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Determination of galacturonic acid content in pectin from fruit juices by liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry

Research Article

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Abstract: A reversed-phase high-performance liquid chromatographic (HPLC) method is applied for the determination of galacturonic acid (GA) of pectins in different commercial fruit juices. The separation was carried out on a C_{18} column using precolumn derivatization with *p*-aminobenzoic acid (*p*-ABA) and UV detection at 304 nm. The identification of GA was confirmed by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) in positive ion mode. The concentration of GA in the samples analyzed ranged from 12.9 ± 0.5 to 49.4 ± 0.5 mg_{GA} L^{-1}. Amongst the samples analyzed, mango juice was found to be richest in GA content, and therefore a good source of pectins. Detection and quantification limits of the described methodology were 1.2 and 3.9 mg L^{-1}, respectively. Quantitative GA recoveries in the beverages had a range between 90 and 98%. The results showed that the HPLC method proposed was precise and suitable for the identification and quantification of GA in commercial fruit juices.

Keywords: *Pectin • Galacturonic acid • p-aminobenzoic acid • Fruit juices • HPLC-ESI-MS/MS*

1. Introduction

Pectic substances, commonly known as pectins, are a group of polysaccharides consisting of repeat units of D-galacturonic acid (GA) linked by α−(1,4) glycosidic bonds creating a linear polymer (Fig. 1). In this main chain, carboxyl groups of GA may either remain as free acids, be esterified with methanol or be neutralized with cations. Furthermore, other neutral sugars such as arabinose, fucose, galactose, glucose, mannose and xylose may occur attached as side-chains [1].

Pectin is an effective and necessary additive to form the structure of food products [2]. It also has medicinal benefits which include lowering low density lipoprotein cholesterol (LDL), lowering glucose levels in diabetic patients as well as lowering the risk of several types of cancer [3]. Many food processes and pectin ingredient suppliers need to determine pectin content to control the quality of their products. In fact, the determination of GA in foods is very important since their presence can affect the chemical and sensorial characteristics of the matrix such as pH, total acidity, microbial stability, sweetness, global acceptability and therefore, provide precious information on the wholesome quality of the food or on the optimization needed to impart select technical features [4].

Several chemical and instrumental methods are available for the determination of pectin content in food

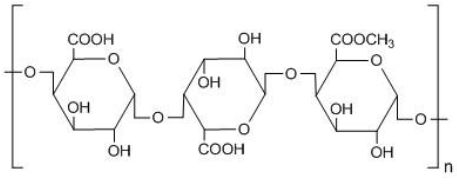

Figure 1. Chemical structure of pectin.

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products. Some of the methods of measuring pectin as GA content are quite sensitive, accurate, reproducible, rapid and well-correlated to pectin content in many samples. Most of them require pectin extraction and hydrolysis prior to analysis [5-7]. More recently, high-performance liquid chromatographic (HPLC) methods have been developed [8-11] and replaced colorimetric methods [5,12]. HPLC is faster and more selective relative to colorimetric methods. Colorimetric methods are also sensitive to the presence of other uronic acids and sugars, which interfere with the GA quantification [13]. However, the lack of chromophores in the structure of GA limits the mode of detection. Refractive index detection and other related methods do not often meet the demands of modern trace level analysis with regard to sensitivity and/or selectivity [11]. Therefore, the derivatization of GA is indispensable to improve the chromatographic properties of the analyte and to improve its efficiency in trace level determination [14]. According to Fischer and co-workers [9], derivatization through reductive amination offers some advantages over other types of derivatizations since compounds of low absorption in the UV/VIS region, such as GA, are transformed into compounds of high absorption. The reagent p-aminobenzoic acid (p-ABA) is one of the popular labels that react with reducing carbohydrate under mild conditions, requiring no acid catalyst and causing no isomerisation [11]. Moreover, the method is easy and requires no special equipment.

