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The Nutritional and Phytochemical Composition of the Indigenous Australian Pindan Walnut (*Terminalia cunninghamii*) Kernels

Liezhou Zhong[1] · Janet F. Bornman[2] · Gangcheng Wu[1] · Aline Hornoff[1] · Koya Ange Pamela Dovi[1] · Hayder AL-Ali[1] · Naveed Aslam[1] · Stuart K. Johnson[1]

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Abstract Nutritional composition of the kernels of two types of Pindan walnut (*Terminalia cunninghamii*), a native nut consumed traditionally by Australian Indigenous peoples, is reported for the first time. Results showed that Pindan walnut kernels contained high levels of fat, protein and ash, approximately 50, 30 and 5% fresh basis, respectively. The levels of minerals in the kernels were much higher than common walnuts and macadamia nuts, especially those of phosphorus, magnesium and zinc. The high amounts of polyphenols in the kernels provided strong hydrophilic antioxidant capacities, of up to 2004 mg Trolox equivalents/100 g fresh basis using the hydrophilic oxygen radical absorbance capacity assay. Both free polyphenol content and hydrophilic antioxidant capacities of the kernels were higher than those of macadamia nuts, although the lipophilic oxygen radical absorbance capacity was lower. These preliminary studies indicate high potential for wider use of the Pindan walnut as a novel, nutritious and health-promoting food.

Keywords Pindan walnut · *Terminalia cunninghamii* · Indigenous Australian nut · Proximate composition · Polyphenols · Antioxidant capacities

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✉ Stuart K. Johnson
S.Johnson@curtin.edu.au

[1] School of Public Health, Curtin University, Perth, Western Australia 6102, Australia

[2] Curtin Business School, Curtin University, Perth, Western Australia 6102, Australia

Introduction

Australia possesses rich and unique flora, including over 25,000 species of indigenous plants, due in part to its geographical isolation and variable climate [1]. Over recent decades, scientific investigations on the nutritional and phytochemical properties of Australian native plants and fruits have offered significant opportunities for their commercial utilisation as novel foods, medicines or cosmetics, and boosted the development of Australia's native foods industry [2]. Several Australian bush plants have been systematically studied for their bioactive compounds, physiological benefits, and commercial food product opportunities [2, 3]. However, very many other endemic plants are still exclusively used by the Indigenous peoples and are largely unexplored or even unknown by others; Pindan walnut is such an example.

Pindan walnut is indigenous to the north-western coast of Australia and locally known as "Pindan quondong" or "kalumburu almond" [4]. Although its large "reddish-purple" fleshy fruits have succulent mesocarps when mature, the indigenous peoples in the area collect them from the wild before maturity for their kernels [5]. After cracking the thick, corrugated nutshells (namely, walnut-like endocarp) using hammers or stones, the raw seeds, which taste like almonds, can be eaten [4]. Pindan walnut was originally described by C.A. Gardner in 1923, and systematically named as *Terminalia cunninghamii* C.A. Gardner. More recently, based on their morphological differences, Barrett refined the taxon into two subspecies with *Terminalia kumpaja* R.L. Barrett, sp. nov. described as the new species [5]. In this paper, however, the Pindan walnut refers to *Terminalia cunninghamii*.

Currently, the major concerns of food security and lack of agro-diversity have led to increased emphasis in the global agriculture sector to incorporate more local food crops into farming systems [6]. In addition, nut consumption has shown

to have multiple health benefits, which are believed to be linked to their high levels of unsaturated fatty acids and phytochemicals [7, 8]. This general health association suggests that Pindan walnut might also have potential health protective effects. Although Pindan walnut has undergone cultivation trials and has been designated food ID (15A10712) by Food Standards Australia New Zealand (FSANZ), to the best of our knowledge, no scientific report has been published that systematically investigated its nutritional and phytochemical composition. Moreover, the nutrient values of Pindan walnut on the FSANZ database require updating [9]. As such, this paper presents preliminary data on its proximate composition and potential health-enhancing components including minerals, dietary fibre, polyphenols, and antioxidant capacities. This new information on Pindan walnut should assist in the development of its indigenous importance and more widespread use as a nutritious food.

