Bioactive compounds and phytonutrients in edible part and nutshell of pecan (Carya illinoensis)

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Bioactive compounds and phytonutrients in edible part and nutshell of pecan (*Carya illinoinensis*)

M.A. Flores-Córdova¹, E. Sánchez Chávez¹*, C. Chávez-Mendoza¹, J.L. García-Hernández² and P. Preciado-Rangel³

**Abstract:** Nuts contain compounds in their mineral composition. Nutrients, and fatty acids, when consumed in sufficient quantities, provide health benefits. In this research the nutritional and functional contribution of bioactive compounds and phytonutrients of the edible part and two varieties of shell of the pecan from the Wichita and Western Schley trees was evaluated. Physicochemical results and the content of mineral elements had high nutrient levels, the fatty acid content showed high levels of 69%, the presence of unsaturated acids such as oleic, linoleic, palmitic was determined, the content of vitamin E was determined by high-performance liquid chromatography with 47.38%. Finally, the range of the Western Schley pecan tree had the highest values in most of the bioactive compounds and phytonutrients.

**Subjects:** Environment & Agriculture; Food Science & Technology; Health and Social Care

**Keywords:** nutrition; bioactive, functional compounds; phytonutrients

1. **Introduction**

As a new millennium is beginning, a new era in food science and nutrition has shown up with major intensity. Obesity and the occurrence of chronic degenerative diseases as a consequence of inappropriate diet and lifestyle are an evident concern of consumers and researchers (Swinburn, 2009). Scientific studies indicate that nut consumption reduces the occurrence of heart diseases, cancer, Type II diabetes and obesity, and it also participates in liver detoxification, as an anticancer agent and as an essential component of enzymes that inactivate the dangerous free radicals.
Moreover, the nutritional composition of the nut has been previously documented due to its functional and nutraceutical properties as it contains polyunsaturated fatty acids such as oleic, linoleic, tocopherols, vitamin E, proteins, fiber and carbohydrates. It also contributes with significant amounts of minerals, such as iron, zinc, selenium, manganese and magnesium (Venkatachalam & Sathe, 2010). Along with other bioactive compounds with antioxidant and functional activities, it meets the nutritional requirements and promotes the consumers’ health when ingested frequently (Kornsteiner, Wagner, & Elmadfa, 2006; Luna Guevara & Guerrero-Beltrán, 2013). The nut is internationally recognized for its caloric and nutritional value, as shown by several nutritional and functional studies (Santos et al., 2013). When consumed as part of a nutritionally adequate diet, within the calorie requirements, they help to reduce cardiovascular risk factors. The lipid fraction of this fruit stands out as one of the food products of great industrial interest. The consumption of healthy fats, such as nuts, causes a satiating effect and it has been shown that a daily consumption of 15 g of nuts helps to lose weight (Mattes, Kris-Etherton, & Foster, 2007). Similarly, Bao et al. (2013) reported that regular consumption of nuts was inversely correlated with mortality in men and women, independently of other predictors of death.

Conversely, in comparison with other dry fruits, nuts are a rich source of mineral compounds, such as calcium, magnesium and potassium. These elements are necessary for human beings who need more than 22 mineral elements. Some of them are needed in high amounts but others, such as Fe, Zn, Cu, I and Se, are needed in trace amounts as they are potentially harmful. However, the deficiency of mineral nutrients, such as Ca, Zn, Se, Fe and I, affects health and productivity of an important sector of the population in developing countries, especially poor women, children and babies (Graham, Welch, & Bouis, 2001). An excessive intake of minerals can have an effect on systemic physiology. Therefore, some researchers have focused their efforts on gathering precise data regarding the minimum and toxic doses of minerals in food (Mares et al., 2013).

From this perspective, the pecan nut has a great potential to contribute to food research, mainly due to its high content of bioactive compounds and phytonutrients. This evidence reveals that nut consumption is an attainable way to prevent several diseases when consumed frequently (Luna Guevara & Guerrero-Beltrán, 2013). Pecan nutshells also possess physiochemical properties and minerals (Pinheiro do Prado, Monalise Aragão, Fett, & Block, 2009). Thus, its characterization is important in order to obtain better alternatives for the exploitation of this fruit.

