Hypolipidemic Effects of Hickory Nut Oil using Cold Pressure Extraction

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Abstract Optimal conditions of hickory nut oil cold press technology were studied. $L_9(3^4)$ orthogonal experiment results showed that optimal conditions were a pressing pressure of 15 MPa, pressing temperature of 50°C, pressing cycle of 4 s, and stop cycle of 9 s. Fatty acid compositions were determined using GC-MS and hypolipidemic effects in mice were investigated. Compared to a high fat diet group, hickory nut oil administration decreased serum and visceral total cholesterol, triacylglycerol, and serum low density lipoprotein cholesterol levels. Serum high density lipoprotein cholesterol values were increased. Hickory nut oil can be used as a valuable bioactive source of natural hypolipidemic compounds.

Key words: hickory nut oil, cold press technology, hypolipidemic effect

Introduction

Hickory (*Carya cathayensis*) is known in China for production of hickory nuts, especially in the northeast Changbai Mountain region. Hickory nuts are consumed generally as a spice in many dishes. Hickory nuts are an oil-bearing wild plant product that contain a 60 to 70% oil content, which is frequently extracted using organic solvent and supercritical carbon dioxide (SC-CO₂) methods (1). However, organic solvent extraction always introduces safety and environmental pollution issues, and a lot of energy is required to run the supercritical carbon dioxide (SC-CO₂) extraction procedure. These factors make the organic solvent and supercritical carbon dioxide (SC-CO₂) extraction methods unsuitable for plant-oil industry production (2). Cold press extraction is a green extraction method conducted under low temperatures and the premise of the maximum to retain the nutrients and active substances (2). However, optimal extraction conditions for hickory nut oil using the cold press method have not been established. Therefore, this study determined the yield of hickory nut oil under different conditions for determination of optimal extraction conditions. Physical properties of hickory nut oil are slightly different under different conditions. Therefore, the physicochemical properties of hickory nut oil produced under optimal conditions were also investigated.

Hickory nut oil contains large amounts of polyunsaturated α -linolenic, α -eleostearic, and linolenic fatty acids, which have received considerable interest due to beneficial health effects associated with consumption, particularly for lowering the risk of hyperlipidemia,

which is the primary risk factor for cardiovascular diseases that are fatal to humans and are believed to be the major cause of death worldwide. Epidemiological research has indicated that hyperlipidemia is greatly affected by dietary habit, especially lipid intake (3). Many studies have shown that intake of a controlled quantity of plant oil contributes to a lower risk for cardiovascular disease based on reduction of blood lipid levels. Tzang *et al.* (4) reported that diets supplemented with flaxseed oil exhibited significant lipid-lowering effects, compared with a control group. High density lipoprotein cholesterol in a serum cholesterol profile was enhanced and the high density lipoprotein cholesterol (HDL-C)/total cholesterol (TC) ratio was lowered with a high fecal cholesterol output. Cactus pear oil was shown to regulate serum and liver lipids (5) based on determination of serum cholesterol, HDL, and triglyceride (TG) levels, and analysis of fatty acid compositions in the liver and in serum cholesterol. Cactus pear oil decreased the incidence of hyperlipidemia risk factors in rats by decreasing the atherogenic index and low density lipoprotein cholesterol (LDL-C) level in the serum. Other studies have shown that the increased dietary intake of Black currant seed oil (6), perilla oil, *Ricinus dendron heutelotii* (Bail.) oil, and *Tetracarpidium conophorum* Müll. Oil (7) may help for reducing blood fat and cholesterol or hypolipidemic.

Consumption of hickory nut oil should alter *in vivo* lipid profile parameters. However, no known research has reported investigation of hypolipidemic effects of hickory nut oil. Therefore, animal experimentation was performed for evaluation of the hypolipidemic potential of hickory nut oil.

Materials and Methods

Materials and Chemicals Hickory nuts were obtained from Zhitao dried fruit market in Siping, Jilin Province, China. According to the retailer, nuts were grown on Changbai Mountain of Jilin Province. Total cholesterol (TC), triacylglycerol (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) standards were purchased from Nanjing Jiancheng Bioengineering Co., Ltd. (Nanjing, China). All the other reagents used were of analytical grade.

