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THE IDENTIFICATION OF PHENOLIC ACIDS BY HPLC METHOD FROM STRAWBERRIES

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Abstract

It is known that the phenolic acids have beneficial effects on health as antioxidants and anticarcinogens. Some phenolic acids (gallic, protocatechuic and p-hydroxy benzoic acid) were analyzed in cultivated strawberries (Fragaria moschata) with and without alkaline and acid hydrolysis. All fractions were quantified by HPLC with DAD and ECD detection. The results of alkaline and acid hydrolysis were calculated as to represent total phenolic acids. The results showed a little amount of free phenolic acids in strawberries but, after the hydrolysis of strawberry samples, an increased content of phenolic acids was noticed. From all analyzed phenolic acids, the one detected in the greatest quantity was p-hydroxy benzoic acid.

***Keywords**: p-hydroxy benzoic acid, phenolic acids, flavonoids, HPLC method, strawberries.*

Introduction

Fruits and vegetables contain high levels of antioxidant compounds that provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart diseases in addition to a number of other health benefits (Cao, 1996). There is a positive correlation between antioxidant activity and total phenolic or anthocyanin content of fruits and vegetables (Velioglu, 1998).

The present study evaluated some phenolic compounds found in strawberries.

The main objective is the extraction, separation and identification of phenolic acids by HPLC method.

By HPLC method (DAD and ECD detectors) it could be obtained information about some phenolic acids (gallic acid, protocatechuic acid, p-hydroxibenzoic acid) that occur in the fresh strawberry. Quantitative determinations were made too. Hydrolyze and extraction of the phenolic acids was made for a comparison by two methods (Schiberl, 2001).

Experimental

Extraction of the free phenolic acids from fresh strawberries was made after the method of Schiberl (2001). Hydrolysis and extraction of phenolic acids were made with the method used by Mattila (2002).

Fruit samples of 5 g were extracted twice with 15 mL of acetone using a Polytron for 1 min. Extracts (30 mL) were combined and concentrated to 1 mL using a Buchler Evapomix in a water bath at 35 C. The concentrated sample was dissolved in 10 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge, which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column while sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% aqueous formic acid. The anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanolic extract was passed through a 0.45-μm membrane filter (Millipore, MSI, Westboro, MA) and 20 μL was analyzed by HPLC. High-performance liquid chromatography (HPLC) was used to separate and determine individual phenolic compounds in strawberry tissue samples. The samples were analyzed using a Waters (Waters Associated, Millipore, Milford, MA) HPLC system equipped with two pumps (600 E system controller) coupled with a photodiode array detector (Waters 990 series). Samples were injected at ambient temperature (20 C) onto a reverse-phase NOVA-PAK C_{18} column with a guard column (NOVA-PAK C_{18},) (Cioroi, 2000).

The chromatograms of free phenolic acids strawberry sample contain many picks. In the base of the standard phenolic acids chromatogram were identified following phenolic acids: gallic acid, protocatechuic acid, p-hydroxibenzoic acid, gentissic acid, cafeic acid.

The quantitative determinations were made by the calibration curves for gallic acid, protocathecuic acid and p-hydroxybenzoic acid.

Statistical Analysis. Data were subjected to analysis of variance using the Tukey-Kramer multiple-comparison test used in NCSS. Differences at $P < 0.05$ were considered significant (NCSS 97, 1997).

Results and Discussions

The chromatograms for free phenolic acids, alkaline hydrolyze and acid hydrolyze were obtained and compared with the chromatograms of standard phenolic acids for DAD-1, DAD-2, and ECD detectors. The qualitative determinations were made for the following compounds: gallic acid, protocatechuic acid, p-hydroxibenzoic acid (see figures 1 - 3).

Total phenolic acids were calculated by HPLC quantification, as results from alkaline and acid hydrolysed samples (table 1).

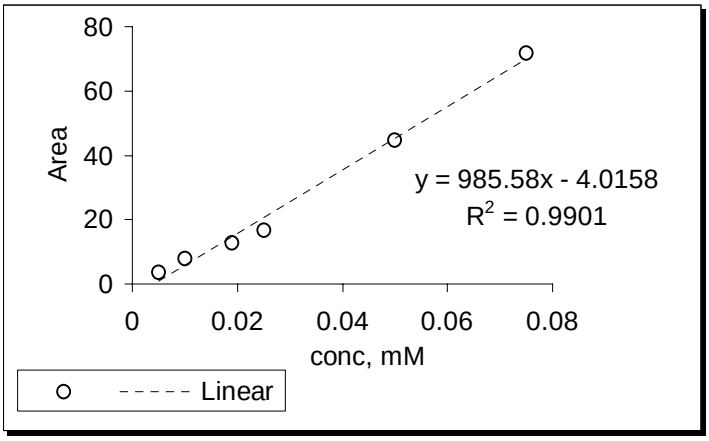

Fig. 1. The calibration curve for gallic acid.

Compounds such as *p*-coumaroylglucose, dihydroflavonol, quercetin 3-glucoside, 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide were detected in strawberries (Shiow 2001). In the present work, single phenolic acids from acid and alkaline hydrolysis, were identified. Their presence in fresh strawberries was validated and quantified using different phenolic acids as standard compounds.

