

<https://doi.org/10.17221/55/2021-CJFS>

Chromatographic separation of mannitol from mixtures of other carbohydrates in aqueous solutions

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Citation: Gillarová S., Henke S., Svoboda T., Kadlec P., Hinková A., Bubník Z., Pour V., Sluková M. (2021): Chromatographic separation of mannitol from mixtures of other carbohydrates in aqueous solutions. Czech J. Food Sci., 39: 281–288.

Abstract: The isolation of mannitol from natural sources, e.g. from plant extracts or broths, requires considerable time and effort. The separation of mannitol from aqueous solutions containing also glucose, fructose, and sucrose was tested using discontinuous preparative anion- and cation-exchange chromatography. The suitability of the application in the separation of carbohydrates and especially mannitol was tested under various conditions and using three different types of ion-exchangers. The effect of sorbent regeneration and modification on the separation was also examined using different concentrations and volumes of chemical agents. The fractions collected after the discontinuous chromatography were analysed on the content of mannitol by the high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method. The successful isolation of pure mannitol fraction, using water as a mobile phase and a combination of sodium chloride and hydroxide for sorbent regeneration, was achieved only on anion-exchange chromatography.

Keywords: sugar alcohol; saccharides; ion chromatography; sorbents

Mannitol is an acyclic sugar alcohol, also known as mannite or manna sugar. It was first obtained by drying of exudate of a manna ash tree (*Fraxinus ornus*) from where it has got its name. It occurs mainly in plants (e.g. pumpkins, onions, celery, grasses, olives and mistletoe) and additionally can be produced by various microorganisms (e.g. fungi, yeast, bacteria and algae). Mannitol in pure form is a white, odourless, crystalline powder. The crystals are shaped as needles or rhombuses. Having a solubility in water at 25 °C of only 18% (w/v), which is much lower than that of other sugar alcohols (Dai et al. 2017), mannitol can be separated using fractional crystallisation. Mannitol is widely used for its physical properties, e.g. negative heat of dissolution, in the food industry, pharmacy, medicine and in various chemical applications. The average consumption of mannitol in the world is 150 000 t per year; this cor-

responds to 11% of the total consumption of all polyols (Dai et al. 2017). Due to the growing global number of patients with diabetes and obesity, the demand for mannitol is rising (Dai et al. 2017). The requirement to produce mannitol in high yield from alternative sources is therefore appropriate. In the food industry, it is used as a functional sweetener and sugar-free substitute for diabetics because the metabolic pathway of mannitol is not insulin-dependent. Furthermore, it is used as an additive because of its low hygroscopicity in crystalline form. This property helps to reduce the crystallisation rate of carbohydrates in foods and thus extends their shelf life. Mannitol also indicates the quality of sugar cane and sugar beet products and by-products. If the amount of mannitol in sugar solutions is high, the technological process has been contaminated with *Lactobacillus bacteria* (Eggleston

2010). In pharmacochimistry and pharmacy, mannitol is used for its stability because it rarely reacts with other drug additives and for its sweet-cold taste. Mannitol is also a synergistic antioxidant due to its free-radical scavenging effects. As a polyol, mannitol acts as a low-energy carbohydrate and does not digest in the oral cavity, preventing caries (Dai et al. 2017). In medicine, it is used as a hyperosmolar diuretic agent to reduce brain oedema (Dai et al. 2017; Glykys et al. 2019; Patil and Gupta, 2019; Sun et al. 2019; Narayan et al. 2020) and as a cough provocation medium in tests for investigating the sensitivity of the cough reflex (Nurmi et al. 2019). A mixture of mannitol and sucrose (and sometimes glycerol) is also used to lyophilise proteins (Johnson et al. 2002; Horn et al. 2018; Kulkarni et al. 2018; Anko et al. 2019).

Nowadays, mannitol is produced by catalytic hydrogenation of glucose (Dai et al. 2017) or using microorganisms on various substrates, such as glycerol, glucose, fructose, and sucrose (Hendriksen et al. 1988; Wisselink et al. 2002; Dai et al. 2017). There are also several methods for isolation of mannitol, for example, extraction (Rupérez and Toledano 2003; Ghoreishi and Shahrestani 2009; Lama-Muñoz et al. 2020) or sorption of various biological materials and ferments with lower mannitol content or from materials of a complex matrix (von Weymarn et al. 2002; Racine and Saha 2007; Khan et al. 2009; Yoshikawa et al. 2014). The enzymatic method for the preparation of mannitol in the presence of mannitol dehydrogenase is based on the conversion of D-fructose to D-mannitol (Maria 2020).

