NOTE

# Alginate Determination in Beer[1]

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ABSTRACT

Tests of a previously used colorimetric method for the determination of alginate in beer showed that the method was of no value. Using this method, we found significant amounts of alginate in beers produced in our pilot plant that had no alginate addition. Consequently, we devised a new procedure by which the alginate is converted into its barium salt and separated, followed by hydrolysis with hydrochloric acid. The resulting lactone of mannuronic acid is then detected by thin-layer chromatography. Because this method is very time-consuming, we attempted to devise a new procedure based on an electrophoretic separation. We tested different foils and gels as separating media, and varied the buffer solution and the pH value to achieve optimum separation. We also tried various staining techniques for the visualization of the separated alginate and determined the limit of detection.

Key words: *Alginate, Beer foam stabilizer, Electrophoresis, Thin-layer chromatography*

Alginate preparations consisting of salts or derivatives of the alginic acid obtained from brown algae are often used in the food industry as gelling and thickening aids. Breweries in several countries use these additives to improve the stability of beer foam. Alginic acid is a linear polysaccharide with a molecular weight of about 20,000–240,000. The monomers of this substance, D-mannuronic and L-guluronic acid, are bound glycosidically.

In many countries, the addition of alginate to beer is forbidden. For the appropriate controls to be made, reliable analytical methods to determine these compounds are required. Whereas alginate can be detected in many foods in relatively high concentrations, beer contains only about 10–100 mg/L. Accurate determinations of these small quantities of alginate are difficult to obtain.

Several different methods of determining alginates exist. Raible and Engelhardt (4) used a procedure in which alginate, after alkaline hydrolysis, was identified as a blue flaky precipitate by addition of calcium ions and the dye night blue. Through previous enrichment on a Sephadex column, they obtained a detection limit of 20 mg/L, corresponding to 2 g/hl of beer. Dark beer, especially, contains substances that may interfere with the determination, to the disadvantage of this low-sensitivity method. Buckee et al (2) used another way. First, they degraded the high molecular weight α-glucans with amyloglucosidase, and then removed the low molecular weight substances by dialysis. They then hydrolized the alginate ester with sodium hydroxide and precipitated the liberated alginate as calcium salt. This was redissolved with sodium hexametaphosphate and identified colorimetrically with orcinol hydrochloric acid iron sulfate reagent. The detection limit of this procedure seems to be 1 mg/L. This method was only slightly modified by Scriban et al (7) and Benard et al (1). Benard et al (1) used a special vacuum-dialysis apparatus with which they were apparently able to identify alginate concentrations down to 1 mg/L. Our own tests (6) with the orcinol method described by Scriban et al led to unusual results. For instance, in beers brewed at our institute that definitely contained no alginate, we found alginate quantities up to 15 mg/L. In beer to which different quantities of alginate were added, we alternately found too much and too little of this substance. We therefore concluded that this method is not specific and hence is very susceptible to interfering components. This method cannot be used for official tests, as may have been done in the past.

All the procedures mentioned are based on a nonspecific reaction. In contrast, Schmolck and Mergenthaler (5) developed a specific gas-chromatographic method for the analysis of gelling and thickening additives, in which the monomers of the different polysaccharides can be identified. This method is suitable for the determination of such additives. According to the procedure, the polysaccharides are hydrolized with methanolic hydrochloric acid, and the formed monomers are separated by gas chromatography after derivatization, eg, as trimethyl-silyl compounds or as alditol-acetates. To the best of our knowledge, this procedure has not been used previously for the determination of alginate in beer. Generally, there is nothing against the use of this procedure, as uronic acids and glucose originating from dextrins and other monomeric sugars can be separated easily by gas chromatography.

