Review

Black Walnuts (*Juglans nigra L.*): Potential as a Health Promoting Food

Cristiane Rodrigues Silva Câmara, M.Sc. [a] **and Vicki Schlegel, Ph.D.** [a,*]

[a] Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, USA

* Corresponding author. Tel.: +1-402-416-0294.
E-mail address: vschlegel3@unl.edu.

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Abstract: Nut consumption has been reported to protect against cardiovascular disease, certain types of cancer, diabetes and others disease states, including neurodegenerative conditions. The nutrient composition of black walnuts indicates that this edible nut has a strong potential on human health promoting. However, scientific studies on the functional properties of black walnut are extremely scarce. Thus, the aim of this review was to evaluate the potential black walnuts consumption on human-health promotion, using recent evidences obtained from English walnuts research, as both classes have comparable bioactive compounds composition. Compared to English walnut, the most marketed and studied walnut, and other classes of nuts, black walnut contains higher content of polyunsaturated fatty acids levels and antioxidants agents, such as polyphenolic compounds and γ-tocopherol, which has been extensively correlated with prevention and/or attenuation of those cited diseases. Moreover, other nutrients present in black walnuts are linked with those benefic effects, , including dietary fiber, folate, phytosterols, vegetable protein, melatonin, among others. The information provided herein provide strong evidence that black walnuts can be readily incorporated into diet as a means to produce multiple beneficial health outcomes.

Keywords: Black walnut; English walnuts; health benefits; PUFAs; antioxidants

1. Introduction

Several epidemiologic and clinical studies have reported remarkable health benefits through the consumption of tree nuts, including effects against cardiovascular diseases (CVDs), diabetes, inflammatory process, oxidative stress, cancer and neurodegenerative conditions (Djoussé et al., 2008; Gonzalez et al., 2006; Hu et al., 1998; Damasceno et al., 2011; Carvalho et al. 2010; Poulose et al. 2012; Pan et al. 2013; Pereira et al., 2007, Willis et al., 2010). The health promoting properties derived from these plant sources have been mainly attributed to their unsaturated fatty acids composition and other bioactive nutrients such as phenolic compounds, tocopherols, phytosterols, high-quality vegetable protein, fiber and minerals (Ros and Mataix, 2006; Brufau et al 2006 ; Segura et al. 2006; Oliveira et al., 2008).

Tree nuts are dry fruits with one seed in which the ovary wall becomes hard at maturity. The most popular edible nuts are almonds (*Prunus amigdalis*), hazelnuts (*Corylus avellana)*, walnuts (*Juglans regia*), pistachios (*Pistachia vera*), pine nuts (*Pinus pinea*), cashews (*Anacardium occidentale*), pecans (*Carya illinoiensis*), macadamias (*Macadamia integrifolia*), and Brazil nuts (*Bertholletia excelsa*) (Ros, 2010).

Walnuts, almonds and pecans are the top three nuts consumed in the United States (USDA/ERS, 2012a). Walnut is the second most produced, with 504,000 tons produced in 2010 (USDA/ERS, 2012b) and standing behind of only almonds (820,000 tons) (USDA/ERS, 2012c). California is the largest producer and processor of 90% of nuts with far exceeding other states (USDA/ERS, 2012a).

The two most produced varieties of walnuts in the U.S. are the English walnuts or common walnut (*Juglans regia* L.) and the Black walnus (*Juglans nigra* L.). English walnuts, the most commercially cultivated, originated in Persian and expanded to U.S. through English settlers. The Black walnut is native to the Midwest and Northeastern United States. These nuts are typically harvested in Missouri from the wild when they fall from trees in forested areas. Black walnut trees produce high-value, hardwood products and flavored edible nuts, however, the commercial value of the latter market is because itssmall kernels andhard and thick shells, which is difficult to hull (USDA /ERS, 2005). English and Black walnuts are typically sold in the market as a snack or as cooking ingredient for candies, cereals, baked goods and others sweets. Ninety percentof walnuts are sold in-shell, as most consumers perfor to crack the nuts (AgMRC, 2007).

