Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 398–402
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Use of the alditol acetate derivatisation for the analysis of reducing sugars in potato tubers

N.P. Brunton *, T.R. Gormley, B. Murray

Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

Received 26 May 2006; received in revised form 16 January 2007; accepted 28 January 2007

Abstract

The alditol acetate derivatisation for determining levels of reducing sugars in ethanolic extracts from potato tubers by gas chromatography was investigated. Standard curves for alditol acetate derivatives of aqueous solutions of allose, glucose and fructose were of acceptable linearity over the concentration range of interest ($R^2 = 0.9933$, 0.9992 and 0.9997, respectively). Reproducibility of replicate derivatisation of the standards was also acceptable [mean relative standard deviation (RSD) was 5.16%]. Fructose was converted to mannitol and glucitol hexa-acetate in a fixed proportion (mean mannitol:glucitol ratio was 0.67) over the concentration range applicable to potatoes. This ratio was used to calculate reducing sugar levels in ethanolic extracts from five varieties of potatoes using allose as an internal standard. Reproducibility for replicate analyses of reducing sugars in potatoes was acceptable (Mean RSD = 6.65%) and values were within the range of those reported previously.

Keywords: Analysis; Gas chromatography; Reducing sugars; Alditol acetate; Potato

1. Introduction

A knowledge of the quantitative distribution of sugars in fruits, vegetables and other foodstuffs is essential as these compounds are involved in many important characteristics such as flavour, authenticity and quality. In general, analyses of mixtures of sugars are carried out using chromatographic techniques such as high performance liquid chromatography (HPLC) and gas chromatography. Gas chromatographic analysis of reducing sugars requires conversion of the sugars into suitably volatile derivatives. Conversion of free sugars into alditol acetates (Bjorndal, Lindberg, & Svensson, 1967; Griggs et al., 1971; Kim, Shome, Liao, & Pierce, 1967; Shaw & Moss, 1964) and trimethylsilyl ethers (TMS) (Davison & Young, 1964; Mateo, Bosch, Pastor, & Jimenez, 1987; Wells, Chin, & Weber, 1964) have been the most widely used methods for GC analysis of sugars. Due to the relative simplicity of the resulting chromatograms in comparison to other methods of derivatisation quantification of reducing sugars as their alditol acetate derivatives remains a widely used method. This method was first published in the 1960s and has been modified and optimised extensively over the years. For example, Blakeney, Harris, Henry, and Stone (1983) recommend reduction of monosaccharides, dissolved in 1 M ammonia, with sodium borohydride in anhydrous dimethyl sulfoxide, rather than water. Using this method the rate of reduction is also increased by use of a high concentration of sodium borohydride (20 mg ml^{-1}) and an elevated temperature. In addition, 1-methylimidazole instead of pyridine is used as a catalyst for acetylation with acetic anhydride. This results in a complete reduction of hydroxyl groups to alditols within 90 min and acetylation in 10 min at room temperature.

Notwithstanding the great success of the alditol acetate method in qualitative analyses of mixtures of sugars, a

* Corresponding author. Tel.: +353 1 8059505; fax: +353 1 8059550.
E-mail address: nigel.brunton@teagasc.ie (N.P. Brunton).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.045

caveat must be noted regarding quantification of the results. Firstly, the response factors, used to correct the peak areas for differences in the response of the flame-ionisation detector (FID) to alditol acetates derived from different sugars, are far from reproducible, especially when a capillary column is used. Therefore, it is essential to include an internal standard. Difficulties arise also when quantifying glucose in the presence of fructose. This is because on reduction fructose yields a mixture of mannitol and glucitol but since glucose also yields glucitol quantitation is difficult (Davison & Young, 1964). Free sugars in potato tubers consist mainly of a mixture of fructose, glucose and sucrose derived mainly from enzymatic hydrolysis of starch (Isherwood, 1976; Marangoni, Duplessis, & Yada, 1997). Therefore, analysis of sugars by GC in potatoes is usually carried out by conversion to TMS ethers. This method has the disadvantage that it may require separation and measurement of as many as four derivatives for each sugar, which result from anomer formation and ring isomerisation (Kim et al., 1967). The principal objective, therefore, of the present study was to investigate the use of the alditol acetate derivatisation procedure for the analysis of reducing sugars in ethanolic extracts of potato tubers.

