ISSN 2377-8350

ADVANCES IN
FOOD TECHNOLOGY AND NUTRITIONAL SCIENCES

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Original Research

The Lincang Walnut's Nutritious Make-Up and Health Advantages

Carolyn E. Lister, PhD[1*]; Yi Xiao, MSc[2]; Jianrong Yang, BSc[3]; Janine M. Cooney, PhD[4]; Dwayne J. Jensen, NZCS[4]; Yongfeng Tang, BSc[2]; Fengrui Xie, BSc[2]; Lijing Chen, BSc[2]; Xuejiang Tie, BSc[2]; Hongxing Tian, BSc[2]

[1]*Food & Health Information Team, The New Zealand Institute for Plant and Food Research Limited, Private Bag 4704, Christchurch, New Zealand*
[2]*Anchor Center for Certification, No.1295 Chuan Qiao Road, Building 2, Site 302, Pudong, Shanghai, China*
[3]*Lincang Municipal Academy of Forestry, Lincang, Yunan Province, China*
[4]*The New Zealand Institute for Plant and Food Research Limited, Ruakura, New Zealand*

***Corresponding author**
Carolyn E. Lister, PhD
Principal Scientist, Food & Health Information Team, The New Zealand Institute for Plant and Food Research Limited, Private Bag 4704, Christchurch, New Zealand; Tel. +64 3 325 9453; Fax. +64 3 325 2074; E-mail: carolyn.lister@plantandfood.co.nz

Article information
Accepted: June 22nd, 2025; **Published:** July 7th, 2025

Cite this article
Lister CE, Xiao Y, Yang J, et al. The Lincang Walnut's Nutritious Make-Up and Health Advantages. *Adv Food Technol Nutr Sci Open J*. 2025; 11(1): 29-41.

ABSTRACT

Background

Although there is considerable information on the nutritional composition of the common walnut, information on the iron walnut is scarce.

Aim

To analyze the nutritional composition of Lincang walnuts (which belong to the species *Juglans sigillata L*., also known as the iron walnut) and if they confer any advantages over Xinjiang or Californian walnuts (both *Juglans regia L*. or common walnut).

Results

Nutrients of particular nutritional significance in Lincang walnuts (both regular and old tree) were protein (5.9 and 5.6 g/30 g serve), dietary fiber (6.5 and 6.7 g/30 g serve), vitamin B6 (0.87 and 0.93 mg/30 g serve), copper (1.65 and 1.63 mg/30 g serve), magnesium (158 and 142 g/30 g serve), manganese (7.33 and 8.41 mg/30 g serve) and phosphorus (437 and 427 g/30 g serve). Despite a high fat content, the fatty acids profile was predominantly polyunsaturated and monounsaturated types. The Lincang walnuts were similar in macronutrient composition to the Xinjiang and Californian-sourced samples, but the Lincang ones were higher in some of the B vitamins (niacin, thiamin, and vitamin B6) and minerals (manganese and to a lesser extent copper, iron, phosphorus, and potassium). The serotonin concentration in Lincang walnuts (7.6 and 7.2 mg/30 g serve) was over double that found in Californian walnuts (2.9 mg/30 g serve) and higher than Xinjiang walnuts (5.5 mg/30 g serve).

Conclusion

The nutrients present in Lincang walnuts confer a range of well-established health benefits. One area of particular interest is the role that walnuts may play in brain function owing to the combination of nutrients and phytochemicals present. In addition to direct consumption of walnuts, there are many options for the development of functional food products, including walnuts as ingredients, to promote regular consumption of walnuts for improvement of health.

Keywords

Walnuts; *Juglans sigillata*; *Juglans regia*; Nutrients; Brain health; Vitamin; Mineral; Fatty acid; Serotonin.

INTRODUCTION

In recent years there has been increasing interest in nut consumption and the impacts on human health.[1-3] Nuts, such as walnuts but also almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, and pistachios, are often regarded as nutrient-dense foods. Each type of nut contributes similar but different profiles of nutrients and other bioactive substances and are often included in healthy eating recommendations. Of particular note are the protein, dietary fiber, healthy fats, vitamins (e.g. vitamin E and selected B vitamins including folate, and thiamin) and minerals (e.g. magnesium, copper, potassium, and selenium) contents. In addition, bioactive components such as phytosterols, phenolics and more recently serotonin and melatonin have attracted considerable interest in terms of their contribution to human health.[4-8]

The genus Juglans includes 21 species, with the common, English or Persian walnut (*Juglans regia L.*) being the most economically important cultivated species. There has been less study of the walnut species *Juglans sigillata L.*, also known as the (Chinese) iron walnut — the species grown in Lincang, which is the focus of this study. It has been debated for some time if the common and iron walnuts are different species or simply different ecological types.[9]

There is considerable information on the nutritional composition of the common walnut, including in the United States (U.S.),[10] Australian[11] and New Zealand National Food Composition Databases[12] (Table 1). In terms of nutritional significance (significant contribution to recommended dietary intakes) the components of note are protein, dietary fiber, biotin, folate, thiamine,

Table 1. *Existing Information on the Nutritional Composition of Raw Walnuts from Major Food Composition Databases. Values are Expressed per 100 g Fresh Weight*