The majority of the published works associating walnut consumption and health benefits are based on English walnuts studies, however similar research on Black walnuts are extremely scarce. Thus, the aim of this review is evaluate the broad range of potential effects of Black walnut on health promotion, taking into account its composition and similarity with the common walnut. The comparisons reviewed herein can stimulate future research in this field and also promote a consumption of Black walnuts by increasing our understanding on the potential health promoting properties of these nuts.

2. Black Walnuts Composition

Black walnuts are nutrient dense foods that contain high levels of lipids, protein (approximately 15% of energy), fiber, vitamins, minerals and also other bioactive molecules, such as phenolic compounds and phytosterols. Table 1 shows the basic nutrient composition relative to Black and English walnuts. As the other nuts, Black walnuts are rich dietary sources of unsaturated fatty acids (Ros and Mataix, 2006), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) accounting for more than 80% of the total lipid content . MUFAs profile is comprised mainly of oleic acid (C18:1n-9) and omega-6 linoleic acid (LA 18:3n-6); omega-3 α-linolenic

Table 1. Black and English walnut nutrient composition (USDA/ARS, 2012).

Nutrient	Unit	Value per 100g*	
Proximates		**Black walnut**	**English walnut**
Water	g	4.56	4.07
Energy	kcal	618	654
Protein	g	24.06	15.23
Total lipid (fat)	g	59.00	65.21
Carbohydrate, by difference	g	9.91	13.71
Fiber, total dietary	g	6.8	6.7
Sugars, total	g	1.10	2.61
Minerals			
Calcium, Ca	mg	61	98
Iron, Fe	mg	3.12	2.91
Magnesium, Mg	mg	201	158
Phosphorus, P	mg	513	346
Potassium, K	mg	523	441
Sodium, Na	mg	2	2
Zinc, Zn	mg	3.37	3.09
Vitamins			
Vitamin C, total ascorbic acid	mg	1.7	1.3
Thiamin	mg	0.057	0.341
Riboflavin	mg	0.130	0.150
Niacin	mg	0.470	1.125
Vitamin B-6	mg	0.583	0.537
Folate, DFE	µg	31	98
Vitamin A, RAE	µg	2	1
Vitamin A, IU	IU	40	20
Vitamin E (alpha-tocopherol)	mg	1.80	0.70
Vitamin E (gamma-tocopherol)	mg	28.48	20.85
Vitamin K (phylloquinone)	µg	2.7	2.7
Lipids			
Fatty acids, total saturated	g	3.368	6.126
Fatty acids, total monounsaturated	g	15.004	8.933
Oleic acid (C18:1)	g	14.533	8.7
Fatty acids, total polyunsaturated	g	35.077	47.174
Linoleic acid (C18:2)	g	33.072	38.2
α-linolenic acid (C18:3)	g	2.006	9.08

* Nutrient values refer to edible portion.

acid (ALA; 18:3n-3) are the major contributors for the PUFAs levels in both types of walnuts (Table 1). Importantly, more than half of the total content of lipids is PUFAs, in Black and English walnuts.

LA and ALA are considered essential fatty acids as they are not synthesized in the human body and are mostly obtained from the diet (Das, 2006). Epidemiologic studies have shown overall improvement in the health of individuals with cardiovascular heart disease (CVD) when fed the ALA (Kris-Etherton, 2003). After absorption into a cell, LA is elongated and desaturated to arachidonic acid (AA) while ALA is converted by a series of elongation and desaturation reactions into eicosapentaenoic acid (EPA) and then into docosahexaenoic acid (DHA) (Leaf et al, 2008; Harris et al., 2008; Willis et al. 2009a). Leukotrienes, prostaglandins, and thromboxanes, metabolites derived from AA, are generally pro-inflammatory and proaggregatory agonists, while those derived from the ALA are able to inhibit platelet aggregation and inflammation. The latter mechanisms are involved in the prevention of CVD, hypertension, type 2 diabetes, chronic obstructive pulmonary disease, among others (Simopoulos, 1999). Thus, the lipid profile of Black walnuts is likely to be an important key factor to beneficial health effects.

Walnuts are also rich sources of antioxidant vitamin E (tocopherol), with γ-tocopherol the predominant isomer present in significant amount in Black walnuts, even more than English walnuts (Table 1). Although γ-tocopherol has not been investigated as intensively as α-tocopherol, recent studies have reported high antioxidant activity of this molecule and correlated its biological function with cancer and CVDs prevention (Wagner et al., 2004).