Taking the latter into account and based on its nutritive and functional potential, the objective of this study was to evaluate the content of bioactive compounds and phytonutrients in the edible part and the nutshell of two varieties of pecan nut: Western Schley and Wichita.

2. Experimental

2.1. Plant material and experimental design

The pecan nut fruits of Western Schley and Wichita varieties were harvested in the 2014 production cycle. They were provided by cooperating producers from the Delicias region (Chihuahua, Mexico). The nuts were transported and stored in refrigeration at −4°C in the Laboratory of Plant Physiology and Nutrition of the Research Center for Food and Development (Centro de Investigación en Alimentación y Desarrollo A.C.), Delicias Unit (CIAD). The nuts were washed and then shelled in order to separate the edible part from the shell. Afterwards, the shells underwent a drying process inside an air oven (Felisa, model FE-291, Zapopan, Jal.) at 40°C. The experimental design was completely randomized with four treatments (two varieties—Western Schley and Wichita nuts and two parts of the fruit—the edible part and the shell) and three repetitions.
2.2. Evaluated variables

2.2.1. Physiochemical properties assessment
The physiochemical composition of the edible part and the shell were determined according to the Association of Official Analytical Chemists (2000) and in compliance with the current Mexican Official Standards (NOM).

2.2.2. Humidity
The humidity percentage was determined by using the AOAC official method 925.40. The humidity content was expressed as a percentage.

2.2.3. Ash
Ash content was determined in compliance with the Mexican Standard NMX-F-066-S-1978 (1978). The content was expressed as a percentage.

2.2.4. Protein
Crude protein content was determined through total nitrogen quantification by the micro-Kjeldahl method (Association of Official Analytical Chemists, 2000) and it was expressed as a percentage.

2.2.5. Fiber
Fiber crude content was determined according to the Mexican Standard NOM-F-90-S-1978 (1978). The content of the crude fiber was shown as a percentage.

2.2.6. Fat
Fat content was determined according to the Goldfish method, Mexican Standard NMX-F-427-1982 (1982) and it was expressed as a percentage.

2.2.7. Carbohydrates
Carbohydrate content was determined according to the Mexican Standard NOM-F-90-S-1978 (1978) and it was calculated by difference. Carbohydrate content was expressed as a percentage.

2.3. Determination of phytonutrients (mineral composition)
The mineral content analysis of the elements Ca, Mg, Fe, Cu, Zn, Mn, K, Na, K, the organic elements N, C, H, S, and protein was assessed using the method previously reported by Uvalle-Bueno (1995). Both determinations were conducted separately using a digestion system with a microwave (Milestone Sk-10, model START, 2011, Italy). The elements were detected by atomic absorption spectrophotometry (Falcon, Thermo Scientific, ICE-300 AA, 2009, China).

2.4. Bioactive compounds
In order to determine fatty acids, a gas chromatograph (GC, Varian 430-GC) was used, equipped with a fused silica capillary column (SP-2560 Supelco EE.UU.) with a length of 100 m, 0.25 mm interior diameter and a polyethylene film of 0.2 mm thickness. The operating conditions were as follows: an injection divided in a 50:1 proportion, temperature 140°C for 5 min; ramp rate to 240°C at 4°C/min using helium as carrier gas; isobaric pressure of 37 μ, linear speed 20 cm/s, helium gas with a 29 ml/mm flow, and both injector and detector at a temperature of 250°C. All samples were analyzed in triplicate and the obtained results are expressed as the mean of the respective values.

