Grapefruit - Drug Interaction: Isolation, Biological Activities of Furocoumarins and Their Variation due to Pre- and Post-harvest Factors

Bhimanagouda S. Patil
Overview

- What is food-drug interaction?
- Grapefruit-drug interaction
- Media: GFJ & drug interaction
- Isolation of furocoumarins
- Inhibition of CYP enzymes by GFJ
- Benefits of furocoumarins (FC)
- Variation of FC by pre and post harvest factors
Drug metabolism

ORAL DOSE

GI TRACT

CYP

PORTAL CIRCULATION

LIVER (CYP3A)

SYSTEMIC CIRCULATION

Greenblatt et. al., Clinical Psychopharmacology 21(4):357-359, 2001
Movement of drugs through the body by:

- Absorption
- Distribution
- Metabolism
- Excretion

Food-drug interaction
Food-drug interaction

- **Food-drug interaction:**
  - Is a broad term that includes interaction of food-drug and their effect.

- **A food-drug interaction can:**
  - prevent a medicine from working the way it should
  - cause a side effect from a medicine to get worse or better
  - cause a new side effect
Food-drug interaction

• Give some examples of food-drug interaction you commonly come across?
Examples of Food-drug interaction

- Vitamin K rich foods → Warfarin
  Examples: spinach, turnip greens

- Monoamine oxidase inhibitors (MAOIs)
  → metabolism of Tyramine
  Examples of MAOI: Resveratrol (wine & grape skin), Curcumin (Turmeric), Harmine alkaloid (Coffee), Catechin (Tea)

Exp. of Food-drug interaction

- Tea and Coffee \(\rightarrow\) Iron supplements
  - Eggs, dairy products

- High fiber diet \(\downarrow\) Absorption of antidepressants
  - Wheat, bran, oatmeal

- Alcohol \(\uparrow\) Produce additive toxicity

*J. Pharmacy Soc. of Wisconsin: NOV/DEC 1998, 28-35*
Grapefruit
Drug Interaction
History

Interaction of citrus juices with felodipine and nifedipine

David G. Bailey  J. David Spence
Claudio Munoz  J. Malcolm O. Arnold

• FIRST REPORT ON GRAPEFRUIT JUICE AND DRUG INTERACTION

• Published in the Journal of LANCET 1991

• Results conclude GFJ increase the bioavailability of tested drugs

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Breakthrough</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEAR</td>
<td>Breakthrough</td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
</tr>
</tbody>
</table>
• GFJ interacts with many prescription drugs which CAN cause potentially serious side effects

• Interaction listed on the medication labels.
• Woman consumed GFJ along with oral contraceptive

• Developed a blood clot in the limb

• Almost risked losing the limb.

• Report published in LANCET and speculated the role of GFJ-drug interaction.

• DRAWBACKS: NO PROOF, Single patient
What is Grapefruit Drug Interaction?

- Concurrent intake of GFJ and certain drugs increases the plasma concentrations of drugs from 1.5 to 15 times.

- GFJ Inhibits the drug metabolizing enzyme system cytochrome P450, P-glycoprotein and OATP.
What is GFJ-Drug Interaction?
Bioavailability of Drugs
Bioavailability of Drugs

Greenblatt et. al., Clinical Psychopharmacology 21(4):357-359, 2001
## Drugs Influenced by GFJ

<table>
<thead>
<tr>
<th>Drug</th>
<th>Increase in Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Felodapine</td>
<td>~3 Fold</td>
</tr>
<tr>
<td>Cisapride</td>
<td>~1.4 Fold</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>~1.5 Fold</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>~2 Fold</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>~2.5 Fold</td>
</tr>
<tr>
<td>Buspirone</td>
<td>~9 Fold</td>
</tr>
<tr>
<td>Lovastatin/Simvastatin</td>
<td>~10 Fold</td>
</tr>
</tbody>
</table>

and....many more

*Greenblatt et. al., Clinical Psychopharmacology 21(4):357-359, 2001*
**In vivo evidence.....**

**OBJECTIVE**: Whether furocoumarins mediate the GFJ felodipine interaction?

**Design**: 18 healthy volunteers ingested felodipine (10 mg) with 1 of the 3 juices (240 mL).

**RESULTS**: conc. of felodipine were significantly greater with consumption of GFJ than with that of orange or furocoumarin-free GFJ.

