to use the continuous cultivation of yeast and not batch cultivation.

There are adequate grounds for considering that lipids of microbial origin and, in particular, yeast lipids are potential substitutes for plant oils used for technical demands. However, in addition to having a high productivity, lipid-forming strains of yeast produce lipids close in their chemical composition to solid plant fats. The main fraction in the lipid strains – active lipid-producing agents – just as in plant oils, consists of triacylglycerols (TAGs), the chemical composition and structure of which determine many of the physicochemical and technological characteristics of the lipids. We have previously studied the chemical compositions of the TAGs of several active lipid-forming strains belonging to various genera [14]. The biomass of these yeasts were obtained under the conditions of batch cultivation.

To obtain microbial lipids it is possible to use successfully the continuous method of cultivation, which ensures the more effective utilization of the organic substrates [2].

Our aim was to establish the stereotypic and molecular-species composition of the TAGs of yeast of various specific rates of growth (μ) of the culture.

We investigated the yeast strain Rhodotorula gracilis VKM u-335, which is capable of accumulating more than 40% of lipids on the dry mass of the cells under conditions of batch cultivation [3]. The investigations were performed in the regime of a chemostat with limitation of the growth of the culture by means of the source of nitrogen at rates of feed of medium to the fermenter of 0.1, 0.075, 0.05, and 0.1 h^{-1}, which under the conditions selected, corresponded to the specific rates of growth of the culture.

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