Nutrient Class	Nutrient	Unit	USA[10]	Australia[11]	New Zealand[12]
Proximates	Energy	kJ	2738	2904	2836
	Protein	g	15.2	14.4	14.5
	Fat, total	g	65.2	69.2	68.8
	-Fatty acids, saturated	g	6.1	4.4	4.9
	-Fatty acids, trans	g	nr	0.02	0.00
	-Fatty acids, monounsaturated	g	8.9	12.1	9.0
	-Fatty acids, polyunsaturated	g	47.2	49.6	50.0
	-Cholesterol	mg	0	0	0
	Available carbohydrate, by difference	g	13.7	11.6	2.0
	Available carbohydrate, measured	g	2.7	3.0	2.6
	Total sugars	g	2.6	2.7	2.5
	Starch	g	0.1	0.3	0.1
	Dietary fibre	g	6.7	6.4	9.0
Vitamins	Biotin	µg	nr	19	nr
	Folate	µg	98	70	86
	Niacin (B3)	mg	1.12	1.40	4.49
	Pantothenic acid	mg	0.57	0.66	nr
	Riboflavin (B2)	mg	0.15	0.18	0.17
	Thiamin (B1)	mg	0.341	0.33	0.35
	Vitamin A, retinol equivalents (RE)	µg	1	4	1.5
	Vitamin B6	mg	0.537	0.43	0.6
	Vitamin B12	ug	0	nr	0
	Vitamin C	mg	1.3	0	0
	Vitamin D	µg	0.00	nr	0.00
	Vitamin E	mg	0.70	2.60	1.01
	Vitamin K	µg	2.7	nr	nr
Minerals	Calcium (Ca)	mg	98	89	78
	Copper (Cu)	mg	1.59	1.40	1.35
	Iodine (I)	µg	nr	nr	0.0
	Iron (Fe)	mg	2.91	2.50	2.70
	Magnesium (Mg)	mg	158	150	165
	Manganese (Mn)	mg	3.41	3.20	3.40
	Phosphorus (P)	mg	346	370	380
	Potassium (K)	mg	441	440	470
	Selenium (Se)	µg	4.9	2	0
	Sodium (Na)	mg	2	3	0
	Zinc (Zn)	mg	3.09	2.53	3.00

nr=not reported

vitamin B6, copper, magnesium, manganese, phosphorus and zinc. Although high in fats, the fatty acid profile is also ideal, with a high proportion of polyunsaturated fatty acids (PUFA). In addition to nutrients, walnuts contain a number of phytochemicals that may contribute to biological activities. The phytochemicals reported as present in walnuts include phenolics (e.g. proanthocyanins, ellagic acid, ellagitannins, flavonoids), phytosterols, sphingolipids, serotonin and melatonin.[4,7,8,13]

Unlike the common walnut, composition data for iron walnut in the international scientific literature are scarce. Zhai et al[14] compared the mineral contents of the common walnut and iron walnut. The order of the selected minerals in the common walnut was Mg>Ca>Zn>Mn>Fe>Cu, while in the iron walnut it was Mg>Ca>Mn>Fe>Zn>Cu. Although there were slight differences in order these may be minimal in terms of nutritional significance but warrant further investigation. These authors also determined the amino acid composition and found the order of components in the common walnut was leucine>isoleucine>valine>phenylalanine>lysine>threonine>methionine, while the order in the iron walnut was leucine>isoleucine>lysine>phenylalanine>valine>threonine>methionine. As with the minerals these slight differences may have limited nutritional significance but do indicate they should be examined in our study to ascertain if there are differences. In a second paper, Zhai et al[15] noted oil from the iron walnut was high in linoleic acid and tocopherols. In another study, Gao et al[16] compared common walnut oil and iron walnut oil and noted differences in their lipid composition: iron walnut oil contained C16:1, C22:0, and C22:1 fatty acids that were not detected in common walnut oil. However, the amounts of these fatty acids were low and the key fatty acids present in larger amounts were similar across both species. In addition, these authors found that concentrations of tocopherols, phytosterols, squalene, and polyphenols in iron walnut oil were significantly lower than those of common walnut oil.

Numerous health benefits have been reported for walnuts from *in vitro* studies through animal studies, epidemiological evidence and human intervention trials.[17-50] It is only the human trials that provide concrete evidence for the potential health benefits, and many recent key trials are summarised in Table 2. Effects of walnut consumption may be seen with acute ingestion as

Table 2. *Summary of Some of the Potential Health Benefits of Walnuts from Recent Human Studies*

