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Food Chemistry 100 (2007) 699–704
Food Chemistry
www.elsevier.com/locate/foodchem

Quercetin content in some food and herbal samples

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Received 5 August 2005; received in revised form 27 October 2005; accepted 27 October 2005

Abstract

Quercetin is a typical flavonoid ubiquitously present in vegetables and fruits, and its antioxidant effect is implied to be helpful for human health. The efficiency of extraction process and acidic hydrolysis parameters for HPLC analysis of quercetin present in glycosides and aglycone forms was investigated. Hydrolysis for 5 min in the presence of 2.8 M HCl as well as for 10 min with 1.1 M HCl efficiently released quercetin from rutin. The method developed in this study was applied for quantitative determination of quercetin in some food (onion, apple) and herbal (*Hypericum perforatum* and *Sambucus nigra*) products.

Keywords: Flavonoids; Quercetin; Hydrolysis; HPLC

1. Introduction

Flavonoids, and particularly quercetin derivatives, have received special attention as dietary constituents during the last few years. The epidemiological studies point out to their possible role in preventing cardiovascular diseases and cancer (Chu, Chang, & Hsu, 2000; Hertog, Hollman, & Katan, 1992a; Hertog, Feskens, Hollmann, Katan, & Kronthout, 1993). This health-promoting activity seems to be related to the antioxidant (free-radical scavenging) activity to flavonoids (Murota & Terao, 2003). Flavonoids are widely distributed in plants and are categorized as flavonol, flavanol, flavanone, flavone, anthocyanidin and isoflavone.

Quercetin, 3,3′,4′,5,7-pentahydroxylflavone, is one of the most abundant flavonoids present in fruits and vegetables. In plants, it occurs mainly in leaves and in the outher parts of the plants as aglycones and glycosides, in which one or more sugar groups is bound to phenolic groups by glycosidic bond. Glucose is the most common sugar, with galactose and rhamnose also frequently found in composition with flavonoids. In general, quercetin glycosides contain a sugar group at the 3-position. A considerable amount of isoquercetin (quercetin-3-*O*-β-glucoside) has been found in apple and pear peels (Schieber, Hilt, Conrad, Beifuss, & Carle, 2002; Spanos & Wrolatad, 1992) as well as in *Hypericum perforatum* leaves or flowers (Urbánek, Blechtová, Pospišilová, & Polášek, 2002). Almost 180 different glycosides of quercetin have been described in nature, with rutin (quercetin-3-*O*-rutinoside) being one of the most common (Hollman & Arts, 2000). In onion, however, phenolic group at the 4′-position is necessarily bound by a sugar group and thus its major glycosides are quercetin 4′-*O*-β-glucoside and quercetin 3,4′-*O*-β-diglucoside (Murota & Terao, 2003). They together account for about 80% of the total content of flavonoids (Bonaccorsi, Caristi, Gargiulli, & Leuzzi, 2005). Vegetables, fruits and beverages are the main dietary sources of quercetin. Onion (*Allium cepa* L.) ranked highest in quercetin content in a survey of 28 vegetables and nine fruits (Hertog et al., 1992a). Amounts of this flavonoid in onions vary with bulb color and type, being distributed mostly in the outer skins and rings (Bonaccorsi et al., 2005; Crozier, Lean, McDonald, & Black, 1997).

Determination of individual flavonoid glycosides in plant materials is difficult, due to their large number. In many cases, for example for the development of useful database of monomeric flavonoid values, the knowledge of the total aglycone content for each flavonoid is required.

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0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.10.028

For the determination of flavonol aglycones, a hydrolysis procedure is required to break the glycosidic bonds. The extraction recovery is dependent on molarity of HCl, hydrolysis time and temperature and the composition of the extraction solvent. Flavonoids are commonly extracted from plant materials with pure methanol, ethanol or their combination with water (Hertog et al., 1992a; Molnár-Perl & Füzfai, 2005; Robards, 2003; Naczk & Shahidi, 2004), but in some cases ethyl acetate (Spanos, Wrolstand, & Heatherbell, 1990) or acetone (Schieber et al., 2002) have been used. In most publications the hydrolysis of flavonoid glycosides from vegetables and fruits is carried out in 1.2 M HCl at 90 °C for 2 h according to a procedure presented by Hertog, Hollman, and Venema (1992b).