Several authors have studied and reported that fruits, especially albedo/flavedo of citrus fruits, are rich in pectins [6,15-17]. However, only a few studies [4,18], have demonstrated that these fruit juices contain GA thereby being a good source of pectins. For these reasons, a need exists to determine the pectin content of these kinds of matrices. This will also be helpful for determining the human consumption of pectins and exploring new functional products rich in pectins due to their pharmacological importance and application in the food industry.

The aim of the present work was the optimization and validation of a simple and reproducible analytical methodology for the determination of GA in different commercial fruit juices by high-performance liquid chromatography with diode array detection (HPLC-DAD). To our knowledge, this is the first study quantifying the presence of GA, and consequently of pectin, in commercial fruit juices. Furthermore, identification of GA was confirmed by tandem mass spectrometry using electrospray ionization (HPLC-ESI-MS/MS). Until now, ESI had not been applied in the study of GA in these matrices. Our investigation was focused on the quantitative determination of pectin monomers in these commercial juices, which could be useful for technological and health reasons.

2. Experimental Procedure

2.1. Chemicals

Sodium cyanoborohydride ($NaBH_3CN$), GA, dimethyl sulfoxide (DMSO), p-ABA, ammonium formate, calcium chloride dihydrate and tris(hydroxymethyl)aminomethane were all p.a. grade and purchased from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Formic acid (HCOOH), sodium hydroxide and hydrochloric acid were purchased from Prolabo (Fontenay-sous-Bois, France). Recombinant pectate lyase was purchased from Megazyme (County Wicklow, Ireland)._High-purity water from a Millipore Simplicity 185 water purification system (Millipore Iberian S.A., Madrid, Spain) was used for all chemical analyses and glassware washing.

2.2. GA standard solution

A stock standard solution (500 mg L^{-1}) of GA (0.0125 g) was prepared by rigorous dissolution in water (25 mL). The standard solution was stored at -20°C and used for further dilutions.

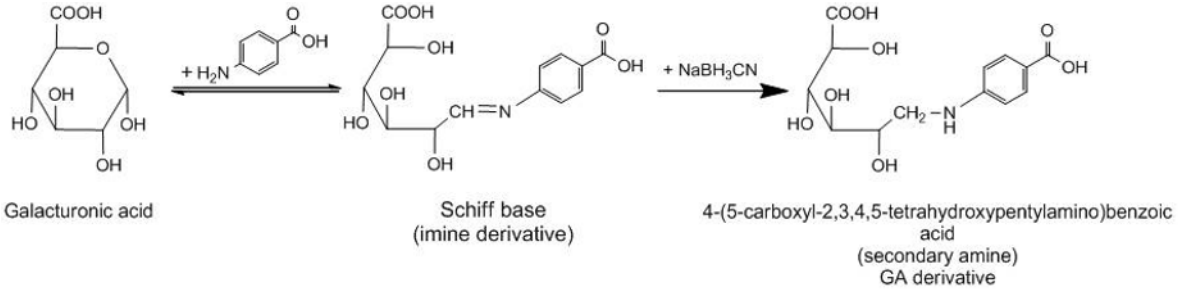
2.3. Sample preparation

Commercial apple, peach, pear, mango and citrus juices were bought from regular supermarkets in Porto, while others were kindly supplied by UNICER – Bebidas de Portugal. Sample analysis was carried out after an enzymatic hydrolysis according to the procedure developed by Hansen and his co-workers [19]. After cleavage of the polygalacturonic acid by a recombinant pectate lyase, samples were filtered through a 0.45 µm Nylon membranes, and GA present in the sample was derivatized as described below.

2.4. Derivatization procedure

The procedure employed for the derivatization of GA with p-ABA was carried out with proper modifications of previously described work [8]. Modifications include changes in mobile phase composition and gradient, making the GA determination simpler without a need to use an ion-pair reagent. In addition, these new conditions allowed the confirmation of pectins' monomer by mass spectrometry, for the first time.

Reductive amination of the analyte with p-ABA (0.35 M) was performed in a DMSO/acetic acid solution



Scheme 1. Derivatization reaction of GA with p-ABA in the presence of reducing agent (reductive amination). The product of the reaction is a secondary amine and will be called by GA derivative.