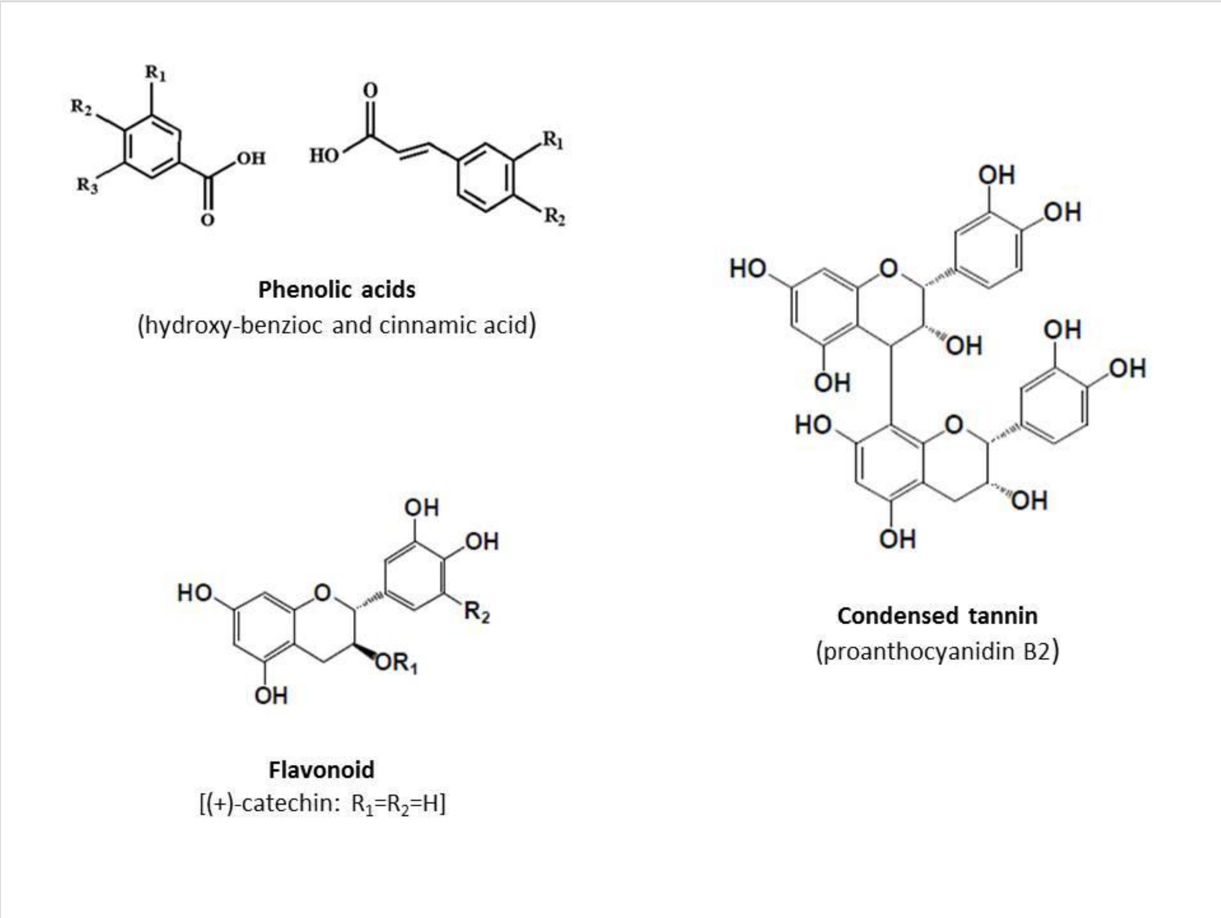
In addition to tocopherol, walnuts are rich in phenolic compounds that increase the antioxidant potential of this food (Blomhoff et al., 2006; Yang et al., 2009). The major phenolic compounds frequently identified in walnuts are phenolic acids and condensed tannins (Labuckas et al., 2008, Ito et al., 2007), including ellagic acid, ellagitannins, gallic acid and hydrocinnamic acids like chlorogenic acid (Anderson et al., 2001; Canales et al., 2007). Phenolic acids are a subclass of the phenolic compounds that contain at least one aromatic ring and one hydroxyl group in their molecule (Tsao, 2010). Condensed tannins or proanthocyanidins are polymeric flavonoids constructed from at least two linked catechin units (Khanbabaee and Ree, 2001). Flavonoids (e.g. catechin) are the largest subclass of polyphenols in the human diet and are characterized by two or more aromatic rings containing at least one hydroxyl group in each (Tsao, 2010). Figure 1 illustrates the basic chemical structure of those cited phenolic compounds.