The aim of this work was to examine the extraction and hydrolysis parameters for determination of quercetin present as glycosides and aglycone forms. The efficiency of these processes was checked out by quantification of quercetin and rutin concentrations. Onion, apple peel, *H. perforatum* and *Sambucus nigra* were chosen as the examples of vegetables, fruits and herbal plants.

2. Experimental

2.1. Apparatus

The separations were carried out with an HPLC system from Merck, which consisted of a gradient pump (type L-7100), an autosampler (L-7250) with a 10-μL loop, and DAD (L-7450) detector. The data was collected and evaluated by the Merck chromatographic software D-7000. The analytical column was a Luna C-18(2) 25 cm × 4.1 mm I.D (Phenomenex, Torrance, CA, US). The mobile phase consisted of 25 mM phosphate buffer at pH 2.5 and methanol. The chromatograph was operated in the gradient mode starting at a mobile phase of methanol-buffer (22:78, v/v), changing within 33 min to 100% methanol. Eluent was delivered at a flow rate of 1 mL/min. Identification was based on retention times and UV–VIS spectra by comparison with commercial standards.

2.2. Reagents

The standards were purchased from Sigma–Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany). Solvent and hydrochloric acid were obtained from Merck (Darmstadt, Germany).

The food samples (white onion and Jonagold apple) were obtained from a local market. *H. perforatum* and *S. nigra* were purchased from Herbapol (Lublin, Poland) and their extracts were prepared from dried leaves or dried flowering tops.

2.3. Extraction and hydrolysis of plant material

Air-dried plant (0.02–0.05 g) was extracted with 1–2 mL of appropriate solvent and heated in a water bath for 30 min. Two 200 μL samples of plant extract were dried in a dessicator under vacuum. To the first sample 200 μL of water/methanol (60:40, v/v) was added, filtered and it was immediately injected to HPLC system. The second sample was dissolved in 2.8 M HCl–methanol (60:40, v/v) mixture and heated in water bath for the next 20 min, dried in a dessicator, dissolved in water/methanol (60:40, v/v), filtered and injected to HPLC.

3. Results and discussion

Determination of compounds in solid foods requires extraction from the sample matrix prior to injection into the HPLC system. Several solvents were investigated, including water, methanol, ethanol, ethyl acetate and dimethylformamide (DMF) to check their efficiency for extraction of quercetin and its glycoside (rutin) from onion skin, apple peel and *H. perforatum* leaves. The content of these compounds (expressed in mg per gram of the dry plant material analysed) is presented in Fig. 1. Each data point represents the average of duplicate analyses. The extract composition was the same in each case, but the flavonoids yields were different. The highest efficiency from

