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Extraction, Analysis, and Study on the Volatiles in Roselle Tea

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The Likens−Nickerson steam distillation procedure was utilized to mimic the preparation of roselle tea. Thermally generated volatiles from roselle were collected and analyzed by GC and GC−MS. There were four differently treated samples: untreated, frozen, hot-air-dried at 50 °C, and hot-air-dried at 75 °C. More than 37 compounds were characterized. They were classified into four groups: fatty acid derivatives, sugar derivatives, phenolic derivatives, and terpenes. Large amounts of the aliphatic C_6 lipid derivative, which contributes to the green note aromas, were in the fresh roselle, while only trace amounts were found in the frozen and air-dried samples. In the air-dried roselles, significant amounts of furfural and 5-methyl-2-furfural were formed, while only minimal amounts were detected in the fresh samples. There were no obvious changes in phenolic derivatives (eugenol) among the four samples. Terpenoid and oxide could also be isolated after distillation extraction. The drying process reduced them dramatically, especially the amount of α-terpineol, linalool oxide, and limonene. Principal component analysis (PCA) confirmed that characteristic roselle tea aromas depend upon a subtle quantitative balance of various components. A combination of the terpene derivatives with fragrance notes and the sugar derivatives with a caramel-like odor are responsible for the roselle aroma.

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) an annual shrub, produces fleshy, red, edible calyces with a unique taste and flavor (Al-Kahtani et al., 1990). The calyces, which contain large amounts of pectin, anthocyanin, ascorbic acid, and malate (Tsai, 1995), can be used for making jellies, jams, preserves, sauces, and beverages.

Roselle extract, having a brilliant red color is a good source of natural food colorants (Esselen et al., 1975). Because the color in roselle is unstable, there have been many studies investigating the pigment change (Pouget et al., 1990a,b; Tsai, 1995). In addition, roselle's unique flavor is very delicate, and trace amounts are too difficult to detect. There are few reports referring to the aroma of roselle. In the studies of Jirovetz et al. (1992), the volatiles in the seed oil of roselle had been identified by GC−MS and GC−FTIR. More then 25 volatiles, mainly unsaturated hydrocarbons, alcohols, and aldehydes predominantly from C_8 to C_{13}, were found in the seed oil of roselle. However, the volatiles of roselle calyces still remain unknown.

The purpose of this study is to identify the volatiles of cooked roselle calyces by the use of GC−MS, and to understand the effect of drying and freezing on them.

MATERIALS AND METHODS

Roselle Samples. Roselle (*H. sabdariffa* L.), bought from Taitung, Taiwan, were collected during November 15 to December 30, 1995. The samples were divided into four parts: fresh, frozen, hot-air-dried at 50 °C, and hot-air-dried at 75 °C. Frozen samples were kept in a freezer at −20 °C, while the dried samples were dried in an oven at 50 or at 75 °C for 36 h, respectively (Tsai, 1995).

Isolation of Volatile Flavor Compounds. Distillation and extraction of roselle volatiles were carried out simultaneously in a Likens−Nickerson apparatus (Nickerson and Likens, 1966). In a sample flask, roselle samples (500 g of undried sample and 50 g of the dried sample) were mixed with 700 mL of distilled water. The solvent flask was filled with 50 mL of diethyl ether. The two solutions were heated under reflux for 3 h. Tridecane (Sigma Chemical Co., St. Louis, MO) was added to the diethyl ether as an internal standard. After dehydration with anhydrous sodium sulfate, the diethyl ether layers were concentrated by condenser to about 100 μL.

Capillary Gas Chromatography (GC). Capillary GC analysis was carried out on a Hewlett-Packard Model 5890A gas chromatograph equipped with a flame ionization detector (FID) and connected to a Hitachi D-2500 Chromato-Integrator. Separation was achieved on a 60 m × 0.32 mm i.d. fused silica capillary column, coated with cross-linked poly(dimethylsiloxane) with a film thickness of 0.25 μm (SPB-1; Supleco, Bellefonte, PA). The oven temperature was programmed from 40 to 200°C at 4 °C/min and held for 20 min. The injector and detector temperatures were 220 and 230 °C, respectively. The nitrogen carrier gas flow rate was 20 m/s with an injection splitter at a slit ratio of 30:1. Retention indices were estimated in accordance with modified Kovats method (Majlát et al., 1974).