(70:30 v/v). Reagent solution was prepared daily. A aliquot (200 µL) of the standard solution or sample, the p-ABA solution (500 µL) and $NaBH_3CN$ (10 mg) were mixed in 10 mL polyethylene vials. The vials were tightly capped and heated for 15 min at 60°C. After that time, the vials were cooled to room temperature and the reaction mixtures were dissolved in water (6.3 mL) containing 0.1% of HCOOH and ammonium formate (20 mmol L^{-1}), filtered through 0.45 µm Nylon membranes from VWR (West Chester, USA) and analyzed by HPLC-DAD. The reaction scheme is shown in Scheme 1.

2.5. HPLC-DAD analysis

The HPLC system (Jasco Corporation, Tokyo, Japan) consisted of a low pressure quarternary gradient unit (model LG-1580-04) with an in-line degasser (model DG-1580-54) and an auto-sampler (model AS-950). The system is equipped with a photodiode array detector (model MD-1510 UV/vis multiwavelength detector). Separations were achieved using a guard column Synergi Hydro C_{18} (10.0 mm × 4.0 mm, 3 µm) and a Synergi Hydro C_{18} column (250 mm × 4.6 mm, 3 µm), both from Phenomenex (Palo Alto, USA). The mobile phase consisted of: (A) water and (B) methanol, both containing 0.1% of HCOOH and ammonium formate (20 mmol L^{-1}) in gradient mode (100% to 65% of A in 40 min followed by an equilibration step to 100% of A in 20 min). The flow rate was 0.3 mL min^{-1} and the detection wavelength of the GA derivative was 304 nm. A total of 20 µL of sample was injected into the column that was kept at room temperature. Analyte in each sample was identified by comparing its retention time ($R_t \approx$ 28 minutes) and UV–vis spectra with those of standard solutions. Peak purity was checked to exclude any contribution from interfering peaks.

2.6. HPLC-ESI-MS/MS analysis

Identification of GA derivative in samples was confirmed by HPLC coupled with electrospray ionization tandem mass spectrometry in positive ion mode. The HPLC system (Finnigan - Thermo Electron Corporation, San Jose, CA, USA) consisted of a low-pressure quaternary pump (model Finnigan Surveyor Plus) and an autosampler (model Finnigan Surveyor Plus with 200-vial capacity sample). Separation was achieved on a Synergi Hydro C_{18} column (250 mm × 4.6 mm, 3 µm) from Phenomenex (Palo Alto, USA) and the mobile phase conditions were the same as those described above to the HPLC-DAD analysis. A 25 µL aliquot of sample was injected into the column that was kept at room temperature.

A quadrupole ion-trap mass spectrometer (Finnigan LCQ Deca XP Plus, San Jose, CA, USA) equipped with a ESI source in positive ion mode and Xcalibur software version 1.4 (Finnigan, San Jose, USA) were used for data acquisition and processing. Optimal operating parameters of the ESI interface and quadrupole/ion trap were found by infusing standard solutions of GA derivative in the mobile phase at 3 µL min^{-1} using a Finnigan syringe pump. The optimum conditions applied for the interface were as follows: source voltage of 5.0 kV; source current of 80.0 µA; capillary voltage of 15.0 V; capillary temperature of 300ºC; sheath gas of N_2 at flow rate of 60 arbitrary units; auxiliary gas of N_2 at flow rate of 15 arbitrary units and collision energy for fragmentation at 30 V. During the chromatographic run, mass spectra of the eluate was recorded from m/z 130 to m/z 1500 and fragmentation experiments were carried out on the eluting substances.