Phenolic compounds are mainly concentrated in the hull (the skin that covers the kernel) and this fraction is reported to have improved human health effects (Fukuda et al., 2003). Importantly, a review study reported that antioxidants in walnuts possess high bioavailability (Manach et al., 2005). In an *in-vitro* study, the total phenolic concentration of English walnuts was higher (550 ± 11 mg catechin equivalents per gram of tannin fraction) than hazelnut (329 ± 7) and almonds (83 ± 2) (Karamac, 2009). Total phenolic analysis of Black walnuts showed a concentration of 2.45 ± 0.01 mg *trans*-cinnamic acid equivalents per gram of kernel (data not published), which was slightly higher in English walnuts (2.14 mg gallic acid equivalents per gram of kernel) reported by Carvalho et al. (2010). In a different study, Rorabaugh et al. (2011) reported that English walnuts contain a greater profile of flavonols, a subclass of flavonoids, compared to Black walnuts. In this study, the compounds identified in both types of walnuts were 5-caffeoylquinic acid, 4-caffeoylquinic acid, quercetin-3- rutinoside, quercetin-3-galactoside, quercetin- 3-pentoside, quercetin-3-arabinoside and quercetin-3-rhamnoside. Thus, the phenolic content of Black walnuts may play an important role in the inhibition of oxidation process leading to health promotion.

Other abundant nutrients in walnuts are folate, melatonin, pectin and some minerals; those have been associated to the improvement of lipid profile, endothelial function and increased plasma antioxidant capacity, and also demonstrated neuroprotective action (Tapsell et al., 2004, Willis et al., 2009a and 2009b, Banel and Hu, 2009, Ma et al., 2010, McKay et al., 2010, Segura et al., 2006). It must be noted that the B vitamin, folate, plays a role in detoxifying homocysteine, a methionine derived amino acid with atherothrombotic properties that accumulates when folate is deficient, as well as vitamin B6 and B12 (Welch and Loscalzo, 1998). Moreover, walnuts contain high levels of arginine, 3.62 g per 100 g of Black walnut and 2.28 g per 100 g of English walnut (USDA/ARS, 2008), which has been linked to lowering blood pressure. This amino acid is a precursor of nitric oxide (NO), an endogenous vasodilator (Huynh and Chin-Dusting, 2006).

Additionally, walnuts composition contain considerable non-cholesterol sterols levels known as plant sterols or phytosterols (Segura et al., 2006). Black walnuts contain higher level of phytosterols than English walnuts, 109 mg/100g to 72 mg/100g of nut (USDA/ARS, 2008). In the intestinal lumen, phytosterols can promote reduction of cholesterol absorption, thus lowering plasma cholesterol levels. The mechanism of action of phytosterols has been associated to their high affinity for micelles than cholesterol. Consequently, cholesterol is displaced from micelles and the amount available for absorption is reduced (Ostlund, 2002; Brauner et al., 2012). In all probability the phytosterol content of Black walnuts may contribute to their cholesterol-lowering effect.

Fig 1. Examples of phenolic acids, condensed tannins and flavonoids structure (adapted from Pandey and Rizvi, 2009; Tsao, 2010)**.**



3. Black walnuts potential to health outcomes

Although the compositional analysis of Black walnuts has revealed potentially health promoting constituents, research remains limited on the effects of this type of nut on the human health. In order to assess the potential health benefits of Black walnuts comparisons with English walnut was conducted in this review, as several studies have linked the consumption of these tree nuts with multible health impacts..

3.1 *Black Walnuts versus Cardiovasculares Diseases*

Decades ofEepidemiological research have shown a strong correlation between nut consumption and reduced risks of heart diseases. Four seminal studies conducted in U.S. showing the relationship between several foods (including nuts) and chronic diseases are presented; The Adventist Health Study, The Iowa Women's Health Study, The Nurses's Health Study and The Physicians' Health Study.

