# Bioflavonoid Profile of Exotic Citrus Species

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**Keywords**: Citron, eriocitrin, diosmin, flavanones, flavones, flavonoids, hesperidin, lemon, mandarin, naringin, narirutin, neoeriocitrin, neohesperidin, papeda, pummelo, sour orange

**Abstract**

**The levels of the flavanones naringin, hesperidin, narirutin, eriocitrin, neohesperidin and neoeriocitrin, and the flavone diosmin were evaluated in fruits of several exotic citrus (citron, pummelo, sour orange, papeda, mandarin and lemon). The highest total flavonoid content was detected in sour oranges and mandarins, while low to non-detectable levels were found in pummelo, papeda, citron, and lemon cultivars. Average total bioflavonoid values on a dry weight basis were between 4-5 % in both sour oranges and mandarins. Individual flavonoids varied among species. Naringin, neohesperidin, and neoeriocitrin were found in some sour orange cultivars. Hesperidin was the only flavonoid detected in mandarin. Pummelos showed a diverse flavonoid profile. Some pummelo varieties had up to 6 different flavonoids (narirutin, naringin, hesperidin, eriocitrin, neohesperidin, neoeriocitrin). Citrus flavonoids have a wide variety of clinical applications. This information should prove useful to the nutraceutical industry in identifying alternative sources of flavonoids.**

## INTRODUCTION

Citrus fruits have been widely recognized for their medicinal applications such as antioxidant, anti-inflammatory, cholesterol-lowering, anticancer, and antiviral among others (Benavente-Garcia, et al., 1997). Flavonoids are key phytochemicals supporting citrus medicinal properties and contributing to the sensory quality of these fruit (Garg et al., 2001; Kris-Etherton et al., 2002; Middleton et al., 2000; Peterson and Dwyer, 1998; Ren et al., 2003; Silalahi, 2002; Tomás-Barberán and Clifford, 2000). Furthermore, they are also important in chemotaxonomy as each species of Citrus is characterized by a particular flavanone glycoside pattern (Berhow et al., 1996; Rouseff et al., 1987). Flavanones such as eriocitrin, narirutin, hesperidin, naringin, and neohesperidin are characteristic of common citrus fruits (Tomás-Barberán and Clifford, 2000) and have their health benefits known as referenced above. Minor flavonoids such as flavones also play an important nutraceutical role (Marín and Del Río, 2001). The main location on the fruit for these flavonoids is on the peel (Tomás-Barberán and Clifford, 2000).

It is desired to find new citrus sources that can provide a broad spectrum and/or a high concentration of flavonoids. For this communication, we have analyzed 12 exotic citrus species and/or cultivars to investigate their potential application in dietary supplements and functional foods based on the total flavonoids content and profile.

Proc. XXVI IHC – Future for Medicinal and Aromatic Plants
Eds. L.E. Craker et al.
Acta Hort. 629, ISHS 2004
Publication supported by Can. Int. Dev. Agency (CIDA)

## MATERIALS AND METHODS

### Plant Material

In most cases, species were selected based on their peel thickness; others were selected based on previous knowledge of their being rich sources of the bioflavonoids of interest (Pusateri, 1998). Samples of each fruit group were harvested at an immature stage in December 2000 from the USDA-ARS Citrus National Clonal Germplasm Repository/ UC Riverside Citrus Variety Collection (Riverside, CA). Table 1 notes the genus species, cultivars, groups and study names of the species tested. Samples were freeze dried and milled into powder using a Universal Mill (Bauermeister, Inc, Memphis, TN) with a 1.5 mm screen.

### Flavonoids Analysis

Seven different flavonoids were evaluated including 6 flavanones (naringin (N), hesperidin (H), narirutin (NR), eriocitrin (E), neohesperidin (NeoH) and neoeriocitrin (NeoE)), and one flavone (diosmin (D)).

Samples were prepared by dissolving 0.25 g of freeze dried powder in 25 ml of extraction solvent (methanol: DMSO 4:1), sonicated at room temperature for 30 min. Liquid sample was filtered through a 0.45 μm filter into an HPLC autosampler vial. The linear range for standard calibration was 5–500 ppm.

The HPLC system consisted of a Hewlett Packard HPLC 1100 (Palo Alto, CA) with a dual absorbance detector (DAD) and a Waters C18 symmetry column (250 x 4.6 mm i.d.) at ambient temperature. Flow rate was 1.0 mL/ min, injection volume 10 μl, detection wavelength 280 nm. A gradient mobile phase was used (Table 2.).