2. Materials and methods

2.1. Materials

Water and methanol (HPLC grade) were obtained from BDH (Merck, Poole, Dorset, UK). Dimethyl sulfoxide, acetic anhydride and glacial acetic acid (Analytical grade) were from BDH. Hypersolv grade ammonium hydroxide and dichloromethane were obtained from BDH. Sodium borohydride (+98%) and 1-methyl imidazole (redistilled, purity +99%) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Hexa-acetate derivatives (mannitol, galactitiol, glucitol and inositol hexa-acetate) dissolved in chloroform (12.5 mg/ml) were obtained from Sigma–Aldrich. Potato samples were homogenised using an Ultra-Turrax T25 tissue homogeniser (Jankel and Kunkel, IKA Labortechnik, Germany). The Mistral 3000i refrigerated centrifuge was from MSE (Leicester, UK). Nylon syringe filters (0.22 μm pore size) were purchased from Phenomenex (Macclesfield, UK).

2.2. Collection of potato samples

Samples of Rooster, Oilean and Record potatoes were obtained from a local supermarket. Lady Clare and Maris Piper samples were sourced from a local supplier (Ivan Curran, Stamullen, Co. Dublin). Samples of Lady Clare were either stored in a commercial potato warehouse at 8 °C or in a refrigerator at 3 °C. All Maris Piper samples were stored at 8 °C in the warehouse. Samples were collected at bi-monthly intervals over the period September–November 2005.

2.3. Extraction of reducing sugars from potato tubers

Prior to chemical analyses, ethanolic extracts of homogenised samples from each variety were prepared. Twenty-five milliliters of 70% ethanol was added to 3 g of finely grated potatoes and homogenised at maximum speed (24,000 RPM) for 70 s. Following mechanical agitation for 20 min at room temperature (900 RPM), the samples were centrifuged for 5 min at 1500*g* at 4 °C. The total volume of supernatant was calculated gravimetrically from both the density and total weight of the recovered supernatant. Finally, the supernatants were filtered through a 0.22 μm nylon membrane (Phenomenex Ltd., UK). A 10 ml aliquot was then stored at −20 °C until further analysis.

2.4. Preparation of alditol hexa-acetate reducing sugar derivatives

Alditol hexa-acetate derivatives were prepared using a modified version of the method of Blakeney et al. (1983). Sample extract (1.5 ml) was centrifuged at 12,000*g* for 10 min at 4 °C and 500 μl of the supernatant was evaporated under a stream of N_2 gas at a temperature of 60 °C. The residue was re-suspended in 200 μl of water and 20 μl allose (1 mg/ml aqueous solution) was added as an internal standard. The pH in all tubes was raised by adding 20 μl of 15 mol/l ammonium hydroxide. Following this 1 ml of a sodium borohydride solution (0.5 M in dimethyl sulfoxide) was added, the tubes were vortexed for 1 minute and incubated at 40 °C for 90 min before leaving overnight at 4 °C, protected from the light. The following day, after vortexing the tubes and incubating them at 40 °C for 5 min, 100 μl of glacial acetic acid was slowly added and the tubes were vortexed again. Two hundred microliters of 1-methylimidazole and 1 ml of acetic anhydride was then added and the tubes were vortexed after the addition of each reagent. The tubes were incubated at 40 °C for 10 min to ensure acetylation proceeded to completion. Finally, 2.5 ml of water and 1 ml of dichloromethane were added. The tubes were vortexed for 5 min and centrifuged at 1000*g* for 10 min at 4 °C. Following this, approximately 80% of the upper aqueous phase was discarded and the majority of the solvent phase was transferred to 3 ml GC vials fitted with PTFE/silicon septa (Phenomenex Ltd., UK), containing a small spatula head of sodium sulphate. Before GC analysis, standard curve samples were diluted 10 times and aliquots (200 μl) were transferred to amber GC vials fitted with 200 μl glass inserts. One microliter of each solution was injected onto the GC. Standard curves for allose, glucose and fructose were prepared from 200 μl aqueous standards of each of the sugars at concentrations of 20, 50, 100, 200 and 400 μg/ml. Alditol hexa-acetate derivatives of standard curve samples were prepared as described above. Hexa-acetate standards were prepared from an authenticated alditol acetate standard mixture to check reproducibility and FID response factors (RF).

