A C T A A L I M E N T A R I A P O L O N I C A
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ANALYSIS OF STARCH AND ITS PRODUCTS

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This paper gives a survey of instrumental methods used in our laboratory in the fied of starch analysis. Chromatographic methods for analysis of polysaccharides (column packing Separon HENA) and oligosaccharides (column packing OSTION) are clearly shown. Capillary isotachophoresis is used for analysis of some ionic constituents of starch.

INTRODUCTION

An analysis plays an important role during the production and processing of starch. Instrumental methods have made progress in this field within the last several years. When we analyze starch or its products we usually characterize either starch substances of admixtures or both. The aim of my paper is to inform you about experiences of our laboratory with HPLC and capillary isotachophoresis (ITP) in starch analysis. We applied HPLC methods for characterization of saccharides (polysaccharides, oligosaccharides and monosaccharides) and ITP for analysis of ionic admixtures.

MATERIALS AND METHODS

CHEMICALS AND INSTRUMENTATION

Metalic chromatography columns Separon HEMA (exclusion limit of HEMA column is designated by number behing the word HEMA; e.g. HEMA 40 has exclusion limit 40 kDa) were purchased from Tessek, (Czechoslovakia); cation exchanger OSTION LG KS OBO3 — 4.2% DVB was obtained from Spolek pro chemickou a hutni vyrobu (Czechoslovakia); standards of dextran with weight average molecular weights varying from 10 do 2000 kDa from Pharmacia (Sweden); the equipment for chromatography consisted of the high pressure pump HPP 4001, sampling valve LCI 30, differential refractometer RIDK 101 and line recorder TZ 4200, all from Laboratorni pristroje, (Czechoslovakia); isotachophoretic analyse AGROFOR equipped with PTFE capillary 300×0.5 mm and conductivity detector, JZD Odry, (Czechoslovakia); inte-

grator SP 4100, Spectra Physics, (USA). Deionized water was used as mobile phase or for preparation of electrolytes and standard solutions.

ANALYSIS OF POLYSACCHARIDES [2.3]

Starch polysaccharides were analysed by the High Performance Size Exclusion Chromatography (HPSEC) on Separon HEMA columns (stainless steel, 250 × 8 mm). The column temperature varied in range 30-85°C. Deionized water or 0.01 M — KOH solution was used as mobile phase at a flow rate of 0.5 ml/min. Each analysis took 20 minutes. Calibration analyses were made with dextran standards. Samples were dissolved in water, DMSO or solution of KOH to 0.5% (w/v) solutions, filtered through 0.5 μm membrane and injected (20 μl) into the chromatograph. Chromatograms were handled by hand or by specjally programmed integrator.

ANALYSIS OF OLIGOSACCHARIDES AND MONOSACCHARIDES [1.4]

Oligosaccharides and some monosaccharides were separated and determined by HPLC on cation exchanger OSTION. The resin was transfered to silver form (70% Ag^+ and 30% H^+) or to calcium form and filled into the metallic column (250 × 8 mm) in our laboratory. Deionized water was used as mobile phase at a flow rate of 0.5 ml/min., the column temperature was kept at 85°C. Samples were dissolved in water to 1-5% (w/v) solutions, of which 20 μl injections were made. The samples were before injection filtrated through 0.5 μm membrane and deashing by mix bed or by the guard column (in case of the resin in silver form). Each analysis required 20 minutes. Chromatograms were handled by the integrator and qualitative analysis was based on retention time of oligosaccharides. The external standard or area normalization methods were used for the quantitative analysis.

ANIONIC ITP ANALYSIS

Anions in starch were determined on isotachophoretic analyser AGROFOR. The anions were separated in PTFE capillary (300 × 0.5 mm) and detected by conductimeter. Applied driving current was 120 μA, which was decreased during detection to 60 μA. A solution of 10 mM — HCL + 18 mM — 6-aminocaproic acid + 0.1% (w/v) hydroxymethylpropylcellulose served as leading electrolyte. A terminating electrolyte was 5 mM — caproic acid. Samples were extracted or dissolved in water (1-10 g/100 ml) and after filtration injected (25 μl) into the analyser. Each analysis required approximately 10 minutes.

CATIONIC ITP ANALYSIS [5]

Some alkali metals (Na, K) and alkaline earth metals (Mg, Ca) were separated and determined on analyser AGROFOR. Cationic analysis of these

metals was performed with a leading electrolyte comprising of 5mM — H_2SO_4 and a terminating electrolyte of 5mM — lithium citrate. Samples of starch were dissolved (gelatinized) in deionized water (1% w/v solution) and injected by valve (25 l) into ITP analyser. Each analysis took 10 minutes.