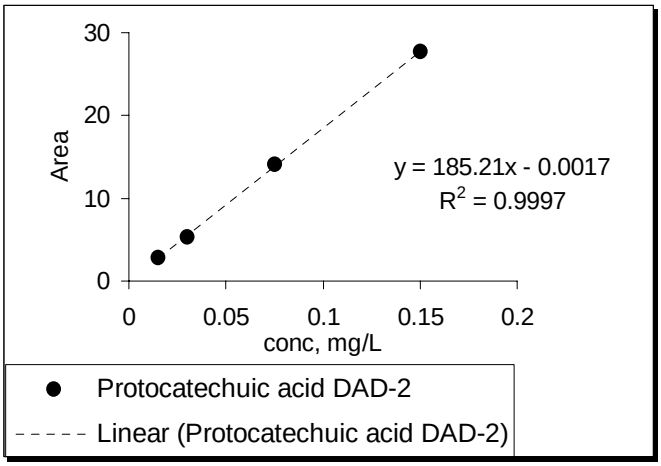

Fig. 2. The calibration curve for protocathecuic acid.

The occurrence of *p*-coumaroylglucose, dihydroflavonol, quercetin 3-glucoside, 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide has been detected in strawberries (Bakker, 1994, Gil, 1997). The quantitative determinations in the present work were made for gallic, p-hydroxybenzoic and protocathecuic acids. The results (µg/g strawberry) are shown in table 1.

Table 1. The quantity of phenolic acids from fresh strawberries

Phenolic acids	Free phenolic acids	Alkaline hydrolyse	Acid hydrolyse	Total hydrolyses	Total phenolic acids
Gallic acid	73.63	231.83	98.01	329.84	403.51
Protocatechuic acid	3.19	128.06	26.02	154.08	157.27
p-hydroxy benzoic acid	5.07	1046.14	576.86	1623.00	1628.07

The most important single group of phenolics in plants is the flavonoids, which consist mainly of catechins, proanthocyanidins, anthocyanidins and flavons, and flavonols and their glycosides. Although catechins seem to be widely distributed among plants, they are abundant only in tea leaves. Proanthocyanidins are polyflavonoid

in nature, consisting of chains of flavan-3-ol units. They are widely distributed in plants such as apple, grape, strawberry, and plum (Macheix, 1990). Proanthocyanidins have relatively high molecular weights and have the ability to complex strongly with carbohydrates and proteins.

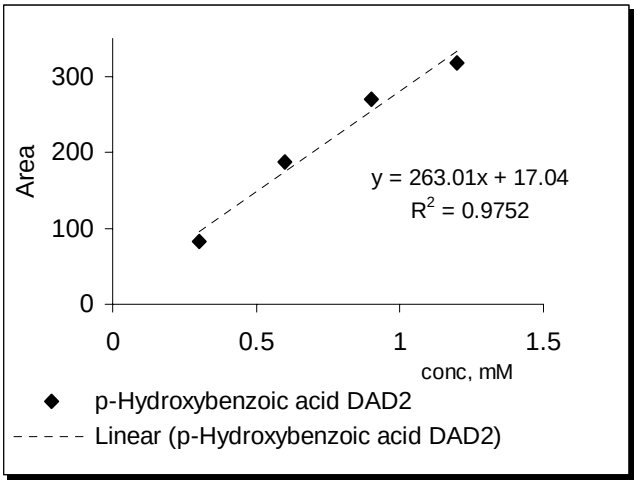

Fig. 3. The calibration curve for p-hydroxybenzoic acid**.**

Conclusions

As we see in the table 1, the free phenolic acids in fresh strawberries are in the low quantity. After alkaline hydrolysis the quantity of p-hydroxy benzoic acid is bigger than of the other two identified phenolic acids. The quantities of phenolic acids in acid hydrolysis are lower than in the alkaline hydrolysis. In our stomach occur some chemical reactions in the presence of HCl that allow the obtaining of free phenolic acids from strawberries. The results reported allow us to evaluate the composition of some polyphenolics compounds identified per kilogram of strawberries.

The main deliverables, which will be obtained, are:

- Optimization of some harvest and post-harvest conditions in order to improve the health promoting capacity of fresh fruits and

vegetables and to match specific requirements for industrial production.

- Establishment of the relationship between chemical and physical stability of food and their intrinsic health properties.
- To identify new strategies in order to obtain high quality of food products able to meet the consumers′ needs.

References

Bakker, J.; Bridle, P.; Bellworthy, S. J. (1994). Strawberry juice colour: a study of the quantitative and qualitative pigment composition of juices from 39 genotypes. *J. Sci. Food Agric*, 64, 31-37.

Cao, G.; Sofic, E.; Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.*, 44, 3426-3441.

Cioroi M, (2000). *The Identification and Estimation of the Volatiles Heterocyclic Compounds in the Glucose-Lysine Model System*, 19th European Colloquium on Heterocyclic Chemistry, Proceedings Volume p. 233, 19-22 July, Aveiro-Portugal.

Gil, M. I.; Holcroft, D. M.; Kader, A. A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem*., 45, 1662-1667.

Macheix, J. J.; Fleuriet, A.; Billot, J. (1990). *Fruit Phenolics*; CRC Press: Boca Raton, FL.

Mattila, P. (2002). *J. Agric. Food Chem.,* 50*, 3660-3667*.

NCSS 97.(1997). *Statistical System for Windows*; NCSS: Kaysville, UT.

Schiber, A., et al. (2001). *Journal of Chromatography* A, 910 265-273.

Shiow Y. Wang, Wei Zheng, (2001). Effect of Plant Growth Temperature on Antioxidant Capacity in Strawberry. *J. Agric. Food Chem.,* 49 (10), 4977 -4982.

Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.*, 46, 4113-4117.