3. Results and Discussion

3.1. Method performance

In order to offset the loss of GA that may occur during the process of sample preparation and test the influence of the matrix, the calibration curve and standard additions method were performed in two different commercial fruit

Table 1. Features of the proposed methodology

Calibration curve equation (y = ax + b)		r^2	LOD	LOQ	Intra-day precision	Inter-day precision
a	b		(mg L^{-1})	(mg L^{-1})	(CV%, n = 5)	(CV%, n = 3)
6.82 ± 0.04	0.29 ± 0.08	0.9997	1.2	3.9	2.7	4.2

Table 2. Recovery studies using two different fruit juices

Fruit juice	GA concentration (mg L^{-1})	Concentration added (mg L^{-1})	Recovery (%) (χ ± SD*)	CV (%)
Apple	27.9 ± 0.8	13.7	91.8 ± 1.4	1.5
		26.8	94.4 ± 1.5	1.6
		42.9	96.7 ± 1.2	1.3
		56.2	95.7 ± 2.5	2.6
Mango	49.4 ± 0.5	23.7	92.1 ± 1.9	2.0
		47.8	93.2 ± 1.1	1.2
		72.9	95.7 ± 2.1	2.0
		97.5	98.1 ± 1.6	1.6

Standard deviation for the average, n = 3

juices (apple and mango juices). By the analysis of the slopes obtained for both methods (data not shown) it was concluded that the matrix has no influence on the determination of GA.

The HPLC method was validated in terms of linearity, precision, limits of detection (LOD) and quantification (LOQ).

The linearity was verified by the analysis of standard solutions in the range of 7.1 to 155.0 mg L^{-1} of GA. A calibration curve was constructed by plotting the instrument response as a function of GA concentration and a good linearity was achieved (correlation coefficient, r^2 = 0.9997) in the tested range. The correlation of the calibration graph was also checked using the correlation test (Student t-test). Furthermore, LOD and LOQ were evaluated on the basis of the signal obtained in the analysis of GA standard solutions (n = 5), following the recommendations of IUPAC [20]. LOD (1.2 mg L^{-1}) and LOQ (3.9 mg L^{-1}) were expressed as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively (Table 1). Moreover, the precision of the method was determined by measuring repeatability (intra-day variability) and intermediate precision (inter-day variability) of peak area for apple and mango juices (concentration level ranging from, approximately, 30 to 50 mg L^{-1}). The precision of the method was calculated as the coefficient of variation (CV) for five repeated analyses of the samples already mentioned in the same day (intra-day precision). The inter-day experiments were performed in 3 days and repeated 5 times for each sample. The results showed that the intra-day CV (2.7%) and the inter-day CV (4.2%) were less than 5% (Table 1).

Recovery experiments were performed in order to study the accuracy of the method. Thus, known amounts of GA were added to two different fruit juices (apple and mango) and the resulting spiked samples were subjected to the entire analytical sequence. Each sample was spiked at four different concentrations and recoveries were calculated based on the difference between the total amount determined in the spiked samples and the amount observed in the non-spiked samples. All analyses were carried out in triplicate. The average recoveries obtained were higher than 90% in all cases (Table 2) and testified to the accuracy of the methodology for analysis of GA in pectin of commercial fruit juices.

3.2. Analysis of GA in fruit juices by HPLC-DAD

This work was aimed at developing a rapid, reproducible and accurate analysis method for the quantification of GA in pectins from different commercial fruit juices.

The optimized procedure was applied to different samples, in order to evaluate the applicability of the

