Bioavailability of Phyto(nutrients) in Orange Juice

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Beneficial effects on human health

• Previous nutritional studies with orange juice
• or isolated citrus flavonoids
  • shown several properties to protect health, such as:
    • Antioxidant effect
    • Lowering blood lipids and hypertension (CVD)
    • Lowering blood glucose and insulin (diabetes)
    • Protection against low grade of inflammation (Met Syn & obesity)

(Snyder et al, 2011; Aptekmann and Cesar, 2013; Basile et al, 2010; Morand et al, 2011; Rizza et al, 2011; Yoshida et al, 2013)
NUTRITIONAL CONTRIBUTION OF ORANGE JUICE

8 oz of orange juice

Bioavailability

USDA Nutrient Database (2005 e 2007) release 2.1b
First clinical trial: Bioavailability of OJ sugars

**NFC OJ**
- 95°C / 15s Stored at -20°C
- Thawed under refrigeration one day before the experiment

**Fresh OJ**
- Extracted with commercial juicer (MJ-20, Mulligan)
- 2h before starting the procedure subjects

**Placebo**
- Water + sugars (2 Suc : 1 Fru : 1 Glu)
- + citric acid (0.75%)
- + malic acid (0.25%)
- + 0.5% fiber + flavor & color with orange essence
- 2h before starting procedure subjects
# Orange Juices: Fresh, NFC and Placebo

<table>
<thead>
<tr>
<th>Composition (8fl oz)</th>
<th>NFC OJ</th>
<th>Fresh OJ</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>120</td>
<td>112</td>
<td>110</td>
</tr>
<tr>
<td>Total Carbohydrate, g</td>
<td>28</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Sucrose, g</td>
<td>15</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Glucose, g</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Fructose, g</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total dietary fiber, g</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100mL)</td>
<td>29</td>
<td>39*</td>
<td></td>
</tr>
<tr>
<td>Citrus Flavonoids (µg/mL)</td>
<td>202*</td>
<td>57</td>
<td>3.5 x</td>
</tr>
<tr>
<td>Naringin (µg/mL)</td>
<td>37*</td>
<td>5.5</td>
<td>6.6 x</td>
</tr>
<tr>
<td>Hesperidin (µg/mL)</td>
<td>155*</td>
<td>47</td>
<td>3.3 x</td>
</tr>
<tr>
<td>Polymethoxyflavones - PMF (µg/mL)</td>
<td>2.3</td>
<td>6.6*</td>
<td></td>
</tr>
<tr>
<td>Antioxidant capacity (µmol Trolox/100mL)</td>
<td>3.6</td>
<td>4.7*</td>
<td></td>
</tr>
</tbody>
</table>

No differences among OJs
Study Design: cross-over within-subjects
18 Healthy man (9) and woman (9), aged 26 ± 8, BMI = 22kg/m²

Phase One
NFC OJ (5 mL/kg weight)
10 oz. / person
Blood collection
Glucose
Insulin
Measures for 5hs

Phase Two
Fresh OJ (5 mL/kg weight)
10 oz. / person
Blood collection
Glucose
Insulin
Measures for 5hs

Phase Three
Placebo juice (5 mL/kg weight)
10 oz. / person
Blood collection
Glucose
Insulin
Measures for 5hs

7 days
7 days
7 days
Glucose blood metabolism (pharmacokinetic)

Glucose clearance after Fresh, NFC and placebo juices intake

[Graph showing glucose clearance over time for Placebo, Fresh OJ, and NFC]
Insulin curves (pharmacokinetic)

*Insulin blood clearance after Fresh, NFC and placebo juices intake*
Plasma bioavailability pharmacokinetics analysis

Bioavailability:
Quantity available of a nutrient inside body compartments: blood, organs, tissues, cell, etc

\[ AUC = \frac{1}{2} \sum_{i=1}^{n-1} (time_{0-24\text{hs}})(\text{Glu}_{0\text{min}} + \cdots + \text{Glu}_{300\text{min}}) \]
Area under the curve – after 5 hours
Sugar metabolites for sweetened drink, NFC and Fresh OJ

