

up to 89% compared to catechins (irrespective of fluoride concentrations). The 6 subjects' salivary amylase was significantly lower after consumption of black or green tea versus water. A Japanese *in vitro* study applied tannins, catechins, caffeine and fluoride in isolation and in combination to tooth enamel exposed to acid<sup>37</sup>. The application of catechins and tannins reduced calcium release from the enamel by 35% and 40% respectively compared to control. Combining tannins and catechins and adding fluoride increased enamel resistance to acid by 98%. A review of the data in 2002 reflected that despite the significant publications on the positive effect of tea and tea flavonoids on oral health, there is lack of standardisation of methodology and emphasises the need for large, well-controlled clinical studies<sup>38</sup>.

### Fat oxidation and increased energy expenditure

A high green tea consumption containing catechins at 375 mg/d and caffeine at 150 mg/d has been shown to help increase energy expenditure and fat oxidation in both animal and human studies<sup>39</sup>. While caffeine by itself may enhance short-term fat oxidation and thermogenesis, the effects are likely to be apparent in individuals who are caffeine-deprived or non-users<sup>40,41</sup>. A number of long term studies have indicated that longer-term consumption of green tea components can have benefits for body weight or fat mass/distribution<sup>42,43</sup>. However a few other well-designed long-term studies have failed to show any effect<sup>44,45</sup>.

### Catechins and Cancer

The evidence for catechins, like many other plant actives e.g. isoflavones, and inverse correlations with cancer, is still in its early stages and much more clinical evidence is needed before any conclusions can be drawn. However, interest in catechins has increased due to their ability to effectively scavenge reactive oxygen species *in vitro* and possibly function indirectly as antioxidants through their effects on transcription factors and enzyme activities. The effects of black tea and green tea catechins on biomarkers of oxidative stress, especially oxidative DNA damage, appear very promising in animal models, but data on biomarkers of *in vivo* oxidative stress in humans are limited. A well-controlled human intervention study in 143 smokers showed that consumption of 4 cups of green tea per day for 4 months resulted in a 31% reduction in oxidative damage markers in the urine. These results suggest that regular green tea consumption may protect smokers from oxidative damage and could potentially reduce cancer risk or other diseases caused by free radicals associated with smoking<sup>46</sup>.

**In conclusion, tea flavonoids - catechins, thearubigins and theaflavins - have been shown to significantly increase plasma antioxidant capacity after acute ingestion and epidemiological evidence points strongly to an inverse association between tea consumption and heart health. Tea flavonoids are complex structures and the biomechanism of action has yet to be identified.**

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Correct at the time of going to press

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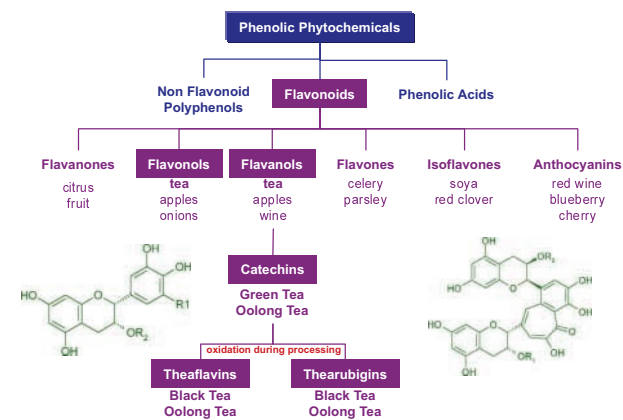
# Tea and Vitality

## Facts

# Tea Flavonoids

Tea remains the most widely consumed drink in the world - after water, but well ahead of coffee, beer, wine and fizzy soft drinks. Green, black and oolong tea is produced from the same plant, *Camellia sinensis*, through different manufacturing processes.

Figure 1. The Polyphenolic Family



The traditional tea beverage is characterised by a high content of polyphenols called flavonoids, with levels for a 235ml cup of black tea of 140 to 300 mg of total flavonoids dependent on the brew time and tea origin<sup>1</sup>.

Polyphenols can be classified into six groups on the basis of structure similarities (Figure 1). Flavonoids are present in fruits, vegetables and beverages such as tea, grape juice and red wine. Over 4,000 different flavonoids have been identified. Many *in vitro* and *ex vivo* studies have demonstrated that tea flavonoids possess strong antioxidant and metal chelating properties and may therefore protect cells and tissues against free oxygen radicals. In addition, flavonoids may also possess non-antioxidant properties which have been associated with various health benefits.

During the meeting of the American Dietetic Association in May 2005, tea was identified as the most significant source of flavonoids in the US diet. The Michigan State University estimated a total of 24 flavonoid compounds within the US diet and found the major flavonoids to be, in

descending order, flavanols, flavonols, flavones, anthocyanins and isoflavones. Tea, which is rich in flavanols, was identified as the most significant source of flavonoids, accounting for 55% of the daily intake (74mg of the 134mg daily flavonoid intake).

