# Concentrations of Hesperidin and Other Orange Peel Flavonoids in Citrus Processing Byproducts

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The concentrations of flavonoids were measured in the peel of different orange (*Citrus sinensis* L.) varieties and compared to the distributions of flavonoids in the byproducts of orange processing. While the flavanone glycoside hesperidin was the main flavonoid in peel, precipitation of this compound during processing resulted in dramatic losses in hesperidin in filtered peel juice and filtered molasses. Yet, hesperidin occurred at very high levels in dimethyl sulfoxide extracts of unfiltered molasses (5718 ppm) and in the centrifuged insoluble solids of orange peel molasses (65 642 ppm). The polymethoxylated flavone aglycons were the only flavonoids in cold-pressed orange peel oil. These compounds also occurred in high concentrations in light-density oil solids and in commercial wax isolated from the cold-pressed peel oil. Thus, the different citrus peel byproducts are selectively enriched with high concentrations of different groups of flavonoid compounds.

**Keywords:** *Citrus sinensis; bioflavonoids; orange peel molasses; peel oil; polymethoxylated flavone*

## INTRODUCTION

The flavonoids in citrus are a major class of secondary metabolites that have significant impact on nearly every aspect of citrus fruit production and processing. Two of the remarkable properties of certain key flavonoids are bitter taste (e.g. naringin in grapefruit and pummelo) and low solubilities in aqueous solutions (Horowitz, 1961). The negligible solubility of hesperidin, the flavonoid in highest concentration in many commercial citrus varieties, contributes to lemon and orange juice cloud; yet, in the peel the solubility of hesperidin is nearly 1000-fold greater than its solubility in water. Other less well characterized properties of citrus flavonoids are the roles of these compounds in plant defense (Koes et al., 1994), the overall flavonoid distribution patterns in citrus, and the use of these compounds as taxonomic markers (Albach and Redman, 1969; Tatum et al., 1978; Kamiya et al., 1979; Gaydou et al., 1987). While the exact roles of flavonoids in plant tissue remain speculative, the biological activities of citrus flavonoids, in particular the polymethoxylated flavones, in mammalian systems are well documented and recently reviewed (Robbins, 1980; Baker, 1992; Brandi, 1992; Middleton and Kandaswami, 1992).

The highest concentrations of flavonoids in citrus fruit occur in the peel (Maurer et al., 1950; Kanes et al., 1993). For oranges, the peel represents roughly half of the fruit mass. Processing of oranges generates 40 000 lb of peel byproduct per 1000 boxes (or 90 000 lb) of fruit (Kesterson and Braddock, 1976). Also included as byproducts of peel are 3092 lb of citrus molasses, 5405 lb of dried citrus pulp, and 256 lb of cold pressed peel oil. Smaller quantities of D-limonene and wax are also generated as orange processing byproducts. Due to the large amounts of peel byproducts and the high concentrations of flavonoids in citrus peel [roughly 1.7−2.0% per dry weight in orange and grapefruit peel (Sinclair, 1972)], citrus processing byproducts represent a rich source of naturally occurring flavonoids. Furthermore, while flavonoids are abundant elsewhere in the plant kingdom, citrus peel is a particularly rich source of flavanones and flavanone glycosides, which are relatively rare in other plants (Horowitz, 1961). Also peculiar to citrus are a number of the polymethoxylated flavones (e.g. nobiletin and tangeritin), which are rarely found in other plants. These latter compounds have been of intense interest for their pharmacological properties (Robbins, 1980).

While it has long been recognized that orange peel represents a promising source of hesperidin (Higby, 1947; Baier, 1948; Bonnell, 1958; Crandall et al., 1977), little attention has been given to the isolation of the other flavonoids in peel and to the possibility of recovering flavonoids from the other citrus peel byproducts. In this paper we report on the quantitation of the flavonoids in different orange processing byproducts and show that by virtue of the extremely high flavonoid concentrations in the different byproducts, these materials are rich commercial sources of several classes of flavonoid compounds.

## MATERIALS AND METHODS

**Sample Preparation.** Flavonoids were extracted from dried, powdered orange peel and peel byproducts as described by Nogata et al. (1994). Orange peel was obtained from 10−15 fresh Valencia, Ambersweet, navel, and Temple oranges. Contamination of the peel with juice and orange segment membranes was avoided. The fresh peel was ground in a coffee grinder and dried at 70 °C. The dried peel was further ground to a fine powder and extracted for 24 h in 100 mg portions three times with 1.5 mL of dimethyl sulfoxide (DMSO)/methanol (1/1 v/v). The volumes of the combined extracts were adjusted to 5 mL and filtered through 0.45 $\mu$m filters. Dried commercial pellets were powdered and extracted in a similar manner.