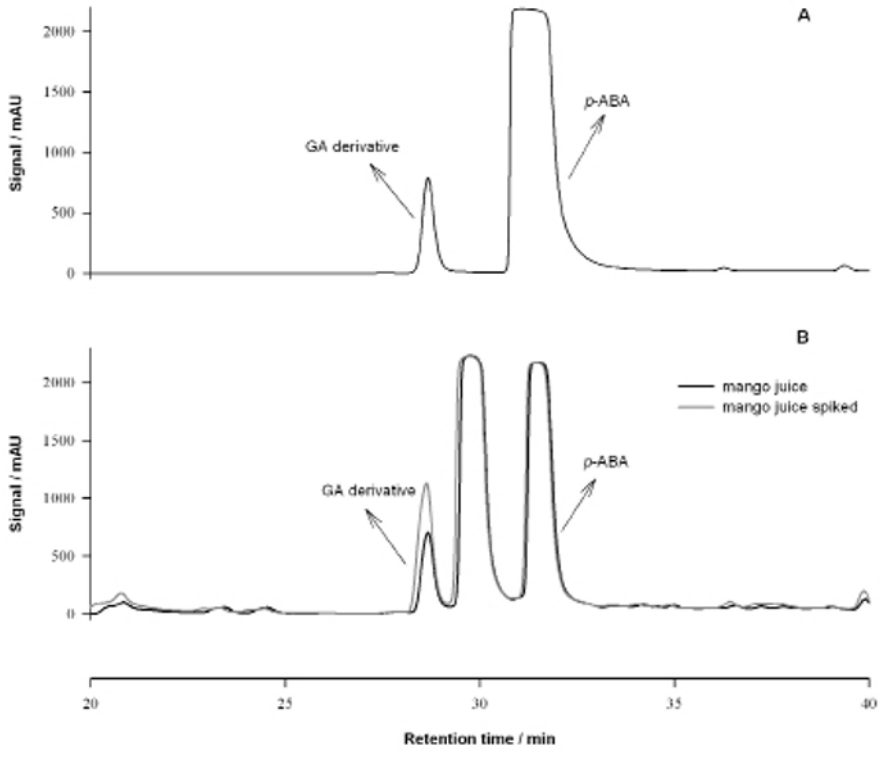


Figure 2. Chromatograms obtained in the HPLC-DAD analysis for a GA standard solution (A) and for a commercial mango juice without and with 16.5 mg L^{-1} of GA standard (B). The HPLC analysis was carried out as described in the Experimental section.

Table 3. Galacturonic acid conten t (mg L^{-1}) in several commercial fruit juices (n = 3)

Fruit juice	GA concentration (mg L^{-1}) (χ ± SD*)	CV / %
Apple 1	27.9 ± 0.8	2.8
Apple 2	43.9 ± 0.9	2.0
Orange 1	24.6 ± 0.8	3.2
Orange 2	26.6 ± 0.7	2.7
Orange/Mango 1	33.1 ± 1.1	3.3
Orange/Mango 2	30.0 ± 0.6	2.0
Clementine	18.9 ± 0.6	3.0
Grapefruit	22.8 ± 0.5	2.0
Pear 1	12.9 ± 0.5	4.1
Pear 2	16.1 ± 0.7	4.2
Peach 1	15.5 ± 0.7	4.6
Peach 2	23.4 ± 0.5	2.2
Mango	49.4 ± 0.5	1.1

Standard deviation for the average, n = 3

proposed methodology. The identification of the peak was carried out by comparing its retention time with standard and by the method of standard additions, as well as by comparing the UV spectra in samples and standard by using a photodiode array detector. The different commercial fruit juices were analyzed in triplicate. Fig. 2 shows a typical chromatogram for the GA standard solution (Fig. 2A) and for the commercial mango juice without and with 16.5 mg L^{-1} of GA standard added (Fig. 2B). The contents of GA for several commercial fruit juices are listed in Table 3.

The amount of GA found in commercial fruit juices range from 12.9 ± 0.5 (pear 1) to 49.4 ± 0.5 (mango) $mg_{GA}\ L^{-1}{}_{juice}$. These values are consistent with results of pectin reported from other sources in the past [17,21-22]. It should be highlighted that most studies are performed in albedo/flavedo of citrus fruits [16,22-23] or in natural juices [21]. To our knowledge, this is the first study confirming and quantifying the presence of GA, and consequently of pectin in commercial fruit juices. Mango juice was seen to be the richest in GA content (49.4 ± 0.5 $mg_{GA}\ L^{-1}{}_{juice}$), indicating it to be a good source of pectins. These results were not unexpected since other authors reported that mango peels are rich in pectins [7,24]. Also, according to our knowledge,

Table 4. HPLC-ESI(+)-MS/MS characteristics of GA standard solution and mango juice

	HPLC retention time / min	Tandem MS			
		Parent ion (m/z)	Fragment ions (m/z)		
		$[MH]^+$	$[MH-H_2O]^+$	$[MH-2H_2O]^+$	$[MH-C_2O_3H_3]^+$
GA standard solution	28.07	316.13	298.11 (100)	280.13 (90)	222.05 (82)
Mango juice	28.11	316.07	298.08 (100)	280.17 (86)	222.07 (78)

Relative abundances of fragment ions are given in parentheses.

