

Taste and flavor compounds in box thorn (Lycium chinense Miller) leaves

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Contents of selected taste components in box thorn leaves collected at different seasons (from mid-May to mid-October) were determined. Volatile flavor compounds in the box thorn leaves were extracted using a simultaneous steam distillation-extraction apparatus, and the compounds were isolated and identified by gas chromatography/mass spectrometry. Box thorn leaves contained fructose (0.58-1.54%), glucose (0.33-1.33%), sucrose (0.23-0.68%) and maltose (0.60-0.98%). Non-volatile organic acids identified were citric acid (162.3-361.3 mg%). oxalic acid (61.1-130.9 mg%), malonic acid (44.9-59.4 mg%), malic acid (15.6-34.3 mg%), succinic acid (3.7-5.1 mg%), fumaric acid (1.3-2.9 mg%), and lactic acid (0.0-1.1 mg%). Eighteen free amino acids were found in the leaves and their contents varied greatly with season. Proline (52.6-267 mg%), histidine (162-244 mg%) alanine (99.5-198 mg%), leucine (51.4-149 mg%), valine (54.5-125 mg%), isoleucine (37.0-119 mg%) and aspartic acid (46.7-111 mg%) were major amino acids in the leaves. Total tannin contents were 0.90-2.10%, showing the highest amount in October. Forty-five volatile flavor components were identified in the leaves for the first time. These included four acids, 15 alcohols, seven aldehydes, two esters, three furans, nine hydrocarbons, two ionones, and three other compounds. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The fruits and leaves of box thorn (Lycium chinense Miller), which is a plant belonging to the family Solanaceae, have been used as foods, tea and/or medicine in the Orient. It is known that box thorn leaves are capable of abating or reducing the risk of certain diseases such as arteriosclerosis, essential arterial hypertension, diabetes and nightblindness (Soga, 1985). Box thorn leaves have also been known for improvement of stamina, tranquillizing activity, thirst-quenching and antiaging activity (Soga, 1985). Nishiyama (1963) reported that the betaine content in box thorn leaves was 1.50% on a dry weight basis. Betaine is known as a preventive phytochemical for reducing or abating the risk of fatty liver. Mizobuchi et al. (1969) reported that box thorn leaves contained a rutin (1.1-2.7%, dry weight basis), a preventive phytochemical for essential hypertension and stroke. Box thorn leaves reportedly contain the antiaging vitamins ascorbic acid and tocopherols (Mizobuchi et al., 1964, 1969; Park, 1995). It is well known that ascorbic acid and tocopherols have antiaging activity due to their scavenging of singlet oxygen, superoxide anion and other active oxygens (Nishikimi, 1975; Jung *et al.*, 1991, 1995*a,b*). Park (1995) reported that water extract or methanolic extract of box thorn leaves showed a strong ability to scavenge superoxide anion radicals. Park (1995) also reported that water extract of box thorn leaves inhibited the activity of angiotensin converting enzyme (ACE). ACE catalyzes the conversion of angiotensin I to angiotensin II and the breakdown of bradykinin. Angiotensin II and bradykinin are hypertensive and hypotensive agents, respectively.

The effective utilization of box thorn leaves for tea or as health food ingredients requires detailed information on their taste and flavor components. However, this information is limited. Free sugars, free amino acids and non-volatile organic acids in Japanese box thorn leaves were reported by Nishiyama (1962) and Yoshimura *et al.* (1969). However, these authors reported only qualitative data on the free sugars and non-volatile organic acids in box thorn leaves; the contents of the free sugars and non-volatile organic acids were not reported. Collection of box thorn leaves can be made from May to October. Thus, information on the

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contents of these compounds in box thorn leaves collected at different seasons is also important, but not available in the literature. Also, the important taste component, tannin, has not previously been reported. Sannai *et al.* (1984) identified four volatile flavor compounds from *Lycium chinense* leaves. These were 3hydroxy-7,8-dehydro- β -ionone, 3-hydroxy-7,8-dehydro- β -ionol, 3-hydroxy- β -ionone, and 3-hydroxy- β -ionol. No other volatile compounds were identified in the study.

