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GAS CHROMATOGRAPHIC DETERMINATION OF POLYSACCHARIDE GUMS IN FOODS AFTER HYDROLYSIS AND DERIVATIZATION

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SUMMARY

A gas chromatograpic method was evaluated for the determination of food grade gums in dairy products, salad dressings and meat sauces. The gums studied were tragacanth, karaya, ghatti, carob, guar, arabic and xanthan gum. The extraction method included removal of fat followed by starch degradation then precipitation of protein. The isolated gums were hydrolysed with trifluoroacetic acid and the resulting neutral monosaccharides converted to their aldonitrile acetate derivatives for determination by gas chromatography. Recoveries from thirteen different commodities averaged 85%. However, the recovery of guar gum from ice cream and cold pack cheese was 42 and 50%, respectively. In a comparison of enzyme hydrolysis and iodine complexation for the removal of starch the former was simpler and provided cleaner extracts than the iodine treatment. Both gave similar results.

INTRODUCTION

Gums are heteropolysaccharides that exhibit solution thickening or gelling effects and have secondary functional properties such as emulsification and stabilization[1]. In the food industry in Canada they are permitted for a number of uses such as emulsifiers in salad dressings, thickening agents in jams, sauces and gravies, and stabilizers in ice cream, to name a few[2]. Unlike starch, gums are not metabolized by humans and therefore are considered non-caloric food ingredients[3].

The determination of gums in foods is a complex and lengthy task and the area has been reviewed[4,5]. For analysis, it is necessary to remove the simple sugars, fat, protein and starch before the gums are hydrolysed to their component sugars for identification. In recent years, gas chromatography (GC) has proven to be very useful for identifying sugars[6–13] and have been used for characterization of commercial gums[14,15]. These methods all involve derivatization of the monosaccharides to volatile products employing a variety of techniques including trimethylsilylation[6,7], trifluoroacetylation[7], methanolysis[8,9,14] and conversion to alditol acetates[10,11] and aldonitrile acetates[7,12,13,15]. The last two methods produce single derivatives which enable simpler identification of the sugars as opposed to the older techniques which

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produce multiple products for the individual monosaccharides. However, aldonitrile acetates are easier to prepare, are more stable and are easily separated by GC[7].

The present work describes the use of aldonitrile acetates to determine gums in both dairy and starch-containing foods. Previous extraction methodology used only for dairy products and desserts[14,15] or for gravimetric analysis[16] was evaluated and extended to cover a variety of food types. Also, a comparison of enzyme hydrolysis and iodine complexation for the removal of starch was carried out.

EXPERIMENTAL

Reagents

Standards of the sugars and gums were purchased from Sigma (St. Louis, MO, U.S.A.). α-Amylase and α-amyloglucosidase were obtained from BDH (Toronto, Canada). All other reagents and solvents were analytical or distilled-in-glass grade materials and used as received.

Gas chromatography

A Varian 2700 gas chromatograph equipped with a flame ionisation detector and a 1 m × 2 mm I.D. stainless-steel column packed with Gas Chrom Q (100–200 mesh) coated with 3% neopentylglyclosuccinate was used for the analyses. Column temperature was programmed from 150–230°C at 2°C/min. Helium carrier gas flow-rate was 25 ml/min. The detector and injector temperatures were 250 and 200°C respectively.

Extraction of dairy products

The method of Glück and Thier[15] was used for ice cream, cottage cheese, processed cheese and cold pack cheese. Briefly, about 10–20 g of sample were dissolved in 70 ml of water at 90°C and diluted to 100 ml. The sample was defatted by precipitation of the gum (plus protein and starch) with dioxane. The precipitate was washed with ethanol–water (70:30), dried and dissolved in 10 ml water at 60°C. Starch was degraded by incubation with α-amylase and α-amyloglucosidase. Protein and the sugars were removed by adding 50% aqueous trichloroacetic acid solution. The resulting precipitate was discarded and the supernatant mixed with ethanol to precipitate the gum. The residue was washed with 70% ethanol, dried then hydrolysed as described later.

