Carbohydrate Polymers 81 (2010) 732–736

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Optimization of process conditions for enzymatic modification of alternan using dextranase from *Chaetomium erraticum*☆

Timothy D. Leathers*, Melinda S. Nunnally, Gregory L. Côté

Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, IL 61604, USA

ARTICLE INFO

Article history:
Received 26 January 2010
Received in revised form 12 March 2010
Accepted 17 March 2010
Available online 25 March 2010

Keywords:
Alternan
Chaetomium erraticum
Dextranase
Gum arabic
Linkage analysis
Modified alternan

ABSTRACT

Alternan is a unique branched glucan with alternating α-(1 → 6) and α-(1 → 3) backbone linkages. We previously described the modification of alternan to a reduced molecular weight form using dextranase from *Penicillium* sp. The solution viscosity properties of this modified alternan resemble those of commercial gum arabic. In this study we optimize process conditions for modification of alternan using commercial dextranase from *Chaetomium erraticum*. This enzyme is considered GRAS (generally regarded as safe) and thus suitable for potential food applications. Optimal conditions were 10% alternan, pH 4.5, 50 °C, and 125 IU dextranase/ml (assayed at 28 °C, pH 5.0). Using these conditions, we scaled up production of modified alternan, permitting for the first time the isolation of separate peaks of modified alternan. Methylation analysis revealed a loss of linear 1,6 linkages during modification. Optimized conditions will be useful to produce modified alternan for applications testing.

Published by Elsevier Ltd.

1. Introduction

Rare strains of *Leuconostoc mesenteroides* secrete the enzyme alternansucrase, which converts sucrose to the unique glucan "alternan" with the release of fructose as a byproduct (Fig. 1). Alternan features a backbone structure of regular, alternating α-(1 → 6) and α-(1 → 3) linkages (Cote & Robyt, 1982; Misaki, Torii, Sawai, & Goldstein, 1980). Native alternan exhibits a molecular weight average of 10^6 Da to 10^7 Da (Cote, 1992; Leathers, Nunnally, & Cote, 2002).

Previously, we isolated novel strains of *Penicillium* sp. that modify alternan to lower apparent molecular weight forms with rheological properties that more closely resemble those of gum arabic (Leathers et al., 2002a; Leathers, Nunnally, & Cote, 2002; Leathers, Nunnally, Ahlgren, & Cote, 2003; Leathers, Nunnally, & Cote, 2006). However, modified alternan lacks the emulsification capacity of gum arabic. Modified alternan is characterized by the formation of heterodisperse peaks at approx. 5–10 × 10^5 Da and 1–5 × 10^4 Da (Fig. 2) (Leathers et al., 2002a).

Subsequently we found that these *Penicillium* sp. strains secrete dextranase during germination on alternan, and that alternan is modified *in vitro* by dextranase from *Penicillium* sp. (Leathers, Nunnally, & Cote, 2009). Dextranase-modified alternan appears to be identical with bioconversion-modified alternan. This result was surprising, since alternan has long been considered to be resistant to dextranase. It was proposed that native alternan may have localized regions of consecutive α-(1 → 6) linkages that serve as substrates for dextranase (Leathers et al., 2009). Dextranase treatment of native alternan, particularly with GRAS (generally regarded as safe) enzymes, may have practical advantages for the production of modified alternan as a gum arabic substitute.

Gum arabic is used in the preparation of confectionaries, beverages, encapsulated flavors, and pharmaceuticals (Whistler, 1993; Williams & Phillips, 2000). Approximately 40,000–50,000 metric tons of gum arabic are produced annually from Acacia trees in Nigeria, Chad, and the Sudan (Williams & Phillips, 2000). However, the price, quality, and availability of gum arabic vary considerably. A replacement for gum arabic would be highly beneficial to the food industry.

In this study we report optimized process conditions for production of modified alternan using commercial dextranase from *Chaetomium erraticum*, a GRAS enzyme.

2. Materials and methods

2.1. Polysaccharides and enzymes

Native alternan was produced as previously described from *L. mesenteroides* strain NRRL B-21297 (Leathers, Ahlgren, et al., 1997;

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author. Tel.: +1 309 681 6377; fax: +1 309 681 6040.
E-mail address: Tim.Leathers@ars.usda.gov (T.D. Leathers).