<table>
<thead>
<tr>
<th>Glucose, 5h</th>
<th>AUC (mg/h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4479</td>
</tr>
<tr>
<td>NFC</td>
<td>4257 (-5%)</td>
</tr>
<tr>
<td>Fresh OJ</td>
<td>3999 (-11%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin, 5h</th>
<th>AUC (mg/h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1878</td>
</tr>
<tr>
<td>NFC</td>
<td>1327 (-28%)</td>
</tr>
<tr>
<td>Fresh OJ</td>
<td>1364</td>
</tr>
</tbody>
</table>
Maximum Concentration (C max) after 5 hours

Control | NFC | Fresh OJ
---|---|---
Glucose, 5h
126 | 104 | 107

-16%

Control | NFC | Fresh OJ
---|---|---
Insulin, 5h
78 | 55 | 54

-30%

Cmax, µmol
Why the bioavailability of glucose from Orange juice is lower than that bioavailability of placebo juice?

8oz = 22g sugars =

5.5g glucose / 5.5g fructose / 11g sucrose
Bioavailability of OJ vs. sugary beverages

**OJ**: has intrinsic sugars that are delivered more slowly as part of the juice matrix

**Sucrase** activity is inhibited by free Glucose, decreasing digestion and absorption of sucrose

**Beverages w/ Sucrose**: the hydrolysis by sucrase releases free Glu/Fru

**HFCS**: Free 55% fructose, 42% glucose and 3% oligosaccharides

**Immediate source of fructose and glucose**

↑ fructose bioavailability and fructose adverse metabolic effects
Conclusions on Bioavailability of OJ Sugars

• NFC or Fresh OJ showed lower levels of glucose and insulin in the blood stream compared to placebo, meaning lower bioavailability of OJ sugars.
Bioavailability of Citrus Flavanoids

Major citrus flavonoids found in foods:
- hesperetin-7-O-rutinoside and 7-O-glucuronide
- naringenin-7-O-rutinoside, 7-O-glucuronide, 4-O-glucuronide

(Mullen et al; 2008)
Pharmacokinetics of Flavanone Glycosides after Ingestion of Single Doses of Fresh-Squeezed Orange Juice versus Commercially Processed Orange Juice in Healthy Humans

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**Purpose:** to determine pharmacokinetic and bioavailability of the NFC and Fresh orange juice flavonoids in healthy human subjects
### Citrus flavonoids and Polymethoxyflavones (PMF)

<table>
<thead>
<tr>
<th>Flavonoids, µg mL(^{-1})</th>
<th>NFC OJ</th>
<th>Fresh OJ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narirutin</td>
<td>36.5 ± 5.5</td>
<td>5.5 ± 0.91</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>154.6 ± 47.2</td>
<td>47.2 ± 4.03</td>
</tr>
<tr>
<td><strong>Polymethoxylyflavones (PMF)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinensetin</td>
<td>0.60 ± 0.03</td>
<td>1.77 ± 0.07</td>
</tr>
<tr>
<td>Nobiletin</td>
<td>0.84 ± 0.04</td>
<td>2.36 ± 0.19</td>
</tr>
<tr>
<td>Tetramethylscutellarein</td>
<td>0.35 ± 0.03</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td>Heptamethoxyflavone</td>
<td>0.35 ± 0.03</td>
<td>1.18 ± 0.10</td>
</tr>
<tr>
<td>Tangeretin</td>
<td>0.11 ± 0.02</td>
<td>0.45 ± 0.04</td>
</tr>
</tbody>
</table>


\(3 \times \uparrow\)
Study Design: cross-over within-subjects
24 Healthy men (12) and women (12), aged 27 ± 6, BMI = 24kg/m^2

Phase One

NFC OJ
(11.5 mL/kg weight)
3 glasses (8oz)/ person
Blood & Urine collection

HSPT
NRGN

Measured for 24hs

Phase Two

Fresh OJ
(11.5 mL/kg weight)
3 glasses (8oz)/ person
Blood & Urine collection

HSPT
NRGN

Measured for 24hs

30 days

Study Design: cross-over within-subjects
24 Healthy man (12) and woman (12), aged 27 ± 6, BMI = 24kg/m²

