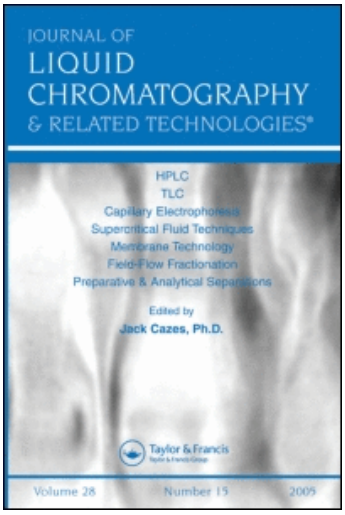

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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R. Plata-Guerrero[a]; E. Guerra-Hernández[a]; B. García-Villanova[a]
[a] Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Campus Universitario de Cartuja, Granada, Spain

To cite this Article Plata-Guerrero, R. , Guerra-Hernández, E. and García-Villanova, B.(2009) 'Determination of Reducing Sugar and Asparagine in Potatoes', Journal of Liquid Chromatography & Related Technologies, 32: 17, 2556 — 2568
To link to this Article: DOI: 10.1080/10826070903249732
URL: http://dx.doi.org/10.1080/10826070903249732

Journal of Liquid Chromatography & Related Technologies®, 32: 2556–2568, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903249732

Determination of Reducing Sugar and Asparagine in Potatoes

R. Plata-Guerrero, E. Guerra-Hernández, and B. García-Villanova

Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Campus Universitario de Cartuja, Granada, Spain

Abstract: Reducing sugars and asparagine levels were determined in four varieties of Spanish potatoes purchased from a local supermarket. A gas chromatography method was used to analyze glucose, fructose, and saccharose, an HPLC technique was used to analyze asparagine, and two unspecific volumetric methods were also used to study reducing sugars and amino acid groups. Two ethanolic extractions were required to obtain maximum recovery. The reducing sugar content ranged from 0.55% to 2.01%, and the asparagine content from 0.13% to 0.17%. Significantly different results were obtained by volumetric and chromatographic methods, but the correlation obtained between them was high, therefore volumetric methods can be used in the food industry for automatic control of raw potatoes used to prepare chips.

Keywords: Asparagine, Fructose, Glucose, Potatoes

INTRODUCTION

Acrylamide is widely used in chemical and environmental applications, including the paper industry, cosmetics, and drinking water treatments, among others. Its presence in the organism is reported to be detrimental due to possible carcinogenic and confirmed neurotoxic effects.[1] Swedish researchers[2] demonstrated its presence in fried and roasted food, but it

Correspondence: E. Guerra-Hernández, Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Campus Universitario de Cartuja, 18012 Granada, Spain. E-mail: ejguerra@ugr.es

has not been detected in raw or boiled foods, indicating that high temperatures are required for its formation. Acrylamide is produced under the following conditions:[3] high asparagine content, high reducing sugar (fructose and glucose) content, humidity below around 30%, and temperatures above around 100°C.

Potatoes are considered a basic food in many countries and represent a major source of carbohydrates, fiber, potassium, and vitamins, especially vitamin C. Researchers in many countries have detected large amounts of acrylamide in fried potatoes, which are cooked at temperatures above 120°C and have a high content of precursors of this compound. Acrylamide content is reported to be indirectly influenced by the variety of potato, by its method of cultivation, including the fertilization used, and by storage conditions, which all have a direct effect on acrylamide precursors, i.e., reducing sugars (glucose and fructose) and asparagine.[4]

Model systems show a marked increase in acrylamide levels when asparagine is heated in the presence of fructose or glucose.[5] The C-backbone of the acrylamide molecule is wholly derived from the asparagine molecule.[6] In a first step, asparagine reacts with a reducing sugar, forming a Schiff's base. Acrylamide is formed from this compound *via* a complex pathway that includes decarboxylation and several elimination reactions. The mechanism of acrylamide formation is based on non-enzymatic browning; which is also responsible for the development of the color, taste, and smell of fried potatoes.[7] It has been shown that each potato variety contains a different amount of reducing sugars and amino acid.[8] It was also reported, in an amino acid study,[9] that the amount of free asparagine and glutamine in potatoes increases (by protein degradation) with longer storage time.