### Tea and its specific flavonoids

The type of flavonoids found in different types of teas will depend on the level of processing the tea leaves undergo. The major step in tea production is to stop the oxidation process at a predetermined stage, depending on the type of tea being aimed for. The process also determines quantity and type of flavonoids present in the tea leaf (Table 1).

Catechins are the main flavonoids produced by the *Camellia sinensis* plant. During the oxidation process, enzymatic activity allows for the catechins to be polymerised and thus alter their structure.

- Typically heated soon after harvesting, **green tea** leaves undergo minimal oxidation. This stops the enzymatic activity, retaining the majority of **catechins**.
- Black tea** receives substantial oxidation under controlled temperatures and humidity. This causes an enzymatic reaction, which changes the colour of the leaves from green to brown, and results in the polymerisation of catechins into **theaflavins** and **thearubigins**.
- Oolong tea**, is a result of oxidation being stopped somewhere in between that of green and black tea and therefore contains flavonoids that are found in both teas.

Table 1. Composition of green and black tea (g per 100g of dry tea)

	Total flavonoids	Catechins	Caffeine
Chinese Green tea	15.7	11.3	2.74
Black tea	17.4	2	3.54

Source: Scientific Information Centre, Lipton

Five **catechin** flavonoids (major flavonoids in the tea plant and green tea) have been investigated and identified as<sup>2</sup>:

- Catechin - C
  - Epicatechin - EC
  - Epigallocatechin - EGC
  - Epicatechin Gallate - ECG
  - Epigallocatechin Gallate - EGCG
- main catechin in *Camellia sinensis* and green tea**

During the black tea process, the catechins are enzymatically polymerised. Two polymer groups of catechins have been identified - **theaflavins** and **thearubigins**. Four theaflavins (TF) structures have been ascertained<sup>2</sup>:

- Theaflavin - TF
- Theaflavin 3-gallate - TF3G
- Theaflavin 3'-gallate - TF3'G
- Theaflavin 3,3'-gallate - TFDG

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## What's in a cup of tea?

A serving of 235 ml black tea contains between 140 and 300 mg of total flavonoids<sup>1</sup>. The composition of these flavonoids in brewed black and green tea is shown in Fig 2.

Levels of flavonoids in a tea brew will depend on a many factors<sup>3</sup> including:

- Type of tea used - loose leaf tea, standard tea bag or pyramid tea bag
- Amount of tea used or present in the tea bag
- The size of the tea leaves
- How long the tea is left to infuse in the water
- Whether the tea is stirred and how many times
- Whether the tea bag is squeezed
- Leaf variety

Most of the flavonoids are infused from the leaf to the brew after four minutes of brewing, or after two minutes of brewing if the teabag is first stirred at least three times and squeezed<sup>4</sup>.

The monomeric **catechins** are the major flavonoids in green tea whilst the **thearubigins** are the major flavonoids in black tea. Small amounts of the flavonol glycosides are present in comparable quantities in black, green and oolong tea (5-15 mg/cup). Chlorogenic acid is present in tea but in minor amounts: 0.3-0.7 mg/cup, based on analysis of seven major brands in the UK market (Unilever internal data).

## Tea Flavonoids and Antioxidant Capacity

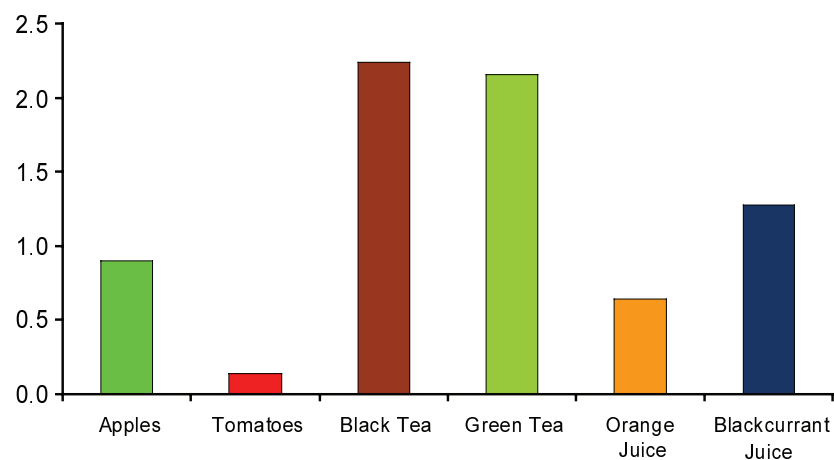
A large number of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neuro-degenerative diseases. Reactive oxygen and nitrogen species are formed in the human body. Endogenous antioxidant defenses are not always sufficient to scavenge them completely. Diet-derived antioxidants may therefore be particularly important in protecting against chronic disease<sup>5,6</sup>.