*Paine et al., Am J Clin Nutr May 2006 vol. 83 no. 5 1097-1105*
In vivo evidence.....
In vivo evidence.....

Plasma felodipine concentration time profile

Drug enters the GI

Drug is not metabolized in the stomach

After absorption, drug enters the portal vein

In the Liver: the drug is further metabolized: 45% is unmetabolized as compared to 15% without GFJ

In the small bowel GFJ inhibits CYP3A4: 90% is Unmetabolized

What are the components in GFJ that influence drug interaction....?
GFJ: Components

- Initial reports speculated role of naringin and naringenin in drug interaction
- *In vitro* tests on human liver models suggest flavonoids do not inhibit CYP3A4

![Graph showing inhibition of CYP3A4](image)

In *in vivo* test similar results were noticed.

**GFJ: Components**

- AUC was measured after administering felodipine and
  - a) Supernatant
  - b) Particulate

<table>
<thead>
<tr>
<th>Component</th>
<th>Naringin (mg)</th>
<th>DHB (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>148</td>
<td>1.85</td>
</tr>
<tr>
<td>Particulate</td>
<td>7</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Fuhr et al., *Clin Pharmacol Therap* 1998: 64, 248-256
GFJ: Components

AUC (0-12 h) (nmol. h/L)

Fuhr et al., Clin Pharmacol Therap 1998: 64, 248-256
Grapefruit and medication: A cautionary note

Grapefruit and grapefruit juice are healthful, providing enough vitamin C, potassium, dietary fiber, and other nutrients to earn the American Heart Association’s “heart-check” mark. That’s the good news. The bad news is that grapefruit juice can interact with dozens of medications, sometimes dangerously.

Doctors are not sure which of the hundreds of chemicals in grapefruit are responsible. The leading candidate is furanocoumarin. It is also found in Seville (sour) oranges and tangelos; although these fruits have not been studied in detail, the guidelines for grapefruit should apply to them as well.

Grapefruit’s culprit chemical does not interact directly with your pills. Instead, it binds to an enzyme in your intestinal tract known as CYP3A4, which reduces the absorption of certain medications. When grapefruit juice blocks the enzyme, it’s easier for the medication to pass from your gut to your bloodstream. Blood levels will rise faster and higher than normal, and in some cases the abnormally high levels can be dangerous.

A variety of medications can be boosted by grapefruit juice; the table below lists some of the most important along with related drugs that are less likely to be influenced.
GFJ: other components

Bioactive Furocoumarins in Grapefruit
6’, 7’ Dihydroxybergamottin

Bergamottin
Paradisin A
Paradisin B
Paradisin C
Epoxy-bergamottin
Bergaptol
Geranylcoumarin
GF-1, GF-2

Imperatonin
Pimpenellin
Isopimpenellin
Isolation and Purification of Furocoumarins from Grapefruit Juice
Extraction of Grapefruit Juice

• Isolation and purification.

• 300 L of juice was extracted with Ethyl acetate to get crude mixture, which was reconstituted in methanol.