RESULTS AND CONCLUSIONS

We have used the HPSEC method for determination of molecular weight distribution of polysaccharides in starchy samples. The Separon HEMA 1000 (d_p = 10 m) column is mostly used for these analyses. Figure 1 shows the calibration curve of this column. Exclusion limit 2000 kDa done by the producer Tessek was exceeded, because there were fractions of samples, which were eluted before dextran D 2000. We analysed dextran D 5000 (molecular weight 5000-40000 kDa), of which retention time was one minute shorter then the retention time of the D 2000. We have analysed by HPSEC method native starches (potato, wheat, corn and rice) and some modified starches (oxidized, hydrolyzed, cross-linked, starch esters, dextrins etc.). The chromatogram of the native potato starch is given in Figure 2. The estimated weight-average molecular weight (M_w) of the first fraction (amylopectin) was 12000 kDa and of the second fraction (amylose) 1800 kDa. Figure 3 shows the chromatogram of commercially produced maltodextrin KMS × 50 (DE = 10—14%) with M_w = 500 kDa and the number — average molecular weight M_n + 2 kDa. This maltodextrin contains four main fractions and by analyses with help of LALLS (Low Angle Laser Light Scattering) detector on line with refractometr we have found that the third fraction is highly branched. Apparent M_w + 9 kDa but M_w determined by LALLS detector was 35 kDa.

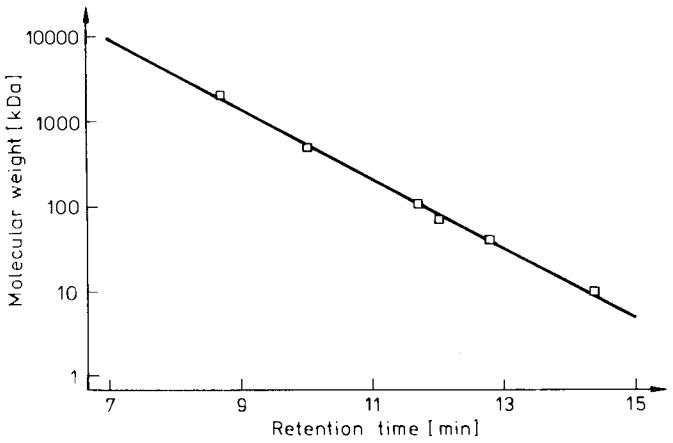

Fig. 1. Calibration curve of Separon HEMA 1000 ($\bar{d}_p$ = 10 μm). Conditions: column temperature 65°C, 0.5 ml of water/min, RI detection, attn $4x10^{-5}$ RIU, 100μl sample loop.

Fig. 2. Chromatogram of native potato starch (0.2% w/v). Conditions as in Fig. 1.

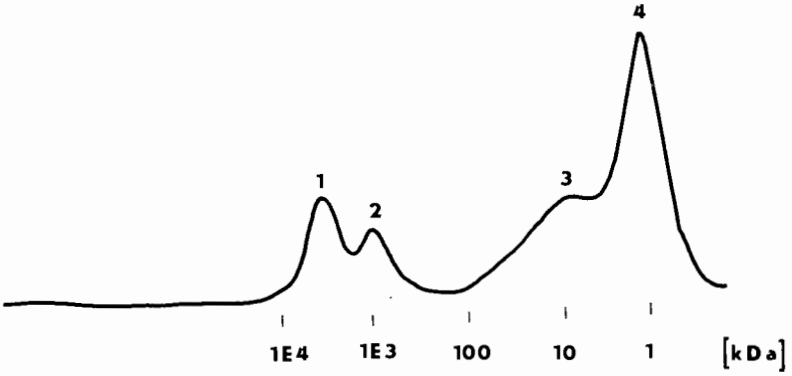

Fig. 3. Chromatogram of maltodextrin KMS X50 (1% w/v). Conditions as in Fig 1.

Another application of HPSEC is analysis of dextrins with photometric detection at 405 nm. Figure 4 gives a chromatograms of dextrins produced from potato starch at 160°C. At the beginning of the dextrinization low molecular weight colour substances are present, from which high molecular weight substances are gradually created.