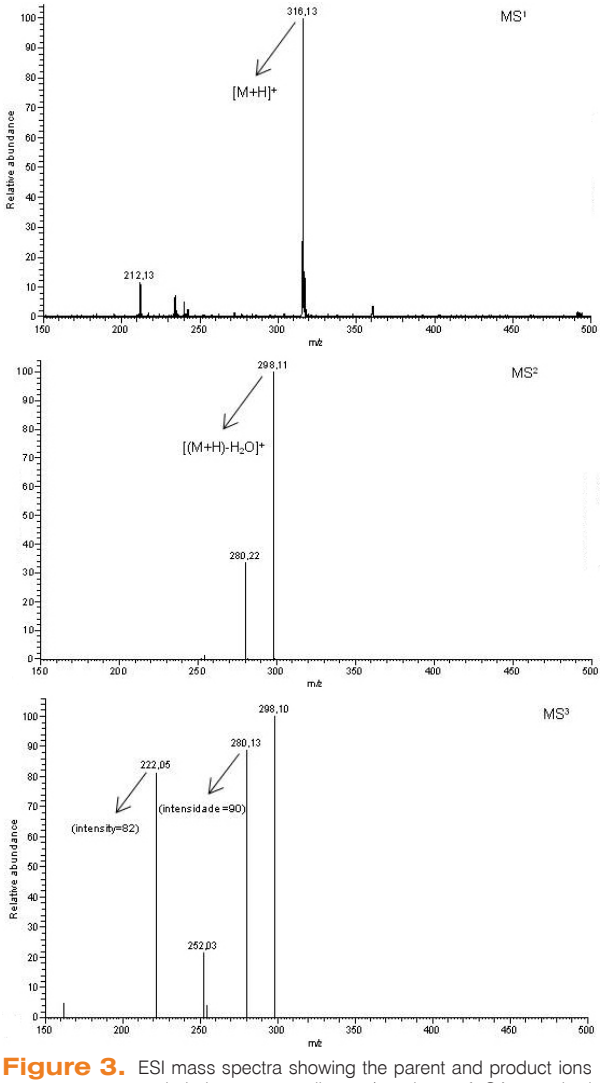


Figure 3. ESI mass spectra showing the parent and product ions and their corresponding *m/z* values of GA standard (25.0 mg L^{-1}).

commercial mango juices are prepared from whole fruit (including the peel), so it is not surprising that pectin content is high in these beverages. On the other hand, pear (12.9 ± 0.5 mg_{GA} L^{-1}_{juice}) and peach (15.5 ± 0.7 mg_{GA} L^{-1}_{juice}) juices have the lowest levels of GA. It should also be noted that commercial juices of citrus fruits (orange, clementine and grapefruit) are not the richest in pectins, contrary to that was previously stated by other authors

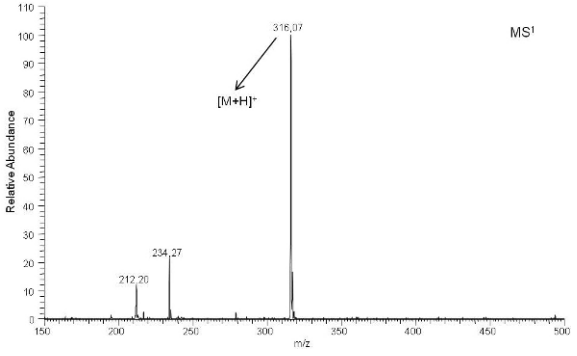


Figure 4. ESI mass spectrum for commercial mango juice ($[M+H]^+$ of *m/z* 316).

