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Short Communication

Flow-injection spectrophotometric method with on-line photodegradation for determination of ascorbic acid and total sugars in fruit juices

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ABSTRACT

This article reports a flow-injection spectrophotometric determination (FIA) with a photodegradation step to determine ascorbic acid and total sugars. The flow-injection system includes a simple ultraviolet photoreactor for the on-line photodegradation. The method is based on the determination of ascorbic acid at 300 nm before the photodegradation step, followed by UV irradiation and measurement of total sugars at 268 nm. The proposed method was used to determine ascorbic acid and total sugars in commercial and natural fruit juice samples. The method was validated by using spiked samples with recoveries in the range 96.4–108.3% for ascorbic acid, and 91.0–113.2% for total sugars.

1. Introduction

Ascorbic acid and sugars such as glucose, fructose and sucrose are usually present in a large variety of commercial fruit juices (Fennema, 2000). Their presence can cause changes in the chemical and sensory characteristics of the food, such as pH, total acidity, sweetness, etc. (Chinnici et al., 2005). Hence the importance of doing an adequate control and quantification of ascorbic acid and sugars to verify the authenticity and quality of the fruits used in food manufacturing. Also, such analysis makes it possible to evaluate the degree of maturity and possible microbiological alteration during storage (Dong et al., 2007; Li et al., 2007; Soga and Serwe, 2000).

Different methods for the analysis of ascorbic acid and total sugars have been documented in the literature, such as high-performance liquid chromatography (HPLC) (Li et al., 2007; Usenik et al., 2008; Xu et al., 2008), gas chromatography (GC) (Füzfai and Molnár-Perl, 2007; Komthong et al., 2007), amperometry (Dong et al., 2007; Surareungchai et al., 2001), flow injection analysis (Liu and Itoh, 2007; Maestre et al., 2005), electrophoresis (Soga and Serwe, 2000; Tang and Wu, 2005), chemiluminescence (Pires et al., 2006), UV/UV procedure (Roig and Thomas, 2003), etc. Nowadays, photochemical reactions are an important field for analytical purposes. Electromagnetic radiation is considered an ideal reagent because it is inexpensive, easy to connect in an FIA manifold and no excess reagent is present during analytical signal detection. The amount of radiation employed depends on the irradiation time and the selectivity can be improved by varying the power and wavelength of the lamp (Demadrille et al., 2004).

Fructose, glucose and sucrose are non-UV absorbing molecules. Under the influence of UV radiation in alkaline medium, they are oxidised to carbonyl compounds which present a maximum absorption band in the UV zone at 268 nm (Roig and Thomas, 2003). Roig and Thomas (2003) established that sugars commonly present in fruit juices such as fructose, glucose and sucrose, display similar behaviour when exposed to UV radiation under alkaline conditions. They found that the slopes of the calibration curves, corresponding to the UV absorbance versus the concentration of everyone of these sugars, were comparable to each other. On the other hand, ascorbic acid has an absorption band at 300 nm, in alkaline medium, which disappears after UV photodegradation.

In this work, we developed an FIA manifold with an on-line UV irradiation step for measuring ascorbic acid and total sugars in natural and commercial orange and grapefruit juices. Ascorbic acid was detected at 300 nm in alkaline medium prior to irradiation of the sample in the FIA manifold. After UV irradiation of the sample for 5 min, under alkaline conditions, total sugars were measured as a function of their photodegradation products at 268 nm.

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2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared daily. Analytical reagent-grade chemicals and ultra pure water of Milli-Q quality (18.3 Ω cm^{-1}) were used. Ascorbic acid (Aldrich) stock solution 100 mg L^{-1} and fructose (Anedra) stock solution 50 g L^{-1} were prepared with ultra pure water.