Extraction of hickory nut oil Dried hickory nut were crushed and pressed by a cold hydraulic press (Model 6YL-190; Changbai Mountain Technology Limited company, Changchun, China). Hickory nut oil extracts were collected as a single sample at the end of each run in 100 mL amber glass bottles. Samples of hickory nut were pressed twice and collected oil was centrifuged (CR20B2; Hitachi, Tokyo, Japan) at 3,000 $\times$ g for 15 min (8). Supernatants were stored in separate amber bottles and were stored at -20°C until use. The pressing temperature (A), pressing pressure (B), pressing cycle (C), and stop cycle (D) of extraction were optimized to obtain a high yield. Each of these parameters was varied at 3 levels (Table 1). Amounts of extracted oil were weighed (AL204; Mettler Toledo Inc., Zurich, Switzerland) to determine the extraction yield, which was calculated as: extraction yield (%) = [(weight of extracted oil)/(weight of hickory nuts)] $\times$ 100.

Fatty acid composition and physicochemical properties of hickory nut oil Fatty acid compositions were determined as methyl esters for obtained oils. Methyl esters were analyzed following the method of Bannon *et al.* (9) and fatty acid compositions were determined following the methodology of American Oil Chemists Society (AOCS) (10). Approximately 0.5 μL (split 1:100) of the *n*-hexane phase was injected into a GC-MS device (Model QP2010; Shimadzu, Kyoto, Japan), equipped with a quartz capillary column (30 $\text{m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$) (Model Rxi-5ms; Shimadzu). The injection temperature was 250°C and gas flow was 1 mL/min. The initial temperature of the column was 100°C for 3 min with a heating program involving a temperature increase of $20^{\circ}\text{C}/\text{min}$ to 200°C and a subsequent increase of $1^{\circ}\text{C}/\text{min}$ to 230°C . The optimized MS instrument parameters were an electron multiplier voltage of 0.97 kV, an electron energy of 70 eV, an ion source temperature of 200°C , a connection temperature of 230°C , and solution mute time of 2.5 min. Each determination was performed in triplicate and results were expressed as a relative percentage of each individual fatty acid present in an oil sample. Peak areas were identified and quantified using external standards and pure fatty acids with methyl ester standard mixtures.

Animals and diets Sixty male Kunming mice weighing 18 to 22 g were purchased from the Laboratory Animal Center of Jilin University (Changchun, China). Mice were raised in an animal room at a

Table 1. Factors and levels of orthogonal experimental design

Independent variables	Independent variable levels		
	1	2	3
A: Pressing temperature ($^{\circ}\text{C}$)	45	50	55
B: Pressing pressure (Mpa)	10	15	20
C: Pressing cycle (s)	4	6	8
D: Stop cycle (s)	7	9	11

temperature of $25 \pm 2^{\circ}\text{C}$ with a relative humidity of $50 \pm 10\%$ and with a 12/12 h light-dark cycle. Mice were randomly divided into 5 groups after acclimatization for 3 days provided with basic commercial diet chow (Jilin University Animal Center) every 12 h for each group and water *ad libitum*. Mice belonging to the same group were housed together in a stainless steel cage and randomly assigned to treatment groups. All of the animal experiments in the present study were conducted in accordance with the regulations of the Jilin University Institutional Animal Care and Use Committee.

Common diet control group (NC): Mice received a basic commercial diet chow (Jilin University Animal Center) for 28 days consisting of 18.0% crude protein, 4.0% crude fat, 10.0% moisture content, 8.0% crude ash, 5.0% crude fiber, 1-1.8% calcium, and 0.6-1.2% phosphorus.

High fat diet group (HF): Mice received a high fat diet chow for 28 days consisting of 76.5% (w/w) basic commercial feed, 10% (w/w) yolk powder, 1.0% (w/w) cholesterol, 0.5% (w/w) sodium cholate, and 12% (w/w) lard.

