Phenolic profile, color parameters and antioxidant activity of walnut kernel extracts as influenced by different time and temperature during extraction

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Introduction

Walnuts have been consumed for centuries as a highly nutritious food in many diets and societies of the world. Recent research has shown that they are helpful to tackle life-style diseases like arteriosclerosis, cardiovascular diseases, and diabetes mellitus. Walnuts contain notable amounts of bioactive compounds such as polyphenols that have positive effects on health. In addition, the polyphenols in walnuts are significantly higher compared to other nuts. Not much research have been done on polyphenols of walnuts. In this study we wanted to develop an accurate and comprehensive process to maximize the extraction of polyphenols and antioxidants.

Material and methods

Hungarian walnut cultivar 'Alsószentiváni 117' was obtained from Pálháza (Hungary) for the research. The walnuts were first deshelled, and the kernels were collected. The kernels were ground, and a fine powder was prepared. This kernel powder was used to extract polyphenols using 100% methanol in 1:5 (w/v) sample:solvent ratio. The extraction was done using four different methods (Figure 1) to find out the one which gives maximum polyphenols. The four different methods are explained in Table 1.

Table 1. Extraction solvent- 100% Methanol; Sample: Solvent- 1:5(w/v); Treatment- Shaking water bath

Method 1	Method 2	Method 3	Method 4
50°C, 30 Minutes shaking	50°C, 30 Minutes shaking; then 5°C, 20 Hours	40°C, 2 Hours shaking	40°C, 2 Hours shaking; then 5°C, 20 Hours

Total Polyphenol Content (TPC) - was determined using Singleton and Rossi Method (1965).
Ferric Reducing Ability of Plasma (FRAP) - values were measured spectrophotometrically according to the Benzie and Strain method (1996). Free radical scavenging activity (DPPH) was performed according to the method described by (Blois 1958).
Color coordinates were determined according to C.I.E.LAB system using a tristimulus colorimeter (Konica Minolta CR 410, Minolta Canada Inc.) and the ΔE * was calculated using (Klimczak, 2017).
The individual polyphenols were quantified using Shimadzu High Performance Liquid Chromatograph (HPLC) (C18 2.6 µm 150×4.6 mm column, the gradient mobile phase was A1% formic acid with HPLC grade water and, B: 1% formic acid with acetonitrile (0–30 min: A 100%–10%, 30–30.1 min: 10%–100%, 30.1–31: A 100%) with a flow rate 1.5 mL/min, the pressure in the column was 4200±10 psi at a column temperature of 30 ºC, between 280 and 310 nm).
Statistical analysis was performed using one factor complete randomized ANOVA using IBM SPSS version 27.

