Polyphenols in beer: the role of xanthohumol and isoxanthohumol

Rosa M. Lamuela
University of Barcelona
Index

• What are polyphenols
• Polyphenols and health outcomes
• Polyphenols from beer
• Health effects of prenylflavonoids from beer
In 1991, key word: polyphenols
Only 23 papers about:

- Antioxidant effects *in vitro*
- Presence in food
- Antitumoral properties

On September 26 2014, Key word: polyphenols
1318 papers in 12 months

- Apoptosis
- Arthritis
- Inflammation
- Effect on testosterone
- Antibactericide
- Cosmoceutical
- ...
Nowadays they are not considered **nutrients** however they are bioactive compounds, since they perform important physiological functions in the organism.

Chiou, Y.-S. et al. 2014
• What are polyphenols
• Polyphenols and health outcomes
• Polyphenols from beer
• Health effects of prenylflavonoids from beer
• Isoxanthohumol reliable biomarker of beer consumption
Polyphenols and Cardiovascular Health

7447
55-80 years
Free of CVD but at high risk

FFQ 1 → FFQ 2 → FFQ n → CUMULATIVE polyphenol intake

502 polyphenols in 452 foods

Polyphenol-Explorer

Database on polyphenol content in foods

Beer and Health
Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study

A. Tresserra-Rimbau a, b, E.B. Rimm c, d, A. Medina-Remón a, b, M.A. Martínez-González b, e, R. de la Torre b, f, D. Corella b, g, J. Salas-Salvadó b, h, E. Gómez-Gracia b, i, J. Lapetra b, j, F. Arós b, k, M. Fiol b, l, E. Ros b, m, L. Serra-Majem b, n, X. Pintó b, o, G.T. Saez b, p, J. Basora b, q, J.V. Sorli b, r, J.A. Martínez b, s, E. Vinyoles b, t, V. Ruiz-Gutiérrez b, u, R. Estruch b, v, R.M. Lamuela-Raventós a, b, * on behalf of the PREDIMED Study Investigators
### Table 2: Association between quintiles of cumulative polyphenol intake (total and main groups) and incident CVD in the PREDIMED study.

<table>
<thead>
<tr>
<th>Total polyphenols (mg/d)</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols (mg/d)</td>
<td>562</td>
<td>701</td>
<td>800</td>
<td>917</td>
<td>1170</td>
<td></td>
</tr>
<tr>
<td>No. of CVD cases</td>
<td>66</td>
<td>49</td>
<td>58</td>
<td>49</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>No. of person years</td>
<td>5312</td>
<td>6668</td>
<td>6905</td>
<td>6629</td>
<td>5554</td>
<td></td>
</tr>
<tr>
<td>Age and sex adjusted</td>
<td>1.00</td>
<td>0.60 (0.38–0.95)</td>
<td>0.62 (0.39–0.97)</td>
<td>0.58 (0.36–0.91)</td>
<td>0.58 (0.36–0.93)</td>
<td>0.04</td>
</tr>
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<td>1.00</td>
<td>0.57 (0.36–0.92)</td>
<td>0.60 (0.38–0.95)</td>
<td>0.54 (0.34–0.87)</td>
<td>0.51 (0.30–0.84)</td>
<td>0.02</td>
</tr>
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<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
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<td>0.51 (0.30–0.84)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lignans (mg/d)</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignans (mg/d)</td>
<td>0.44</td>
<td>0.57</td>
<td>0.67</td>
<td>0.77</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>69</td>
<td>57</td>
<td>53</td>
<td>44</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>No. of person years</td>
<td>4625</td>
<td>6122</td>
<td>6899</td>
<td>6692</td>
<td>6530</td>
<td></td>
</tr>
<tr>
<td>Age and sex</td>
<td>1.00</td>
<td>0.61 (0.40–0.95)</td>
<td>0.55 (0.36–0.86)</td>
<td>0.57 (0.35–0.91)</td>
<td>0.51 (0.31–0.84)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.65 (0.41–1.01)</td>
<td>0.55 (0.35–0.87)</td>
<td>0.61 (0.37–0.99)</td>
<td>0.50 (0.29–0.85)</td>
<td>0.007</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00</td>
<td>0.84 (0.41–0.99)</td>
<td>0.54 (0.34–0.85)</td>
<td>0.60 (0.36–0.97)</td>
<td>0.51 (0.30–0.86)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* HR (95% CI).
* Additionally adjusted for smoking, BMI, alcohol, physical activity, family history of CVD, aspirin use, antihypertensive drugs, cardiovascular drugs, diabetes status, and total energy intake.
* Additionally adjusted for intake of protein, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and cholesterol.
## FLAVONOIDS AND CARDIOVASCULAR HEALTH