Sodium hydroxide solutions at different concentrations 0.1 and 0.2 mol L^{-1} were prepared by dissolving appropriate amounts of sodium hydroxide (Anedra) in pure water. Hydrochloric acid solution pH 2.5 (3.0×10^{-3} mol L^{-1}), was prepared with an suitable volume of hydrochloric acid (Anedra) in ultra pure water. Britton Robinson buffer solution, pH 6.5, was prepared by mixing an appropriate volume of acid solution (0.04 mol L^{-1} of acetic (Mallinckrodt), phosphoric (Tejon) and boric (Sigma) acids) and basic solution (1 mol L^{-1} NaOH (Cicarelli)).

2.2. Apparatus

A Hewlett Packard 8452A diode array spectrophotometer with a Hellma 178-010-QS flow cell with an inner volume of 18 μL and 10 mm light path was used. A Gilson Minipuls 3 peristaltic pump and a Rheodyne 5041 injection valve were used. All reaction coils were made of PTFE tubing (inner diameter: 0.8 mm).

The photoreactor was a PTFE tubing (0.8 mm i.d., length 120 cm) helically coiled around a-15 W-low mercury UV lamp (Phillips) with maximum emission at 254 nm (typical germicide lamp). The photoreactor-lamp assembly was housed into a box in which the internal walls were covered with aluminium foil to increase photon flux by reflection. A Ross Sure Flow 8172 ion selective electrode was used for potentiometric measurements of pH.

All measurements were done at room temperature (around 23 °C).

2.3. Procedure

The flow injection system (Fig. 1) was simple and had only one line. The sample was injected by means of a rotary valve into the carrier stream through the photoreactor assembly. When the lamp was switched off, the ascorbic acid signal was detected at 300 nm. Afterwards, another aliquot of the sample was injected. When this sample plug reached the photoreactor, the pump was stopped and the lamp was switched on for 5 min. Then, the flow was restored, the lamp was turned off, and total sugars were detected at 268 nm.

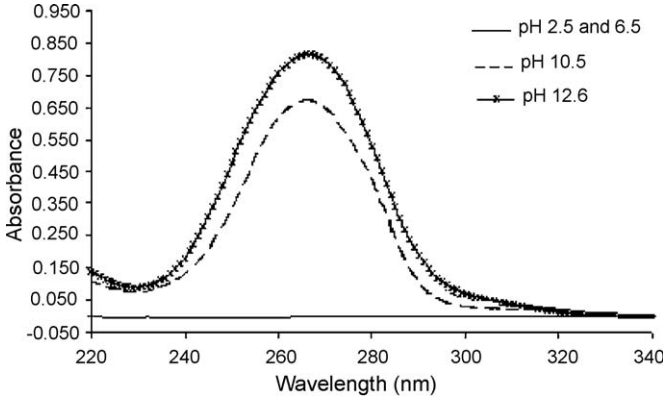

Fig. 1. FIA manifold for ascorbic acid and total sugars determination. PP: peristaltic pump; I.V.: injection valve; *q*: flow rate: 1.46 mL min^{-1}; injection volume: 50 μL, PR: photoreactor (UV lamp with the reactor coil, wavelength lamp: 254 nm, coil length: 120 cm); D: spectrophotometric detector.

2.4. Analysis of fruit juices

Four commercial fruit juices (orange and grapefruit) and two natural fruit juices that were prepared in the laboratory (orange and grapefruit) were analysed by the proposed method.

Commercial fruit juices were homogenized and filtered through 0.45 μm Nylon glass fibre filters. Natural juices were squeezed and then filtered through 0.45 μm Nylon glass fibre filters to remove fibre or pulp. Appropriate dilutions of fruit juice samples were made for the analysis of each analyte.

3. Results and discussion

3.1. Photodegradation study. Selection of experimental conditions

In order to determine total sugars by using the photodegradation step and taking into account that these sugars, commonly present in this kind of samples, have the same behaviour when are exposed to UV irradiation, we selected fructose as standard to study the best conditions for photodegradation and to make calibration curves.

The photodegradation is affected by the pH and time of irradiation. Both variables were selected in order to obtain the best sensitivity and reproducibility of FIA signal, before irradiation for ascorbic acid (300 nm) and after irradiation at 268 nm for total sugars.

