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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF SUGARS IN RAW AND PROCESSED POTATOES[1]

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ABSTRACT

A precise liquid chromatographic method was developed to determine sucrose, glucose and fructose content in raw, precooked, steam cooked, moist and dry potato granules from the Add-Back (A-B) and Freeze-Thaw (F-T) processes. The sugars were extracted from samples with a mixture of 95% ethanol, water and concentrated H_2SO_4 (v/v) and purified by removal of organic acids with an onion exchange formate resin column. The final filtered sample was analized for sugars using an HPLC system with an Aminex HPX-87H column, 0.01 N H_2SO_4 as the mobile phase and a differential refractometer detector. The extraction and purification procedure provided sugar recovery of over 98%. The analytical method was highly reliable with standard curves of the sugars having a minimum correlation coefficient of 0.998. It was found that Shepady potatoes stored at 4°C contained the highest quantities of glucose and fructose, 5.35 and 3.95% (d.b.), respectively, as compared to Kenebec, Norgold, California Rose, Norchip and Norland. Shepady lost 8, 22 and 13% of sucrose, glucose and fructose, respectively, after precooking and produced A-B granules with about 50% of original reducing sugars. When Norgold and Norland were processed to granules with the F-T process, its reducing sugars were reduced by approximately 24 to 31% in the dry granules.

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استفاده از دستگاه کروماتوگراف مایع (HPLC) برای تجزیه قندهای سیب زمینی خام و فرآیندشده

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به ترتیب استادیار صنایع غذایی دانشگاه صنعتی اصفهان و دانشیاران و استاد صنایع غذایی دانشگاه آلبرتا، ادمونتون، کانادا.

خلاصه

جهت دقیق کروماتوگراف مایع برای تعیین مقدار ساکارز، گلوکز و فروکتوز سیب زمینی خام، نیم‌پز، کاملا پخته، پودر مرطوب و خشک سیب زمینی فرآیندشده با دوروش (ا ـ بی) و (اف ـ تی) ابداع گردید. قندهای سیب زمینی با حلالی مخلوط از اتانول ۹۵% آب و اسید سولفوریک غلیظ استخراج شده و با کمک ستونی از رزین تبادل یونی از نوع فرمات تخلیص گردیدند. نمونه‌های خالص شده قندها به دستگاه کروماتوگراف مایع که با ستون آمینکس با مدل HPX - 87H و انکسار سنج تعبیه شده بود تزریق گردیدند و با کمک محلول رقیق اسیدسولفوریک ۰/۰۱ نرمال تجزیه شدند. متد استخراج و تخلیص موجب بازیابی قندها بیش از ۹۸ درصد گردید. بین روش تجزیه قندها و منحنی های استاندارد ضریب همبستگی ۰/۹۹۸ وجود داشت که نشانگر قابلیت اعتماد بالا در روش تجزیه بود. غده‌های سیب زمینی شپودی که در ۴ درجه سانتیگراد انبار شده بودند بیشترین مقدار گلوکز و فروکتوز که به ترتیب ۵/۳۵ و ۳/۹۵ درصد در ماده خشک بودند نسبت به سایر ارقام نشان دادند. قطعات سیب زمینی درضمن پختن در آب مقدار قابل توجهی از ساکارز، گلوکز و فروکتوز خود را از دست دادند. درمتدهای تولید پودر سیب زمینی با روشهای (ا ـ بی) و (اف ـ تی) به ترتیب ۵۰ درصد و ۲۴ تا ۳۱ درصد قندهای احیا در حین عملیات کاهش یافته بودند.

INTRODUCTION

The quantitation of sugars, particularly reducing sugars such as glucose and fructose, is of importance in the potato processing industries. The color and flavor of potato products such as chips, French fries and dehydrated potatoes can be influenced by the reducing sugars originally present in potato tubers. The deterioration of these products may be attributed to the accumulation of reducing sugars (6) which react with amino acids in the Maillard reaction to form melanoidins with undesirable color and flavor (3, 7). Therefore, reducing sugar content in raw tubers as well as some finished products is routinely determined to evaluate the suitability of the potatoes