<table>
<thead>
<tr>
<th>Table 3</th>
<th>The relationship between CVD and cumulative flavonoids subclasses intake (in quintiles) in participants from the PREMID study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Q1</td>
</tr>
<tr>
<td><strong>Anthocyanins (mg/d)</strong></td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>69</td>
</tr>
<tr>
<td>No. of person years</td>
<td>5375</td>
</tr>
<tr>
<td>Age and sex</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.95 (0.65−1.40)*</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.15 (0.74−1.79)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.18 (0.76−1.84)</td>
</tr>
<tr>
<td>Dihydrochalcones (mg/d)</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>47</td>
</tr>
<tr>
<td>No. of person years</td>
<td>5036</td>
</tr>
<tr>
<td>Age and sex</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.73 (0.51−1.67)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.25 (0.78−1.99)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.24 (0.78−1.99)</td>
</tr>
</tbody>
</table>

| Flavanols (mg/d) | | | | | |
| No. of cases | 69 | 51 | 59 | 59 | 35 |
| No. of person years | 4841 | 6409 | 7058 | 6860 | 5900 |
| Age and sex | | | | | |
| Model 2 | 0.64 (0.43−0.94) | 0.65 (0.44−0.95) | 0.55 (0.37−0.82) | 0.33 (0.21−0.53) | <0.0001 |
| Model 3 | 0.65 (0.41−1.02) | 0.70 (0.44−1.09) | 0.57 (0.36−0.91) | 0.36 (0.20−0.63) | 0.0004 |
| Model 4 | 0.70 (0.44−1.10) | 0.77 (0.49−1.21) | 0.66 (0.41−1.05) | 0.40 (0.23−0.72) | 0.003 |

- **HR (95% CI)**
- **Model 2** - age, sex, smoking, BMI, alcohol, energy, physical activity, family history of CVD, aspirin use, antihypertensive drugs, cardiovascular drugs, and diabetes status.
- **Model 3** - model 2 plus intake of proteins, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and cholesterol.
## Phenolic Acids and Cardiovascular Health

Table 4: The relationship between CVD and cumulative phenolic acids subclasses intake (in quintiles) in participants from the PREDIMED study.

<table>
<thead>
<tr>
<th>Phenolic acids</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxybenzoic acids (mg/d)</td>
<td>6.9</td>
<td>12.9</td>
<td>17.8</td>
<td>24.1</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>69</td>
<td>62</td>
<td>47</td>
<td>55</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>No. of person years</td>
<td>5398</td>
<td>6603</td>
<td>6734</td>
<td>6853</td>
<td>5480</td>
<td></td>
</tr>
<tr>
<td>Age and sex</td>
<td>1.00</td>
<td>0.80 (0.54–1.17)</td>
<td>0.60 (0.40–0.90)</td>
<td>0.54 (0.36–0.82)</td>
<td>0.46 (0.29–0.71)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.82 (0.52–1.29)</td>
<td>0.65 (0.40–1.06)</td>
<td>0.59 (0.36–0.97)</td>
<td>0.37 (0.20–0.66)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00</td>
<td>0.91 (0.57–1.43)</td>
<td>0.74 (0.46–1.22)</td>
<td>0.73 (0.44–1.21)</td>
<td>0.47 (0.26–0.86)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hydroxycinnamic acids (mg/d)</td>
<td>138</td>
<td>207</td>
<td>252</td>
<td>316</td>
<td>422</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>61</td>
<td>50</td>
<td>42</td>
<td>59</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>No. of person years</td>
<td>5632</td>
<td>6486</td>
<td>6869</td>
<td>6914</td>
<td>5167</td>
<td></td>
</tr>
<tr>
<td>Age and sex</td>
<td>1.00</td>
<td>0.80 (0.53–1.20)</td>
<td>0.55 (0.35–0.87)</td>
<td>0.92 (0.62–1.36)</td>
<td>1.08 (0.72–1.63)</td>
<td>0.40</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.81 (0.51–1.29)</td>
<td>0.57 (0.34–0.96)</td>
<td>0.91 (0.58–1.42)</td>
<td>0.99 (0.62–1.58)</td>
<td>0.71</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00</td>
<td>0.79 (0.49–1.25)</td>
<td>0.58 (0.35–0.97)</td>
<td>0.86 (0.55–1.36)</td>
<td>0.93 (0.58–1.49)</td>
<td>0.93</td>
</tr>
<tr>
<td>Other phenolic acids (mg/d)</td>
<td>0.1</td>
<td>2.5</td>
<td>4.6</td>
<td>8.6</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>58</td>
<td>66</td>
<td>47</td>
<td>62</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>No. of person years</td>
<td>5100</td>
<td>5225</td>
<td>6571</td>
<td>7787</td>
<td>5385</td>
<td></td>
</tr>
<tr>
<td>Age and sex</td>
<td>1.00</td>
<td>1.11 (0.75–1.64)</td>
<td>0.69 (0.45–1.08)</td>
<td>0.79 (0.52–1.21)</td>
<td>0.73 (0.46–1.14)</td>
<td>0.10</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.31 (0.83–2.09)</td>
<td>0.75 (0.44–1.28)</td>
<td>0.88 (0.54–1.42)</td>
<td>0.74 (0.45–1.24)</td>
<td>0.11</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00</td>
<td>1.39 (0.87–2.22)</td>
<td>0.82 (0.48–1.39)</td>
<td>0.92 (0.57–1.51)</td>
<td>0.82 (0.49–1.39)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* HR (95% CI).