Figure 1. Preparation of extract from walnut

Results

Figure 2. TPC of Extracts

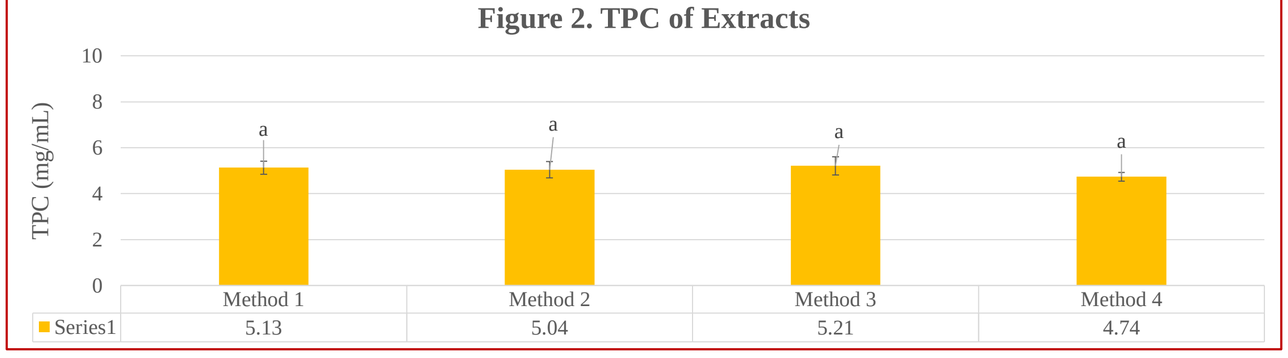

Figure 3. FRAP of Extracts

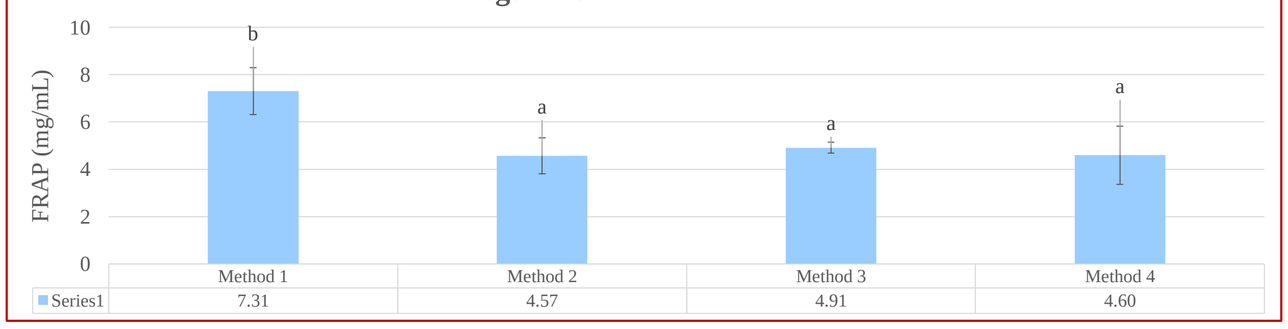

Figure 4. DPPH of Extracts

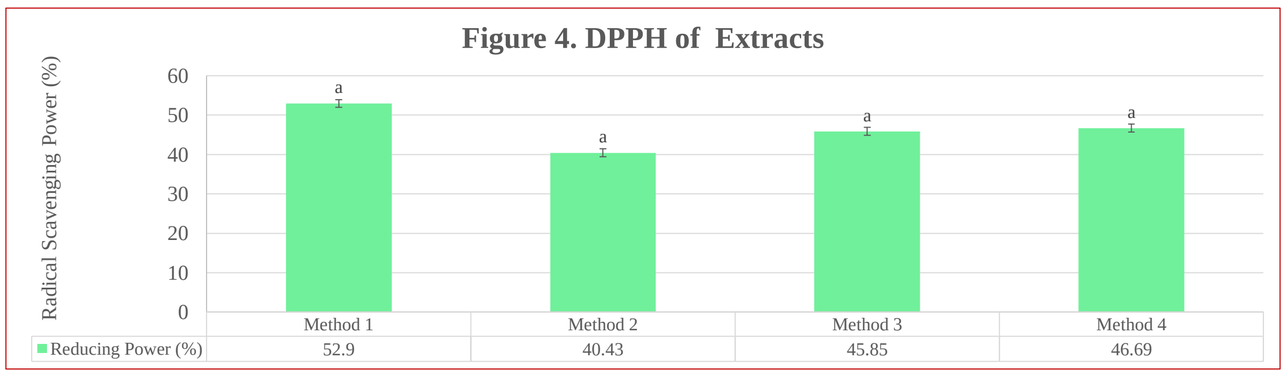

Table 2. Color values of extracts

	L*	a*	b*
Method 1	25.98 ± 0.31[a]	-0.35 ± 0.10[b,c]	6.96 ± 0.32[b]
Method 2	28.28 ± 1.83[a]	-0.73 ± 0.09[a]	5.35 ± 0.39[a]
Method 3	26.63 ± 0.57[a]	-0.15 ± 0.05[c]	5.93 ± 0.11[a]
Method 4	27.59 ± 0.81[a]	-0.48 ± 0.23[ab]	5.68 ± 0.15[a]