In summary, the vast majority of mannitol preparation methods result in a product that also contains other carbohydrates or sugar alcohols. Thus, our work focused on the application of chromatographic separation as an efficient method for the isolation of pure mannitol from plant extracts. The experiments were performed with aqueous solutions of mannitol, glucose, fructose, and sucrose with a representation of the individual components as in the case of celery extract (Rupérez and Toledano 2003). The individual frac-

tions collected at the end of the column were analysed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using Dionex DX-600 (Dionex, USA). For discontinuous chromatography, we used an XK16/40 column (General Electric, USA) for three types of sorbents. The strongly basic anion exchange sorbent Lewatit MonoPlus M 500 KR (Lanxess, Germany) and Dowex 550A Monosphere (Dow, USA) sorbent, as well as the strongly acidic cation exchange sorbent Amberjet 1500H (Rohm & Haas, Germany) were tested by effective column chromatography.

MATERIAL AND METHODS

Mannitol, D-glucose, D-fructose, and sucrose (all Lachema, Czech Republic) were used for the feed of the preparative chromatography; demineralised water (AquaOsmotic Ltd., Czech Republic), NaCl, NaOH, and CaCl₂ (all Penta, Czech Republic) were used for anion- and cation-exchanger regeneration and modification. Ultrapure water (Millipore, USA), NaOH 50% (Sigma-Aldrich, USA) and high purity standards of carbohydrates – sucrose, glucose, fructose, galactose, mannose, and mannitol (Sigma-Aldrich, USA) were used for analytical chromatography. Table 1 summarises the sorbents used for preparative chromatography.

We tested the efficiency of mannitol separation using three different sorbents and examined the effect of various sorbent regenerations steps that involved different volumes and concentrations of sodium hydroxide. Preparative discontinuous chromatography (Figure 1) was used for the separation of mixtures. The equipment consisted of a pump DME-8 (Danfoss, France), 3/2-way switching valve (Regada, Slovakia), thermostat U2 (MLW, Germany) and the three columns XK 16/40 (General Electric, USA), each packed with different sorbent. The XK 16/40 columns are made of borosilicate glass, with an inner diameter of 16 mm, variable bed height up to 40 cm, jacketed to 40 °C, and 500 kPa of maximum pressure. One of these columns

Table 1. Used sorbents

Sorbent	Marking	Type	Particle size (µm)	Capacity (mol L ⁻¹)
Amberjet 1500H (Rohm and Haas, Germany)	cation-exchanger	strong acidic	650 ± 50	2.0
Dowex 550A Monosphere (Dow, USA)	anion-exchanger I	strong basic	550 ± 50	1.4
Lewatit MonoPlus M 500 KR (Lanxess, Germany)	anion-exchanger II	strong basic	640 ± 50	1.1

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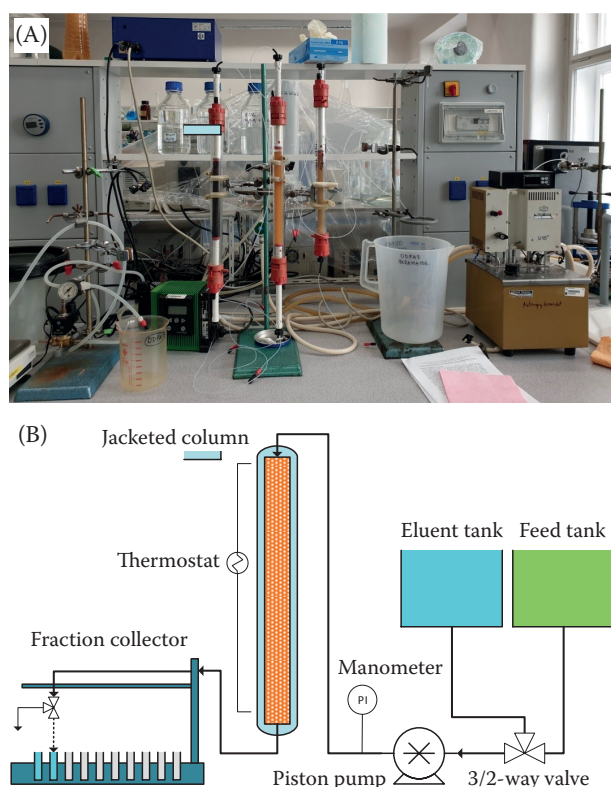