2.5. GC analysis of alditol hexa-acetate derivatives

Analysis of alditol hexa-acetate reducing sugar derivatives (glucitol and mannitol) was carried out on a Varian GC 3400 CX gas chromatograph fitted with a Varian 8200 CX auto-sampler and ZB-50 semi-polar capillary column (15 m × 0.25 mm (i.d.), 0.25 μm film thickness; Phenomenex Ltd., UK). Helium was used as a carrier gas. On-column injection and FID detection was used. The injection temperature was 270 °C and the column temperature was ramped from 100 °C to 280 °C at a rate of 3 °C min^{-1}. For ethanolic extracts of potato samples, Eqs. (1) and (2) below were used to calculate levels of fructose and glucose respectively in μg/g fresh weight (FW).

$$[\text{Fructose}\ (\mu g/g\ FW)] = \frac{\left(\left(\frac{(PA_M)+(PA_M\times 0.67)}{(PA_A)}\right)\times[\text{I.S.}]\times 2\times(\text{extract volume})\times RF\right)}{\text{weight of potato sample}} \quad (1)$$

$$[\text{Glucose}\ (\mu g/g\ FW)] = \frac{\left(\left(\frac{(PA_G)-(PA_M\times 0.67)}{(PA_{Al})}\right)\times[\text{I.S.}]\times 2\times(\text{extract volume})\times RF\right)}{\text{weight of potato sample}} \quad (2)$$

where PA_M, PA_G and PA_A are the respective peak areas for glucitol (G), mannitol (M) and allitol (A) hexa-acetate. [I.S.] is the concentration of the internal standard (20 μg/ml). RF is the response factor of hexa-acetate derivative relative to allitol hexa-acetate, for fructose RF is the mean of mannitol and glucitol hexa-acetate response factors. Two extractions were carried out for each potato sample and duplicate runs were performed for each sample.

3. Results and discussion

Derivatisation of aqueous solutions of allose, glucose and fructose was carried out to yield standard curves for their alditol acetate derivatives in the concentration range 2–400 μg ml^{-1}. Since as outlined in the introduction, derivatisation of fructose yields both glucitol hexa-acetate and mannitol hexa-acetate, the standard curve was prepared by summing the peak areas of the two derivatives. Linearity over the concentration ranges was acceptable with R^2 values of 0.9997 (±0.0345), 0.9992 (±0.005) and 0.9933 (±0.002) for glucose, fructose and allose respectively. Relative standard deviations (RSD) for replicate analyses of allose, glucose and fructose aqueous standards were 4.17%, 4.71% and 6.61% respectively. Potato tubers contain both glucose and fructose and, therefore, derivatisation of ethanolic extracts from potato samples resulted in a peak for glucitol hexa-acetate derived from both fructose and glucose. Therefore, in order to ensure accurate quantification of both sugars, it was essential to investigate if fructose was converted to mannitol and glucitol hexa-acetate in a fixed proportion over the concentration range applicable to potatoes. Table 1 presents peak areas and ratios for glucitol and mannitol hexa-acetate derivatives prepared from fructose standards with concentrations from 2 to 400 μg ml^{-1}. It is evident that the ratio between mannitol and glucitol peaks remains relatively constant (range 0.65–0.71). The mean mannitol:glucitol ratio was 0.67 and this figure was used to calculate the proportion of glucitol hexa-acetate derived from fructose in a standard analysis of reducing sugars in potato samples as outlined in Eqs. (1) and (2) in Section 2.4. Analysis of ethanolic extracts of five varieties of potatoes (Rooster, Record, Oilean, Maris Pier and Lady Clare) gave values for individual and total reducing sugars within the range of those previously reported (Carey & Cronin, 1990; Granda, Moreira, & Tichy, 2004; Pedreschi, Kaack, & Granby, 2004; Wicklund et al., 2006). In order to ensure that both reducing sugars were maximally extracted under the conditions described in Section 2.3, extraction times between 5 and 60 min were examined. Yields of reducing sugars increased for extraction times of up to 10 min but levelled off thereafter and small increases in peak areas for alditol acetate derivatives occurred with extraction times greater than 20 min. Therefore we considered an extraction time of 20 min to be a good compromise between maximising the yield of reducing sugar and shortening the overall analysis time. Mean RSD values for replicate analyses of glucose and fructose in potatoes were 6.8% and 6.5%, respectively. The limit of detection (defined as the concentration of analyte that gives a response three times as large as the standard deviation of baseline noise) was 1 μg/ml fresh weight for both reducing sugars. The limit of quantification (defined the concentration of analyte that gives a response 10 times as large as the standard deviation of baseline

Table 1
Peak areas and ratios for gas chromatographic analysis of hexa-acetate derivatives of aqueous standards of fructose