Materials and Methods

Collection and Preparation for Analysis of Pindan Walnuts Two types of Pindan walnut fruits, "large/round" (PWL) and "small/oval" (PWS), were collected in 2015 along Broome Highway and Broome Parkland Western Australia, respectively. The outer dried fruit layers were removed and the resulting stones carefully cracked to retrieve the intact kernels using a nutcracker (Supplemental Figure). Subsequently, the kernels were stored at −20 °C and ground into a paste just before analyses.

Proximate Composition, Minerals and Dietary Fibre Moisture, ash and fat of the Pindan walnuts were determined using standard methods, AOAC 925.40, 950.49 and 948.22, respectively. Protein content (N × 5.3) was determined by a Dumas combustion method. An inductively coupled plasma-optical emission spectrometer was used to determine specific minerals (*i.e.*, Fe, Ca, P, Mg, Cu, Na, K and Zn) at the National Measurement Institute (Department of Industry Innovation and Science, Kensington WA, Australia).

A total starch test kit (K-TSTA, Megazyme, Ireland) based on the amyloglucosidase-thermostable α-amylase method (AOAC 996.11) was used to determine total starch content. Total dietary fibre was quantified by the enzymatic-gravimetric method (AOAC 985.29) using a total dietary fiber assay kit (K-TDFR, Megazyme, Ireland). Energy (kJ) was calculated using the following factors (kJ/g): carbohydrate: 17; unavailable carbohydrate (including dietary fibre): 8; fat: 37; and 17 for protein [10]. The available carbohydrate was calculated by difference from the sum of the values of moisture, fat, protein, ash and dietary fibre.

Sugar Composition Analysis Defatted samples were extracted for sugars with 30 mL of distilled water using a vortex mixer for 5 h at ambient temperature, followed by centrifugation at 2700×*g* for 6 min at 20 °C. After washing twice, the resulting supernatants were pooled and made up to 50 mL with distilled water, then filtered through 0.45 mm membrane filter. An Agilent (Santa Clara, CA, USA) 1200 HPLC system equipped with evaporative light scattering detector 2000ES and a Prevail Carbohydrate ES (250 × 4.6 mm × 5 μm) (Grace Davison Discovery Scinces, Deerfield, IL, USA) column was used to identify individual sugars. For the detector, a flow rate of 2 L/min of nitrogen was used as nebulising gas, and the drift tube temperature was set to 80 °C. HPLC-grade water and acetonitrile (25:75, *v*/v) were the mobile phases. A flow rate of 1 mL/min and injection volume of 20 μL were used. Fructose, glucose, and sucrose standard solutions were run to obtain the standard curves.

Extractions of Free and Bound Polyphenols Free and bound polyphenols were extracted as described by Wu et al. [11] with some modifications. 0.5 g of Pindan walnut kernel was accurately weighed and mixed with 10 mL hexane. After thoroughly shaking for 10 min at ambient temperature, the mixture was centrifuged at 2700×*g*, 4 °C for 5 min. The hexane layer, which contained free lipophilic antioxidants was collected and extraction repeated twice. The combined lipid extract was later used for determination of lipophilic antioxidant capacity. After hexane evaporation under nitrogen flow, 25 mL of methanol/water (80:20, *v*/v) were added to the remaining residue and vortexed for 10 min. The sample was then centrifuged at 2700×*g*, 4 °C for 5 min. The supernatant was collected, the wash process repeated twice and the resulting clear supernatants collected. The supernatant was subsequently evaporated to dryness at 45 °C with a rotary vacuum evaporator, and re-suspended in 10 mL of methanol. This fraction was stored at −20 °C before analysis of the free polyphenols.

For extraction of bound polyphenols, the residue from the free polyphenols extraction was mixed with 20 mL of 2 mol/L NaOH. The mixture was flushed with nitrogen for 2 min and then sealed. After digestion for 60 min at ambient temperature with rotational-shaking, the suspension was acidified to pH 2 by concentrated HCl (12 mol/L). Then hexane was used again to extract lipids as described above, which was kept for lipophilic antioxidant capacity assay. Ethyl acetate (20 mL) was added to the residue, and mixed by rotational-shaking for 10 min at ambient temperature. After centrifugation at 2700×*g*, 4 °C for 5 min, the resulting organic layer was collected. The ethyl acetate extraction was repeated five times. The combined organic supernatant containing bound polyphenols was then evaporated to dryness in vacuo using a rotary evaporator, and finally re-dissolved in 10 mL of methanol and stored at −20 °C until analysis.

