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Improved HPLC Determination of Phenolic Compounds in Cv. Golden Delicious Apples Using a Monolithic Column

Fabio Chiniaci,[†] Anna Goiani,[§] Nadia Natali,[†] Claudio Riponi,[†] and Sergio Galassiti

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Received for review June 24, 2003. Revised manuscript received September 25, 2003. Accepted September 29, 2003.

Abstract:

A rapid HPLC-DAD determination of phenols in apple using an RP monolithic column is reported. Because of the hydrodynamic advantages offered by this kind of column and the use of acidified acetonitrile as eluent, assays of apple extracts can be performed in <21 min. Assays of pulp and peel extracts were carried out without the need for time-consuming sample pretreatment except filtration. Several flavanols, hydroxycinnamic acids, dihydrochalcones, and six quercetin glycosides were identified and quantified. A seventh quercetin derivative, two chalcone-related compounds, and three hydroxycinnamic derivatives were also found. Peels proved to be richer in phenols than pulps, the former being composed mainly of (-)-epicatechin, procyanidin B2, chlorogenic acid, phloridzin, hyperin, and avicularin. In pulps, where the chlorogenic acid was the principal phenolic compound, quercetin glycosides were found in very low amounts.

Keywords: HPLC-DAD; monolithic column; phenolics; apple; Golden Delicious; analysis

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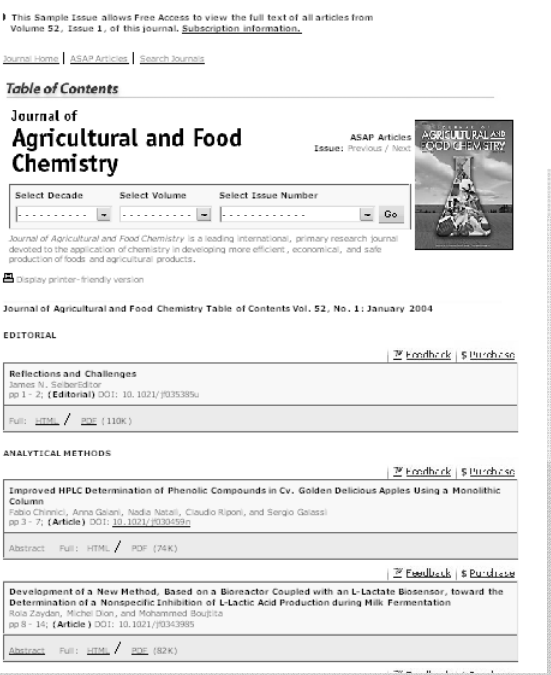
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Improved HPLC Determination of Phenolic Compounds in Cv. Golden Delicious Apples Using a Monolithic Column

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Keywords: HPLC-DAD; monolithic column; phenolics; apple; Golden Delicious; analysis

Introduction

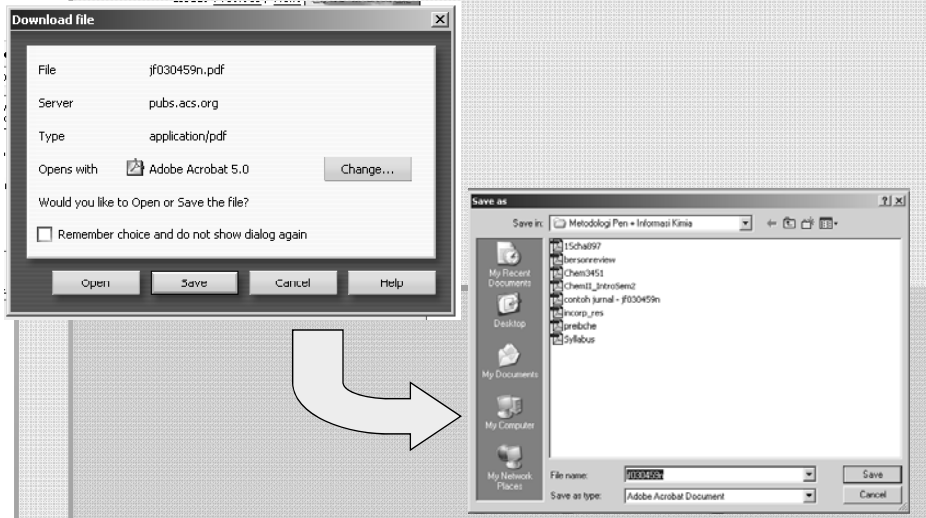
During the past decade, apple phenols have been subjected to a number of investigations due to their presence in the human diet (1) and beneficial effects on health (2). Moreover, phenols are involved in apple browning, which is one of the main problems in juice processing (3,4). Phenols are also important because of their contribution to the color, taste, and flavor of the fruits (5).

