

A Previous Study of Phenolic Profiles of Quince, Pear, and Apple Purees by HPLC Diode Array Detection for the Evaluation of Quince Puree Genuineness

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An HPLC/DAD methodology for separating 13 phenolic compounds is described. This methodology is applied to the definition of qualitative and quantitative profiles of quince, pear, and apple purees and a blending of these purees. Two different extraction methods were needed for the complete definition of their profiles, one of them including an Amberlite XAD-2 cleaning step. The analysis of phenolics of each fruit puree profile and that of a blending seems to indicate that this methodology can be applied to the detection of apple and/or pear fraudulently added to quince puree. The presence of apple can be detected by phloretin 2'-xylosylglucoside and phloretin 2'-glucoside, while that of pear is detected by arbutin. In addition, 3-*O*-caffeoylquinic acid is present at a considerable relative amount (~23.4%) in quince puree, while the sample of pear puree contains only 8.2%; this compound is absent in apple puree.

Keywords: HPLC; phenolics; quince; purees; adulteration

INTRODUCTION

Quince is the fruit of a deciduous tree, *Cydonia oblonga* Miller (var. *maliformis* or *piriformis*). Ripe quince fruit have a strong floral odor, most of the essential oil responsible for this odor being located in the peel (Umano et al., 1986). Although quince fruit is not edible raw because of its hardness, bitterness, and astringency, it is appreciated for its jam "marmelada" [made exclusively with quince puree and sugar (50:50)]. Quince jam has a characteristic and strong flavor. Some compounds responsible for this flavor are not present in the fruits but are formed during technological processes (Guldner and Winterhalter, 1991). When quince production is low, quince jam is easily adulterated by apple (*Malus communis* Lamk) and pear (*Pirus communis* Lin.) due their low cost and their texture, which is similar to that of quince. Besides, the strong odor of quince jam easily covers the flavors of apple and/or pear.

Because sensory evaluation often fails in detecting the mentioned adulteration, a methodology based on chemical markers is needed. Phenolic compounds are widely distributed in nature and have been successfully used for the determination of genuineness of some fruit jams and juices (Tomás-Lorente et al., 1992; Simón et al., 1992). The phenolic compositions of apple and pear are fairly well known (Dick et al., 1987; Lee and Wrolstad, 1988; Oleszek et al., 1988, 1994; Burda et al., 1990; Spanos et al., 1990; Spanos and Wrolstad, 1990, 1992; Simón et al., 1992; Tomás-Lorente et al., 1992; Tomás-Barberán et al., 1993; Vallés et al., 1994; Oleszek et al., 1994), but as far as we know, studies on quince are related, mainly, to terpenoid composition (Guldner and Winterhalter, 1991; Lutz and Winterhalter, 1992).

To prevent speculative commerce, either with purees or with the respective jams, we tried to study the phenolic profiles (qualitative and quantitative) of quince, apple, and pear purees to determine if they could be a tool for the search of apple and/or pear puree in the authentication of quince puree.

To study the mentioned profiles, a methodology was developed to separate, identify, and quantify 13 phenolic compounds usually described in apple and/or pear (Spanos and Wrolstad, 1992), including several classes of compounds [cinnamic acids and their derivatives, catechins, dihydrochalcones, flavonol glycosides, and a simple phenol glycoside (arbutin)].

For an accurate quantification of all phenolics identified, two different extraction methods were needed.

MATERIALS AND METHODS

Standards and Reagents. The standards were from Sigma (St. Louis, MO) and from Extrasynthèse (Genay, France). 3-*O*-Caffeoylquinic acid was not commercially available, so it was prepared by transesterification of 5-*O*-caffeoylquinic acid using tetramethylammonium hydroxide (Murata et al., 1995). HPLC grade methanol and formic acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Samples. Quince, apple, and pear purees were obtained from fruits provided directly by the farmers. The varieties of apple and pear used were those more abundant in the Portuguese market (Golden Delicious and Rocha, respectively). The fruits were placed in boiling water and boiled for 15 min. Fruits were separated, and the cores were removed and then pulped with a Moulinex mixer.

A blending of quince, apple, and pear purees was prepared in the proportions 60:20:20, respectively. The purees were frozen (-20 °C) until their analysis.