Figure 1. Apparatus for discontinuous preparative ion-exchange chromatography; (A) photo, (B) scheme

was packed with Amberjet 1500H cation exchange sorbent (Rohm and Haas, Germany), and two columns were packed with anion exchange sorbent, one with Dowex 550A (Dow, USA) and the other with Lewatit MonoPlus M 500 KR (Lanxess, Germany). They will be hereinafter referred to as anion-exchanger I (Dowex 550A) and anion-exchanger II (Lewatit MonoPlus M 500 KR). The volume of both anion exchange sorbents in the columns was 47.2 mL, the cation exchange column contained 39.2 mL of the sorbent.

Preparation of the feed solution. For the preparative separation, we prepared a model solution that simulated the sugar content in the extract from celery

stems, as described in (Rupérez and Toledano 2003). This solution had a higher content of mannitol than the extract from the leaves (Rupérez and Toledano 2003). For a sufficient number of replicate measurements, 500 g of stock solution was prepared. The calculations were based on a dry matter content of petiole, which was 16%.

The first column of Table 2 shows the content of carbohydrates and sugar alcohol taken from the literature source (Rupérez and Toledano 2003), which is the percentage of ingredients in dry matter. In the second column of the table, the data from the first column are expressed as percentages of a given component in the extract with a considered dry matter of 16%. The third column shows the conversion of the content of carbohydrates from the second column into units of g (500 g)^{-1} , i.e. the amount of the weighed component to prepare a 500 g solution.

Analytical chromatography. HPAEC-PAD was used for the analyses. The analytical system Dionex DX-600 consisted of a quaternary gradient pump GS50 (Dionex, USA), TCC-100 thermostat (Dionex, USA), autosampler 234 (Gilson, France), CarboPac PA1 ($2 \times 250 \text{ mm}$) column (Dionex, USA) packed with sorbent with quaternary ammonium functional groups, and detector ED-50 (Dionex, USA). The Chromeleon 6.3 software (Dow, USA) operated the system and provided the data evaluation.

Carbohydrates in samples were analysed in duplicates using a Dionex DX-600 instrument. The analyses were performed at 25°C with the mobile phase flow rate of 0.25 mL min^{-1} . The mobile phase composition was: the first 30 min of isocratic elution in 16 millimolar (mM) NaOH (analysis), followed by 10 min of column regeneration in 200 mM NaOH, and finally 15 min of re-equilibration in 16 mM NaOH. The injection volumes were $2 \mu\text{L}$ or $10 \mu\text{L}$. The potential on the measuring electrode of the ED-50 detector was set as follows: 0.05 V at the time of 0.00 s,

Table 2. Contents of mannitol and other carbohydrates in model solutions simulating the extract from celery petiole (Rupérez and Toledano 2003)

Carbohydrates	Percentage in dry matter (%) (Rupérez and Toledano 2003)	Percentage in sample (%) (Rupérez and Toledano 2003)	g (500 g)^{-1} solution
Mannitol	15.240	2.438	12.192
Glucose	11.740	1.878	9.392
Fructose	12.800	2.048	10.240
Sucrose	5.680	0.909	4.544
Other	54.540	8.726	0.000
Total	100.000	16.000	36.368

0.05 V (integration begins) at 0.20 s, 0.05 V (integration ends) at 0.40 s, 0.75 V at 0.41 s, 0.75 V at 0.60 s, –0.15 V at 0.61 s, and –0.15 V at 1.00 s.

The concentration of analytes was calculated from calibration curves measured with external standards containing five carbohydrates (glucose, fructose, sucrose, galactose, and mannitol) at various concentrations.