3.1.1. Effect of pH

The photodegradation step was carried out at different pH, using the following solutions, 0.2 mol L^{-1} NaOH (pH 13.1), 0.1 mol L^{-1} NaOH (pH 12.6), 3×10^{-4} mol L^{-1} NaOH (pH 10.5), Britton Robinson buffers (pH 10.5 and 6.5) and 3×10^{-3} mol L^{-1} HCL (pH 2.5).

No changes were observed in the absorbance spectrum of fructose when UV radiation was carried out for 5 min at acidic or neutral pH (pH < 7.0), while a distinct band was detected at 268 nm under alkaline conditions. Maximum absorbance of photodegradation products of fructose was obtained with 0.1 mol L^{-1} NaOH (Fig. 2). Also, ascorbic acid showed an absorption band at 300 nm in this medium, when UV radiation was not performed (Fig. 3).

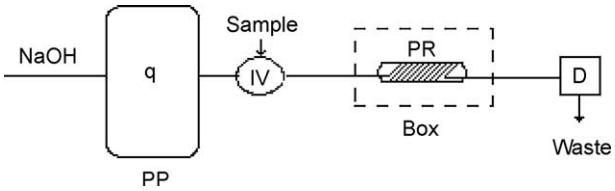

Fig. 2. Absorption spectra of fructose (0.352 g L^{-1}) in 3×10^{-3} mol L^{-1} HCL (pH 2.5), Britton Robinson buffer (pH 6.5), 3×10^{-4} mol L^{-1} NaOH (pH 10.5) and 0.1 mol L^{-1} NaOH (pH 12.6), after 5 min of UV radiation exposure.

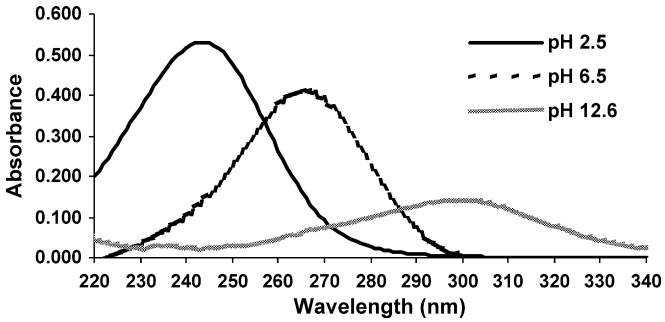

Fig. 3. Absorption spectra of ascorbic acid (7.27 mg L^{-1}) in 3×10^{-3} mol L^{-1} HCL (pH 2.5), Britton Robinson buffer (pH 6.5) and 0.1 mol L^{-1} NaOH (pH 12.6), before UV radiation.