[6,22]. These results are not entirely unexpected since commercial juices of orange, clementine and grapefruit are produced without the albedo/flavedo. Albedo of citrus fruits has essential oils that, when during pasteurization of the juice releases terpenes that are responsible for the bitterness of the beverage [25]. Therefore, juice producers do not include the citrus peel, which is very rich in pectins [17,23], hence, as is to be expected, it is not surprising that pectin content is not high. Besides the previously mentioned factors the source of pectins, the state of maturation, as well as the variety of fruit used in the production of beverages and the extraction conditions have a deep impact on pectin content [7,26].

3.3. Confirmation of GA in fruit juices by HPLC-ESI-MS/MS

Identification of GA in commercial fruit juices was confirmed by HPLC–ESI–MS. The combination of retention time and MS spectral information of GA in fruit juice with those of pure standard can be a useful tool for a reliable identification and confirmation of this compound in commercial fruit juices. Until now, ESI has not been applied in the study of GA in these matrices.

The positive ion full-scan ESI-MS spectrum of GA pure standard (25.0 mg L^{-1}) was characterized by the presence of the parent ion $[M+H]^+$ at m/z 316, as shown in Fig. 3. In order to obtain structural information on the ion m/z 316, ESI–MS–MS studies were carried out. The collision-induced dissociation of the ion at m/z 316,

caused by its interaction with the nitrogen damping gas in the ion trap, resulted in three main fragments: m/z 298 (intensity ≈ 100), m/z 280 (intensity ≈ 90) and m/z 222 (intensity ≈ 82). The most abundant signal (m/z 298) corresponds to the molecular ion, $[MH\text{-}H_2O]^+$ obtained after loss of H_2O. The fragment at m/z 280 can be explained as the rearrangement of two hydrogen atoms of the fragment with m/z 298 and subsequent loss of one water molecule forming a stable fragment. The appearance of the fragment with m/z equal to 222 may be attributed to the loss of the substituent OHC^+HCOOH after rearrangement of a hydrogen atom and cleavage of the linkage between the C4'–C5'. The fragmentation of GA derivative follows the mechanism proposed for the Mass Frontier 1.0 software program (data not shown).

As reported in Fig. 4, HPLC-ESI(+)-MS mass spectrum of GA for mango juice was characterized by the presence of the protonated molecule $[M+H]^+$ at m/z 316. On the other hand, the observed fragment ions of this compound matches to those of the standard (data not shown), confirming the existence of GA in commercial fruit juices.

In Table 4 the characteristics ratios of mass to charge (m/z) as well as their fragment ions obtained for the standard solution and for the mango juice sample are summarized.

4. Conclusions

In this work, a HPLC method with UV detection was optimized and validated for quantification of GA in commercial fruit juices. The proposed HPLC method provides a rapid, repeatable, accurate and economic alternative for the analysis of this compound in fruit juices. The method was characterized by good precision, linearity and accuracy. During the derivatization reaction of the sample, recovery studies performed on the different fruit juices, have shown percentage recoveries above 90% for the pectins' monomer. No significant interferences from the sample matrix were observed for the determination of the GA. The commercial mango juice was revealed to be the richest in GA content (49.4 ± 0.5 mg L^{-1}) relative to the pear (12.9 ± 0.5 mg L^{-1}) and peach juices (15.5 ± 0.7 mg L^{-1}). The confirmation of GA in commercial fruit juices was performed by HPLC-ESI-MS/MS and the fragment ions observed matched with those of the standard. In addition, the HPLC method proposed was shown to be precise and suitable for the identification and quantification of GA in different commercial fruit juices. Previous studies have shown that consumption of pectin may have direct health benefits [3,27]. Although further studies on the absorption of pectin by the cells is needed, our results seems to suggest that the consumption of commercial fruit juices, especially mango juices, is expected to result in higher intake of pectin and may have enhanced health benefits.