Discontinuous chromatography. During all steps, the temperature of the chromatography column was kept by the thermostat at 45 °C. Regeneration was performed at a flow rate of 1 000 mL h^{–1} from reservoirs connected to the feed line. Initially, the column was washed with 250 mL of deionised water. The regeneration of both anion-exchangers was carried out as follows: anion-exchanger I was regenerated with 250 mL of 200 mM NaCl (5.3 mL of regenerant per mL of anion exchange resin) and subsequently modified with 250 mL of 10 mM NaOH. The anion-exchanger II was regenerated with 250 mL of 200 mM NaCl (10.6 mL of regenerant per mL of anion exchange resin) and subsequently modified in three ways: 250 mL of 10 mM NaOH, 500 mL of 5 mM NaOH, and 500 mL of 10 mM NaOH. The cation-exchanger was regenerated with 250 mL of 1% CaCl₂ (6.4 mL of regenerant per mL of cation-exchanger). In the final phase, all columns were washed again with 250 mL of deionised water. The separation was carried out at a flow rate of 150 mL h^{–1}. Before the measurements, the valve was switched to the position allowing the mobile phase to enter the column. Then the injection of 1 mL of the sample was performed at a given flow rate from

the sample container via the injection branch. After 24 s, the valve was switched again to allow a flow of pure mobile phase through the column. Samples at the outlet of the column were taken manually at certain time intervals. The first ten samples were taken at 3 min intervals, then twenty samples at 5 min intervals and the last six samples were taken every 20 min. The total withdrawal time for each column was 250 min. Thus, 36 samples were obtained from each measurement and then analysed directly by HPAEC-PAD.

RESULTS AND DISCUSSION

Separation experiments. The results of the discontinuous separation of the solution containing mannitol, glucose, fructose, and sucrose on anion-exchanger I (Dowex 550A) showed that the mass fraction of mannitol in dry matter was above 50% in fractions collected between 15 min and 27 min (Figure 2).

The results of the discontinuous separation of mannitol, glucose, fructose, and sucrose on anion-exchange II (Lewatit MonoPlus M 500 KR) showed that the fractions containing more than 50% of mannitol in the dry matter were collected between 15 min and 27 min (Figure 3). This is similar to separation on anion-exchanger I (Figure 2); however, the maximum achieved concentration of mannitol in the dry matter in the region between 12 min and 27 min was slightly below 75%, whilst on the anion-exchanger I, it was around 60%.

We observed the same elution order of mannitol and glucose as that reported in the literature (Saska and Chen 2002) and a similar retention time of mannitol when tak-