### Sugar Analysis

A Perkin-Elmer HPLC system (Wellesley, MA) equipped with a refractive index detector, an amino propyl column (250 x 4.6 mm i.d.) (Altech, Deerfield, IL) with thermostat at 25 $^{o}$C was used. Mobile phase was isocratic with a mixture of 70 % acetonitrile and 30 % water and flow rate was 1 mL/min.

## RESULTS

The total flavonoids identified in this study are presented in Figure 1. Species with the highest total flavonoids (% w/w) were sour orange ‘myrtifolia’ with 5 % and sour orange cv. ‘Gadadehi’ and mandarin hybrid cv. ‘Nova’ both with 4.6 % each. Pummelo hybrids followed with 2.6 %, 2.4 % ad 1.9 % for cvs. ‘Pong Yau’, ‘Yuma Ponderosa’, and ‘Tresca’, respectively. Papeda Hybrid cv. ‘Ichang Lemon’ and papeda cv. ‘Honghe’ had 2.4 % and 1.8 %, respectively. Citron species were 1.5 %, 0.6 % and 0.5 % for cvs. ‘Bengal’, ‘Odorata’, and ‘Diamante’, respectively. Last, Lemon Hybrid cv. ‘Ponderosa’ registered 0.7 %.

The breakdown of individual bioflavonoids is noted on Table 2. Pummelo Hybrid cv. ‘Yuma Ponderosa’ had the broadest variety of flavonoids with a total of 6 (1.4 % NeoH, 0.6 % NeoE, 0.2 % N, 0.1 % H, and 0.04 % each for E and NR). Sour orange ‘myrtifolia’ contained 3 flavonoids (2 % NeoH, 1.6 % NeoE, and 1.5 % N). Species containing 2 flavonoids were citron cv. ‘Bengal’ (1.2 % NeoH and 0.3 % N); papeda cv. ‘Honghe’ (1.6 % N and 0.2 % E); pummelo cv. ‘Tresca’ (1.2 % N and 0.7 % NR); citron cv. ‘Diamante’ (0.3 % H and 0.2 % D); and citron cv. ‘Odorata’ (0.4% H and 0.3 % D). Single flavonoid species were: sour orange cv. ‘Gadadehi’ (4. 6% N), mandarin hybrid cv. ‘Nova’ (4.6 % H) and lemon hybrid cv. ‘Ponderosa’ 0.7 % NH.

Total sugar content is displayed in Figure 2. Values were as low as 22 % for both papeda hybrid cv. ‘Ichang Lemon’ and pummelo hybrid cv. ‘Yuma Ponderosa’ and as high as 46 % for citron cv. ‘Odorata’. With the exception of mandarin hybrid cv. ‘Nova’, glucose and fructose were the dominant sugars in immature fruit (Table 3).

## DISCUSSION

Total flavonoid results show some potential for species such as sour orange 'myrtifolia' (5 %), sour orange cv. 'Gadadehi' and mandarin hybrid cv. 'Nova' (both at 4.6 % each) as potential new sources of flavonoids. Although harvested green, their level of immaturity was unknown.

Total flavonoid content for the remaining varieties tested was similar to those of some commonly consumed citrus fruits. Botero-Omary et al. (2000) reported total flavonoid levels at commercial maturity in grapefruits (cv. 'Ruby red'), oranges (cv. 'Valencia') and mandarins (cv. 'Fairchild') at 0.9 %, 0.4 % and 3 %, respectively.