Determination of Total Polyphenol and Total Flavonoid Content Total polyphenol content (TPC) of each extract was quantified using a modified Folin-Ciocalteu colorimetric assay and gallic acid as standard as previously described by Wu et al. [11]. The total flavonoid content (TFC) was determined using an aluminum chloride colorimetric assay and catechin as standard described by Wu et al. [11].

DPPH and ABTS• + scavenging Assays 150 μL of diluted free or bound polyphenol extracts were mixed with 2850 μL of freshly prepared 2–2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS•+) solutions, respectively [11]. The resulting solutions were left to stand for 8 h and 2 h in the dark at room temperature prior to the absorbance at 515 and 734 nm, respectively. Results were expressed as mg of Trolox equivalents (TE)/100 g fresh basis.

Hydrophilic and Lipophilic Oxygen Radical Absorbance Capacity Assay Hydrophilic (H-$ORAC_{FL}$) and lipophilic (L-$ORAC_{FL}$) oxygen radical absorbance capacity assays were performed on 96-well black-walled, clear-bottom polypropylene microplates essentially basing on the procedures described by Huang et al. [12]. For H-$ORAC_{FL}$ assay, 25 μL of the extracts in triplicates and 150 μL of 0.084 μmol/L fluorescein were added into wells and mixed thoroughly using a microplate vortex mixer. After incubation at 37 °C for 30 min, 25 μL of 153 mmol/L freshly prepared AAPH was quickly added to initiate the reaction. Fluorescence intensity was monitored kinetically at 37 °C with an excitation wavelength of 485 and 538 nm emission for every minute during a total of 120 min, using a multi-detection micro-plate reader (BioTek, VT, USA). The areas under the average fluorescence-reaction time kinetic curves (AUC) were integrated. 75 mmol/L phosphate buffer (pH 7.0) was used to further dilute the extracts when necessary, and to dissolve fluorescein, AAPH and Trolox standard.

For L-$ORAC_{FL}$ assay, the hexane extracts were blow-dried with nitrogen then re-dissolved in 250 μL of acetone and diluted with 70 g L^{-1} randomly methylated-β-cyclodextrin in aqueous acetone (1:1, *v*/v) to 1 mL. The assay was performed in the same manner as that for H-$ORAC_{FL}$, except that the randomly methylated-β-cyclodextrin solution was used for sample dilutions, and to prepare Trolox standard solutions.

Statistical Analysis At least duplicate analyses were used for all variables and all results were expressed as mean ± standard deviation on fresh basis. SPSS Statistics V 23 (IBM, NY, USA) was used for data analyses, and $p < 0.05$ was considered significant (*t*-test). Published data and USDA Food Composition Databases on macadamia nuts and common walnuts were used for comparisons.

Results and Discussion

Proximate Composition Table 1 shows the results of proximate composition of the two Pindan walnut types. Pindan walnuts in this study were collected from the wild in the hot season without further drying. As such, they had relatively higher levels of moisture (57.57–58.44 g kg^{-1} fresh basis) than commercially dried common walnuts and macadamia nuts at 31–41 and 14–22 g kg^{-1} fresh basis, respectively [13]. However, a much higher moisture content (97 g kg^{-1}) in Pindan walnut was reported by Brand-Miller et al. [9]. The drying characteristics of in-shell Pindan walnut kernels are still unknown and need to be determined in future. Generally, for most of in-shell nuts, drying to appropriate moisture content is essential to obtain desirable kernel recovery ratio during de-shelling, as well as the desired kernel flavour and texture after processing and bulk storage. Moreover, high moisture content in Pindan walnut may accelerate the deterioration of kernel qualities through toxigenic mold development, enzymatic lipid oxidation and browning. For macadamia nuts, drying to lower than 100 g kg^{-1} on farm and further to 30 g kg^{-1} before cracking are recommended, but the optimal drying strategy for Pindan walnut requires further study [14].