In the Adventist Health Study (Fraser et al., 1992), 27,000 California Seventh-day Adventists provided information on their dietary and lifestyle habits during six years whilecoronary heart disease (CHD) data was also obtained. Among the enquired foods, nut consumption presented the greatest inverse relationship with the risk of non-fatal myocardial infarction (MI) or death from CHD. Individuals who ate nuts five or more times per week presented with 48% less risk of MI and 38% less risk of death from CHD compared to the individuals who consumed nut less than once per week.

The consumption of 127 foods, including nuts, was correlated with the risk of CHD in The Iowa Women's Health Study (Kushi et al., 1996). Similar to the previous study, a food frequency questionnaire was applied and lifestyle habits were also. After 7 years of follow-up, data from 19,411 women who were not taking vitamin supplements showed a 40% reduction of fatal CHD risk in the group with highest nut consumption (more than 4 times per month) compared to the group with the lowest consumption (rarely or never).

The results from The Nurses's Health Study (Hu et al. 1998) also reported a cardio-protective effect of nuts consumption. During 14 years, data from 86,016 nurses were collected and analyzed regarding to the intake of 61 foods, including nuts, lifestyle and occurrence of CHD. In the group with high intake of nuts (≥5 times per week), the risk of fatal and non-fatal MI decreased by 39% and 32%, respectively, compared to the group eating nuts less than once per month. The Physicians' Health Study showed comparable with the previous works, but it had less impact. Male physicians totaling 22,017 participated of this study during 17 years of follow up. The group who consumed nuts more than once per week presented with 20% less risk of sudden cardiac death, however no reduction was detected for non-fatal MI, which was attributed to the low frequency of nut consumption in this studied population (Albert et al., 2002).

Consistent with those prospective studies, Estruch et al. (2013) published a recent study that included 7447 subjects (57% women) with a high cardiovascular risk were randomized to one of three diets, a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts (50% of walnuts), or a control diet, during 4.8 years. In summary, the results showed that the experimental diets led to a reduction of the major cardiovascular events (myocardial infarction, stroke, or death from CHD).

The cardiovascular effects of nuts consumption can be mainly attributed to their rich content of omega-3 PUFAs and antioxidants, as several epidemiologic studies have demonstrated significant associations between the intake of those nutrients and a reduced risk of CVDs (Banel and Hu, 2009; Scalbert et al, 2005; Vanschoonbeek et al., 2003). Omega-3 fatty acids are related to improvements in plasma lipids and endothelial function (West et al. 2005; Steer et al., 2003). English walnuts contain the highest amount of α-linolenic acid among all edible plants and their antioxidant polyphenols content is noteworthy (Crews et al., 2005; Fukuda et al, 2003). Accordingly, several studies have been performed with the purpose of investigating the cardio-protective effect of English walnuts.

West et al. (2010) assessed the effects of ALA on the vascular endothelial function in 20 hypercholesterolemic subjects using a randomized crossover study. Individuals were fed an average American diet, which contained 8.7% energy from PUFA (7.7% LA, 0.8% ALA), LA diet containing PUFA from whole walnuts and walnut oil that provided 16.4% of energy (12.6% LA, 3.6% ALA) and ALA diet containing 17% energy from PUFA from walnuts, walnut oil, and flax oil (10.5% LA, 6.5% ALA). It was reported that experimental diets (ALA and LA) significantly reduced diastolic blood pressure (-2 to -3 mm Hg) and total peripheral resistance (-4%). Flow-mediated dilation and arginine-vasopressin were increased after intake of the ALA diet. Data from this study suggest novel mechanisms for the cardio-protective effects of English walnuts.

Nergiz-Ünal et al. (2013) demonstrated that the intake of English walnut influenced plasma lipids levels and atherosclerotic plaque formation through an intervention study using mice as animal model. Proatherogenic Apoe(-/-) mice were fed during eight weeks with a high fat diet supplemented with walnuts (rich in omega-3 PUFA and antioxidants), walnut oil (with omega-3 PUFA only) or sunflower oil (control group). The consumption of whole walnuts, but not walnut oil, led to a 55% reduction in atherosclerotic plaque formation in the aortic arch compared to the control group. In addition, triglycerides, cholesterol and prothrombin serum levels decreased in the first group by 36%, 23% and 21%, respectively. Moreover, increased plasma antioxidant capacity was detected. The atheroprotective effect of walnuts were attributed to the combination of n-3 PUFA with other antioxidants components, likely polyphenols.

