

## Review

# The phytochemical composition and antioxidant actions of tree nuts

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In addition to being a rich source of several essential vitamins and minerals, mono- and polyunsaturated fatty acids, and fiber, most tree nuts provide an array of phytochemicals that may contribute to the health benefits attributed to this whole food. Although many of these constituents remain to be fully identified and characterized, broad classes include the carotenoids, hydrolyzable tannins, lignans, naphthoquinones, phenolic acids, phytosterols, polyphenols, and tocopherols. These phytochemicals have been shown to possess a range of bioactivity, including antioxidant, antiproliferative, anti-inflammatory, antiviral, and hypocholesterolemic properties. This review summarizes the current knowledge of the carotenoid, phenolic, and tocopherol content of tree nuts and associated studies of their antioxidant actions in vitro and in human studies. Tree nuts are a rich source of tocopherols and total phenols and contain a wide variety of flavonoids and proanthocyanidins. In contrast, most tree nuts are not good dietary sources of carotenoids and stilbenes. Phenolic acids are present in tree nuts but a systematic survey of the content and profile of these compounds is lacking. A limited number of human studies indicate these nut phytochemicals are bioaccessible and bioavailable and have antioxidant actions in vivo.

**Key Words:** tree nuts, phytochemicals, flavonoids, resveratrol, antioxidants

## INTRODUCTION

Tree nuts appear to have an impact on antioxidant status<sup>1,2</sup> and inflammatory pathways<sup>3,4</sup> that may underlie, in part, their putative benefits in reducing the risk of cardiovascular disease,<sup>5,6</sup> type 2 diabetes,<sup>7,8</sup> and some types of cancer.<sup>9</sup> Evidence for this relationship has been sufficiently compelling that nuts have been incorporated into recommended dietary guidelines in the United States, Canada, and Spain<sup>10</sup> and a qualified health claim for reducing the risk of heart disease is allowed by the U.S. Food and Drug Administration.<sup>11</sup> As the nutrient composition of different tree nuts can vary markedly, there is unlikely to be a single or even a few constituents that contribute principally to these outcomes. Thus, it would be worthwhile to consider the potential interactions between the essential nutrients (including amino acids, fatty acids, minerals, and vitamins), fiber, and phytochemicals (including carotenoids, phytosterols, and polyphenols like flavonoids and stilbenes) most commonly present in tree nuts.<sup>12</sup> Recently, several review articles have been published describing the health benefits and related aspects of tree nuts.<sup>1,2,7,13,14</sup> This review focuses on carotenoids, tocopherols, and the polyphenols found in tree nuts and their impact on antioxidant capacity.

## CAROTENOIDS

Carotenoids are polyisoprenoids with differing degrees of conjugated double bonds. They are abundant in most colorful plant foods, e.g., with 7.6 and 6.6 mg/100 g  $\beta$ -carotene and lutein found in carrots and spinach, respectively. In contrast, tree nuts contain very low amounts of

carotenoids with  $\alpha$ - and  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, and zeaxanthin found in  $\mu\text{g}/100\text{ g}$  concentrations when present at all. Pistachios present a modest exception with a mean  $\beta$ -carotene and lutein content of 0.21 and 4.4 mg/100 g dry weight, respectively.<sup>15</sup>

## TOCOPHEROLS

Tree nuts are among the richest sources of vitamin E, the principal lipid-soluble vitamin, along with seeds and vegetable oils. Table 1 lists the  $\alpha$ - and  $\gamma$ -tocopherol content of tree nuts. Traces of  $\delta$ -tocopherol at  $<0.5\text{ mg}/100\text{ g}$  (except for walnuts at  $<4\text{ mg}/100\text{ g}$  extracted oil) are found in many tree nuts. Barreira et al.<sup>16</sup> report the presence of tocotrienols in chestnuts, particularly  $\gamma$ -tocotrienol at 0.03 mg/100 g. Kornsteiner et al.<sup>15</sup> report a several-fold range in vitamin E content of some types of tree nuts, though it is not clear whether this result is from differences in the harvest year, source, variety or agricultural and environmental factors. It is noteworthy that the absorption of vitamin E is influenced by the amount of fat in a meal or snack and the food matrix,<sup>17</sup> making nuts with their high fat content, a particularly

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**Table 1.** Mean vitamin E content of tree nuts (mg/100 g)<sup>15,16,68</sup>

Tree Nut	$\alpha$ -Tocopherol	$\gamma$ -Tocopherol
Almonds	25.9	0.9
Brazils	5.7	7.9
Cashews	0.9	nd <sup>†</sup>
Chestnuts	0.005	0.4
Hazelnuts	15.0	nd
Macadamia	0.6	nd
Pecans	4.1	8.1
Pines	9.3	11.2
Pistachios	1.9	22.5
Walnuts	0.7	20.8

<sup>†</sup>nd: not detected

bioavailable food source. Jambazian et al<sup>18</sup> found increasing almond intake from 0 to 10 and 20% energy significantly elevated plasma  $\alpha$ -tocopherol status by 12 and 15%, respectively.