Table 3. ΔE* values of extracts

Method 1-2	Method 1-3	Method 1-4	Method 2-3	Method 2-4
2.83	1.23	2.06	1.84	1.04

Table 4. HPLC analysis of individual polyphenols (mg/mL)

Extraction Method	Chlorogenic acid	Catechin	Epicatechin	Rutin	Juglone
Method 1	2.54 ± 0.15[b]	2.80 ± 0.93[a]	3.22 ± 0.82[a]	3.61 ± 0.11[a]	0.06 ± 0.00[a]
Method 2	2.18 ± 0.13[a]	3.59 ± 0.07[a]	2.00 ± 0.03[a]	3.25 ± 0.05[a]	0.09 ± 0.00[a]
Method 3	1.95 ± 0.08[a]	3.53 ± 0.13[a]	2.32 ± 0.68[a]	3.11 ± 0.04[a]	0.06 ± 0.00[a]
Method 4	2.06 ± 0.10[a]	3.38 ± 0.06[a]	2.58 ± 0.79[a]	3.35 ± 0.42[a]	0.06 ± 0.00[a]

Table 5. Correlation among the dependent variables

	FRAP	DPPH	L*	a*	b*	chlorogenic acid	cathecin	epicathecin	rutin	juglone
TPC	0.376	0.802	-0.515	0.460	0.390	0.185	-0.125	0.029	-0.143	0.040
FRAP		0.758	-0.867	0.409	0.990	0.834	-0.964	0.925	0.812	-0.498
DPPH			-0.925	0.782	0.808	0.374	-0.566	0.565	0.235	-0.522
L*				-0.808	-0.926	-0.453	0.754	-0.807	-0.458	0.760
a*					0.529	-0.159	-0.254	0.405	-0.105	-0.830
b*						0.749	-0.941	0.932	0.749	-0.604
chlorogenic acid							-0.875	0.731	0.919	-0.007
cathecin								-0.969	-0.929	0.489
epicathecin									0.863	-0.683
rutin										-0.253

Discussion

Insignificant differences were found in amounts of TPC in extracts (P = 0.343) of all four methods (Figure 2). Highest values of FRAP (7.31 mg/mL) were seen in extracts of Method 1 (Figure 3). According to ANOVA this was significantly different (P<0.05) from other methods.
The values of DPPH for all the extracts were not significantly different (P = 0.487) from each other in all the extraction methods (Figure 4). Similar results for polyphenols and their activities were reported by (Bujdosó et al. 2014; Kumar et al. 2022).
The extraction methods had no significant effect on L* color values of the extracts (P = 0.107). b* values were highest for Method 1. There was significant difference in the results of a* (P<0.005) and b* (P<0.0001) color values (Table 2). ΔE* color difference between Method 1-3 and Method 2-4 is "hardly noticeable (Table 3). However, the difference between Method 1-2, 1-4 and 2-3 is "Noticeable" by human eye.
When the individual polyphenols were quantified. Chlorogenic acid was present in significantly higher amount (P<0.005) in Method 1 compared to others (Table 4). We found 2.54 mg/g chlorogenic acid in walnut kernels using Method 1. The other polyphenols like rutin (P = 0.111), catechin (P = 0.233), epicatechin (P = 0.195) were also more in samples extracted using Method 1 but they were not significantly higher than the samples extracted using other methods. Earlier (Bolling, McKay, and Blumberg 2010; Bujdoso and Cseke 2021) had found similar results. Strong correlation can be shown between TPC and DPPH (R^2=0.802), FRAP was strongly correlated with chlorogenic acid (R^2=0.834), epicatechin (R^2=0.925), and rutin (R^2=0.812) (Table 5).

Conclusion

More focus should be given on walnuts bioactive compounds and polyphenols beyond the fatty acids. Our results showed that Method 1 could be better for extracting more polyphenols with high antioxidant power. However, there is a requirement of more research on stability of polyphenols and further experiments should be done to evaluate their efficacy on human health.

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