Fat contents in Pindan walnuts did not significantly differ between the two types. Nevertheless, they were lower than those of macadamia nuts and commercial walnuts varieties, 640–760 and 620–740 g kg^{-1} fresh basis, respectively [15, 16]. The high level of fat in the kernels indicated the need to study its fatty acid profile to determine levels of essential, polyunsaturated, monounsaturated and saturated fatty acids, and hence determine the nutritional and health value of this lipid fraction. There may be high potential to use Pindan walnut as a novel health-promoting dietary oil source depending on this fatty acid profile [8]. Further research is also required to understand the oxidative stability, including sensory acceptability by consumers, of Pindan walnut lipids during post-harvest storage, food processing and food storage.

Of the two types of Pindan walnut kernels, PWS had less amount of protein than PWL. However, compared to common walnuts and macadamia nuts, protein content in Pindan walnut was approximately 1.5–4 times higher, indicative of its potential as a valuable plant protein source. Similarly, ash contents in both Pindan walnut types were much higher than those in common walnuts and macadamia nuts (Table 1). Based on data from the USDA database, Pindan walnut had similar contents of carbohydrate (138.6–148.5 g kg^{-1} fresh basis, by difference) and total dietary fibre to walnuts and macadamia nuts [16]. Conversely, Pindan walnut had a lower level of energy than the other two nuts mainly due to the much lower level of fat. The Pindan walnut energy value may, however, be overestimated, since it has been reported that

Table 1 Comparisons of proximate composition (g kg^{-1} fresh basis) of the two types of Pindan walnuts with common walnuts and macadamia nuts

Component	PWS	PWL	Walnuts[a]	Macadamia nuts[a]
Energy (kJ)	26,010 ± 217.37a	25,110 ± 1253.22a	*27,380*	*30,040*
Moisture	57.57 ± 0.13a	58.444 ± 1.15a	*40.7*	*13.6*
Ash	48.30 ± 0.14a	48.3 ± 0.00a	*17.8*	*13.4*
Fat	501.96 ± 9.42a	452.15 ± 63.09a	*652.1*	*757.7*
Protein	253.55 ± 6.78a	290.58 ± 4.27b	*152.3*	*79.1*
Total dietary fibre	66.30 ± 3.96a	74.83 ± 4.27b	*43.6*	*86*
Starch	Negligible	Negligible	*0.6*	*0*
Sugars	43.15 ± 2.67a	73.20 ± 23.25b	*26.1*	*45.7*
Glucose	ND	ND	*0.8*	*0.7*
Fructose	3.18 ± 0.69a	3.00 ± 0.57a	*0.9*	*0.7*
Sucrose	35.89 ± 3.16a	60.32 ± 22.91b	*24.3*	*24.3*

Means for each component marked with different letters in each row are significantly different ($p < 0.05$)

PWS, Pindan walnut small/oval; *PWL*, Pindan walnut large/round, *ND*, not detected

[a] Adapted from USDA Food Databases except where noted, values are for edible portion

digestibility of macronutrients in nuts such as almond, is significantly lower than that of other foods [17].

Both types of Pindan nuts contained negligible levels of starch. Pindan walnuts had slightly higher levels of sucrose (ranging from 35.89 to 60.32 g kg^{-1} fresh basis) than walnuts (up to 26.1 g kg^{-1} fresh basis) and macadamia nuts (up to 45.7 g kg^{-1} fresh basis). Correspondingly, total sugars contents of the three nuts were in the same manner. This indicated that Pindan walnut may be vulnerable to browning reactions after harvest, since sucrose may be hydrolysed to the reducing sugars which can facilitate the browning process during storage and processing [18].

Minerals and Trace Elements Only the PWS type was analysed for minerals due to lack of sample size of the PWL type. Pindan walnut generally contained high levels of minerals and trace elements (Table 2) compared to walnut and macadamia nut, in accordance with its higher ash content (Table 1). Particularly, the amount of magnesium in 100 g of Pindan walnut was almost double that of the recommended dietary intake (310–420 mg/day for Australian adults) [19]. Of all the three nuts, Pindan walnut contained the highest amount of calcium, iron and zinc, suggesting that Pindan walnut could be a valuable dietary source of these essential minerals. However, phosphorus was the most abundant element in Pindan walnut and was 4–5-fold higher than that of walnut and macadamia nut, which may be due to a higher level of phytates. Generally, tree nuts have considerable amount of phytates, for example, up to 6.69 g/100 g in walnut and 2.62 g/100 g in macadamia nut [20, 21]. Phytates are considered an anti-nutritional factor due to their capacity to chelate cations, in particular calcium, iron and zinc, to form insoluble complexes and then reduce their bio-availability [20]. Further work is needed to evaluate phytates content and their effects on minerals bio-availabilities of the Pindan walnut.

