Quantification by LC-MS/MS of individual sugars in fruit juice consumed in Portugal

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Abstract. To address the sugar contents in fruit juices, consumed in Portugal, a mass spectrometer coupled to a liquid chromatographer (UPLC-MS/MS) method was developed and optimized. The method offers good sensitivity and excellent linearity with a correlation coefficient above 0.995. The optimized method was applied to the analysis of fruit juice in terms of their sucrose, fructose and glucose content. The highest content of total sugars was found in red berries juice and pear juice, in contrast with multi-fruit juice. The results were lower than those previously published in the Portuguese Food Composition Table, indicating that the national campaign, presently in course, to reduce the amount of sugar in beverages is effective.

1. Introduction

Human diet must have all nutrients required for the metabolic process and life support. One of the relevant nutrients belongs to the carbohydrate group namely sugars [1]. However, it has been identified a relation between dietary sugar overconsumption and health problems such as obesity, non-insulin dependent diabetes mellitus, cardiovascular disease and dental caries. In Portugal, the national sugar consumption is about 90 g/day, where 95% of the Portuguese population consume sugars above the limit recommended by the World Health Organization (WHO) [2-3]. Also, the WHO recommends the limitation of free sugar intake to a maximum of 10% of energy [3]. Recently, the Portuguese government launched the campaign “Sugar hidden in food” where promote and alert for the importance of the food labels information about the sugar content. So, the sugar composition analysis is essential to know the sugar concentration and consequently reduce them in foodstuff. A variety of analytical methods have been developed to separate and analyse the sugars in food, such as gas chromatography (GC) [4], high-performance liquid chromatography using refractive index detection (HPLC-RJ) or an evaporative light scattering detector (ELSD) [5-6] and ultra-performance liquid chromatography-mass spectrometry (LC-MS/MS) [7]. GC methods require the sample derivatization and take a long time to present the results. In case of HPLC, the use of ultraviolet detector is not possible, due to the lack of chromophore in sugars, however, the refractive index detector is a simple, fast and economical method, yet this detector has limitations in sensitivity and reproducibility. So, the UPLC-MS/MS seems to be the most appropriate method, due to advances in sensitivity and specificity without affecting the speed and the simplicity of the method implementation.

The aim of the present study was the determination of sugars, namely sucrose, fructose, glucose in fruit juices through the development and optimization of the chromatographic method by UPLC-MS/MS.

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2. Materials and methods

2.1 Materials and reagents

D-glucose was obtained from the Supelco, and the D-sucrose and D-fructose were purchased from Dr. Ehrenstorfer. Also, the following reagents were used: Acetonitrile LC-MS grade from Merck, Ammonium hydroxide (Avantor Performance Materials). The water was purified with a Milli-Q water purification system (Millipore Corporation, Saint-Quentin, France).

2.2 Sample preparation

The samples studied included juices acquired from local supermarkets and stored at ambient temperature until further analysis. The juices were diluted in the same solvent as the standards (80:20 ACN:H₂O) and filtered using 0.2 μm GHP syringe filter, before injection into the system. All the tests were performed in triplicate.

2.3 Standard preparation

The sugar stock solutions (fructose, glucose, sucrose) were prepared in 80:20 ACN:H₂O, and stored at -20°C. The range used was for fructose 2.5 to 25 µg/ml, sucrose, 2.5 to 15 and for glucose 6 to 25 µg/ml. Results were expressed as the mean and standard deviation of three replicates in mg/100 ml of the edible portion on a fresh weight basis.

2.4 LC-MS/MS method

The LC-MS/MS method was performed with a Waters mass spectrometer with electrospray ionization in negative ion mode equipped with a Waters Acquity ultra performance liquid chromatography system. The standard elution was carried out on an ACQUITY UPLC BEH Amide 1.7µm, 2.1 x 150 mm column from Waters maintained at 35ºC. The mobile phase was a mixture of A 10:90 acetonitrile: 0.1% ammonium hydroxide in water and B 90:10 acetonitrile: 0.1% ammonium hydroxide in water applied at a flow rate of 0.2 ml/min in a 35 min linear gradient as follows: 0 - 30 min, 100-70% B; 30 - 30.1, min 70 - 60% B; 30.1 - 33 min, 60 - 80% B; 33 - 35 min, 80 -100 % B. The injection volume was 10 µl, the column was set at 35ºC, and the sample manager temperature was set at 15 ºC. The mass spectrometer detector conditions were set as follows: Capillary voltage, 2.80 kV, Source temperature 120 ºC, desolvation temperature, 350 ºC, desolvation gas flow 500 ml/h and cone gas flow, 50 ml/h.

2.5 Traceability

All the equipment used during experiments were calibrated according to equipment and approved calibration procedures. Calibration values for the analytical balance and pipettes were registered, and corrections were made when necessary.