In a crossover study, the effects on serum lipids and other markers of CVDs risk were evaluated in 18 hypercholesterolemic patients (9 women and 9 men) randomized into sequences of diets containing 40% fat from English walnuts, virgin olive oil or almonds, during 4 weeks each (Damasceno et al., 2011). The results showed that LDL-cholesterol (LDL-c) was significantly decreased by 7.3%, 10.8% and 13.4% after the olive oil, English walnut and almond diets, respectively, when compared to baseline. Similarly, total cholesterol and LDL/HDL ratios were significantly reduced, but only after nut diets. This study is consistent with those reporting the well-established cholesterol lowering properties of nut consumption.

Also performing a randomized crossover study, Torabian et al. (2010) investigated a long duration consumption of English walnuts by 87 individuals (38 men and 49 women) with normal to moderate high plasma total cholesterol. The participants were assigned = a walnut-supplemented diet (approximately 12% of total daily energy intake) or habitual diet (control group) for six months each. The results showed that walnuts supplementation decreased total cholesterol and triglyceride levels, which was more significant in the high plasma total cholesterol group (at baseline). They also detected a high degree of dietary compliance (95%), indicating that people can maintain a walnut-rich diet for long periods. Those effects of English walnuts on lipid profile were consistent with the previous commented studies and others (Banel and Hu, 2009; Almario et al., 2001, Olmedilla-Alonso et al., 2008).

Despite the potentially health-promoting content, studies involving Black walnuts and their effect on CVDs or other disease states are limited. Fitschen (2010) investigated the cardiovascular effect of Black versus English walnuts consumption during 28-30 days. 29 participants (13 men and 16 women) were randomly selected to Black or English walnuts consumption and after a 12-week washout period they switched to the other type of walnut and consumed 30 g daily for more 28-30 days. The results showed that the walnuts intake led to a 2.7% reduction of total cholesterol and 4.2% of LDL-cholesterol. Importantly, the author reported that Black walnut consumption resulted in significantly improvement of the blood lipids than English walnuts. In another study, Rorabaugh et al. (2011) compared the antioxidant activity of English versus Black walnuts, through the ability to prevent oxidation of LDL-c, which was assessed *in-vitro* using walnuts extracts and *ex-vivo* after walnuts intake during 28 days by 36 subjects randomized to consume their habitual diets supplemented with either with 30 g of English or Black walnuts. The results revealed that both walnuts improved markers of oxidation, with English walnuts exhibiting a higher antioxidant capacity via an *in-*

vitro assay. However, the consumption of walnuts (30g daily/28days) was not sufficient to improve protection of LDL oxidation.

Based on these few studies and considering that Black walnuts are rich in many bioactive compounds that are present in English walnuts, such as PUFAs, polyphenols, vitamin E, fiber and arginine,]] (Dreher and Maher, 1996; Huynh and Chin-Dusting, 2006), Black walnuts consumption also could provide protective benefits against CVDs. However, more research using black walnuts is needed to determine its cardiovascular health promoting properties.

3.1 *Black Walnuts versus Cancer*

Epidemiological studies have shown a significant association between regular consumption of fruits, nuts and vegetables, and a lower incidence of certain types of cancer (Reddy et al., 2003; Mathew et al., 2004; Gonzales et al 2006). Some bioactive compounds presnet nuts are reported to poccess potent antioxidant, anti-inflammatory or chemopreventive properties, including omega-3 fatty acids (Berquin et al., 2008), tocopherol (vitamin E), phytosterols, folate and polyphenols (Segura et al. 2006, Gonzales et al 2006). Importantly, Black and English walnuts are excellent sources of these components (Table 1). Moreover, walnuts contain the highest content of antioxidants, mainly polyphenolic compounds and tocopherols (Blomhoff et al., 2006) compared to other nuts.