[Fructose] (μg ml^{-1})	% Mannitol	% Glucitol	Ratio
2	59	40	0.68
5	59	41	0.69
10	58	39	0.71
20	61	39	0.65
40	58	42	0.71
50	62	38	0.65
100	61	40	0.65
200	60	40	0.67
400	61	39	0.65

Table 2
Reducing sugar contents (μg g^{-1} fresh weight) in ethanolic extracts from a selection of potato cultivars

Cultivar	Fructose	Glucose	Total
Rooster	252	228	480
Record	211	196	407
Oilean	183	184	367
Maris Piper	254	260	514
Lady Clare (8 °C)	146	168	312
Lady Clare (3 °C)	301	322	623

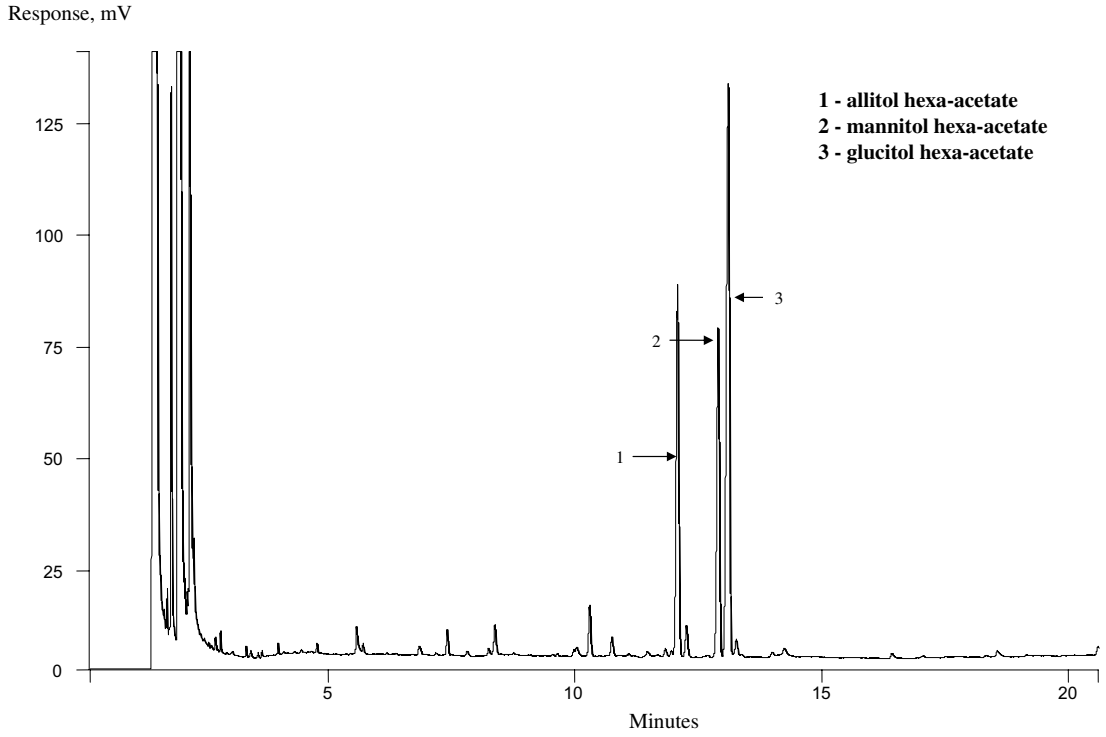

Fig. 1. Typical chromatogram of alditol acetate reducing sugar derivatives derived from ethanolic extracts of potato tubers.

noise) was 3.3 μg/ml for both reducing sugars. Glucose and fructose levels for each potato variety were similar (Table 2). Other authors have also reported that glucose and fructose levels were similar in potatoes (De Wilde et al., 2005, 2006; Elmore, Koutsidis, Dodson, Mottram, & Wedzeicha, 2005; Williams, 2005). A sample chromatogram of alditol hexa-acetate derivatives derived from an ethanolic extract of potato tubers with the relevant peaks indicated is shown in Fig. 1. Mean FID response factors derived from replicate injections of the standard hexa-acetate mixture were 1.11 and 1.05 for mannitol and glucitol hexa-acetate respectively. Reproducibility of response factors between runs was acceptable giving mean RSD values of 2.1% and 2.4% for glucitol and mannitol hexa-acetate, respectively.