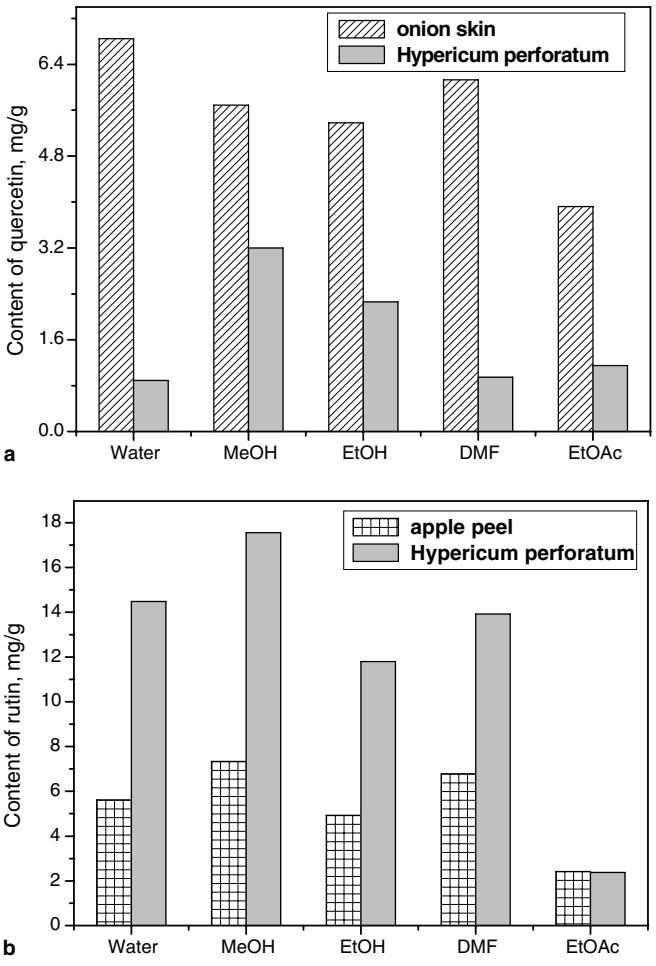

Fig. 1. The efficiency of extraction of: (a) quercetin and (b) rutin using different solutions.

onion samples was obtained when water and DMF, the most polar solvents, were used for extraction of quercetin in its aglycone form (Fig. 1(a)). At the subcellular level, phenolic compounds are located mainly in the vacuoles. Their occurrence in soluble or suspended forms, and in combination with cell wall components, may have a significant impact on their extraction (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). Extraction of quercetin as well as rutin from *H. perforatum* with methanol gave the highest efficiency, that confirmed earlier results (Brolis et al., 1998; Urbánek et al., 2002). With dried plant materials, low polarity solvents such as ethyl acetate, simply leach the sample whereas alcoholic solvents or water presumably rupture cell membranes and enhance the extraction of endocellular materials (Robards, 2003). Extraction from apple peel using methanol and DMF gave also maximum rutin yields. Because methanol is more agreeable as a component of mobile phase in HPLC analysis, we continued our experiments with methanol as an extraction solvent checking its concentration.

Adequate extraction of quercetin was achieved with 40% (v/v) methanol for onion and 50–60% for *H. perforatum* leaves (Fig. 2(a)). When the percentage of methanol was reduced from 80%, rutin yield in apple decreased (Fig. 2(b)). In contrast, the efficiency of extraction from the herb sample was similar different solvents (40–100%). This difference may be explained by the action of polyphenol oxidase. Polyphenol oxidase is an enzyme, widely distributed in plants, which catalyzes the oxidation and polymerization of flavonoids when the cells are ruptured (Pinelo, Manzocco, Nuňez, & Nicoli, 2004). Methanol reduces activity of this enzyme. It is therefore possible that extraction with low methanol content does not completely inactive polyphenol oxidase in fruit sample.

The effect of hydrolysis conditions was examined for pure rutin. Hydrochloric acid was chosen for this process as it yielded a higher efficiency compared to H_2SO_4 (Hertog et al., 1992b). After addition of different concentration of HCl in 40% methanol, the samples were refluxed at 90 °C for a appropriate time (5–20 min) The amount of quercetin increased with time due to release from its glycosides (Fig. 3). Hydrolysis for 5 min in the presence of 2.8 M HCl as well as for 10 min with 1.1 M HCl efficiently released quercetin from rutin. For most flavonoid glycosides, the 2 h refluxing at 80 °C with 1.2 M HCl and no antioxidant addition was proposed. However, the extended exposure time to HCl could cause degradation of quercetin (Hertog et al., 1992b; Nuutila, Kammiovirta, & Oksman-Caldentey, 2002). Generally for the hydrolysis process, optimum compromise is to achieve complete release of aglycones and to minimize degradation reactions of compounds involved. For this purpose, a central composite experimental design was described (Careri, Elviri, Mangia, & Musci, 2000). Applying a multiple regression analysis on the data set, it was possible to obtain a mathematical model that took linear, quadratic and cross-product terms into account. According to this mathematical approach optimum