Amrein et al.[8] studied asparagine and reducing sugar content in potatoes of different varieties and cultivation methods in Switzerland and always detected larger amounts of free asparagine than of sugars. They also observed a considerable variation in sugar and free amino acid content as a function of the season in which they were harvested. Becalski et al.[10] found that the amount of sugars (glucose, fructose and sucrose) and free amino acids in raw potatoes were correlated with their post frying acrylamide levels. Sugar levels can be useful as an indicator of acrylamide levels, regardless of free asparagine levels, even in potatoes that are very rich in free amino acids. Studies on precursors in different potato cultivars found no correlation between asparagine and reducing sugar levels. Asparagine levels are similar among different cultivars whereas fructose and glucose levels vary widely,[11] indicating that acrylamide formation mainly depends on the amount of reducing sugars. Consequently, the acrylamide content in our diet could be reduced by consuming potatoes with low sugar levels.[12] As has been pointed out, a

low sugar content was previously associated with product quality and is now also linked to health,[14] and the reducing sugar content of potatoes used for frying should be as low as possible.

The main objective of this study was to develop methods to quantify the amount of reducing sugars (glucose and fructose) and asparagine in potatoes of different varieties as a means of predicting the acrylamide content of the cooked product.

EXPERIMENTAL

Samples

Samples of the following varieties of raw Spanish potatoes were purchased for analysis: Monalisa, sold for frying or boiling; Agata, for boiling or roasting; Bintje, non-specific culinary use; and Red Pontiac, for stewing. Monalisa, Agata, and Bintje varieties were purchased at a supermarket (in 3 kg or 5 kg bags), and Red Pontiac was purchased at a local greengrocer's.

Reagents and Chemicals

Reducing sugars were analyzed using the following reagents: sodium thiosulfate solution (0.1 N) (24.82 g sodium thiosulfate $5H_2O$ [Panreac] and 3.8 g sodium borate $10H_2O$ [Panreac] dissolved in 1 L of distilled water); 10 N sulfuric acid solution (Panreac); 30% potassium iodide solution (Panreac); Fehling A solution (34.64 g copper sulfate $5H_2O$ [Probus] in 500 mL of distilled water); Fehling B solution (173 g sodium-potassium tartrate $4H_2O$ [Panreac] and 50 g sodium hydroxide [Panreac]) dissolved in 500 mL of distilled water; and soluble starch (Panreac).

The following reagents were used for the sugar analysis by GC: internal standard solution (0.9935% myoinositol and 0.6752% trehalose [Sigma]) in methanol:water (60:40); 5% hydroxylamine hydrochloride (Panreac) dissolved in pyridine (Panreac); Hexamethyldisilazane (Sigma); trifluoroacetic acid (Merck); and hexane (Panreac).

The amino acid analysis used phenolphthaleine (Panreac), 0.01 N NaOH (Panreac), and formaldehyde (Panreac).

The HPLC asparagine analysis used: acetonitrile (Panreac); phosphate buffer at pH 7.2 (2.34 g monosodium phosphate $2H_2O$ [Probus] and 2.13 g disodium phosphate [Probus] dissolved in 1 L of milliQ water; 0.4 M borate buffer at pH 9.5 (3.814 g sodium borate $10H_2O$ [Probus] dissolved in 100 mL of milliQ water; OPA (50 mg of o-phthaldialdehyde reagent [Sigma], 1 mL of methanol, 3 mL 0.4 M borate buffer pH

9.5, 50 μL mercaptoethanol [Aldrich], and 2 mL borate buffer. The mixture was used diluted 50:50 with borate buffer.

Instruments

Mono- and disaccharide analyses were carried out using an Autosystem XL GC chromatograph (Perkin-Elmer) with a Chrompack 25 CP-Sil 5CB 25 m × 0.25 mm × 0.25 μm column (Sugelabor) and 1 μL Hamilton microsyringe. Data were analyzed with Totalchrom software (Perkin-Elmer). Asparagine was determined by HPLC with a Varian Prostar chromatograph, Varian Prostar model 230 pump, Varian Prostar model 363 fluorescence detector, Varian Prostar model 500 column valve, and a 150 mm × 4.60 mm × 5 μm Phenomenex column C18[2] (Jasco). The sample was injected using a 25 μL Hamilton microsyringe.

Methods

Reducing Sugars

Extraction. Potatoes were peeled and grated using a common grater. Approximately 10 g of sample were weighed and placed in centrifuge tubes, adding 40 mL of 80° alcohol. The extraction was performed for 1 hr in a 80°C water bath, followed by centrifugation of samples to gather the extract, and then a second 1 hr extraction and centrifugation. The two extracts were mixed, and 80° alcohol was added to a final volume of 100 mL. All samples were analyzed in duplicate.

