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ORIGINAL ARTICLE

Examining Effects of Storage Duration on the Different Forms of Tocopherol and Peroxide Value in Walnut Kernel and Powder

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K E Y W O R D S

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A B S T R A C T

The walnut kernel (WK), which has high oil content and contains many polyunsaturated fatty acids (FFA), is considered a valuable nut with a protective effect against cardiovascular diseases. This product is sensitive to oxidation and has a short shelf life. Fat oxidation is the crucial parameter that reduces the economic value and has a negative side for consumers during storage time. The objective of present research was the evaluation of Vit E types ($\alpha - \beta - \gamma - \delta$) and peroxide indexes during storage time (6 months) for walnut kernel and walnut powder (WP). The analysis of peroxide indicated that during storage time of this parameter increased to kernel and powder, significantly ($p < 0.05$). Moreover, testifier samples had the highest total tocopherol value. According to the results of the statistical analysis, the effect of the storage time on the tocopherol and peroxide index was bilateral. Meanwhile, there is a direct relationship between the reduction of tocopherol content and the increase of peroxide value.

Introduction

Walnut have bioactive compounds, including caffeic acid, ferulic acid, and phenolic compounds like juglon, which have been demonstrated to improve lipid profiles, blood pressure, endothelial function, inflammation, oxidative stress, and thrombosis (Jahanbani *et al.*, 2016, 2018, 2021). The kernel of walnuts has a high nutrient value food because of its high concentration of proteins, unsaturated fatty acids, triglycerides, tocopherols, vitamins minerals, and phenolic acids such as Gallic acid (Hassankhah *et al*., 2017; Habibie *et al.*, 2019, 2021a, 2021b; Sarikhani *et al*., 2021; Habibi et al., 2022, 2023; Quyingyang *et al*., 2024). Carbohydrates such as pectic compounds and other polyphenols are abundant in these nuts, contributing to their overall nutritional profile (Rashki *et al*., 2025). Unsaturated fatty acids in walnuts have been shown to provide health benefits, such as cardio-protective effects including lowering blood cholesterol levels and providing antioxidant protection (Richard *et al*. 2008). Peroxide number is measured as the amount of peroxide in the oil (Chatrabnous *et al*., 2018, 2019). Heat, oxygen, and metal (Cu) are among the factors that intensify oxidation and it also found that this index shows a good correlation with organoleptic properties (Pal *et al*., 2025). There various research papers indicated that application of walnut powder as a novel, functional and rich in nutrition value natural ingredient to apply in food formulations to enhances physicochemical and sensory evaluation properties (Nosirov *et al*., 2024;

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Ifeh *et al*., 2024;). In the case of study, Scientifics stated that the peroxide number of butter replacement by walnut powder showed lower values than the standard of the Iranian organization (Emami, *et al*., 2011; Pakrah *et al.*, 2021, 2022). Therefore, this study was performed to the effect of different walnut types (kernel and powder) on tocopherol and peroxide number properties, during storage time at laboratory temperature (20ºC for 180 days).

Materials and Methods

Tocopherol measurement

About $10mg$ of the extracted oil was dissolved in $1\,ml$ of heptane and 10 ml was injected into the HPLC device. Tocopherol with a particle size of 5μ and a mobile phase mixture of Lichrosphere 100 $NH2$ filled with LichroCART 250-4 column of Heptane: Tert butyl ether and Methanol: tetrahydrofuran (0.2:0.89; 20:79) at a speed of $1ml$ separated per minute. For determination, the fluorescence detector was used at the wavelength of 294 nm and $320nm$ respectively for excitation and emission. Based on the retention time of tocopherol in the oil samples, the foreign standard method was used to determine the amount.

Peroxide number

$5gr$ Of the sample was weighed in an Erlenmeyer sandblasted lid. $30ml$ chloroform acetic acid mixture and then $0.5ml$ of saturated potassium iodide solution were added to it and placed in a cover after adding potassium iodide, the resulting mixture was kept in the dark for 1 minute. After this step, $30ml$ of distilled water and $0.5ml$ of starch solution were added and titrated with sodium thiosulfate $0.1N$. The peroxide number was calculated using the following equation (Pascoalino *et al*., 2025).

$$\text{Peroxide number} = \frac{0.1\times N\times 1000}{m} \qquad (1)$$

Where N is the volume of the sodium thiosulphate used a sample in ml and m is the mass of the walnut sample in g. Measurements (in triplicate) were conducted within the period 6 months (Tahmasbian *et al*., 2021).