Sample Preparation. Phenolic Extraction via the Amberlite XAD-2 Step. Each fruit puree (~40 g) was thoroughly mixed with 5 parts of water (pH 2 with HCl) until completely

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Table 1. Phenolic Composition of Quince, Apple, and Pear Purees and a Blending of These Fruit Purees (Milligrams of Phenolic Compound per Kilogram of Puree) Obtained by Amberlite XAD-2 Extraction^a

	retention time ^b (min)	quince	apple	pear	mixture of quince/apple/pear (60:20:20)
(1) arbutin	4.52 (0.05)				2.9 (0.4) ^c
(2) 3- <i>O</i> -caffeoylquinic acid	9.55 (0.07)	2520 (819)		440 (2)	2009 (4.2)
(3) (+)-catechin	10.19 (0.07)		1.5×10^{-3} (1.4×10^{-6})	nq ^d	nq
(4) <i>p</i> -hydroxybenzoic acid	14.57 (0.10)			1.6×10^{-2} (9.2×10^{-6})	nq
(5) 5- <i>O</i> -caffeoylquinic acid	16.17 (0.12)	6770 (736)	690 (130)	4260 (78)	6812 (43)
(6) (-)-epicatechin	17.08 (0.12)		0.4 (2.2×10^{-3})	3.5×10^{-3} (5.9×10^{-6})	5×10^{-2} (2.5×10^{-3})
(7) <i>p</i> -coumaric acid	25.02 (0.20)		nq		
(8) phloretin 2'-xylosylglucoside	35.61 (0.23)		12 (1.1×10^{-2})		2.5 (4×10^{-3})
(9) phloretin 2'-glucoside	38.09 (0.25)		16 (1.5×10^{-3})		4 (3.5×10^{-3})
(10) rutin	42.04 (0.27)	20 (3)	4.7 (0.1)		20.9 (0.4)
(11) quercetin 3-galactoside	42.32 (0.27)	20 (1)		3.6 (6×10^{-4})	27.3 (0.03)
(12) quercetin 3-xyloside	44.32 (0.29)	203 (0.7)	1300 (0.6)		377 (0.9)
(13) quercetin 3-rhamnoside	45.49 (0.30)	1460 (114)	991 (96)	130 (70)	189 (1.1)

^a Values are expressed as mean (standard deviation) of three assays for each sample. ^b Values are expressed as mean (standard deviation) of three assays for each sample. ^c Arbutin was determined by the simplified technique. ^d nq, not quantified.

fluid and filtered through cotton wood to remove solid particles. The filtrate was then passed through a column (25 × 2 cm) of Amberlite XAD-2 (Fluka Chemicals; pore size 9 nm, particle size 0.3–1.2 mm), as reported previously (Ferrerres et al., 1994). Sugars and other polar compounds were eluted with the aqueous solvent. The column was washed with water (pH 2 with HCl, 100 mL) and subsequently with distilled water (~300 mL). The phenolic fraction remained in the column and was then eluted with methanol (~300 mL). The methanolic extract was evaporated to dryness under reduced pressure (40 °C) and redissolved in methanol (1.5 mL), and 20 μL was analyzed by HPLC.

Phenolic Extraction via the Simplified Technique. Each puree (~40 g) was thoroughly mixed with 100 mL of methanol (24 h). The volume of solvent and time of maceration were previously tested to guarantee a complete extraction of phenols (negative reaction with NaOH). The extract was then filtered and evaporated to dryness under reduced pressure (40 °C) and redissolved in methanol (1.5 mL), and 20 μL was analyzed by HPLC.

HPLC Analysis of Phenolic Compounds. Separation of phenolics was achieved with an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 × 0.46 cm; 5 μm, particle size) column. The solvent system used was a gradient of water/formic acid (19:1) (A) and methanol (B). After trying different solvent gradients and flow velocities, the best resolution was obtained at a solvent flow rate of 0.9 mL/min, starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min, and 80% B at 60 min. Detection was achieved with a diode array detector, and chromatograms were recorded at 350 and 280 nm.

The different phenolic compounds were identified by their UV spectra recorded with the diode array detector and by chromatographic comparisons (retention times) with authentic standards.

Phenolic quantification was achieved by the absorbance recorded in the chromatograms relative to external standards of phenolic compounds with detection at 350 nm for *p*-coumaric acid, 3- and 5-*O*-caffeoylquinic acids, quercetin 3-galactoside, quercetin 3-xyloside, quercetin 3-rhamnoside, and rutin and at 280 nm for the others.