Capillary Gas Chromatography−Mass Spectrometry (GC−MS). Elector impact mass spectrometric data were collected on a Shimadzu GCMS-QP2000 gas chromatograph-mass spectrometer interfaced to Shimadzu BC-14A gas chromatograph. The helium carrier gas flow rate was 20 cm/s with an injection splitter at a slit ratio of 10:1. The column and chromatographic conditions were the same as described for GC analysis. The mass spectrometer was operated at an ionization voltage of 70 eV and ion source temperature of 250 with a scanning rate of 1 scan/s.

Fatty Acid Analysis. The fatty acid of the roselle was extracted using the Folch method (3 h). Methyl esterification

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Table 1. Identification of Volatile Compounds of Roselle (*Hibiscus sabdariffa* L.)

peak no.	volatile components	RI[a]	MW	ID[b]	mass data (*m*/*z*)			
1	2,3-dimethylbutane		86	MS	43 (100)	42 (69)	41 (27)	71 (10)
2	acetic acid		60	MS	43 (100)	45 (87)	60 (57)	42 (14)
3	2-ethylfuran	682	96	MS, GC	81 (100)	96 (40)	53 (28)	39 (16)
4	2,2-dimethylhexanal	758	128	MS	57 (100)	43 (16)	72 (10)	41 (9)
5	hexanal	773	100	MS, GC	44 (100)	56 (60)	41 (57)	43 (42)
6	furfural	799	96	MS, GC	96 (100)	95 (82)	39 (61)	38 (17)
7	(*E*)-2-hexenal	824	98	MS, GC	41 (100)	55 (62)	42 (59)	69 (53)
8	(*Z*)-3-hexenol	836	100	MS, GC	41 (100)	67 (57)	55 (29)	72 (25)
9	2-hexenol	847	100	MS, GC	57 (100)	41 (46)	43 (26)	56 (26)
10	1-hexanol	857	100	MS, GC	56 (100)	38 (63)	37 (47)	55 (47)
11	heptanal	873	114	MS, GC	44 (100)	43 (74)	70 (51)	41 (50)
12	(*E*)-2-heptenal	923	112	MS, GC	41 (100)	57 (65)	55 (65)	83 (64)
13	5-methyl-2-furaldehyde	926	110	MS, GC	110 (100)	109 (76)	53 (52)	54 (34)
14	2-ethenyltetrahydro-2,6,6-trimethyl-2*H*-pyran	960	154	MS	43 (100)	71 (79)	68 (73)	69 (55)
15	2-pentylfuran	973	138	MS, GC	81 (100)	82 (25)	53 (17)	138 (15)
16	octanal	976	128	MS, GC	67 (100)	43 (98)	55 (83)	68 (76)
17	1,4-cineole	998	154	MS, GC	43 (100)	71 (58)	111 (47)	55 (42)
18	1,8-cineole	1012	154	MS, GC	43 (100)	81 (35)	71 (31)	84 (27)
19	limonene	1015	136	MS, GC	68 (100)	67 (72)	93 (55)	41 (23)
20	linalool oxide (a)[c]	1054	170	MS, GC	59 (100)	43 (58)	55 (38)	94 (34)
21	tetrahydro-2,2-dimethyl-5-(1-methylpropyl)furan	1065	156	MS	81 (100)	43 (94)	99 (77)	71 (44)
22	linalool oxide (b)	1069	170	MS, GC	59 (100)	43 (56)	94 (35)	55 (35)
23	nonanal	1075	142	MS	43 (100)	41 (90)	57 (86)	56 (51)
24	linalool	1079	154	MS, GC	71 (100)	43 (79)	41 (75)	72 (75)
25	2-methyl-6-methylene-7-octen-2-ol	1093	154	MS	59 (100)	43 (50)	68 (40)	79 (39)
26	1-methyl-4-(1-methylethyl)-3-cyclohexenol	1112	154	MS	81 (100)	43 (70)	82 (44)	58 (37)
27	2,6-dimethyl-5,7-octadien-2-ol	1139	154	MS	93 (100)	59 (73)	81 (67)	43 (45)
28	α,4-dimethyl-3-cyclohexyl-1-acetaldehyde	1152	152	MS	94 (100)	79 (30)	68 (25)	67 (16)
29	methyl salicylate	1160	152	MS	120 (100)	152 (49)	92 (47)	121 (35)
30	α-terpineol	1167	154	MS, GC	59 (100)	43 (44)	93 (42)	121 (28)
31	α-terpinyl acetate	1172	196	MS	121 (100)	93 (82)	43 (75)	136 (53)
32	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol acetate	1222	196	MS	93 (100)	121 (67)	43 (64)	108 (57)
33	eugenol	1324	164	MS, GC	164 (100)	77 (31)	55 (30)	149 (28)
34	caryophyllene	1406	204	MS, GC	41 (100)	69 (77)	93 (64)	91 (56)
35	α-farnesene	1486	204	MS, GC	41 (100)	93 (98)	55 (79)	69 (81)
36	*exo*-2-hydroxycineole	1586	170	MS	43 (100)	108 (71)	111 (65)	71 (48)
37	6,10,14-trimethyl-2-pentadecanone	1822	268	MS, GC	43 (100)	58 (85)	59 (48)	71 (35)