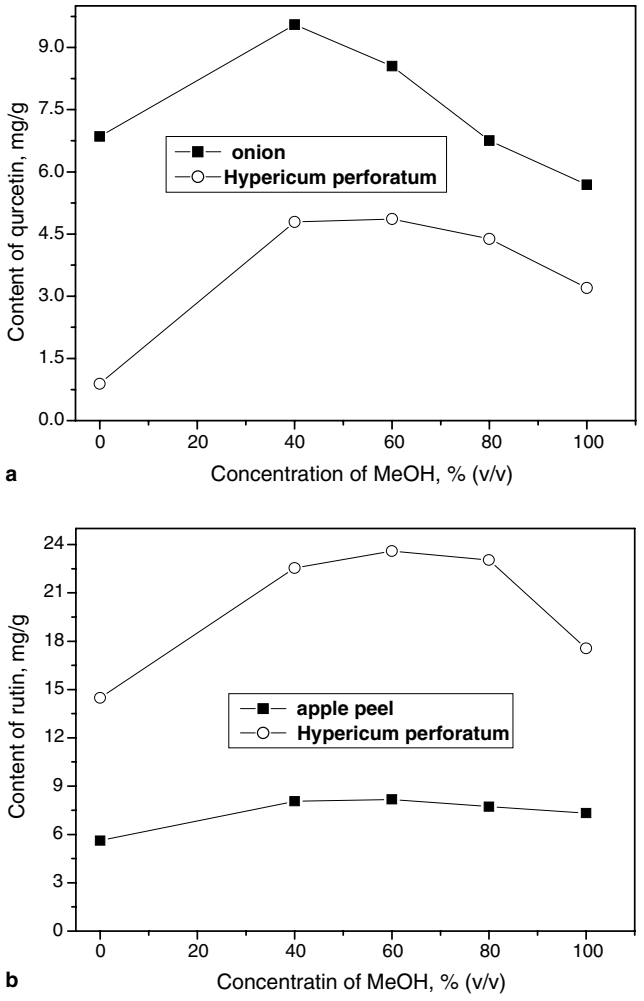

Fig. 2. The effect of methanol concentration on the extraction yield of quercetin (a) and rutin (b).

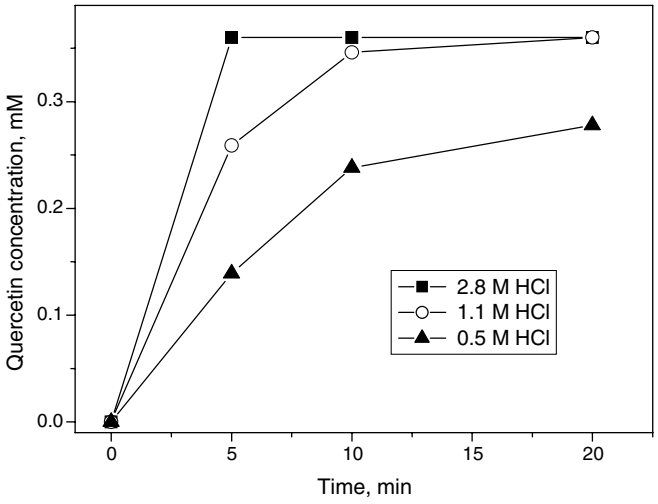

Fig. 3. Hydrolysis of rutin as a function of time and acid concentration.

conditions for rutin hydrolysis in orange juice corresponded to HCl concentration of 1.5 M and a hydrolysis time of 1 h. However, identical optimum hydrolysis conditions for

flavonol glucosides in different vegetables or fruits could not be achieved as they are dependent on the binding site of the sugar on the flavonoid nucleus (Hertog et al., 1992b). The study presented by Lombard (2005) indicated that the main quercetin glucosides present in onion were relatively heat stable, thus, higher temperature and shorter time for their hydrolysis could be employed.