0144-8617/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2010.03.030

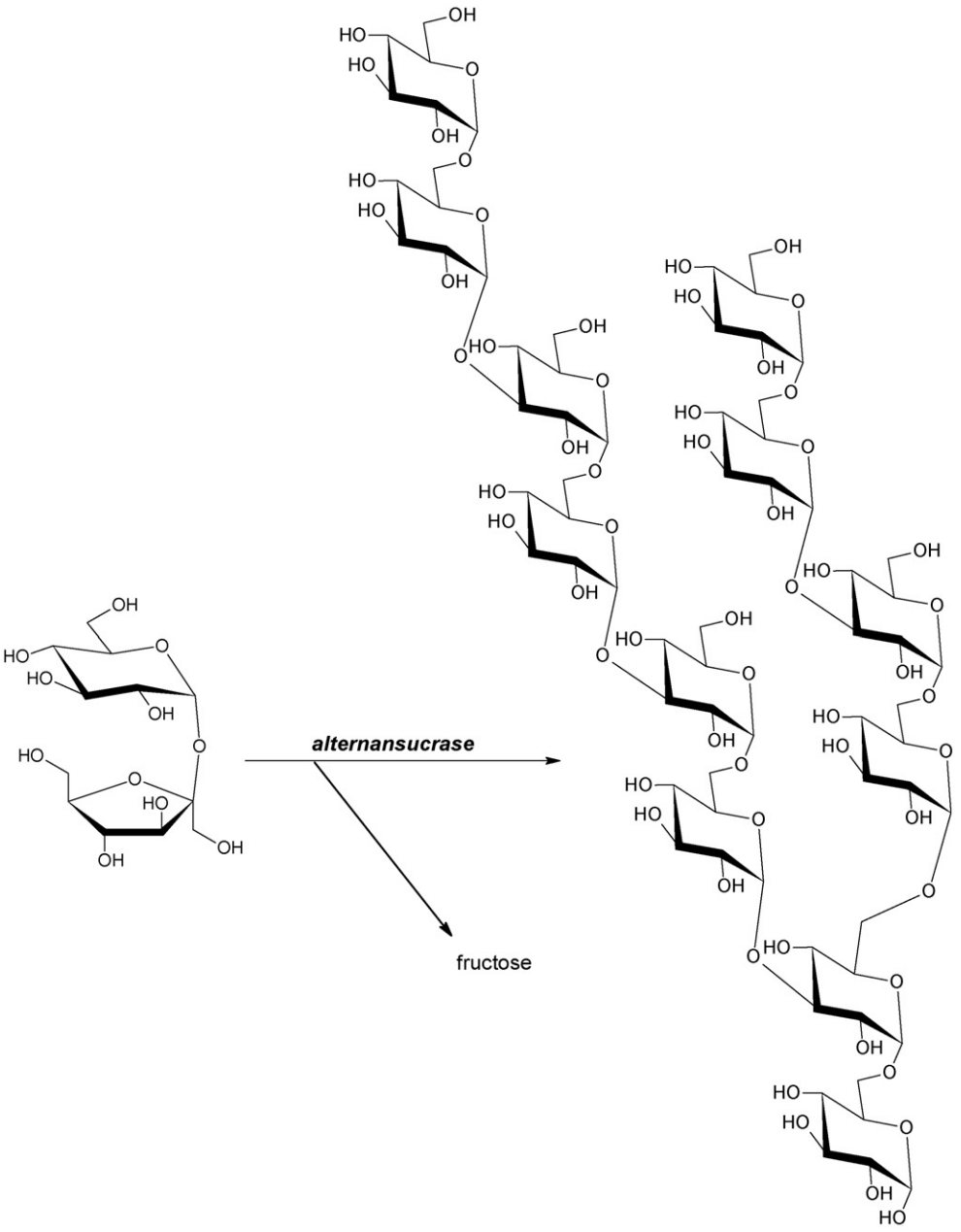

Fig. 1. Biosynthesis and structure of native alternan.

Leathers, Hayman, & Cote, 1995; Leathers, Hayman, et al., 1997). Commercial dextranase was from *C. erraticum* (Bio-Cat Inc., Troy, VA).