[a] Calculated Kovat's indices[83] on an SPB-1 column. [b] MS: Identification by comparing the mass spectra. GC: Identification by comparing the retention indices (RI). [c] Linalool oxide(a) and (b) are cis- and trans- linalool oxide of furanoid type.

of the fatty acid was completed using a BF_3−methanol reagent (Metcalfe et al., 1961) before GC analysis (by a Shimadzu GC-7A gas chromatograph). Separation was achieved on a 30 m × 0.25 mm i.d. fused silica capillary column coated with cross-linked poly(80% bis(cyanopropyl)−20% [(cyanopropyl)phenyl]-siloxane), with a film thickness of 0.20 μm (SP-2330; Supleco, Bellefonte, PA). The oven temperature was programmed from 100°C to 230°C at 4°C/min and held for 16 min, with an injector temperature of 230°C. The nitrogen carrier gas flow rate was 20 cm/s with an injection splitter at a slit ratio of 30:1. Esters of palmitic acid, stearic acid, oleic acid, linoleic acid, and lineolenic acid with Sigma grade were used as lipid standards.

Statistical Analysis. Principal component analysis (PCA) was conducted using the SAS statistical software (SAS, 1988). The percentage peak areas of the GC peaks identified by GC−MS were used as variables.

RESULTS AND DISCUSSION

The Likens−Nickerson distillation apparatus was set up to mimic the process of roselle tea preparation. To characterize the aromas of roselle tea, samples were prepared from fresh, frozen, and dried (50 and 75 °C) calyces for gas chromatographic analysis. More than 37 volatile compounds were identified by GC and GC−MS (Table 1). The MS spectra of these analyses were compared with library spectra, and the retention indices (GC−FID) of the compounds were used to aid identification. The compounds were separated and classified according to their source: (1) fatty acid derivatives; (2) sugar derivatives; (3) phenolic derivatives; (4) terpene with its thermal degradation derivatives; (5) miscellaneous (Table 2).