RESULTS AND DISCUSSION

To optimize the HPLC conditions (concerning column, gradient, and flow rate of solvents) for the analysis of phenolic compounds in quince, apple, and pear purees, an artificial mixture was prepared containing 13 phenolic compounds usually described in apple and pear (Spanos and Wrolstad, 1992). Under the conditions described under Materials and Methods, the retention

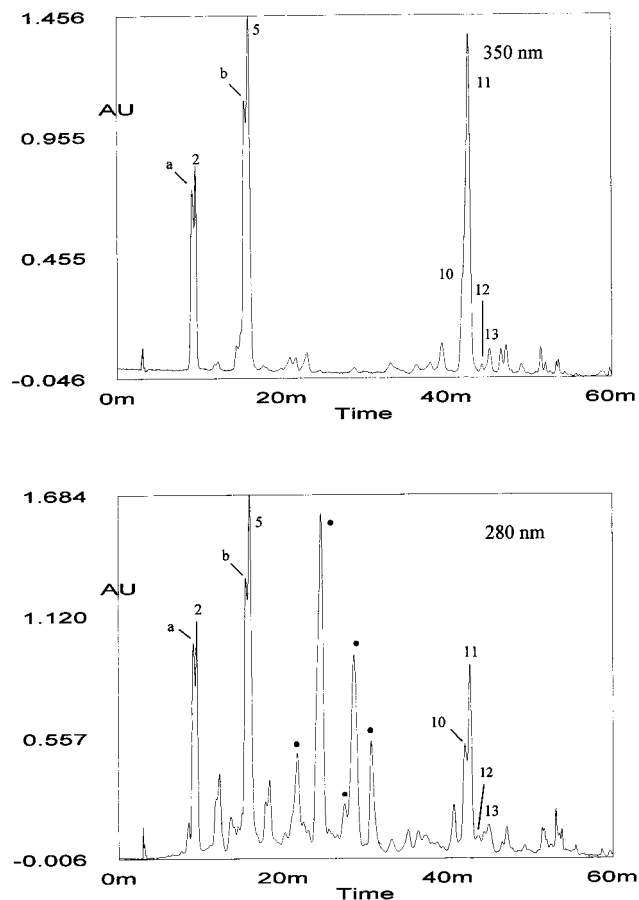


Figure 1. HPLC profile of quince puree phenolics obtained by Amberlite XAD-2 extraction: (a) 3-*O*-caffeoylquinic acid isomer; (2) 3-*O*-caffeoylquinic acid; (b) 5-*O*-caffeoylquinic acid isomer; (5) 5-*O*-caffeoylquinic acid; (10) rutin; (11) quercetin 3-galactoside; (12) quercetin 3-xyloside; (13) quercetin 3-rhamnoside; (●) unidentified characteristic phenolic compounds of quince puree.

times obtained were those indicated in Table 1. The repeatability of the method was high, with respect to both retention times (Table 1) and peak areas.

The optimized HPLC methodology was then applied to the analysis of phenolic compounds in quince, apple, and pear purees (Figures 1–4) and then to the blending of these purees (Figure 5).

The compounds in each sample were identified by comparing their retention times and UV-vis spectra in the 200–400 nm range with the library of spectra previously compiled by the authors.

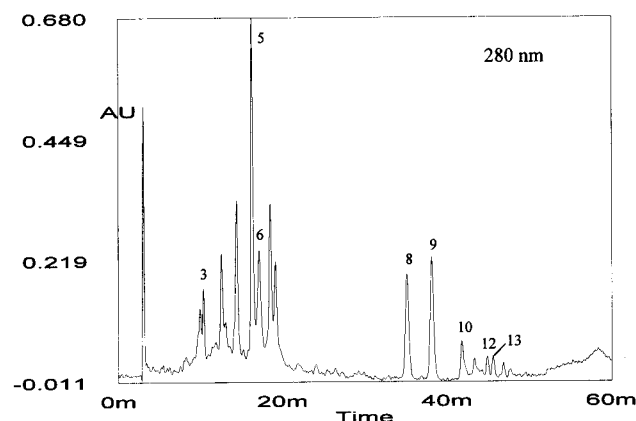
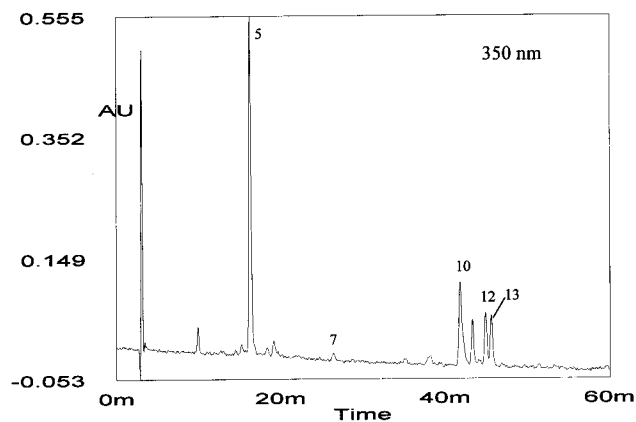