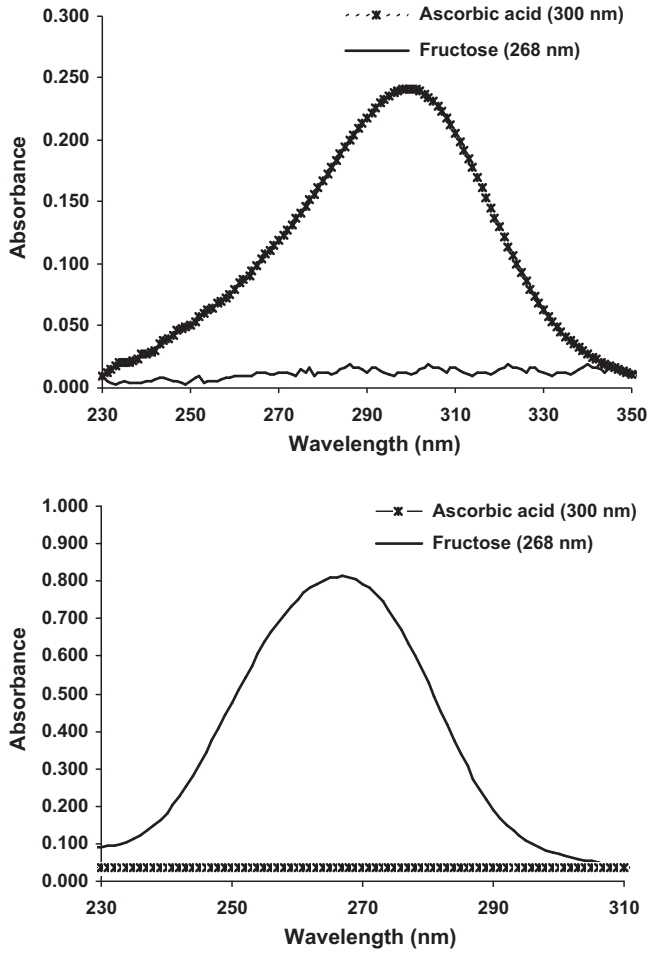

Fig. 4. Ascorbic acid (14.12 mg L^{-1}) and fructose (0.352 g L^{-1}) spectra before photodegradation step (a) and after 5 min of UV photodegradation (b).

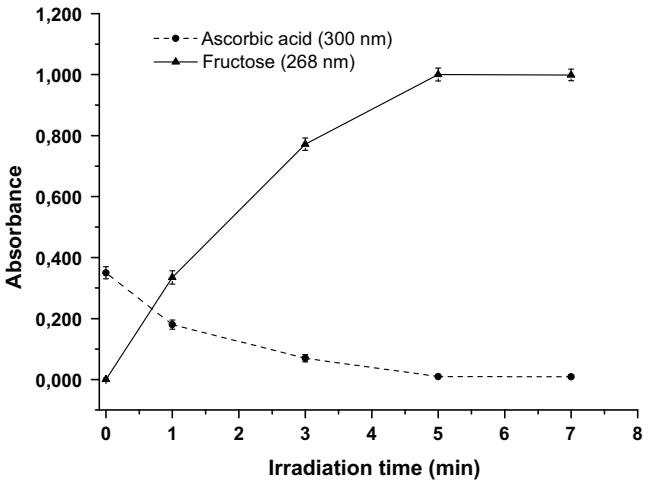

Fig. 5. Effect of irradiation time on the absorbance of ascorbic acid (19.60 mg L^{-1}) and fructose (0.412 g L^{-1}). The concentrations of ascorbic acid and fructose were selected taking into account the upper limits of concentrations of the calibration curves.

3.1.2. Influence of irradiation time

The effect of irradiation time on ascorbic acid and fructose was studied from 0 to 5 min. Before the photodegradation step ascorbic acid showed an absorption band at 300 nm while sugars did not absorb (Fig. 4a). On the other hand, when fructose was exposed to UV radiation, an absorption band at 268 nm appeared. As irradiation time increased, the absorption band at 268 nm became higher and the ascorbic acid signal (300 nm) diminished (Fig. 5).

Fig. 4(b) shows the effect of UV radiation on ascorbic acid and fructose when they were exposed to UV radiation during 5 min. At this time the ascorbic acid signal (300 nm) disappeared and a maximum signal for fructose was obtained.

Therefore, ascorbic acid analysis was carried out before the photodegradation step (irradiation time: 0 min) and fructose quantification after 5 min of UV radiation exposure.

3.2. Influence of FIA variables

The hydrodynamic parameters of the system, sample volume injected and flow rate were optimised by univariant method, in order to achieve a compromise between sensitivity, sample throughput and reproducibility. The studied range was 30–100 μL and 1.00–3.50 mL min^{-1} for volume of sample injected and flow rate respectively. The optimum values were, 50 μL and 1.46 mL min^{-1}, for ascorbic acid and total sugars.

3.3. Analytical parameters

Under the experimental conditions described above, the calibration graph was linear over the range 4.18–20.8 mg L^{-1} of ascorbic acid and 0.05–0.50 g L^{-1} of sugars. The calibration line is $A = (0.0146 \pm 0.0007)\ C + (0.0439 \pm 0.0086)$, $R^2 = 0.9914$ for ascorbic acid and $A = (2.391 \pm 0.048)\ C - (0.010 \pm 0.002)$, $R^2 = 0.9984$ for total sugar, where A is the absorbance and C the concentration of ascorbic acid in mg L^{-1} and total sugars expressed as g L^{-1} of fructose.