This study pointed to naringin as the main flavonoid for two types of sour oranges, 2 types of papedas and two types of pummelos. Benavente-García, et al. (1997) cite N among the principal flavonoids in grapefruits (*C. paradisi*) and sour oranges (*C. aurantium*). Del Río and Ortuño (1994) reported pummelos (*C. grandis*) as also being high in N. In contrast, Berhow et al. (1996) reported only traces of N in leaves of papeda *C. wilsonii* (cv. 'Ichang lemon'). A significant source of H in this evaluation was the mandarin hybrid (cv. 'Nova') only. In the literature sweet oranges (*C. sinensis*) are cited among the primary source. However, Botero-Omary et al. (2000) reported H as the main flavonoid during the development of mandarins (cv. 'Fairchild'). NeoE is normally reported as being one of the most common flavonoids in sour oranges (*C. aurantium*). However, this study indicated that this was true for only one type of sour orange ('Myrtifolia') in addition to citron (cv. 'Bengal') and pummelo hybrid (cv. 'Yuma Pondersosa'). There were no significant sources of E, NR and D in this study. Lemons are common sources of E, while sweet oranges (*C. sinensis*) and Rangpur limes (*C. limonia*) are good sources of D (Benavente-García, et al., 1997). Relevant sources of NeoH were sour orange (cv. 'Myrtifolia') and pummelo hybrid cv. 'Yuma Ponderosa'. Tomás-Barberán and Clifford (2000) cite neohesperidin as significant in sour oranges (*C. aurantium*).

Based on this preliminary screening, future analytical work will be conducted on selected species/cultivars from fruit setting to maturity.


## ACKNOWLEDGEMENTS

The authors extend their appreciation to Mrs. Leno Wong and Mrs. Chung Hyun at Nutrilite, Division of Access Business Group for assistance with flavonoid and sugar analyses.


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## Tables

Table 1. *Genus species*, cultivars, groups and study names of species tested.

| ***Genus species*** | **Cultivar** | **Group** | **Study Name** |
|---|---|---|---|
| *Citrus medica* | 'Bengal' | Citron | Citron cv. 'Bengal' |
| *Citrus medica* | 'Diamante' | Citron | Citron cv. 'Diamante' |
| *Citrus medica* | 'Odorata' | Citron | Citron cv. 'Odorata' |
| *Citrus pyriformis* | 'Ponderosa' | Lemon Hybrid | Lemon Hybrid cv. 'Ponderosa' |
| *Citrus reticulata* | 'Nova' | Mandarin | Mandarin Hybrid cv. 'Nova' |
| *Citrus hongheensis* | 'Honghe' | Papeda | Papeda cv. 'Honghe' |
| *Citrus hybrid* | 'Ichang Lemon' | Papeda hybrid | Papeda Hybrid cv. 'Ichang Lemon' |
| *Citrus hybrid* | 'Yuma Ponderosa' | Pummel Hybrid | Pummelo Hybrid cv. 'Yuma Ponderosa' |
| *Citrus maxima* | 'Pong Yau' | Pummelo | Pummelo cv. 'Pong Yau' |
| *Citrus maxima* | 'Tresca' | Pummelo | Pummelo cv. 'Tresca' |
| *Citrus aurantium* | 'Gadadehi' | Sour orange | Sour Orange cv. 'Gadadehi' |
| *Citrus myrtifolia* | | Sour orange | Sour Orange 'Myrtifolia' |

Table 2. Evaluated soil samples constituents.

| Time (min) | 0.2 % $H_3PO_4$/water (%) | Methanol (%) | Acetonitrile (%) |
|---|---|---|---|
| 0 | 69 | 19 | 12 |
| 12 | 51 | 34 | 15 |
| 18 | 20 | 40 | 40 |
| 25 | 69 | 19 | 12 |
| 35 | 69 | 19 | 12 |

Table 3. Individual flavonoid concentration of exotic citrus tested[12].

| **Species** | **NR (%)** | **H (%)** | **E (%)** | **NRR (%)** | **NH (%)** | **NE (%)** | **DM (%)** |
|---|---|---|---|---|---|---|---|
| Citron cv. 'Bengal' | 0.261± 0.001 | | | | | 1.235± 0.005 | |
| Citron cv. 'Diamante' | | 0.249± 0.008 | | | | | 0.24± 0.003 |
| Citron cv. 'Odorata' | | 0.371± 0.001 | | | | | 0.269± 0.001 |
| Lemon Hybrid cv. 'Ponderosa' | | | | | 0.693[3] | | |
| Mandarin Hybrid cv. 'Nova' | | 4.57[3] | | | | | |
| Papeda cv. 'Honghe' | 1.595± 0.005 | | 0.209± 0.002 | | | | |
| Papeda Hybrid cv. 'Ichang Lemon' | 2.43[3] | | | | | | |
| Pummelo Hybrid cv. 'Yuma P'. | 0.231± 0.002 | 0.135± 0.000 | 0.042± 0.001 | 0.038± 0.001 | 1.385± 0.005 | 0.58± 0.006 | |
| Pummelo cv. 'Pong Yau' | 2.59± 0.01 | | | | | | |
| Pummelo cv. 'Tresca' | 1.23± 0.02 | | | 0.707± 0.003 | | | |
| Sour Orange cv. 'Gadadehi' | 4.64± 0.06 | | | | | | |
| Sour Orange 'Myrtifolia' | 1.47± 0.00 | | | | 2.015± 0.015 | 1.585± 0.005 | |