2.5. Vitamin E concentration
Vitamin E concentration was determined according to the method reported by Hess, Keller, Oberlin, Bonfanti, and Schuep (1991) through reverse phase high-performance liquid chromatography (HPLC), with a 1,252 model chromatograph with a binary pump and a C18 column (Waters, New Braunfels, TX). The mobile phase was of 100% methanol and the measurement flow was 1 ml/min at 40°C, quantification was performed at a 280 nm wavelength.
3. Results and discussion

3.1. Physicochemical properties assessment

The chemical composition of the edible portion of the pecan nut reveals its contribution and nutritional quality for human consumption (Luna-Guevara & Guerrero-Beltrán, 2010). Table 1 shows the values of physicochemical composition obtained from both varieties, Wichita and Western Schley. Their edible portions displayed high caloric values of 704 and 702 kcal for the Wichita and Western varieties, respectively. The obtained values correlate with the reported values in the literature. The protein content was lower in the Wichita variety by 7.32%, but it was higher in the Western Schley variety with a 9.99% value. Regarding humidity content, it was 2.94% for the Wichita variety and 3.70% for the Western Schley variety. Conversely, fiber content was of 5.26% for the Wichita variety and 6.75% for the Western Schley variety. The ash content was 1.60% for the Wichita variety and 1.40% for the Western. Fatty acid content was higher in the Western Schley variety, reporting 68.67, and 70% of its weight, and they were identified as monounsaturated and polyunsaturated, as well as omega 3 and omega 6 fatty acids.

Regarding the nutshell, the fiber content of the Wichita variety was of 58.82 and 57.91% for the Western Schley variety. The humidity was 7.06 and 6.55%, the protein 1.57 and 1.64%, ash 2.10 and 1.98%, carbohydrates 30.16 and 31.36%, lipids 0.26 and 0.52% and 129.36 and 136.78 kcal in Wichita and Western Schley varieties, respectively.

Various authors as, Oro, Ogliari, Castanho, Barrera-Arellano, and Mara (2008) obtained values of 726.7 kcal. Mares et al. (2013) obtained values of 658.73 kcal, whereas Ros (2010) obtained 690.02 kcal in pecan nuts but in different species, indicating that nut consumption could provide a high level of energy value input as it represents a significant percentage of the daily calorie requirement for a 2,000 kcal diet (Santos et al., 2013). The result in protein is similar to the 9.9% result obtained by Oro et al. (2008). The values reported by other authors range between 7.8 and 10.43%. Protein accumulation occurs when cotyledon expands and it is completed three weeks after shell lignification (Singanusong, Mason, & D’Arcy, 2003). These proteins are generally rich in arginine, lysine and threonine. Thus, the accumulation process was more favorable for the Western Schley variety. The optimal percentage is considered 4%. When values below 2.5% are attained, it becomes fragile, brittle and susceptible to oxidation due to oxygen penetration as consequence of cracking. If it contains 5% or more, they tend to be spongy and susceptible to fungal growth and rotting during storage (Montoya, Garcia, Martinez, Vázquez, & Robles-Ozuna, 2010). Another of the favorable parameters, in the nut content is the fiber which to maintain the normal peristaltic movement in the intestinal tract,

| Table 1. Physicochemical composition of pecan nut shells and edible portions of the Wichita and Western Schley |
| Composition (g/100 g) |
| Shell | Edible portion |
| Wichita | Western | Wichita | Western |
| Fiber | 58.82 ± 0.93a | 57.91 ± 0.41a | 5.23 ± 2.03a | 6.75 ± 0.71a |
| Protein | 1.57 ± 0.14a | 1.64 ± 0.08a | 7.32 ± 1.55a | 9.99 ± 0.89a |
| Lipids | 0.26 ± 0.36a | 0.52 ± 0.45a | 68.67 ± 1.95a | 69.96 ± 0.52a |
| Humidity | 7.06 ± 0.12a | 6.55 ± 0.09a | 2.94 ± 0.02a | 3.70 ± 0.02a |
| Ash | 2.10 ± 0.03a | 1.98 ± 0.07a | 1.60 ± 0.02a | 1.60 ± 0.04a |
| Carbohydrates | 30.16 ± 0.96a | 31.36 ± 2.55a | 14.21 ± 0.48a | 8.22 ± 2.27a |
| kcal | 129.36 ± 2.62a | 136.78 ± 3.14a | 704 ± 17.29a | 702 ± 10.88a |

Notes: Data represent the mean value of three repetitions ± standard deviation. Means with the same letter within a column indicates no significant difference between varieties at $p < 0.05$.

