Carbohydrate Research, 63 (1978) 253–256

Note

Separation of xanthan gums of differing pyruvate content by fractional precipitation with alcohol*

PAUL A. SANDFORD, PAUL R. WATSON, AND CLARENCE A. KNUTSON

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture†, Peoria, Illinois 61604 (U.S.A.)

(Received August 17th, 1977; accepted for publication, September 23rd, 1977)

Xanthan gum is the exocellular, acidic polysaccharide (PS) produced[1,2] by *Xanthomonas campestris* NRRL B-1459. Pyruvic acid, a constituent of PS B-1459, is linked as a 4,6-acetal to terminal D-mannosyl residues in side chains[3-5]. The pyruvate content is usually in the range of 2.5–4.5%, but, theoretically, it can vary from 0 to >8% (assuming all pyruvate is appended to terminal D-mannosyl residues)[6]. Recently, Cadmus *et al.*[7,8] found that pyruvate levels in PS B-1459 vary with both the substrain and the growth medium used; it was also noted that the content of pyruvate in the PS B-1459 formed in the initial stages of the fermentation was lower than that in the final product. This observation implies that the final product may be a mixture of high- and low-pyruvate types. The importance of pyruvate content stems from its correlation[6] with the rheological behavior of PS B-1459. As more alcohol is required for complete precipitation of PS B-1459 high in pyruvate than for gum of low pyruvate content, we were led to precipitate PS B-1459 fractionally with ethanol.

EXPERIMENTAL

Ethanol precipitation[9]. — To solutions (0.05–0.1%) of xanthan in 1% aqueous KCl was added sufficient absolute ethanol to give a mixture containing 34% of ethanol. The mixture was kept overnight at 20°, and the precipitate (if any) was collected by centrifugation (50,000 *g* for 30 min), and dissolved in water. A sample of the solution was removed for carbohydrate analysis by the phenol–sulfuric acid method[10], and the rest was dialyzed against de-ionized water, and then freeze-dried.

*Presented at the American Chemical Society Meeting, Chicago, Illinois, August 28–September 2, 1977.

†The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

To the original alcohol–water solution was added enough alcohol to make it 35% in ethanol. This precipitate was collected as just described. Addition of alcohol in increments, and collection of precipitates, was continued until no precipitation occurred on further addition of alcohol. Nonprecipitable material in the supernatant liquor was also recovered for analysis.

Cetavlon precipitation[11]. — To 0.1% xanthan solutions, an aqueous solution (0.1%) of Cetavlon (hexadecyltrimethylammonium bromide) was added slowly, below the surface, with vigorous stirring. A precipitate began forming almost at once, and the addition of Cetavlon was stopped at an arbitrary point. The mixture was kept overnight at 25°, and centrifuged at 50,000 *g* for 30 min. The pellet was dissolved in 2M KCl, reprecipitated with ethanol, dialyzed, and then freeze-dried. Another portion of Cetavlon was added to the clear, supernatant liquid, and the procedure repeated until all of the polysaccharide was recovered.

Pyruvate analysis. — Pyruvic acid in acid hydrolyzates (M HCl for 3 h at 100°) was measured colorimetrically by a method[9,12] that utilizes the enzyme lactate dehydrogenase.

RESULTS AND DISCUSSION

Various samples of purified PS B-1459 in the potassium salt form, whose overall pyruvate values ranged from 1.3 to 5.9%, were fractionally precipitated by addition of increasing amounts of ethanol. In Fig. 1, the size of each fraction, as determined by the phenol–sulfuric assay, is plotted against the percentage of alcohol required to precipitate it from solution. Sample A contained 1.3; B, 3.2; C, 4.9;

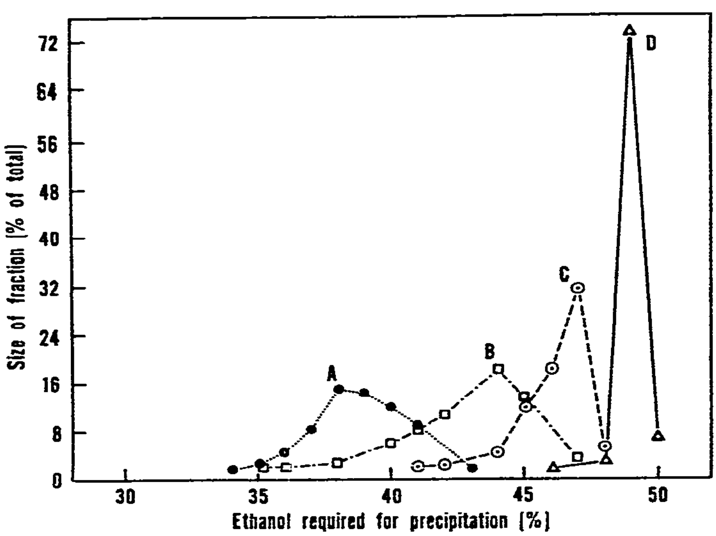

Fig. 1. Fractional precipitation of four PS B-1459 products with ethanol. [Size of fraction *vs.* ethanol (%) required for its precipitation. 0.1% of PS B-1459, 1% of KCl, and increasing amounts of ethanol. Pyruvate content (%) of unfractionated PS B-1459 material: A, 1.3; B, 3.2; C, 4.9; D, 5.9.]