2.2. Analytical methods

Polysaccharide molecular weight distributions were analyzed by size exclusion chromatography. Samples were filtered through Nanosep MF 0.45 μm spin tubes (Pall Gelman Laboratory, Ann Arbor, MI), applied to a Shodex KB-806 M high-performance size exclusion chromatography (HPSEC) column (Showa Denko, Tokyo, Japan) and eluted with 0.05 M sodium nitrate at a flow rate of 0.5 ml/min. The mobile phase was filtered through a 10 μm stainless steel inlet filter. Separations were monitored using a Shodex OR-1 optical rotation detector (Showa Denko K.K.). The column and detector were at room temperature. Molecular weights are reported as weight-average molecular weights. The column was calibrated with a set of eight pullulan molecular weight standards ranging from 5.8×10^3 Da to 1.66×10^6 Da (Showa Denko). A semi-log plot of these standards exhibited a linear regression correlation coefficient (r^2) of 0.997 (data not shown). However, it should be noted that the use of this standard curve to predict alternan values assumes similar conformational behavior. Dextranase activity was calculated using a modification of the dinitrosalicylic acid method as previously described (Leathers, 1986) at 28 °C and pH 5.0, with 1 IU representing the release of 1 μmol isomaltose (measured as maltose) per min. Solution viscosities were measured by the method of Cote (Cote, 1992), using a Brookfield LVTDV-1 digital viscometer. Methylation analysis was carried out as previously described (Leathers et al., 2009). The permethylated derivatives were analyzed by capillary GC–MS as the peracetylated aldononitrile (PAAN) derivatives (Seymour, Plattner, & Slodki, 1975).

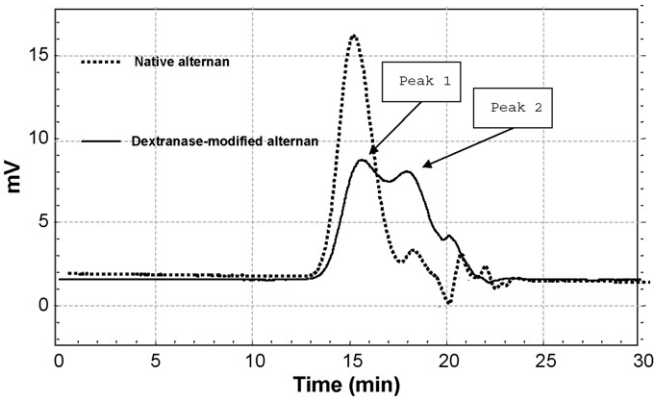

Fig. 2. Molecular weight distributions of native alternan and modified alternan as determined by high-performance size exclusion chromatography (HPSEC). Adapted from Leathers et al. (2009).

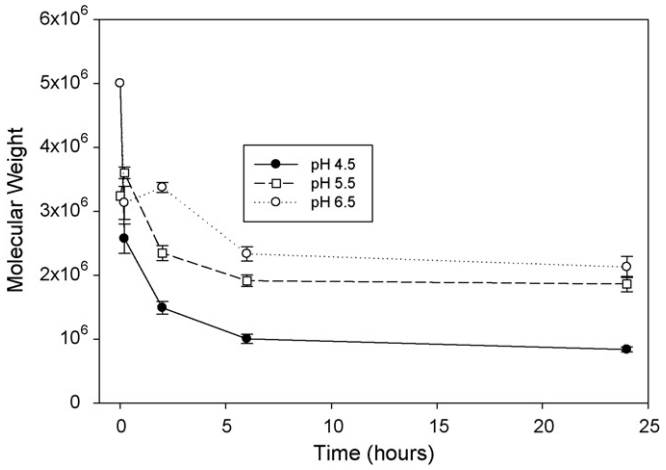

Fig. 3. Time course of alternan modification at 28 °C, pH 4.5–6.5, monitored by molecular weight distribution.

3. Results and discussion

3.1. Optimization of substrate concentration and enzyme amount

In previous studies, native alternan was modified using an *in vivo* bioconversion process in which living spores of *Penicillium* sp. were germinated in a culture medium containing 1.0% (w/v) alternan as a sole carbon source (Leathers et al., 2002a, 2002b, 2003, 2006). Subsequently, we discovered that these strains of *Penicillium* secrete dextranase, and that dextranase from *Penicillium* sp. modified alternan under identical conditions (Leathers et al., 2009). Although 1.0% (w/v) solutions were preferred for the *in vivo* bioconversion process, it is desirable to employ higher concentrations for the *in vitro* enzymatic modification process, since removal of water in downstream processing is an expensive step. Native alternan is highly soluble, but difficult to make up in solutions greater than 12–15% (w/v) due to high viscosity (Cote, 1992). In this study, 5.0% and 10% solutions were easily prepared, but 15% solutions were obtained only with difficulty.

