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Determination of the effective components in the various parts of *Luffa cylindrica* (L.) Roemer and development to remove the flesh from its fruit : (I)

Determination of chemical components in the various parts of *Luffa cylindrica*(L.) Roemer by GC and GC/MS

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Abstract : The volatile components, normal hydrocarbons, organic acids, and nonvolatile fatty acids in the various parts of *Luffa cylindrica*(L.) Roemer(sponge-gourd) were analyzed by GC and GC/MS. More than 150 volatile components were separated and thirty seven components were identified and quantified. The contents of essential oil were 0,05%, 0.05%, and 0.08% on a dried weight base in leaves, stem, and seeds respectively. Fruit juice and sap contained 0.06% and 0.03% oil on the fresh weight base. Twenty six components of normal hydrocarbons in leaves, stem, seed, and unripe fruit were separated, identified, and quantified. The total concentrations of the hydrocarbons were 75.5 μg/g in leaf, 52.0 μg/g in stem, 46.6 μg/g in fruit juice, and 32.8 μg/g in seed fractions. The major hydrocarbons in leaves, stem, and fruit juice were nC_{25}, nC_{27}, nC_{29}, and nC_{31}, nC_{16}, nC_{17}, nC_{18}, and nC_{19} were abundant in seeds mainly. The concentration of malonic acid among the five organic acids was highest in leaves, stem, and flowers. Unripe fruit contained 24.5 mg/g of the five organic acids and malic and citric acids were higher. The concentrations of palmitic, linoleic, and linolenic acids were higher concentration in the various parts of sponge-gourd and palmitic acid was distributed in the most parts. The concentrations of organic and fatty acids in the sap were negligible(Received November 21, 1991, accepted December 23, 1991).

Sponge-gourd [*Luffa cylindrica*(L.) Roemer] is a useful plant used multipurposely by human beings[6]; young fruit as food,[2] leaves and stem extract[3,6] as a source of cosmetics and Chinese medicine, seeds[2,6,15,17] as a source of oil and Chinese medicine, and net fiber[2,6] as a raw material of medicine. Especially, the net fiber[4,32] is used as materials for dish washing, bath, oil and gas filter, and many kinds of decorations.

Sponge-gourd was experimented to find optimum managements of cultivation such as varity test, seeding and transplanting times, level of N source, plant space, and fruit number per plant.[13,19,21,23,28]

Also, it is a point of interest in determining the chemical components.[33] Recently, it was found that callus was formed from cotyledon protoplast[10] and elastic fiber-rubber[8] was made from sponge-gourd. It was reported by Kawashima *et al*[18] that the ext-

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raction of ginsenosides and lucosides from *Luffa cylindrica* was treated on skin disoders around the anus in patients with hemorrhoid. Fiber contents of sponge-gourd and their in vitro binding capacity of bile acids were studied by Chen *et al.*[9]

On the other hand, some sterols,[17] saponins,[29,30] fatty acids,[19] and sugars[12] in sponge-gourd were found by isolation and characterization. Ten flavonoid glycosides were isolated by Schilling and Heiser in 1981.[24] It was found in 1989 that a protein with a molecular weight of about 30,000 in *Luffa cylindrica* inhibited cell free translation.[25] Several reports have been published *Luffa cylindrica*(L.) Roemer used as medicinal herb[18,22,32] and health food.[16,34,35,36] It is important to use all the parts such as leaf, fruit, stem, extract, and sap of sponge-gourd. In this research, the volatile oil, hydrocarbons, organic and fatty acids in the various parts of sponggourd were determined to find the useful components related to flavor, cosmetics, and any medicines.

Materials and Methods

Materials

By the results of experiments of variety test, seeding and transplating times, level of fertilizers, plant space and fruit number per plant, Cheongwon variety[23,28] was cultivated in the field of Chungnam provincial, RDA located in Taejon. Seeding and transplating times of sponge-gourd were on April 1 and May 15.[20] The rate of fertilizers[21] was 25-20-25-10-1000 kg/10a as N-P_2O_5-K_2O-Ca-manure. Plant space was 150×90 cm and 4-6 fruits per plant were harvested.[14] The physico-chemical characteristics of the test field was shown in Table 1.