Determination of Reducing Sugars. Fehling A, 10 mL, 10 mL Fehling B, and 10 mL distilled water were added to 20 mL ethanolic extract in an Erlenmeyer flask, followed by some glass pearls. The mixture was boiled for 2 min on a Bunsen burner and quickly cooled in an ice bath. Then, 10 mL 30% potassium iodide, 10 mL 10 N sulfuric acid, and drops of starch were added. The evaluation was performed with 0.1 N sodium thiosulfate up to the disappearance of brown color. A blank was done with 20 mL of the 80° alcohol mixture instead of sample extract. A calibration curve was performed using anhydrous glucose (Sigma) $Y = 0.0036X + 0.0007$ $r^2 = 0.9975$.

Gas Chromatography Determination. Internal standard (1 mL) was added to 10 mL ethanolic extract and then evaporated under reduced pressure

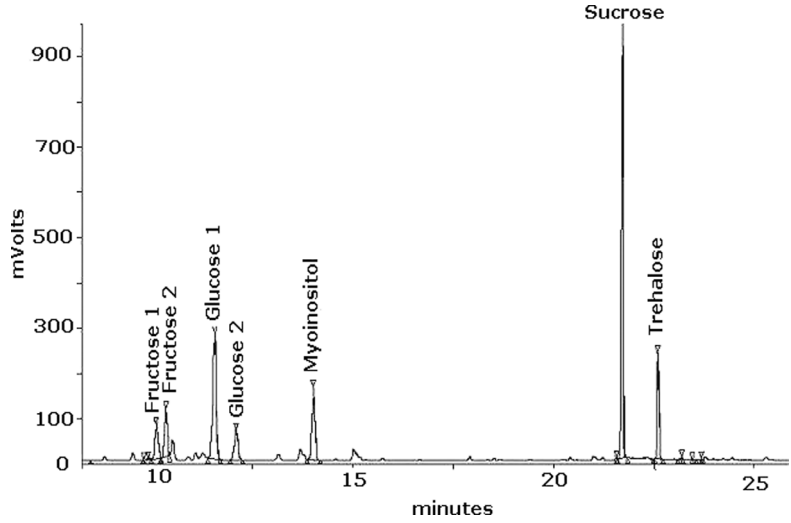

Figure 1. Typical GC chromatogram of sugars in potatoes.

at room temperature. The evaporated sample was dissolved in 2 mL of 5% hydroxylamine hydrochloride and incubated for 30 min at 75°C. Next, 1 mL was transferred to a glass tube, and 1 mL hexamethyldisilazane and 100 μL trifluoroacetic acid were added, followed by incubation of the tube for 1 hr at 65°C. Distilled water and 100 μL hexane were then added to dissolve the sample, and 1 μL of the upper layer was taken for the chromatograph injection. The initial temperature was 180°C and was increased at 2°C/min to 206°C, and then at 10°C/min to 300°C, which was maintained for 5 min. The internal standard method was used for the quantitation. Calibration curves were: $Y = 1.5487X - 0.0879$ $r^2 = 1$ for glucose; $Y = 1.7145X - 0.047$ $r^2 = 1$ for fructose; and $Y = 0.816X + 0.0065$ $r2 = 0.9964$ for sucrose. Figure 1 shows a typical chromatogram.

Amino Acids

Extraction. Potatoes were peeled and grated as described above. Approximately 10 g of sample were weighed and placed in centrifuge tubes, adding 40 mL of different degrees of alcohol for the extraction. The extraction was performed for 1 hr at room temperature, followed by centrifugation of samples to collect the extracts. A second extraction was performed, and the extracts of both procedures were mixed and

brought to a final volume of 100 mL with 33° alcohol. Samples of each variety were analyzed in duplicate.

Determination. Amino acids: were determined by blocking the amino group with formaldehyde. Drops of phenolphthalein were added to 10 mL of sample, then adding NaOH until a light pink color was obtained. The same procedure was followed using 5 mL formaldehyde. After their neutralization, the two solutions were mixed together, with disappearance of the pink color. The evaluation was performed with 0.01 N NaOH until the mixture again showed a pink color (molecular weight of asparagine: 132.13).

