Food Chemistry 37 (1990) 113–121

Naringin Content in Local Citrus Fruits

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(Received 13 September 1989; accepted 25 September 1989)

ABSTRACT

The content of bitter component (naringin) from the skin, juice and seed of musk lime, mexican lime, rough lime, pummelo and mandarin orange was determined by the high-performance liquid chromatographic (HPLC) method. Naringin could only be found in pummelo and rough lime but could not be detected in musk lime, mexican lime and mandarin orange. The skin of pummelo contained a higher amount of naringin (3910 μg/g fresh weight) than the juice (220·0 μg/g fresh weight) whereas the amounts of naringin obtained from the skin, juice and seed of rough lime were 517·2 μg/g, 98·4 μg/g and 29·2 μg/g fresh weight, respectively. Sensory analysis further confirmed that the juices extracted from pummelo and rough lime were bitter while those extracted from musk lime, mexican lime and mandarin orange were not bitter. The correlation coefficient (r) for bitterness using both techniques (sensory and HPLC) was 0·97.

INTRODUCTION

Bitterness in certain citrus fruit juices is one of the major problems of the citrus industry worldwide, and this has had significant economic impact (Hasegawa *et al.*, 1983). Generally, bitterness is considered objectionable in citrus products; however, for grapefruit a certain amount is desirable.

In citrus fruits, the bitter principles of greatest significance are limonin and naringin (naringenin 7β-neohesperidose) (Cook, 1983). Naringin is also the primary bitter component in grapefruit, pummelo, sour orange, trifoliate orange and kamquat (Horowitz & Gentili, 1969). It could be found in the

Food Chemistry 0308-8146/90/$03·50 © 1990 Elsevier Science Publishers Ltd, England. Printed in Great Britain

peel, seed and flesh of the citrus fruits. The other bitter component, limonin, is primarily in the albedo and the segment walls. Maier and Margileth (1969) reported that limonin is naturally present in the fruit tissues as a salt of limonoic acid A-ring lactone. Only when the fruit is macerated, does the combined action of juice acids and an enzyme convert Limonoic Acid A-ring Lactone to limonin.

Generally, the amount of naringin depends on the maturity of the fruit. Higher amounts of naringin are found in the immature fruit. Therefore, fruit maturity is an important factor that is always considered in juice processing, especially in grapefruit juices where bitterness in very pronounced. An acceptable balance must be established between juice yield and naringin levels. Being water soluble (Harborne, 1973) the component is easily extracted into the juice. Therefore, the degree of damage done to the albedo, membranes and pith determines the amount of bitter component extracted. This can be monitored by controlling the pressure used in squeezing the juice.

Since citrus fruits are currently gaining in importance locally for the production of juices, this study is conducted to determine the contents of the bitter component, naringin, in a variety of local citrus. The initiation of this work stems from the fact that prewashing of citrus fruits with hot water is routinely carried out in the local musklime juice-making industry in the belief that such a procedure helps to reduce bitterness of the juice produced. The findings from this work can aid the industry in deciding whether to retain or remove such an operation from the processing line.

MATERIALS AND METHODS

Materials

Pummelo and rough lime fruits were obtained from the Fruits Unit, Horticulture Section, UPM. Mexican lime, musk lime and mandarin orange were purchased from the local market. The fruits chosen were at the mature stage with firm texture, uniform colour and with no sign of spoilage. The fruits were washed thoroughly under running tap water. They were cut into halves and the juice was extracted by squeezing each half into a beaker. The seeds were separated and both the skin and seeds were washed again to remove the remaining juice. These were used to extract naringin.

Methods

Extraction of naringin from juice, skin and seeds

Twenty millilitres (known weight) of juice were diluted with 20 ml of

methanol and filtered through a Whatman No. 4 filter paper into a 100 ml round bottomed flask. The methanol extract was then evaporated *in vacuo* to about 10 ml. The concentrated extract was transferred into a 25 ml volumetric flask and made up to volume with methanol.

The extraction of naringin from the skin and attached membranes was done using 20 g of chopped materials. The sample was macerated in a Waring Blender with methanol for 5 min. The mixture was filtered and the residue was re-extracted twice with methanol. After evaporation of the combined filtrate *in vacuo*, the concentrate was transferred quantitatively into a separating funnel and washed three times with a total volume of 15 ml of petroleum ether. The aqueous fraction was retained and diluted with methanol to 25 ml and the ether fraction was discarded.

Ten grams of seeds were used for extraction of naringin. They were ground finely with a mortar and pestle using acid-purified sand to facilitate grinding. The extraction and purification steps taken were similar to those carried out for the skin sample.