• 3g of extract was loaded on to Prep-HPLC column and eluted with aqueous methanol.
GFJ Concentrate
  ↓ Diluted with water
  ↓ Extracted with ethyl acetate
Crude extract
  ↓ Column chromatography
  ↓ Eluted with HEX, HEX:EtOAc & EtOAc

Prep- HPLC
Eluted with MeOH and H₂O

Fraction -1 2 3 4 5 6 7
  ↓ 1 2 3
  ↓ (340 mg) (19 mg) (310 mg)
Isolation of Furocoumarins by PREP HPLC

Grapefruit Concentrate Juice (1L) + Distilled Water (2.4L)

Grapefruit juice + Ethyl Acetate (1:2)

Leave for 2 Hours

Organic layer + Juice Sediment

Twice

Concentrate by Rotary Evaporation

Reconstitute in Methanol

Elute with Methanol

Prep HPLC

LCMS

NMR
HPLC Profile of Crude mixture

Retention Time

Minutes

mAU

0 10 20 30 40 50 60

mAU

0

1000

2000

3000

DHB

Bergaptol

Bergamottin

Paradisin A
Preparative HPLC separation

Paradisin A

Paradisin B

Bergamottin
Crude Mixture

Dihydroxybergamottin

Paradisin A

Bergamottin
NMR Profile of Paradisin A

H⁺ NMR

¹³C NMR

2D NMR
Bergapten
Mass spectrum of bergapten
$^1$H and $^{13}$C NMR spectra of Bergamottin
Mass spectra of bergamottin

Voyager Spec #1 [BP = 339.2, 10766]

Bergamottin

217.22
242.39
299.29
+H
321.29
339.25

Bergapten
Heptamethoxyflavone

% Intensity
Inhibitory Property of GFJ and Furocumarins on Human Cytochrome P450 Enzymes
CYP P450 3A4 enzymes

- Involved several reactions such as Phase I.
- Multiple forms exists
- Isoforms interact with the phytochemicals
- Inhibited reversibly and/or irreversibly by grapefruit juice components.
CYP P450 enzymes system

• 60% of the marketed drugs are metabolized by CYP 3A4, 2C9 and 2D6

Rendic and Di Carlo 1997
Inhibition of CYP3A4 by GFJ and pummelo

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
Inhibition of CYP2C9 by GFJ and pummelo

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
Inhibition of CYP2C9 by GFJ and pummelo

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
Inhibition of CYP3A4 by furocoumarins

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
Inhibition of CYP2C9 by furocoumarins

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
Inhibition of CYP2D6 by furocoumarins

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
**IC$_{50}$ values (µm) of furocoumarins for major drug metabolizing enzymes**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CYP3A4</th>
<th>CYP2C9</th>
<th>CYP2D6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHB</td>
<td>9.77</td>
<td>1.58</td>
<td>5.63</td>
</tr>
<tr>
<td>Paradisin</td>
<td>0.11</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>Bergamottin</td>
<td>22.91</td>
<td>4.508</td>
<td>11.74</td>
</tr>
<tr>
<td>Bergaptol</td>
<td>25.82</td>
<td>9.923</td>
<td>37.33</td>
</tr>
<tr>
<td>Geranylcoumarin</td>
<td>53.47</td>
<td>21.51</td>
<td>56.21</td>
</tr>
</tbody>
</table>

Girennavar et al., J FOOD SCI. Vol. 72, Nr. 8, 2007
GRAPEFRUIT JUICE & BREAST CANCER Controversy
Eating grapefruit every day could raise the risk of developing breast cancer by almost a third, US scientists say.
Prospective study: Role of GFJ in breast cancer

• **Rationale:**
  
  – CYP P450 3A4 (CYP3A4) is involved in the metabolism of oestrogens.
  
  – GFJ is an inhibitor of CYP3A4, increases plasma oestrogen concentrations.
  
  – Well established that oestrogen is associated with breast cancer risk

*Does consumption of GF influence breast cancer in postmenopausal woman?*

Prospective study: Role of GFJ in breast cancer

Prospective cohort:

• Study size 50,000 postmenopausal women

• 1657 breast cancer cases

• GF (1/4th fruit /day) intake increased risk of breast cancer

• RR=1.30, 95% CI 1.06-1.58

• Similar trend noticed in oestrogen + progestin therapy

Are there any beneficial properties of furocoumarins?
Citrus: Breeding for reducing furocoumarins

Furocoumarins: The

GOOD

• Antiproliferative
• Enhances bioavailability of certain low bioavailable drugs
• Inhibits biofilm
• Used in treatment of psoriasis

&

BAD

• Inhibits human intestinal enzyme cytochrome P450
• Potential for toxicity
• Toxic to fish
• Early clinical trial, study conducted at University of Chicago Medical Center

• 8 oz of GFJ intake along with the drug Rapamycin increase the drug levels allowing lower dose administration.