Table 1
Determination of ascorbic acid and total sugars in real samples and recovery study.

Sample	Analyte	Labelled (g L^{-1})	Found (g L^{-1})	Added[a] (g L^{-1})	Found (g L^{-1})	Recovery %
CFJ 1	Ascorbic acid	nl[b]	0.733 ± 0.005	0.168	0.908 ± 0.003	104.2
	Global sugars	135.0	126.6 ± 1.8	50.0	175.7 ± 2.1	98.2
CFJ 2	Ascorbic acid	nl[b]	0.651 ± 0.008	0.168	0.827 ± 0.005	104.8
	Global sugars	120.0	132.0 ± 2.4	50.0	177.5 ± 2.0	91.0
CFJ 3	Ascorbic acid	nl[b]	0.579 ± 0.009	0.168	0.761 ± 0.02	108.3
	Global sugars	115.0	111.3 ± 2.3	50.0	159.5 ± 2.2	96.4
CFJ 4	Ascorbic acid	nl[b]	0.488 ± 0.006	0.168	0.650 ± 0.009	96.4
	Global sugars	120.0	128.5 ± 2.1	50.0	181.9 ± 4.7	106.8
NOJ	Ascorbic acid	0.28–0.86[c]	0.678 ± 0.03	0.180	0.861 ± 0.02	101.7
	Global sugars	60–110[c]	98.4 ± 2.0	50.0	155.0 ± 1.4	113.2
NGJ	Ascorbic acid	0.25–0.50[c]	0.432 ± 0.07	0.180	0.608 ± 0.05	97.8
	Global sugars	50–83[c]	81.9 ± 1.9	50.0	130.7 ± 2.0	97.6

CFJ 1, 3: commercial orange liquid fruit juice. CFJ 2, 4: commercial grapefruit liquid fruit juice. NOJ: natural orange juice. NGJ: natural grapefruit juice.
[a] Fructose.
[b] nl: not labelled.
[c] H.-D. Belitz, W. Grosch. "Química de los alimentos". Cap. 18, pg. 913. 2° edición. Ed. Acribia S.A., Zaragoza (España).

The detection limit estimated as signal (S)/noise (N) = 3 was 2.26 mg L^{-1} for ascorbic acid and 0.02 g L^{-1} for total sugar and the quantification limit (S/N = 10) was 7.5 mg L^{-1} for ascorbic acid and 0.09 g L^{-1} for total sugar. The sample throughput for ascorbic acid and fructose were 257 h^{-1} and 11 h^{-1} respectively.

The precision was expressed as percentage of the relative standard deviation of replicate (n) measurements and it was calculated by using standard solutions. The obtained values were 3.4% (n = 11, 10.0 mg L^{-1}) for ascorbic acid, and 2.8% (n = 11. 0.25 g L^{-1}) for total sugars.

3.4. Analysis of commercial and natural fruit juices

Four different commercial liquid fruit juices and two natural fruit juices were used to prove the method. Commercial fruit juices composition includes sacarose, high fructose corn syrup, ascorbic acid, citric acid and sodium eritorbate. In this method no interference of potential interferences as citric acid and sodium eritorbate was found (relative error $< 5\%$ on the signal).

In order to validate the proposed method a recovery study was done. For this purpose, fruit juice samples were spiked and then they were analysed by the proposed method. The obtained results were acceptable and are shown in Table 1.

4. Conclusion

In this work a novel FIA-on-line-photodegradation system with direct UV spectrophotometric detection was developed for sequential determination of ascorbic acid and total sugars in liquid fruit juice samples. This method is an important contribution to quantitative analysis based on photoreactive analytes in food samples.