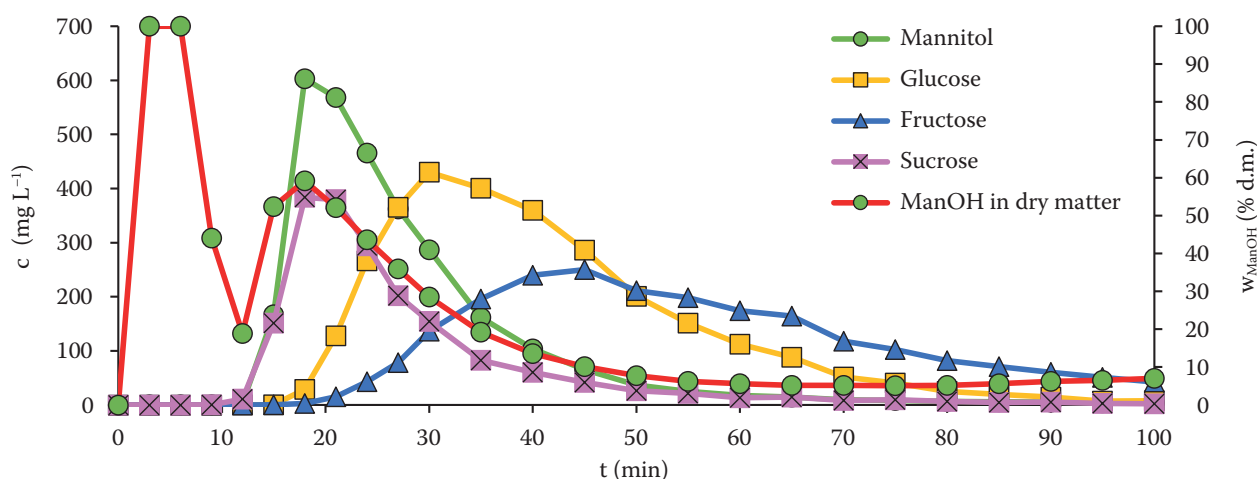


Figure 2. Time dependence of mannitol, glucose, fructose, and sucrose concentrations at the column exit (left y-axis) and time dependence of mannitol content in the dry matter at the column exit (right y-axis) for discontinuous anion-exchange I separation (Dowex 550A), regeneration with 250 mL of 10 mM NaOH

c – concentration (mg L^{–1}); w_{ManOH} – the content of ManOH in the dry matter of fraction (% d.m.); t – time (min)

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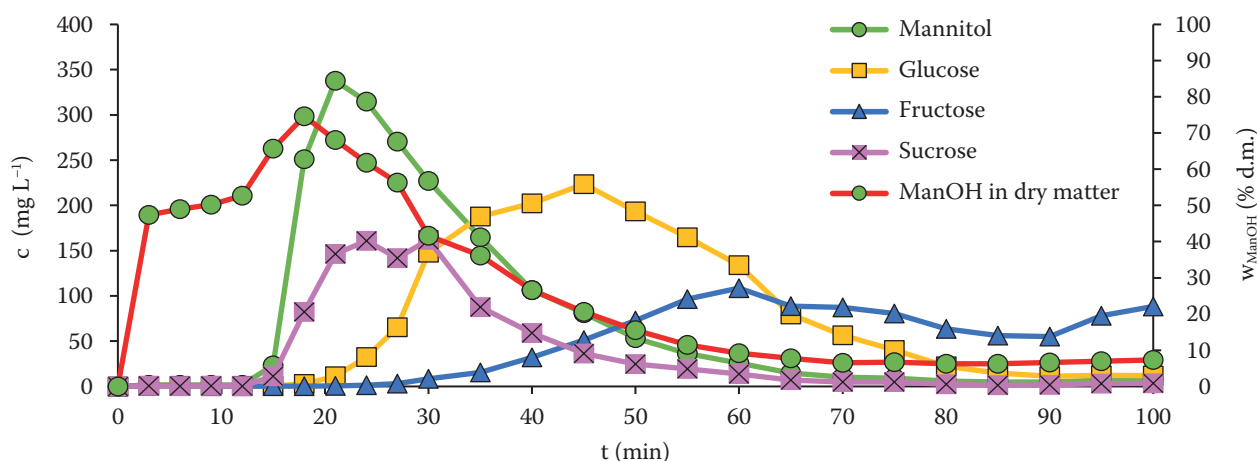


Figure 3. Time dependence of mannitol, glucose, fructose, and sucrose concentrations at the column exit (left y-axis) and time dependence of mannitol content in the dry matter at the column exit (right y-axis) for discontinuous anion-exchange II separation (Lewatit MonoPlus M 500 KR), regeneration with 250 mL of 10 mM NaOH

c – concentration (mg L⁻¹); w_{ManOH} – the content of ManOH in the dry matter of fraction (% d.m.); t – time (min)

ing into consideration that the sorbents were regenerated with 250 mL of 10 mM NaOH. The mixed fractions taken from 0 min to 21 min containing both mannitol and sucrose could be used for lyophilisation of proteins after thickening of the solution to the desired concentration, as reported elsewhere (Johnson et al. 2002).

The results of separation of mannitol, glucose, fructose, and sucrose solutions on both anion-exchange resins I and II (Dowex 550A and Lewatit MonoPlus M 500 KR) were almost identical due to very similar parameters of both sorbents (sorbent layer height, particle size, capacity) (Table 1) and maintaining the same measurement conditions (separation tem-

perature 45 °C, mobile phase flow rate 150 mL h⁻¹) and regeneration.

The results of the separation with anion-exchanger II (Lewatit MonoPlus M 500 KR) that was previously modified with 500 mL of 5 mM NaOH showed that the fractions containing more than 50% of mannitol in the dry matter were collected in the time interval between 3 min and 30 min (Figure 4).