Extraction of non-starch containing dressings

The AOAC gravimetric procedure[16] was used for French and English dressings, a calorie reduced dressing and tartar sauce. Briefly, the sample was mixed with water and heated to 65–70°C. A volume of 50% trichloroacetic acid was added and the mixture heated until the emulsion began to separate. The mixture was centrifuged and the lower aqueous layer removed to a clean beaker where the gum was precipitated with 70% ethanol. The residue was removed, redissolved in hot water and precipitated a second time with ethanol. The dried precipitate was then hydrolysed as described later.

Extraction of starch-containing sauces

The AOAC procedure for starch-containing foods[16] was evaluated for samples of a beef baking sauce, a chicken sauce, a barbecue sauce and an instant poowdered drink all of which had label declarations indicating added starch. Briefly, fat was removed by extraction of the sample (50 g) with light petroleum (b.p. 40–60°C) until the organic layer became colorless. Starch was degraded using iodine complexation. An aliquot of the final filtrate equivalent to 5 g of sample was taken for precipitation of the gum. The purified gum was then hydrolysed as described below.

For comparison purposes starch degradation was also carried out by incubation with α-amylase and α-amyloglucosidase as described for dairy products. However, only 5–10 g of sample were taken for this which still yielded enough gum for identification purposes.

Hydrolysis of gums

About 15–20 mg of gum were mixed with 2–5 ml of 0.5 *M* trifluoroacetic acid and heated for 4 h at 100°C in a PTFE-lined screw-capped reactivial. After cooling, the mixture was extracted with 3 × 1 ml of a 20 g/l solution of Amberlite LA-2 (BDH) in diethyl ether. The organic phases were discarded and the aqueous phase extracted with 4 × 1 ml of diethyl ether which were discarded. The aqueous phase was then evaporated to dryness by rotary evaporation at 60°C.

Derivatization of sugars and hydrolysed gums

About 30 mg of carbohydrate (standard sugars or gum hydrolysate) were dissolved in 0.5 ml of pyridine containing 30 mg of hydroxylamine hydrochloride in a 4 ml PTFE-lined screw-capped reactivial. The mixture was heated at 100°C for 1 h and then cooled. A volume of 2 ml of acetic anhydride was then added and the mixture heated for another hour at 100°C. The contents were then cooled and excess pyridine removed by rotary evaporation at 60°C. The aldonitrile acetates were then dissolved in 1–2 ml of acetone for analysis.

Recovery studies

Recoveries of the gums through the complete analytical procedures were determined by adding known quantities of the gums to selected samples at the beginning of the determination.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of a mixture of standard sugars as their aldonitrile acetates. These represent the monosaccharides that would be expected from the hydrolysis of the seven gums studied. The separation is similar to that reported elsewhere[15]. Some typical gum patterns are shown in Fig. 2. The gums are identified by the fingerprint patterns produced by their constituent neutral sugars. In some cases such as gum tragacanth, arabic, karaya and ghatti, the hydrolysis and derivatization yield very characteristic patterns permitting easy identification of the gums. However, gums such as guar and carob (locust bean) each yield only mannose and galactose as their component monosaccharides and thus identification becomes more difficult being based on the relative proportions of each sugar. Fig. 2 illustrates typical results for the two gums.

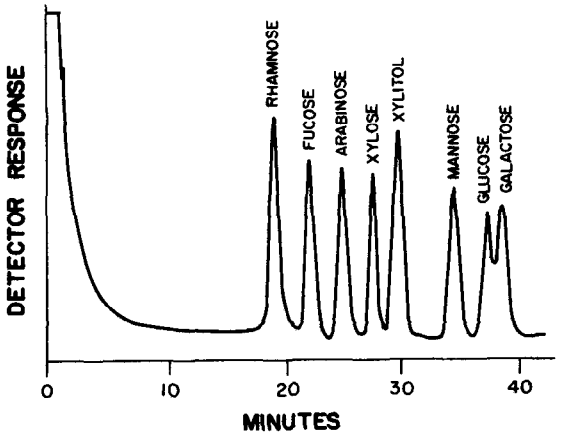

Fig. 1. Separation of the aldonitrile acetates of a mixture of sugars. Xylitol is used as an internal standard.