for processing and the quality of the product

Several methods for sugar analysis in potatoes have been suggested (5, 8, 15, 16, 18, 21) and compared (11) for their accuracy, time required, costs of equipment and chemicals and level of skill of the operators. The use of ion-exclusion chromatography for the separation of non-ionic from ionic materials has been discussed by many authors (9, 17, 19, 20). HPLC analysis of organic acids in potatoes using the ion exclusion principle (4, 13) revealed the ease and accuracy of this method with respect to sample preparation and quantitation of the components. For juice extraction from potato tubers a solvent consisting of 95% ethanol, water and concentrated H_2SO_4 in the ratio of 60:40:0.2 (v/v/v) was suggested because of its non-foaming and rapid filtering characteristics (4). For the separation of mixtures of sugars and organic acids such as those in fermentation processes, HPX-87H ion exclusion column using 0.01 N H_2SO_4 as the mobile phase and refractive index detection have been suggested (1).

This study describes an improvement to the HPLC analysis of sugars through a modified sample extraction and purification procedure where organic acids were removed with ion exchange chromatography prior to injection into the HPLC system. The method was used to analyse sucrose, glucose and fructose in raw potatoes as well as in samples taken from various processing steps of either the A-B (14) or F-T (12) potato granule process.

MATERIALS AND METHODS

Raw and Processed Potatoes

Shepady, Kenebec, Norgold and California Rose cultivars of potatoes were supplied by I & S Produce Ltd. and Edmonton Potato Growers (1971) Ltd., Edmonton, AB, Canada. Before use, the potatoes were stored at 4°C for about three months.

Dehydrated potato granules, commercially used as instant

mashed potatoes or as an ingredient in products such as croquettes or fabricated potato chips, were prepared in the Department of Food Science pilot plant. The add-back granule process is the only process currently used by the industry to produce potato granules. In this process, about 90% of the previously dried granules is recycled to be mixed with freshly cooked potato to reduce its moisture content while granulating it to fine particles. Therefore, the granules are recycled through the system 8-10 times before they are discharged as product. To simulate this process in the laboratory, two kg of potatoes were washed, peeled, sliced to 0.8-1.5 cm thick, precooked at 70° C for 20 min, cooled, steam cooked, hot mash-mixed with 80-90% commercial granules (Vauxhall Foods Ltd., Vauxhall, AB) and 0.4% (dry weight) monoglycerides at 45° C for 40 min in a Hobart mixer. The moist mix was dried in a modified Manesty Petrie fluid bed dryer Model MP.10.E (Manesty Machines Ltd., Speke, Liverpool) to produce granules with about 7% moisture. The product was sifted through an 80 mesh screen and stored in a polyethylene bag.

The freeze-thaw granule process is a recently developed process which minimizes the recycling of dry granules, thus improving the product quality and increasing the production output. To produce the freeze-thaw granules, the process described by Ooraikul (12) was used where potatoes were peeled, sliced, washed, steam cooked for 35 min, hot mashed and frozen at -18° C. The frozen mash was thawed to 0° C, pre-dried in the modified Manesty Petrie fluid bed dryer with gentle stirring, granulated with fast stirring action and dried to about 10% moisture. The product was sifted through 80 mesh screen and stored in a polyethylene bag.

Sugar Analysis

HPLC system and chemicals. Sugars were analysed with an HPLC system which consisted of Beckman model 110 A pump, Waters model R401 differential refractometer, either a Terochem

recorder (Terochem Lab., Edmonton, AB) or a Hewlett Packard model 3388 A integrator, 20 µL Rheodyne loop injector, and Bio-Rad 300x7.8 mm I.D. Aminex HPX-87H column protected by an analytical grade 40x4.6 mm micro-guard cation H^+ refill cartridge.

HPLC grade water was prepared with Milli-RO and Milli-Q systems (Millipore Corp., Bedford, MA). H_2SO_4 was A.C.S. grade (Fisher Scientific, Fair Lawn, NJ). Analytical grade anion exchange resin, AG 1-X4, 100-200 mesh, formate form (Bio-Rad Labs, Richmond, CA) was packed into a 10 ml polyethylene syringe to a bed volume of 3-4 ml over glasswool. Ethyl alcohol, 95%, was obtained from the Department of Chemistry, the University of Alberta.

Standard sugars, fructose (BDH Chem., Toronto, Ont.), sucrose (Fisher Scientific, Fair Lawn, NJ) and glucose (Terochem Lab., Edmonton, AB) were dissolved in HPLC grade water as stock solutions (20 mg ml^{-1}). Lower concentrations were freshly prepared when required.