Primary citrus flavonoids

HSPT
NRGN

Transformation of primary compounds in Metabolites

As glucuronides and sulfates of HSPT and NRGN
Plasma concentration of flavanones metabolites: glucuronide HSPT, glucuronide HSPT sulfate, glucuronide NRG, and NRG sulfate

AUC$_{0-24h}$ (nmol/h/L) flavonoids metabolites in human blood

**HSPT GLUCURONIDE**
- Fresh: 192

**HSPT GLUC-SULFATE**
- NFC: 121
- Fresh: 71

**HSPT SULFATE**
- 15

**NGIN GLUCURONIDE**
- NFC: 245
- Fresh: 209

- 25% more abundant metabolites
- 4X
- 2 more abundant metabolites
### $C_{\text{max}}$ (nmol/L) Flavanoids Metabolites in Human Blood

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>NFC</th>
<th>Fresh OJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSPT Glucuronide</td>
<td>25</td>
<td>93</td>
</tr>
<tr>
<td>HSPT Gluc-Sulfate</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>HSPT Sulfate</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>NGIN Glucuronide</td>
<td>29</td>
<td>142</td>
</tr>
</tbody>
</table>

**Significance:**
- *: Statistically significant

**Note:**
- Fresh OJ vs NFC: 4 X for HSPT Glucuronide, 9 X for NGIN Glucuronide
Flavanones metabolites (nmol) in human urine 24h after consumption of OJ

Less excretion of metabolites from Fresh OJ vs. NFC
Estimates of Absorption and Bioavailability of flavanoids from NFC and Fresh Orange Juice

<table>
<thead>
<tr>
<th>Citrus Flavonoids</th>
<th>Dose</th>
<th>Blood</th>
<th>Urine</th>
<th>Absorption (AUC_{blood}/Dose)</th>
<th>Bioavailable (Absorbed-urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NFC OJ</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hesperitin, mg</td>
<td>47.5</td>
<td>0.37</td>
<td>1.95</td>
<td>3.80</td>
<td>1.85</td>
</tr>
<tr>
<td>Naringenin, mg</td>
<td>10.5</td>
<td>0.11</td>
<td>0.40</td>
<td>1.16</td>
<td>0.76</td>
</tr>
<tr>
<td>Total Flavanones</td>
<td>= 58.0</td>
<td>= 0.48</td>
<td>= 2.35</td>
<td>= 4.96</td>
<td>= 2.61</td>
</tr>
<tr>
<td><strong>Fresh OJ</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hesperitin, mg</td>
<td>14.4</td>
<td>0.13</td>
<td>0.55</td>
<td>1.30</td>
<td>0.75</td>
</tr>
<tr>
<td>Naringenin, mg</td>
<td>1.6</td>
<td>0.09</td>
<td>0.14</td>
<td>0.94</td>
<td>0.80</td>
</tr>
<tr>
<td>Total Flavanones</td>
<td>= 16.0</td>
<td>= 0.22</td>
<td>= 0.69</td>
<td>= 2.24</td>
<td>= 1.55</td>
</tr>
</tbody>
</table>
Citrus flavonoids bioavailable after consumption NFC and Fresh OJ (3 glasses 8oz.)
Conclusions: Bioavailability of Total Flavanones
NFC vs. Fresh OJ

- Total flavanones from NFC are 3.6 x higher than Fresh juice
- Absorption from NFC is 2.2 x higher than Fresh juice
- Bioavailability in NFC is 1.7 x higher than Fresh juice
Final thoughts about citrus sugars and flavonoids from OJ

- There is an influence of digestive factors plus gut microbiome on the time release of glucose to the blood stream, affecting the metabolic response of glucose and insulin.

- In addition, the presence of flavonoids in OJ, and absence of them in the placebo beverage, suggests that citrus flavonoids may be responsible for decreasing response of glucose and insulin, as previous studies have shown.

Hanhineva et al, 2010; Bock et al, 2012
Clinical Trials

Polymethoxyflavones

Gut Microbiome

Bioavailability
Thank you!