Health Area	Study Findings	Reference
Cancer (breast)	A non-placebo clinical trial with 57 g walnuts per day pre- and post-surgery altered gene expressions related to tumor growth, survival, and metastasis in breast cancer patients.	43
Cancer (prostate)	Walnuts (75 g per day for 8 weeks) increased plasma γ-tocopherol and free prostate-specific antigen (PSA)/total PSA.	44
Cardiovascular health	A randomized, crossover trial with 65 g walnuts per day for 4 weeks significantly reduced low-density lipoprotein (LDL)-cholesterol, total cholesterol, and LDL/high-density lipoprotein (HDL) ratio. Plasma polyunsaturated fatty acids (PUFA) were higher.	45
	A 2 year intervention with walnuts at 15% energy intake (average 42.5 g walnuts per day) reduced systolic blood pressure in elderly subjects, particularly in those with mild hypertension.	46
	Consumption of 56 g walnuts per day for 8 weeks improved endothelial function in a randomized, controlled crossover trial.	18
	A single-blind, controlled crossover trial with type 2 diabetic individuals showed consumption of 56 g walnuts per day for 8 weeks improved endothelial function.	47
	Intervention with 21 or 42 g walnuts per day for 6 weeks decreased total cholesterol and LDL-cholesterol.	40
	Walnuts at 28-64 g per day for 6 months decreased total cholesterol and triglycerides.	48
Cognitive function	In a cross-sectional assessment consumption of walnuts was associated with improved working memory in an older female cohort at high cardiovascular disease risk.	31
	A double-blind, randomized, placebo-controlled study demonstrated walnuts (60 g per day in banana bread for 8 weeks) improved mood in healthy, young males but no significant changes observed with both genders combined or females.	30
Diabetes/glucoregulation	Epidemiological data showed consumption of walnuts was associated with a lower risk for diabetes compared with non-nut consumers.	23
	Data from two large cohort studies showed walnut consumption (≥ twice/week) was associated with a lower risk of developing type 2 diabetes.	26
	A randomized parallel group trial with 30 g walnut per day for 1-year reduced fasting insulin and increased PUFA intake.	27
Improvement in diet quality	Walnuts (average of 42.5 g/day for 2-years) increased beneficial nutrient intake (e.g. protein, PUFA) while reducing saturated fatty acids and sodium in a prospective, parallel design trial.	21
	A randomized, controlled crossover trial with 75 g/day of walnuts for 8-weeks provided beneficial nutrients, such as monounsaturated fatty acids (MUFA), PUFA and dietary fiber.	22
Metabolic syndrome (MetS)	Intervention with 48 g walnuts/day for 4 days increased satiety and sense of fullness but had no impact on insulin resistance.	24
	A randomized, crossover study with 45 g walnuts/day or 16-weeks increased the concentration of HDL-c and decreased fasting glucose.	49
	A single walnut enriched meal increased adiponectin.	25

well as with chronic consumption.[1] The largest body of evidence is for a reduction in cardiovascular disease risk, which may happen through a variety of mechanisms including improvement in blood lipid profile, blood pressure, and cholesterol reduction, moderating inflammation and/or improving endothelial function.[17-20] Walnut consumption seems to be a strategy to improve diet quality by increasing the intake of important dietary nutrients and replacing less nutritious foods in the diet.[21,22] Walnuts may also provide benefits for overweight individuals and diabetics/pre-diabetics through a variety of mechanisms.[23-27] Including walnuts in the diet has led to increased dietary intake of some nutrients associated with a lower risk of developing type 2 diabetes and other cardiometabolic risk factors.[28,29]

An area of particular interest is the role walnuts may play in brain health. One small human intervention study showed some effects on mood, although only in males.[30] A small cross-sectional study showed improvements in working memory in an older female cohort at high cardiovascular disease risk.[31] However, walnut supplementation for 2-years was shown not to affect cognition in healthy, free-living elderly people.[32] However, in this study, there were some indications that walnuts might delay cognitive decline in subgroups at higher risk for dementia. In addition to human trials, animal trials indicate other potential health benefits, including other effects on brain function. Walnut diets improved motor performance and cognitive ability in older mice, but effects were dependent on amounts consumed, with the moderate intake being optimal.[33] These authors subsequently showed anti-inflammatory effects of walnuts in microglia, which could have benefits in protecting neurodegeneration.[34] Poulose et al[35] also showed that the polyphenols in walnuts may reduce inflammation and oxidant load in brain cells, improve signaling between neurons, increase neurogenesis, and enhance sequestration of insoluble toxic protein aggregates. The effect of walnuts on learning and memory function in rats was also investigated by Haider et al,[36] who showed enhanced performance and reduced anxiety. A further study with pregnant and lactating mice revealed that their adult pups showed improvements in memory and learning.[37]

Walnuts have often been shown to possess significant antioxidant activity *in vitro*.[38] How this translates to benefits *in vivo* remains uncertain. There has been considerable debate in recent years about the relevance of antioxidant activity measures and the US Department of Agriculture (USDA) removed the oxygen radical absorbance capacity (ORAC) database.[39] Many questions regarding the antioxidant capacity of foods, and whether or not those measurements have any bearing on effectiveness in the human body, remain unanswered and research is ongoing. With specific regards to walnuts, consumption did not significantly change the plasma antioxidant capacity of healthy, well-nourished older adults.[40] Other studies have shown positive changes in plasma oxidative stress measures after walnut consumption.[41,42] However, what this may mean to human health outcomes remains uncertain. The aim of this study was to determine the composition of Lincang walnuts (regular and old tree), to determine which health benefits may be able to be promoted and if they confer any advantages over Xinjiang or Californian walnuts.

MATERIALS AND METHODS

Samples

Because the analysis reported here was intended to distinguish the nutritional differences between walnuts from three different locations, we selected the largest producing regions, which are Lincang (Yunnan Province), Xinjiang and California. Four composite samples were analyzed as follows:

- Sample A: Lincang old-tree walnuts (trees>100-years-old); *Juglans sigillata*
- Sample B: Lincang regular walnuts; *Juglans sigillata*
- Sample C: Xinjiang walnuts; *Juglans regia*
- Sample D: Californian walnuts; *Juglans regia*.