In group I, the volatiles hexanol (GC peak 5), (*E*)-2-hexenal (GC peak 7), (*Z*)-3-hexenol (GC peak 8), 2-hexenol (GC peak 9), and hexanol (GC peak 10), which contribute to the green note aromas (Olías et al., 1993), belong to the aliphatic C_6 aldehydes and alcohols. This may be the result of lipoxygenase action on unsaturated fatty acids (Schlozhauer et al., 1996; Luning et al., 1995), especially during the disruption of cell structure in the presence of oxygen (Olías et al., 1993). For example, (*Z*)-3-hexenol (32.87 mg/kg) and 2-hexenol (27.72 mg/kg) were the major volatile constituents in the fresh roselle. According to the research of Luning et al. (1995), these two compounds might be formed from linolenic acid by biogenetic pathways. Further analysis of the fatty acid showed that linolenic acid reached about 0.1022 mg/g (dry wt) in the fresh calyces. Other fatty acids including palmitic acid (0.1712 mg/kg), stearic acid (0.0217 mg/kg), oleic acid (0.0409 mg/kg), and linoleic acid (0.1837 mg/kg) were found in the fresh calyces as well. It is therefore possible that the green note of the fresh roselle might be induced by enzymatic reaction. In the sample prepared from fresh roselle, the high concentration of (*Z*)-3-hexenol (grassy, green odor) and 2-hexenol (fruity, sweet fresh note) (Hatanaka, 1993) obviously preponderate the other aromas of roselles, while in the frozen and oven-dried samples, they declined considerably. This revealed that freezing

Table 2. Classification of Volatile Constituents in Roselle Clayx by Source

volatile compound	fresh[a]	frozen	oven dried 50 °C	oven dried 75 °C
Fatty Acid Derivatives[b]				
2-ethylfuran	0.776	0.179		
hexanal	4.287	1.291	1.337	0.353
(*E*)-2-hexenal	6.465	3.265	0.534	
(*Z*)-3-hexenol	32.870	1.899	1.192	0.898
2-hexenol	27.721	6.650		
1-hexanol	21.936			
heptanal	0.295	0.278	0.791	
(*E*)-2-heptenal	0.246	0.359		
2-pentylfuran	1.308	1.391	1.347	
octanal				0.349
nonanal	3.887	5.548	3.335	0.617
Sugar Derivatives				
furfural	0.292	0.641	23.006	43.606
5-methyl-2-furaldehyde			0.931	1.915
Phenolic Derivatives				
eugenol	9.128	9.089	9.052	9.014
Terpene Components				
2-ethenyltetrahydro-2,6,6-trimethyl-2*H*-pyran	2.577	2.728	1.258	
1,4-cineole	0.626	0.552		
1,8-cineole	1.546	2.584		
limonene	2.288	2.229		
linalool oxide (a)[c]	6.380	7.110	4.797	1.436
tetrahydro-2,2-dimethyl-5-(1-methylpropyl)furan	0.522	0.596	0.653	0.213
linalool oxide (b)	3.039	3.581	2.086	0.759
linalool	1.007	1.498	0.384	0.349
2-methyl-6-methylene-7-octen-2-ol	1.750	1.612	0.525	0.390
1-methyl-4-(1-methylethyl)-3-cyclohexenol	3.423	4.101	0.833	0.383
2,6-dimethyl-5,7-octadien-2-ol	3.125	4.009	0.600	0.131
α,4-dimethyl-3-cyclohexene-1-acetaldehyde	3.661	2.800	1.271	0.495
α-terpineol	12.322	18.014	2.002	1.450
α-terpinyl acetate	3.369	3.560	1.074	0.684
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol acetate	3.267	3.122	0.633	0.311
caryophyllene	4.510	2.329	0.213	0.517
α-farnesene	4.075	3.313	1.315	1.906
exo-2-hydroxycineole	2.758	2.427	1.366	1.596
Miscellaneous				
2,3-dimethylbutane	0.246	1.003	2.633	0.499
acetic acid	1.095	2.511	12.670	6.512
2,2-dimethylhexanal	1.198	0.516		
methyl salicylate	0.744		0.447	0.968
6,10,14-trimethyl-2-pentadecanone	2.073	1.052	0.955	0.867

[a] Concentration by GC-FID (mg/kg dry wt). [b] The class of volatiles by source of reaction. [c] Linalool oxide (a) and (b) are *cis*- and *trans*-linalool oxide of the furanoid type.

and hot-air-drying seemed to inactivate the lipoxygenase activity (Belitz et al., 1986).