Apple phenolic composition appears to be largely made up of flavanols, hydroxycinnamic acids, flavonol glycosides, and dihydrochalcones (6-10). For their determination, RP-HPLC has proved to be the most appropriate technique, being sufficiently sensitive and precise. Unfortunately, HPLC run times are often excessive and sometimes preceded by a time-consuming sample cleanup step (6,10,12). Only recently has a fast separation of apple polyphenols been reported (8,13), the determination of the main phenols taking <25 min using a conventional RP C18 column.

However, nonconventional monolithic supports for column packing are increasingly attracting the interest of researchers (16,17). Due to their rigid and porous structure, they enable higher solvent flows, shorter assay times, and fast column re-equilibration between runs (18). The present paper reports a simple and fast method for the determination of the largest number of phenolic compounds in apple peels and pulps using a monolithic column and a diode array detector.

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Improved HPLC Determination of Phenolic Compounds in
 Cv. Golden Delicious Apples Using a Monolithic Column

FABIO CHENNICI,^{1,2} ANNA GALANI,¹ NAUKA NATALI,¹ CLAUDIO REINISI,¹ AND
 SERGIO GALASSI¹

Dipartimento di Scienze degli Alimenti and Dipartimento Culture Arboree, Università di Bologna,
 40127 Bologna, Italy

A rapid HPLC-DAD determination of phenols in apple using an RP monolithic column is reported. Because of the hydrodynamic advantages offered by this kind of column and the use of acified acetonitrile as eluent, storage of apple extracts can be performed in 20 min. Acetate of pulp and peel extracts were carried out without the need for time-consuming sample pretreatment except filtration. Several flavonols, hydroxycinnamic acids, dihydrochalcones, and six quercetin glycosides were identified and quantified. A new class of quercetin derivatives, two chalcone-related compounds, and three hydroxycinnamic derivatives were also found. Peels proved to be richer in phenols than pulp, the former being composed mainly of (-)-epigallocatechin, procyanidin B2, chlorogenic acid, phloretin, hyperin, and naringenin. In pulp, where the chlorogenic acid was the principal phenolic compound, quercetin glycosides were found in very low amounts.

KEYWORDS: HPLC-DAD; monolithic column; phenolics; apple; Golden Delicious; analysis

INTRODUCTION

During the past decade, apple phenols have been subjected to a number of investigations due to their presence in the human diet (1) and beneficial effects on health (2). Moreover, phenols are involved in apple browning, which is one of the main problems in juice processing (3, 4). Phenols are also important because of their contribution to the color, taste, and flavor of the fruit (5). Apple phenolic composition appears to be largely made up of flavonols, hydroxycinnamic acids, dihydrochalcones, and dihydrochalcones (6-10). For their determination, RP-HPLC has proved to be the most appropriate technique, being sufficiently sensitive and precise. Unfortunately, HPLC run times are often excessive and acetonitrile presented by a time-consuming sample cleanup step (6, 10-14). Only recently has a fast separation of apple polyphenols been reported (9, 15), the determination of the main phenols taking $\sim 25\text{ min}$ using a conventional RP-C18 column.

However, nonconventional monolithic supports for column packing are increasingly attracting the interest of researchers (16, 17). Due to their rigid and porous structure, they enable higher solvent flows, shorter assay times, and fast column re-

Table 1. Standard Compounds, Concentration Ranges, and Wavelength Used for Operation of the Gradient Method

compound	chemical class	concentration range	assay wavelength (nm)
gallic acid	hydroxybenzoic acid	200	0.8-40.0
(-)-catechin	flavanol	200	0.8-40.0
chlorogenic acid	hydroxycinnamic acid	200	0.8-40.0
procyanidin B2	flavan dimer	200	1.0-50.0
p-coumaric acid	hydroxycinnamic acid	200	2.5-50.0
(-)-epigallocatechin	flavanol	200	0.8-40.0
hyperin	flavonol glycoside	200	0.8-40.0
quercetin	flavonol glycoside	200	1.0-50.0
phloretin	dihydrochalcone	200	0.8-40.0

equilibration between runs (18). The present paper reports a simple and fast method for the determination of the largest number of phenolic compounds in apple peels and pulp using a monolithic column and a diode array detector.

MATERIALS AND METHODS

Reagents and solvents. Solvents were from Merck and of analytical or HPLC grade. Flavonol, hydroxycinnamic acid, and dihydrochalcones (Table 1) were from Sigma (St. Louis, MO), whereas procyanidin B2, hyperin, quercetin-3-glucuronide, rutin, quercetin-3-rutinoside, quercetin (quercetin-3-rutinoside), and procyanidin B2-galactoside

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