ORIGINAL ARTICLE

ASSESSMENT OF FLAVONOIDS CONTENT IN CITRUS JUICES USING A LC/MS METHOD

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Abstract

Functional foods like citrus fruits contain a range of nutrients and phytochemicals like flavonoids that are important for human health. Using a LC/MS method, this study investigates the concentration of naringin and naringenin in 36 citrus juices (orange, tangerine, pomelo, white, pink, and red grapefruit) that are commercially available or freshly prepared in the laboratory. Among the freshly prepared grapefruit juices, the red grapefruit juice prepared from pulp and albedo exhibited the highest concentration of naringin (332.02 mg/L). In commercially available juices, the average naringin content was 89.23 ± 107.80 mg/L. In conclusion, both citrus juices prepared in the laboratory and commercially available present a great variability of naringin content.

Rezumat

Alimentele funcționale, precum citricele, conțin o serie de nutrienți și compuși fitochimici precum flavonoidele care sunt importante pentru menținerea stării de sănătate. Acest studiu și-a propus determinarea prin LC/MS a concentrației naringinei și naringeninei din 36 sucuri de citrice (portocale, mandarine, pomelo, grapefruit alb, roz și roșu) care au fost preparate în laborator sau se găsesc în comerț. Sucul de grapefruit roșu preparat în laborator din pulpă și albena prezintă cea mai mare concentrație de naringină dintre sucurile de grapefruit preparate în laborator (332,02 mg/L). În probele achiziționate din rețeaua comercială conținutul de naringină a fost în medie de 89,23 ± 107,80 mg/L. În concluzie, sucurile de citrice proaspăt preparate în laborator sau achiziționate din rețeaua comercială prezintă o mare variabilitate a conținutului de naringină.

Keywords: flavonoids, grapefruit juice, naringin, LC/MS

Introduction

Functional foods like citrus fruits contain a range of nutrients and phytochemicals like furanocoumarins and flavonoids that are important for human health [12]. Naringin and its aglycone, naringenin which are the most common flavonoids in the grapefruit juice, that for a long time have been recognized to possess anti-inflammatory, anti-oxidative, anti-atherosclerotic and anti-tumoural activity [3, 9, 11, 16].

The interaction between grapefruit juice and certain calcium channel blockers (felodipine and nifedipine) was highlighted in 1991 [2]. As compared to furanocoumarins that inhibit CYP3A4 and P-glycoprotein with consecutive increase of drug bioavailability, flavonoids like naringin inhibit intestinal organic anion transporting polypeptides (OATPs) which are uptake transporters that facilitate drug absorption [1, 4, 16]. As shown in the case of the antihypertensive agent aliskiren, the decrease of the concentration of the drug due to this pharmacokinetic interaction is associated with potential therapeutic failure [5, 14].

The purpose of this paper was to determine the concentrations of naringin and naringenin in commercial citrus juice products available internationally and fresh squeezed citrus juices using a LC/MS method.

Materials and Methods

Sample preparation. The samples that were subjected to analysis consisted in industrially prepared (commercially available) grapefruit (white, pink and red), orange, and tangerine juices or fresh squeezed juices prepared in the laboratory (using a juice extractor). The citrus juices prepared in the laboratory were available in three variants: juice from pulp (P), pulp and albedo (PA) and whole fruit (WF). The industrial prepared juices were purchased from the supermarkets in Iași, Romania. The juices were diluted 1/100 with purified water and centrifuged for 3 minutes at 12,000 rpm; 2 μL of supernatant were injected into the chromatographic system. The results obtained for each sample are presented as the average of three determinations.

Reagents. Standards for naringin (4,5,7-trihydroxy-flavanone-7-b-L-rhamnoglucoside-(1,2)-a-D-gluco-pyranoside) 96.6% and naringenin (4,5,7-tri-hydroxyflavanone) 99%, were purchased from Extrasynthese, Genay, France. All other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany); throughout the experiment, Milli-Qultrapure water was used.

Apparatus. The LC/MS analysis was performed on an Agilent chromatographic system 1100 series (Agilent Technologies) equipped with a G1379A degasser, a G1312A binary pump, a G1329A autosampler, and a G1316A column thermostat. The LC was coupled with an Agilent Ion Trap 1100 SL mass detector.

