Hesperidin in *Citrus* Species, Quantitative Distribution During Fruit Maturation and Optimal Harvesting Time

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Abstract

The experiment was done in the Citrus Research Organisation located in Ramsar (North of Iran) and was conducted through two successive seasons of 1997 and 1998. We have studied the effect of harvest time on fruit growth and hesperidin content of 4 *Citrus* species. Weight, diameter and peel thickness were characteristic to the species. The highest hesperidin content in different *Citrus* species, used in those investigations, was obtained 50 to 60 days after full bloom. *Citrus* species had been differentiated according to their hesperidin content. As the local sweet orange is cultivated on large scale in the North of Iran, therefore it is a suitable source to produce hesperidin.

INTRODUCTION

A well-documented characteristics of the *Citrus* genus is the accumulation of high amounts of flavonone glycosides in the fruits (Ortuno et al., 1997, Maier and Hasegawa, 1970). Some of these flavonone glycosides are of commercial interest because they are used in the pharmaceutical and food industries. Hesperidin is indicated as oedema protective as well as in the prevention of vein blood stagnation and the regeneration of the normal wall capillary permeability (Gabor, 1998, Ortuno et al., 1997). Several studies have shown that the accumulation of flavonoids in various *Citrus* species is related to certain stages of fruit growth, the highest levels of these secondary metabolites being detected in very young tissues of the fruit (Castillo et al., 1992, Del Rio and Ortuno, 1994, Ortuno et al., 1995). Therefore, this study was oriented to determine the optimal time of harvest of the immature fruits, to obtain the maximum yield of hesperidin.

MATERIALS AND METHODS

Plant Material

The flavonone glycoside hesperidin was isolated and quantitatively evaluated in local sweet orange (*Citrus sinensis*), Thomson navel orange (*Citrus sinensis*), clementine mandarin (*Citrus reticulata*) and unshiu (satsuma) mandarin (*Citrus unshiu*). The statistical design used was a factorial examination on the basis of complete randomised design. The experiment was done in the Citrus Research Organisation located in Ramsar (North of Iran) and was conducted through two successive seasons of 1997 and 1998. The main characteristics of the chosen samples were: immature fruits of local sweet orange from 25 years old trees with fruit diameter varying from 12 up to 42 mm., immature fruits of Thomson navel orange from 17 years old trees with fruit diameter varying from 12 up to 47 mm, immature fruits of clementine mandarin from 25 years old trees with fruit diameter varying from 10 up to 29 mm and immature fruits of unshiu (satsuma) mandarin from 17 years old trees with fruit diameter varying from 10 up to 38 mm. The fruits were harvested at 44, 60, 72, 88 days and 30, 40, 53, 63, 74, 90 days after full bloom in 1997 and 1998, respectively.

Crude Hesperidin Extraction

After the collection the immature fruits were immediately dried at 50 °C then
grounded. After homogenisation, 100 g from each powder sample were exhaustively extracted in a Soxhlet apparatus in two steps, first with petroleum ether (60°C for 24h) to remove the lipophilic fraction, then with methanol (70°C for 48h) to obtain the total extract.

Crude hesperidin was completely precipitated by cooling the methanolic extract in the refrigerator. After filtration and washing with cold methanol, the crude hesperidin was dried in an oven at 50°C.

Analysis of Hesperidin Content

The purity of the crude hesperidin was determined by HPLC. A µ Bondapak C_{18} (300×3.9 mm) reverse phase column with an average particle size of 10 µm protected by a C_{18} (10 µm) guard column cartridge was used (Fischer, 1978). HPLC grade solvents were used for the mobile phase in an isocratic mode with acetonitrile 21.5% and water 78.5% at 2ml/min flow rate. The absorbency was monitored at 258 nm with a U.V. detector.

All samples were prepared as follows: 5 mg crude hesperidin powder were dissolved in 6 ml dimethyl sulfoxide (DMSO), then the volume was adjusted to 100 ml with methanol. The obtained solutions were filtered through a 0.45 µm single use filter.

From each sample, 5µl was injected into the chromatograph. Pure hesperidin standard (97 %) was obtained from Aldrich Co.

RESULTS

Fruit Growth

Results showed that the increase of the weight (Fig. 1) and diameter (Fig. 2) of different tested Citrus species were very fast. However it was even faster for the navel orange (Citrus sinensis).

Fruit peel thickness at the first step of growth increased then slowly decreased. The highest peel thickness was observed around 50 days after full bloom (Fig. 3).

Crude Hesperidin Yield

It was proved by the results that at the first stage of fruit growth, the total dry extract (crude hesperidin) of citrus species increased then decreased afterwards (Fig. 4). This process took place between 50 and 60 days after full bloom. In that period, fruit diameter for local orange, navel orange, clementine and satsuma were 32.5; 32.5; 22 and 25 mm respectively in 1997 and 32; 35; 17 and 26 mm respectively in 1998. Results show, that the highest yield of crude hesperidin was obtained in 1997 and 1998 (1.67 and 1.73% respectively) from clementine and the lowest one (0.74 and 0.77%) from navel orange (Table 1).

Hesperidin Content

Results show (Fig. 5) that the highest hesperidin content was found in local orange and clementine. The hesperidin content of local orange and clementine increased until 40 days after full bloom.

Results (Table 2) indicated that species had significant effect on the hesperidin content. The highest hesperidin content was obtained from local orange and navel orange in 1997 and clementine in 1998 (61.67, 61.00 and 61.33% respectively).

DISCUSSION

It was proved, that the hesperidin content depends upon the harvest time, significantly. The highest flavonone content was found in the immature fruits. In this early stage of fruit development, the synthesis of that compounds seems to be rather intensive. The hesperidin content was the highest between 50 to 60 days after full bloom in the case of 4 tested species, and decreased afterwards. This statement is in harmony with results concerning Citrus fruits published earlier (Del Rio et al., 1995, Kuppusamy and Das, 1993, Jourdan et al., 1981). The flavonone content was significantly affected by species
We found that local orange, navel orange (in 1997) and clementine (in 1998) had the highest hesperidin content. Because of the local orange cultivated in large scale in the north of Iran, it is a suitable source to produce hesperidin.

Literature Cited


Maier, V.P. and Hasegawa, S. 1970. L-phenylalanine ammonia-lyase activity and naringenin glycoside accumulation in developing grapefruit. Phytochemistry 9:139-44.


### Tables

#### Table 1. Effect of species on crude hesperidin of Citrus dry peel.

<table>
<thead>
<tr>
<th>Species</th>
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#### Table 2. Effect of species on hesperidin content of Citrus dry peel

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Figures

Figure 1. Fruit weight variation of Citrus species

Figure 2. Fruit diameter variation of Citrus species
Figure 3. Fruit peel thickness variation of *Citrus* species

Figure 4. Effect of fruit growth on total extract (crude hesperidin) of *Citrus* species
Figure 5. Effect of fruit growth on hesperidin content of *Citrus* species