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References

[1] B.R. Thakur, R.K. Singh, A.K. Handa, Critical Reviews in Food Science and Nutrition 37, 47 (1997)
[2] N.M. Ptichkina, O.A. Markina, G.N. Rumyantseva, Food Hydrocolloids 22, 192 (2008)
[3] A.P. Gunning, R.J.M. Bongaerts, V.J. Morris, FASEB Journal 23, 415 (2009)
[4] F. Chinnici, U. Spinabelli, C. Riponi, A. Amati, Journal of Food Composition and Analysis 18, 121 (2005)
[5] I. Meseguer, V. Aguilar, M.J. González, C. Martínez, Journal of Food Composition and Analysis 11, 285 (1998)
[6] Y. Liu, H. Ahmad, Y. Luo, D.T. Gardiner, R.S. Gunasekera, W.L. McKeehan, B.S. Patil, Journal of Agricultural and Food Chemistry 49, 3051 (2001)
[7] B.B. Koubala, G. Kansci, L.I. Mbome, M.J. Crépeau, J.F. Thibault, M.C. Ralet, Food Hydrocolloids 22, 1345 (2008)
[8] A. Meyer, C. Raba, K. Fischer, Analytical Chemistry 73, 2377 (2001)
[9] K. Fischer, M. Wacht, A. Meyer, Acta hydrochimica et hydrobiologica 31, 134 (2003)
[10] H. Garna, N. Mabon, K. Nott, B. Wathelet, M. Paquot, Food Chemistry 96, 477 (2006)
[11] Y. Lv, X. Yang, Y. Zhao, Y. Ruan, Y. Yang, Z. Wang, Food Chemistry 112, 742 (2009)
[12] C.-Y. Hou, Y.-S. Lin, Y. Tai Wang, C.-M. Jiang, M.-C. Wu, Journal of Food Composition and Analysis 21, 410 (2008)
[13] M.A. Monsoor, U. Kalapathy, A. Proctor, Food Chemistry 74, 233 (2001)
[14] D. Gomis, D. Tamayo, J. Alonso, Analytica Chimica Acta 436, 173 (2001)
[15] G. Mandalari, R.N. Bennett, G. Bisignano, A. Saija, G. Dugo, R.B. Lo Curto, C.B. Faulds,

K.W. Waldron, Journal of Agricultural and Food Chemistry 54, 197 (2005)
[16] O. Kurita, T. Fujiwara, E. Yamazaki, Carbohydrate Polymers 74, 725 (2008)
[17] Y.-C. Wang, Y.-C. Chuang, H.-W. Hsu, Food Chemistry 106, 277 (2008)
[18] G. Saccani, S. Gherardi, A. Trifirò, C. Soresi Bordini, M. Calza, C. Freddi, Journal of Chromatography A 706, 395 (1995)
[19] K.M. Hansen, A.B. Thuesen, J.R. Soderberg, Journal of AOAC international 84, 1851 (2001)
[20] Thompson, Ellison, Wood, Pure Applied Chemistry 74, 835 (2002)
[21] N. Grigelmo-Miguel,O. Martín-Belloso, Food Research International 31, 355 (1999)
[22] Y.-C. Wang, Y.-C. Chuang, Y.-H. Ku, Food Chemistry 102, 1163 (2007)
[23] S. Yeoh, S. Zhang, J. Shi, T.A.G. Langrish, Chemical Engineering Communications 195, 511 (2008)
[24] V. Prasanna, T.N. Prabha, R.N. Tharanathan, Journal of the Science of Food and Agriculture 84, 1731 (2004)
[25] M. Uenojo,G.M. Pastore, Química Nova 30, 388 (2007)
[26] K. Rumpunen, M. Thomas, N. Badilas, J.F. Thibault, Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 35, 490 (2002)
[27] D. Sánchez, B. Muguerza, L. Moulay, R. Hernández, M. Miguel, A. Aleixandre, Journal of Agricultural and Food Chemistry 56, 3574 (2008)