Carvalho et al. (2010) investigated the antiproliferative and antioxidant activities of English walnut in human renal cancer cell lines A-498 and 769-P and the colon cancer cell line Caco-2. The results showed that walnuts extract were able to inhibit the growth of those cells in a dose-dependent manner. This inhibition was not correlated with the total phenolic contents in the extracts tested, suggesting that a specific or a class of phytochemical in extracts may be responsible for their antiproliferative activities. In addition, after induced oxidative hemolysis of human erythrocytes the walnut extracts significantly protected their membrane from hemolysis in a time- and concentration-dependent manner. Data from this work suggest that walnuts are an excellent source of antioxidantatvie and anti-cancer agents.

Polyphenols may act as anti-cancer agents through the following mechanisms: (a) suppression the activation of nuclear factor-kB (NF-kB), which regulates the expression of genes involved in inflammation and carcinogenesis; (b) inhibition of the activator protein-1 (AP-1) transcription factor, which has increased function by carcinogenesis-promoting agents; (c) suppression of mitogen activated protein kinases (MAPK); (d) suppression of the protein kinases PKC; (e) suppression of growth-factor receptor (GFR)-mediated pathways; (f) cell cycle arrest and induction of apoptosis; (g) antioxidant and anti-inflammatory effects; and (h) suppression of angiogenesis (Bonfili et al., 2008; Fresco et al.,2006).

In a study using mice as animal models, Nagel et al. (2012) demonstrated that dietary walnuts inhibited colorectal cancer by suppressing angiogenesis. HT-29 human colon cancer cells were injected in female mice, which were thenrandomized to diets containing approximately 19% of total energy from English walnuts, flaxseed oil, or corn oil (control group) for 25 days. Walnut and flaxseed diets were responsible for slowing tumor development compared to the control group by 27% and 43%, respectively, while tumor weight was also reduced. In addition, plasma expression levels of angiogenesis factors were significantly reduced in mice fed on experimental diets. Angiogenesis was significantly decreased only in walnut diet compared to the control. The authors suggest that more studies should be performed in order to verify if walnuts supplementation have similar effects on colon cancer in humans.

As previously exposed, Black walnuts contains a sizeable content of γ-tocopherol (Table 1), a nutrient linked to protection against risks for prostate cancer (Chan et al., 1998;

Helzlsouer et al., 2000). For example, γ-tocopherol inhibited tumor cells growth for a variety of cancer cells including prostate cancer cells (Gysin et al., 2002). In a clinical study, Spaccarotella et al. (2008), aimed to evaluate the effects of English walnuts supplementation on markers of prostate cancer. Twenty one men at risk for prostate cancer were randomized into their usual diet with or without 75 g of walnuts during eight weeks. Walnuts supplementation led to an increased plasma γ-tocopherol and increased ratio of free prostate specific antigen (PSA): total PSA compared to the control diet. In this study, the results confirmed the potential anti-cancer effect of γ-tocopherol present in English walnuts.

Thus, Black walnuts have a promising potential on cancer prevention as it has similar chemopreventive agents to English walnuts, especially polyphenolic compounds and γ-tocopherol vitamin E, which are present even in higher amounts in the black walnuts (Table 1).

3.2 *Black walnuts versus Diabetes and Neurodegenerative Diseases*

Recent evidence suggests that the type of lipids is more important for type 2 diabetes development than the total intake of fat (Risérus et al., 2009). Studies have reported a relationship between the higher intake of MUFAs and PUFAs and lower intake of saturated fat and *trans*-fat with a decrease in the risk of type-2 diabetes (Risérus et al., 2009). Thus, nut consumption may play an important role on type-2 diabetes as the saturated fatty acid content of this food is low (4-16%) and the unsaturated fatty acids levels are high (Ros and Mataix, 2006). As showed in Table 1, Black and English walnuts composition alighn t with this information. Compared to English walnuts, Black walnuts contain less saturated fat and more MUFAs content; although the PUFAs profile is lower in Black walnuts, it is still higher than in the other commercial edible nuts (Ros, 2010). Walnuts also contain several other bioactive components that may be associated with the beneficial effects on type-2 diabetes, such dietary fiber, sterols, vegetable protein, and antioxidants (Pan et al., 2013).