Low dose group (LD): Mice received a high fat diet chow for the first week, then received a diet consisting of a high fat diet chow supplemented with 5% (w/w) hickory nut oil for the next 3 weeks (9).

Medium dose group (MD): Mice received a high fat diet chow for the first week, then received a high fat diet chow supplemented with 10% (w/w) hickory nut oil for the next 3 weeks.

High dose group (HD): Mice received a high fat diet chow for the first week, then received a high fat diet chow supplemented with 15% (w/w) hickory nut oil for the next 3 weeks.

Biological analysis Serum total cholesterol (TC), triacylglycerol (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were determined by the prescribed corresponding analytical kits with the detector, ELISA reader (Synergy HTX; BioTek Instruments, Inc., Winooski, VT, USA) (11).

Statistical analysis All the data were expressed as mean $\pm$ SD. Statistical evaluation of values obtained was performed using SPSS version 10.0. An unpaired Student's *t*-test and a one-way analysis of variance (ANOVA) were used for analysis of significance at $p < 0.05$.

Results and Discussion

Cold press technology for recovery of hickory nut oil Since various parameters potentially affect the hickory nut oil extraction yield, the

optimization of the experimental conditions represents a critical step in the development of a press technology. Effect of the 4 independent variables pressing temperature (A), pressing pressure (B), pressing cycle (C), and stop cycle (D) on the hickory nut oil extraction yield (Y) were evaluated. An orthogonal experimental design was used to study the main effects of extraction process components on the yield of hickory nut oil (Table 2). The range (R) of pressure, pressing cycle, and stop cycle were more variable than the pressing temperature, indicating that these factors had important effect on the extraction yield of hickory nut oil. Results indicates that the influential order of the 4 factors on the extraction yield is stop cycle >pressing cycle> pressing pressure>pressing temperature (D>C>B>A) (Table 2A). The order is in agreement with the order based on the values of F in variance analysis (Table 2B). According to extreme difference analysis, the optimum extraction condition of hickory nut oil was deduced as: pressing temperature 50°C, pressing pressure 15 MPa, pressing cycle 4 s, and stop cycle 9 s (Table 2A). Unfortunately, the optimal combination of variables was not included in the orthogonal test. To reconfirm this deduced optimum condition, 3 additional tests

under optimal conditions were conducted and the average extraction yield of hickory nut oil reached 58.3%. So this deduces condition was rationally confirmed to be the best combination for hickory nut oil extraction of different parameters.

Results of variance analysis of orthogonal analysis are shown in Table 2B. According to variance analysis, both the contributions of the pressing cycle ($p<0.1$) and stop cycle ($p<0.1$) and pressing pressure ($p<0.25$) for the extraction yield of hickory nut oil, whereas pressing temperature is not significant factors.

Fatty acid composition Fatty acid constituents and physicochemical properties of hickory nut oil are shown in Table 3. The dominate fatty acids of hickory nut oil were 62.94% linoleic acid (C18:2, *n*-6) and 16.23% oleic acid (C18:1, *n*-9). Other fatty acids present were 9.38% linolenic acid, 6.18% palmitic acid, and 2.58% stearic acid. Amounts of oleic acid, palmitic acid, and stearic acid were similar to pecan nuts from Brazil, except for the content of linoleic acid (C18:2, *n*-6) and oleic acid (C18:1, *n*-9) (12). Differences may have resulted from different conditions in cultivation areas. Hickory nut oil contained