## POLYPHENOLS

The classes of polyphenols identified in tree nuts include anthocyanins, flavonoids, lignans, naphthoquinones, phenolic acids, proanthocyanidins, stilbenes, and hydrolyzable tannins. However, a substantial number of nut polyphenols remain to be identified. Importantly, the profiles and content of polyphenols are affected not only by the type of nut and its cultivar but also the harvest year, orchard location, processing steps, and storage. The dietary sources and intake of polyphenols is of interest because of their putative contribution to health promotion and the reduction of risk of chronic disease. However, limited information is available regarding the intake of polyphenols from tree nuts. In a Spanish population, Saura-Calixto et al.<sup>19</sup> estimated an intake of 102-121 mg polyphenols from daily consumption of 5.9 g nuts. They calculated that 107 mg of these polyphenols were bioaccessible. Almond polyphenols have been found to be bioaccessible, bioavailable, and subsequently metabolized by phase 2 conjugating enzymes and gut microflora.<sup>20</sup> Vari-

ous studies suggest that it is the biotransformed polyphenols that are the bioactive compounds.

## Total phenols

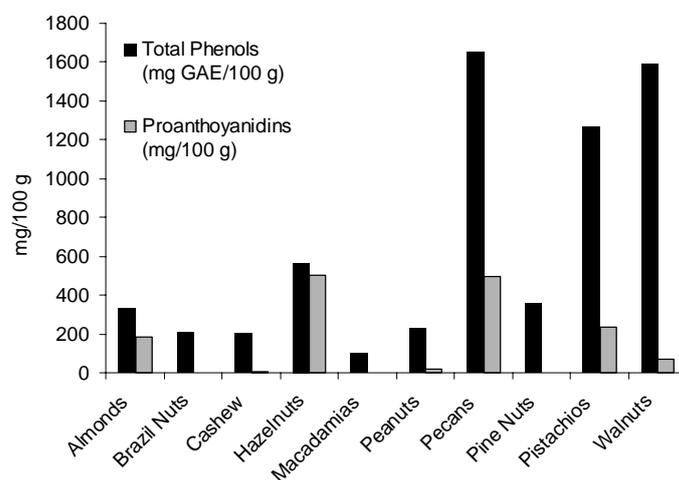
The Folin-Ciocalteu reaction has been widely used to estimate of the content of total phenols in plant foods, including nuts. The phenolic content of nuts ranges from 103-1650 mg Gallic Acid Equivalents (GAE)/100 g, with pecans, walnuts, and pistachios having the highest values<sup>15,21</sup> (Figure 1). The total phenols in nuts are within the range of fruit such as blueberries (531 mg GAE/100 g), plums (367 mg GAE/100 g), and raisins (1065 mg GAE/100 g).<sup>22</sup>

## Proanthocyanidins

Proanthocyanidins are flavan-3-ol oligomers linked through carbon-carbon bonds. Type A proanthocyanidins consist of a C<sub>4</sub> to C<sub>6</sub> or C<sub>8</sub> interflavan bond and a C<sub>2</sub>-ether bond to the flavanol extension. Type B proanthocyanidins have single C<sub>4</sub> to C<sub>6</sub> or C<sub>8</sub> interflavan bonding. Nut proanthocyanidins mainly consist of (+)-catechin and (-)-epicatechin, but also include afzelechin (almonds and peanuts) and epigallocatechin (hazelnuts, pecans, pistachios).<sup>23-25</sup> To date, the A-type proanthocyanidins have been found only in almonds and peanuts. The majority of nut proanthocyanidins are highly polymerized (>10-mers).<sup>24</sup> However cashews and peanuts contain proanthocyanidins of 1-4 mers.<sup>24,26</sup> Proanthocyanidins are the predominant polyphenols in almonds, hazelnuts, peanuts, pecans, and pistachios. Hazelnuts and pecans have the highest proanthocyanidin content with 501 and 494 mg/100 g, respectively.<sup>24</sup> No proanthocyanidins have been reported in Brazil nuts, macadamias or pine nuts.<sup>24</sup> Roasting generally decreases the proanthocyanidin content of nuts. Roasting decreased peanut skin proanthocyanidin monomers, trimers, and tetramers 54-29%, but increased monomers 29% from raw skin.<sup>26</sup> Toasting pistachios decreases their proanthocyanidin to 12% of that found in the raw nut.<sup>27</sup>