For the determination of oligosaccharides in starch, hydrolysated HPLC on cation exchanger OSTION was used. The resin in silver form (70% Ag^+, 30% H^+) enables separation of oligosaccharides with degree of polymerization Dp1-12, and in calcium form up to Dp7. A better separation between glucose and Dp2 on the resin in calcium form was achieved. By this HPLC method we analysed starch hydrolysates with DE in range 3-96% and used this one for studying the time course of starch hydrolysis. Some chromatograms on the resin in silver form are shown for the illustration. The chromatogram of commercially produced maltodextrin KMS × 50 (α-amylase hydrolysis of potato starch), is shown in Figure 5. The chromatograms of two maltose sirup are given in Figure 6. and 7. The former is produced by enzyme/enzyme hydrolysis of the potato starch (DE = 40%), the latter by combined acid/enzyme hydrolysis of the same raw starch (DE + 50%). According to the dextrose equivalent the latter sirup should be more hydrolyzed and thus should contain more maltose (the saccharification degree is sometimes referred to maltose content). However, the results of chromatographic analysis showed that the content of maltose in the

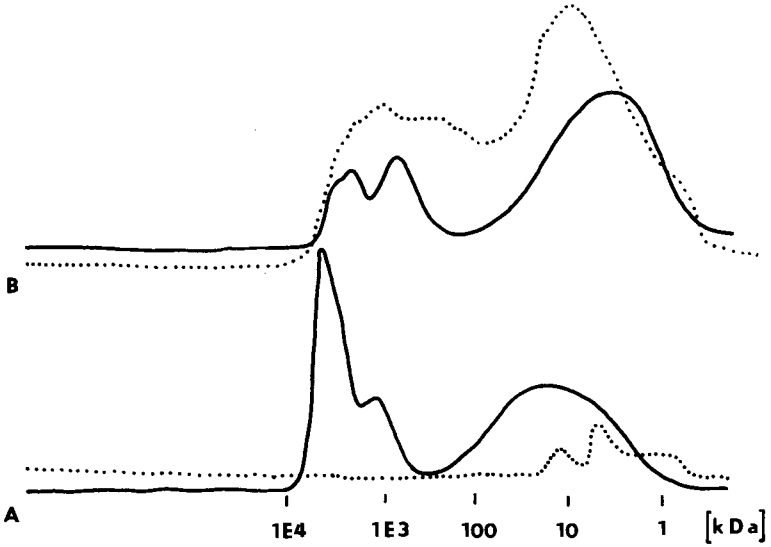

Fig. 4. Chromatograms of dextrins (1% w/v). Dextrinization of potato starch at 160°C for 1 h (sample A) and 5 h (B). Conditions as in Fig 1., full line — RI, dotted line — A_{405}

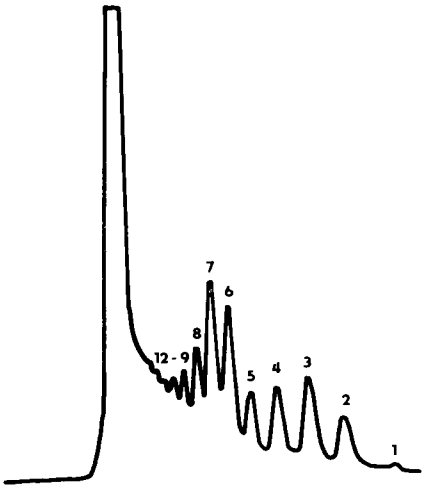

Fig. 5. Chromatogram of maltodextrin KMSx50 on cation exchanger OSTION in silver from. Conditions: column temperature 85°C, 0.5 ml of deionized water/min, RI detection, attn 1.8×10^{-4} RIU, 20 μl sample loop. The number above the peak means the degree of polymerization

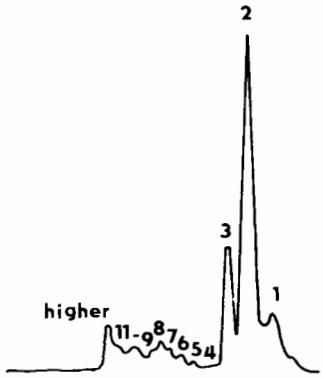

Fig. 6. Chromatogram of the potato maltose sirup (enzyme/enzyme hydrolysis, DE =40%). Chromatographic conditions as in Fig. 5.

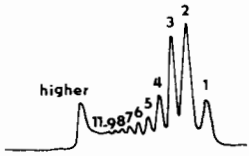

Fig. 7. Chromatogram of maltose sirup (acid/enzyme) hydrolysis of potato starch, DE = 50%). Conditions as as in Fig. 5.