Table 2. Results of an Orthogonal experiment

(A) Orthogonal test design and result

Test	A: Pressing temperature (°C)	B: Pressing pressure (MPa)	C: Pressing cycle (s)	D: Stop cycle (s)	Extraction yield (%)
1	1	1	1	1	58.00
2	1	2	2	2	57.60
3	1	3	3	3	55.90
4	2	1	2	3	56.20
5	2	2	3	1	57.70
6	2	3	1	2	57.90
7	3	1	3	2	58.20
8	3	2	1	3	57.40
9	3	3	2	1	55.90
K ₁ ¹⁾	57.17	57.47	57.77	57.20	
K ₂	57.27	57.57	56.57	57.90	
K ₃	57.17	56.57	57.27	56.50	
R ²⁾	0.10	1.00	1.20	1.40	
Optimization levels	A ₂	B ₂	C ₁	D ₂	
Primary and secondary factors	D,C,B,A				
Excellent-level collocation	A ₂ B ₂ C ₁ D ₂				
Optimal combination	A ₂ B ₂ C ₁ D ₂				

¹⁾K_i is the average extraction yield under the corresponding factor level (i=1, 2, 3).

²⁾R is the range among K_i values.

(B) Variance analysis of orthogonal test

Source of variance ¹⁾	Square of the deviance	Degrees of Freedom	Mean square	The ratio of F values	Significant ²⁾
B	4.31	1	4.31	45.79	*
C	5.15	1	5.15	54.73	**
D	6.96	1	6.96	73.96	**
Error	0.05	1	0.05		
Sum	16.46	4			

¹⁾B: Pressing pressure, C: Pressing cycle, D: Stop cycle.

²⁾** $p<0.1$ ($F>F_{0.1}(2,1)=49.50$); * $p<0.25$ ($F>F_{0.25}(2,1)=7.50$).

$F_{0.25}(2,1)=7.50$, $F_{0.1}(2,1)=49.50$, $F_{0.05}(2,1)=199.50$, $F_{0.01}(2,1)=4999.50$.

Table 3. Fatty acid compositions and physicochemical properties of hickory nut oil

Fatty acid composition					Physicochemical parameters	
Number	Determination	Molecular formula	Mw	Relative weight (%)	Parameter	Analytical results
1	Stearic acid (C18:0)	C ₁₈ H ₃₆ O ₂	284	2.58	Acid value (KOH mg/g)	1.94
2	Palmitic acid (C16:0)	C ₁₆ H ₃₂ O ₂	256	6.18	Peroxidation value (mmol/kg)	2.37
3	Linolenic acid (C18:3, <i>n</i> -3)	C ₁₈ H ₃₀ O ₂	278	9.38	Iodine value (g/100 g)	164.26
4	Oleic acid (C18:1, <i>n</i> -9)	C ₁₈ H ₃₄ O ₂	282	16.23	Saponification value (KOH mg/g)	188.85
5	Linoleic acid (C18:2, <i>n</i> -6)	C ₁₈ H ₃₂ O ₂	280	62.94		

Table 4. Effects of diet supplementation with hickory nut oil

Items	Groups ¹⁾				
	NC	HF	HD	MD	LD
Initial body weight (g)	25.54±0.98 ^{a2)}	26.10±0.10 ^a	25.55±0.78 ^a	25.60±0.97 ^a	25.88±0.49 ^a
Final body weight (g)	33.08±2.22 ^b	32.57±0.45 ^b	33.72±1.42 ^b	34.59±1.63 ^b	33.12±1.67 ^b
Body weight gain (g)	7.76±1.38 ^a	7.08±0.38 ^a	8.11±1.42 ^a	9.00±1.92 ^a	7.16±1.52 ^a
Absolute liver weight (g)	2.01±0.19 ^c	1.62±0.01 ^c	1.65±0.12 ^c	1.89±0.16 ^c	1.65±0.19 ^c
Relative liver weight (%) ³⁾	5.00±0.10 ^a	5.73±0.25 ^b	4.90±0.16 ^a	5.16±0.23 ^a	4.96±0.35 ^a
Absolute kidney weight (g)	0.53±0.07 ^d	0.63±0.11 ^d	0.54±0.59 ^d	0.52±0.02 ^d	0.57±0.07 ^d
Relative kidney weight (%) ⁴⁾	1.641±0.25 ^a	1.80±0.27 ^c	1.59±0.11 ^a	1.42±0.01 ^b	1.70±0.13 ^a

¹⁾NC, HF, HD, MD, and LD are normal control group, high fat group, hickory nut oil high dose group, medium dose group, and low dose group, respectively.