Young leaves and stem were collected, dried, and pulverized. Unripe fruit without fiber structure was extracted to get fruit juice. The fruit juice was cleared by vacuum filtration through a bed of celite 545. Sap was collected in the bottle from the cut stem after harvest.

Isolation of the various components

Volatile components[11,27]

Fifty grams of dry sample were blended with 1000ml distilled water in a Waring Blender at low speed for three minutes. The slurry was transferred to a 2000ml round-bottomed flask connected in simultaneous steam distillation-extraction(SDE) apparatus modified by Flath *et al*[11] and the distillate was collected in cold ethyl ether for eight hours. The collected fraction was dehydrated through anhydrous Na_2SO_4 and concentrated to ca 1 m*l* on a Vigreaux column by nitrogen blowing and injected into GC column.

Normal hydrocarbons[13,31]

Forty grams of pulverized sample were placed in the cylindrical filter paper and 100 m*l* n-hexane was added to weighed 250 m*l* round flask, which was connected to soxhlet extractor. Normal hydrocarbons in the sample were extracted in sand bath for 13 hours at 75℃. The components collected in n-hexane were dried over anhydrous Na_2SO_4, and concentrated to 1ml volume by using a Vigreaux column after evaporation of the solvent in vacuum evaporator.

Organic and nonvolatile fatty acids[7]

For methylation of the acids, 5g of dried sample was shaked with 75 m*l* of H_2SO_4: MeOH(1 : 6.5) mixture and 25 m*l* glutaric methyl alcohol as an internal standard(1.25 mg/m*l*). The exact volume of 50 m*l* from the methylated sample was placed to 250 m*l* separate funnel and extracted five times

Table 1. Physico-chemical characteristics of the test soil

Location	pH	OM	T-N	P_2O_5	Ex Cation(me/100g)			CEC
	(1 : 5)	(%)		(ppm)	K	Ca	Mg	me/100g
Taejon	6.8	1.0	0.2	100.1	0.3	1.8	1.2	9.0

with 100 ml H_2O and 10 ml CH_3Cl.

Gas chromatography and mass spectrometry(GC/MS)

Volatile components

GC analysis of the volatile components was carried out on a Hewlett-Packard(HP) 5880A GC equipped with a flame ionization detector(FID) and a 30 m×0.25 mm(ID) fused silica capillary column coated with SE-54. The oven temperature was programmed from 50℃ to 230℃ at a rate of 3℃/minute and held at 230℃ for 40 minutes. The injector and detector temperatures were 250℃. Nitrogen gas was used as a carrier gas at a flow rate of 1 ml/minute. 1ul of the sample was injected in the split mode with a split ratio of 1 : 100. GC/MS spectra were recorded by Varian MAT 212 system, SS MAT 188 data system equipped with the same column described above. The ion source was EI 70 eV and the ion source temperature was kept at 220℃. The column temperature was programmed from 50℃ to 250℃ at a rate of 2℃/minute and the injector and detector temperatures were 250℃. Nitrogen gas was used as a carrier gas at a flow rate of 1 ml/minute with a split ratio of 1 : 300, The components were quantified by area index of GC chromatogram.

Normal hydrocarbons

Normal hydrocarbons were analyzed with a HP 5880A GC equipped with FID. A fused silica capillary column, 30 m×0.25 mm ID(0.25 mm thickness) coated SPB-1, was used to separate the components. The column oven temperature was programmed from 50℃ to 270℃ at a rate of 3℃/minute. The injuctor and detector temperatures were kept at 270℃. Nitrogen was used as carrier gas at a flow rate of 1 ml/minute with a split ratio of 1 : 100. The components were identified and quantified with the known concentrations of 26 standard normal hydrocarbons by GC.