Determination of naringin by HPLC

A Hewlett–Packard 1084 B liquid chromatograph equipped with a Model 79875 A RI detector and a Model 79850 B LC terminal recorder was used. A reverse phase C-18 column (Merck Co.) of 10 μm particle size was used. The running solvent was acetonitrile and distilled water (20:80; v/v) programmed at a flow rate of 2·0 ml/min. Naringin was detected at 280 nm.

The chromatographic standard used was naringin (naringenin-7-rhamnosidoglucoside, Sigma Co.). A series of naringin standard solutions (200–1000 μg/ml) was prepared by dissolving the flavonoid in methanol. A rectilinear curve was obtained ($r = 0{\cdot}993$). Prepared naringin extracts were filtered through a C-18 cartridge and a 0·45 μm membrane filter prior to injection into the HPLC column. The injection volume was 10 μl. The naringin content in a sample was calculated by comparing the naringin peak area of the sample with that of the standard. The reliability of the procedure was determined by a series of recovery experiments.

Sensory evaluation of juice

Fruits used for the evaluation were washed properly with tap water before extraction of juice. For mexican lime, musk lime and rough lime, the fruits were cut into halves and the juice was obtained by squeezing the fruits by hand into a container.

Due to the difficulty in extracting the juice from pummelo, the flesh was blended until fine and filtered. The juice obtained was diluted five times with water. Before testing for bitterness, the total soluble solids content of the juice was made up to 9·0°Brix, similar to a commercial grapefruit juice which

was used as a reference (R). The tasting panel consisted of ten people. They were requested to rate the bitterness of the juice samples in comparison to the commercial sample using the following scale:

1 = Extremely less bitter than R
2 = Less bitter than R
3 = Equal to R
4 = More bitter than R
5 = Extremely more bitter than R

Data obtained from the organoleptic test were analysed using the Analysis of Variance (Larmond, 1977).

RESULTS AND DISCUSSION

The naringin standard was eluted at 280 nm at 7·5 min retention time. Naringenin was also eluted but had a longer retention time and required a greater concentration ($> 1000\ \mu g/ml$) before detection was possible. Besides naringin and naringenin, other possible constituents that are present in citrus fruits which could be co-extracted with naringin during the extraction process were also tested to see if they could also be detected under the chromatographic conditions employed. These were limonin, fructose, glucose and citric acid. However, it was found that none of these was detected. As a check on the efficacy of the extraction procedure, a grapefruit juice (available commercially) which is known to have high contentrations of naringin was analysed. Indeed, a peak which coincided with that of the naringin standard was obtained. Recovery studies carried out on samples produced values at the 97% level.

A study of the chromatograms obtained for the juices, peel and seeds of all the citrus fruit samples analysed showed that naringin was present only in the pummelo and rough lime (Figs 1 and 2, respectively) but was not detected in the musk lime (Fig. 3) or mexican lime (Figs 4 and 5). Naringin was also not detected in mandarin orange. The results obtained showed that the pummelo had a higher naringin content than the rough lime. In both cases, the peel contained the highest proportion of naringin when compared to the juice or seeds. Figure 6 shows the distribution of naringin in pummelo and rough lime and the values given (g/g fresh weight) represent the average of three determinations. The studies by Jourdan *et al.* (1983) showed that, for grapefruit, the pith contained the highest concentration of naringin followed by the peel together with the membrane, the seeds and the juice.

As can be seen in Figs 3–5, several peaks were obtained for the juices of musk lime and mexican lime but none of the peaks coincided either with naringin or naringenin. It was also observed that the chromatographic