Orange peel juice and molasses were obtained from three commercial sources designated A, B, and C. The °Brix values for the molasses from these sources were 51.1, 52.3, and 55.2, respectively. The isolation of the molasses solids was accomplished by the centrifugation of molasses at 12000$g_{max}$ for 1 h at 15 °C. The recovered, pelleted solids were dried at 70 °C and extracted as described above. Filtered molasses was obtained as the clear filtrate passed through Whatman No. 1 filter paper.

The light-density solids from cold-pressed orange peel oil were obtained by partial gravity filtration of the solids from the oil/water interface. To remove the cold-pressed peel oil from the solids, 100 mL of the thick slurry of solids was

**Table 1. Quantitation of Flavonoids in Valencia, Navel, Ambersweet, and Temple Orange Peel[a]**

| compound | Valencia | navel | Temple | Ambersweet |
|---|---|---|---|---|
| narirutin 4′-*O*-glucoside 16.965 min | 835 ± 16 | 499 ± 8 | 675 ± 16 | 663 ± 17 |
| eriocitrin 19.837 min | 1010 ± 57 | 870 ± 40 | 1649 ± 54 | 1278 ± 6 |
| narirutin 23.320 min | 1998 ± 41 | 2673 ± 118 | 2344 ± 85 | 1676 ± 16 |
| hesperidin 24.938 min | 19170 ± 595 | 31749 ± 1422 | 27781 ± 1005 | 20549 ± 286 |
| isosakuranetin rutinoside 30.370 min | 1858 ± 31 | 1806 ± 56 | 316 ± 10 | 3361 ± 80 |
| sinensetin 41.112 min | 305 ± 8 | 468 ± 26[b] | 475 ± 15 | ND[c] |
| HQ/HG[d] | 82 ± 4 | | 247 ± 12 | ND |
| nobiletin/TETRA[e] | 513 ± 4 | 576 ± 2 | 1127 ± 36 | ND |
| HEPTA[f] | 225 ± 3 | 415 ± 2 | ND | ND |
| T/5-OH[g] | 109 ± 4 | 74 ± 2 | 142 ± 2 | 51 ± 7 |

[a] Elution times of individual flavonoids are also listed. Results of analyses, run in triplicate, are reported as ppm. [b] Values for sinensetin and HQ/HG are combined. [c] None detected. [d] Hexa-*O*-methylquercetagetin/hexa-*O*-methylgossypetin. [e] Tetra-*O*-methylscutellarein. [f] 3,5,6,7,8,3′,4′-heptamethoxyflavone. [g] Tangeritin/5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone.

extracted in a large separatory funnel with 200 mL of hexane. The mixture was vigorously shaken and the hexane/oil phase removed. This was repeated until the hexane phase was colorless (signifying the complete removal of the peel oil). The hexane-extracted solids were recovered as the material that sedimented between the hexane and water phases after centrifugation of the sample at 12000$g_{max}$. Resuspension of the solids in 200 mL of water and recentrifugation removed most of the remaining hexane. The resulting solids were initially air-dried and further dried at 70 °C.

Flavonoids in commercially produced wax (Koster Keunen Inc., Sayville, NY) from cold-pressed orange peel oil were extracted from a 2-propanol mixture of the wax solids. Three grams of wax was shaken in 25 mL of 2-propanol overnight and then mixed for 12 h with 25 mL of DMSO/methanol (1/1 v/v). The flavonoid-containing extract was recovered as the supernatant after centrifugation at 12000$g_{max}$ for 15 min.

**Chromatography System**. The flavonoids were analyzed by a Shimadzu LC-600 high-performance liquid chromatography (HPLC) system with a CR601 Chromatopac Integrator and SPD6AV variable-wavelength UV−vis detector. The column used to separate the individual flavonoids was a Whatman Partisil 5 ODS 3 analytical column. Elution was accomplished by a two-solvent gradient system as previously described by Kanes et al. (1993). The initial solvent was 80% 0.01 M phosphoric acid in 20% methanol (v/v). The final solvent was 100% methanol. The linear gradient was run over 60 min at 1.0 mL min$^{-1}$. The column was operated at room temperature. The flavonoids were detected at 285 nm. The flavonoid levels were calculated by comparing the integrated peak areas of the individual compounds to that of a standard curve prepared from the corresponding standards. Assignments of the flavonoid peaks in the HPLC chromatograms of the peel, molasses, and peel oil extracts were further verified by comparison of the UV spectra of the individual peaks to those of the flavonoid standards. UV spectra were obtained by monitoring the chromatograms with a Hewlett-Packard Series 1050 photodiode array detector with an attached Hewlett-Packard analysis computer and data storage system. Flavonoid standards were obtained from authentic flavonoid samples isolated by R. Horowitz and B. Gentili, USDA, Pasadena, CA, and J. Tatum, USDA, Winter Haven, FL.