Chromatographic conditions. The separation was performed using a reverse phase column Kinetex C18 (50 x 2.1 mm i.d., 2.6 μm). The mobile phase was a mixture of acetonitrile: 2 mM ammonium acetate in the ratio 30:70 for naringenin and 25:75 respectively for naringin. The flow rate of 0.3 mL/min, at a temperature of 40ºC was used and the injection volume was 2 μL. An in-line filter of 0.5 μm was used.

MS conditions. The MS was equipped with Turbo-Ionspray (electrospray ionization (ESI)) interface, negative ion mode. ESI settings were: negative ionisation, ion source temperature 350ºC, gas: nitrogen, flow rate 10 L/min, nebuliser: nitrogen at 40 psi pressure, capillary voltage 3000 V. Full scan mass spectra were measured at *m/z* 579.1 for naringin and MS/MS *m/z* 271 > *m/z* 151.7 for narigenin.

The mass spectrum of a standard solution of naringin and chromatogram of a standard solution of naringin (analyte retention time: 1.25 minutes) are shown in Figure 1 and respectively in Figure 2. As one cannot find a true blank matrix (e.g. grapefruit juice naringin free) to be used for preparation of calibration curves, those were prepared in distilled water. Naringin calibration curve was performed in the range of 63 to 6300 ng/mL (Figure 3). For the construction of the calibration curves, the quadratic model was used ($y = ax^2 + bx + c$).

Matrix effect analysis. The matrix effect on the ionization yield of the analyte was carried out by adding a certain amount of naringin (final concentration three times lower quantification limit, 200 mg/mL) to matrix (grapefruit juice). The naringin content was then quantified in both matrix and matrix enhanced with analyte, and the difference in content was expressed as percent from the amount added. A difference no more than ± 15%, is indicating a non-significant matrix effect.

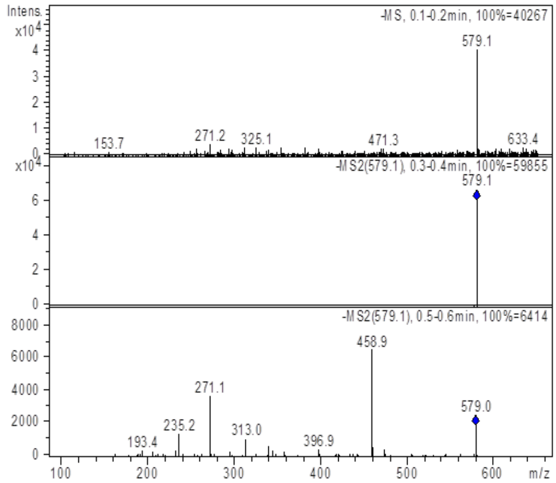

Figure 1.
Naringin mass spectra obtained through electrospray ionization, negative mode: up - full-scan spectrum; middle - isolation; down – fragmentation

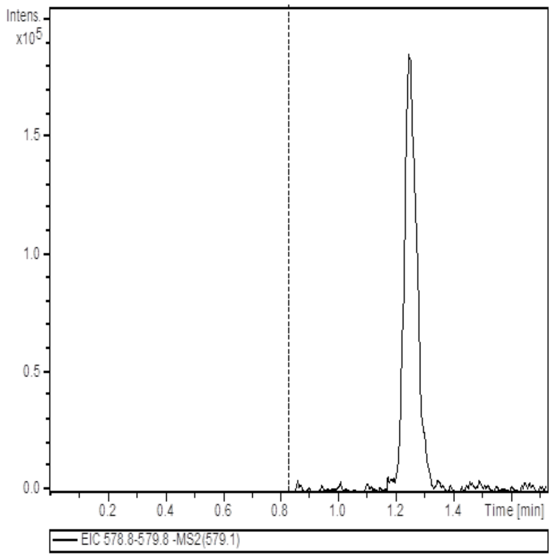

Figure 2.
The chromatogram of a standard solution of naringin

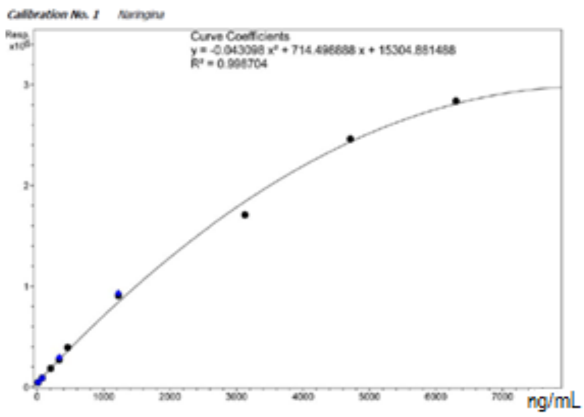

Figure 3.
Naringin calibration curve

Results and Discussion

Naringenin was not identified in any of the analysed samples. This can be explained by the fact that naringenin (the aglycone found in the structure of naringin) is not found in this chemical form in fruits. Flavanoids (naringin being the most abundant) are found in citrus juices in the form of glycosides and after ingestion are transformed into aglycones and sugar by the intestinal flora [2].