• Similar combination can be used for treating various types of cancer.
Furocoumarins: beneficial properties

1) Increases bioavailability of drugs
2) Antiproliferative
3) Attenuates TNF-α-stimulated endothelial molecule expression.
4) Inhibit biofilm in bacteria
5) Induce GST
GFJ increases bioavailability of low bioavailable drugs

- **Saquinavir**: Is a potent HIV protease inhibitor drug
- **Problem:**
  - Very low bioavailability
  - Expensive

GFJ increases bioavailability of low bioavailable drugs

Grapefruit Juice (400 mL)  Water (400 mL)

Intravenous (12 mg)  ORAL (600 mg)

Serial blood samples

Intravenous (12 mg)

Plasma concentration ng/mL

ORAL (600mg)

Water
GFJ

2 Fold increase

Furocoumarins: HL-60 Differentiation-Inducing Compounds

Furocoumarins: HL-60 Differentiation-Inducing Compounds

Bergamottin attenuates TNF-α-stimulated endothelial molecule expression

Effects of orally administered imperatorin and isopimpinellin on GST activity.

Kleiner et al., Carcinogenesis 2001;22:73-82
GFJ & furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria

Inhibition of AI-1 activity

GFJ & furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria

GFJ & furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria

<table>
<thead>
<tr>
<th>Treatment</th>
<th>E. coli O157:H7</th>
<th>S. typhimurium</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total biofilm (OD&lt;sub&gt;590&lt;/sub&gt;)</td>
<td>% Inhibition</td>
<td>Total biofilm (OD&lt;sub&gt;590&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Control</td>
<td>1.32 ± 0.11</td>
<td></td>
<td>1.16 ± 0.03</td>
</tr>
<tr>
<td>DHB</td>
<td>0.39 ± 0.06</td>
<td>71.9</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td>Bergamottin</td>
<td>0.55 ± 0.08</td>
<td>58.3</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Rio red juice</td>
<td>0.49 ± 0.16</td>
<td>64.7</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td>Marsh white juice</td>
<td>1.18 ± 0.01</td>
<td>10.6</td>
<td>1.13 ± 0.05</td>
</tr>
</tbody>
</table>

Probable clinical applications of GFJ

• Grapefruit juice may provide a non-toxic and inexpensive alternative to drugs that are used to reduce cyclosporin dose. (Yee GC, Lancet. 1995 Apr 15;345(8955):955-6.)


• Super pill?
Furocoumarins:
Variation due to Pre- and Post-harvest Factors
Pre harvest and Post harvest

- Variation: commercial products
  varieties
  location
  Season
- Storage
- Packaging
- Degreening
- Conditioning
- Processing techniques