## Flavonoids

Flavonoids have been identified in all nuts except macadamias and Brazil nuts. Flavan-3-ols, flavonols, and anthocyanins are the main flavonoids in nuts, with fla-



**Figure 1.** Total Phenols and Proanthocyanidin Content of Nuts<sup>15,21,24</sup>

**Table 2.** Flavonoid content of nuts (mg/100 g)<sup>28</sup>

Tree Nut	Flavan-3-ols	Flavanones	Flavonols	Anthocyanins	Isoflavones	Sum
Almonds	4.47	0.38	7.93	2.46	0.01	15.25
Brazils	nd <sup>†</sup>	nd	nd	nd	nd	nd
Cashews	1.98	nd	nd	nd	0.01	1.99
Chestnuts	0.01	nd	nd	nd	- <sup>‡</sup>	0.01
Hazelnuts	5.25	nd	nd	6.71	0.03	11.99
Macadamias	nd	nd	nd	nd 0	-	nd
Peanut	0.66	nd	nd	nd	0.26	0.68
Pecans	15.99	nd	nd	18.02	nd	34.01
Pines	0.49	nd	nd	nd	-	0.49
Pistachios	6.85	nd	1.46	6.06	3.63	18.00
Walnuts (English)	nd	nd	nd	2.71	0.03	2.74

<sup>†</sup>nd, not detected. <sup>‡</sup>- not determined

vanones and isoflavones found in lesser amounts (Table 2). Pecans have the highest total flavonoid content among nuts at 34 mg/100 g, consisting mostly of flavan-3-ols and anthocyanins.<sup>28</sup> Hazelnuts and almonds also have an appreciable content of flavonoids with 18 and 15 mg/100 g, respectively. To date, flavanones have only been found in almonds. Almonds and pistachios are the only nuts that contain flavonols, mainly as isorhamnetin and quercetin. Pistachios have the highest isoflavone content of nuts at 3.63 mg/100 g, more than 100-fold greater than levels of other nuts; in contrast, peanuts contain 0.26 mg isoflavones/100 g.<sup>29</sup>

### Resveratrol

The stilbene resveratrol is found in pistachios and peanuts at comparable levels. Resveratrol in peanuts ranges from 3 to 192 µg/100 g, with Cerezlik cultivars as the highest producers.<sup>30</sup> In pistachio, resveratrol content ranges from 9 to 167 µg/100 g, with the most produced in Ohadi and Siirt cultivars.<sup>30</sup> In comparison, the resveratrol content of red wine ranges from 98 to 1800 µg/100 mL.<sup>31</sup> Recent research suggests that resveratrol may contribute to neuroprotection, immune regulation, and cancer chemoprevention.<sup>32</sup>

### Other Phenolic Compounds

Tree nuts contain a variety of other phenolic compounds which may contribute their bioactivity. Phenolic acids are

present in almonds<sup>33</sup> but a systematic survey of the content and profile of these compounds in nuts is lacking. Walnuts contain considerable amounts of syringic acid (34 mg/100 g) and the naphthoquinone juglone (12 mg/100 g).<sup>34</sup> Ellagitannins are also found in walnut, hazelnut, and cashews.<sup>35</sup> Nuts also contain lignans, which have phytoestrogenic activity. Pistachios have the highest amount of lignans among nuts, with 0.2 mg/100 g.<sup>36</sup>

### ANTIOXIDANT CAPACITY OF TREE NUTS IN VITRO

In vitro assessment of the antioxidant capacity of tree nuts has largely been conducted by examining the ability of extracts to increase the resistance of human plasma or low density lipoprotein (LDL) to oxidation. Extracts of walnuts,<sup>37</sup> almond and almond skins,<sup>38-40</sup> pistachios,<sup>27</sup> and hazelnuts<sup>41</sup> have been found to increase the lag time to oxidation of LDL. Walnut extracts have been reported to inhibit lipid peroxidation reactions as well in human plasma.<sup>37</sup> Pistachios have also been shown to inhibit lipid peroxidation in bovine liver microsomes.<sup>27</sup> Protection of DNA from oxidative injury has also been demonstrated in vitro for extracts of almonds<sup>40</sup> and hazelnuts.<sup>41</sup>