The objectives of our research were (1) to study the seasonal changes in the contents of free sugars, organic acids, free amino acids and tannin, and (2) to identify the volatile flavor compounds in box thorn leaves.

MATERIALS AND METHODS

Box thorn leaves were collected once a month from 15 May 1993 to 15 October 1993 at a box thorn farm located in Chungyang, Chungnam Province, Korea. The collected leaves were washed with 0.001% acetic acid solution to remove possible pesticide residues and rinsed with water. The washed leaves were dried with hot air at 60°C and ground to pass a 60 mesh sieve. The ground leaves were flushed with nitrogen and stored at -18° C until used. These prepared samples were used for all investigations except for the volatile analysis. For analysis of volatile constituents in the leaves, the leaves collected on 15 May were washed as described. The washed leaves were freeze-dried and ground to avoid artifacts that may arise during hot air treatment.

Free sugar analysis

To extract free sugars, purified water was added to the samples. They were then heated for 2 h at 80°C and filtered (Lee & Sheo, 1986). The filtered solution was passed through a SEP-PAK C-18 cartridge and 0.45 μ m membrane filter for high-performance liquid chromatographic (HPLC) analysis. The HPLC system consisted of a Waters 501 pump, refractive index detector, and Waters 746 integrator (Waters, Milford, MA). A carbohydrate column (Waters) was used. The flow rate of the mobile phase (acetonitrile–water, 75:25) was 1 ml min⁻¹.

Non-volatile organic acid analysis

Non-volatile organic acids were extracted from the samples and methylesterified according to the method of Court & Hendel (1978). The methylated sample was injected into a gas chromatograph (HP 5890 Series II; Hewlett Packard, Avondale, PA) equipped with a flame ionization detector. The column used was a DB-Wax fused silica capillary column (30 m×0.25 mm i.d.; J & W Scientific, Folsom, CA). The temperatures of injection port and detector were 210°C and 230°C, respectively.

The oven temperature was held at 60° C for 3 min, then programmed at 3° C min⁻¹ to 230° C.

Free amino acids

Free amino acids were extracted and purified through an Amberlite IR column according to Kim *et al.* (1982). The contents and compositions of free amino acids were analyzed using an automatic amino acid analyzer (LKB 4150 alpha amino acid analyzer; LKB, Bromma, Sweden). Under the analytical conditions, glutamine and threonine were coeluted at the retention time of 26.69 min. To determine the contents of glutamine and threonine, the purified free amino acid sample was hydrolyzed with 2 N HCl at 100°C for 3 h. This hydrolysis process converts all the glutamine to glutamic acid (Kim *et al.*, 1982). The glutamine content was calculated by the difference in peak area of glutamic acid before and after hydrolysis.

Total tannin

Total tannin content was determined according to AOAC methods (AOAC, 1985).

Extraction of volatile components

The freeze-dried box thorn leaves collected at mid-May were prepared by stirring 300 g of leaves with 1 liter of distilled water in a Waring blender. The sample was then transferred to a 3 litre capacity round bottle and flavor compounds were extracted with 50 ml of double-distilled diethyl ether in a simultaneous steam distillation-extraction apparatus for 2 h at atmospheric pressure according to Schultz *et al.* (1977).

The extract was dehydrated under anhydrous sodium sulphate at 4°C and then filtered. Then 5 ml of n-dodecane (25 μ g ml⁻¹) as an internal standard was added to the extract, which was then concentrated to 0.2 ml by nitrogen flushing.

Isolation and identification of volatile compounds

The extracted sample was injected into a gas chromatograph equipped with a flame ionization detector for the isolation and relative quantitation of the individual volatile components. The column used was DB-Wax fused silica capillary column (30 m×0.25 mm i.d.; J & W Scientific). The temperatures of injection port and detector were 210°C and 230°C, respectively. The oven temperature was held 50°C for 3 min, then programmed at 3°C min⁻¹ to 230°C.

For identification, a Varian 3700 gas chromatograph coupled to a mass spectrometer (Varian Mat 212 system) was used. Mass spectra were obtained by electron ionization at 70 eV and a temperature of 220°C. The spectra were recorded on a Varian SS MAT 188 data system.