aCarbohydrate content was calculated by difference.

bProtein (N × 5.46).
therefore low-fiber diets can promote constipation and lead to colon diseases (Akinhanmi, 2008). Ashes determine the content of organic compounds. Thus, when compared to other studies, in which values between 1.05 and 3.32% were obtained, it is indicated that they are within the reported values. Fatty acids reported by Ros (2010) in which a 72.0% value was obtained, whereas our results are below this level. However, Sze-Tao and Sathe (2000) obtained 66.90%, Santos et al. (2013) 67.20%, and Oro et al. (2008) 69.40%. Thus, the results obtained in this study are within the ideal values for fatty acid content, all of them having protective function through blood clot prevention and the reduction of coronary heart diseases (Mares et al., 2013). These values were compared to those in the study.

Pinheiro do Prado et al. (2009) reported the values of pecan nutshell as follows: protein 2.58, humidity 7.95, lipids 0.42, minerals 1.63, fiber 46.11 and carbohydrates 41.41; thus displaying similar values, excepting that of fiber. The differences observed in the shell composition can be explained by the different extraction techniques used to obtain the compounds and the different evaluated species, as well as the year of production. On the other hand, genetic factors, soil composition and maturation can also have a significant influence on nutritional composition (Malik et al., 2009).

### 3.2. Phytonutrients, macro- and micro-nutrients

Vitamins and minerals represent three-quarters of the biosubstances needed for life. Their assessment is an important part of nutrition (Cabrera, Lloris, Giménez, Olalla, & López, 2003). In Table 2, the results obtained from phytonutrient (mineral composition) determinations of the edible portion and the nutshell of pecan nuts are shown. Significant differences were observed ($p > 0.05$) in the edible portion regarding Ca, Cu, Mn, Ni and P. In the shell, there were significant differences in K, Cu and Ni. The edible portion of the Western Schley variety showed the highest Mg values: 186 mg g$^{-1}$. Other authors such as Senter (1976) reported the lowest Mg, Ca, Zn, Cu, Mn and Ni values, down to 130 mg g$^{-1}$. The Wichita variety exhibited higher values of K, Fe, Na and P. The Western Schley variety was therefore better as it displayed higher values of the majority of evaluated elements. Regarding the shell, the highest values of the Western Schley variety were those of the elements Cu, Mn and Ni, whereas for the Wichita variety, the elements K Ca Fe, Mn and P exhibited the highest values. Other authors, such as Senter (1976), Wakeling, Mason, D’Arc, and Caffin (2001) and Singanusong et al. (2003) obtained Mg values of 130, 126 and 152 mg g$^{-1}$, respectively. The latter values were lower compared to those observed in the Western variety, but higher regarding those of the Wichita variety. The same authors reported K values of 370, 477 and 457 mg g$^{-1}$, respectively, these being higher compared to the results obtained in this study.

Conversely, Senter (1976) reported a Ca value of 5.3 mg g$^{-1}$ which is lower, compared to that reported by Wakeling et al. (2001) and Singanusong et al. (2003) (61 and 64 mg g$^{-1}$, respectively) but similar to the values obtained in this study, except for the Western Schley variety having a higher value (73 mg g$^{-1}$). Regarding Zn, the highest reported values were 8.21, 6.90 and 6.0 mg g$^{-1}$. The highest $p$ values were 430, 325 and 378 mg g$^{-1}$, except for the Wichita, which showed a 324 mg g$^{-1}$ value of $p$, similar to that obtained by Senter (1976). Thus, the obtained results show that the edible portion and the shell are a rich source of macro- and micronutrients essential for human health,
representing an important factor for magnesium intake, which is recommended as a daily dose of 200 mg in adults. If the Mg content is 186 mg g⁻¹, as measured in this study, and if a person consumes 100 g nuts per day, 90% of the recommended intake value would be covered and this would help to prevent heart diseases. Cu, Cr, Fe and Zn are important for human metabolism as their combined supply is required to maintain the energy supply. Cu deficiency is rare, however when it is concomitant with Zn deficiency it promotes cardiovascular damages. Thus, they are both important as part of a balanced diet (Cabrera et al., 2003). K is essential to neural function as the transmitter of nerve impulses and it also participates during muscle contraction in the active heart (Martinez-Ballesta, Dominguez-Perles, Moreno, Muries, & Alcaraz-López, 2010). An elevated Ca, Mg and K intake, along with a low sodium intake, is considered protective against bone demineralization, arterial hypertension, insulin resistance and cardiovascular risk in general (Cordain et al., 2005). Finally, macronutrient, micronutrient and non-nutrient components of nuts have been reported to contribute to lowering the risk of coronary heart diseases and related to metabolic disorders. Based on the aforementioned reasons, nuts can be considered natural health capsules in which the whole is always better than the individual parts (Jacobs Jr., Gross, & Tapsell, 2009).