Because we were often faced with the problem of determining alginate in beer, and as our experiences with the nonspecific methods mentioned were not satisfactory, we attempted to develop a reliable procedure (6). For immunological tests, we linked alginic acid and bovine serum albumin, and the resulting conjugate was injected into rabbits according to a detailed program. However, since the animals had not formed even traces of antibodies after 25 injections, the use of immunology was abandoned. An alternative to the specific determination of the alginate components by gas chromatography is thin-layer chromatography. The alkaline hydrolysis of the alginate ester was taken from earlier published work. Trials to reduce beer components of high molecular weight with amyloglucosidase, α-amylase, pectinase, endo-β-glucanase, and protease produced no positive results, so we abandoned this preliminary treatment and confined our work to the separation of the low molecular weight substances by dialysis. We changed the concentration of the added calcium chloride for the separation of calcium-alginate, but produced no satisfactory results. Barium chloride (2% concentration) proved to be much better for the precipitation. Furthermore, we ceased redissolving the barium

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alginate with sodium-hexamethaphosphate and attempted to hydrolyze the precipitate directly. The best way to degrade the alginate into its monomers would be through enzymatic hydrolysis by means of appropriate alginases. Unfortunately, these kinds of preparations are not available in the trade and had to be isolated from appropriate microbiological cultures. For hydrolysis, we first used 2*N* trifluoroacetic acid, but realized that the major part of the mannuronic acid formed was decomposed under the given reaction conditions. A large series of tests was performed in which the type of acid, the time of reaction, and the temperature were varied. Best results were achieved with 2*N* hydrochloric acid, a reaction time of 2.5 hr, and a temperature of 90°C. The mannuronic acid formed (in acid solutions as mannuronic acid lactone) could be easily separated from the other substances on sheets of silica gel. To make the substances visible on the thin-layer chromatogram, a solution of 0.4% orcin sulfuric acid was suitable. The L-guluronic acid, the second monomer of the alginate, could not be identified because we had no corresponding reference substance. A great part of the mannuronic acid is decomposed when heated because of the relatively fast decomposition of uronic acids under the hydrolysis conditions. A semiquantitative evaluation can be made, however, when a defined quantity of alginate, eg, 5, 10, 20 mg/L, is added to a beer that is known to be free of alginate and when the whole procedure is performed with such beers. If some samples contain alginate, the range can be estimated with three reference chromatograms. Besides this, it is usually sufficient to demonstrate with certainty that alginate was added to beer; whether this was done at a level of 10 or 30 mg/L is less important. With this thin-layer chromatographic procedure, alginate concentrations of 1 mg/L can be detected. The additives used to obtain a real improvement in the foam stability are, however, at least 10 times higher. According to a recent publication, Fey and Mack (3) used thin-layer chromatography for the determination of alginate. They added calcium chloride for the precipitation step, and then performed the hydrolysis with concentrated sulfuric acid.

As thin-layer chromatographic methods require much work and time, we looked for alternatives for the determination of alginate. In alkaline solution, the alginic acid is present as alginate-anion. It migrates as other ions do in an electric field. By choice of appropriate conditions, it may be identified through electrophoretic separation. Alginate esters, therefore, must first be hydrolyzed with sodium hydroxide. Because of the low quantity of alginate in beer, concentration of this substance, as in the previously described methods, cannot be avoided. The analysis can be divided into four steps: alkaline hydrolysis, concentration of the alginate, electrophoretic separation, and identification of the separated substances by staining. The methodology for the alkaline hydrolysis and for the concentration step was taken from the thin-layer chromatographic procedure, but the compounds with lower molecular weight were not separated by dialysis, which meant that significant time was saved. After precipitating the alginate as barium salt, this substance had to be redissolved for the electrophoresis. We first tried this with hydrochloric acid, but the results were not satisfactory. A satisfactory way was the addition of sodium sulfate. Because barium sulfate has very low solubility, the barium alginate is transformed with sodium sulfate to nonsoluble barium sulfate and soluble sodium alginate. For the succeeding electrophoresis, an alkaline-saturated sodium sulfate solution was used. With this procedure, the alginate was dissolved in very small volume so that the sensitivity of detection was improved. For the electrophoresis, we used agarose gel sheets made in our laboratory, and commercially available cellulose acetate foils. With the first type, the alginate could barely be separated from the other compounds. In further trials, only cellulose acetate foils were used. We obtained good separations with a 0.2*M* borate buffer of pH 10. A modification of the pH between 9 and 11 showed virtually no difference concerning separation behavior. Best results were reached with the adjustment of the power supply of the electrophoresis apparatus to a voltage of 200V and a current density of 1 mA/cm of foil width. Under these conditions, the electrophoresis took 40 min. When the voltage was considerably increased, the separation was worse; smaller voltages did not lead to any improvement, and only increased the separation time.