Statistical analysis

All contents measurements in box thorn leaves were carried out in duplicate or triplicate. Statistical analysis was accomplished with a Statistical Analysis Systems package (SAS, 1985). Duncan's multiple range test was used to ascertain the seasonal changes in the various components of the box thorn leaves (Kim *et al.*, 1994; Jung *et al.*, 1995b).

RESULTS AND DISCUSSION

Free sugars

Table 1 shows the free sugar contents in box thorn leaves collected at different seasons. Total free sugar contents in the leaves varied with collecting season: in May, June, July, August, September and October they were 3.76%, 2.87%, 2.76%, 2.99%, 3.04% and 3.06%(w/w), respectively. The free sugars found were fructose (0.58-1.54%), glucose (0.33-1.33%), sucrose (90.23-0.68%) and maltose (0.60-0.98%). Yoshimura *et al.* (1969) identified glucose, maltose, fructose, raffinose and sucrose in Japanese box thorn leaves by paper chromatography. However, raffinose was not found in the box thorn leaves in our study. Yoshimura *et al.* (1969) reported glucose and sucrose contents in box thorn leaves of 1.06% and 0.52%, respectively, but did not report the contents of other sugars. The reported contents of glucose and sucrose were within the range of the present data. The fructose content in May was 0.77%, and then it increased greatly to a maximum (1.54%) in June (P < 0.05); thereafter it gradually decreased, showing the lowest amount (0.58%) in October (P < 0.05). The glucose content was highest in May (1.33%) and lowest in July (0.33%) (P < 0.05). Sucrose and maltose were also highest in May and lowest in June. Even though the total sugar content was not high in box thorn leaves, the variation in amounts of individual sugars in the leaves might affect, at least to some extent, the sweet taste of leaves collected at different seasons since sweetness intensity greatly depends on the types and concentrations of sugars.

Non-volatile organic acids

Seven non-volatile organic acids were identified in the leaves as shown in Table 2. These include citric acid (162–362 mg%), oxalic acid (61.1–13 mg%), malonic acid (44.9–59.4 mg%), malic acid (15.6–38.3 mg g⁻¹), succinic acid (3.7–5.1 mg%), fumaric acid (1.3–2.9 mg%), and lactic acid (trace–1.1 mg%). Yoshimura *et al.* (1969) identified succinic acid, pyroglutamic acid, oxalic acid, glycolic acid, malic acid and citric acid in Japanese box thorn leaves, but did not report quantitative data on the non-volatile organic acids in the leaves. In our study, pyroglutamic acid and glycolic acid were not found in the box thorn leaves.

Free sugars			Free sugar con	ntents (% w/w)	orn leaves	
rice sugars	15 May	15 June	15 July	15 August	15 September	15 October
Fructose	0.77 ^d	1.54ª	1.47 ^b	1.40 ^b	1.19°	0 58 ^e
Glucose	1.33 ^a	0.47 ^d	0.33 ^e	0.50 ^d	0.66 ^c	0.91 ^b
Maltose	0.98 ^a	0.60 ^d	0.73 ^c	0.84 ^b	0.94 ^{ab}	0.95 ^{ab}
Sucrose	0.68 ^a	0.26 ^b	0.23 ^b	0.25 ^b	0.25 ^b	0.62 ^a
Total	3.76	2.87	2.76	2.99	3.04	3.06

Table 1. Seasonal variation in contents of free sugars in box thorn leaves

Percent free sugar contents were calculated on a dry weight basis.

Means in the same row with different superscript letters are significantly different at P < 0.05.