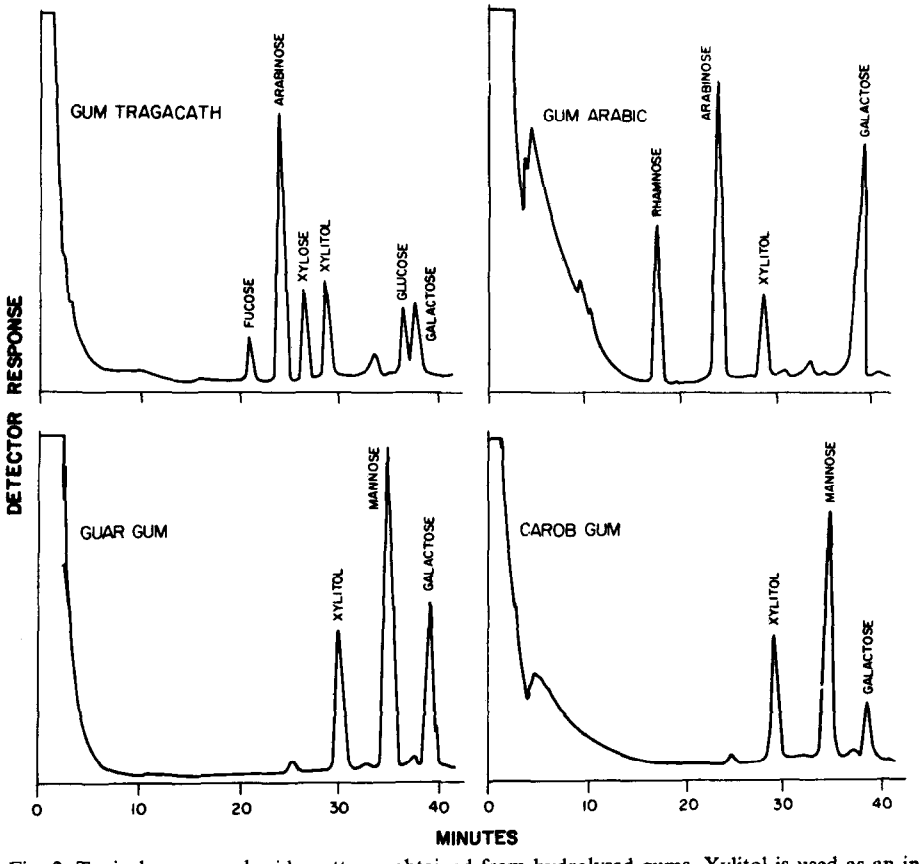

Fig. 2. Typical monosaccharide patterns obtained from hydrolysed gums. Xylitol is used as an internal standard.

Fig. 3 compares the results of a processed cheese sample containing no added gums with the same sample spiked with guar gum. No interferences from the sample components were observed in the determination of the gum. Several chromatograms of food analyses are shown in Fig. 4. Gum tragacanth is easily identified in the English dressing. The cottage cheese sample (Fig. 4D) shows the presence of mannose and galactose derived from carob gum which was indicated on the label. However, because carageenan was also listed as an ingredient the carob gum pattern is altered since carageenan yields galactose upon hydrolysis. Thus the galactose peak is larger than would be expected in relation to the mannose peak. In such cases where more than one gum is added to a product quantitation is best accomplished by using peaks which are unique to a particular gum. Thus for the cottage cheese sample mannose would be used to quantitate the carob gum. In the present work, carageenan was not quantitatively studied.

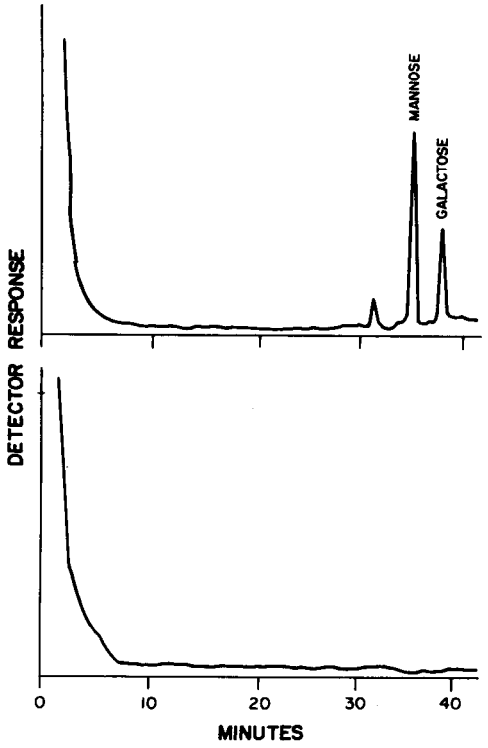

Fig. 3. Chromatographic results obtained for processed cheese (bottom) and process cheese spiked with 0.25% guar gum (top).