Therefore, by modifying the anion-exchange column with 500 mL of 5 mM NaOH, mannitol can be separated from carbohydrate mixtures at the beginning of the elution as the fractions already contain more than 50% of mannitol. The effect of the concentration of the

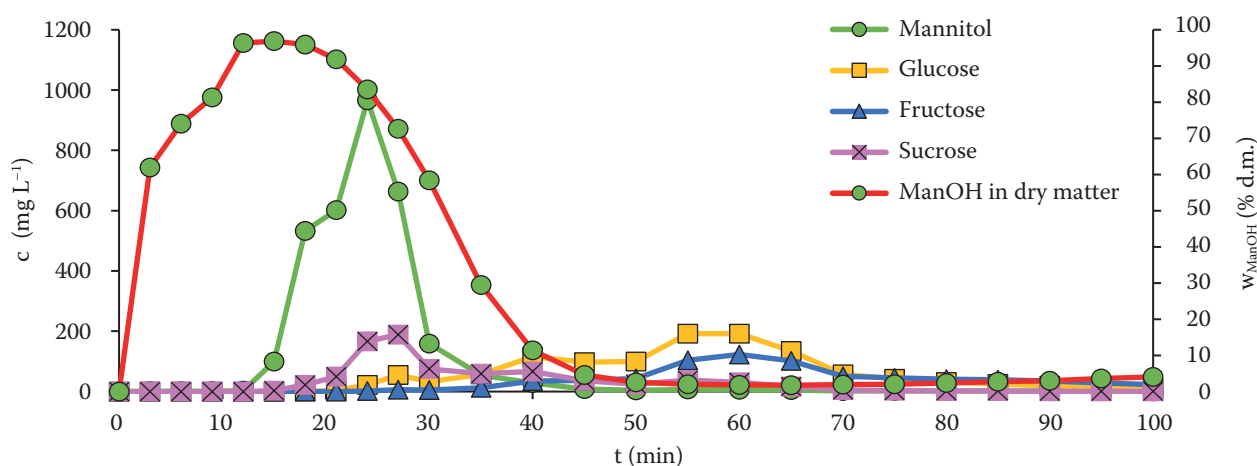


Figure 4. Time dependence of mannitol, glucose, fructose, and sucrose concentrations at the column exit (left y-axis) and time dependence of mannitol content in the dry matter at the column exit (right y-axis) for discontinuous anion-exchange II separation (Lewatit MonoPlus M 500 KR), regeneration with 500 mL of 5 mM NaOH

c – concentration (mg L⁻¹); w_{ManOH} – the content of ManOH in the dry matter of fraction (% d.m.); t – time (min)