Another possible way that the group I volatiles may be formed is by thermal decomposition of the fatty acid. As reported, 2-pentylfuran, 2-ethylfuran, and octanal may be produced from the thermal decomposition of hydroperoxides or cyclic peroxides of linoleate (Min et al., 1989; Belitz et al., 1986). Although only trace amounts of these compounds were detected in the dried sample, thermal degradation of the fatty acid in the dried roselle is possible. In this study, both enzymatic and thermal degradation reactions may occur during the distillation process.

As for group II aromas, a significant variation was observed between the dried and undried samples. A large amount of furfural (23.01 and 43.61 mg/kg for 50 and 75 °C hot-air-dried samples, respectively) was found in the dried roselles. That was also noticed by Tsai (1995) since only a minute amount of furfural was generated from either the fresh or frozen roselle calyces. It is proposed that furfural was formed mainly during the drying process. Thermal processing through air-drying will produce a caramel-like aroma. It is well-known that furfural and 5-methyl-2-furfural could be formed from sugar degradation (Kroh, 1994) and their formation could be accelerated by decreasing the moisture content at a water activity of 0.3−0.7 (Eichner et al., 1972). In this study, after drying at 50 and 75 °C for 36 h, the water activity of the roselle became 0.64 and 0.55, respectively.

For group III, eugenol (GC peak 27), one of the phenolic derivatives, was found in all four of the samples at similar levels. Eugenol, besides being one of the major components in roselle, is also an important component of cloves (Fisher et al., 1992), *Umbelliferous* Vegetable (Roshdy et al., 1992), and *Piper betle* (Hwang et al., 1992). Eugenol originates from phenylalanine by enzymatic synthesis in plants (Belitz et al., 1986). Eugenol is thermally stable during the drying treatment.

In group IV, the terpene components, including limonene (GC peak 19), linalool (GC peak 24), α-terpineol (GC peak 30), 1,4-cineole (GC peak 17), 1,8-cineole (GC peak 18, *cis*- and *trans*-linalool oxide (GC peak 20, 22), 2-vinyltetrahydro-2,6,6-trimethyl-2*H*-pyran (GC peak 14), α-terpinylacetate (GC peak 31), caryophyllene (GC

Table 3. Eigenvalues and Cumulative Percentage of Total Variance of Principal Component of Roselle

factor	eigenvalue	variance (%)	CPTV (%)[a]
1	13.27	35.88	35.88
2	4.17	11.28	47.16
3	3.18	8.58	55.74
4	2.24	6.04	61.79
5	1.94	5.25	67.04
6	1.82	4.92	71.96
7	1.52	4.12	76.08
8	1.24	3.34	79.42
9	1.10	2.97	82.39

[a] CPTV: cumulative percent of total variance.

peak 34), and α-faranosene (GC peak 35). α-terpineol, limonene, and linalool oxide are three of the dominant aromas in roselle calyces. Significant amounts of limonene, 2.288 mg/kg in fresh roselle and 2.229 mg/kg in the frozen roselle, were found. However, no detectable amount of limonene could be isolated in the air-dried roselle. Linalool oxide showed a similar tendency (6.38 mg/kg in fresh roselle and 7.11 mg/kg in the frozen roselle), although trace amounts, 4.797 mg/kg and 1.436 mg/kg in the 50 °C air-dried and 75 °C air-dried roselle, respectively, were found in this experiment. The drying process reduced the amount of α-terpineol dramatically, as shown in Table 2 (12.322, 18.014, 2.002, and 1.450 mg/kg at fresh, frozen, 50 °C air-dried, and 75 °C air-dried roselle, respectively).