-Breeding strategies
### Levels of FC in different commercial products

<table>
<thead>
<tr>
<th>Commercial product</th>
<th>n</th>
<th>White/Pink</th>
<th>GF-I-1 (ng/ml)</th>
<th>GF-I-2 (ng/ml)</th>
<th>GF-I-4 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropicana</td>
<td>8</td>
<td>White</td>
<td>363.3±34.1</td>
<td>5455.1±492.2</td>
<td>292.4±41.5</td>
</tr>
<tr>
<td>Dole</td>
<td>5</td>
<td>White</td>
<td>348.9±15.8</td>
<td>5105.8±98.5</td>
<td>288.8±19.5</td>
</tr>
<tr>
<td>Sunkist</td>
<td>5</td>
<td>White</td>
<td>346.4±24.7</td>
<td>5868.1±278.3</td>
<td>299.6±29.5</td>
</tr>
<tr>
<td>Welch</td>
<td>3</td>
<td>White</td>
<td>352.0±90.5</td>
<td>6991.7±1961.8</td>
<td>351.4±131.4</td>
</tr>
<tr>
<td>Kagome</td>
<td>3</td>
<td>White</td>
<td>202.8±173.6</td>
<td>4416.3±2812.6</td>
<td>250.4±167.7</td>
</tr>
<tr>
<td>Sunpokka</td>
<td>1</td>
<td>White</td>
<td>392.0</td>
<td>7400.3</td>
<td>374.6</td>
</tr>
<tr>
<td>Berri</td>
<td>1</td>
<td>White</td>
<td>312.5</td>
<td>9985.2</td>
<td>436.4</td>
</tr>
<tr>
<td>Takano</td>
<td>1</td>
<td>White</td>
<td>210.0</td>
<td>4136.6</td>
<td>162.7</td>
</tr>
<tr>
<td>Liby</td>
<td>1</td>
<td>White</td>
<td>54.3</td>
<td>4922.1</td>
<td>164.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>28</td>
<td>White</td>
<td><strong>321.4±95.2</strong></td>
<td><strong>5641.2±1538.1</strong></td>
<td><strong>296.3±84.9</strong></td>
</tr>
</tbody>
</table>

| Welch              | 1  | Pink       | 138.4          | 3888.5         | 272.2          |
| Sunpokka           | 1  | Pink       | 296.0          | 5452.1         | 308.5          |
| Texun              | 1  | Pink       | 120.1          | 3694.2         | 178.2          |
| Liby               | 1  | Pink       | N.D.³          | 1925.2         | 77.6           |
| **Total**          | 4  | Pink       | 184.8±96.7 b   | 3740.0±1443.3  | 209.1±103.5    |

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*Fukuda et al., J Chromatogr B, 741 (2000) 195–203*
Levels of DHB in Different Varieties of GFJ and Pummelo

Levels of Paradisin A in Different Varieties of GFJ and Pummelo

Levels of Bergamottin in Different Varieties of GFJ and Pummelo

Influence of Location and Varieties on Levels of DHB

Influence of Location and Varieties on Levels of Paradisin A

Influence of Location and Varieties on Levels of Bergamottin

Variation of DHB During Season

Seasonal Variation of Paradisin A

Seasonal Variation of Bergamottin

![Graph showing seasonal variation of Bergamottin concentration](image)

Effect of Storage and Processing on Furocoumarins

- Commercial GFJ production often involves processing at high temperature and mechanical peeling.

- Objective was to quantify the changes if any during processing.

GFJ Processing Plant at Mission, Texas
Levels of Furocoumarins in Hand Squeezed and Processed GFJ

## Effect of Different Storage Temperatures on Furocoumarins Level in Rio Red GFJ

Mean ± standard deviation, n=15

<table>
<thead>
<tr>
<th>Condition</th>
<th>Days</th>
<th>Levels of Furocoumarins (µg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DHB</td>
<td>Paradisin A</td>
</tr>
<tr>
<td>9 °C</td>
<td>0</td>
<td>1.201 ± 0.061&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.137 ± 0.078</td>
<td>0.088 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.108 ± 0.069</td>
<td>0.087 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>1.098 ± 0.068</td>
<td>0.076 ± 0.006</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>0</td>
<td>1.201 ± 0.061</td>
<td>0.09 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.091 ± 0.055</td>
<td>0.077 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.998 ± 0.014</td>
<td>0.069 ± 0.007</td>
</tr>
</tbody>
</table>

<sup>a</sup>Girennavar et al., Food Chem. 111 (2008) 387–392
Effect of Different Storage Temperatures on Furocoumarins Levels in Marsh White GFJ

<table>
<thead>
<tr>
<th>Condition</th>
<th>Days</th>
<th>Levels of Furocoumarins (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>DHB</strong></td>
</tr>
<tr>
<td>9 °C</td>
<td>0</td>
<td>1.704 ± 0.043&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.689 ± 0.076</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.618 ± 0.091</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>1.587 ± 0.068</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>0</td>
<td>1.704 ± 0.043</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.593 ± 0.099</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.467 ± 0.065</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± standard deviation, n=15

