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Chemical composition of walnuts (*Juglans regia* L.) grown in New Zealand

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Abstract. Walnuts *(Juglans regia* L.) were collected during the 1997 harvest from 12 different cultivars of trees grown in a replicated trial in an experimental orchard at Lincoln University. Two US commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, G139, G120) and eight New Zealand selections (Rex, Dublin's Glory, Meyric, Stanley, 150, 151, 153) were evaluated. The total oil content ranged from 62.6 to 70.3% while the crude protein ranged from 13.6 to 18.1%. Dietary fiber ranged from 4.2 to 5.2% while the starch content made up no more than 2.8% of the remaining portion of the kernel. The amino acid content of the walnuts was similar between cultivars and the patterns of essential amino acids were characteristic of a high quality protein.

Key words: Amino acids, Ash, Crude protein, Dietary fiber, Oil content, Starch, Walnuts

Introduction

In a prospective cohort study, regular consumption of nuts has been associated with a reduced risk of both fatal coronary heart disease and non-fatal myocardial infarction [1]. These results are consistent with an earlier epidemiological study [2] which showed that people who consumed nuts five or more times a week had a 50% reduced risk of coronary heart disease relative to those who never consumed nuts. A similar reduction in relative risk was observed in a cohort of women [1] from the Nurses' Health Study [3].

Although walnuts are rich in fat, a diet supplemented with walnuts had a beneficial effect on blood lipids, lowering blood cholesterol and lowering the ratio of serum concentrations of low density lipoprotein:high density lipoprotein by 12% [4]. The positive results of these experiments have been confirmed in cross-sectional surveys on the effect of walnut consumption on blood cholesterol [5].

Nuts may protect against coronary heart disease through a number of mechanisms [4, 6] and up to eight constituents might contribute to the positive nutritional benefits of nuts [1]. Most nuts are rich in arginine, a precursor

of nitric oxide, a potent vasodilator which can inhibit platelet adhesion and aggregation [7]. Walnuts contain about 10% α-linolenic acid which has been associated with reduced risk in several prospective studies possibly due to antithrombotic and antiarrhymic effects of α-linolenic acid [8, 9]. Other proposed benefits of nuts include their high content of magnesium, copper, folic acid, protein, potassium, fiber and vitamin E [1].

As interest in tree nuts, especially walnuts, is now growing, it is important to investigate the composition of the nuts commonly grown in each country. Studies in Italy and New Zealand have shown that the total fat and the individual fatty acid contents of different cultivars varied widely [10, 11]. The α-linolenic contents of walnuts grown under the same conditions in New Zealand ranged from 8.0–13.8% while the α-linolenic contents of walnuts grown in Italy ranged from 12.8–15.3% [10, 11]. A later study on a wider range of walnut cultivars confirm that the total oil and linolenic acid contents varied widely between cultivars [12]. This study also showed that another key constituent, vitamin E also varied between cultivars grown at the same location.

Ruggeri et al. [10] were the first to present the chemical composition of named cultivars of walnuts. Their data highlight the range of composition between different cultivars. While it is clear that walnuts have a positive role in human nutrition, it will not be easy to identify which constituents have the more important effects. It is possible that some cultivars may have a better effect on human metabolism because of their different nutritional composition. The aim of this study was to gain more in-depth knowledge on the proximate composition, carbohydrates and amino acid content of 12 of the most potentially useful walnut lines or cultivars commonly grown in New Zealand.