Several in vitro assays that are particularly sensitive to polyphenols have been developed to assess "total antioxidant capacity" in foods. Four assays have been applied to testing tree nuts, the Oxygen Radical Absorbance Capacity (ORAC), Ferric Reducing Antioxidant Power (FRAP),

**Table 3.** "Total Antioxidant Capacity" of tree nuts<sup>21,69</sup>

Tree Nut	ORAC (L + H) <sup>†</sup> µmol TE <sup>‡</sup> /g	FRAP µmol Fe <sup>2+</sup> /g	TRAP µmol TE/g	TEAC µmol TE/g
Almonds	44.5	41.3	6.3	13.4
Brazils	14.2	- <sup>§</sup>	-	-
Cashews	20.0	-	-	-
Hazelnuts	96.5	42.3	6.9	12.0
Macadamias	17	-	-	-
Pecans	179.4	-	-	-
Pines	7.2	13.4	1.5	5.3
Pistachios	79.8	192.7	25.9	61.5
Walnuts	135.4	453.9	31.9	137.0

<sup>†</sup>Lipophilic (L) and hydrophilic (H) ORAC assay values combined. <sup>‡</sup> Trolox Equivalents. <sup>§</sup>not determined.

Total Radical-trapping Antioxidant Parameter (TRAP), and Trolox Equivalent Antioxidant Capacity (TEAC) (Table 3). The relevance of these assays in whole foods to *in vivo* antioxidant actions is not always clear as they cannot account for factors such as bioaccessibility, bioavailability, metabolism, and distribution of the phytochemical ingredients. However, these assays may prove useful in experiments directed to the understanding the potential interactions between nut components and constituents and other nutrients.

### ANTIOXIDANT ACTIONS OF TREE NUTS IN HUMAN STUDIES

Data from large observational studies show that regular nut consumption is associated with a reduced risk of several conditions in which oxidative stress may play a role, including coronary heart disease,<sup>42-44</sup> hypertension,<sup>45</sup> type 2 diabetes,<sup>46</sup> inflammation and endothelial dysfunction.<sup>3,47</sup> To date, no observational studies have directly correlated nut intake with plasma total antioxidant activity or biomarkers of oxidative stress as these measures are not routinely assessed in large cohorts.

The acute bioavailability of polyphenols from both walnuts and almonds, as well as a concomitant reduction in plasma lipid peroxidation and increased antioxidant capacity following their consumption, was demonstrated by Torabian et al.<sup>48</sup> in a randomized, placebo-controlled crossover study of 13 healthy adults. The total phenolic content of plasma significantly increased 30 min after consuming either 81 g walnuts or 91 g almonds (blended in water), while no changes were observed following an isocaloric control meal. Peak phenolic concentrations coincided with a significant reduction at 90 min in plasma thiobarbituric acid reactive substances, a measure of lipid peroxidation. Values from the FRAP and both lipophilic and hydrophilic ORAC assays also increased significantly following the consumption of both nut meals, with peak capacity observed at 150 min. No changes in plasma total antioxidant capacity were observed following the control meal.

The effects of nut consumption on antioxidant status and biomarkers of oxidative stress have been reported in a limited number of human intervention studies. Lopez-Uriarte et al.<sup>2</sup> and Ros<sup>13</sup> reviewed a total of 21 clinical studies in which the potential effects of tree nuts on biomarkers of oxidation or antioxidant activity were evaluated. As most of these studies used whole nuts, rather than nut components (such as the skins which contain much of the polyphenol content or the kernels where the tocopherols are found), or their individual phytochemical constituents, the contribution of these bioactive compounds to these results is unknown. However, when the reported outcomes of these nut studies are considered, it is clear that their effects cannot be due entirely to their polyunsaturated fatty acid (PUFA) or monounsaturated fatty acid (MUFA) content alone.

Early intervention studies comparing a walnut-enriched, high PUFA diet with a walnut-free, lower PUFA diet showed no differences in the prevention of LDL oxidation<sup>49-52</sup> or total antioxidant capacity<sup>53</sup> between diets. While the shift to more PUFA in the diet and plasma with walnut consumption could be predicted to increase plasma

biomarkers of lipid peroxidation, the absence of an effect may be due to the concurrent intake and bioavailability of walnut phytochemicals, including phenolic compounds and tocopherols. More recently, Davis et al.<sup>54</sup> reported no change in plasma antioxidant capacity following the consumption of 63-108 g/d walnuts or cashews for 8 wk in metabolic syndrome patients. Interestingly, a significant reduction in erythrocyte lipid peroxidation was observed in subjects at increased risk for cardiovascular disease following a diet with 21.4 g/d walnuts for 5 wk, when compared to a control diet,<sup>55</sup> but this effect was modulated according to the subjects' particular paraoxonase (PON1) polymorphism.<sup>56</sup>