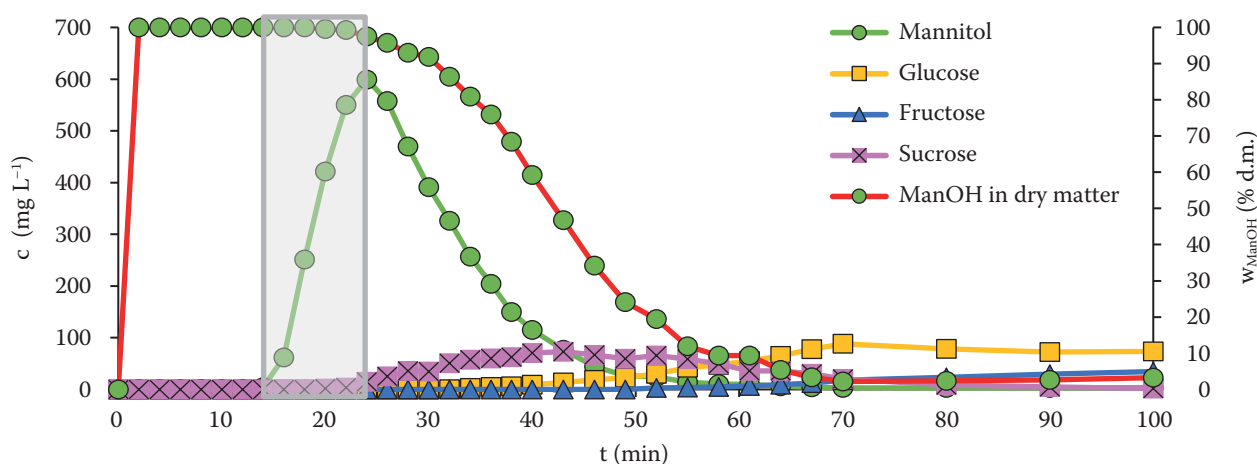


Figure 5. Time dependence of mannitol, glucose, fructose, and sucrose concentrations at the column exit (left y-axis) and time dependence of mannitol content in the dry matter at the column exit (right y-axis) during discontinuous anion-exchange II separation (Lewatit MonoPlus M 500 KR) – detail of the first 100 min of analysis with marked band between 14 min and 24 min, regeneration with 500 mL of 10 mM NaOH

c – concentration (mg L⁻¹); w_{ManOH} – the content of ManOH in the dry matter of fraction (% d.m.); t – time (min)

NaOH regenerative agent (in this case, the concentration reduction) on the separation of carbohydrates and sugar alcohols is also reported in the literature (Saska and Chen 2002).

As detailed in Figure 5, one can see that increasing both the concentration and the volume of NaOH (500 mL of 10 mM NaOH was applied in this case) used for the anion-exchange II (Lewatit Mono-Plus M 500 KR) modification improved the mannitol separation and it was possible to obtain nearly pure mannitol containing only residues of other carbohydrates in fractions collected in the time interval from 14 min to 24 min.

The mannitol content in dry matter is higher than 97.6%; the maximum is 99.6%. Figure 5 also demonstrates that from the 3rd min to 41st min, it is possible to collect fractions containing more than 50% of mannitol.

The results of the discontinuous separation of the mannitol, glucose, fructose, and sucrose solution on the cation-exchange resin (Amberjet 1500 H) in Ca form indicate that the cation-exchange column was not able to separate individual carbohydrates. This is because the affinity of the functional groups on the surface of the cation-exchange sorbent was the same for all the present carbohydrates (Figure 6).

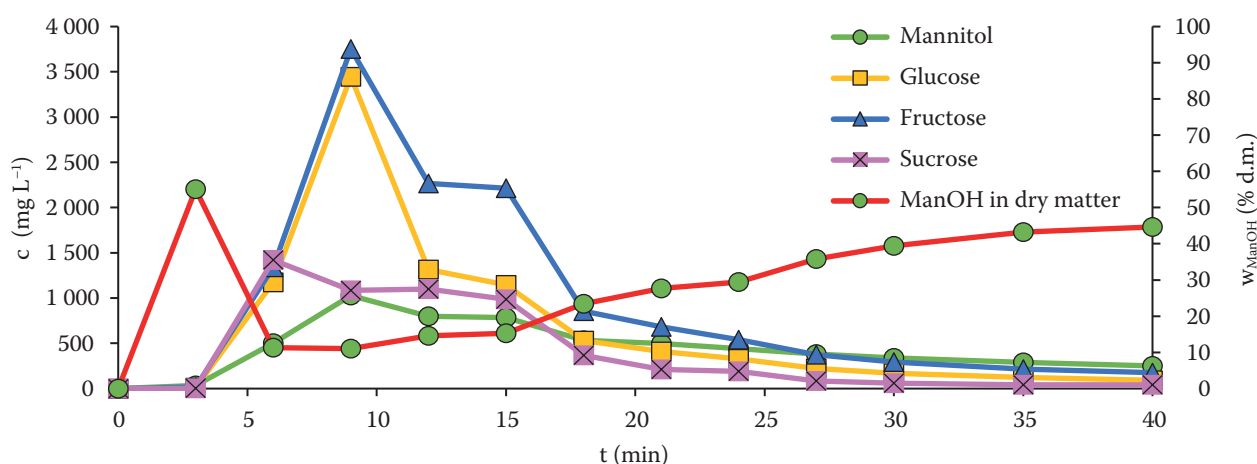


Figure 6. Time dependence of mannitol, glucose, fructose, and sucrose concentrations at the column exit (left y-axis) and time dependence of mannitol content in the dry matter at the column exit (right y-axis) during discontinuous cation-exchange separation (Amberjet 1500 H), regeneration with 250 mL of 1% CaCl₂

c – concentration (mg L⁻¹); w_{ManOH} – the content of ManOH in the dry matter of fraction (% d.m.); t – time (min)

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Comparison with other feeds and methods.

