Analysis of flavonols in the peels of vegetables by High Performane Liquid Chromatography.

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Abstract: Flavonoids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Quercetin, myricetin and kaempferol were used as standards. Eight vegetables were selected for the study. These vegetables were purchased from the local market in JAN 2009. Flavonols were analyzed using HPLC. Acidified 50% acetonitrile was used as mobile phase. %ages of flavonols were compared in different vegetables. Three different extraction solvents were used to extract flavonols. The purpose of this study was to analyze the better extraction condition for the utilization of vegetable peels as the source of flavonols. Then overall comparison between three type of extracted samples showed that MEOH: H_2O (80; 20) was best solvent for the extraction of flvonols from the peels of vegetables under study. [New York Science Journal. 2009;2(5):27-31]. (ISSN: 1554-0200).

Key Words: Flavonols, Vegetable peels, HPLC

INTRODUCTION:

Among dietry sources, fruits rich in flavonoids include apples (*Pyrus malus*), red fruits, and citrus fruits [1, 2]. In general the more colorful components of the food, like the skins of fruits contain the highest concentration of flavonoids. An exception to this rule, however, is the white pulpy inside of oranges [3]. Pome trees, apple, pear, and quince, are classified into the subfamily Pomoideae, belonging to the Rosaceae family. Quince (*Cydonia oblongo* Miller), followed by 'Red Delicious', peel extracts shows the highest phenolic content (160.33 and 110.90 mg/100 g of fresh weight). Red skin apple and quince peels are of great interest as important antioxidant and antimicrobial polyphenol sources [4]. Total amount of glycosilated flavonols was higher in the whole berries of red grapes where the most abundant phenolic compound was quercetin 3-O -glucoside [5]. Tomato plants can be engineered to produce isoflavones without comprising the levels of endogenous flavonols, which are also health-beneficial, but it may be necessary to enhance the expression levels of chalcone isomerase simultaneously to achieve significant yields in edible tissues such as fruit peels [6]. Flavonol O- and xanthone C-glycosides are extracted from mango (*Mangifera indica L.*) peels. Seven quercetin O-glycosides, one kaempferol O-glycoside, and four xanthone C-glycosides are found in mango. On the basis of their fragmentation pattern, the latter were identified as mangiferin and isomangiferin and their respective galloyl derivatives. A flavonol hexoside was identified as a rhamnetin glycoside.The results obtained in study confirm that peels originating from mango fruit processing are a promising source of phenolic compounds that might be recovered and used as natural antioxidants or functional food ingredients [7]. In the present study peels of eight commonly used vegetables were selected for their flavonol contents.

MATERIALS AND METHODS Chemicals

All reagents were of analytical grade and were used as received. Quercetin (3,3,4,5,7-tetrahydroxyflavonol), myricetin (3,3,45,5,7-hexahydroxyflavone), kaempferol were purchased from sigma Aldrich. Acetonitrile was from Merck.

Instrumentation

The HPLC system (Waters) consisted of a pump (1500 series), a UV detector (2487) was used in the study. Column was a C18, 250 x 4.6 mm, 5 mm particle sizes. Water was HPLC grade and acidified with 1 % acetic acid. Qualitative analysis was made with samples, in isocratic mode, with acetonotrile/water 1:1 at a flow- rate of 1 mL min-1. The injection volume was 10 *uL* and the elute was monitored at 254 nm. The filtered samples of vegetables were injected under these conditions, as well as a mixture of authentic standards of myricetin, quercetin and kaemferol was also injected.

Sample preparation

All vegetables were purchased from a local market. The vegetable and fruits were dried at room temperature and for analysis the weighed portions of the dried sample were homogenized into powder. Ultrasonic extraction was performed using a mixture of methanol and water. For the extraction 15 g of the ground vegetable was weighed and 30 mL of the extraction mixture was added. The sample was left at room temperature for 60 min and in an ultrasonic bath at room temperature for 20 min. The extract was filtered through a 0.45 μm filter and stored at + 4 °C in dark.

RESULTS:

Table: 1 Details of vegetables under study

Sr no	Name	Scientific name	Colour	Date of collection	Date of analysis
1	Ginger	*Zingiber offocinarum*	Yellow	8.1.09	9.1.09
2	Carrot	*Daucus carota*	Red	8.1.09	9.1.09
3	Garlic	*Allium sativum*	Whitish-yellow	5.1.09	6.1.09
4	Onion	*Allium cepa*	White/Pink	8.1.09	9.1.09
5	Pumpkin	*Cucurbita pepo*	Light green	5.1.09	6.1.09
6	Turnip	*Brassica napus*	White/Purple	5.1.09	6.1.09
7	Potato	*Solanum tuberosum*	Brown	2.1.09	3.109
8	Tomato	*Lycopersicum esculentum*	Red	2.1.09	3.109