<table>
<thead>
<tr>
<th>Days</th>
<th>Levels of Furocoumarins (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHB</td>
</tr>
<tr>
<td>0</td>
<td>2.065 ± 0.081a</td>
</tr>
<tr>
<td>15</td>
<td>1.985 ± 0.092</td>
</tr>
<tr>
<td>30</td>
<td>1.885 ± 0.015</td>
</tr>
<tr>
<td>45</td>
<td>1.772 ± 0.064</td>
</tr>
<tr>
<td>60</td>
<td>1.598 ± 0.109</td>
</tr>
<tr>
<td>75</td>
<td>1.499 ± 0.089</td>
</tr>
<tr>
<td>90</td>
<td>1.411 ± 0.037</td>
</tr>
</tbody>
</table>

\( ^a \)Mean ± standard deviation, n=15

Cardboards

*Mean ± standard deviation, n=15*

<table>
<thead>
<tr>
<th>Days</th>
<th>Levels of Furocoumarins (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHB</td>
</tr>
<tr>
<td>0</td>
<td>2.125 ± 0.073&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>1.985 ± 0.092</td>
</tr>
<tr>
<td>30</td>
<td>1.818 ± 0.108</td>
</tr>
<tr>
<td>45</td>
<td>1.789 ± 0.089</td>
</tr>
<tr>
<td>60</td>
<td>1.5612 ± 0.089</td>
</tr>
<tr>
<td>75</td>
<td>1.4511 ± 0.061</td>
</tr>
<tr>
<td>90</td>
<td>1.4198 ± 0.183</td>
</tr>
</tbody>
</table>

Cartons

*Mean ± standard deviation, n=15

<table>
<thead>
<tr>
<th>Days</th>
<th>Levels of Furocoumarins (µg/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>DHB</td>
<td>Paradisin A</td>
<td>Bergamottin</td>
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<td>0.1009 ± 0.006</td>
<td>1.8655 ± 0.091</td>
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<td>15</td>
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<td>0.0981 ± 0.013</td>
<td>1.6813 ± 0.086</td>
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<tr>
<td>30</td>
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<td>45</td>
<td>1.8212 ± 0.064</td>
<td>0.0769 ± 0.017</td>
<td>1.3193 ± 0.045</td>
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<tr>
<td>60</td>
<td>1.7598 ± 0.109</td>
<td>0.0685 ± 0.009</td>
<td>1.2168 ± 0.021</td>
</tr>
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</table>

Degreening

- Ethylene gas used
- Early season citrus fruits and regreened Valencia oranges
- Destruction of chlorophyll and accumulation of carotenoids.
- Attractive fruit color
Effect of degreening on furocoumarins
Temperature Conditioning

- Low temperature used to maintain quality and slow respiration and metabolic activity after harvest

- Grapefruits are susceptible to chilling injury when stored at low temperature (\(< 10^\circ C\)) for prolonged period

- Chilling injury symptoms – browning of flavedo, pitting, surface lesions, water soaked tissues

- Temperature conditioning – 7 days at 16°C then stored at low temperature

- Other treatments used – Intermittent warming, waxes and vegetable oils, modified atmosphere packaging
House hold processing techniques

- Blending
- Juicing
- Squeezing
Furocoumarins

Uckoo et al., Journal of food science 77 (9), C921-C926
Citrus: Breeding for reducing furocoumarins

Diploid hybrids identified with low content of furocoumarins

Conclusions

• Various furocoumarins were isolated and characterized from GFJ

• GFJ and furocoumarins are potent inhibitors of CYP3A4, CYP2C9 and CYP2D6 isoenzymes. The order of inhibition potential is CYP2D6>CYP3A4>CYP2C9

• Furocoumarins levels are affected by pre- and post-harvest factors.