Organic and nonvolatile fatty acids

The extracted samples were dried over anhydrous Na_2SO_4, and the concentrated extract was analyzed by a HP 5890A GC equipped with FID and a 30 m×0.32 mm ID fused silica capillary column coated SP-2340. The oven temperature was programmed from 120℃ to 230℃ at 2℃/minute and then held at 230℃ for 40 minutes. The injector and detector temperatures were 230℃. The samples were injected in the split mode with a split ratio of 1 : 100. The carrier gas was nitrogen at a flow rate of 2 ml/minute. The organic and nonvolatile fatty acids were quantified compared with an internal standard, glutaric methyl alcohol.

Results and Discussion

Growth characteristics and quality components of sponge-gourd

Growth characteristics and quality components of sponge-gourd produced for the experiment were shown in Table 2.

Volatile components

Total contents of essential oil were 0.05%, 0.05%, and 0.08% in leaves, stems, and seeds of sponge-gourd on a dried weight base. Contents of essential oil in fruit juice and sap were 0.06%, and 0.03% on a fresh weight base.

More thon 150 volatile components were separated and thirty seven components including one alcohol, two aldehydes, one ketone, one ester, one phenol, three epoxids, five acids, eight aromatic hydrocarbons, two cyclic hydrocarbons, and thirteen

Table 2. Growth characteristics and quality components of sponge-gourd used for chemical analysis

Variety	Trans-planting (month/day)	Flowering stage (month/day)	Fruit diameter (cm)	Fruit length (cm)	Fruit weight (g)	Fiber weight (g)	Rate of marketing(%)			Yield
							>40cm	39-20cm	20cm<	per/10a
Cheongwon	4/12	6/28	8.1	33	451	27	17	57	26	5,500

aliphatic hydrocarbons, were identified and quantified by GC and GC/MS.

The compounds of No. 1, 2, 3, 4, 5, 8, 14, and 16 were distributed to various parts of sponge-gourd and the concentrations of No. 1, 2, 5, and 16 compounds were comparatively high. In addition,

Table 3. Concentration of volatile components in the various parts of sponge-gourd

No.	R. T. Min.	Compound name	Parts of sponge-gourd				
			Leaves	Stems	Seeds	Fruit juice	Sap
			(mg/100g)			(mg/1000ml)	
1.	4.13	Methyl benzene	2.44	2.46	2.76	2.18	0.07
2.	5.59	Tetrachloro ethene	6.74	6.25	3.24	5.42	0.34
3.	6.72	Ethyl benzene	1.40	-	0.28	0.03	-
4.	7.62	1,2-dimethyl benzene	0.32	0.30	0.62	0.39	-
5.	8.83	Bicyclo[4,2,0]octa-1,3,5-triene	1.88	2.21	4.90	1.81	-
6.	8.92	Dimethyl benzene	-	-	-	0.09	-
7.	9.70	Cyclohexanone	-	-	-	-	-
8.	10.68	1-methylethyl benzene	0.81	0.81	1.31	0.95	-
9.	13.61	2-pentyl furan	0.50	-	-	-	-
10.	15.85	3a,4,7,7a-tetrahydro-4,7 -methano-1H-idene	0.59	0.21	0.41	-	-
11.	*	2-(1-methylethyl) phenol					
12.	19.93	Methyl propyl benzene	0.57	-	-	-	-
13.	21.03	Benzenethanol	0.48	-	-	-	0.04
14.	25.77	Tetrahydro-4-methyl-1-prophenyl-2H-Pyran	1.61	0.76	-	3.45	0.37
15.	26.27	Azulene	-	-	-	-	-
16.	29.28	4,4-dimethyl-8-methylene,1-oxaspiro[2,5] octane	1.18	1.10	10.41	8.22	9.78
17.	31.72	5-methyl-2-(1-methy-ethyl)-2-cyclohexen-1-one	-	-	-	-	0.05
18.	*	Bicyclo[2,2,2]octane					
19.	36.79	Decanoic acid	1.65	-	-	-	0.32
20.	38.05	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-(E)-2-buten-1-one	0.11	-	-	-	0.27
21.	38.72	Tetradecane(nC_{14})	0.68	-	-	-	0.37
22.	41.81	Pentadecane(nC_{15})	0.57	0.71	-	-	0.22
23.	*	Lauric acid					
24.	45.42	Hexadecane(nC_{16})	0.43	-	-	-	-
25.	*	Heptadecane(nC_{17})	-	-	-	-	-
26.	51.99	Octadecane(nC_{18})	-	-	-	-	-
27.	*	Myristic acid					
28.	54.78	Nonadecane(nC_{19})	0.45	0.63	-	0.27	-
29.	*	Palmitic acid methy ester					
30.	*	Palmitic acid					
31.	*	Eicosane(nC_{20})	-	-	-	-	-
32.	61.70	Heneicosane(nC_{21})	0.50	0.33	-	-	-
33.	*	Linoleic acid					
34.	*	Docosane(nC_{22})	-	0.29	-	-	-
35.	68.01	Tricosane(nC_{23})	0.59	0.24	-	0.85	0.22
36.	70.44	Tetracosane(nC_{24})	-	0.14	0.14	-	-
37.	73.43	Pentacosane(nC_{25})	0.57	0.57	0.34	0.54	0.44