Materials and methods

Sources of the walnuts. Two replicated walnut variety trials were planted at Lincoln University in 1985 and 1987, respectively, by the Walnut Action Group, (Rex Baker Memorial Trial). The trees were grafted clonal material on seedling rootstock (primarily *J. regia* but with some trees on *J. nigra* to obtain adequate rootstock numbers). There were six replicates of each cultivar or selection. Two US commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, G139, G120) and seven New Zealand selections (Dublin's Glory (143), Meyric (1199), Stanley (Ble 300), Rex (152), 150, 151, 153) were evaluated. The numbers in brackets are the Walnut Action Group's Accession Number. The New Zealand seedling selections were made by the Walnut Action Group. The trials were planted in Tem-

pleton Silt Loam and the entire block was surrounded by guard trees to act as pollinizers. Details of the management practices and climate conditions [13] and the harvesting and drying conditions [14] followed standard methods. The walnuts analyzed in this experiment were harvested in April and May 1997 with harvests at three-day intervals. The walnuts were collected from the ground and were then washed and dried in a forced air drier at 30 °C to a moisture content of approximately 9%. The nuts were then opened and the kernels were vacuum packed in plastic bags and stored at –70 °C until analyzed. Chemical analysis was carried out on bulk harvested samples. Representative samples of each cultivar were then finely chopped using a stainless steel knife. Dry matter, ash, total protein and fat contents were determined on representative samples of finely chopped material. Starch, soluble sugars and total dietary fiber contents were determined on defatted material. Twenty g of finely chopped nuts were placed in a cellulose extraction thimble and extracted with hexane at 60 °C in a Tecator Soxtec system HT 1043 extractor for 6 h. Excess solvent was allowed to evaporate from the defatted meal, which was then ground to a fine powder using a water cooled IKA M20 mill. The finely ground material was stored at room temperature (15 °C in a desiccator) until analysis.

Chemical analysis. All chemical analyses on the samples of nuts were carried out in triplicate. Moisture, ash, total fat and protein contents (N $\times$ 6.25) were determined in accordance with AOAC methods [15].

Starch. The starch content of the defatted walnut meals were determined using a Boehringer assay kit No 207748 (Boehringer Mannheim, Germany). Triplicate samples (0.5 g) of defatted walnut meal were weighed into 100 ml conical flasks and hydrolyzed with 20 ml dimethyl sulphoxide and 5 ml 8 M HCL for 30 min at 60 °C in a shaking water bath. When cool, 50 ml of distilled water were added and the pH of the solution adjusted to 7.0 $\pm$ 0.5 using NaOH and made up to 100 ml in a volumetric flask. The glucose content of the filtered solution was determined following incubation with amyloglucosidase to degrade the hydrolyzed starch. The released glucose was assayed using hexokinase and glucose-6-phosphate dehydrogenase to release NADPH which was measured at 340 nm.

Total dietary fiber. The total dietary fiber content of the defatted walnut meals were determined using a Sigma assay kit (TDF 100A) (Sigma Chemical Company, St Louis, USA).

Acid and neutral detergent fiber. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined using Van Soest and Wine

methods [16]. Two g of defatted walnut meal were weighed and treated with either 100 ml neutral detergent or acid detergent fiber solution. The residues from each process were collected in a sintered glass crucible and dried to a constant weight at 105 °C. This residue was then ashed in a muffle furnace (McGregor, New Zealand) at 550 °C to correct for ash retained in the dried fiber material.

Amino acid analysis. Duplicate samples (20–25 mg) of defatted walnut meal were hydrolyzed with 5 ml 6 M HCI containing 2 mg/ml reagent grade phenol and 5000 μ moles norleucine for 24 h at 110 °C in thoroughly evacuated and sealed Pyrex tubes. Following removal of the acid at 50 °C on a Büchi rotary evaporator (R110, Switzerland), the hydrolysates were taken up in sodium citrate buffer, pH 2.2, and suitable aliquots were analyzed with a LKB-Model 4151 Alpha Plus amino acid analyzer using the standard protein hydrolysate program with sodium citrate buffers and ninhydrin detection. Data collection was done with a Shimadzu CR2AX integrator; results were normalized on the basis of the added internal standard. The values for threonine and serine were corrected for hydrolysis loss using the standard recovery factors 0.96 and 0.90, respectively.

Statistical analysis. Descriptive statistics were calculated. Results are expressed as mean $\pm$ SE.