In certain samples, particularly those that contained spices or other added flavourings such as the beef baking sauce, tartar sauce, barbecue sauce and chicken sauce, glucose was always evident. In Fig. 4B carob gum could still be identified since the glucose does not interfere. However, in Fig. 4C glucose interferes in the identification of xanthan gum in tartar sauce since xanthan gum normally yields both mannose and glucose in a ratio of about 1–1.3. In the samples where glucose was

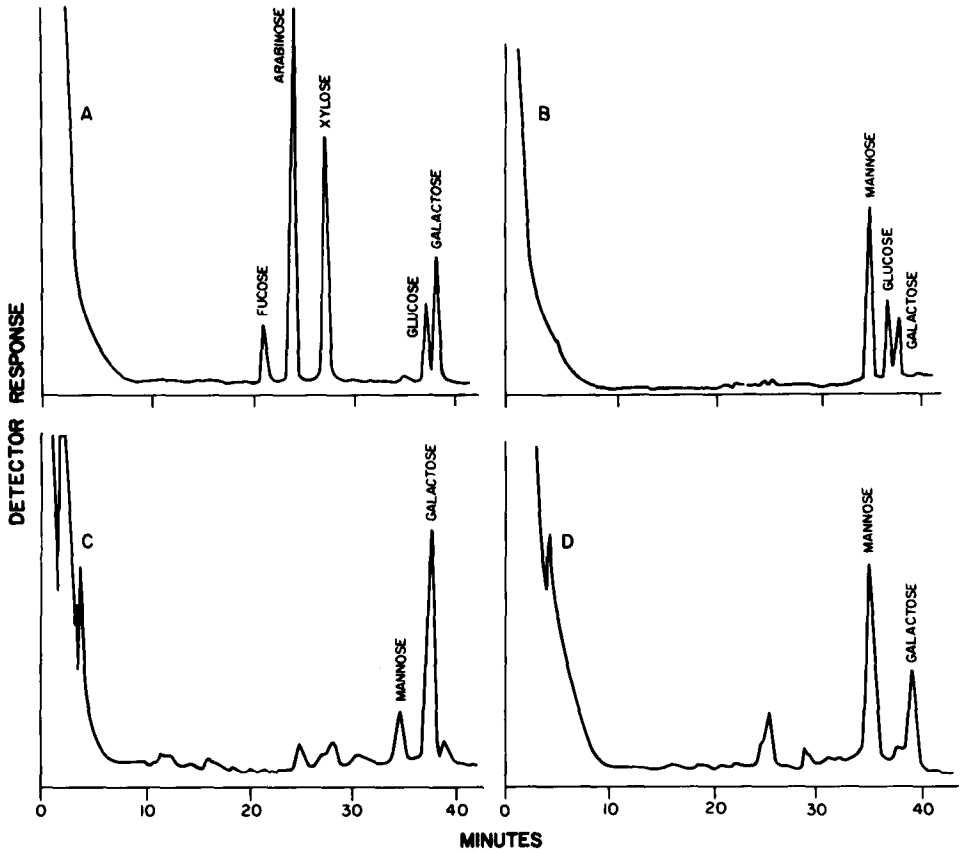

Fig. 4. Chromatographic results of selected food commodities. (A) English dressing contains tragacanth; (B) barbecue sauce, carob gum; (C) tartar sauce, xanthan gum and (D) cottage cheese, carob gum plus carageenan.

unexpectedly present the extraction procedures were repeated to ensure that the interference was not from the failure to remove starch. It has been reported that certain spices can yield interferences in some types of samples[16] particularly if the quantity of added gum is relatively low.