No significant matrix effect on the ionization yield of naringin was observed in three different matrix tested.

The chromatogram of naringin from sample 1 (fresh red grapefruit juice from pulp) is shown in Figure 4. The naringin concentrations determined in juice samples prepared in the laboratory are presented in Figure 5 and those determined in commercially available juice samples are shown in Figure 6.

From the analysis of the grapefruit juices which were prepared in the laboratory, higher values of naringin were found in the juice prepared from red grapefruit as compared to the juice prepared from pink or white grapefruit.

Naringin content was higher in the PA red grapefruit juice (332.02 mg/L) than in WF (292.24 mg/L) juice, the lowest level of flavonoid being determined in P juice (225.32 mg/L).

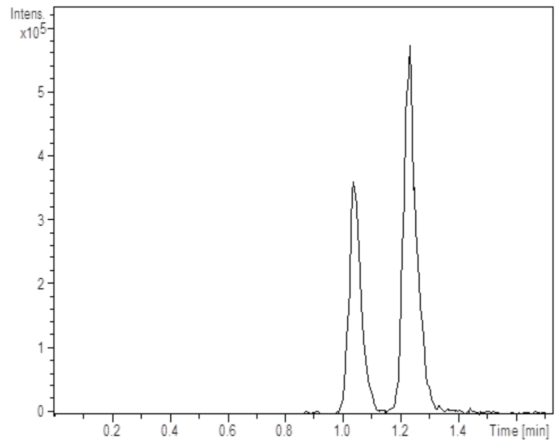

Figure 4.
The chromatogram of naringin (sample 1)

The orange juices presented much lower concentrations of naringin ranging from 67.41 mg/L in P juice to 94.12 mg/L in PA juice. With respect to the tangerine juice, the concentrations of naringin varied between 81.65 mg/L and 116.23 mg/L.

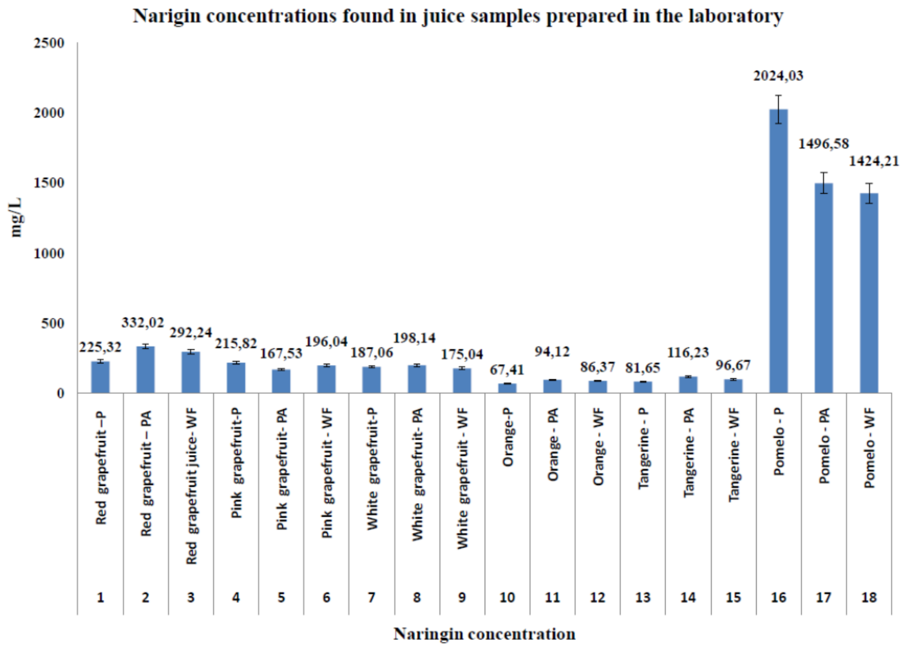

Figure 5.
Naringin concentrations found in juice samples prepared in the laboratory (average concentration of 3 determinations)

For pomelo, the naringin concentrations were significantly elevated as compared to the grapefruit, orange and tangerine juice; thus, the P pomelo juice contained the highest concentration of naringin of all types of juice (2024.03 mg/L).