The strong antioxidant properties of tea flavonoids may be beneficial for humans. This will however depend on absorption, metabolism and tissue distribution following consumption. Due to analytical and ethical limitations, the various aspects of bioavailability are poorly understood. Due to the complex structure of thearubigins and the wide range of molecular weights in this group, the analysis of these compounds are very difficult<sup>7</sup>. The bioavailability and metabolism of tea flavonoids have been reviewed in detail by Hollman et al<sup>8</sup>.

*In vitro* studies measuring antioxidant capacity of tea using oxygen radical absorbance capacity (ORAC) and Trolox Equivalent Antioxidant Capacity (TEAC) assays, demonstrate the antioxidant capacity of a cup of black or green tea to be over three times more effective than a serving of most common vegetables and over two times more effective than a serving of common fruits (Figure 3)<sup>9,10</sup>. Assessment of antioxidant power with the TEAC assay gives similar results with green and black tea<sup>12</sup>. No significant difference is seen between the TEAC and ORAC assays.

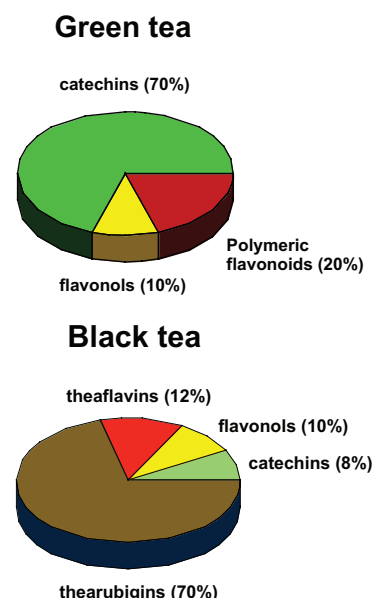
These are *in vitro* studies and do not take into account the bioavailability and biomechanism in humans.

Figure 3. Antioxidant activity of tea and some fruits & vegetables (Modified to US serving sizes from Panganga et al., 1999)



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Figure 2. Composition of green and black tea beverages



## Human Antioxidant Studies

The majority of the human intervention studies in which biological antioxidant properties of tea polyphenols have been studied, demonstrate an increase in plasma antioxidant potential after the consumption of either green or black tea.

A consistent increase in antioxidant capacity of plasma after tea ingestion has been demonstrated in independent studies with a variety of methods indicating that tea flavonoids enter the body and have antioxidant function in the short term (Table 2).

Small but significant increases in plasma antioxidant capacity (2-4%) were found in studies by Leenen (FRAP), Benzie (FRAP) and Van het Hof (TEAC) after green tea consumption<sup>12-14</sup>. Leenen found a small but significant increase (2%) with the FRAP assay after ingestion of black tea<sup>12</sup>. A substantial increase in the antioxidant potential of plasma after consumption of green tea (50%) and black tea (40%) was found by Serafini using the TRAP assay<sup>15</sup>.



Table 2 Effect of tea consumption on plasma antioxidant capacity in human intervention studies

Study	Assay	Plasma AOX capacity
Serafini, 1996 (15)	TRAP	+ 34% (GT), +29% (BT)
Maxwell, 1996* (19)	ECL	no effect (BT)
Van het Hof, 1997 (14)	TEAC	+3% (GT), no effect (BT)
McAnlis, 1998** (20)	ECL	no effect (BT)
Benzie, 1999 (13)	FRAP	+4% (GT)
Leenen, 2000 (12)	FRAP	+3% (GT), +2% (BT)
Serafini, 2000 (21)	TRAP	+40% (GT), +52% (BT)
Sung, 2000 (17)	TEAC	+12.7% (GT)
Langley-Evans, 2000 (16)	FRAP	+76% (BT)
Hodgson, 2000 (18)	TRAP	[+4% (GT), +3% (BT)] ns
Duffy, 2001 (22)	FRAP ORAC	[+12% (BT)] ns [+11% (BT)] ns

BT = black tea, GT = green tea, ns = non-significant \* no control \*\* coffee as a control

TRAP = Total Radical-trapping Antioxidant Potential ECL = Enhanced Chemiluminescence  
FRAP = Ferric Reducing Ability of Plasma ORAC = Oxygen Radical Absorbance Capacity  
TEAC = Trolox Equivalent Antioxidant Capacity

Langley-Evans reported an increase in plasma antioxidant capacity of 76% after black tea consumption, as measured by FRAP assay<sup>16</sup>. An increase of 12% was found by Sung after ingestion of green tea measured with the TEAC assay<sup>17</sup>. A small non-significant improvement of antioxidant status was found by Hodgson after green and black tea ingestion using the TRAP assay<sup>18</sup>. Maxwell and McAnlis did not find any change in antioxidant potential after consumption of black tea<sup>19,20</sup>. The study by Maxwell however, was a low power study (10 subjects) without a control treatment<sup>19</sup>. In the study by McAnlis, coffee was taken as a control. The high level of chlorogenic acids in coffee may explain the lack of positive results in this study<sup>20</sup>. Due to the nature of the applied assays and the different approaches to the expression of the results, it is at present difficult to make a quantitative comparison of the TEAC, TRAP, FRAP and ECL results.