Samples A and B were provided by the Lincang Forestry and Grassland Bureau. Sample A was provided on August 3, 2019 and was produced in July 2019. Sample B was provided on August 26, 2019 and was produced in August 2019. Sample C was purchased over the internet. Its production date was July 18, 2019, the production company was Fenyang Yuankang Native Product Co., Ltd. in Changsha, Hunan, China, and the walnut production location is Akesu City, Xinjiang Province, China. Sample D was also purchased *via* the internet on August 15, 2019, its "best before" date was March 15, 2020 and the walnut production location was California, USA. Based on purchase dates and "best before" dates, the walnuts were of a similar age.

Analyses of composite samples were conducted using the methodology outlined below. Samples were analyzed blind using the letter codes. Data were expressed per 100 g but also on a per serve basis, to put the data in a nutritional context of typical dietary intake. Serve has been set as 30 g, consistent with the international recommendations. To determine nutritional significance, the nutrient data were calculated as percentages of the recommended nutrient intakes (RNIs) for adult males aged 18-49-years in China.[50]

Nutritional Analysis

Nutritional analysis was conducted by Anchor Center for R&D and Certification (ACC Labs, China) using the methodology outlined in Table 3. Analysis of each nutrient was carried out in duplicate.

Serotonin and Melatonin Analysis

The concentration of serotonin in walnuts was determined in triplicate by liquid chromatography mass spectrometry (LCMS) with ion trap detection following a recently published method[8] and using methanol:water 1:1 (v/v) as the extraction solvent.

As melatonin was not detected in the above method, a more targeted extraction for melatonin in walnuts (1 g) was under taken. This followed a published method[51] using methanol as the extraction solvent and with the addition of evaporation and solve-

Table 3. *Details of Methods Used for Nutritional Analysis*

Nutrient	Test method
Energy	Calculated
Protein	GB 5009.5-2016 Part I
Amino acids	GB 5009.124-2016
Fat	GB 5009.6-2016 Part 2
Fatty acids	GB 5009.168-2016 Part I
Cholesterol	GB 5009.128-2016 Part I
Carbohydrate	Calculated
Sugars	GB 5009.8-2016 Part I
Dietary Fiber	GB 5009.88-2014 6.3
Folate	GB 5009.211-2014
Niacin (B3)	GB 5009.89-2016 Part 2
Thiamin (B1)	GB 5009.84-2016 Part I
Vitamin A	GB 5009.82-2016 Part I
Vitamin B_6	GB 5009.154-2016 Part I
Vitamin C	GB 5009.86-2016 Part 2
Vitamin E	GB 5009.82-2016 Part I
Calcium	GB 5009.268-2016 Part I
Copper	GB 5009.268-2016 Part I
Iron	GB 5009.268-2016 Part I
Magnesium	GB 5009.268-2016 Part I
Manganese	GB 5009.268-2016 Part I
Phosphorus	GB 5009.87-2016 Part I
Potassium	GB 5009.268-2016 Part I
Sodium	GB 5009.268-2016 Part I
Zinc	GB 5009.268-2016 Part I

nt partition steps to improve the limit of detection of analysis. Extractions using the Reiter methodology were repeated but ethanol was used as the extraction solvent. Concentrated samples were analyzed by LCMS with triple quad detection.

RESULTS AND DISCUSSION

Macronutrients

The composition of macronutrients in the four walnut samples is given in Table 4. For the most part, the differences between the four samples were relatively small. The Lincang walnuts were a little higher in monounsaturated fatty acids. There are some differences in the nutrient values reported in this study compared with the literature and international food composition tables (Table 1). Energy values were slightly lower but this is probably because of the lower fat contents reported here (although they may also in part because of calculation methods, as there are numerous ways in which energy is calculated depending on regulatory requirements in each country). On the other hand protein values in this study were slightly higher than given in Table 1 and dietary fiber was significantly higher here. Analysis of walnuts is complex because of the high fat content. This is demonstrated by large variations in available carbohydrate measures depending if determined by difference or using analytical values. This is evident in the data shown in Table 1, with United States (U. S.) and Australian data having significant differences.

The fatty acid composition of the four walnut samples is shown in Table 5, with linoleic acid being the predominant fatty acid in all four samples. This result is in agreement with the values reported in international databases[10-12] and reported by Gao et al[16] for iron walnut oil. The profiles of the Lincang walnuts and the other two samples were similar, but α-linolenic acid content was lower in the Lincang samples. However, the Lincang samples con-

Table 4. *Proximate Content of the Four Types of Walnut Analyzed in this Study (Results are the Average of Duplicate Composite Samples)*