As previously described, the amounts of bioactive compounds in citrus juices depend on variety of the fruit (white, pink, red), geographical region, climate, soil conditions, harvest date, storage, low-dose irradiation, and different method of preparing juices (pulp, pulp and albedo, whole fruit) [7].

Regarding the naringin content determined in the commercially available grapefruit juice, the variability of the concentrations was much higher, depending on the proportion of juice used in preparation. While, in some samples (samples 11 and 13) the concentration of naringin was below the method's detection limit, in others (sample 9), the average naringin content was 420.96 mg/mL (Figure 6).

Our results are consistent with the ones provided by literature data [5-7, 15, 19, 21]. Thus, Haleblian *et al.* reported average values of naringin in grapefruit juice varying between 36.6 mg/100 mL and 134.7 mg/100 mL juice and the absence of naringenin in the analysed samples [8]. Zhang reported 10.1 - 86.7 mg/100 mL concentration of naringin in commercial juices and 7.3 - 30.7 mg/100 mL naringin in hand-extracted ones. The albedo exhibited the highest concentration of naringin 490 - 4100 mg/100 mL [20]. Similarly, Peterson *et al.* reported different naringin values for grapefruit juice, depending on the type of grapefruit: white, from 0 to 43.22 mg/100 mL and red from 3.28 and 48.89 mg/100 mL [13].

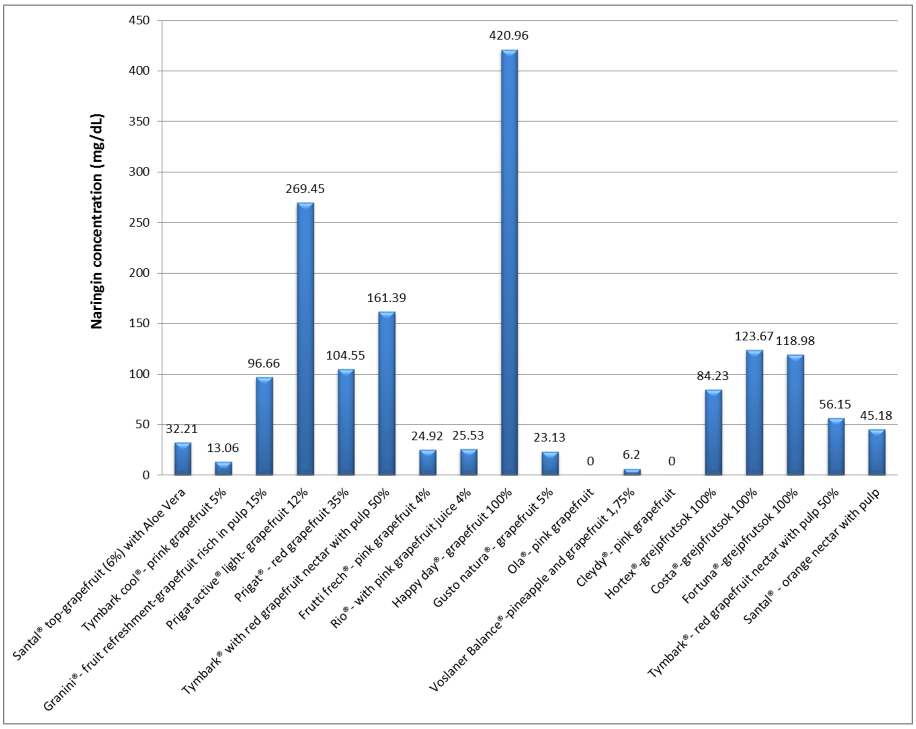

Figure 6.

Naringin concentrations determined in commercially available juices (average concentration of 3 determinations)

The results of Vandermolen show that, in five different grapefruit juices, four of which were commercially available products, the naringin concentration ranged from 309 - 1182 µM [18]. Silva L.C. reported an average of naringin content in orange juices between 0.01 and 0.30 mg/100 g [17].

Conclusions

The potential for clinical significant drug interaction emphasizes the importance of flavonoids determination in commercially and fresh-squeezed juices. Our results, which are consistent with the literature data, show a great variability of results that can be explained both by the origin of the samples, and by the method used for preparing citrus beverages and concentrates.

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