In previous studies, enzymatic modification of 1.0% alternan solutions required a minimum of 5.0 IU/ml of dextranase from *Penicillium* sp. (Leathers et al., 2009). In the current study, we used commercial dextranase from *C. erraticum* (a GRAS enzyme) at a basal level of 12.5 IU/ml (assayed at 28 °C, pH 5.0) and at an elevated level proportional to the concentration of alternan tested. Thus, a 10% alternan solution was tested at both 12.5 IU/ml and 125 IU/ml. Tests were performed on 5.0%, 10%, and 15% alternan solutions at pH 4.5 and at 37 °C. Qualitatively, it was noted that as soon as the dextranase was introduced into the alternan, that there was an immediate noticeable change in the opalescence and viscosity of the samples. HPSEC analysis showed that 5% alternan was modified equally well by dextranase at 12.5 IU/ml and 62.5 IU/ml. However, alternan solutions at 10% and 15% were only partially modified by dextranase at 12.5 IU/ml (data not shown). Furthermore, 15% solutions required dilution before HPSEC analysis. For these reasons, subsequent studies were performed with 10% alternan solutions treated with 125 IU/ml dextranase.

3.2. Optimization of process pH and temperature

Dextranase from *C. erraticum* reportedly exhibits activity at pH 4.0–6.5 and 40–65 °C when acting on dextran (Bio-Cat Inc., Troy, VA). However, the enzyme is reported to become unstable above approximately 50 °C and below approximately pH 4.5 (Bio-Cat Inc., Troy, VA). Therefore, activity on alternan was tested between pH 4.5 and pH 6.5 and between 28 °C and 50 °C.

A 50 mM buffer solution of sodium acetate was adjusted to the desired pH before dissolving native alternan at 10% (w/v). Solutions were equilibrated at the desired temperature, and commercial dextranase from *C. erraticum* was then added to 125 IU/ml. Samples were taken at time 0 (prior to enzyme addition) and at 12 min, 2 h, 6 h, and 24 h, briefly boiled to stop the reaction, and then analyzed by HPSEC as described above.

As shown in Fig. 2, native alternan exhibits a symmetrical peak with a weight-average molecular weight range of 10^6 Da to 10^7 Da. During the process of modification, this peak is gradually converted to a more heterodisperse form characterized by peaks with weight-average molecular weight ranges of $5–10 \times 10^5$ Da (peak 1) and $1–5 \times 10^4$ Da (peak 2) (Fig. 2). The modification process can be conveniently followed in terms of the shift in the molecular weight of the native alternan peak toward peak 1 of modified alternan. The molecular weight of modified alternan represented by peak 2 does not change over time, but its relative proportion increases as the modification process proceeds from 0 h to 24 h. Assays were performed at 28 °C (Fig. 3), 37 °C, (Fig. 4), and 50 °C (Fig. 5).

At 28 °C, samples at all pH levels showed significant modification within 12 min (Fig. 3). At pH 4.5 modification was complete within 24 h, while modification was incomplete at higher pH levels. Presumably, pH 4.5 represents the optimal pH for the action of dextranase from *C. erraticum* against alternan. However, it was subjectively noted that native alternan at pH 6.5 appears to be slightly

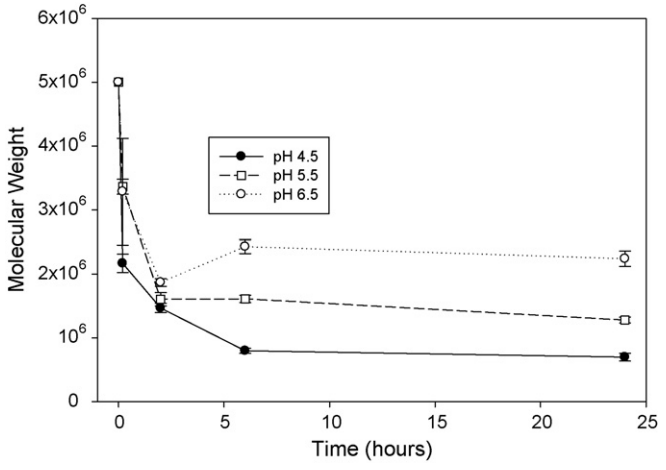

Fig. 4. Time course of alternan modification at 37 °C, pH 4.5–6.5, monitored by molecular weight distribution.