Table 2: Detail of the codes of fruits and vegetables

Sr no	Name of vegetable/fruit	Codes50%	80%	90%
1	Ginger Peel	G.P.01	G.P.02	G.P.O3
2	Carrot Peel	C.P.01	C.P.02	C.P.03
3	Garlic Peel	Ga.P.01	Ga.P.02	Ga.P.03
4	Onion Peel	O.P.01	O.P.02	O.P.03
5	Pumpkin Peel	Pu.P.01	Pu.P.02	Pu.P.03
6	Turnip Peel	T.P.01	T.P.02	T.P.03
7	Potato Peel	Po.P.01	Po.P.02	Po.P.03
8	Tomato Peel	To.P.01	To.P.02	To.P.03

Extraction solvent: MEOH**: H_2O 50:50**

Table: 3 Qualitative and quantitative analysis of flavonols in peels of vegetables

Sr.no	Vegetable	Flavonol aglycons Mg/kg			Total mg/kg
		Quercetin	Myricetin	Kaemfherol	
1	G.P.01	0.97±0.3	2.04±0.3	0.35±0.3	3.36±0.3
2	C.P.01	0.31±0.3	3.02±0.2	0.13±0.3	3.46±0.2
3	Ga.P.01	1.16±0.4	8.4±0.3	1.11±0.2	10.67±0.3
4	O.P.01	0.53±0.2	7.24±0.4	0.075±0.4	7.845±0.2
5	Pu.P.01	2.22±0.2	3.55±0.3	0.311±0.4	6.081±0.4
6	T.P.01	0.97±0.4	0.88±0.2	0.311±0.2	2.161±0.4
7	Po.P.01	0.04±.03	1.95±0.2	0.088±0.3	2.078±0.4
8	To.P.01	0.14±.02	0.53±0.3	0.88±0.3	1.55±0.3

 Each reading is the mean of three HPLC readings. *****
Sum of three flavonols.

Extraction Solvent: MEOH: H_2O 90:10

Table:4 Qualitative and quantitative analysis of flavonols in peels of vegetables

Sr.no	Vegetable	Flavonol aglycons mg/kg			Total mg/kg
		Quercetin	Myricetin	Kempherol	
1	G.P.O3	1.53±0.2	3.46±0.2	0.75±0.3	5.74±0.3
2	C.P.03	1.13±0.3	7.33±0.3	0.66±0.2	9.12±0.2
3	Ga.P.03	1.68±0.3	3.95±0.3	1.733±0.3	7.363±0.2
4	O.P.03	14.2±0.2	41.3±0.4	1.86±0.4	57.36±0.3
5	Pu.P.03	2.25±0.4	38.2±0.4	0.44±0.4	40.89±0.4
6	T.P.03	0.66±0.3	21.89±0.3	0.17±0.3	22.72±0.4
7	Po.P.03	0.39±0.3	0.66±0.3	0.04±0.2	1.09±0.2
8	To.P.03	0.84±0.4	0.84±0.2	0.62±0.2	2.3±0.3

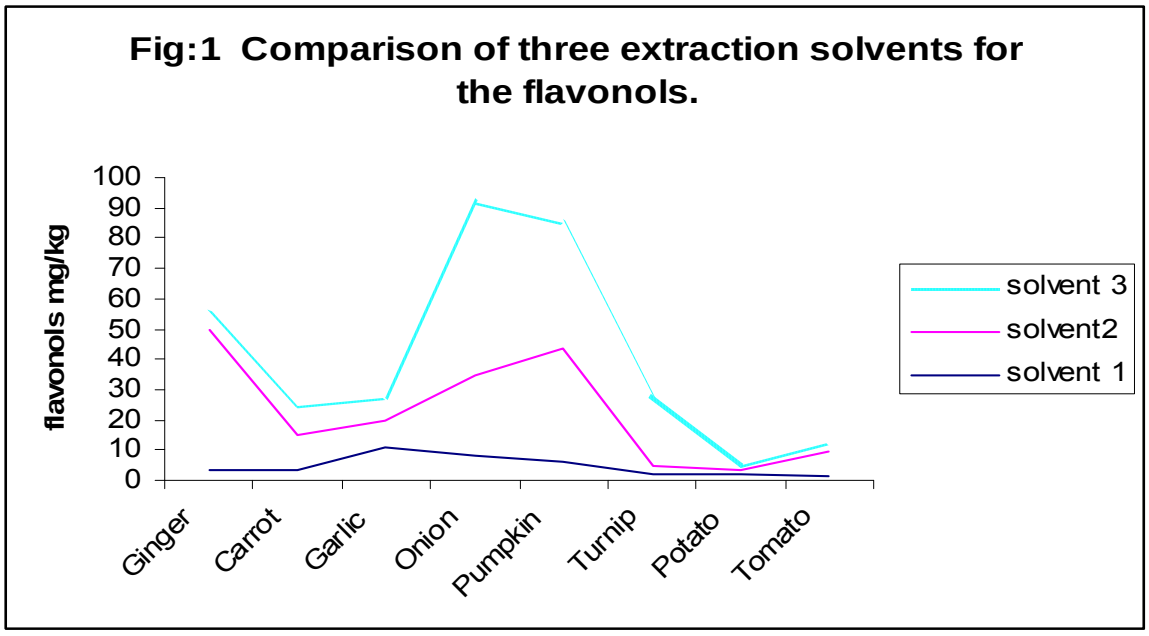
 Each reading is the mean of three HPLC readings.
* Sum of three flavonols.