Nutrient	Units	Values in mg Per 100 g				Values in mg per 30 g Serve			
		Lincang Old Tree	Lincang Regular	Xinjiang	California	Lincang Old Tree	Lincang Regular	Xinjiang	California
Energy	kJ	2331	2391	2306	2337	699	717	692	701
Protein	g	19.6	18.5	19.2	17.7	5.9	5.6	5.8	5.3
Fat, total	g	46.2	49.5	45.6	47.9	13.9	14.9	13.7	14.4
- Fatty acids, saturated	g	4.56	4.94	4.73	4.51	1.37	1.48	1.42	1.35
- Fatty acids, trans	g	0.053	0.057	0.055	0.079	0.016	0.017	0.017	0.024
- Fatty acid, monounsaturated	g	8.8	11.1	6.84	6.75	2.64	3.33	2.05	2.03
- Fatty acid, polyunsaturated	g	32.4	32.4	33.7	34.4	9.7	9.7	10.1	10.3
Cholesterol	mg	nd	nd	nd	nd	nd	nd	nd	nd
Available carbohydrate, by difference	g	6.8	3.9	6.2	3.6	2.0	1.2	1.9	1.1
Total sugars	g	2.6	1.7	2.1	2.1	0.78	0.51	0.63	0.63
- Sucrose	g	1.9	1.1	1.6	2.1	0.57	0.33	0.48	0.63
- Glucose	g	0.36	0.32	0.26	nd	0.11	0.10	0.08	nd
- Fructose	g	0.363	0.29	0.23	nd	0.11	0.09	0.07	nd
- Lactose	g	nd	nd	nd	nd	nd	nd	nd	nd
- Maltose	g	nd	nd	nd	nd	nd	nd	nd	nd
Dietary Fiber	g	21.6	22.3	23.4	25.3	6.5	6.7	7.0	7.6

nd=not detected

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Table 5. *Fatty Acid Composition of the Four Types of Walnut Analyzed in this Study (Results are the Average of Duplicate Composite Samples)*

Fatty Acid Name	Lincang Old Tree	Lincang Regular	Xinjiang	California
Saturated fatty acids				
Butyric acid (C4:0)	nd	nd	nd	nd
Caproic acid (C6:0)	nd	nd	nd	nd
Caprylic acid (C8:0)	nd	nd	nd	nd
Capric acid (C10:0)	nd	nd	nd	nd
Lauric acid (C12:0)	nd	nd	nd	0.01
Tridecanoic acid (C13:0)	nd	nd	nd	nd
Myristic acid (C14:0)	0.13	0.08	0.13	0.07
Pentadecanoic acid (C15:0)	0.01	0.01	0.01	0.01
Palmiticacid / Hexadacanoicacid (C16:0)	3.17	3.49	3.21	2.99
Heptadecanoic acid (C17:0)	0.03	0.03	0.03	0.02
Stearic acid / Octadecanoic acid (C18:0)	1.15	1.25	1.27	1.34
Arachidic acid (C20:0)	0.05	0.04	0.04	0.04
Henicosanoic acid (C21:0)	nd	0.01	0.01	0.01
Behenic acid (C22:0)	0.02	0.02	0.02	0.02
Tricosanoic acid (C23:0)	nd	nd	nd	nd
Lignoceric acid (C24:0)	0.01	0.01	0.01	nd
Monounsaturated fatty acids				
Myristoleic acid (C14:1)	nd	nd	nd	nd
cis-10-Pentadecenoic acid (C15:1)	nd	nd	nd	nd
Palmitoleic acid (C16:1)	0.06	0.08	0.04	0.03
cis-10-Heptadecenoic acid (C17:1)	nd	nd	nd	nd
Elaidic acid (C18:1n9t)	0.02	0.11	0.05	0.09
Oleicacid / Octadecenoicacid (C18:1n9c)	8.65	10.86	6.60	6.44
cis-11-Eicosenoic acid (C20:1)	0.06	0.09	0.13	0.17
Erucic acid (C22:1n9)	0.02	0.01	0.02	0.02
Nervonic acid (C24:1n9)	nd	nd	nd	nd
Polyunsaturated fatty acids				
Linolelaidic acid (C18:2n6t)	nd	0.01	nd	0.01
Linoleic acid (C18:2n6c)	28.73	28.70	27.51	27.67
γ-Linolenicacid (C18:3n6)	nd	nd	nd	nd
α-Linolenicacid (C18:3n3)	3.65	3.72	6.16	6.73
cis-11,14-Eicosadienoic acid (C20:2)	0.01	0.01	0.02	0.02
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	nd	nd	nd	nd
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	nd	nd	nd	nd
Arachidonic acid (C20:4n6)	0.01	0.01	0.01	0.01
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	nd	nd	nd	nd
cis-13,16-Docosadienoic acid (C22:2n6)	nd	nd	nd	nd
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	nd	nd	nd	nd

nd=not detected

tained significantly more oleic acid and slightly more linoleic acid. Previously Gao et al[16] noted differences in the lipid composition of common walnut and iron walnut oil, with the latter containing C16:1, C22:0, and C22:1 fatty acids, which were not detected in common walnut oil. In this study, these three fatty acids were detected in very low concentrations in all samples.