## RESULTS

The flavonoids in orange peel are comprised primarily of flavanone glycosides (narirutin 4′-*O*-glucoside, eriocitrin, narirutin, hesperidin, isosakuranetin rutinoside) and polymethoxylated flavone aglycons (sinensetin, hexa-*O*-methylquercetagetin, nobiletin, hexa-*O*-methylgossypetin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, tetra-*O*-methylscutellarein, tangeritin, and 5-hydroxy-3,7,8,-3′,4′-pentamethoxyflavone) (Horowitz and Gentili, 1977). As shown in Table 1, the flavanone glycosides occur in the highest concentrations, with hesperidin, the main flavonoid in orange peel, occurring at approximately 2% (19 000−21 000 ppm) dry weight in Valencia and Ambersweet peel. Moderately higher concentrations of hesperidin occurred in navel orange peel. While the Valencia and navel orange peel flavonoid compositions were qualitatively similar, differences were observed in the late-eluting polymethoxylated flavones in the Temple and Ambersweet peel. The only polymethoxylated flavone that was detected in the Ambersweet peel was tangeritin. The flavone aglycons in the Temple orange peel were similar to those in Valencia peel with the exception of the absence of 3,5,6,7,8,3′,4′-heptamethoxyflavone in the Temple peel extract.

**Table 2. Quantitation of Flavonoids in DMSO/Methanol-Extracted, Filtered, Valencia Peel Juice and Filtered, Nonextracted Valencia Peel Molasses[a]**

| compound | extracted Valencia peel juice | filtered molasses |
|---|---|---|
| narirutin 4′-*O*-glucoside | 60 (0.071)[b] | 933 ± 19 (1.117) |
| eriocitrin | 145 (0.144) | 787 ± 86 (0.78) |
| narirutin | 116 (0.058) | 2050 ± 189 (1.03) |
| hesperidin | 408 (0.021) | 961 ± 88 (0.050) |
| isosakuranetin rutinoside | 27 (0.015) | 256 ± 21 (0.138) |
| sinensetin | 69 (0.226) | 271 ± 24[c] (0.89) |
| HQ/HG[d] | 18 (0.220) | |
| nobiletin/TETRA[e] | 98 (0.191) | 331 ± 29 (0.65) |
| HEPTA[f] | 42 (0.187) | 110 ± 10 (0.49) |
| T/5-OH[g] | 12 (0.110) | 31 ± 3 (0.28) |

[a] Results of analyses, run in triplicate, are reported as ppm. [b] Ratio of flavonoid levels in extracted peel juice vs levels in dry Valencia orange peel. [c] Combined value for sinensetin + HQ/QG. [d] Hexa-*O*-methylquercetagetin/hexa-*O*-methylgossypetin. [e] Tetra-*O*-methylscutellarein. [f] 3,4,5,6,7,3′,4′-Heptamethoxyflavone. [g] Tangeritin/5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone.

The flavonoid levels of filtered Valencia orange peel juice, generated during the pressing and processing of peel byproduct, are listed in Table 2. The ratios of the levels of the individual flavonoids in the peel juice, relative to the levels in the fresh peel, show that there were significant losses in the water-insoluble glycosides (narirutin, hesperidin, and isosakuranetin rutinoside). In contrast, the relative levels of the more soluble flavone aglycons were constant and probably reflected the sample dilution occurring during in the peel-to-juice conversion. Similarly, hesperidin levels in filtered orange peel molasses (concentrated, limed peel juice) were also sharply lower (Table 2) in relation to the other flavonoids in orange peel.

