Chemistry of Natural Compounds, Vol. 32, No. 1, 1996

ISOLATION OF BEET PECTIN ON SOLID-PHASE FERMENTATION

M. A. Khodzhaeva, M. T. Turakhozhaev, É. L. Kristallovich, N. D. Abdullaev, and M. N. Azimkhodzhaeva

UDC 547.917

Beet pectin has been isolated by the solid-phase fermentation method; its physicochemical characteristics are given.

It is known that pectin substances (PcSs) make up 1/3 of the primary coat of plants, together with cellulose and hemicellulose. A considerable change in the cell wall of plants takes place under the action on its polymers of enzymes produced by various microorganisms [1-4].

A capacity for the synthesis of pectolytic enzymes has also been detected in fungi. From deuteromycetes as producers of these enzymes, highly productive strains of *Aspergillus avamori* Nakazawa, *Aspergillus foetidus* Them. et Raper, *Rhizopus arrhizus* Fischer, *Cylindrocarpon radicicola* Wr., *Fusarium oxysporum* Sugd. et Hang., *Venturia rumicus* Wint, and *Penicillium fellutanum* Biourge have been selected [5-10].

There is information in the literature on the effective use of *Pleurotus ostreatus* Kumm. for the degradation of lignin in lignocellulose plant substrates [11-14].

In view of the high specificity of pectin-cleaving enzymes (PECs), it is desirable to obtain them under the conditions of solid-phase fermentation (SPF), which is effective in the isolation of pectin substances from plant raw material.

SPF is characterized by the fact that the growth of the microorganisms proceeds on the surface or in the thickness of a particle in a mass of comminuted solid substrate. The advantage of SPF consists in its low consumption of energy and of labor. The technology of obtaining enzymes on cultivation by this method is simple because of the low water content of the fermenting mass, and a smaller working space is used.

To obtain native pectin, beet pulp (industrial sample) was subjected to SPF under the influence of the macromycetes *Flamulina velitipes* Singer, *Pleurotus ostreatus* Kumm., *Phanerochaete chrysosporium* Berk., and *Panum tigrinus* Fries., which possess the above-mentioned properties. From deuteromycetes as producers of these enzymes we selected highly productive strains of *Aspergillus flavus* Link and *Aspergillus ustus* Thom. Before contact with these fungi, the beet pulp was treated with water to eliminate low-molecular-mass carbohydrates and accompanying substances.

The filtered-off raw material was subjected to biodegradation by the fungi. After the end of the action of the fungi, the samples were inactivated and the pectin substances were extracted with water. All the samples of PcSs isolated formed light-cream-colored powders readily soluble in water. The quantitative yields of the pectin substances isolated and their qualitative characteristics are given in Table 1.

As can be seen from the figures given in Table 1, depending on the nature of the fungus the yield of PcSs varied over a wide interval (8.5-25.3%), and PcSs with the optimum yield and a relatively high degree of esterification were obtained on the use of the fungus *Flamulina velitipes* Singer.

The molecular masses of the pectins were established from viscosity determinations.

While the main index of the PcSs — the amount of methoxylated groups — ranged over a wide interval (6.2-8.4%), there was a considerable variation in the galactic acid (GalA) content — from 32.0 to 39.9%.

Thus, SPF gives a good yield of PcSs with a relatively high degree of esterification (λ) (apart from sample 2 — see Table 1) as compared with the pectin of an industrial sample.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 3-6, January-February, 1996. Original article submitted May 29, 1995.

0009-3130/96/3201-0001$15.00

TABLE 1. Yields of Pectin Substances and Their Characteristics

Species of fungus	Yield of PcSs, %	Mol. mass	λ	K_f	K_m	Amount of La_1A	Ash content
			%				
1. Aspergillus flavus Link.	8.5	85550	84.9	1.5	8.4	32.0	3.5
2. Aspergillus ustus Thom.	21.0	39450	55.4	3.4	6.2	39.0	3.2
3.Pleurotus ostreatus Kumm.	23.5	32320	76.0	2.3	7.3	35.8	3.0
4. Penicillium chrysogenum Thom.	15.0	32900	79.9	2.0	7.7	39.9	4.7
5. Penicillium notatum Westl.	20.8	33700	77.7	2.0	7.0	33.6	2.3
6.Phanorochaete chrysosporium Berk.	23.8	34450	80.2	1.2	7.5	32.7	2.9
7. Panus tigrinus Fries.	11.5	38420	83.2	1.5	7.3	33.1	3.5
8. Flamulina velitipes Singer.	25.3	38480	88.2	1.0	7.5	32.0	2.6
9. (industrial sample) Beet pectin	12.0	31000	61.0	3.8	6.0	40.3	2.9

Note. K_f) amount of free carboxy groups; K_m) amount of methoxylated groups.

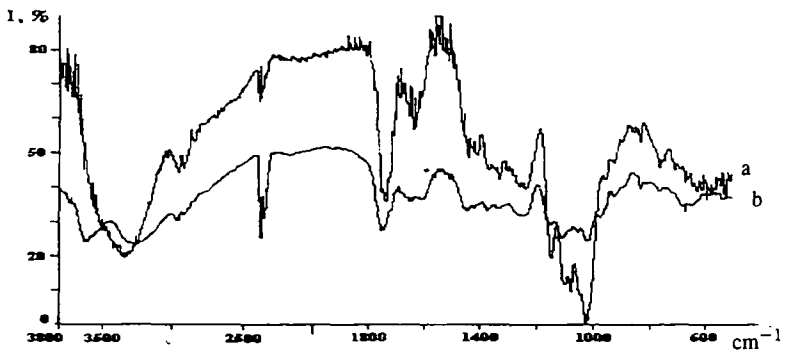

Fig. 1. IR spectra of beet pectin: a) fermentation pectin; b) industrial sample.