The FIA manifold is extremely simple and easy to implement for the routine analysis owing to the fact that a spectrophotometric detector is generally available in many laboratories. Moreover, the germicide lamp for the photoreaction is inexpensive and easy to connect on-line. This method offers the advantages of rapid analysis, low contamination risk, low consumption of reagents. Sample preparation is very simple because it only requires filtration and dilution. Also, this method is an important contribution to the quality control of fruit juices.

The accuracy was tested with spiked samples, and the recoveries obtained were in the range 96.4 and 108.3% for ascorbic acid, and 91.0 and 113.2% for total sugars.

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References

Chinnici, F., Spinabelli, U., Riponi, C., Amati, A., 2005. Optimization of the determination of organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. Journal of Food Composition and Analysis 18, 121–130.

Demadrille, R., Rabourdin, A., Campredonc, M., Giusti, G., 2004. Spectroscopic characterisation and photodegradation studies of photochromic spiro[fluorene-9,3′-[3′H]-naphtho[2,1-b]pyrans]. Journal of Photochemistry and Photobiology A: Chemistry 168, 143–152.

Dong, S., Zhang, S., Cheng, X., He, P., Wang, Q., Fang, Y., 2007. Simultaneous determination of sugars and ascorbic acid by capillary zone electrophoresis with amperometric detection at a carbon paste electrode modified with polyethylene glycol and Cu_2O. Journal of Chromatography A 1161, 327–333.

Fennema, O.R., 2000. Química de los Alimentos, 2nd ed. Acribia (Chapter 16), p. 1126.

Füzfai, Z., Molnár-Perl, I., 2007. Gas chromatographic–mass spectrometric fragmentation study of flavonoids as their trimethylsilyl derivatives: analysis of flavonoids, sugars, carboxylic and amino acids in model systems and in citrus fruits. Journal of Chromatography A 1149, 88–101.

Komthong, P., Igura, N., Shimoda, M., 2007. Effect of ascorbic acid on the odours of cloudy apple juice. Food Chemistry 100, 1342–1349.

Li, J., Chen, M., Zhu, Y., 2007. Separation and determination of carbohydrates in drinks by ion chromatography with a self-regenerating suppressor and an evaporative light-scattering detector. Journal of Chromatography A 1155, 50–56.

Liu, J., Itoh, J., 2007. Kinetic method for determination of ascorbic acid on flow injection system by using its catalytic effect on the complexation reaction of an ultra sensitive colorimetric reagent of porphyrin with Cu(II). Spectrochimica Acta Part A 67, 455–459.

Maestre, E., Katakis, I., Narváez, A., Domínguez, E., 2005. A multianalyte flow electrochemical cell: application to the simultaneous determination of carbohydrates based on bioelectrocatalytic detection. Biosensors and Bioelectronics 21, 774–781.

Pires, C.K., Lavorante, A.F., Marconi, L.M.T., Meneses, S.R.P., Zagatto, E.A.G., 2006. A multi-pumping flow system for chemiluminometric determination of ascorbic acid in powdered materials for preparation of fruit juices. Microchemical Journal 83, 70–74.

Roig, B., Thomas, O., 2003. Rapid estimation of global sugars by UV photodegradation and UV spectrophotometry. Analytica Chimica Acta 477, 325–329.

Soga, T., Serwe, M., 2000. Determination of carbohydrates in food samples by capillary electrophoresis with indirect UV detection. Food Chemistry 69, 339–344.

Surareungchai, W., Deepunya, W., Tasakorn, P., 2001. Quadruple-pulsed amperometric detection for simultaneous flow injection determination of glucose and fructose. Analytica Chimica Acta 448, 215–220.

Tang, Y., Wu, M., 2005. A quick method for the simultaneous determination of ascorbic acid and sorbic acid in fruit juices by capillary zone electrophoresis. Talanta 65, 794–798.

Usenik, V., Fabcic, J., Stampar, F., 2008. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (Prunus avium L.). Food Chemistry 107, 185–192.

Xu, G., Liu, D., Chen, J., Ye, X., Maa, Y., Shi, J., 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chemistry 106, 545–551.