**Table 3. Quantitation of Flavonoids in DMSO/Methanol-Extracted Valencia Orange Peel Juice Molasses from Three Commercial Sources[a]**

| compound | elution time (min) | A | B | C |
|---|---|---|---|---|
| narirutin 4′-*O*-glucoside | 17.208 | 631 ± 28 | 1130 ± 16 | 982 ± 88 |
| eriocitrin | 20.103 | 494 ± 16 | 926 ± 57 | 1011 ± 59 |
| narirutin | 23.428 | 1350 ± 69 | 2460 ± 109 | 1772 ± 115 |
| hesperetin 7-*O*-glucoside | 24.293 | 4770 ± 219 | | 1142 ± 59 |
| hesperidin | 25.072 | 4840 ± 376 | 8017 ± 650 | 7218 ± 242 |
| | 29.463 | 233 ± 21 | | |
| isosakuranetin rutinoside | 30.398 | 340 ± 23 | 805 ± 117 | 471 ± 10 |
| naringenin | 31.003 | 304 ± 26 | | |
| hesperetin | 32.163 | 160 ± 33 | 341 ± 36 | |
| | 33.39 | 169 ± 33 | 455 ± 55 | |
| sinensetin/HQ/HG[b] | 41.177 | 362 ± 7 | 307 ± 16 | 511 ± 49 |
| nobiletin/HEPTA/TETRA[c] | 43.737 | 510 ± 13 | 516 ± 19 | 729 ± 74 |
| T/5-OH[d] | 46.033 | 78 ± 5 | 56 ± 4 | 82 ± 6 |

[a] Results of analyses, run in triplicate, are reported as ppm. [b] Hexa-*O*-methylquercetagetin/hexa-*O*-methylgossypetin. [c] 3,4,5,-6,7,3′,4′-Heptamethoxyflavone/tetra-*O*-methylscutellarein. [d] Tangeritin/5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone.

Extraction of the nonfiltered molasses, including the solids distributed in the molasses with DMSO/methanol (1/1) resulted in the solubilization and recovery of most of the insoluble hesperidin that was unaccounted for in the peel juice and filtered molasses (Table 3). A compound, hesperetin 7-*O*-glucoside, not originally present in orange peel, was present in two of the commercial molasses samples. This compound was generated from either chemical or enzymatic hydrolysis of the terminal rhamnose from the hesperidin molecule. The total concentrations of hesperidin and hesperetin 7-*O*-glucoside (8662 ± 684 ppm) were similar in each of the molasses samples. Small increases occurred in the levels of flavone aglycons in the molasses, thus demonstrating the presence of the orange peel flavone aglycons in the insoluble portions of the orange peel molasses.

In addition to the presence of hesperetin 7-*O*-glucoside in molasses, there were low levels of several other compounds with retention times (31.0, 32.2, and 33.4 min) expected for flavanone aglycons that occurred in molasses (Table 3) and peel juice samples (data not shown). These flavonoids were not originally present at these levels in orange peel, and the presence of these compounds is further evidence of glycosidic hydrolysis occurring during peel juice extraction and molasses production.

Centrifugation of orange peel molasses resulted in the recovery of a large portion of the molasses solids. Analysis of the DMSO/methanol extracts of this material from the three commercial sources showed a dry weight concentration of 5.9−7.3% hesperidin (Table 4). Also in Table 4 are the flavonoid levels of the final, commercially prepared dried peel byproduct containing the dried, pressed peel recombined with the peel molasses.

The composition of the flavonoids of Valencia peel cold-pressed oil and wax is listed in Table 5. In the cold-pressed peel oil the flavanone glycosides are absent, whereas the more hydrophobic flavone aglycons were preferentially extracted. The commercial wax, recovered from the cold-pressed oil, reflects the oil composition and is, in fact, extremely high in the flavone aglycons, particularly nobiletin. In a separate cold-pressed orange peel oil sample, the solids present in the oil were exhaustively extracted with hexane to remove contamination by the oil. Analysis of the hexane-extracted oil solids showed extremely high concentrations of nobiletin (32%) and other flavone aglycons (Table 5). The partial loss of these compounds, shown by the large differences in the composition of dried peel pellet and molasses (Table 4), can be linked to the accumulation of the flavone aglycons in the cold-pressed oil products.

**Table 4. Quantitation of Flavonoids in Molasses Solids and Commercial Dried Orange Peel Pellet[a]**

| compound | molasses solid | peel pellet |
|---|---|---|
| narirutin-4′-*O*-glucoside | 1292 ± 82 | 680 ± 16 |
| eriocitrin | 1465 ± 60 | 576 ± 24 |
| narirutin | 4785 ± 155 | 2153 ± 17 |
| hesperetin-7-*O*-glucoside | 6620 ± 181 | 10185 ± 254 |
| hesperidin | 65642 ± 2137 | 18090 ± 104 |
| isosakuranetin rutinoside | 3966 ± 258 | 1230 ± 167 |
| naringenin | 623 ± 55 | 616 ± 8 |
| hesperetin | 956 ± 328 | |
| sinensetin/HQ/HG[b] | 800 ± 33 | 281 ± 8 |
| nobiletin/HEPTA/TETRA[c] | 1741 ± 38 | 498 ± 17 |
| T/5-OH[d] | 464 ± 16 | 99 ± 5 |