b Model 2: age, sex, smoking, BMI, alcohol, energy, physical activity, family history of CVD, aspirin use, antihypertensive drugs, cardiovascular drugs, and diabetes status.

c Model 3 - model 2 plus intake of proteins, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and cholesterol.
Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial

Anna Tresserra-Rimbau¹,², Eric B Rimm³, Alexander Medina-Remón²,¹⁷, Miguel A Martínez-González²,⁴, M Carmen López-Sabater¹,², María I Covaś²,⁵, Dolores Corella²,⁶, Jordi Salas-Salvadó²,⁷, Enrique Gómez-Gracia²,⁸, José Lapetra²,⁹, Fernando Arós²,¹⁰, Miquel Fiol²,¹¹, Emili Ros²,¹², Lluís Serra-Majem²,¹³, Xavier Pinto²,¹⁴, Miguel A Muñoz²,¹⁵, Alfredo Gea²,⁴, Valentina Ruiz-Gutiérrez²,¹⁶, Ramón Estruch²,¹⁷, Rosa M Lamuela-Raventós¹²* and on behalf of the PREDIMED Study Investigators
Polyphenol Intake and Mortality Risk

Product-Limit Survival Estimates

Survival

follow-up years

intake low medium high
Total polyphenol intake was significantly associated with a decrease (almost 40%) in all-cause mortality, after adjusting for all confounders. The dose-response trend suggested an L-shaped relationship.
Polyphenol Intake and Mortality Risk

Hazard ratios (95% CI) of total mortality for the highest vs. lowest quintiles of polyphenol intake.

*Isoflavones*
- HR 0.49; CI 0.28 to 0.84; P-trend=0.009

*Stilbenes*
- HR 0.48; CI 0.25 to 0.91; P-trend=0.04

*Lignans*
- HR 0.60; CI 0.37 to 0.95; P-trend=0.03
• What are polyphenols
• Polyphenols and health outcomes
• Polyphenols from beer
• Health effects of prenylflavonoids from beer
We analyzed the beer polyphenolic profile by High Resolution Mass Spectrometry (LC-LTQ-Orbitrap).

- 3 types of Beer: Marzen Bier, Pilsen and Lager.
- Full Scan Mode and Tandem MS/MS experiments with accurate mass measurements of the ions and the fragments.
- 47 phenolic compounds were detected, 7 of them were reported for the first time.
### Malt Polyphenols

**Hydroxybenzoic acids**
- Gallic acid
- Protocatechuic acid-\(O\)-hexoside
- Dihydroxybenzoic acid
- Protocatechuic acid
- Hydroxybenzoic acid
- Vanillic acid

**Hydroxycinnamic acids**
- Caffeic acid \(O\)-hexoside
- Caffeic acid
- 1-caffeoylquinic acid
- 3-caffeoylquinic acid
- 4-caffeoylquinic acid
- 5-caffeoylquinic acid
- Coumaric acid \(O\)-hexoside
- Feruloylquinic acid
- Sinapic acid-\(O\)-hexoside
- Sinapic acid
- Ferulic acid

**Alkylmethoxyphenols**
- 4-vinylguaiacol

**Hydroxyphenylacetic acids**
- Hydroxyphenylacetic acid

**Polyphenols**
- 70-80% of the beer polyphenols come from malt
Flavanols
- Catechin
- Catechin-<i>O</i>-hexoside
- Catechin-<i>O</i>-dihexoside
- Epicatechin