Free, Bound and Total Polyphenols The polyphenol contents of the two types of Pindan walnuts were significantly different, with PWS having higher levels (Table 3). TPC and TFC in free extracts of PWS was 2- and 4-fold greater than those of PWL as were the total polyphenol contents. However, the values for bound extracts of PWS were just slightly higher than those of PWL. Overall, the results were in agreement with the observed appearance of the colour of the ground PWS paste, which appeared much darker than the PWL paste. Polyphenols can contribute to colour as well as the slightly astringent flavour of nuts [22].

Table 2 Comparison of minerals and trace elements content (mg/100 g fresh basis) of Pindan walnuts with common walnuts and macadamia nuts

Mineral	Pindan walnut*	Walnuts[a]	Macadamia nuts[a]
Calcium, Ca	133.33 ± 5.77	*98*	*85*
Magnesium, Mg	526.67 ± 5.77	*158*	*130*
Potassium, K	910.00 ± 0.00	*441*	*368*
Sodium, Na	8.47 ± 0.21	*2*	*5*
Phosphorus, P	1033.33 ± 57.74	*346*	*188*
Zinc, Zn	6.00 ± 0.10	*3.09*	*1.3*
Iron, Fe	4.80 ± 0.00	*2.91*	*3.69*
Copper, Cu	1.30 ± 0.00	*1.59*	*0.76*

* "small/oval" type only

[a] Adapted from USDA Food Databases, values are for edible portion

Table 3 Polyphenol contents and antioxidant capacities of the two types of Pindan walnuts

Fractions	Types	TPC[a]	TFC[b]	DPPH[c]	ABTS·+[c]	H-ORAC$_{FL}$	L-ORAC$_{FL}$	H-ORAC$_{FL}$ (%)
Free	PWS	214.94 ± 7.03a	141.60 ± 4.89a	298.36 ± 5.96a	420.80 ± 12.13a	1420.12 ± 22.55a	34.93 ± 0.61a	97.60
	PWL	104.87 ± 4.00b	40.76 ± 3.39b	131.04 ± 2.22b	187.72 ± 5.23b	866.46 ± 9.04b	20.98 ± 1.82b	97.64
Bound	PWS	51.46 ± 0.59c	37.29 ± 2.78c	55.33 ± 3.52c	95.23 ± 4.73c	583.57 ± 31.40c	16.58 ± 1.11c	96.65
	PWL	41.47 ± 2.50d	28.70 ± 1.14c	57.21 ± 4.55c	82.54 ± 4.22c	468.52 ± 6.62d	20.23 ± 0.12b	96.58
Total	PWS	266.40 ± 7.03e	178.89 ± 7.67e	353.69 ± 9.48e	516.03 ± 16.87e	2003.70 ± 8.86e	51.51 ± 0.50e	97.49
	PWL	146.34 ± 1.51f	69.46 ± 4.52f	188.25 ± 6.77f	270.26 ± 9.45f	1334.98 ± 2.42f	41.21 ± 1.70f	97.01

Means of fraction marked in each column with different letters are significantly different ($p < 0.05$)

PWS, Pindan walnut small/oval; *PWL*, Pindan walnut large/round

[a] Total phenolic content, mg gallic acid equivalents/100 g fresh basis

[b] Total flavonoid content, mg catechin equivalents /100 g fresh basis

[c] mg Trolox equivalents/100 g fresh basis

The free polyphenols of Pindan walnuts were 3–6 times higher than that of macadamia nut (36.2 mg GAE/100 g dry basis), although much lower than common walnut (up to 1325 mg GAE/100 g dry basis) [7]. Likewise, of the three nuts, the total free flavonoid contents were macadamia nuts (9.4 mg CE/100 g dry basis) < Pindan walnuts (38.3–133.1 CE/100 g dry basis) < common walnuts (535.4 CE/100 g dry basis) [7]. Interestingly, according to the results reported by Yang et al. [7], macadamia nuts had significantly higher contents of bound polyphenols than common walnuts and Pindan walnuts. But it worth noting that the bound, insoluble polyphenols may be less bioavailable than the free soluble form [23]. In summary, the high levels of polyphenols compared to macadamia nut imply that Pindan walnut could be a good source of these potentially health protective phytochemicals [21]. However, future research is required to identify and quantify its individual polyphenols, since different individual poyphenolics can have different bioactivities [24].