Results and discussion

Results obtained for proximate composition of the 12 cultivars of walnuts are shown in Table 1. The total oil content of the kernels ranged from 62.6 to 70.3%; crude protein ranged from 13.6 to 18.1%. The European and New Zealand cultivars, in general, had a higher crude protein content than the two American walnut cultivars Vina and Tehama. The American cultivars also contained lower dietary fiber contents than the remainder of the walnuts. These two cultivars often have a softer flesh and have less crunch when eaten. The remaining cultivars of walnuts had dietary fiber contents that tends to be preferred in the New Zealand market because they have a more satisfying bite.

The proximate composition of the walnuts reported in this study are comparable to the composition of different cultivars of walnuts grown in Italy [10]. The crude protein content of the walnuts grown in Italy were marginally higher than those grown in New Zealand, once allowance for the different conversion factor (N $\times$ 5.30) used by Ruggeri et al. [10] was taken into account. Other nutrient contents were comparable, except that the dietary fiber

Table 1. Proximate chemical composition (mean ± SE) (g/100 g DM) of walnuts grown under the same conditions in New Zealand

Cultivar & origins	Dry matter	Crude protein	Lipid	Ash	Starch	Dietary fiber	ADF	NDF
European & US								
Esterhazy	93.5 ± 0.75	15.4 ± 0.78	70.1 ± 0.12	2.2 ± 0.13	2.8 ± 0.01	4.3 ± 0.19	2.9 ± 0.13	4.4 ± 0.24
G139	93.6 ± 0.30	16.8 ± 0.19	65.3 ± 0.11	2.1 ± 0.05	1.6 ± 0.28	5.2 ± 0.23	3.2 ± 0.39	3.8 ± 0.26
G120	93.3 ± 0.98	14.3 ± 0.14	69.9 ± 0.50	2.1 ± 0.16	1.6 ± 0.28	3.6 ± 0.08	2.7 ± 0.13	3.3 ± 0.22
Tehama	93.7 ± 0.61	13.6 ± 0.38	68.5 ± 0.11	2.0 ± 0.09	2.7 ± 0.02	3.1 ± 0.28	2.5 ± 0.28	3.3 ± 0.24
Vina	93.7 ± 0.62	14.2 ± 0.38	67.8 ± 0.26	1.9 ± 0.04	2.3 ± 0.16	3.8 ± 0.20	2.2 ± 0.22	3.4 ± 0.24
New Zealand								
Rex	93.8 ± 0.71	18.1 ± 0.22	67.2 ± 0.16	2.1 ± 0.08	1.5 ± 0.18	4.3 ± 0.22	2.8 ± 0.27	3.4 ± 0.17
Dublin's Glory	93.9 ± 0.17	16.6 ± 0.77	62.6 ± 0.27	2.4 ± 0.13	1.1 ± 0.08	4.2 ± 0.23	2.6 ± 0.41	3.4 ± 0.98
Meyric	93.9 ± 0.60	15.5 ± 0.05	69.1 ± 0.47	2.0 ± 0.12	1.8 ± 0.13	4.2 ± 0.29	2.2 ± 0.17	3.4 ± 0.40
Stanley	93.7 ± 0.93	14.1 ± 0.12	70.3 ± 0.17	2.1 ± 0.06	1.8 ± 0.06	4.9 ± 0.14	2.2 ± 0.11	3.9 ± 0.35
150	94.9 ± 0.86	15.2 ± 0.08	68.6 ± 0.24	1.9 ± 0.04	1.8 ± 0.02	4.6 ± 0.15	3.1 ± 0.37	4.0 ± 0.43
151	93.2 ± 0.94	16.7 ± 0.09	68.6 ± 0.24	1.9 ± 0.06	1.6 ± 0.03	4.4 ± 0.32	3.2 ± 0.36	3.8 ± 0.46
153	94.5 ± 0.54	15.9 ± 0.14	69.6 ± 0.04	1.9 ± 0.13	1.8 ± 0.21	4.9 ± 0.30	3.8 ± 0.24	4.4 ± 0.48

Results are mean values of triplicate determinations.

content of the New Zealand grown nuts appear to be marginally lower and the starch higher than Italian nuts.