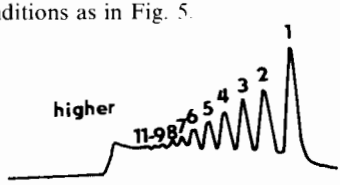

Fig. 8. Chromatogram of potato starch sirup (acidic hydrolysis, DE = 38%). Conditions as in Fig. 5

former sirup was more than double that found in the latter. Figure 8. shows chromatogram of the starch sirup manufactured by acidic hydrolysis of the potato starch (DE = 38%).

We have used the resin in calcium form for the determination of monosaccharides such as glucose and fructose and of alditols (mannitol and sorbitol).

The connection of Separon HEMA and OSTION columns in series appeared to be useful for analyses of maltodextrins and for the studying of the liquefaction step of starch hydrolysis. The chromatogram of the maltodextrin KMS × 50 analysed on coupling columns Separon HEMA 1000 and the cation exchanger OSTION in silver form are shown in Figure 9.

We have determined by capillary isotachophoresis some ionic substances present in starch. The absence of disturbing effect of saccharides from ITP analysis is the great advantage of this method. It is possible to make direct ITP

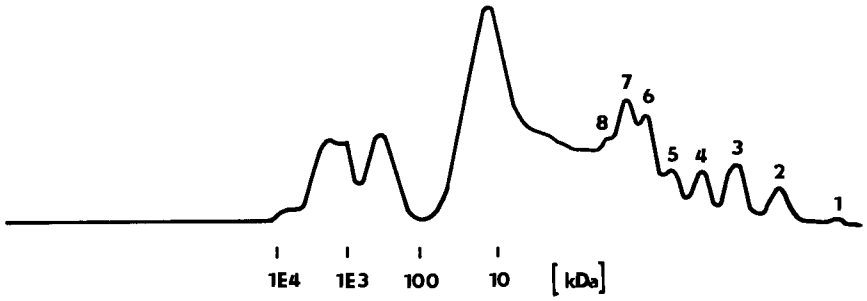

Fig. 9. Chromatogram of the maltodextrin KMS X50 on coupled columns Separon HEMA 1000 with the OSTION (Ag^+)

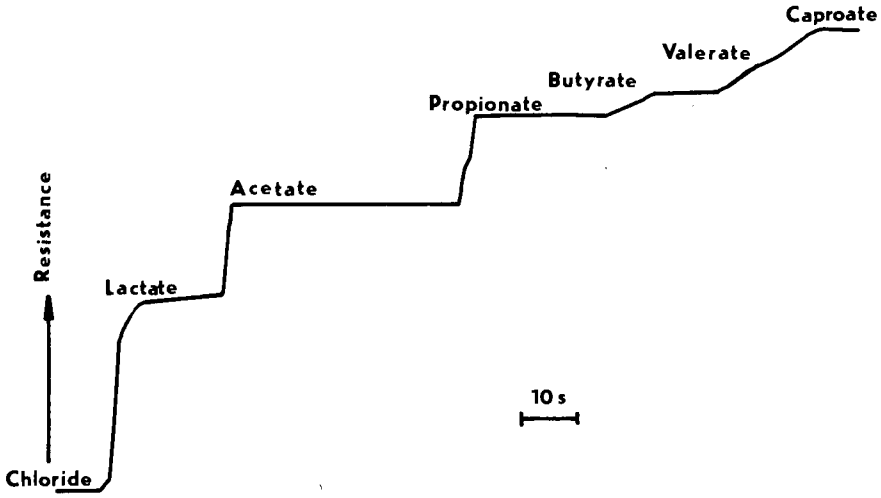

Fig. 10. Anionic isotachophoretic analysis of wet potato starch. Isotachophoretic conditions as in text

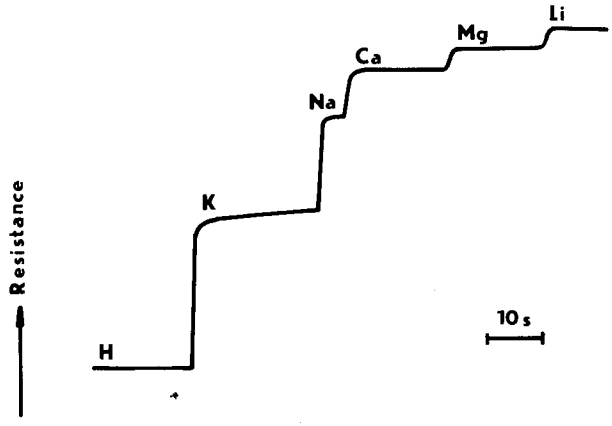

Fig. 11. Cationic isotachophoretic analysis of the native potato starch. Conditions as in text

analysis in the presence of saccharides in the relatively high concentration (up to 50% w/v). We used the ITP method for the determination of organic acids in wet starch during its storing. The isotachopherogram of the anionic analysis of wet potato starch stored at 25°C after 3 weeks is given in Fig. 10. We identified lactic, acetic, propionic and butyric acids in this sample. We used the ITP method also for the analysis of some starch derivatives such as esters or ethers. For example, we determined free and bounded acids in citrate and acetate esters of potato starch. The free scids we determined after gelatinization of starch in water and the bounded acids after deesterification in alkali solution. We applied the JTP method for the determination of the content of chloroacetic and glycollic accids in carboxymethylether of potato starch.