Extraction Solvent: MEOH: H_2O 80:20

Table: 5 Qualitative and quantitative analysis of flavonols in peels of vegetables

Sr.no	Vegetable	Flavonol aglycons mg/k			Total mg/kg
		Quercetin	Myricetin	Kempherol	
1	G.P.02	1.9±0.3	42.6±0.3	1.68±0.3	46.18±0.2
2	C.P.02	1.13±0.4	9.55±0.2	1.06±0.2	11.74±0.3
3	Ga.P.02	3.9±0.3	4.75±0.3	0.62±0.4	9.27±0.2
4	O.P.02	3.63±0.4	22.2±0.4	0.97±.03	26.8±0.3
5	Pu.P.02	3.99±0.2	33.77±0.4	37.76±0.4	75.52± 0.2
6	T.P.02	1.95±0.2	0.88±0.2	0.11±0.3	2.94±0.3
7	Po.P.02	0.17±0.2	1.28±0.3	0.088±0.2	1.538±0.2
8	To.P.02	3.11±0.3	1.955±0.3	3.11±0.3	8.175±0.3

 Each reading is the mean of three HPLC readings. *
Sum of three flavonols.



Discussion:

In the present study three extraction solvents were used to study the extraction and analysis of flavonols in the vegetable peels. Eight vegetables were used for analysis. Their details are given. **[Table: 1]**. Analysis of flavonols in solvent system 1(50:50 MEOH: H_2O) in peels of vegetables showed that garlic peels were richest in flavonols and contained flavonols (10.67 mg/kg) greater than any other vegetable peels. Minimum amount was present in tomato peels (1.32 mg/kg). Onion and pumpkin peels also contained reasonable amounts of flavonols (7.845 mg/kg and 6.081 mg/kg respectively). **[Table: 3]**

Analysis of flavonols in vegetable peels in this extraction system 2(90:10 MEOH: H_2O) showed that ginger peels were richest in flavonols and contained flavonols (46.18 mg/kg) greater than any other vegetable peels. Pumpkin and onion peels also contained a reasonable amount of flavonols (37.76 mg/kg and 26.8 mg/kg respectively). The lowest amount of flavonols was present in potato peels (1.538 mg/kg) **[Table: 4]**. Analysis of flavonols in vegetable peels, during this method of extraction (80:20 MEOH: H_2O),

showed that onion peels were richer in flavonols (57.36 mg/kg) than any other vegetable peels. Minimum amount was present in potato peels (1.09 mg/kg). Pumpkin also contained great amount of flavonols (40.89 mg/kg) **[Table: 5].** It was observed that solvent system 3(80:20 MEOH: H_2O) was best for the extraction of flavonols from the most vegetables because these give high amount of flavonols in this system **[FIG: 1]**

CONCLUSION:

It is concluded from the study that a good amount of flavonols was present in peels of vegetables, so they could be used as a source of flavonols.

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REFFERENCES:

1. **Arts I C.W, van de putte B, Hollman P.C.H**. Catehin contents of foods commonly consumed in Netherlands. 2. Tea, wine, fruit juices, and chocolate mil. J Agric Food Chem 2000;48:1736-1751.
2. **Gil MI, Ferreres F, Tomas-Barberan FA**. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. J Agric Food Chem 1999; 47(6):2213-7.
3. **Divi RL, Chang HC, Deorge DR**, Anti thyroid isoflavonoes from soybean: isolation, characterization, and mechanisms of action. Biochem Pharmacol 1997;54(10):1087-1096.
4. **Fattouch S, Caboni P, Coroneo V, Tuberoso C, Angioni A, Dessi S, Marzouki N, Cabras P**. Comparative analysis of polyphenolic profiles and antioxidant and antimicrobial activities of tunisian pome fruit pulp and peel aqueous acetone extracts. J Agric Food Chem. 2008; 56(3):1084-90.
5. **Nicoletti I, Bello C, De Rossi A, Corradini D** Identification and quantification of phenolic compounds in grapes by HPLC-PDA-ESI-MS on a semimicro separation scale. Nicoletti I, Bello C, De Rossi A, Corradini D J Agric Food Chem. 2008 Oct 8;56(19):8801-8.
6. **Shih CH, Chen Y, Wang M, Chu IK, Lo C**. Accumulation of isoflavone genistin in transgenic tomato plants overexpressing a soybean isoflavone synthase gene. J Agric Food Chem. 2008 56(14):5655-61.
7. **Schieber A, Berardini N, Carle R**. Identification of flavonol and xanthone glycosides from mango (Mangifera indica L. Cv. "Tommy Atkins") peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Agric Food Chem. 2003 51(17):5006-11.

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