Fig. 4 presents the chromatograms of extracts obtained with and without acidic hydrolysis of apple peel and *H. perforatum* leaves. Identification of compounds was based on retention times and their comparison with standards as well as UV–VIS spectra. The extracts without hydrolysis contained only traces of free flavonoid (Fig. 4(a)). Comparing the profile of the obtained chromatograms allowed quantification of quercetin in the hydrolyzed samples.

Quantitative estimates of quercetin as aglycone in different part of white onion, apple peel and some herbal samples are presented in Table 1. The content of quercetin after hydrolysis in onion bulbs was consistently high (2604 mg/kg of dry weight) and comparably with earlier findings (Crozier et al., 1997; Hertog et al., 1992b; Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005). Differences found may be due to varietal or seasonal differences. The leaves of spring onion contained much lower levels of quercetin than the edible parts. We found that dry outer skin of onion also had a significant amount of quercetin, even higher than that in the leaves of spring onion. Suh, Lee, Cho, Kim, and Chung (1999) reported that onion skin extract exhibits superoxide anion and hydroxyl radical scavenging activity. Regardless of quercetin content, a large amount of onion skins remains unused after onion processing. This vegetable is commonly consumed alone or in prepared foods after being subjected to a wide variety of heat-treatment processing methods. Boiling resulted in a fall in quercetin content, while losses occurring during frying were less substantial (Crozier et al., 1997; Lombard et al., 2005). This could be due to more effective extraction of quercetin glycosides by hot water than with hot sunflower oil (Fig. 1(a)). In contrast to the findings of Bilyk, Cooper, and Sapers (1984), kaempferol was not detected in any of the onion extracts that were analyzed.

Quercetin in apple skin is mostly bounded with sugar moiety as much higher its amounts of it was found after acid hydrolysis (Table 1). Apple is one of the main sources for flavonoid intake in the European diet. Its attractiveness to consumers is determined by appearance and by internal attributes of firmness, taste and health benefits. Our results