The subsequent fixing of the alginate is done by immersion in ethanol for 10 min. To make the alginate visible, we used two different staining techniques: periodic acid and Schiff's reagent (PAS) (Fig. 1) and toluidine-blue, which specifically stains polysaccharides containing an acidic group (Fig. 2). To clarify whether or not pectins, which are always present in beer, interfere with the detection, we added sodium pectate in concentrations of up to 100 mg/L to the beers and treated the samples in the same manner. As the electrophoretogram shows clearly (Fig. 1), the pectin on the foils migrated considerably less than the alginate. Moreover, it was only slightly colored by the PAS reagent.

From a series of additional tests, a detection limit of approximately 5 mg/L was obtained. Further tests, in which we worked with larger samples, did not result in any increase of sensitivity.

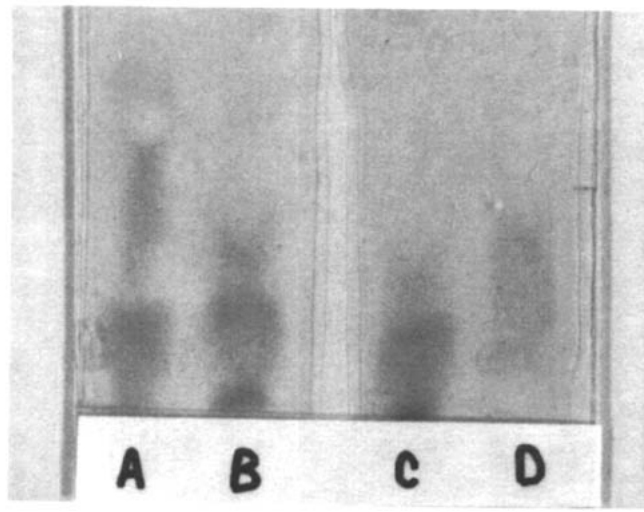


**Fig. 1.** Electrophoretogram using period acid and Schiff's reagent for staining. **A** = beer + 100 mg/L alginate; **B** = beer with no additive; **C** = beer + 50 mg/L pectin; and **D** = water + 100 mg/L pectin.

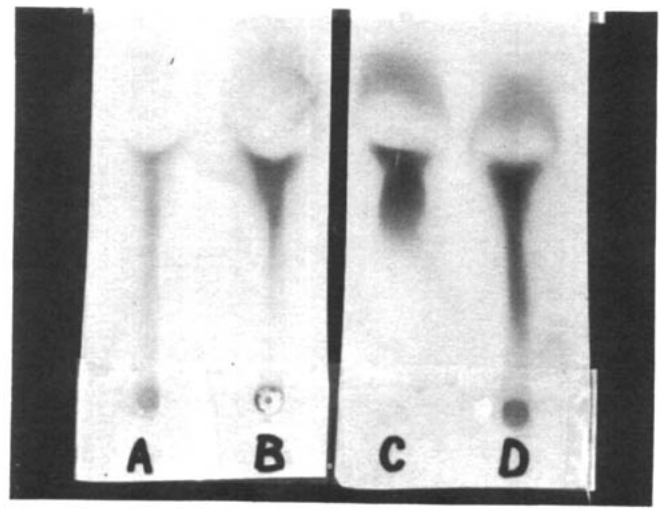


**Fig. 2.** Electrophoretogram using toluidine blue 0 for staining. **A** = beer; **B** = beer + 5 mg/L alginate; **C** = water + 10 mg/L alginate; and **D** = beer + 10 mg/L alginate.

## SUMMARY

The new method developed for determination of alginate in beer incorporates alkaline hydrolysis of the beer, barium chloride precipitation, and sodium sulfate addition, followed by electrophoresis and staining. Electrophoresis utilized cellulose acetate foil, a 0.2*M* borate buffer at pH 10, a voltage of 200V with a current density of 1 mA/cm, and staining with periodic acid Schiff's reagent or with toluidine blue.


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