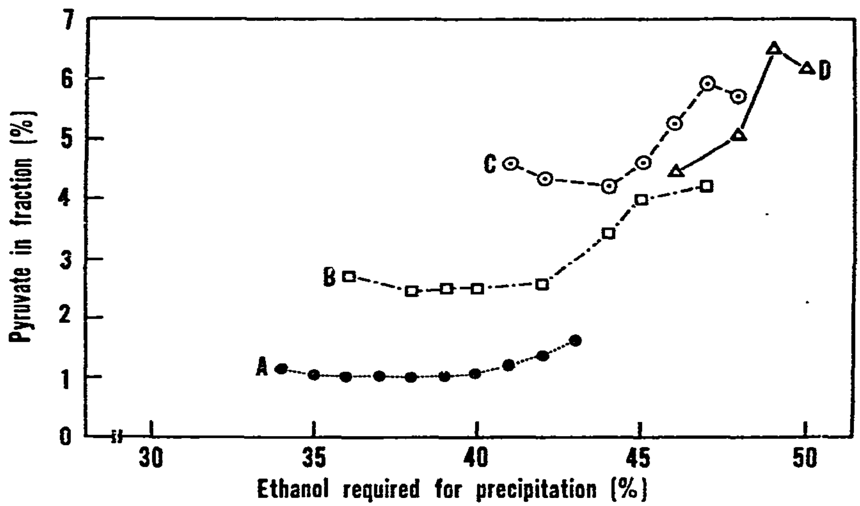

Fig. 2. Fractional precipitation of four PS B-1459 products with ethanol. [Pyruvate content (%) of fractions *vs.* ethanol (%) required for its precipitation. 0.1% of PS B-1459, 1% of KCl, and increasing amounts of ethanol. Pyruvate content (%) of unfractionated PS B-1459 material: A, 1.3; B, 3.2; C, 4.9; D, 5.9.]

TABLE I

FRACTIONATION OF PS B-1459. FINAL PRODUCTS OF DIFFERING PYRUVIC ACID CONTENT

		Analysis of fractions				
				Main fraction		
Sample	*Pyruvate content of parent material* (%)	*Highest pyruvate value* (%)	*Lowest pyruvate value* (%)	*Pyruvate content* (%)	*EtOH required for precipitation*[a] (%)	*Size of fraction*[b] (%)
A	1.3	1.6	1.0	1.0	38	15
B	3.2	4.2	2.4	3.4	44	13
C	4.9	5.9	4.2	5.9	47	31
D	5.9	6.5	4.0	6.5	49	73

[a]1% of KCl present; % = proportion of absolute ethanol in mixture. [b]Percentage of unfractionated material.

and D, 5.9% of pyruvate. Each sample gave a differently shaped distribution curve. In Fig. 2, the pyruvate content of each fraction is plotted against the proportion of alcohol required to precipitate it. As may be seen in Figs. 1 and 2, each final product is heterogeneous with regard to pyruvate types; *i.e.*, each sample, regardless of its original pyruvate analysis, was separated into fractions, some of which had pyruvate contents above, and some below, that of the unfractionated, parent material. Table I summarizes the characteristics of these PS B-1459 products and their fractions. For products low in pyruvate content, a broader distribution curve was noted, and the main fraction represented a smaller percentage of the total material. The main fraction of sample D, which had the highest pyruvate analysis (6.5%), appeared to be of narrow distribution of molecular weight, as, after isolation, refractionation did not increase its pyruvate content. It is not known whether there is a distribution of many pyruvate types, or whether the precipitation with alcohol fails to separate two main pyruvate types, *i.e.*, a low- and a high-pyruvate PS B-1459. Other factors may affect the precipitation behavior; for example, it is not known why each of the 42%-ethanol precipitates from samples A, B, and C (see Fig. 2) have three different contents of pyruvate. It is possible that each final product contains polysaccharide molecules of differing molecular weight.

Attempts to fractionate, with Cetavlon, the parent PS B-1459 samples used in the alcohol-precipitation studies were not successful.

ACKNOWLEDGMENT

We thank M. C. Cadmus for providing the culture broths from which the exopolysaccharides were isolated.

REFERENCES

1 *Xanthan Gum/Keltrol/Kelzan: A Natural Biopolysaccharide for Scientific Water Control*, 2nd edn., Kelco Company, San Diego, California, 1975, pp. 1–36.
2 *The Merck Index*, 9th edn., Merck and Company, Rahway, New Jersey, 1976, p. 1297.
3 J. H. Sloneker and D. G. Orentas, *Nature*, 194 (1962) 478–479.
4 J. H. Sloneker and D. G. Orentas, *Can. J. Chem.*, 40 (1962) 2188–2189.
5 P. E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.
6 P. A. Sandford, J. E. Pittsley, C. A. Knutson, P. R. Watson, M. C. Cadmus, and A. Jeanes, *Am. Chem. Soc. Symp. Ser.*, 45 (1977) 192–210.
7 M. C. Cadmus, S. P. Rogovin, K. A. Burton, J. E. Pittsley, C. A. Knutson, and A. Jeanes, *Can. J. Microbiol.*, 22 (1976) 942–948.
8 M. C. Cadmus, C. A. Knutson, A. A. Lagoda, J. E. Pittsley, and K. A. Burton, *Chem. Inst. Can.–Am. Chem. Soc. Joint Conf.*, Montreal, 1977.
9 A. Jeanes, P. Rogovin, M. C. Cadmus, R. W. Silman, and C. A. Knutson, *Polysaccharide (Xanthan) of* Xanthomonas campestris *NRRL B-14599: Procedures for Culture Maintenance and Polysaccharide Production, Purification, and Analysis*, ARS-NC-51, USDA, November, 1976.
10 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
11 W. J. Albrecht, S. P. Rogovin, and E. L. Griffin, *Nature*, 194 (1962) 1279.
12 M. Duckworth and W. Yaphe, *Chem. Ind. (London)*, 23 (1970) 747–748.