Table 1
Methylation analysis of alternan before and after modification with dextranase.

Sample	2,3,4,6-tetra-*O*-Me	2,4,6-tri-*O*-Me	2,3,4-tri-*O*-Me	2,4-di-*O*-Me
Native	8	31	49	12
Total modified	7	36	46	11
Modified peak 1	8	34	45	13
Modified peak 2	8	39	41	13

Mole percent of methylated products.

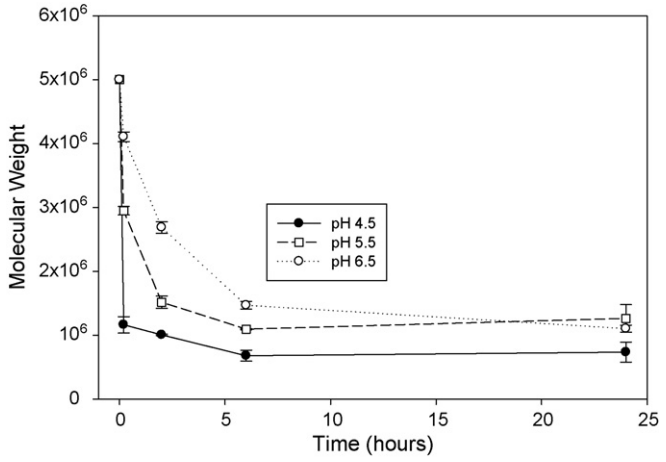

Fig. 5. Time course of alternan modification at 50 °C, pH 4.5–6.5, monitored by molecular weight distribution.

less viscous than at lower pH levels, and this difference was confirmed by quantitative viscosity testing (Fig. 6). It is conceivable that pH exerts an effect on the conformation of alternan in solution, which could in turn affect its susceptibility to dextranase. However, no effect of pH on the apparent molecular weight distribution of alternan was observed.

Modification at 37 °C was somewhat more rapid than at 28 °C, particularly for samples at pH 5.5. These intermediate conditions might be useful to achieve controlled partial modification of alternan for fine control of product viscosity. Modification at 50 °C was even more rapid, particularly at pH 4.5. These are optimal conditions for production of modified alternan.

3.3. Methylation analysis of native and modified alternan

Optimization of process conditions for the modification of alternan permitted convenient scale-up of product concentrations and yields, allowing for the first time the preparative separation of modified alternan peaks 1 and 2 (Fig. 2). Native alternan, total modified alternan, and isolated modified alternan peaks 1 and 2 were compared by methylation analysis for differences in linkages. Alternan has long been considered to be resistant to dextranase because of its regular, alternating α-(1 → 6) and α-(1 → 3) linkage structure. However, it is possible that high molecular weight alternan includes limited regions with short stretches of consecutive α-(1 → 6) linkages. As Table 1 shows, native alternan contained approximately 49% linear 1,6 linkages. Total modified alternan contained approximately 46% linear 1,6 linkages, and modified alternan peaks 1 and 2 contained approximately 45% and 41% linear 1,6 linkages, respectively. The results of methylation analysis are somewhat variable, but overall results suggest that the loss of linear 1,6 linkages may result from hydrolysis of contiguous sequences by dextranase. Furthermore, the reduction in molecular weight suggests that these contiguous sequences are distributed throughout the chains, rather than at the non-reducing ends. Improved production of modified alternan will facilitate more detailed structural studies.

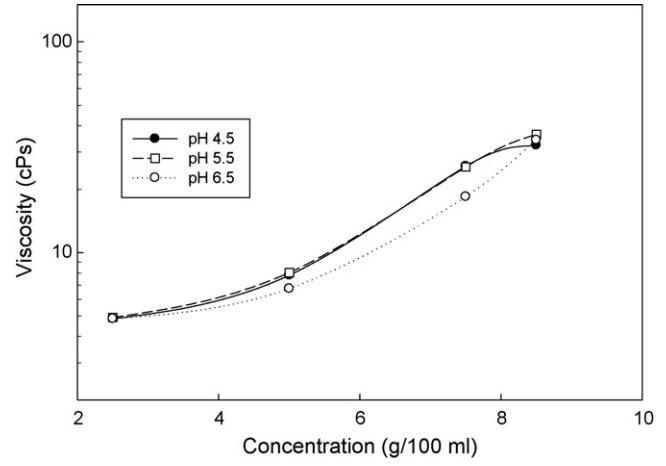

Fig. 6. Solution viscosities of native alternan as a function of concentration, pH 4.5–6.5. All solutions were tested at a shear rate of 39.6 s^{-1}.