3.3. Bioactive compounds

Fats are the main component in nuts and they have been shown to be potentially beneficial for maintaining good health (Santos, Corrêa, Soares, Gioielli, & Costa, 2012). Thirteen fatty acids were identified and quantified in the edible portion of the Wichita and Western Schley varieties. Table 3 shows the fatty acid content profile. The most relevant are unsaturated fatty acids, especially oleic acid (omega 9) which displays the highest value in the Wichita variety (45.38%), and the lowest value (38.24%) in the Western variety. However, the linoleic acid (omega 6) content was the highest in the Western Schley variety (47.0%) and the lowest in the Wichita variety (36.57%). A similar pattern was observed for the following acids: palmitoleic acid 10.83 and 8.27%, palmitic 0.36 and 0.22%, elaidic 2.96 and 2.05%, linolelaidic 1.2 and 0.97%, gamma-linolenic 0.91 and 1.60%, undecanoic 0.21 and 0.34%, myristoleic 0.33 and 0.09%, myristic 0.25 and 0.33%, pentadecanoic 0.20 and 0.43%, cis-10-heptadecanoic 0.52 and 0.09%, heptadecanoic 0.33 and 0.12% respectively. These results were compared to those obtained in the study performed by Montoya et al. (2010), in which the oleic acid value was 39.01% and the linoleic value was 58.00%. This correlates with the Western Schley variety, although this same study evaluated the Wichita variety with no correlation at all. Santos et al. (2013) observed contents of 36.26% oleic acid and 37.53% linoleic. Wakeling et al.

| Fatty acids          | Wichita      | Western      |
|----------------------|--------------|--------------|
| Oleic                | 45.38 ± 1.3a | 38.24 ± 1.32a|
| Linoleic             | 36.57 ± 1.4a | 47.0 ± 1.41a |
| Palmitic             | 0.22 ± 0.006a| 0.36 ± 0.01a |
| Palmitoleic          | 10.83 ± 0.34a| 8.27 ± 0.16a |
| Elaidic              | 2.96 ± 0.03a | 2.05 ± 0.06a |
| Linolelaidic         | 1.2 ± 0.06a | 0.97 ± 0.08a |
| Gamma-linolenic      | 0.91 ± 0.04a | 1.60 ± 0.07a |
| Undecanoic           | 0.21 ± 0.002a| 0.34 ± 0.004a|
| Miristoleic          | 0.33 ± 0.01a | 0.09 ± 0.01a |
| Miristic             | 0.25 ± 0.007a| 0.33 ± 0.001a|
| Pentadecanoic        | 0.20 ± 0.02a | 0.43 ± 0.03a |
| Cis-10-heptadecanoic | 0.52 ± 0.02a | 0.09 ± 0.01a |
| Heptadecanoic        | 0.33 ± 0.01a | 0.12 ± 0.005a|

Notes: Mean values ± standard deviation. Means with the same letter within column indicates no significant difference between varieties at p < 0.05.
(2001) evaluated the Wichita and Western varieties and reported the following fatty acid composition: palmitic 6.56 and 6.65%, oleic 57.28 and 53.88%, and linoleic 31.50 and 53.88% respectively. 