4. Conclusion

Standard curves for alditol acetate derivatives of aqueous solutions of allose, glucose and fructose were of acceptable linearity over the concentration range of interest. Reproducibility of replicate derivatisation of the standards was also acceptable. Fructose was converted to mannitol and glucitol hexa-acetate in a fixed proportion (mean mannitol:glucitol ratio was 0.67) which facilitates use of the assay for the estimation of reducing sugars in samples containing glucose and fructose. Use of the alditol acetate derivatisation, using allose as an internal standard for the determination of glucose, fructose and total reducing sugars in five potato varieties gave values for reducing sugars within the ranges previously reported for other potato cultivars. Reproducibility for replicate ethanolic extractions from the tubers was also acceptable.

Acknowledgements

We thank the Irish Department of Agriculture and Food who funded this project under the Food Institutional Research Measure (FIRM) as part of the Irish National Development Plan. We also acknowledge the assistance of Ivan Curran who provided us with potato samples and Tommy Walshe for his assistance in the analysis.

References

Bjorndal, H., Lindberg, B., & Svensson, S. (1967). Mass spectrometry of partially methylated alditol acetates. *Carbohydrate Research, 5*(4), 433–440.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research, 113*(2), 291–299.

Carey, A. T., & Cronin, D. A. (1990). A comparative study of cold-sweetening in two potato cultivars. *Irish Journal of Food Science and Technology, 14*(2), 95–107.

Davison, P. K., & Young, R. (1964). Gas chromatography of carbohydrates the quantitative determination of the free sugars of plants as their trimethylsilyl ethers. *Journal of Chromatography A, 41*, 12–21.

De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., et al. (2005). Influence of storage practices on acrylamide formation during potato frying. *Journal of Agricultural and Food Chemistry, 53*(16), 6550–6557.

De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., et al. (2006). Influence of fertilization on acrylamide

formation during frying of potatoes harvested in 2003. *Journal of Agricultural and Food Chemistry, 54*(2), 404–408.

Elmore, J. S., Koutsidis, G., Dodson, A. T., Mottram, D. S., & Wedzeicha, B. L. (2005). Measurement of acrylamide and it's precursors in potato, wheat and rye model systems. *Journal of Agricultural and Food Chemistry, 53*, 1286–1293.

Granda, C., Moreira, R. G., & Tichy, S. E. (2004). Reduction of acrylamide formation in potato chips by low-temperature vacuum frying. *Journal of Food Science, 69*(8), E405–E411.

Griggs, L. J., Post, A., White, E. R., Finkelstein, J. A., Moeckel, W. E., Holden, K. G., et al. (1971). Identification and quantitation of alditol acetates of neutral and amino sugars from mucins by automated gas–liquid chromatography. *Analytical Biochemistry, 43*(2), 369–381.

Isherwood, F. A. (1976). Mechanism of starch–sugar interconversion in *Solanum tuberosum*. *Phytochemistry, 15*(1), 33–41.

Kim, J. H., Shome, B., Liao, T.-H., & Pierce, J. G. (1967). Analysis of neutral sugars by gas–liquid chromatography of alditol acetates: application to thyrotropic hormone and other glycoproteins. *Analytical Biochemistry, 20*(2), 258–274.

Marangoni, A. G., Duplessis, P. M., & Yada, R. Y. (1997). Kinetic model for carbon partitioning in *Solanum tuberosum* tubers stored at 2 °C and the mechanism for low temperature stress-induced accumulation of reducing sugars. *Biophysical Chemistry, 65*(2–3), 211–220.

Mateo, R., Bosch, F., Pastor, A., & Jimenez, M. (1987). Capillary column gas chromatographic identification of sugars in honey as trimethylsilyl derivatives. *Journal of Chromatography A, 410*, 319–328.

Pedreschi, F., Kaack, K., & Granby, K. (2004). Reduction of acrylamide formation in potato slices during frying. *Lebensmittel-Wissenschaft and Technologie, 37*(6), 679–685.

Shaw, D. H., & Moss, G. W. (1964). Quantitative estimation of neutral sugars by gas–liquid chromatography. *Journal of Chromatography A, 41*, 350–357.

Wells, W. W., Chin, T., & Weber, B. (1964). Quantitative analysis of serum and urine sugars by gas chromatography. *Clinica Chimica Acta, 10*(4), 352–359.

Wicklund, T., Ostlie, H., Lothe, O., Knutsen, S. H., Brathen, E., & Kita, A. (2006). Acrylamide in potato crisp–the effect of raw material and processing. *LWT – Food Science and Technology, 39*, 571–575.

Williams, J. S. E. (2005). Influence of variety and processing conditions on acrylamide levels in fried potato crisps. *Food Chemistry, 90*(4), 875–881.