* : in GC/MS spectral data, - : trace

dimethylbenzene, 1,2-dimethyl-benzene, and methylbenzene are used for manufacture of medicine and flavor. 1-methyl-ethyl benzene is applicated to promotion of oxidation. Capric, lauric, myristic, palmitic, and linoleic acid have been the frequent use of raw material as cosmetic and flavor.

Table 4 showed twenty six of normal hydrocarbons identified from the various parts of sponge-gourd and quantified by GC analysis. Twenty three and twenty two components were distributed in the fractions of leaves and seeds, 21 in stems, and 17 in fruit juice. The total concentrations of the hydrocarbons were 75.5 μg/g in leaf, 52.0 μg/g in stem, 46.6 μg/g in fruit juice, and 32.8 μg/g in seed fractions. The major components among twenty six hydrocarbons in leaves, stem, and fruit juice were nC_{25}, nC_{27}, nC_{29}, and nC_{31}. nC_{16}, nC_{17}, nC_{18}, and nC_{19} were contained in seeds mainly and the considerable content of nC_{31} compound was distributed in leaf, stem, and fruit juice.

Table 4. Concentration of normal hydrocarbons in the various parts of spɔnge-gourd (unit : ug/g)

No.	R. T. Min.	Compound name	Parts of sponge-gourd Leaves	Stems	Fruit juice	Seeds
1.	15.87	Decane(nC_{10})	trace	trace	trace	0.81
2.	21.27	Undecane(nC_{11})	0.77	0.23	trace	2.45
3.	26.51	Dodecane(nC_{12})	0.16	trace	trace	0.24
4.	31.47	Tridecane(nC_{13})	trace	trace	trace	trace
5.	38.98	Tetradecane(nC_{14})	0.10	0.12	trace	0.34
6.	40.54	Pentadecane(nC_{15})	1.26	0.27	trace	1.10
7.	44.70	Hexadecane(nC_{16})	0.52	0.63	trace	2.78
8.	48.64	Heptadecane(nC_{17})	0.66	1.07	0.05	4.33
9.	52.39	Octadecane(nC_{18})	1.00	1.54	0.06	4.85
10.	55.94	Nonadecane(nC_{19})	1.04	1.73	1.44	3.73
11.	59.36	Eicosane(nC_{20})	1.23	2.10	0.26	2.75
12.	62.62	Heneicosane(nC_{21})	1.14	1.97	1.61	1.51
13.	65.76	Docosane(nC_{22})	0.82	1.79	0.26	0.64
14.	68.74	Tricosane(nC_{23})	1.44	1.67	1.11	0.75
15.	71.62	Tetracosane(nC_{24})	1.00	1.88	1.09	0.74
16.	74.41	Pentacosane(nC_{25})	2.80	1.85	9.29	1.06
17.	77.06	Hexacosane(nC_{26})	1.20	1.48	1.07	0.77
18.	79.74	Heptacosane(nC_{27})	6.25	2.68	9.66	0.95
19.	82.71	Octacosane(nC_{28})	2.76	1.47	0.75	0.54
20.	86.84	Nonacosane(nC_{29})	6.84	2.66	4.99	0.56
21.	90.66	Triacontane(nC_{30})	2.99	1.70	0.90	0.37
22.	95.87	Hentriacontane(nC_{31})	35.56	21.59	12.74	0.98
23.	102.62	Dotriacontane(nC_{32})	2.10	1.24	0.54	0.26
24.	110.54	Tritriacontane(nC_{33})	3.76	2.24	0.66	0.19
25.	120.70	Tetratriacontane(nC_{34})	tarce	trace	trace	trace
26.	133.45	Pentatriacontane(nC_{35})	trace	trace	trace	trace