HPLC separation conditions. The mobile phase was 0.01N H_2SO_4, pH 2.15, prepared freshly each run with HPLC grade water and degassed with a vacuum aspirator as it was filtered through 0.45 µm filter membrane. The flow rate of the system was kept constant at 0.8 ml min^{-1} at ambient temperature (23± 1°C).

Extraction of sugars. The extraction solvent was prepared by mixing 95% ethanol with water and concentrated H_2SO_4 in the ratio of 60:40:0.2 (v/v) according to Bushway *et al.* (4). For raw potatoes, 6 tubers were taken randomly to represent small, medium and large tuber size. They were washed, peeled, diced to 0.5x0.5x0.5 cm cubes, and mixed thoroughly before a 50 g sample was weighed into a 4 oz (113 g) container of an Osterizer blender. The sample was homogenized with 100 ml of extraction solution for 2 min and vacuum filtered through No. 4 Whatman filter paper on a Buchner funnel. The filtrate was centrifuged at 25,000x*g* (Beckman Model J-21B Centrifuge,

Beckman Instruments, Palo Alto, CA) in 20 ml polycarbonate tubes with caps for 10 min. Ten ml of the supernatant were passed through the anion exchange formate resin column and the sample was collected in a 25 ml volumetric flask. The column was washed with water into the flask until the volume of the sample was up to the mark. Five ml of the sample were then filtered through a 0.45 µm Millipore membrane and injected into the 20 µl injector loop of the HPLC.

Samples of precooked and cooked potatoes, moist (before drying, about 40% moisture) and dry granules were also taken for analysis. For precooked and cooked potatoes, 50 g representative samples were taken and proceeded as with raw potatoes. For moist and dry granules, 25 and 10 g, respectively, were used and the extraction procedure was the same as above.

Standard curves and recovery studies. Five standard solutions, 0-2%, were prepared from the stock solutions with Millipore-filtered water. A mixture of 1:2 parts of standard solution:extraction solvent was homogenized for 2 min. Other steps of preparation were the same as those in the extraction of sugars from potatoes, and 20 µl of each standard was injected into the HPLC.

For the sugar recovery studies, 250 and 500 mg of standard sugars were added to 50 g diced potato samples and extraction of sugars from the samples proceeded as before. Another 50 g sample of diced potatoes from the same batch was used as the control. Peak areas of the chromatograms were used to quantitate the sugars.

RESULTS AND DISCUSSION

The HPLC separation of sucrose, glucose and fructose in standard solution or potato samples is demonstrated in Fig. 1. In less than 10 min the three sugars were eluted and separated on the Aminex column with the retention time of 5.7 min for sucrose, 6.8 min for glucose and 7.6 min for fructose. Total analysis time for each sample, including extraction and

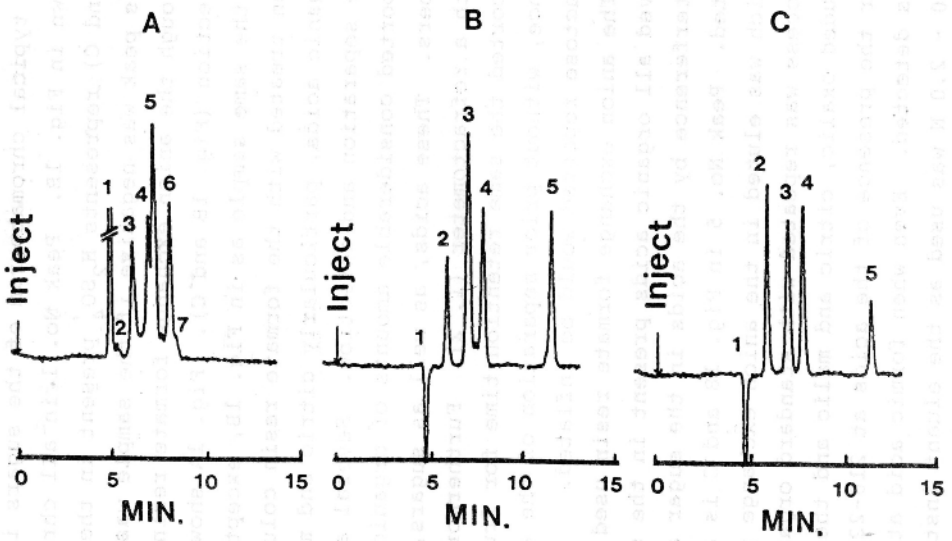

Fig. 1. HPLC chromatograms of sugars with 0.01N H_2SO_4 (pH 2.15) as the mobile phase, flow rate 0.8 ml min^{-1} and chart speed of 0.34 cm min^{-1}.