Amino acid composition of the four walnut samples is provided in Table 6. The Lincang walnuts did have a slightly higher total amino acid content, particularly those from old trees, and this would be the result of the slightly higher protein content. However, the proportions of individual components were not significantly different. In all four walnut types, the predominant amino acids were glutamic acid, arginine, aspartic acid, and leucine, which is in agreement with international database values,[10-12] but differs from data published by Zhai et al.[14]

Table 6. *Amino Acid Composition of the Four Types of Walnut Analyzed in this Study (Results are the Average of Duplicate Composite Samples)*

Amino Acid	Unit	Lincang Old Tree	Lincang Regular	Xinjiang	California
Alanine	g/100 g	0.78	0.73	0.66	0.70
Arginine	g/100 g	2.36	2.21	2.08	2.08
Aspartic acid	g/100 g	1.83	1.78	1.60	1.66
Cystine	g/100 g	0.28	0.25	0.24	0.24
Glutamic acid	g/100 g	3.28	3.00	2.89	2.96
Glycine	g/100 g	0.89	0.83	0.79	0.82
Histidine	g/100 g	0.55	0.50	0.48	0.49
Isoleucine	g/100 g	0.67	0.63	0.59	0.61
Leucine	g/100 g	1.25	1.16	1.06	1.12
Lysine	g/100 g	0.55	0.51	0.51	0.54
Methionine	g/100 g	0.13	0.13	0.12	0.11
Phenylalanine	g/100 g	0.77	0.72	0.66	0.69
Proline	g/100 g	0.85	0.79	0.72	0.76
Serine	g/100 g	0.89	0.84	0.77	0.80
Threonine	g/100 g	0.65	0.62	0.57	0.59
Tryptophan	g/100 g	0.25	0.23	0.21	0.21
Tyrosine	g/100 g	0.54	0.51	0.48	0.48
Valine	g/100 g	0.77	0.72	0.67	0.70
Total amino acids	g/100 g	17.29	16.16	15.10	15.56

Table 7. *Vitamin and Mineral Content of the Four Types of Walnut Analyzed in this Study (Results are the Average of Duplicate Composite Samples)*

Vitamin/ Mineral	Units	Values in mg Per 100 g				Values in mg per 30-g Serve			
		Lincang Old Tree	Lincang Regular	Xinjiang	California	Lincang Old Tree	Lincang Regular	Xinjiang	California
Folate	µg	88.3	88.3	84.5	88.3	26.5	26.5	25.4	26.5
Niacin (B3)	mg	2.13	2.12	1.31	1.61	0.64	0.64	0.39	0.48
Thiamin (B1)	mg	0.105	0.107	0.073	0.063	0.032	0.032	0.022	0.019
Vitamin A, RE	mg	nd	nd	nd	nd	nd	nd	nd	nd
Vitamin B6	mg	0.869	0.934	0.752	0.707	0.261	0.280	0.226	0.212
Vitamin C	mg	nd	nd	nd	nd	nd	nd	nd	nd
Vitamin E	mg	1.73	1.56	1.65	1.84	0.52	0.47	0.50	0.55
Calcium	mg	76	65.7	96	107	22.8	19.7	28.9	32.1
Copper	mg	1.65	1.63	1.25	0.86	0.50	0.49	0.38	0.26
Iron	mg	2.61	2.71	2.46	2.04	0.78	0.81	0.74	0.61
Magnesium	mg	158	142	154	132	47.4	42.6	46.2	39.6
Manganese	mg	7.33	8.41	3.01	3.06	2.20	2.52	0.90	0.92
Phosphorus	mg	437	427	349	399	131	128	105	120
Potassium	mg	521	488	405	427	156	146	122	128
Sodium	mg	nd	nd	0.8	nd	nd	nd	0.24	nd
Zinc	mg	2.28	2.40	2.74	2.70	0.68	0.72	0.82	0.81

nd=not detected; RE=Retinol Equivalents

Vitamins and Minerals

The vitamin and mineral contents are shown in Table 7. The values for the vitamins are largely in agreement with the composition given in Table 1, except for those for thiamin, which was lower in all samples analyzed in this study and vitamin B6, which was slightly higher. Lincang walnuts contained more of some of the B vitamins (niacin, thiamin, and B6) than the other two samples. Mineral contents were within the range generally reported in Table 1, although Lincang walnuts contained significantly more manganese and to a lesser extent copper, iron, phosphorus, and potassium. In some cases, mineral content may be influenced by

Table 8. *Serotonin and Melatonin Concentrations the Four Types of Walnut Analyzed in this Study (Results are the Mean of Triplicate Samples±Standard Error of the Mean)*

Component	Values in mg per 100 g				Values in mg per 30 g serve			
	Lincang Old Tree	Lincang Regular	Xinjiang	California	Lincang Old Tree	Lincang Regular	Xinjiang	California
Serotonin	25.2±0.9	23.9±0.5	18.4±1.7	9.8±0.3	7.6±0.3	7.2±0.2	5.5±0.5	2.9±0.1
Melatonin	nd	nd	nd	nd	nd	nd	nd	nd

nd=not detected

Table 9. *Nutrients of Dietary Significance in Lincang Walnuts (Percentage Recommended Nutrient Intakes (RNIs) are Based on Chinese Values for an Adult Male, 18-49-Years-Old[50])*

Nutrient	Unit	Lincang Old Tree			Lincang Regular		
		Amount Per 100 g	Amount Per 30 g Serve	%RNI Per Serve	Amount Per 100 g	Amount Per 30 g Serve	%RNI Per Serve
Protein	g	19.6	5.9	na	18.5	5.6	na
Dietary fibre	g	21.6	6.5	na	22.3	6.7	na
Vitamin B6	mg	0.869	0.261	19%	0.934	0.280	20%
Copper	mg	1.65	0.50	62%	1.63	0.49	61%
Magnesium	mg	158	47	14%	142	43	13%
Manganese	mg	7.33	2.20	49%	8.41	2.52	56%
Phosphorus	mg	437	131	18%	427	128	18%

na=not applicable

the mineral content in the soil where the trees are grown.