Figure 2. HPLC profile of apple puree phenolics obtained by Amberlite XAD-2 extraction: (3) (+)-catechin; (5) 5-*O*-caffeoylquinic acid; (6) (–)-epicatechin; (7) *p*-coumaric acid; (8) phloretin 2'-xylosylglucoside; (9) phloretin 2'-glucoside; (10) rutin; (12) quercetin 3-xyloside; (13) quercetin 3-rhamnoside.

For all fruit purees, except for the pear puree, both extraction techniques described under Materials and Methods lead to the same phenolic profiles (Figures 1–4; Table 1). The HPLC profile of phenolics from pear puree obtained according to the simplified technique (Figure 4) presented arbutin, which did not occur when the Amberlite XAD-2 step was used (Figure 3). This could be explained by taking into account the polarity of arbutin, which could be eluted with the sugars and other polar compounds when the Amberlite XAD-2 column was washed with aqueous solvent. To test the recovery of arbutin by the simplified technique extraction, a known quantity of arbutin was added to a weighted portion of pear puree. The sample was analyzed in duplicate before and after the addition of arbutin. The recovery rate of arbutin according to this simplified technique was ~96%.

With the Amberlite XAD-2 cleaning step a phenolic extract without sugars and other polar compounds was obtained. The chromatograms appeared somewhat cleaner than those obtained with the simplified technique extraction and, as a general rule, the amount of each phenolic compound extracted was higher. Due to the low recovery rate of arbutin when the Amberlite XAD-2 was used, the simplified technique is needed for its quantification in pear puree and in the blending.

Apple puree (Figure 2) was characterized by the presence of (+)-catechin, 5-*O*-caffeoylquinic acid, (–)-epicatechin, *p*-coumaric acid, phloretin 2'-xylosylglucoside, phloretin 2'-glucoside, rutin, quercetin 3-xyloside,

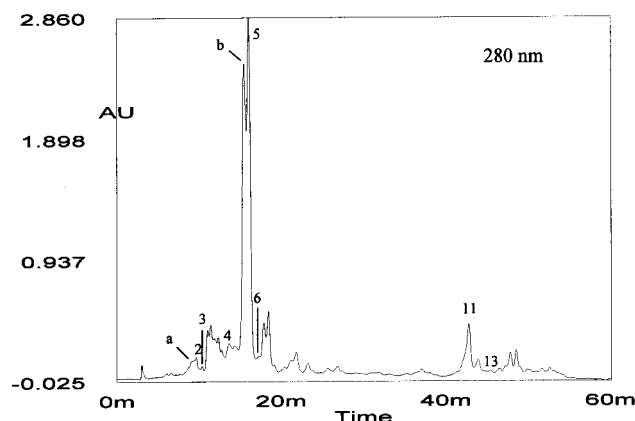
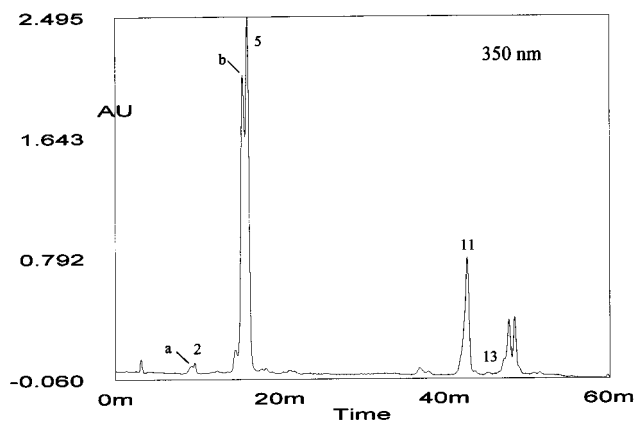