[a] Results of analyses, run in triplicate, are reported as ppm. [b] Hexa-*O*-methylquercetagetin/hexa-*O*-methylgossypetin. [c] 3,4,5,-6,7,3′,4′-Heptamethoxyflavone/tetra-*O*-methylscutellarein. [d] Tangeritin/5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone.

**Table 5. Quantitation of Flavonoids in Cold-Pressed Peel Oil, Commercial Peel Oil Wax, and Hexane-Extracted Oil Solids**

| compound | hexane-extracted oil solids | cold-pressed peel oil | commercial peel wax |
|---|---|---|---|
| sinensetin | 62113 ± 871 | 399 ± 78 | 1953 ± 81 |
| HQ/HG[b] | 679 ± 97 | 3578 ± 215 | |
| nobiletin/TETRA[c] | 316162 ± 7355 | 2710 ± 246 | 17656 ± 1679 |
| HEPTA[d] | 4547 ± 558 | 44473 ± 4706 | |
| T[e] | 14873 ± 1037 | 812 ± 91 | 13238 ± 706 |
| 5-OH[f] | 9304 ± 1045 | 419 ± 44 | 4264 ± 253 |

[a] Results of analyses, run in triplicate, are reported as ppm. [b] Hexa-*O*-methylquercetagetin/hexa-*O*-methylgossypetin. [c] Tetra-*O*-methylscutellarein. [d] 3,4,5,6,7,3′,4′-Heptamethoxyflavone. [e] Tangeritin. [f] 5-Hydroxy-3,7,8,3′,4′-pentamethoxyflavone.

## DISCUSSION

Annual orange juice processing in Florida generates approximately 3.3 million tons of peel (Florida Citrus Processors Association, 1994). This is converted to a number of commercial byproducts, most of which bring marginal profit to the citrus industry. For the bulk of the material, this conversion is largely done to facilitate disposal of the peel. In spite of this current practice, it has long been recognized that orange peel represents a rich source of natural products important to human health and nutrition. Nutritional products already recovered from peel include pectin, citric acid, and ethanol from the fermentation of peel molasses. Recent approaches to developing additional value-added products from processed orange peel have placed renewed emphasis on the recovery of carbohydrates, other than pectin, from the peel (Grohmann and Balwin, 1992; Grohmann et al., 1994) and on the recovery of a number of potentially important secondary metabolites, including primarily limonoid and flavonoid compounds. The limonoid compounds in citrus peel and seeds have recently been found to have important pharmacological properties (Lam and Hasegawa, 1989; Lam et al., 1989a,b; Miller et al., 1989, 1992) as well as potential in the use as an insect antifeedant for agricultural crops

(Alford and Bentley, 1986). While these compounds are found in the highest concentrations in seeds, high levels also occur in the peel and in the orange peel molasses (Maier et al., 1980, Hasegawa et al., 1989).

The analysis of the flavonoid levels in orange peel byproducts reported here shows that high flavonoid concentrations exist in the byproducts and that distinctive distributions of the two main flavonoid classes occur in the different byproducts. The flavanone glycosides occur in relatively high concentrations in the peel, commercial dried peel pellets, and insoluble molasses solids. The presence of these compounds, particularly hesperidin, in the molasses solids is due to the extremely limited solubility and, hence, precipitation during peel processing. The precipitation of hesperidin results in the enrichment of hesperidin in the molasses solids, and this consequent enrichment represents a potential commercial source of hesperidin. The low levels of the polymethoxylated flavones in the peel and molasses have discouraged the commercial recovery of these compounds from these sources. However, this obstacle is alleviated by the fact that these compounds are selectively enriched in the cold-pressed peel oil and, in particular, the wax. The light-density solids recovered from the cold-pressed oil contained nearly 32% nobiletin after hexane extraction. Flavonoids are of tremendous interest as phamacological agents in human health and nutrition, as well as components of other manufacturing applications. The recovery of the diverse flavonoids in citrus represents an economically important source of these naturally occurring compounds, and the results shown in this paper demonstrate that, as a result of the chemical and solubility properties of the different sets of orange peel flavonoids, the recovery of these compounds in peel byproducts is greatly facilitated.

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