Determination of Asparagine by HPLC. In order to obtain the derivatization sample, a 200 μL sample was added to 400 μL borate buffer with 50 μL OPA reagent. This was injected after a 5 min interval. Separation was performed at room temperature using a gradient program at a flow rate of 1 mL/min. Eluent A was 100% phosphate buffer pH 7.2 and eluent B acetonitrile. Gradient expressed as solvent A was: 0–10 min 85%, 10–15 min 84%, 15–20 min 80%, 20–25 min 75%, 25–30 min 65%, 30–35 min 50%, 35–43 min 30%, and 43–55 min 85%. Detection was carried out at 415 nm and 345 nm emission and excitation wavelengths, respectively. Figure 2 shows the asparagine peak. The calibration curve was $Y = 5737X + 2.5753$ ($r^2 = 0.9991$).

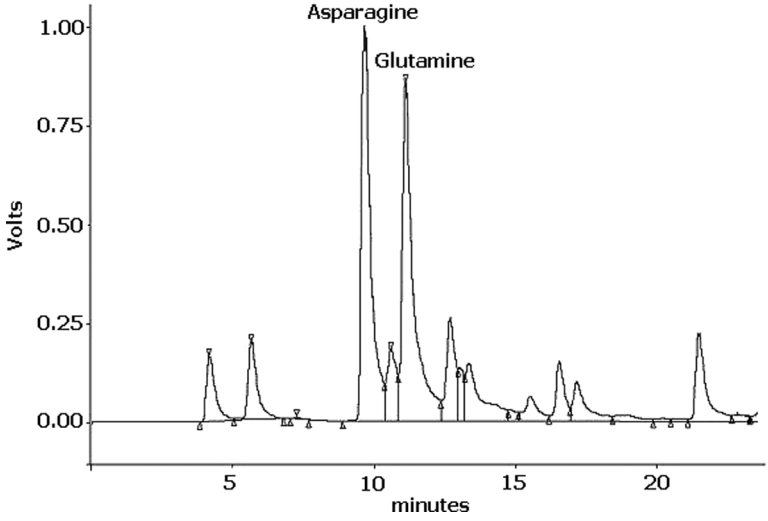

Figure 2. Typical HPLC chromatogram of asparagine in potatoes.

Table 1. Reducing sugars and amino acid content (g/100 g fresh weight) obtained according to number of extractions

No extractions	Reducing sugars content (%)	Amino acid content (%)
1	0.33	0.56
2	0.71	0.70
3	0.73	0.71

RESULTS

Extraction Methods

Reducing Sugars

Various numbers of extractions were performed to determine how many were required to obtain the maximum sugar concentration. Table 1 shows the reducing sugars obtained after one, two, and three extractions. Fructose, glucose, and sucrose were separately determined by GC in each extract (Table 2). Different samples were used for the two methods.

Amino Acids

Two studies were performed: one with distilled water to determine the number of extractions needed (Table 1), and another to study the effectiveness of different dissolvents (Table 3).

Precision Study

The relative standard deviation for the volumetric method was 0.12% (n = 7) for a sample with a mean reducing sugar value of 0.22% (0.22 g/100 g sample). The deviations for the GC method were 0.13%

Table 2. *Percentage of individual sugars and total sugars* obtained in 1st, 2nd and 3rd extraction

Sugar	1st extraction (%)	2nd extraction (%)	3rd extraction (%)	Total (%)
Fructose	0.38	0.05	0.017	0.45
Glucose	0.46	0.05	0.016	0.53
Sucrose	0.36	0.05	0.012	0.42

Table 3. Percentage of total amino acids (expressed in g/100 g fresh weight in asparagine) obtained with different degrees of alcohol

Extraction medium	Distilled water	33° Alcohol	64° Alcohol	80° Alcohol
Amino acids (%)	0.41	0.54	0.35	0.15

(n = 5) for glucose, 0.18% (n = 5) for fructose, and 0.03% (n = 5) for sucrose in samples with 0.11%, 0.07%, and 0.76% of glucose, fructose, and sucrose, respectively. The relative standard deviation for total amino acids (volumetric method) was 0.06% (n = 7) for a sample with a mean content of 0.48%, and for asparagine (HPLC), it was 0.13% (n = 6) for a sample with a mean content of 0.15%.

Precursor Determination in Different Varieties

Reducing Sugars

Results obtained by the volumetric method and gas chromatography for the reducing sugars in each variety are shown in Table 4.

Amino Acids

Results obtained by the volumetric method for total amino acid and by HPLC for asparagine in each variety are shown in Table 4.