Figure 3. HPLC profile of pear puree phenolics obtained by Amberlite XAD-2 extraction: (a) 3-*O*-caffeoylquinic acid isomer; (2) 3-*O*-caffeoylquinic acid; (3) (+)-catechin; (4) *p*-hydroxybenzoic acid; (b) 5-*O*-caffeoylquinic acid isomer; (5) 5-*O*-caffeoylquinic acid; (6) (–)-epicatechin; (11) quercetin 3-galactoside; (13) quercetin 3-rhamnoside.

and quercetin 3-rhamnoside, which agrees with previous studies (Dick et al., 1987; Lee and Wrolstad, 1988; Oleszek et al., 1988; Burda et al., 1990; Spanos et al., 1990; Spanos and Wrolstad, 1992; Simón et al., 1992; Tomás-Lorente et al., 1992; Tomás-Barberán et al., 1993; Vallés et al., 1994). Pear puree (Figure 3) contained 3-*O*-caffeoylquinic acid (cis and trans isomers), (+)-catechin, *p*-hydroxybenzoic acid, 5-*O*-caffeoylquinic acid (cis and trans isomers), (–)-epicatechin, quercetin 3-galactoside, quercetin 3-rhamnoside, and arbutin (Figure 4). These results are in accordance with those reported previously (Spanos and Wrolstad, 1990, 1992; Simón et al., 1992; Tomás-Lorente et al., 1992; Oleszek et al., 1994). On the other hand, quince puree (Figure 1) is characterized by the presence of 3-*O*-caffeoylquinic acid (cis and trans isomers), 5-*O*-caffeoylquinic acid (cis and trans isomers), rutin, quercetin 3-galactoside, quercetin 3-xyloside, quercetin 3-rhamnoside, and several unidentified phenolic compounds.

Upon analyses of fruit puree we could say they were different from one another in terms of the presence of compounds that could be considered as chemical markers: *p*-coumaric acid, phloretin 2'-xylosylglucoside, and phloretin 2'-glucoside for apple; arbutin and *p*-hydroxybenzoic acid for pear. In quince puree only caffeoylquinic acids and quercetin derivatives were identified and, as far as we know, this is the first report of these compounds in quince. Two striking characteristics are remarkable in the phenolic profile of quince: one of

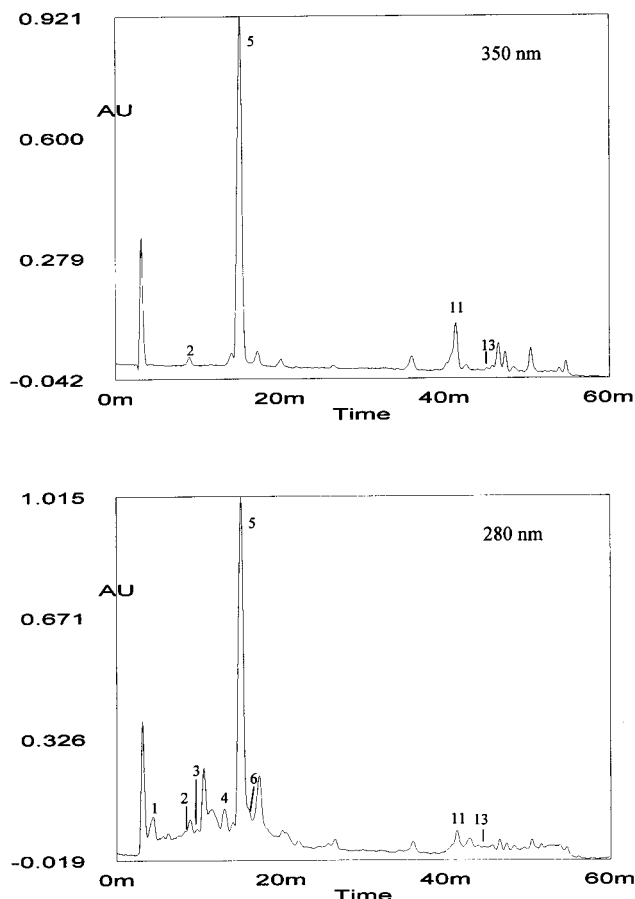


Figure 4. HPLC profile of pear puree phenolics obtained by simple extraction with methanol: (1) arbutin; (2) 3-*O*-caffeoylquinic acid; (3) (+)-catechin; (4) *p*-hydroxybenzoic acid; (5) 5-*O*-caffeoylquinic acid; (6) (–)-epicatechin; (11) quercetin 3-galactoside; (13) quercetin 3-rhamnoside.