The IR spectra of the PcSs obtained by the SPF method were all similar, and we therefore give a single spectrum (Fig. 1a) of the beet pectin (sample 8, *P. tigrinus* Fries., in Table 1).

As can be seen from Fig. 1a, in the IR spectrum of beet pectin obtained by the fermentation method the presence of an absorption band at 3599 cm^{-1} witnesses the presence of free OH groups. An absorption band at 3253 cm^{-1} confirms the formation of hydrogen bonds between OH and C=H groups. For comparison we give the IR spectrum of an industrial sample of beet pectin (Fig. 1b) [16], which had a lower solubility (see Table 1, sample 9).

As can be seen from Fig. 1b, in this case there were no free hydroxy groups, while an absorption band at 3211 cm^{-1} characterizes associated OH groups, i.e., the formation of strong hydrogen bonds between OH and C=O groups. The presence of a maximum at 1040 cm^{-1} in each of the IR spectra of the samples studied shows that they contained the pyranose rings that are the basis of the pectins [17, 18].

Thus, pectin obtained by the fermentation method shows a relatively small fractional participation of OH groups in the formation of hydrogen bonds as compared with pectin obtained by the acid method, which is reflected in the relative solubilities of the pectins.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer model 2000 Fourier IR spectrometer. The samples were prepared by molding into tablets with KBr.

The viscosities of the pectin samples were determined with the aid of an AKL2.842 capillary viscometer [15].

The titrimetric results were obtained by the standard method [19].

Solid-phase fermentation was conducted in 100-ml flasks containing the substrate — beet pulp (particle size 0.5-2.0 ml). The beet pulp was first washed with water at 40°C to eliminate low-molecular-mass carbohydrates and accompanying substances. The substrate was wetted with water in a ratio of 1:2.

The culture was added in a proportion of 1:10 with respect to the medium. The seed material used was a 5- to 6-day culture of the fungus containing 300,000 fungal germs per ml.

The samples were sterilized in an autoclave at 100°C for 30 min and were kept in a thermostat at 27°C for 3-4 days.

After completion of the partial degradation of the cell walls of the beet liquor, water was added to the flask containing the biomass and it was kept in a thermostat at 45 ± 5°C for 1 h. The flask contents were filtered through a Büchner funnel, and the extracts were concentrated by evaporation and poured into alcohol. For all the samples, the resulting precipitates, after drying, consisted of light-cream-colored powders readily soluble in water.

REFERENCES

1. A. E. Arinze and C. M. Smith, Physiol. Plant Plant Pathol., **14**, 141 (1979).
2. T. P. Stark, M. S. Mount, T. Berman, and T. B. Buddard, Phytopathology, **70**, 267 (1980).
3. T. R. Whitaker, Enzyme Microb. Technol., 341 (1984).
4. A. G. Lobanok, R. V. Mikhailova, and M. I. Sapunova, Dokl, Akad. Nauk BSSR, **31**, No. 10, 941 (1987).
5. N. V. Sergeeva and A. A. Desyatnik, The Enzymes of Fungi and their Use in the National Economy [in Russian], Shtiintsa, Kishinev (1975), p. 22.
6. N. V. Sergeev, N. V. Mustyatsa, and A. A. Desyatnik, Izv. Akad. Nauk MSSR, Ser. Biol. Khim. Nauk, No. 1, 32 (1977).
7. A. A. Min'ko and A. V. Mel'nik, A Comparative Study of the Pectolytic Enzymes of Some Species of Fungi [in Russian], Trudy In-ta Biol. Mold. Fil. Akad Nauk SSSR, No. 1, 32 (1960).
8. N. I. Danilyak, V. D. Semichaevskii, L. G. Dudchenko, and I. A. Trutneva, The Enzyme Systems of Higher Basidomycetes [in Russian], Naukova Dumka, Kiev (1989), p. 135.
9. G. B. Bravova, Advances in the Production and Use of Enzymes [in Russian], VNIIbiosinteza Belkovykh Veshchestv. [in Russian], Moscow (1980), p. 28.
10. K. Berker and T. Walker, Phytopathology, **52**, No. 11, 1119 (1962).
11. D. S. Bisaria and S. K. Saxena, Appl. Biochem. Biotechnol., **9**, No. 4, 341 (1984).
12. T. Hiroi and K. E. Erihsson, Appl. Biochem. Biotechnol., **79**, No. 5, 151 (1976).
13. S. S. Kahlon and A. J. Manimder, Food Sci. Technol., **23**, No. 5, 164 (1987).
14. H. Schmitz and G. Eger Hummel, Adv. Biotech. [Proc., 6th Int. Ferment. Symp.], Toronto, Vol. 2 (1981), p. 505.
15. S. Ya. Kovalenko and O. D. Kurilenko, Ukr. Khim. Zh., **31**, No. 2, 175 (1965).
16. M. A. Khodzhaeva and M. T. Turakhozhaev, Khim. Prir. Soedin., 635 (1993).
17. M. P. Filippov and A. V. Buzher, Izv. Vyssh. Ucheb. Zav., Pishch. Tekhnol., No. 4, 61 (1971).
18. M. P. Filippov and G. A. Shkolenko, Prikl. Mikrobiol., **12**, No. 2, 203 (1976).
19. G. B. Aimukhamedova, D. É. Alieva, and N. P. Shelukhina, The Properties and Use of Pectin Sorbents [in Russian], Ilim, Frunze (1984), p. 61.