Table 2.	Seasonal	variation i	in non-	volatile	organic	acid	contents	in box	thorn	leaves
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Organic acid	Organic acid content (mg%)							
	15 May	15 June	15 July	15 August	15 September	15 October		
Citric acid	362ª	249°	179 ^e	162 ^f	209 ^d	264 ^b		
Fumaric acid	1.7ª	1.3 ^a	2.9 ^a	2.3ª	2.1ª	2.6ª		
Lactic acid	1.1ª	0.9 ^a	0.0 ^b	0.7ª	0.0 ^b	1 1ª		
Malic acid	33.5 ^b	15.6 ^d	34.3 ^b	32.7 ^b	29.4°	38.3ª		
Malonic acid	59.4ª	51.5 ^b	46.8 ^{bc}	44.9°	59.1ª	51.2 ^b		
Oxalic acid	61.1 ^e	65.6 ^d	131.9 ^a	101,9°	98.2°	114		
Succinic acid	5.1ª	5.1ª	4.8 ^{ab}	4.7 ^{ab}	4.9 ^{ab}	3.7 ^b		

Organic acid contents (mg%) were calculated on a dry weight basis.

Means in the same row with different superscript letters are significantly different at P < 0.05.

The results showed that citric acid is the major organic acid found in box thorn leaves regardless of the collecting season. The citric acid, malic acid and oxalic acid contents in the leaves varied greatly with the seasons, but the contents of fumaric, lactic, malonic and succinic acid were relatively stable throughout the seasons. The citric acid content was highest in May (361.6 mg%) (P < 0.05), after which it gradually decreased to its lowest value in August (162 mg%) (P < 0.05). THe oxalic acid content was low in May and June; the highest level was observed in July (P < 0.05). The malic acid content was lowest in June (P < 0.05).

Free amino acids

Free amino acids play an important role in the taste of tea and vegetables. Thus, the qualitative and quantitative analysis of free amino acids in box thorn leaves is indispensable for the effective utilization of box thorn leaves. Table 3 shows the seasonal variation of free amino acid contents in box thorn leaves. Eighteen free amino acids were found in the leaves and their contents greatly varied with season. Proline (52.6-267 mg%), histidine (162-244 mg%), alanine (99.5-198 mg%), leucine (51.4-149 mg%), valine (54.5-125 mg%), isoleucine (37.0-119 mg%) and aspartic acid (46.7-111 mg%) were the major amino acids in the leaves. Nishiyama (1962) identified eight free amino acids in Japanese box thorn leaves-isoleucine, leucine, valine, alanine, lysine, glycine, glutamic acid and asparaginebut did not report their contents. Yoshimura et al. (1969) identified 18 free amino acids in Japanese box thorn leaves using paper chromatography; the major ones were proline, aspartic acid, serine and glutamic acid. However, our present results show that serine and glutamic acid are not major free amino acids.

The total free amino acid contents in the leaves collected at different seasons varied greatly (689-1649 mg%), with the highest and lowest levels in May and August, respectively. Glycine and L-alanine reportedly elicit a strong sweet taste (Belitz & Grosch, 1987), thus the high alanine content (99.5-198 mg%) might affect the sweet taste of the box thorn leaves. It has been reported that isoleucine, leucine and histidine elicit a bitter taste (Belitz & Grosch, 1987). The leaves contained considerable amounts of these bitter amino acids (Table 3). Proline reportedly has sweet and bitter tastes (Belitz & Grosch, 1987). The proline contents in the box thorn leaves were high (52.6–267 mg%). Our organoleptic test showed that the tea prepared with box thorn leaves had a slightly sweet and bitter taste. This could partly be explained by the high contents of alanine, histidine, isoleucine, leucine and proline in the leaves. L-Glutamic acid reportedly imitates a meat broth flavor at high concentrations, while at low concentrations it enhances the characteristic flavor of a given food. The most important umami substances of green tea are glutamic acid and L-threonine, which is an ethylamide derivative of glutamic acid (Kato et al., 1989). However, the contents of glutamic acid and threonine in box thorn leaves were low.

Total tannin contents

Box thorn leaves contained low levels of tannin, as shown in Fig. 1. The total tannin content was in the range 0.90-2.10%, the highest level being observed in October. Tannin is a important component for astrin-