A. Sugars in Shepady potato without removal of organic acids: 1. H_2SO_4 (mobile phase), 2. unknown, 3. sucrose, 4. citric acid, 5. glucose, 6. fructose, 7. unknown.

B. Same as A, but organic acids were removed with anion exchange resin before injecting into the HPLC: 1. mobile phase, 2. sucrose, 3. glucose 4. fructose, 5. formate (from the anion exchange column).

C. Sugars of standard solutions which had been cleaned with anion exchange resin: 1. mobile phase, 2. sucrose, 3. glucose, 4. fructose, 5. formate.

purification, was less than 30 min; of this 19 min was for the HPLC analysis. Duplicate samples were analyzed each time. The Aminex column was cleaned and regenerated (2) after every 100 injections to maintain its performance.

A typical chromatogram of the sugars in Shepady tuber is shown in Fig. 1B. Peak No. 1 in all chromatograms (Fig. 1A, B and C) represents H_2SO_4 present in the extraction solvent. This peak was negative if the sample was chromatographed through the anion exchange formate resin column prior to the injection (Fig. 1B and C). Fig. 1A shows the chromatogram of the same sample as in Fig. 1B, except the sample had not been treated with the formate resin column and, therefore, organic acids, particularly citric and malic, interfered with the separation and detection. Several authors (4, 13) have reported considerable amounts of organic acids in potato tubers. These acids, as well as sugars, could be detected with a refractometer (1, 2). Furthermore, Bushway, *et al.* (4) reported the same retention time for fructose and malic acid. Hence, without prior separation of the acid the amount of fructose reported would be inflated.

The anion exchange formate resin used in this method removed all organic acids present in the samples and, therefore, interference by the acids in the sugar analysis was eliminated. Peak No. 5 in Fig. 1B and C is a by-product, formate, which was eluted in the anion exchange process. The same process was repeated with standard organic acids which included oxalic, citric and malic and the eluate was examined for the presence of the acids at 210-220 nm (4, 10, 13); none was detected. Even when formic acid at the concentrations of 1.0 - 2.0 N was used as the eluant instead of water, no organic acids were released from the column. This confirmed that all organic acids present in sugar samples were retained in the formate resin column. This resin was found to be the best when compared with other resins such as Amberlite IRA-400 chloride form (Terochem Lab., Edmonton, AB) and Dowex-1

chloride form 1x8-100 (Sigma Chem., St. Louis, MO) for the purpose of organic acid removal from potato samples. Three ml bed volume of the resin was suitable for five samples after which it had to be regenerated.

A minimum of 98% recovery was obtained when sugars were eluted through the formate resin column (Table 1). At both concentrations (250 and 500 mg 50 g^{-1} potato) of sugars added to diced potato prior to extraction, practically all was recovered. Wilson, *et al.* (21) used a μ Bondapak HPLC column with actonitrile:water (75:25) as the mobile phase and reported a minimum of 93% recovery. Reliability of the analytical method was very high as indicated by the linearity of the plots between standard sugar concentrations and peak areas from the chromatograms, with the minimum correlation coefficient of 0.998.

Table 1. Sugar recovery in raw Shepady potatoes after extraction and purification†.

Sugar	mg added per 50 g potato	Content originally present in 50 g potato	Total found (mg)	Recovery (%)
Sucrose	250.0	130.0	387.5	103.0
Glucose	250.0	460.0	720.0	104.0
Fructose	250.0	425.0	670.0	98.0
Sucrose	500.0	128.0	618.0	98.0
Glucose	500.0	465.0	997.0	106.0
Fructose	500.0	375.0	896.0	104.0

† Average of duplicate analyses.

Sucrose, glucose and fructose contents of potatoes, analised with the method just described, varied greatly among the cultivars (Table 2). Shepady exhibited the highest sugar contents, particularly the total reducing sugar of 8.60 g 100 g^{-1} dry matter, and produced very dark potato chips as compared with other cultivars with low reducing sugars e.g. California Rose and Norchip.