²⁾Values with different letters in a row show values that are significantly different at $p < 0.05$.

³⁾Relative liver weight (%) = absolute liver weight (g)/final body weight (g).

⁴⁾Relative kidney weight (%) = absolute kidney weight (g)/final body weight (g).

91.24% unsaturated fatty acids in this study, in agreement with a report of Venkatachalam *et al.* (13). Hickory nut oil acid, peroxidation, iodine, and saponification values were 1.94 KOH mg/g, 2.37 mmol/kg, 164.26 g/100 g, and 188.85 KOH mg/g, respectively (Table 3), indicating that the quality of hickory nut oil obtained using the cold press method under optimal conditions was good.

Animal health and feed intake During the 24 day experimental period, daily observations made of all test animals revealed that all animals had stable chow consumption and all steadily gained weight. All mice remained healthy from the beginning to the end of the experimental period; however, the fur became wet towards the end of the experiment, perhaps resulting from ingestion of large amounts of lipids (14).

Body weight, organ weight, and body weight gain Mice body weights in each group increased during the study, but no significant ($p > 0.05$) differences were found for body weight gains between mice of the 5 treatment groups (Table 4). Absolute liver and kidney weights of mice in the 3 hickory nut-treatment groups were not significantly ($p > 0.05$) different from for mice in the NC group.

The relative organ weight was determined as organ weight/body weight. Both relative liver weight and kidney weight of HF group mice were significantly ($p < 0.05$) higher, compared with NC group mice. Compared with HF group mice, the relative kidney weight of HD, MD and LD group mice were significantly ($p < 0.05$) lower. Thus, consumption of a high fat diet increased both relative liver and kidney weight. However, mice that consumed chow supplemented

with hickory nut oil displayed significantly ($p < 0.05$) reduced liver and kidney weights, compared with HF group mice. But relative liver weight of the 3 hickory nut-treatment groups were not significantly ($p > 0.05$) with each other.

Serum parameters Serum parameters determined from 60 experimental mice are shown in Table 5. High levels of total cholesterol (TC) and high concentrations of triglycerides (TG) are independent risk factors of cardiovascular disease (15). HDL-C acts to prevent coronary heart disease via carrying extra cholesterol from tissues to the liver (16). However, LDL-C is capable of combining cholesterol with the vascular wall, which was negatively correlated with the bio-function of HDL-C (16). Throughout 24 test days in this study, TC and LDL-C values were significantly ($p < 0.05$) increased in HF group mice, compared with NC group mice. A significant ($p < 0.05$) decrease of HDL-C levels in HF group mice, compared with NC group mice, was also observed.

The Atherogenic Index (AI) is a marker used for identification of a risk for development of cardiovascular disease (17). The higher the risk, the higher the AI value (18). The AI value was calculated as: $AI = (Total\ cholesterol - HDL\ cholesterol) / HDL\ cholesterol$ (17). AI values of HF group mice was significantly ($p < 0.05$) higher than for mice in all other groups. Therefore, a hyperlipidemia condition was successfully induced using a high fat diet. When compared with HF group mice, TC concentration values of mice in the 3 hickory nut oil treated groups showed significant ($p < 0.05$) lower than the HF group mice. TG values for HD group mice were have no significant ($p > 0.05$) difference for HF group mice. Whereas a significant ($p < 0.05$)