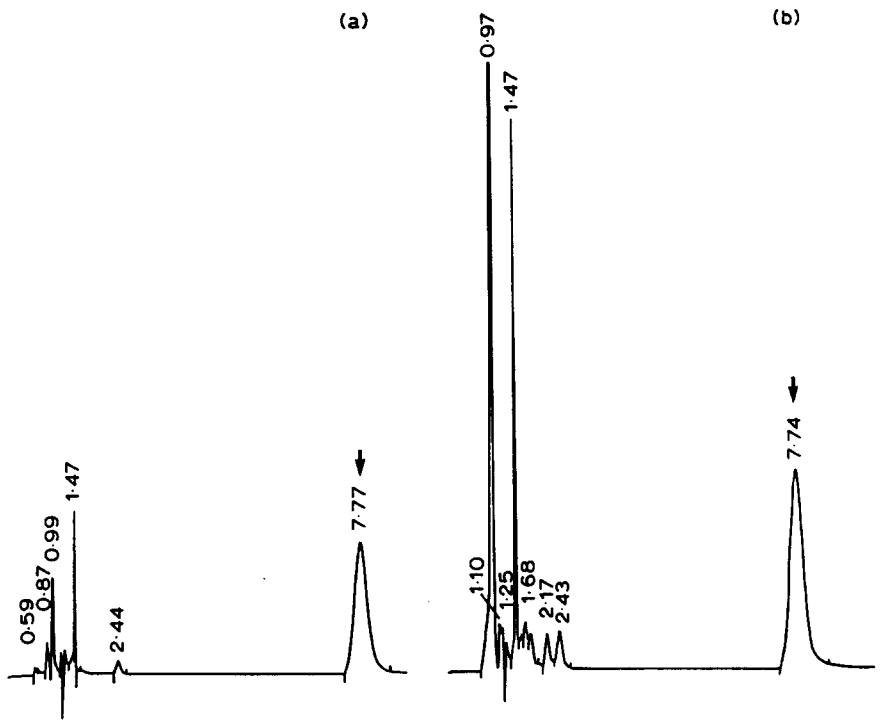

Fig. 1. Chromatogram for (a) pummelo peel (diluted × 25) and (b) pummelo juice. The naringin trace is indicated by an arrow. The numbers indicate retention time in minutes.

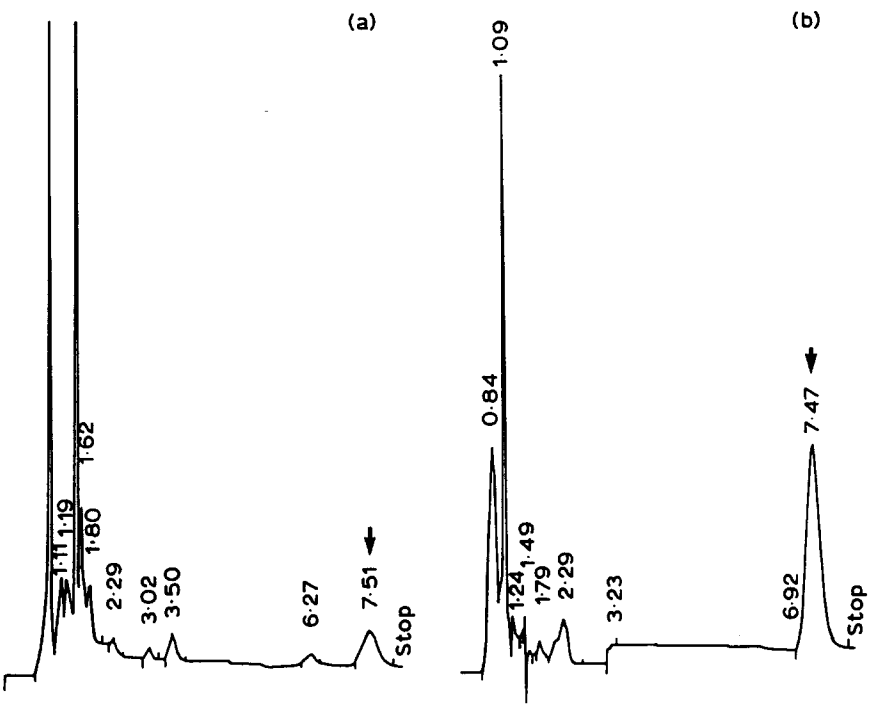

Fig. 2. Chromatogram for (a) rough lime peel (diluted × 25) and (b) rough lime juice. The naringin trace is indicated by an arrow. The numbers indicate retention time in minutes.

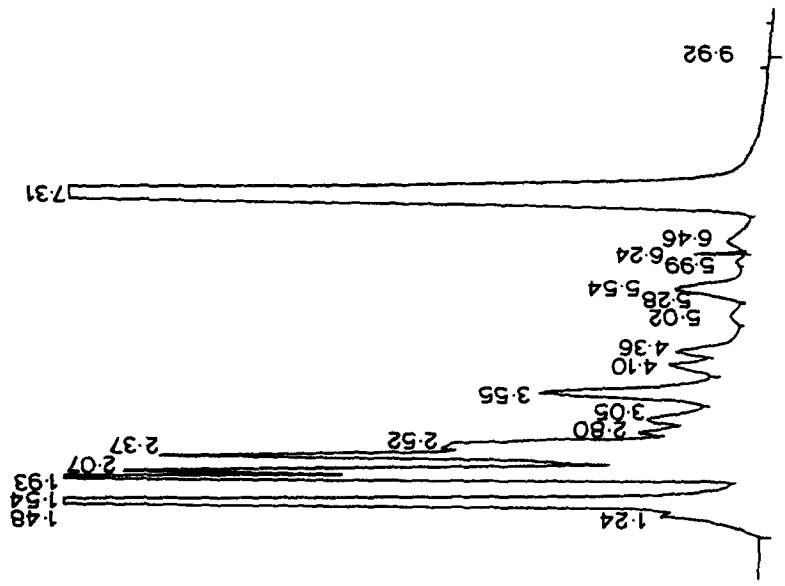

Fig. 3. Chromatogram for musk lime peel.

Fig. 4. Chromatogram for mexican lime peel.