Statistical analysis

Each experiment was carried out in triplicates with at least 3 independent cultures with comparable results. Data are reported as mean± SD in at least three experiments. Comparisons between groups were made by student test $t-test. p<0.05$ was considered statistically significant.

Results

Analysis of tocopherols

The results of tocopherol and peroxidate value of WK and WP during storage times are displayed in Tables 1 - 5. Compared to testifiers before storage times, the concentration of tocopherols decreased by increasing storage of time and differences were significant ($p<0.05$).

Table1. α-Tocopherol concentration during storage time.

	$\alpha-$ **Tocopherol**						
	Before storage	**30 Days**	**60 Days**	**90 Days**	**120 Days**	**150 Days**	**180 Days**
Walnut kernel	375.12 ± 0.14^{a}	370.1 ± 0.22^{a}	367.27 ± 0.29^{b}	360.14 ± 0.36^{b}	351.41 ± 0.33^{c}	340.40 ± 0.20^{c}	329.60 ± 0.11^{d}
Walnut powder	369.11 ± 0.22^{a}	360.47 ± 0.27^{a}	359.78 ± 0.33^{b}	349.10 ± 0.10^{b}	338.11 ± 0.24^{c}	329.20 ± 0.33^{c}	316.11 ± 054^{d}

Different letters within the same row represent significant differences ($p<0.05$).Means ±standard deviation

Table 2. β-Tocopherol concentration during storage time.

	$\beta-$ Tocopherol						
	Before storage	**30Days**	**60 Days**	**90 Days**	**120 Days**	**150 Days**	**180 Days**
Walnut kernel	20.99±0.22[a]	1910±0.97[a]	18.17±0.36[a]	17.87±0.39[a]	15.22±0.36[b]	14.12±0.39[b]	13.28±058[b]
Walnut powder	18.33±0.35[a]	17.77±0.99[a]	16.90±0.37[a]	15.97±0.64[a]	14.44±0.49[b]	13.11±0.98[b]	11.10±0.39[b]

Different letters within the same row represent significant differences (p<0.05).Means ±standard deviation

Table 3. γ-Tocopherol concentration during storage time.

	$\gamma-$ Tocopherol						
	Before Storage	**30 Days**	**60 Days**	**90 Days**	**120 Days**	**150 Days**	**180 Days**
Walnut kernel	17. 22±0.88[a]	16.55±0.21[a]	15.77±0.25[a]	14.25±087[b]	13.58±0.55[b]	12.22±0.98[c]	10.11±0.25[c]
Walnut powder	15.50±0.59[a]	14.40±0.32[a]	13.30±0.19[a]	12.20±0.80[b]	11.10±0.98[b]	9.30±0.39[b]	7.20±0.39[c]

Different letters within the same row represent significant differences (p<0.05).Means ±standard deviation

Table 4. δ-Tocopherol concentration during storage time.

	δ-Tocopherol						
	Before storage	**30 Days**	**60 Days**	**90 Days**	**120 Days**	**150 Days**	**180Days**
Walnut kernel	27.11±0.22[a]	20.11±0.10[a]	17.22±0.23[b]	14.11±011[b]	12.32±0.28[c]	11.11±0.65[c]	10.24±0.74[d]
Walnut powder	25.02±0.30[a]	19.13±0.79[a]	16.25±0.69[a]	14.98±0.35[a]	11.21±0.25[c]	9.98±0.20[c]	8.99±0.25[d]

Different letters within the same row represent significant differences (p<0.05).Means ±standard deviation

Table 5. Total tocopherol concentration during storage time.