Serotonin and Melatonin

The concentration of serotonin in walnuts was determined by LCMS and the results are shown in Table 8 (results presented are the average of triplicate composite samples). Lincang walnuts were significantly higher in serotonin, particularly compared with the Californian sample. The concentrations reported here for the Californian and Xinjiang walnuts are similar to those reported by Yilmaz et al,[8] but higher than those reported by Feldman et al,[52] Reiter et al[51] or Tapia et al[53] for common/English walnuts. Feldman et al[52] did report higher concentrations of serotonin in black walnuts (30.4 mg/100 g) or butternuts/*Juglans cinerea* (39.8 mg/100 g). Thus, the Lincang walnuts may confer some advantage in this area over the common walnut.

Using the initial methodology, no melatonin was detected and thus a more targeted extraction was undertaken following a published method.[51] Using this methodology no melatonin was detected. Matrix spikes at concentrations consistent with those previously reported in raw walnut of 350 ng/100 g[51] were observed. Using this latter method, dopamine was detected but not melatonin. It is possible that the method needs some further validation of the clean-up step before there is much chance of success. However, even then it is unlikely that the concentration of melatonin in walnut is of dietary significance, but the presence of tryptophan and serotonin in walnuts may mean that our body can generate melatonin after walnut ingestion.

Reasons for Differences in Composition

There may be several reasons for the differences in the composition of the four walnut samples, including the species/variety and environmental factors (e.g. climate and soil). The climatic factors in the main producing areas of iron walnut in the Yunnan province have been reported.[54] The geographical coordinates of Lincang City are 98°40'-100°34' E, and 23°05'-25°02' N. The Tropic of Cancer runs through the southern part of Lincang and it has a subtropical low-latitude mountain monsoon climate. Lincang is located on the watershed boundary of the Pacific Ocean and the Indian Ocean and the climate is affected by the two oceans and in particular the warm and humid air currents of the Indian Ocean and the southwest monsoon. The average annual precipitation is 906-1584 mm; the sunshine duration is long (annual average sunshine time is more than 2000-hours). The frost period is short and in some areas, there is no frost all year round and the annual average temperature is 16.6-19.5 °C.

Soil composition may also affect the nutritional composition and the nature of Lincang soils has been studied.[55,56] Carbonatite is the main soil in the Yunnan walnut planting area, especially brick-red soil and limestone. The brick-red soil has good hydrothermal conditions, and the parent materials are mostly granite, phyllite, gneiss, sand shale, and old alluvial laterite. The limestone soil is rich in organic matter and nutrients, with a good structure.

The planting area of Lincang seems to be particularly suitable for the growth of walnuts and the unique climatic environment appears to have a positive effect on walnut quality to some degree. These factors should be explored further to determine the main influences on nutritional composition and in particular the high serotonin content.

Significance of Walnut Composition for Health

To determine which nutrients were of nutritional significance the concentrations were calculated in terms of percentage of how much a serve of walnuts contributed to the Chinese RNIs. Those nutrients that were of significance, i.e. delivered at least 10% of the reference intake in a server, are shown in Table 9. In terms of macronutrients, the values cannot be expressed this way as the Chinese guidelines express these in terms of percentages of energy intake. However, internationally, the protein content of 5 g in serve is often regarded as of significance, and likewise for dietary fiber of 2 g in a serve, and walnuts deliver in both cases. On the other hand, walnuts are high in fats and this can be regarded as negative. However, walnuts deliver large amounts of polyunsaturated and monounsaturated fatty acids, and diets containing regular consumption of walnuts do lead to a reduction in cardiovascular risk, with an improvement in plasma lipid profile.[1]

Naturally, if a serve of greater than 30 g is consumed, other nutrients may reach nutritional significance. For example, when increasing the serve to 45 g, folate, iron, and potassium reach nutritional significance for the Lincang walnuts.

There are numerous well-recognized health benefits of those nutrients of dietary significance and these are often referred to as structure/function claims (Table 10). In addition to the nutrients listed above, other components in walnut may con-

Table 10. *The Main Well-Established Health Benefits Recognized Internationally for Each of the Key Nutrients Present in Walnuts*