Pan et al. (2013) assessed the relationship between English walnut consumption and the prevalence of type-2 diabetes in two seminal studies, the Nurses' Health Study (NHS) 1 and 2. Women (58,063 aged 52–77 years and 79,893 aged 35–52 years) without diabetes, cardiovascular disease, or cancer at baseline, participated in NHS 1 and NHS 2, respectively, during 10 years of follow-up. Consumption of walnuts and other edible nuts was investigated every 4 years by applying food frequency questionnaires. After multivariate adjustment for traditional risk factors, walnut consumption was associated with a lower risk of type 2 diabetes, which persisted after adjustment for other lifestyle factors. This study indicates that frequent ingestion of walnuts may be responsible for reducing risk of type-2 diabetes in women. However, further studies are needed in order to confirm their protective role..

The benefial effects promoted by walnuts intake also extends to brain health. Recent studies have demonstrated that animals fed diets rich in walnuts presented with improved indices of memory, cognition and motor functions (Joseph et al., 2005; Joseph et al., 2009; Willis et al., 2009c). α-linolenic acid rich foods are associated with brain health , namely EPA and DHA, play a crucial role in brain health by maintaining synaptic plasticity, neuronal membrane stability, gene expression, mitigation of oxidative stress and regulation of immune function (Willis et al., 2009a and 2009b). In addition, other nutrients, such as vitamin E, folate, phenolic compounds, melatonin and other micronutrients, have also been related to neuroprotective effects (Willis et al., 2009b and 2009c).

Poulose et al. (2012) investigated whether English walnut diet could activate autophagy function to maintain protein homeostasis, thus preventing the accumulation of polyubiquitinated proteins in brain of aged rats. The accumulation of these toxic proteins in brain increases with age, in part due to increased oxidative and inflammatory stresses and is a biomarker of several neurodegenerative diseases. After consuming a 6% or 9% walnut diet, 19-month-old rats presented with significantly reduced aggregation of polyubiquitinated proteins. Also, those animals exhibited up-regulation of autophagy. The Theresults revealed that a walnut-rich diet is effective on activation of the autophagy function in brain and also antioxidant and anti-inflammatory benefits were demonstrated.

In an *in-vitro* study, Willis et al. (2010) examined the effects of English walnut extract on LPS-induced activation in BV-2 microglial cells. Microglial activation can result in the production of cytotoxic intermediates, being correlated with a variety of age-related and neurodegenerative diseases. The ensuing data showed that cells treated with walnut extract produced lower amounts of nitric oxide and reduced expression of inducible nitric oxide synthase. Walnut supplementation also induced a reduction in tumor necrosis-alpha (TNF-α) generation and internalization of the LPS receptor toll-like receptor 4. This first study showing the anti-inflammatory effects of walnuts in microglia indicates that this edible nut may play an important role in the prevention/treatment of neurodegenerative conditions and the most likely nutrients involved in this function would be essential fatty acids.

The similar fatty acids composition of Black walnuts compared to English walnuts and also the presence of other bioactive agents suggests that the Black walnuts may also positively affect diabetes and neurodegenerative conditions through the same mechanisms cited above. However, future research using this particular type of edible walnut needs to be accomplished to consolidate these estimated results.

4. Conclusion

Black walnuts are the second most produced walnut in U.S., but their commercial value is not as high as for English walnuts because of the difficulty in processing of their seeds. However, the nutritional composition of Black walnut shows that this edible nut is a rich source of bioactive compounds capable of promoting multiple health benefits. The purpose of this review was to estimate the health-promoting potential through the consumption of Black walnuts based on data obtained from English walnuts research, as such studies with Black walnuts are almost inexistent. For example, Black walnuts contain high levels of polyunsaturated fatty acids (i.e. α-linolenic acid) and antioxidant compounds, as polyphenols and γ-tocopherol. These compounds have been associated with English walnuts consumption in terms of their protection against multiple diseases, including cardiovascular health, certain cancers, diabetes, neurodegenerative conditions, and others disease states related to oxidative and inflammatory stress. In addition, others agent present in Black walnuts have been linked to health promoting, including dietary fiber, folate, phytosterols, arginine, melatonin, among others. In summary, data showing health benefits through the English walnuts intake supports the characterization of Black walnuts as effective functional foods. However, future research using specifically Black walnut is needed in order to understand it function in human body and promote its inclusion in the diet.

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