Amino acid	Free amino acid content (mg%)							
	15 May	15 June	15 July	15 August	15 September	15 October		
Alanine	198ª	149 ^b	132°	99.5 ^f	112 ^e	122 ^d		
Arginine	67.7ª	63.8 ^b	33.1c	27.4 ^e	28.1e	30.5 ^d		
Aspartic acid	111	75.2 ^b	56.2 ^c	46.7°	53.3°	49.5°		
Cvsteine	14.3ª	13.4 ^a	9.8 ^b	4.9°	9.1 ^b	0.0 ^d		
Glutamine	10.6 ^b	4.2 ^e	5.3 ^d	2.1 ^r	7.0 ^c	19.7ª		
Glutamic acid	13.3 ^a	8.3°	5.1 ^d	7.6 ^c	9.7 ^b	10.3 ^b		
Glycine	12.8 ^a	13.0 ^a	6.5 ^b	6.9 ^b	6.4 ^b	5.6 ^b		
Histidine	22 4 ª	208 ^ь	191°	173 ^e	180 ^d	162 ^f		
Isoleucine	119 ^a	77.4 ⁶	56.8°	37.3°	45.4 ^d	38.2 ^e		
Leucine	149 ^a	113 ^b	75.7°	51.4 ^e	62.5 ^d	52.0 ^e		
Lysine	88.1 ^a	63.5 ^b	43.1°	25.0 ^e	32.0 ^d	25.1°		
Phenylalanine	85.5ª	81.8 ⁶	34.4 ^f	45.8 ^d	58.7°	40.6 ^e		
Proline	267ª	136°	100 ^d	52.6 ^e	139°	231 ^b		
Serine	20.4 ^b	18.4 ^{bc}	14.5 ^c	15.2°	27.2ª	5.8 ^d		
Threonine	18.3ª	16.9 ^a	7.3 ^b	3.5 ^b	4.5 ^b	5.2 ^b		
Fryptophan	38.9 ^a	18.8 ^b	19.6 ^b	9.0 ^d	11.8°	10.5 ^{cd}		
Tyrosine	85.8ª	58.8 ^b	39.9°	27.0 ^{de}	31.5 ^d	23.4 ^e		
Valine	125 ^a	99.2 ^ь	67.5°	54.5 ^d	60.5 ^d	57.6 ^d		

Table 3. Seasonal variation in free amino acid contents in box thorn leaves

Free amino acid contents (mg%) were calculated on a dry weight basis.

Means in the same row with different superscript letters are significantly different at P < 0.05.



Fig. 1. Seasonal variation in tannin contents of box thorn leaves (bars with different letters are significantly different at P < 0.05).

gency in tea. The tannin contents in box thorn leaves were not greatly different from May to August. Thereafter the content greatly increased, reaching a maximum in October. The tannin content of 0.90-2.10% in box thorn leaves was very low compared to that in tea leaves (11.8%, dry weight basis) (Kim *et al.*, 1983). Our organoleptic test showed that the tea prepared with box thorn leaves had a much lower astringency than green tea. This could be explained by the low level of tannin in the box thorn leaves.

Volatile flavor compounds

The gas chromatogram of volatile components in box thorn leaves is shown in Fig. 2. The volatile components identified in the leaves are shown in Table 4. Forty-five volatile flavor components were identified in the leaves for the first time. These included four acids, 15 alcohols, seven aldehydes, two esters, three furans, nine hydrocarbons, two ionones, and three others.

Four acids in the leaves were identified, representing only 2.16% of the total volatiles. These were 2,4-pentadienoic acid, 3-methyl-1-butanoic acid, hexanoic acid and 2-ethylhexanoic acid. Of these, hexanoic acid has been reported to have cheese, fatty and rancid odors in strawberry jams (Guichard *et al.*, 1991).

Quantitatively, alcohols were the most abundant class of components identified, representing as much as 33.4%. Phytol, which is derived from chlorophyll, contributed 23.7% of the total volatile components in the leaves. Menthol (2.17%), which gives a characterstic minty flavor, was identified in the leaves. The leaves contained leaf alcohol, 3-hexene-1-ol (1.58%), which is considered to give a 'green' flavor. A characteristic mushroom flavor component, 1-octene-3-ol (0.61%), was also found. Benzyl alcohol, which gives a 'floral' flavor, was also found (0.23%). Nerolidol (peanut flavor) and *trans*-geraniol (orange and rosy odor) were also identified in the leaves.