Nutrient	Health benefit
Protein	Helps build and repair body tissues Necessary for normal growth and development of bone in children Contributes to the growth of muscle mass Contributes to the maintenance of muscle mass Contributes to the maintenance of normal bones Necessary for normal growth and development in children Contributes to the maintenance of normal bones
Dietary fiber	Contributes to regular laxation
Vitamin B_6	Necessary for normal protein metabolism Necessary for normal iron transport and metabolism Contributes to normal growth and development in children Contributes to normal cysteine synthesis Contributes to normal energy metabolism Contributes to the normal functioning of the nervous system Contributes to normal homocysteine metabolism Contributes to normal glycogen metabolism Contributes to normal psychological function Contributes to normal red blood cell formation Contributes to normal immune system function Contributes to the reduction of tiredness and fatigue Contributes to the regulation of hormonal activity
Copper	Contributes to normal connective tissue structure Contributes to normal iron transport and metabolism Contributes to cell protection from free radical damage Necessary for normal energy production Necessary for normal neurological function Necessary for normal immune system function Necessary for normal skin and hair coloration Contributes to normal growth and development in children
Magnesium	Contributes to normal energy metabolism Necessary for normal electrolyte balance Necessary for normal nerve and muscle function Necessary for teeth and bone structure Contributes to a reduction of tiredness and fatigue Necessary for normal protein synthesis Contributes to normal psychological function Necessary for normal cell division Contributes to normal growth and development in children
Manganese	Contributes to normal bone formation Contributes to normal energy metabolism Contributes to cell protection from free radical damage Contributes to normal connective tissue structure Contributes to normal growth and development in children
Phosphorus	Necessary for normal teeth and bone structure Necessary for the normal cell membrane structure Necessary for normal energy metabolism Contributes to normal growth and development in children Needed for the normal growth and development of bone in children

fer similar or additional health benefits. For example, the amino acid tryptophan is naturally found in animal and plant proteins (and is present in walnuts, Table 6). L-tryptophan is considered an essential amino acid because our bodies cannot make it. It is important for the development and functioning of many organs in the body. After absorbing L-tryptophan from food, our bodies convert it to 5-HTP (5-hydroxytryptophan), and then to serotonin, melatonin, and niacin. Serotonin may have some important roles in the human body, including regulation of appetite, anxiety, sleep, mood and blood pressure, and decreasing amounts or depletion of its synthesis might cause several diseases, including depression, obesity, and schizophrenia (reviewed in Yilmaz et al[8]). Thus, a higher-serotonin walnut, such as the Lincang ones, may confer these health advantages and warrants further investigation. At present, there is insufficient human clinical trial evidence to confirm the exact benefits of high-serotonin walnuts.

It is also likely that the particular combinations of nutrients and phytochemicals in walnuts may confer the health benefits, such as those shown in Table 2, rather than individual components being responsible. One area of particular interest is the role that walnuts may play in brain function due to the combination of nutrients and phytochemicals present in Lincang walnuts. B vitamins, tryptophan and serotonin all play important roles in maintaining brain function. Likewise, regulation of appetite, enhancing nutrient intake and metabolism may be important given increasing obesity rates.

Internationally there have been two claims permitted by key regulators for walnuts concerning heart health. Firstly, the US Food and Drug Administration (FDA) approved: "*Supportive but not conclusive research shows that eating 1.5 oz of walnuts per day, as part of a low saturated fat and low cholesterol diet, and not resulting in increased caloric intake may reduce the risk of coronary heart disease*".[57] Note 1.5 oz of walnuts equals ~43 g. Secondly, the European Food Safety Authority (EFSA) approved: "*Walnuts contribute to the improvement of the elasticity of the blood vessels as part of a balanced diet and a healthy lifestyle.*"[58] This health effect is through the improvement of endothelium-dependent vasodilation. To obtain the claimed effect, 30 g of walnuts should be consumed daily. Under this same opinion, EFSA rejected more general claims around heart health, and in another opinion, claims for nuts (including walnuts) around weight maintenance were also rejected.[59]

CONCLUSION

Lincang walnuts are a valuable inclusion to a healthy diet and may contribute numerous health benefits. Of particular nutritional significance are protein, dietary fiber, vitamin B6, copper, magnesium, manganese, and phosphorus. The macro nutrient composition of the Lincang walnuts was similar to the Xinjiang- and Californian-sourced samples. Lincang samples contained significantly more oleic acid and slightly more linoleic acid. In addition, the Lincang walnuts were higher in some of the B vitamins (niacin, thiamin and vitamin B6) than the other two samples. In terms of the minerals, Lincang walnuts were significantly higher in manganese and to a lesser extent copper, iron, phosphorus, and potassium. The serotonin concentration in Lincang walnuts is particularly notable and much higher than that in Californian walnuts. The high serotonin concentration in Lincang walnuts is of particular significance and may offer additional health benefits to the nutrients. In addition to direct consumption of walnuts, there are many options for the development of functional food products, including walnuts as ingredients, to promote regular consumption of walnuts for improvement of health.

ACKNOWLEDGMENTS

The funding of the project was provided by the Lincang government through the Anchor Center for Certification. Thank you to Limei Feng (The New Zealand Institute for Plant and Food Research Limited) who assisted with the literature review plus Liverpool Zhang (The New Zealand Institute for Plant and Food Research Limited) and Stella Si (Anchor Center for Certification) for project support.

INSTITUTIONAL REVIEW BOARD (IRB)

The paper has been through the Plant & Food Research's Science Publication Tracking System which is a compulsory internal process for all scientific documents produced by our scientists. The tracking number is: 19105. It has also been approved for submission by Stella Si, Anchor Center for Certification.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest. The agreement signed for funding agreed that the results would be provided as-is, even if Lincang walnuts showed inferior results. The samples were analyzed blind to avoid any potential bias.

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