Flavonols
- Quercetin-<i>3</i>-<i>O</i>-glucoside
- Kaempferol-<i>O</i>-hexoside
- 3,7-dimethylquercetin

Miscellaneous
- Indole-3-carboxylic acid

Flavones
- Apigenin-<i>C</i>-hexoside-<i>O</i>-hexoside
- Apigenin-<i>C</i>-hexoside-pentoside
- Apigenin-<i>C</i>-hexoside
Hop Polyphenols

**α-acids and β-acids**
- co-Humulone
- ad-Humulone
- n-Humulone
- Iso-α-cohumulone
- Iso-α-adhumulone
- Iso-α-nhumulone
- Lupulone

**Prenylflavanoids**
- Isoxanthohumol
- Xanthohumol
- DesmethyIxanthohumol
- 8-prenylNaringenin
- 6-prenylNaringenin

30-20% of the beer polyphenols come from hops.

Beer and Health
Polyphenol Intake from one Drink

- 330 mL: 92 mg
- 150 mL: 48 mg
- 150 mL: 300 mg
Beer and Wine: a Metanalisi

Reduction of cardiovascular risk
- Wine: -32%
- Beer: -22%

Circulation 2002
• What are polyphenols
• Polyphenols and health outcomes
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• Health effects of prenylflavonoids from beer
Isomerization in brewing

95% of the prenylflavanoids found in hops are xanthohumol, desmethylxanthohumol, and 3-geranylchalconenaringenina. (Stevens et al, 1999)

High content in isoxanthohumol (prenylflavanone) and low content in xanthohumol (Stevens et al 1999)

Isoxantohumol is converted to 8-prenylnaringenin by an O-desmethylation catalyzed by gut microbiota. (Hanske L et al 2010, Possemiers S et al 2005)
Biological Activity of Beer Prenylflavanoids

**8-prenylNaringenin**

- Most potent phytostrogen known until date. It showed similar binding characteristics to ERα and ERβ *in vitro*. The oestrogenic activity of 8-prenylNaringenin was greater than that of established phyto-oestrogens such as coumestrol, genistein and daidzein. *(Milligan et al. 2000)*

- Owing to this phytoestrogenic activity, 8PN has been proposed as a treatment for menopausal symptoms, such as hot flashes and osteoporosis.
**Biological Activity of Beer Prenylflavanoids**

**Xanthohumol and Isoxanthohumol**

- Xanthohumol and isoxanthohumol have shown weak or no estrogenic activity.
- IX is considered to be a source of 8-prenylnaringenin, because it is metabolized to 8-prenylnaringenin in the intestinal tract by gut microbiota.
Urinary Isoxanthohumol as a Biomarker of Beer Consumption


Urinary Isoxanthohumol Is a Specific and Accurate Biomarker of Beer Consumption

Paola Quíser-Rada,1,4,5 Miriam Martínez-Huélamo,4,5 Gemma Chiva-Blanch,5,6 Olga Jáuregui,7 Ramon Estruch,5,6 and Rosa M. Lamuela-Raventós4,5,8

1Department of Nutrition and Food Science-XARTA-INSIA, School of Pharmacy, University of Barcelona, Barcelona, Spain; 5CIBER Physiopathology of Obesity and Nutrition, Institute of Health Carlos III, Madrid, Spain; and 6Department of Internal Medicine, Hospital Clinic, Institute of Biomedical Investigation August Pi i Sunyer, and 7Scientific and Technological Center, University of Barcelona, Barcelona, Spain
Urinary Isoxanthohumol as a Biomarker of Beer Consumption

Dose-response, randomized, cross-over clinical trial

20 male volunteers
0 mL
330 mL
660 mL
990 mL

20 female volunteers
0 mL
330 mL
495 mL
660 mL

5 days washout period before each intervention

8 hours after
Urinary excretion of Isoxanthohumol in male (A) and female (B) volunteers 8 hours after the intake of different volumes of beer.
In Free Living Population in PREDIMED

IX is really a good boomeraker that can be used in epidemiological studies to evaluate beer health outcomes.

... two beers, or not two beers...
• Polyphenols consumption decrease all cause of mortality and CVD
• Beer has been shown to be a good source of polyphenols and it is one of the main food contributor to hydroxybenzoic acid intake in the European Prospective Investigation into Cancer and Nutrition cohort study.
• High intake of hydroxibenzoics decrease cardiovascular risk by 53% in the PREDIMED population
• IX is a good biomarker that may be used in epidemiological studies to evaluate beer health outcomes
We would like to thank:
Visit our web site:

http://www.polyphenolresearch.com

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Collaborators

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THANK YOU
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