DISCUSSION

Reducing Sugar Extraction

Using the volumetric method, the amount of sugar recovered from potatoes in two extractions was almost double that recovered in a single extraction (Table 1), with a third extraction only recovering a further 2.8%. In the determination of reducing sugars (fructose and glucose) and sucrose by gas chromatography (Table 2), 85% of sugars were recovered in the first extraction, with the second extraction obtaining a further 9–12% and the third extraction recovering only 3% of the total. Based on these findings, sugar extraction was performed with only two extractions.

Most authors have carried out a single extraction using ethanol[10,13–16], water[9,12,17,18], or acetonitrile.[11]

Table 4. Percentage of precursors in different varieties (g/100 g fresh weight)

Variety	Reducing sugars (volumetric)	Glucose (GC)	Fructose (GC)	Sucrose (GC)	Reducing sugars (Glucose + Fructose)	Total amino acids (volumetric)	Asparagine (HPLC)
Monalisa	1.46%	1.23%	0.78%	0.27%	2.01%	1.03%	0.16%
Bintje	0.43%	0.28%	0.27%	0.45%	0.55%	0.99%	0.13%
Agata	0.95%	0.75%	0.90%	0.30%	1.65%	1.41%	0.17%
Red pontiac	1.25%	0.81%	0.78%	0.26%	1.59%	0.93%	0.15%

Amino Acid Extraction

The amounts of total amino acids obtained in one, two, and three extractions were compared (Table 1), finding that two extractions recovered the maximum amount, with a third extraction only increasing the amount extracted by 0.01% (i.e., 1.4% of the total). Therefore, we used only two extractions. Although amino acids are traditionally extracted with water, we performed extractions with different alcohol solutions. The results in Table 3 show that the highest amino acid extraction was obtained using 33° alcohol, which extracted 0.13% more amino acids compared with distilled water extract (i.e., 25% greater extraction). Nevertheless, the percentage of amino acids obtained with 80° alcohol was very low, indicating that amino acids are not soluble in ethanol. Hence, it is not possible to use the same extraction method for sugars and amino acids.

All studies consulted in the literature used only one extraction to obtain free amino acids.[9–19]

Determination of Acrylamide Precursors in Different Potato Varieties

The small coefficient of variation for each method allows them to be used to analyze differences between precursor levels in these samples.

Reducing Sugars

Volumetric and GC methods produced very different results for reducing sugar content in the same potato extracts (Table 4). The volumetric method is non-specific and can determine other reducing compounds (e.g., vitamin C). However, it can be useful as a sensitive measurement method, since a high correlation was obtained with the chromatographic method ($r^2 = 0.8995$).

Levels of fructose and glucose reported here fall within the ranges of those reported elsewhere for other varieties.[13,16] Mean levels of total reducing sugars were higher in Monalisa, Agata, and Red Pontiac than in Bintje potatoes. High reducing sugar can be a property of the variety or the result of storage conditions. Temperatures of 3–4°C produce an accumulation of reducing sugars.

Amino Acids

A high correlation was obtained between the total amino acid content by the volumetric method and asparagine content by HPLC ($r^2 = 0.8981$). For this reason, the volumetric method, which is much simpler to perform, can be useful to control the amino acid precursor of acrylamide.

Asparagine contents were very similar among the different varieties analyzed, whereas other authors reported differences among some varieties.[13] In our varieties, the asparagine content would not influence the acrylamide amount contained in the chips. The asparagine contents reported fall within the range of other reports.[8,9,19]

Finally, the varieties with the highest content in precursors are Monalisa and Agata, therefore these potatoes can be expected to have the highest acrylamide content after frying. Monalisa is specifically sold for frying, as stated on the pack label, whereas the Agata variety is recommended for baking. Although Bintje (unspecified) and Red Pontiac (stewing or baking) are not sold for frying, they have a smaller content of reducing sugars and chips made from these varieties would carry a lower risk of acrylamide intake. It can be deduced that the definition of the culinary use of a potato variety is not based on its precursor content. It would be valuable if potatoes with lowest precursor contents were identified to reduce the consumption of acrylamide in fried potato chips.

ACKNOWLEDGMENTS

This study was supported by the Comisión Interministerial De Ciencia y Tecnología (Project AGL 2006 12656/ALI). Eroski Foundation provided a scholarship to Rosa Plata-Guerrero for the accomplishment of this work. The authors would like to thank Richard Davies for assisting with the translation.

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Received February 27, 2009
Accepted April 14, 2009
Manuscript 6503