[1]NR=Naringin, H=Hesperidin, E=Eriocitrin, NRR=Narirutin, NH=Neohesperidin, NE=Neoeriocitrin and DM=Diosmin. [2]Dry weight basis values. [3]Values are the average of two samples ± standard error, except for Lemon Hybrid cv. 'Ponderosa', Mandarin Hybrid cv. 'Nova', and Papeda Hybrid cv. 'Ichang Lemon' where only single samples were tested.

## Figures

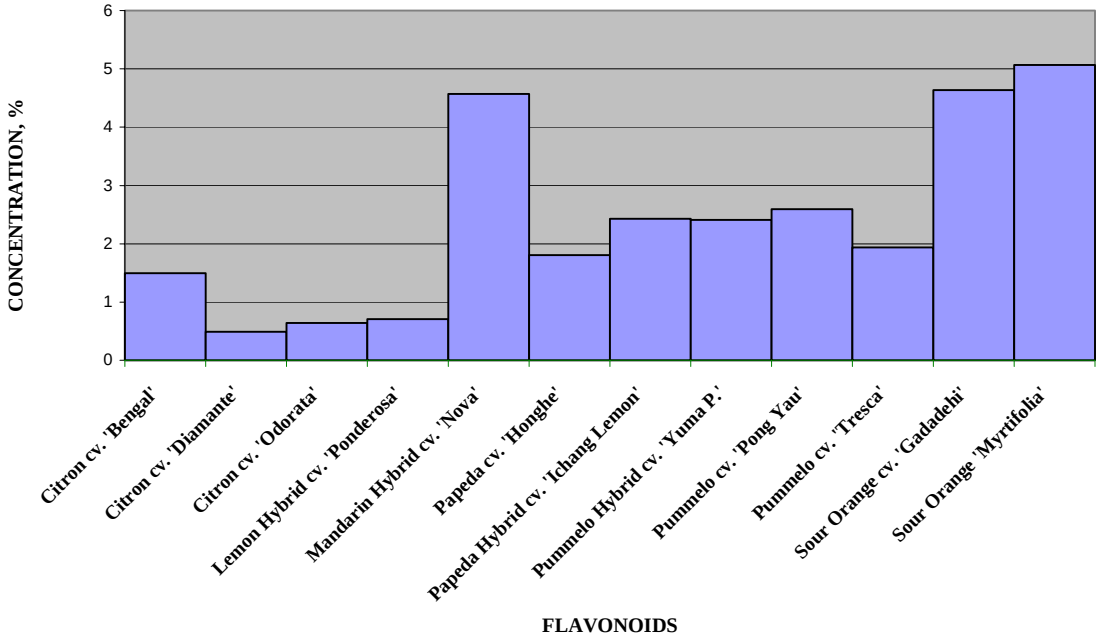


Fig. 1. Total flavonoids in exotic citrus species tested. Each value is the mean of two multi-fruit replications, except for Lemon Hybrid cv. 'Ponderosa', Mandarin Hybrid cv. 'Nova' and Papeda Hybrid cv. 'Ichang Lemon' where only single samples were tested.