them concerns the presence of several unidentified compounds (not found in apple and/or pear) with identical UV spectra when recorded with a diode array detector (identical shape and maximum at 269.3 nm). The possibility of being glycosides of procyanidin polymers is not excluded in accordance with the Porter et al. (1985) study, their chromatographic behavior, and their UV spectra.

The other characteristic is the considerable relative amount (~23.4%) of 3-*O*-caffeoylquinic acid, while the sample of pear puree contains only 8.2%, and this compound is absent in apple puree (Table 1).

On a quantitative level (Table 1), there are other differences: 3-*O*-caffeoylquinic acid, quercetin 3-galactoside, and quercetin 3-rhamnoside are more abundant in quince, while quercetin 3-xyloside is much higher in apple.

When the methodology described herein is applied to the analysis of the artificial blending prepared for this specific purpose (Figure 5), *p*-coumaric and *p*-hydroxybenzoic acids cannot be used as chemical markers because they were present in small but undetectable amounts.

In conclusion, the addition of apple and pear to the quince puree was easily detected by the presence of their characteristic compounds, phloretin 2'-xylosylglucoside and phloretin 2'-glucoside for apple and arbutin for pear (Figure 5 and Table 1). This study suggests that the technique described herein is quite useful for the analysis of phenolic compounds in samples of fruit

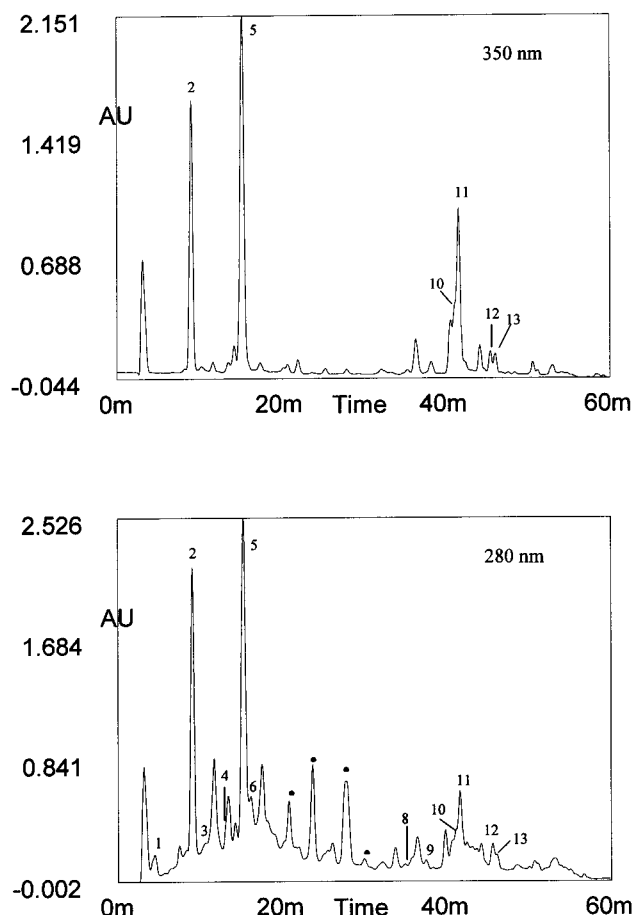


Figure 5. HPLC profile of mixture of quince/apple/pear purees (60:20:20) obtained by simple extraction with methanol: (1) arbutin; (2) 3-*O*-caffeoylquinic acid; (3) (+)-catechin; (4) *p*-hydroxybenzoic acid; (5) 5-*O*-caffeoylquinic acid; (6) (–)-epicatechin; (8) phloretin 2'-xylosylglucoside; (9) phloretin 2'-glucoside; (10) rutin; (11) quercetin 3-galactoside; (12) quercetin 3-xyloside; (13) quercetin 3-rhamnoside; (●) unidentified characteristic phenolic compounds of quince puree.

purees, allowing the detection of apple and/or pear in quince purees and probably their respective jams.

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