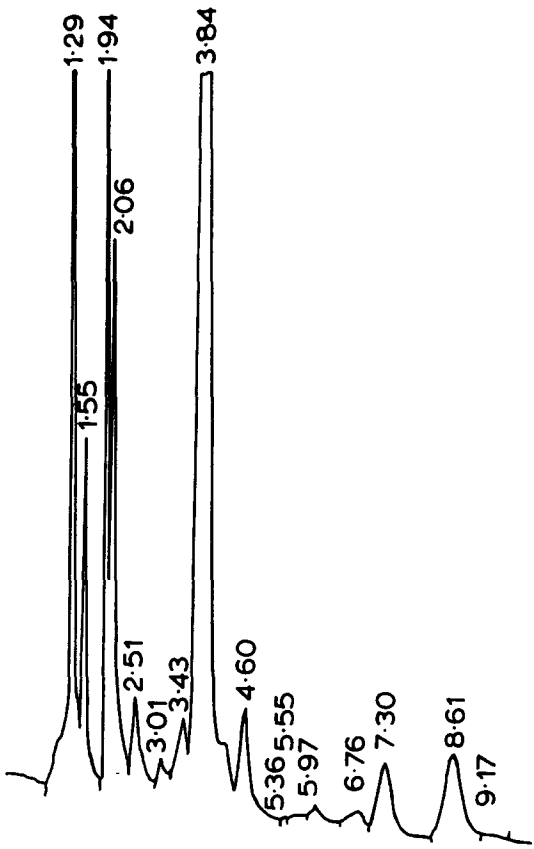

Fig. 5. Chromatogram for mexican lime juice.

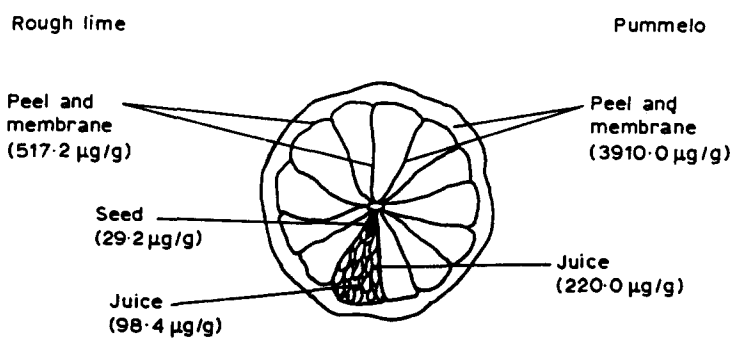

Fig. 6. Distribution of naringin in pummelo and rough lime.

profiles of the juices of these fruits differed from those of the peel and seeds indicating differences in the existence and distribution of the unknown components. The peaks obtained may belong to other bitter components such as neohesperidin, nomillin and/or poncirin. Since sensory analysis showed the juices to be non bitter (Table 1) it is possible that no bitter component is present or, if present, it is below the threshold level for sensory detection. The juices of musk lime and mexican lime were reported by the panelists to be acutely acid in taste and this could mask any bitterness.

Comparisons made between the local citrus fruit juices with a commercial (imported) grapefruit juice showed that there were significant differences ($p < 0.05$) in bitterness between musk lime and pummelo, mexican lime and pummelo and between rough lime and pummelo but there were no significant differences among musk lime, mexican lime and rough lime. From the mean score values (Table 1), it was observed that the pummelo juice was most bitter. The degree of bitterness for pummelo was slightly more than grapefruit juice (reference sample). It was also found that the panelists could not detect the bitter taste in musk lime and mexican lime. However, some of them could detect the bitterness in rough lime juice. The correlation coefficient (r) for sensory bitterness and the level of naringin detected was 0·97.

TABLE 1
Mean Score of Citrus Juices[a]

Attribute	*Types of fruits*			
	Pummelo	*Rough lime*	*Musk lime*	*Mexican lime*
Bitterness	3·4*	1·8**	1·2**	1·1**

[a] Means with different superscripts are significantly different ($p < 0.05$).

CONCLUSIONS

The results obtained indicated that the citrus fruits which are normally used for juice processing (musk lime, mexican lime and mandarin orange) were not bitter. Therefore, the current practice in the local food industry to reduce bitterness in musk lime by washing with hot water before processing may not be necessary. In fact, if washing is done excessively, it might cause a loss of limonin, which is the flavour and aroma compound for citrus products. Pummelo contained a high amount of naringin. The content was higher in the skin (3910·0 μg/g) than in the juice (220·0 μg/g). Rough lime contained less naringin than pummelo. The content was also high in the skin

(517·2 μg/g) and lower in the juice (98·4 μg/g) and seeds (29·2 μg/g). Due to their relatively dry nature, both pummelo and rough lime are not processed into juice, so the presence of naringin in these fruits does not pose any problem.

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