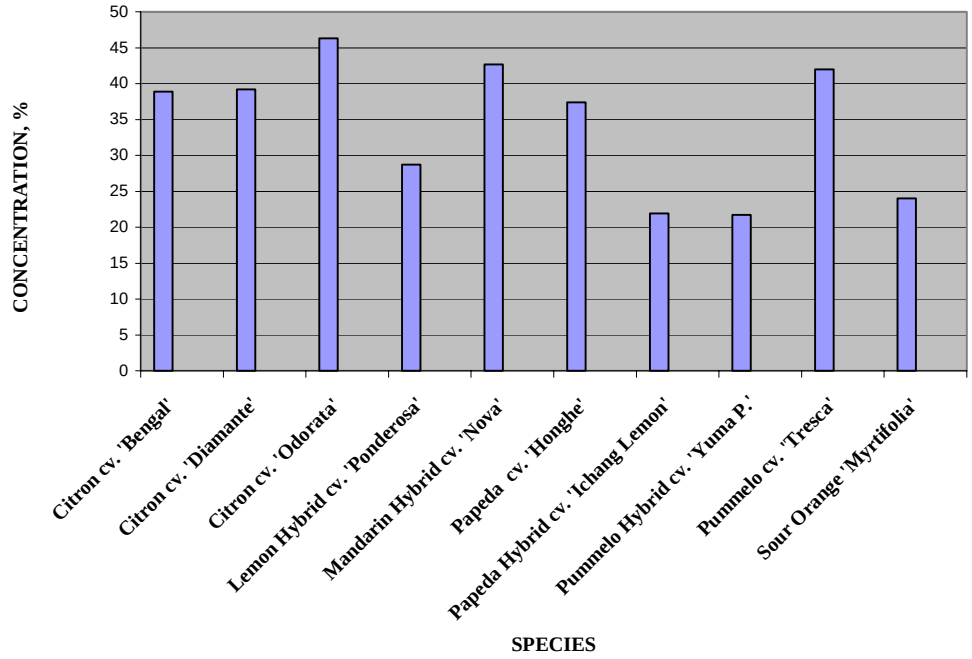


Fig. 2. Total sugar content of immature exotic citrus. Each value resulted from a single sample and duplicate injection. Sugar results are unavailable for pummelo cv. 'Pong Yau', and sour orange cv. 'Gadadehi' species.

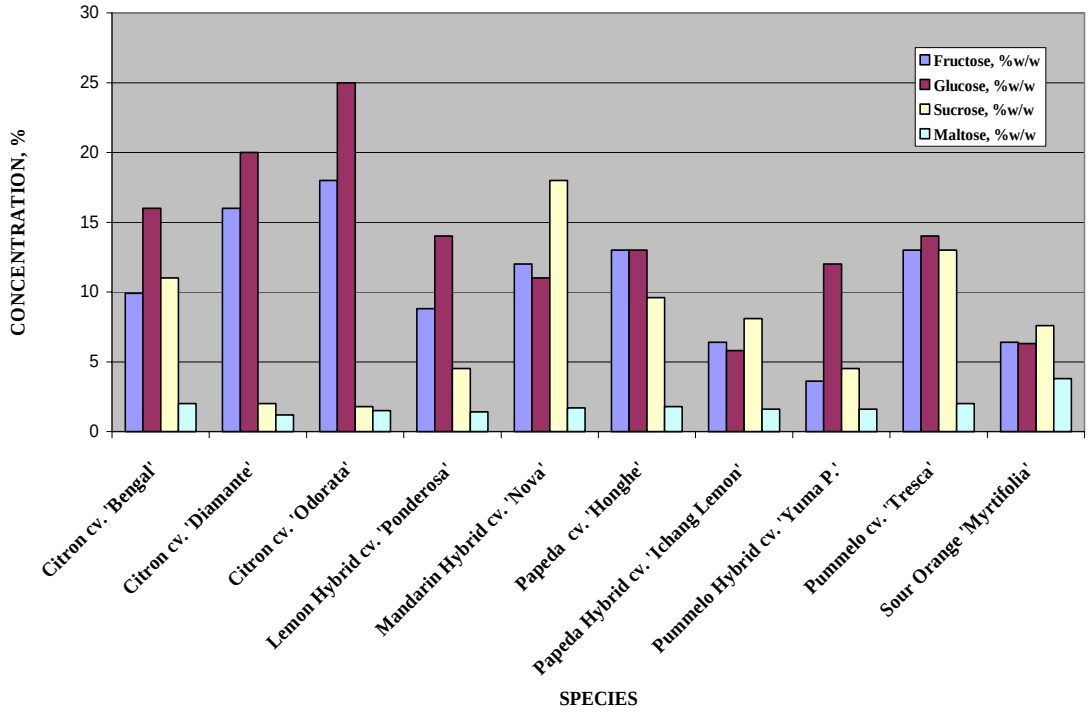


Fig. 3. Sugar profile of exotic citrus tested. Each value resulted from a single sample and duplicate injection. Sugar results are unavailable for pummelo cv. ‘Pong Yau’, and sour orange cv. ‘Gadadehi’ species.