Table 5. Effects of diet supplementation with hickory nut oil on serum lipids

Items	Groups ¹⁾				
	NC	HF	HD	MD	LD
Total cholesterol (mmol/L)	4.01±0.26 ^{a2)}	5.16±0.23 ^b	4.48±0.08 ^c	4.81±0.31 ^d	4.31±0.19 ^c
Triglyceride (mmol/L)	0.50±0.02 ^a	0.79±0.08 ^b	0.71±0.07 ^b	0.64±0.07 ^c	0.53±0.05 ^a
HDL cholesterol (mmol/L)	2.70±0.12 ^a	2.20±0.25 ^b	3.22±0.19 ^c	3.52±0.36 ^c	2.68±0.27 ^d
LDL cholesterol (mmol/L)	1.03±0.09 ^a	2.75±0.11 ^b	1.12±0.05 ^c	1.26±0.04 ^d	1.53±0.16 ^e
Atherogenic Index (AI)	0.49±0.03 ^a	1.35±0.10 ^b	0.56±0.09 ^c	0.54±0.11 ^c	0.61±0.06 ^c

¹⁾NC, HF, HD, MD, and LD are abbreviations for normal control group, high fat group, hickory nut oil high dose of group, medium dose group, and low dose group, respectively.

²⁾Values with different letters in a row are significantly different at $p<0.05$.

Table 6. Effects of supplementation with hickory nut oil on liver and kidney lipid levels

Items ¹⁾	Groups ²⁾				
	NC	HF	HD	MD	LD
Liver TC (mmol/L)	0.5±0.12 ^{a3)}	5.20±0.05 ^b	2.76±0.19 ^c	1.00±0.09 ^d	0.56±0.08 ^a
Liver TG (mmol/L)	2.04±0.16 ^a	12.39±0.50 ^b	8.32±0.38 ^c	3.28±0.10 ^d	1.90±0.03 ^a
Liver TC/TG	0.25±0.06 ^a	0.42±0.02 ^b	0.33±0.05 ^c	0.31±0.01 ^c	0.29±0.03 ^c
Kidney TC (mmol/L)	1.21±0.14 ^a	3.15±0.26 ^b	1.78±0.24 ^a	1.53±0.19 ^a	1.64±0.27 ^a
Kidney TG (mmol/L)	2.93±0.24 ^a	5.28±0.55 ^b	3.25±0.32 ^a	4.02±0.17 ^a	3.70±0.19 ^a
Kidney TC/TG	0.41±0.03 ^a	0.60±0.06 ^b	0.55±0.06 ^b	0.38±0.04 ^c	0.44±0.05 ^a

¹⁾TC is the abbreviation for total cholesterol; TG is the abbreviation for triglyceride.

²⁾NC, HF, HD, MD, and LD are abbreviations for normal control group, high fat group, hickory nut oil high dose of group, medium dose group, and low dose group, respectively.

³⁾Values with different letters in a row are significantly different at $p<0.05$.

decrease of 19.90% was observed in MD group mice and a significant ($p<0.05$) decrease of 32.0% was observed in LD group mice, compared with HF group mice.

Chow supplemented with hickory nut oil significantly ($p<0.05$) increased the HDL-C level in LD, HD and MD group mice, and decreased the LDL-C level compared with HF group mice. Mice received different doses of the hickory nut oil, with a dose-dependent decrease in the LDL-C level. Mice in the LD group showed higher LDL-C levels than mice in the MD group, which showed higher levels than mice in the HD group. AI values significantly ($p<0.05$) decreased for mice in treatment groups fed hickory nut oil, compared with HF group mice.

Liver and kidney parameters Changes in liver and kidney parameters are shown in Table 6. Liver TC, TG, TC/TG and kidney TC, TG, TC/TG of HF group mice showed significant ($p<0.05$) higher than NC group mice. In HD, MD, and LD group mice, liver TC, TG, and kidney TC, TG levels were significantly ($p<0.05$) lower than in HF group mice. Mice of 3 oil-treated groups exhibited significantly ($p<0.05$) decreased liver TC/TG ratios, compared with HF group mice. No significant ($p>0.05$) differences in liver TC/TG ratios were revealed among mice of the 3 hickory nut oil treatment groups. There was a tendency for the value of liver TC/TG ratios to decrease with decreased dosages of hickory nut oil. Kidney TC/TG ratios of MD and LD group mice were significantly ($p<0.05$) lower, compared with HF group mice. Consumption of chow with hickory nut oil was

advantageous for prevention of changes in kidney and liver lipid contents.

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