Aldehydes constituted 13.8% of total volatile compounds in the leaves, and were the second largest group of volatiles. Among the seven aldehydes identified, an important flavor component, 2-methylbutanal was found; this compound has been known as a toasted coffee flavor. Some other aldehydes were considered to be lipid oxidation products.

The three furans identified constituted only 0.78% of the total volatiles identified. 2-Ethylfuran, 2-pentylfuran and 2,3-dihydrobenzofuran were found in the leaves. 2-Ethylfuran has been reported to have a warm sweet aroma (Fors, 1983). 2-Pentylfuran has been reported as



Fig. 2. Gas chromatogram of volatile constituents in box thorn leaves.

Table 4. Volatile compounds identified in box thorn leaves

Peak no.	Compound	Peak area (%		
Acids				
20	2,4-Pentadienoic acid	0.42		
21	3-Methyl-1-butanoic acid	0.52		
28	Hexanoic acid	0.08		
33	2-Ethylhexanoic acid	1.14		
Alcohols				
11	2-Pentene-1-ol	0.28		
12	l-Hexanol	0.40		
13	3-Hexene-1-ol	1.58		
14	2-Hexene-1-ol	0.34		
15	1-Octene-3-ol	0.61		
24	α ,4-Dimethylbenzenemethanol	0.13		
25	Nerolidol	0.63		
26	trans-Geraniol	0.08		
27	Menthol	2.17		
29	Ionol	2.18		
30	Benzyl alcohol	0.23		
38	2,3-Dihydro-2,2-dimethyl-	0.42		
	7-benzofuranol			
39	9,12-Octadecadien-1-ol	0.42		
40	9-Octadecen-1-ol	0.36		
45	Phytol	23.65		
Aldehydes				
1	2-Methylbutanal	9.37		
2	3-Methylbutanal	0.42		
4	2,4-Dimethylpentanal	0.17		
5	4-Pentenal	0.08		
19	Benzeneacetaldehyde	1.46		
23	2,4-Nonadienal	0.16		
43	9,12,12-Octadecatrienal	2.10		
Esters				
37	Dodecanoic acid, methyl ester	2.65		
41	Linoleic acid, methyl ester	2.65		
Furans		<u> </u>		
3	2-Ethylfuran	0.18		
7	2-Pentylfuran	0.39		
42	2,3-Dihydrobenzofuran	0.21		
Hydrocarbons	Limanana	0.18		
0	Limonene	0.10		
9	1-Pentene	0.13		
10	2,2,0-1 finethylcyclonexane	0.07		
17	5-Ethyl-1,4-hexadiche	0.43		
18	1 (2.4.6 This sthat hand) but	0.43		
22	1,3-diene	0.32		
35	2.6.11-Trimethyldodecane	0.13		
36	6,10-Dimethyl-2-undecane	0.18		
44	3-Hexadecene	8.34		
Ionones				
31	β-Ionone	0.77		
34	trans- β -lonon-5,6-epoxide	0.06		
Others				
s s	2-(1 1-Dimethylathyl)_3-math_	0.06		
U	vloxirane	0.00		
16	1 2-Dimethyl-1H-imidazole	0.30		
32	Benzeneacetonitrile	0.04		
	_ monous contrine	0.01		

Relative percent was determined by calculating the relative peak area of known components to the total volatiles on the gas chromatogram.

Identification of compounds was based on a standard mass spectra library.

an off-flavor in fats and oils, imparting a beany, greasy odor characteristic of the reversion flavor of soybean oil (Krishnamurthy *et al.*, 1967).

Hydrocarbons constituted 10.4% of the total volatiles in the leaves. 3-Hexadecene (8.34%) was the most abundant of its class. Limonene, which gives a sweet citrus flavor, was also found in the leaves.

Two ionones were identified in the box thorn leaves. These were β -ionone and *trans*- β -ionone, representing 0.77% and 0.66%, respectively. β -Ionone has been known to give a pleasant violet, floral flavor to black tea. Sannai *et al.* (1984) identified four volatile flavor compounds—3-hydroxy-7,8-dehydro- β -ionone, 3-hydroxy- β -ionone, and 3-hydroxy- β -ionol—in *Lycium chinense* leaves. However, these components were not identified in the present study.

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