According to Ros,<sup>13</sup> the available evidence suggests that while PUFA-rich nuts confer a neutral or minimal effect on oxidative status, the effects of MUFA-rich nuts are more moderate. Indeed, Fito et al.<sup>57</sup> reported a significant reduction in circulating oxidized LDL levels among asymptomatic adults, age 55-80 y, 3 mo after consuming a Mediterranean diet including 30 g/d whole nuts mixed at 50% walnuts, 25% almonds, and 25% hazelnuts. Moreover, chronic feeding studies using low PUFA nuts, including pecans,<sup>58</sup> hazelnuts,<sup>59</sup> macadamia nuts,<sup>60</sup> pistachios,<sup>61</sup> almonds,<sup>62,63</sup> and brazil nuts<sup>64</sup> all showed either an improvement in oxidation status or increased antioxidant enzyme activity. It is plausible that with less PUFA intake, the need to protect this oxidizable substrate is reduced and a higher proportion of the nut bioactives are available for other functions.

Other studies show the antioxidant effects of nuts are not limited to reduced lipid peroxidation and improved plasma antioxidant capacity. Jenkins et al.<sup>65</sup> reported a significant postprandial increase in serum protein thiol concentrations, reflecting less oxidative damage to proteins, following a meal with 60 g almonds in a study of 15 healthy young adults. Jia et al.<sup>66</sup> and Li et al.<sup>67</sup> both reported significant reductions in oxidative DNA damage among smokers following 4 wk of almond consumption at 84 g/d. Li et al.<sup>67</sup> evaluated the effects of almonds vs. pork (120 g/d) in a cohort of 60 male smokers serving in the Chinese army, and compared these effects with 30 nonsmokers who consumed the control (pork) diet. After 4 wk, the amount of DNA strand breaks and urinary 8-hydroxy-deoxyguanosine were significantly lower in the almond-supplemented smokers, compared with the pork-supplemented smokers. Significantly lower urinary malondialdehyde, a biomarker of lipid peroxidation, and higher serum  $\alpha$ -tocopherol status and activities of superoxide dismutase and glutathione peroxidase were also observed in the almond group. No changes in these biomarkers of oxidative stress were observed in the pork-supplemented nonsmokers. Although the authors did not measure the specific contribution of the nut polyphenols or antioxidants to these outcomes, this study does suggest that almonds confer an antioxidant benefit.

### SUMMARY

Tree nuts are a complex whole food containing an array of essential nutrients as well as phytochemicals, including carotenoids, polyphenols, and tocopherols that possess antioxidant functions as well as other bioactivity. These compounds may contribute to the health benefits associ-

ated with nut consumption but elucidating this relationship requires additional research that characterizes their content as well as the impact of agricultural practices and food processing in addition to human studies investigating their bioaccessibility, bioavailability, metabolism, and functional impact in vivo.

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#### AUTHOR DISCLOSURES

The authors declare no conflicts of interest.

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## Review

# The phytochemical composition and antioxidant actions of tree nuts

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## 木本堅果的植化素成份及抗氧化作用

大部分的木本堅果，除了是許多必需維生素、礦物質、單元不飽和脂肪酸、多元不飽和脂肪酸及纖維的豐富來源之外，它們也提供有益於健康的一系列的植化素。雖然尚有許多組成未完全被辨識及確認，堅果所含的大類成份有：類胡蘿蔔素、可水解單寧、木酚素、萜醌、酚酸、植物固醇、多酚以及生育酚。這些植化素已被證明具有多項生物活性：抗氧化、抑制增生、抗發炎、抗病毒以及降低血膽固醇的特性。本篇回顧概述目前對木本堅果的類胡蘿蔔素、酚酸和生育酚的知識，以及它們在體外與人體中抗氧化作用的相關研究。木本堅果是生育酚和所有酚類的豐富來源，並含有多樣的類黃酮及前花青素。相反地，大部份的木本堅果並非類胡蘿蔔素及二苯乙烯類的良好飲食來源。酚酸存在於木本堅果中，但是目前缺乏對這類化合物的含量及資料的系統化測量及調查。由有限的人體研究資料顯示，這些堅果的植化素是人體可攝取的且具生物可利用性，在體內亦具有抗氧化作用。

**關鍵字：**木本堅果、植化素、類黃酮、白藜蘆醇、抗氧化物