4. Conclusions

Modified alternan is a reduced molecular weight derivative of alternan that exhibits solution viscosity properties similar to those of gum arabic. Conditions were optimized for production of modified alternan, using commercial dextranase from *C. erraticum*, a GRAS enzyme. Using these process conditions, production of modified alternan was conveniently scaled up, allowing for the first time isolation of separate modified alternan peaks. Methylation analysis of these peaks revealed a loss of linear 1,6 linkages, supporting the idea that alternan includes consecutive regions of linear 1,6 linkages as a minor structural feature that is susceptible to dextranase. This optimized method will be useful to prepare modified alternan in quantities sufficient for product testing, particularly as new food ingredients.

Acknowledgment

The authors thank Suzanne M. Unser for expert technical assistance.

References

Cote, G. L. (1992). Low-viscosity α-D-glucan fractions derived from sucrose which are resistant to enzymatic digestion. *Carbohydrate Polymers*, *19*, 249–252.
Cote, G. L., & Robyt, J. F. (1982). Isolation and partial characterization of an extracellular glucansucrase from *L. mesenteroides* NRRL B-1355 that synthesizes an alternating (1→6), (1→3)-α-D-glucan. *Carbohydrate Research*, *101*, 57–74.
Leathers, T. D. (1986). Color variants of *Aureobasidium pullulans* overproduce xylanase with extremely high specific activity. *Applied and Environmental Microbiology*, *52*, 1026–1030.
Leathers, T. D., Hayman, G. T., & Cote, G. L. (1995). Rapid Screening of *Leuconostoc mesenteroides* mutants for elevated proportions of alternan to dextran. *Current Microbiology*, *31*, 19–22.
Leathers, T. D., Ahlgren, J. A., & Cote, G. L. (1997). Alternansucrase mutants of *Leuconostoc mesenteroides* strain NRRL B-21138. *Journal of Industrial Microbiology and Biotechnology*, *18*, 278–283.
Leathers, T. D., Hayman, G. T., & Cote, G. L. (1997). *Microorganism strains that produce a high proportion of alternan to dextran*. US Patent 5,702,942.

Leathers, T. D., Nunnally, M. S., & Cote, G. L. (2002a). Modification of alternan by novel *Penicillium* spp. *Journal of Industrial Microbiology and Biotechnology*, *29*, 177–180.

Leathers, T. D., Nunnally, M. S., & Cote, G. L. (2002b). *Penicillium isolates for modifying alternan*. US Patent 6,479,275.

Leathers, T. D., Nunnally, M. S., Ahlgren, J. A., & Cote, G. L. (2003). Characterization of a novel modified alternan. *Carbohydrate Polymers*, *54*, 107–113.

Leathers, T. D., Nunnally, M. S., & Cote, G. L. (2006). *Modified alternan*. US Patent 7,049,105.

Leathers, T. D., Nunnally, M. S., & Cote, G. L. (2009). Modification of alternan by dextranase. *Biotechnology Letters*, *31*, 289–293.

Misaki, A., Torii, M., Sawai, T., & Goldstein, I. J. (1980). Structure of the dextran of *Leuconostoc mesenteroides* B-1355. *Carbohydrate Research*, *84*, 273–285.

Seymour, F. R., Plattner, R. D., & Slodki, M. E. (1975). Gas–liquid chromatography–mass spectrometry of methylated and deuteriomethylated per-*O*-acetyl-aldononitriles from D-mannose. *Carbohydrate Research*, *44*, 181–198.

Whistler, R. L. (1993). Exudate gums. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial gums. Polysaccharides and their derivatives* (3rd edition, pp. 309–339). San Diego: Academic Press.

Williams, P. A., & Phillips, G. O. (2000). Gum arabic. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 155–168). Cambridge, UK: CRC Press.