As reported, two types of oxide isomers can be derived from linalool (cis and trans), each with the furanoid type and pyranoid type under the catalysis of an enzyme (Bock et al., 1986), while only the furanoid type can be produced under acid and heat conditions (Williams et al., 1980). In this research, only the furanoid type of the cis and trans isomer of linalool oxides was found (peaks 20 and 22) in all four samples. This result revealed that linalool oxides came from acid and heat degradation in roselle rather than from biogenetic pathways. Subsequent ring-opening and dehydration lead to the further formation of 2-methyl-6-methylene-7-octen-2-ol and 2,6-dimethyl-5,7-octadien-2-ol (peaks 25 and 27) (Engel et al., 1983; Williams et al., 1980). As compared with the frozen sample, the peaks of these terpene oxides mentioned above (peaks 20, 22, 25, and 27) were decreased with an increased temperature in the hot-air-dried samples. For example, the peak of linalool oxide (a) was 7.11 mg/kg in the frozen sample and 4.79 and 1.43 mg/kg in the hot-air-dried samples, while the 2,6-dimethyl-5,7-octadien-2-ol peaks were 4.009, 0.6, and 0.13 mg/kg, respectively. This reveals that oven drying results in the loss of some terpenoid denatures.

Principal component analysis (PCA) provides a better understanding of the variation among volatile compositions of samples and has been widely used to evaluate food flavors (Kawakami et al., 1990). Each principal component of the axis is a linear combination of the original variable. In this study, eigenvalues and their cumulative proportions are shown in Table 3. The first nine principal components had eigenvalues greater than 1.0 and accounted for about 82.39% of the total variance. Figure 1 shows a plot of the values for the first two principal components, which together account for 47.16% (35.88% + 11.28%) of the variation in the data.

Along the positive direction of the factor 1 axis, peaks 19, 25, 27, 28, 30, and 32 were terpene derivatives, while in the negative direction, peaks 6 and 13 were related

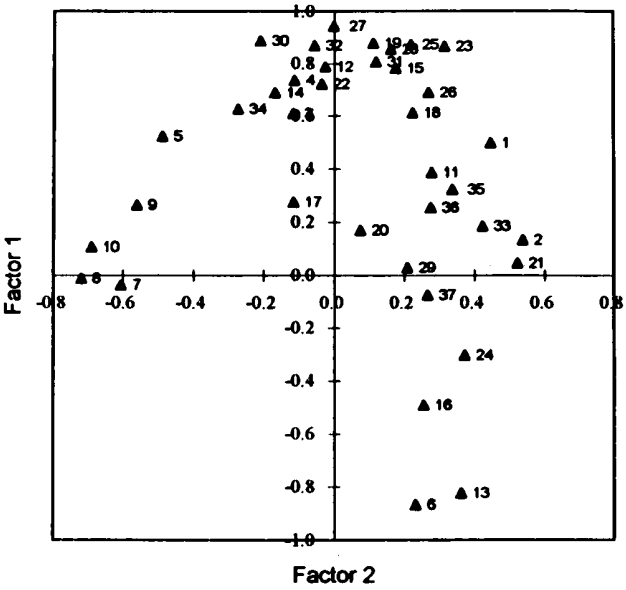

Figure 1. Principal component analysis of roselle volatiles.

to the sugar derivatives. As to the factor 2 axis, in the negative direction, peaks 5, 7, 8, 9, and 10 were aliphatic C_6 lipid derivatives and contributed to the green notes aroma.

Over 37 compounds were characterized in the volatile extracts of the cooked roselle, but none with the unique odor typical of roselle tea has been evidenced. It seems likely that the characteristic roselle tea aromas depend upon a subtle quantitative balance of various components. A combination of the terpene derivatives with fragrance notes and the sugar derivatives with a caramel-like odor are responsible for the roselle aroma. On the other hand, the evolution of aliphatic C_6 lipid derivatives may mask the overall aroma of roselle tea. More efforts will be aimed at the volatiles isolated by using the purge and trap method, which is reported to be the method that best resembles what is involved when we use our sense of smell.

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