The amino acid compositions of the nuts (Table 2) were consistent among the 12 different cultivars, except for the two American cultivars, Tehama and Vina, which showed lower amino acid contents. These lower values reflect the lower crude protein contents of these two cultivars (Table 1). The amino acid pattern of the 12 different cultivars reported in this study show some variations from the values reported for the Sorento cultivar grown under different conditions in Italy [10]. Ruggeri et al. [10] confirm that the ratio of lysine/arginine was very low (0.19). The results in Table 2 confirm the earlier observation [10] that walnuts contain a relatively low content of lysine and high levels of arginine. The mean lysine/arginine ratio for these samples was 0.24 SE $\pm$ 0.01 which is much lower than other common proteins [5]. The high levels of arginine in walnuts has already been identified as a positive feature [7] because arginine can be converted into nitric cixide a potent vasodilator which can inhibit platelet adhesion and aggregation. A low ratio of lysine/arginine in protein has also been identified as a positive feature in the reduction of the development of atherosclerosis in laboratory animals [17].

Conclusions

The data reported in this paper confirm that walnuts are a rich source of a number of important nutrients that appear to have a very positive effect on human health. Further experiments on the effects of feeding walnut diets to humans would be of great interest to understand the mechanisms of all the nutrients in walnuts. Despite the need for further research it is clear that prudent consumption of walnuts has played and can continue to play an important role in a healthy diet.

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Table 2. Mean amino acid composition of walnut protein (g/100 g protein) of 12 different cultivars grown under the same conditions in New Zealand

Cultivar	Esterhazy	G139	G120	Tehama	Vina	Rex	Dublin's Glory	Meyric	Stanley	150	151	153
Aspartic acid	7.93	7.14	6.68	5.87	6.20	5.57	6.66	6.23	8.32	7.90	7.89	8.62
Threonine	2.96	2.68	2.69	2.28	2.51	2.10	2.56	2.37	2.96	3.01	2.89	3.23
Serine	4.63	4.29	4.08	3.48	3.67	3.31	4.03	3.72	4.94	4.70	4.58	5.11
Glutamic acid	15.84	14.38	13.92	11.68	12.35	11.44	13.51	12.60	16.45	15.94	15.92	17.37
Proline	2.97	2.68	2.96	2.48	2.30	2.26	2.85	2.48	3.30	3.16	3.20	3.42
Glycine	4.35	3.92	3.98	3.34	3.84	3.11	3.78	3.70	4.58	4.31	4.01	4.74
Alanine	3.34	3.15	3.01	2.54	2.79	2.42	2.93	2.75	3.55	3.36	3.30	3.57
Valine	4.00	3.61	3.50	3.13	3.30	2.83	3.37	3.21	4.08	3.95	3.91	4.21
Methionine	1.01	1.01	0.94	0.74	0.82	0.95	0.87	0.75	1.01	1.07	1.07	1.14
Isoleucine	3.18	3.05	2.87	2.51	2.68	2.33	2.72	2.56	3.28	3.24	3.25	3.57
Leucine	5.83	5.36	5.12	4.47	4.83	4.15	4.96	4.66	6.07	5.78	5.76	6.37
Tyrosine	2.96	2.67	2.58	2.20	2.49	2.14	2.53	2.35	2.99	2.92	2.94	3.13
Phenylalanine	3.76	3.43	3.32	2.89	3.05	2.73	3.17	3.01	3.92	3.78	3.71	4.08
Histidine	2.16	1.96	1.99	1.65	1.81	1.59	1.82	1.76	2.22	2.16	2.17	2.39
Lysine	2.80	2.50	2.62	2.36	2.59	2.01	2.42	2.32	2.91	2.75	2.55	2.84
Arginine	11.82	11.15	10.07	8.38	9.12	8.96	9.98	9.18	12.04	11.71	11.94	13.15

Results are mean values of duplicate determinations.

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