Recoveries of the gums studied averaged 85% (range 73–95%) when spiked in various samples at 0.25–0.50% by weight. The only exception was guar gum which was recovered at 42% in ice cream and 50% in cold pack cheese. The reason for this is not clear since similar results were obtained upon repeating the work. Table I lists some of the results obtained in a variety of food products examined. Although xanthan gum was listed as an ingredient of the orange drink powder, only traces were found. The gums in the other products could be identified and quantitated.

Initially enzyme hydrolysis was used only for the dairy and low-starch containing salad dressings. However, by taking a small quantity of high-starch sample as outlined in the experimental, starch removal could be effected as efficiently as with iodine complexation. Although it has been reported that low levels of mannose may

TABLE I

LEVELS OF GUMS IN SELECTED FOODS

Food	*Gum**	*Found (%)*
Ice cream	Guar	0.051
Cottage cheese	Carob	0.055
Cold pack cheese	Guar	0.10
Cold pack cheese	Guar	0.10
Processed cheese	none	none
Tartar sauce	Xanthan	0.044
French dressing	Xanthan	0.79
Calorie reduced dressing	Xanthan	0.08
Salad dressing		
English dressing	Tragacanth	0.12
Chicken sauce	Carob gum	0.10
Beef baking sauce	Xanthan	0.064
Barbecue sauce	Carob gum	0.2
Orange drink powder	Xanthan	trace

* As indicated on the package label and identified by GC.

originate from the enzymes[14] we found no significant increase of this peak compared to results obtained by iodine complexation for the levels of gums found in the foods examined. With both types of treatment small amounts of glucose occasionally appeared in some samples, particularly in the meat sauces as mentioned earlier. Thus this peak was not reliable for identification or quantitation of gums.

CONCLUSION

This work demonstrates that aldonitrile acetates are suitable derivatives for the GC determination of gums in dairy products, salad dressings and meat sauces. Gums such as tragacanth, arabic, karaya and ghatti are easily identified by their characteristic monosaccharide patterns. Xanthan, guar or carob gum which yield combinations of mannose, glucose or galactose are more difficult to determine especially if they are in combination with other gums.

REFERENCES

1 M. Glockman, *Gum Technology in the Food Industry*, Academic Press, New York, 1969.
2 *Canadian Food and Drug Regulations, Table IV, Food Additives That May Be Used as Emulsifying, Gelling, Stabilizing and Thickening Agents*, Dept. Supply and Services, Ottawa, 1977, p. 67-5.
3 J. Fleischer, in R. L. Whistler (Editor), *Industrial Gums, Polysaccharides and Derivatives*, Academic Press, New York, 1959, p. 311.
4 H. D. Graham, *Food Colloids*, Avi Publishers, Westport, CT, (1977).
5 E. Mergenthaler and H. Scherz, *Z. Lebensm.-Unters.-Forsch.*, 162 (1976) 25.
6 M. Chapin, *Anal. Biochem.*, 123 (1982) 336.
7 K. Bryn and E. Jantzen, *J. Chromatogr.*, 240 (1982) 405.
8 G G. S. Dutton, *Adv. Carbohydr. Chem.*, 28 (1973) 14.
9 H. Sherz and E. Mergenthaler, *Z. Lebensm.-Unters.-Forsch.*, 170 (1980) 280.
10 J. Lehrfeld, *Anal. Chem.*, 57 (1985) 346.
11 J. H. Sloneker, *Methods Carbohydr. Chem.*, 6 (1972) 20.

12 R. Varma, R. S. Varma and A. H. Wardi, *J. Chromatogr.*, 77 (1973) 222.
13 G. O. Guerrant and C. W. Moss, *Anal. Chem.*, 56 (1984) 633.
14 A. Preuss and H. P. Thier, *Z. Lebensm.-Unters.-Forsch.*, 176 (1983) 5.
15 U. Glück and H. P. Thier, *Z. Lebensm.-Unters.-Forsch.*, 170 (1980) 272.
16 S. Williams (Editor), *Official Methods of Analysis*, Assoc. Offic. Anal. Chem., Washington, DC, 14th ed., 1984, p. 569.