**Thus, in a variety of *in vitro* and *ex vivo* systems, the tea flavonoids are consistently found to be effective scavengers of reactive oxygen species and free radicals. Tea could therefore be considered an important source of dietary antioxidants. However, more clinical studies are required to establish the true physiological relevance of tea flavonoid antioxidants.**

## Tea and Heart Health

Epidemiological studies overall point to a positive relationship between tea consumption and heart health. Peters meta-analysis of 17 epidemiological studies concluded that for every 3 cups (3 240mL) of tea consumed per day, the relative risk for Myocardial Infarction reduces by 11%<sup>23</sup>. However, the complexity of heart disease and the poor control of diet, lifestyle factors, lack of accurate and detailed flavonoid/tea specific diet analysis and publication bias needs to be taken into consideration. To try and explain the tea heart health benefits, some authors have begun to investigate the effect of tea on specific CVD risk factors in randomised placebo controlled clinical studies.

Interest is growing for the effect of tea and tea flavonoids on:

- **Improved endothelial function.** Four clinical studies have shown a significant improvement in endothelial function (brachial artery and coronary artery flow) by 15% - 77% after consumption of 450mL - 1.25L black tea providing 477mg - 1,463mg of tea flavonoids<sup>24-27</sup>.
- **Reduction in total and LDL-cholesterol.** Studies investigating the impact of tea flavonoids on plasma cholesterol levels are conflicting. However, two well controlled clinical studies have shown significant reductions in total and LDL cholesterol. One study using 5 cups of black tea providing approx. 722mg tea flavonoids showed a reduction in total and LDL-cholesterol of 6.5% and 11.1% respectively<sup>28</sup>. A second study using tea extract capsules providing 300mg of tea flavonoids per day, found total and LDL cholesterol to reduce significantly, after 12 weeks intervention, by 11.3% and 16.4% respectively<sup>29</sup>.
- **Inhibition of platelet aggregation** has also been suggested, however, the clinical evidence is currently small.

For further details see our Heart Health Factsheet

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## Other Health Benefits Requiring Further Clinical Evidence

### Tea and Human Immunity

A number of studies (predominantly *in vitro* or animal) have demonstrated tea to have anti-microbial and anti-inflammatory effects<sup>30</sup>.

*Helicobacter pylori* infection and upper gastrointestinal diseases have been investigated significantly. Scientists from Japan investigated the effect of different green tea catechins and black tea theaflavins on *H. pylori in vitro* and in animals. The results showed Epigallocatechin gallate (most prominent catechin in green tea) to significantly inhibit *H. pylori in vitro*<sup>31</sup>. A clinical study by Yee and colleagues investigated Chinese tea consumption in 72 patients undergoing their first upper endoscopy, and found tea consumption to be inversely associated to *H. pylori*<sup>32</sup>. A more recent *in vitro* study from Korea showed EGCG to attenuate *H. pylori*-induced host cell signaling and significantly reduce pro-inflammatory mediators<sup>33</sup>.

Other *in vitro* and animal studies have shown EGCG to have an immunomodulatory effect by enhancing macrophage activity against *L. pneumophila* infection and anti-inflammatory effect on the colon of IL-2 deficient mice<sup>34,35</sup>.

Early investigations are promising for tea's immunological and antibacterial role, however, there is a need for further long-term large scale clinical studies.

### Tea and Dental Health

Tea is a significant source of fluoride in the diet and more so when tea is made with fluoridated water. The tea fluoride contribution has been associated with better dental health.

In addition to the fluoride effect, some studies have demonstrated tea flavonoids to possess antibacterial properties and the ability to inhibit human salivary amylase, resulting in a reduction of cariogenic potential of starch-containing foods. A US study applied 10 different tea brews (3 green, 7 black) to an amylase assay with and without *Streptococcus mutans* strains. It also investigated the effect of water or tea post carbohydrate consumption on salivary amylase of 6 healthy volunteers<sup>36</sup>. The *in vitro* results demonstrated tannins (theaflavins and thearubigins from black tea) to be most effective in inhibiting amylase activity by