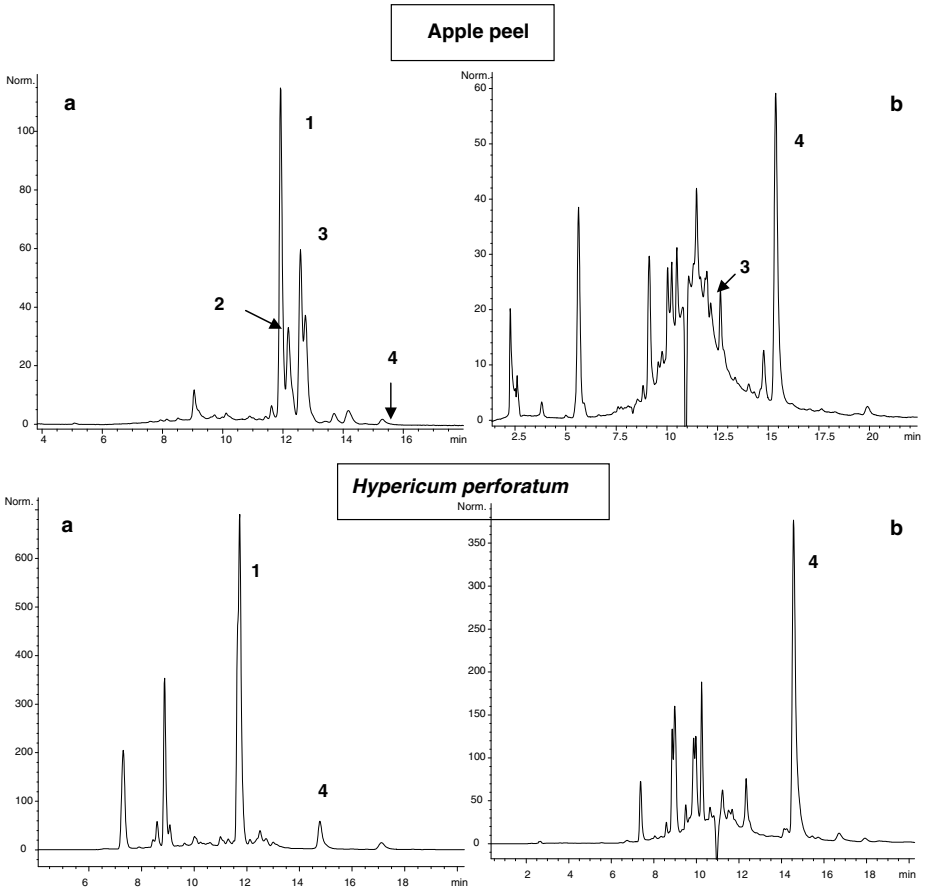

Fig. 4. Chromatograms of: (a) aqueous extracts of investigated samples and (b) after acid hydrolysis. Peaks: 1 quercetin-3-*O*-rutinoside, 2 quercetin-3-*O*-rhamnoside, 3 quercetin-3-*O*-galactoside, 4 quercetin.

Table 1
Content of quercetin (mg/kg dry weight) in some food and herbal samples before and after hydrolysis

Sample	Before hydrolysis	After hydrolysis
White onion bulls	2604 ± 10	50 ± 9
Spring onion leaves	450 ± 7	841 ± 8
Dry outher skin	960 ± 9	1530 ± 5
Apple peel	21 ± 2	250 ± 4
Hypericum perforatum leaves	1.8 ± 0.5	13.3 ± 1.2
Sambucus nigra flowering tops	0.4 ± 0.05	7.9 ± 0.7

confirmed earlier report that quercetin in apples as glycoside or aglycone is exclusively found in the skin (Awad, de Jager, & van Westing, 2000). Since the skin is such an important source of quercetin, any promotion of apple consumption should imply the skin.

H. perforatum (St. John's Wort) and *S. nigra* (elderberry) have commonly been used as herbal treatment for various health problems. *H. perforatum* is considered to be an effective alternative to synthetic treatment of mild-to-moderate depression and a series of bioactive compounds, including flavonols, has been detected in the crude material (Butterweck, 2003). Our study showed that its leaves contained a significant amount of quercetin, mainly present as glycosides (Table 1). *S. nigra*, in addition to high concentrations of anthocyanins, also contains a complex mixture of other flavonoids and has a rich history in folk medicine; from cold and flu remedies to diuretic and anti-rheumatic activities (Wightman, 2004). The content of free quercetin in this herbs after hydrolysis is lower than in St. John's Wort and equals to 7.9 mg/kg of dry weight. The aglycone form of quercetin account for only about 5% of the total flavonoid content.

Several reports have suggested the beneficial nutritional an physiological effect of flavonoids, thus interest in vegetables, fruits and some herbs as important sources of bioactive plant phenolic compounds, has increased. Although a dietary guideline for consumption of flavonoids has not yet been reported, there are many studies on the average intake of flavonoids (Aherne & O'Brien, 2002; Hollman, Hertog, & Katan, 1996; Karakaya & EL, 1999). Hollman et al. (1996) reported that the average intake of these compounds was 23 mg/day, of which quercetin contributed 16 mg/day. The major sources of flavonoids varied substantially between countries. In Japan green tea is a major source, while red wine in Italy accounts as a main source of flavonoids. In parts of Greece and the Middle East, where traditional diets are consumed, edible wild greens and fermented foods are the main staple diets. Onions and apples are the predominant sources of flavonoids in the United States, Finland, Greece and the former Yugoslavia, however, there may exist varietal differences in the composition, concentration and beneficial activities of flavonoids. Dietary flavonoid glucosides may be hydrolyzed in the oral cavity by saliva to deliver the biologically active aglycones (Walle, Browning, Steed, Reed, & Walle, 2005).

Acknowledgements

This work was supported by Warsaw University Grant BW 167/1/04.

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