Oro et al. (2008) reported higher oleic acid values: 62.55%. Meritxell, Mar, and Sánchez-Muñiz (2004) mentioned that the beneficial effect of dry fruits on the prevention of cardiovascular disease is related to its lipoprotein profile and it can be explained by their fatty acid composition. However, there are other compounds that enhance or induce these cardioprotective effects. Kris-Etherton et al. (2002) conducted a study with volunteers, providing a diet lacking in nuts but supplemented with the same fatty acids contained in them. LDL decrease would be the predicted output due to the nut’s lipid composition. However, it was observed that the actual value obtained after nut supplementation decreased LDL value by 25% compared to the predicted value. It was concluded that nuts contained other compounds that enhanced this positive effect on LDL.

Trox et al. (2010) mentioned that a polyunsaturated fatty acid diet has a protective effect against diseases, such as type II diabetes, hyperlipidemia or cardiovascular diseases. They also show promising positive effects on inflammatory diseases, cancer and osteoporosis. Thus, the obtained results show that nuts are an excellent source of energy with health benefits due to their total lipid content. In Figure 1, the respective elution times and orders of each fatty acid contained in the edible portion of the Wichita and Western pecan nuts are observed.

Figure 1. Chromatogram showing elution times and orders for each one of the fatty acids contained in the edible portion of the pecan nut. In different retention times.
3.4. Vitamin E content

In addition to fatty acids, the pecan nut also contains considerable amounts of vitamin E, which is considered an antioxidant (Blomhoff, Carlsen, Andersen, & Jacobs, 2006). In Figure 2 shows the Vitamin E content determined in the edible portion of the Wichita and Western Schley varieties of the pecan nut. There were no significant differences ($p < 0.05$). The vitamin E content of the Wichita was 45.57 mg/100 g and that of the Western Schley was 47.38 mg/100 g. The latter variety presented the highest value. Authors such as Montoya et al. (2010) reported a vitamin E value of 15.4 mg/100 g, similar to the 16.46 mg/100 g value reported by Taipina, Lamardo, Rodas, and del Mastro (2009). However, the result obtained in this study was higher, probably because these authors used conservation methods which caused a considerable loss of vitamin E. This vitamin has been identified as one of the main components in the nut (Sayago, Marín, Aparicio, & Morales, 2007). Regarding nutrition, its increased intake seems to have a protective effect against degenerative diseases, according to the theory explaining its cardioprotective effect, which seems to be related to the tocopherol-mediated inhibition of LDL oxidation, proposed as the key role in the atherogenic process. Moreover, vitamin E has a double antioxidant action: it displays an in vivo effect, preserving cellular lipids against oxidation, but it also has an in vitro effect, protecting oils and food from oxidative rancidity. It can also act synergistically with other antioxidants constituting a network capable of eliminating free radicals and reducing oxidative damage to cellular biomolecules, such as lipids, proteins and DNA (Alasalvar & Shahidi, 2009). The deficiency of this vitamin is very rare and it is circumscribed to the less developed or developing countries. The symptoms of this deficiency are related to the lack of antioxidant protection offered by this vitamin. The most significant sign is erythrocyte lytic tendency as well as other hematological, neurological, muscular and ophthalmic signs (Mataix & Ochoa, 2002). Regarding vitamin E, the recommended daily intake for adults is based on age, sex and physiological state, being 20 mg of tocopherols expressed as alpha-tocopherol (U.S. Department of Agriculture, Agricultural research service, 2004).

The identification of the vitamin E chromatography peaks was carried out by HPLC. They were displayed in a typical chromatogram in which were shown the results obtained from the analyzed samples of the edible portion of the pecan nuts (Wichita and Western Schley). The observed result of vitamin E had a retention time of 20.80 min (Figure 3).
4. Conclusions
The physiochemical characterization of the edible portion and the shell of pecan nuts revealed their nutrient and functional potential to be included in diets. They could also be applied to several agro-industrial activities, because of their elevated content of kcal and fatty acids (70%). In addition, it was
demonstrated that nuts are rich in mineral compounds micro and macronutrients. Nuts also supply vitamin E. Finally, the Western Schley variety exhibited the most elevated contents of bioactive compounds and phytonutrients.

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**Competing Interest**
The authors declare no competing interest.

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