Sodium, potassium, magnesium and calcium were determined in starch by cationic ITP analysis with H^+ as leading cation. We made determinations of the cations mentioned above in different ionic type of starch (sodium, potassium etc.). We compared obtained results by ITP and by atomic absorption spectrometry (AAS). In Table the results of these analyses are summarized. It follows from these results that the ITP method can be replaced by the AAS method.

Table. Results of determination of some cations in diffcrent type of potato starch by ITP and by AAS. Conditions as in text.

Sample	The content of element [mg/kg d.s.]							
	K		Na		Ca		Mg	
	ITP	AAS	ITP	AAS	ITP	AAS	ITP	AAS
Native starch	626	583	134	37	252	217	173	123
H — starch	10	10	12	5	16	11	4	3
K — starch	1271	1203	58	76	13	12	2	3
Na — starch	14	12	1342	1225	5	9	<0.5	3
Ca — starch	5	7	5	5	983	788	7	13

From this paper, it emerges that analyses of polysaccharides on Separon HEMA, oligosaccharides on cation exchanger OSTION and the determination of some ionic substances preesent in starch by capillary ITP are usable methods in starch analysis.

LITERATURE

1. Čopikova J. et al.: Ion exchanger OSTION LG KS C802 (4.2% DUB) and separation of saccharides. Collected papars from the 4th annual American — Eastern European Symposium on Liquid Chomatography, Hungary 1984, 809.
2. Kadlec P. et al.: The study of distribution of molecular weight of the modified starches. Research report for the starch industry. Institute of Chemical Technology, Praque 1987.

3. Kvasnička F. et al.: Cel permeation chromatography of maltodextrins. Bulletin of the Czechoslovak Academy of Agriculture 1987, **5**, 295.
4. Kvasnička et al.: High performance liquid chromatography of starch hydrolysates. Scientific papers of the Praque Institute of Chemical Technology 1985, **E59**, 75.
5. Kvasnička F. et al.: Use of the capillary isotachophoresis in the sugar factory analysis. Listy cukrovarnické 1988, **104**, 255.

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ANALIZA SKROBI I JEJ PRODUKTÓW

Wyższa Szkoła Chemiczno-Technologiczna, Praga

Streszczenie

Podano przegląd metod instrumentalnych stosowanych w laboratorium Katedry Chemii i Technologii Węglowodanów w zakresie badań skrobi. Polisacharydy oznaczono metodą HPSEC na wypełnieniu Separon HEMA z detekcją refraktometryczną. Limit wykluczenia Separon-u HEMA 1000 był większy niż 2000 kDa. Jako fazę ruchomą stosowano wodę lub 0,01 M roztwór KOH z szybkością przepływu 0,5 ml/min. Czas pojedynczej analizy wynosił ok. 20 min. Oligosacharydy rozdzielono metodą HPLC na wymieniaczu kationowym OSTION pracującym w formie srebrowej lub wapniowej. Jako fazy ruchomej używano wody zdemineralizowanej o szybkości przepływu 0,5 ml/min. Oligomery z Dp 1-Dp 12 były rozdzielone na żywicy OSTION w formie srebrowej w czasie 20 min. Substancje jonowe obecne w skrobi analizowano przez izotachoforezę. Analiza zmianowa niektórych kwasów organicznych została przeprowadzona z elektrolitem prowadzącym, składającym się z 10 mM HCl + 18 mM kwasu σ-aminokapronowego i elektrolitem kończącym 5 mM kwasu kapronowego. Zawartość Na, K, Mg i Ca w skrobi określono analizą kationową z 5 mM H_2SO_4 jako elektrolitem prowadzącym i 5 mM cytrynianem litu jako elektrolitem kończącym (terminator). Kationowa analiza izotachoforezy (ITP) może zastąpić metodę AAS przy oznaczaniu zawartości sodu, potasu, magnezu i wapnia w skrobi i jej produktach.