	Total- Tocopherol						
	Before storage	**30 Days**	**60 Days**	**90 Days**	**120 Days**	**150 Days**	**180 Days**
Walnut kernel	408.01±0.02[a]	403.02±0.01[a]	393.02±0.41[b]	389.50±0.09[b]	380.77±0.21[c]	370.14±0.71[c]	361.17±0.11[d]
Walnut powder	405.25±0.10[a]	400.08±0.2[a]	392.11±0.91[b]	387.29±0.81[c]	377.22±0.01[c]	366.36±0.50[c]	356.67±0.31[d]

Different letters within the same row represent significant differences ($p < 0.05$).Means ±standard deviation

Peroxide number

Table 6 shows the peroxide value of treatments after 6 months of storage. Results of the current study showed that there was a continued increase in tocopherol peroxide level during storage time. WK and WP samples before storage had the lowest peroxide value compared to samples after storage time. Significant differences (p > 0.05) among treatments at each time point of the storage time. So, the increase in peroxide value attributed to temperature and storage time.

Table 6. Peroxide value of WK and WP during storage time

	Peroxide value						
	Before storage	**30 Days**	**60 Days**	**90 Days**	**120 Days**	**150 Days**	**180 Days**
Walnut kernel	1.70±0.22[a]	2.22±0.29[a]	2.90±0.59[a]	3.19±0.87[a]	3.90±0.17[a]	4.19±0.98[b]	4.59±22[b]
Walnut Powder	1.90±0.29[a]	2.23±0.88[a]	2.99±0.47[a]	3.59±0.29[b]	4.19±0.87[b]	5..49±0.78[b]	6.10±0.80[c]

Different letters within the same row represent significant differences (p<0.05).Means ±standard deviation

Discussion

Tocopherols, especially α and γ tocopherols, are the most abundant natural antioxidants in vegetable oils (Park *et al*., 2008). The data in the tables indicated that walnut kernel had the excellent amount

of tocopherol and amount of this vitamin was from 408 -361 μg g^{-1} for kernel walnut and 405-356 μg g^{-1} for walnut powder during storage time. These values are higher than those reported with researchers in other walnut varieties (Akbari & Shahidi, 2008). Moreover, present results were in good agreement by previous scientific research (Schwrtz*et al*., 2008 and Kornsteiner *et al*, 2006). Scientific reports stated that in France and the USA, the content of Vit E in walnuts decreased during storage time. They emphasized contents of β tocopherol and γ tocopherol reduced in fresh and stored kernels for 3 momth at 4℃. They found that gamma-tocopherol is the major tocopherol in walnut oil. The quantity of tocopherol depends on some parameters such as variety and cultivation conditions in agriculture like genotype and mutation. Meanwhile, researchers pointed out that some phenomena like cultivation in the warm season (spring and summer) could positively affect the tocopherol concentrations in almonds by warmer temperatures. In addition, the increase in tocopherol concentration content might be attributed to the preservation of plant oxidation damage in drought conditions (Kodad *et al*., 2018). The present study tries to investigate the effort of different parameters on the peroxide value of WK and WP samples. In WP samples, moisture enters the oil from oxygen and accelerates the oxidation.An Oxygen molecule acts as an electron acceptor in oxidation and causes the transfer of electrons from oils, which leads to the formation of free radicals of atoms with extra electron. Present radicals, react with other chemical compounds in walnut unsaturated fatty acids (Linoleic acid and oleic acid) and can begin oxidation chains. In brief, humidity is one of the most important factors affecting the oxidation of nuts. One of the substances produced by the oxidation of fats are peroxides, which are compounds containing two oxygen molecules that can react with fats and fatty acids in cells and produce a substance called Malondialdehyde (MDA), which is a Diketone compound it's one of the common mutagen and cancer factors for health (Roozban *et al.*, 2006). In addition, peroxide values in vegetable oils are $10\leq 0$ (Asadi *et al*, 2022). The present results were in good agreement with the scientific report.

Conclusions

Walnut oils are made up of a myriad of fatty acids, which include polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SUFA). In summation, the storage period of walnuts hurts their quality in terms of an increase in peroxide values and a decrease in the tocopherol content. Walnuts also possess multiple health-related bioactive compounds, including anticancer, antibacterial, cardiovascular, and antibiotic activities. Simultaneously, in the field of agricultural science, several natural and environmental factors like genotype, cultivar, and cultivation origin directly influence physicochemical properties of the end product. Further, a remarkable variation exists in terms of quality and quantity regarding the composition of tocopherol and fatty acid among different varieties.

Conflict of interests

No conflict.

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