Hydrophilic Antioxidant Capacities Following the trends in the results of polyphenol contents, antioxidant capacities of free polyphenols extracts were much higher than those of bound extracts using the three hydrophilic antioxidant assays (Table 3). Furthermore, for both free and bound extracts, antioxidant capacities of PWS were stronger than those of PWL. Common walnuts had shown the greatest antioxidant activity among tree nuts, with the total value of the ABTS· + assay reaching 3429.36 mg TE/100 g fresh basis [25]. A similar pattern was observed in total H-ORAC$_{FL}$ values with Pindan walnuts being significantly lower than that of common walnuts (3268.03 mg TE/100 g fresh basis), but relatively higher than found in macadamia nuts (361.17 mg TE/100 g fresh basis) [26]. Comparisons and interpretations on these antioxidant capacities of the three nuts were difficult due to differences in methods, such as use of different extraction solvents or chemical assays. However it can be postulated that the hydrophilic antioxidant capacities of the three nuts were: common walnut > Pindan walnut > macadamia nut.

Lipophilic Oxygen Radical Absorbance Capacity (L-ORAC$_{FL}$) Nuts are important dietary sources of oil, which also benefits human health in diverse ways [27]. Considerable amounts of lipophilic bioactive components, *e.g.*, tocopherols, carotenoids, polyphenols and fatty acids, can serve as antioxidants to have both health benefits and protecting the nut lipids from oxidation [28]. L-ORAC$_{FL}$ values of PWS and PWL free fractions were much lower than the corresponding values of walnuts and macadamia nuts which were reported to be 121.14 and 63.07 mg TE/100 g fresh basis, respectively [26]. This may be partly due to their higher fat levels than Pindan walnuts. Although H-ORAC$_{FL}$ and L-ORAC$_{FL}$ are not necessarily associated, it was surprising that the L-ORAC$_{FL}$ value of the PWS bound fraction was slightly lower than that of PWL. The most interesting finding was the quite high L-ORAC$_{FL}$ values of bound lipophilic extracts of both Pindan walnut types, because most lipid, along with those lipophilic antioxidants were assumed to have been removed during the free lipophilic extraction. Probably the incomplete extraction and the release of bound and/or trapped lipophilic constituents from Pindan walnut matrix were contributors.

Conclusions In conclusion, this compositional analysis of Pindan walnut kernels revealed that they contain a variety of nutritionally valuable compounds, which are in most cases comparable to or at enhanced levels compared to common walnuts and macadamia nuts. The high levels of protein,

lipids (presuming that they are primarily unsaturated), minerals and phytochemicals may provide benefits for human nutrition and health. They are also indicative of the high potential for development of novel food or cosmetic products using Pindan walnut. However, more research is required to substantiate the initial findings and to investigate the composition of the nut in far more detail, including fatty acid, amino acid profiles, phytochemicals (*e.g.*, phytates and individual polyphenols), potential allergens and contaminants (mainly aflatoxins). This should also include investigation of a wider range of samples to understand the effects of genetic and environmental factors on the composition of this nut.

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Compliance with Ethical Standards

Conflict of Interest All of the authors declare that they have no conflict of interest.

Abbreviations *ABTS·+*, 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt; *CE*, Catechin equivalent; *DPPH*, 2-2-diphenyl-1-picrylhydrazyl; *GAE*, Gallic acid equivalent; *H/L-ORAC$_{FL}$*, Hydrophilic/ Lipophilic oxygen radical absorbance capacity; *PWL/PWS*, Pindan walnut "Large/round" type/ "Small/oval" type; *TE*, Trolox equivalent; *TFC*, Total flavonoid content; *TPC*, Total polyphenol content

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