Table 2. Sucrose (S), glucose (G) and fructose (F) content in Shepady (22% solids), Norgold (21% solids), California Rose (21% solids) and Norchip (21% solids) potatoes in both wet and dry basis.

Potato Cultivar	Sugar Content, g 100 g^{-1}†					
	Wet basis			Dry basis		
	S	G	F	S	G	F
Shepady	0.37	1.10	0.79	1.68	5.00	3.59
Kenebec	0.24	0.66	0.73	1.20	3.30	3.65
Norgold	0.37	0.54	0.72	1.76	2.57	3.43
California Rose	0.53	0.18	0.09	2.52	0.86	0.43
Norchip	0.17	0.07	0.18	0.81	0.33	0.86

†Average of duplicate analyses.

Tables 3 and 4 show sugar content of the product, using Shepady, Norgold and Norland potatoes, at various steps of granule processes. Sugar loss during the preparation steps was appreciable. In the A-B process, Shepady slices lost 8% sucrose, 22% glucose and 13% fructose after precooking at 70°C for 20 min. The loss of 18% sucrose, 45% glucose and 56% fructose in the final dry granules was due to the fact that the freshly cooked potatoes were mash-mixed with 80%

(dry basis) of commercial granules which contained only 1.6% total reducing sugar (dry basis).

Table 3. Sucrose, glucose and fructose content in g 100 g^{-1} dry solids of Shepady potatoes (22% total solids) during the Add-Back granule process†.

Sugar	Unwashed slices	Precooked slices	Moist granules (55% solids)	Dry granules (96% solids)
Sucrose	1.18	1.09	0.98	0.97
Glucose	3.32	2.60	1.71	1.83
Fructose	3.04	2.66	1.60	1.34

†Average of duplicate analyses.

Table 4. Sucrose, glucose and fructose content in g 100 g^{-1} dry solids of Norgold (21% solids) and Norland (17% solids) during the Freeze-Thaw granule process†.

Sugar	Unwashed	After washing	After steam cooking	Dry granules
Norgold				
Sucrose	2.00	1.50	2.00	1.60 (90% solids)
Glucose	2.90	2.60	2.70	2.10 "
Fructose	3.60	3.30	3.50	2.90 "
Norland				
Sucrose	2.75	-	2.57	1.63 (96% solids)
Glucose	3.00	-	3.18	2.09 "
Fructose	3.05	-	3.07	2.07 "

†Average of duplicate analyses.

Cold water washing of Norgold slices in the F-T process resulted in 25% loss of sucrose, 10% of glucose and 8% of fructose. However, the sugar contents appeared to regain the original level, or even slightly increase in the case of Norland, after steam cooking for 35 min. This may be attributed to thermal hydrolysis of starch during the cooking. Further reduction of sugars might occur during thawing and predrying, with some drip loss, resulting in the loss of 20, 28 and 19% for Norgold and 41, 30 and 32% for Norland of sucrose, glucose and fructose, respectively, in the final dry granules.

One of the important concerns of sugar analysis using this analytical method is the possible hydrolysis of sucrose during sample preparation and storage (4°C). To evaluate the problem, standard sucrose solutions at various concentrations were made, either in extraction solvent or in the mobile phase, and were subjected to the same extraction and cleaning procedures and kept at 4°C for one week before injecting into the HPLC system. No hydrolysis of sucrose was detected as there was no loss of the sugar, nor was there any glucose or fructose peak on the chromatogram. This was attributed to the fact that even though the pH of the extraction solvent was 1.8, it was raised to about 4.0 after the extraction, purification and dilution steps which was not sufficiently low to hydrolyze sucrose in the samples at either 4°C or ambient temperature.

CONCLUSION

Results of this study indicate that sugar analysis after sample extraction with a mixture of 95% ethanol, water and concentrated H_2SO_4, sample purification with a formate resin column and quantitation of sugars using the HPLC system with an Aminex HPX-87 H column and diluted H_2SO_4 as the mobile phase, is suitable for potato and its products. As a research tool or for routine analysis it is simple, quick and accurate.

For quality control purposes, however, equipment and operation costs may be prohibitive for some industries.

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