The concentration of mannitol (12.192 g L^{-1} in the feed solution) (Table 2), which corresponds to the celery extract (Rupérez and Toledano 2003), is similar to the concentration of mannitol obtained by fermentation processes (Bhatt et al. 2013), depending on the micro-organisms and conditions used.

Mannitol can also be produced and separated from the carbohydrate mixture by methods other than a combination of extraction and chromatography. The literature (Saha and Racine 2011) describes a technology based on the cooling crystallisation of mannitol from a fermentation medium, which can also produce pure mannitol (> 99.5%) without any other carbohydrate admixtures, i.e. with the similar result as the chromatographic separation using the anion-exchanger Lewatit MonoPlus M 500 KR and regeneration with 250 mL of 200 mM NaCl followed by modification with 500 mL of 10 mM NaOH. The advantage of the chromatographic method could be the possibility of direct drying of the pure mannitol fraction. In order to achieve a purity of mannitol of 99.5% in crystals, which is the purity obtained by chromatography, the preconcentration of the solution using reverse osmosis or evaporation and also recrystallisation steps are needed in the technology based on crystallisation as described in von Weymarn et al. (2003). On the other hand, the disadvantage of chromatography in comparison with crystallisation is the necessity of using chemicals for regeneration and elution. This could be overcome by using continuous chromatography, which comes in most cases with low eluent consumption.

CONCLUSION

For convenience and direct use, isolation of mannitol was performed with a model mixture having the same composition of carbohydrates as the extract of natural material. The composition of the mixture was chosen in order to correspond to the content of major carbohydrates in an aqueous celery petiole extract with a very high mannitol content of up to 2.5%. The solution containing mannitol, glucose, fructose, and sucrose was separated using anion-exchanger I, Dowex 550A Monosphere (Dow, USA), anion exchanger II, Lewatit MonoPlus M 500 KR (Lanxess, Germany), and cation-exchanger, Amberjet 1500 H (Rohm and Haas, Germany) at a temperature of 45°C and a mobile phase flow rate of 150 mL h^{-1} . The results from the separation on cation-exchange resin modified with 250 mL of 1% CaCl_2 (5.3 mL reagent per mL of cation-exchange

resin) showed no separation at all, so the next measurements were performed exclusively with anion-exchange columns. In case of anion-exchangers I and II, and pre-separation regeneration of sorbent by 250 mL of 200 mM NaCl, followed by modification with 250 mL of 10 mM NaOH (5.3 mL of reagent per mL of anion-exchanger), we achieved 59.2% of mannitol for anion-exchanger I and 74.7% for anion-exchanger II, both being a mixed fraction with sucrose.

In the case of pre-separation regeneration of the anion-exchanger II with 250 mL of 200 mM NaCl and subsequent modification with 500 mL of 5 mM NaOH (10.6 mL of NaOH per mL of anion-exchange resin), the maximum mannitol fraction of 96.9% in the dry matter was gained in 15 min. The anion-exchanger II regenerated with 250 mL of 200 mM NaCl and subsequently modified with 500 mL of 10 mM NaOH (10.6 mL of NaOH per mL of anion-exchanger) provided a higher than 99.6% content of mannitol in the dry fraction.

The benefit of this work is the successful isolation of pure mannitol using regenerated anion-exchange sorbent and water as a mobile phase, resulting in obtaining highly purified mannitol in an aqueous solution that did not contain any other carbohydrates or impurities. In the future, this technology may replace the current production of mannitol using a less environmental-friendly chemical way with metal catalysts and energy-demanding crystallisation.

The next step following this work is to carry out measurements with solutions of other, usually higher concentrations, a wider range of temperatures and different flow rates of the mobile phase in order to increase the separation efficiency. The columns with different dimensions will also be tested with regards to the number of theoretical plates. Current research and development are focused on the identification of mathematical models of the chromatographic separation process of mannitol mixtures, computer simulation of continuous chromatography, and searching the optimal operational parameters and conditions. In the future, we will verify the results of the simulations in a continuous preparative chromatography plant.

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Received: February 25, 2021

Accepted: June 17, 2021