Table 5. Concentration of organic acids in the various parts of sponge-gourd (unit : mg/g)

No.	R. T. Min.	Components	Leaves		Strems		Unripe fruit	Fruit juice	Sap	Seeds	Flowers
			Young	Old	Young	Old					
1.	3.9	Oxalic acid	1.70	2.39	2.73	2.23	2.20	0.70	0.043	trace	3.97
2.	4.7	Malonic acid	13.56	2.99	6.36	0.89	3.29	trace	−	trace	5.73
3	5.9	Succinic acid	0.59	0.11	0.48	0.22	1.29	6.80	−	0.29	5.54
4.	13.1	Malic acid	trace	0.86	1.16	0.78	10.19	trace	−	0.49	trace
5.	21.0	Citric acid	0.71	1.64	3.11	2.02	7.48	trace	−	0.73	trace

Table 6. Concentration of fatty acids in the various parts of sponge-gourd (unit : mg/g)

No.	R. T. Min.	Components	Leaves		Strms		Unripe fruit	Fruit juice	Sap	Seeds	Flowers
			Young	Old	Young	Old					
1.	8.9	Myristic acid	0.11	0.18	trace	0.10	trace	trace	–	0.18	0.16
2.	11.9	Myristic acid	5.72	2.47	4.62	3.38	9.60	3.32	0.06	26.61	20.06
3.	14.7	Stearic acid	1.21	0.35	0.70	0.63	1.48	trace	–	12.31	3.64
4.	15.4	Oleic acid	0.70	0.63	1.15	2.48	0.73	0.17	–	104.87	1.62
5.	16.5	Linoleic acid	1.59	0.53	3.59	1.81	3.38	2.68	–	83.59	10.90
6.	17.8	Linolenic acid	21.91	3.26	9.61	1.78	14.72	1.94	–	0.37	29.38

Organic and fatty acids

Five organic acids and six fatty acids were found in the various parts of sponge-gourd. Among them, citric, succinic, and malic acids are chemicals used as a raw material of cosmetic, medicine, and foods. Palmitic and stearic acid have the free use of cosmetic materials. Myristic and oleic acids make good use of medicine, cosmestic, and surfactant. The concentration of malonic acid was high in leaves, stems, and flowers, and higer in young than old leaves and stems. Unripe fruit contained 24.5 mg/g of the total concentration of the five organic acids and high concentration of malic and citric acids. The concentration of succinic acid was higher than any other organic acid in fruit juice. Palmitic acid was distributed in all parts of the plant. The concentrations of oleic, linoleic, and palmitic acids were hightest in seeds. On the average, the contents of palmitic, linoleic and linolenic acids were higher in seeds, and the contents of myristic acid was poor. The concentrations of organic and fatty acids in the sap were negligible.

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수세미외의 部位別 有效成分 調査 및 絲瓜絡中 肉質除去 方法 開發 硏究 : (I)
수세미외의 부위별 화학성분 분석

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초록 : 관행법에 의해 재배된 수세미외의 잎, 줄기, 미숙과즙, 종실, 수액 등 각 부위에 함유된 휘발성 정유성분, 탄화수소, 유기산 및 비휘발성 지방산을 추출, 분리하였다. 150여개의 분리성분중 37개 정유 성분을 확인한 후 각 부위별로 정량하였다. 또한, 26종의 탄화수소, 5종의 유기산 및 6종의 지방산도 분리정량하여 부위별 분포와 함량을 비교하였다.