3. Results and discussion

3.1 Method development

The chromatographic and analytical procedures were developed to be applied in the determination of sugars (sucrose, glucose, and fructose) in fruit juice. Direct infusions were performed in the mass detector in order to select, for each sugar, the appropriate fragment ions, cone voltage and collision energy. Before infusion, standards were dissolved in 50:50 acetonitrile:water and mass detector were set as follows: capillary voltage 2.80 kV, source temperature 120ºC, desolvation temperature 350ºC, desolvation gas flow 500 ml/h and gas flow 50 ml/h, were applied. These parameters were based on a Waters application note [8]. After selecting mass detector conditions, the method for identification and quantification of sugars by UPLC-MS/MS was developed. First, the standards diluted in 50:50 acetonitrile/water were injected and passed through an ACQUITY UPLC BEH Amide 1.7µm, 2.1 x 50 mm column at 35ºC, with an injection volume of
5 µL and a sample manager temperature of 20°C. The selected mobile phase was a mixture of (A) 80:20 acetonitrile:water and (B) 30:70 acetonitrile:water applied at a flow rate of 0.3 ml/min in a 10 min gradient as follows: 0 - 5 min, 100 - 40% A; 5 - 10 min 40 - 100% A. With these conditions, the chromatogram is shown in figure 1. (A) was obtained and can be observed a very close retention times among the monosaccharides and disaccharides. In addition, peak distortion in almost all the sugar except for sucrose is observed, which it may be related to several factors, the mobile phase composition and the possibility of formation of reducing sugar anomers. In consequence, the addition of a buffer, ammonium hydroxide at 0.1%, to the mobile phase was tested in order to raise the pH. So, in the next tests, with the same chromatographic conditions, along with the individual standards the mixture of all of them was injected. However, no separation among the standards, when injected as a mixture, is achieved.

In order to increase the resolution, a new column with a longer length was acquired (ACQUITY UPLC BEH Amide 1.7 µm, 2.1 x 150 mm). Besides, with all the changes applied to the chromatographic conditions, direct infusion of all standards in 80:20, phase B: phase A, were performed again, and new mass conditions (collision energy and cone voltage) for each sugar were obtained. After a few tests, it was verified that a longer run time was ideal to achieve a better separation between the standards even when injected as a mixture. The new mobile phase tested was a mixture of (A) 10:90 acetonitrile:0.1% ammonium hydroxide in water and (B) 90:10 acetonitrile:0.1% ammonium hydroxide in water applied at a flow rate of 0.2 ml/min in a 45 min gradient as follows: 0 - 40 min, 100-95% B; 40 - 40.1 min 95 - 60% B; 40.1 - 43 min, 60 - 60% B; 43 - 45 min, 60 -100 % B. The sample manager temperature was decreased to 15°C, and the injection volume was set at 10 µL. With those changes, the retention time of sucrose changed due to the slow gradient, but a good peak layout and separation was achieved. The best results were obtained when using the chromatographic conditions presented in the subchapter LC-MS/MS method, figure 1.(B).

The analysis of the chromatogram presented in figure 1.(B), demonstrated an adequate separation between all sugars and a desirable peak layout. The following step was applying the selected conditions to real samples.

3.2 Occurrence

The method developed was applied to the most consumed fruit juices in Portugal, to evaluate the sugar profile and compare with the levels indicated on the label. The calibration curves of the three sugars and the correlation coefficient ($R^2$) were presented in figure 2, where it was accepted a $R^2 > 0.995$.

The sugars levels determined in the analyzed samples are given in figure 3. The fructose was highest in pear juice (6.7 g/100 ml), followed by red berries juice (5.5 g/100 ml). Instead, the sugar with the lowest levels was the sucrose in 3 of 4 samples. The total sugars results of the
samples analyzed were similar to the described on packaging label in case of multi fruits juice, peach juice, and pear juice, however, the results of total sugars in red berries juice (10.98 g/100ml) was higher than the expressed in the label, 5.2 g/100ml. Comparing our results with the Portuguese and American food composition databases there is a decrease of total sugars in the juices [9-10]. In case of pear juice, the value in databases is ranged from 11 to 15 g/100g and in this work was obtained a lower concentration (figure 3).

![Figure 3. Sugar concentration (g/100ml) in fruit juices](image)

Nevertheless, in relation to individual sugars of the pear juice, the level of fructose is higher when compared to the values published in the USDA. Chinnici et al. determined the sugar concentration in fruit juices and reported higher levels of sugars in pear juice (4.8 g sucrose/100 ml; 3.5 g glucose/100ml; 4.6 g fructose/100ml) and peach juice (5.4 g sucrose/100ml; 4.0 g glucose/100ml; 4.3 g fructose/100 ml) than our results [11]. The differences between results can be related to the variety of fruits and maybe with added sugars.

4. Conclusions

A selective method for analysis of individual sugars has been developed and optimized on UPLC-MS/MS and applied to quantify sucrose, glucose, and fructose in fruit juices. The relatively short analysis time made UPLC-MS/MS a well-suited method for routine analyses with a large number of samples. This is quite relevant because the level of sugar in fruit juice is linked to major health problems. Furthermore, when we applied this method to the real samples, fructose was the predominant sugar followed by glucose, being sucrose, the saccharide presented in lower amounts. The results of this study indicated that the levels of sugars observed were lower when compared with the levels reported in previous national studies. This data will be combined with national consumption data, in the near future, to estimate the sugar intake of the Portuguese population and to differentiate the role of each sugar in childhood obesity.

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