Analysis of Glycosidically Bound Aroma Precursors in Tea Leaves. 3. Change in the Glycoside Content of Tea Leaves during the Oolong Tea Manufacturing Process

Dongmei Wang,[†,§] Kikue Kubota,*[,†] Akio Kobayashi,[†] and I-Ming Juan[‡]

Laboratory of Food Chemistry, Department of Nutrition and Food Science, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan, and Taiwan Tea Experiment Station, Taoyuan-ken, Taiwan, Republic of China

A direct qualitative and quantitative determination of the glycosides of tea aroma compounds at the four stages of the oolong tea manufacturing process (plucking, solar withering, indoor withering, and oolong tea product) was carried out by a capillary gas chromatographic−mass spectrometric analysis after trifluoroacetyl derivatization of the glycosidic fractions. Sixteen glucosides and primeverosides were identified and quantified in cv. Chin-shin-oolong and cv. Chinhsuan-oolong. A comparison of the glycosides in dried fresh leaves between the two cultivars showed significant differences. During the manufacturing process, the amounts of most of these glycosides increased from the solar-withering stage, reaching the highest level at the final stage of oolong tea production. It was noted that no glycoside decreased in its content during the manufacturing process, this being quite different from the manufacture of black tea. In addition, the contents of these alcoholic aroma compounds in the free aroma concentrate from each cultivar remained almost unchanged or slightly decreased, and they constituted only about 12 and 17% in amount of the whole oolong tea aroma compounds. However, jasmine lactone and indole were markedly higher in the final oolong tea products.

Keywords: *Oolong tea; aroma formation; glycoside quantification; primeveroside; glucoside*

INTRODUCTION

Oolong tea, a semifermented type of tea, is produced mainly in Fujian, Guangdong, and Taiwan provinces of China. As with green tea and black tea, the aroma is one of the most important factors in determining the quality of oolong tea. The particular manufacturing process used gives oolong tea a unique floral, fruity, and jasmine-like aroma. Although various processing methods are known for different kinds of oolong tea, they generally involve tea leaf plucking, solar withering, indoor withering, parching, rolling and drying (*1*). For solar withering, the freshly picked leaves are exposed to sunlight to lose 10−20% of their weight and grassy odor and give off a light fragrance. Then the leaves are moved indoors for withering while being turned over at adequate intervals. The turn-over treatment is a special operation unique to the processing of oolong tea. It causes the friction between leaves, disrupts the cellular organization at the edge of the leaves, and brings about a limited degree of fermentation. During the solar withering and indoor withering stages, enzymatic reactions are activated and directly affect the flavor of oolong tea. Several studies have reported the effect of the general withering process on the formation of aroma components in pouchong tea, a lightly fermented type of oolong tea (*2*, *3*).

Among the various aroma components of oolong tea, the alcoholic compounds, which impart the floral fruity aroma, are known to be important aroma components of both oolong tea and black tea. They are known to be present as glycosides in fresh leaves and to be released by endogenous glycosidases during the manufacturing process. Glycosides such as β-D-glucoside (Glc), β-primeveroside (Prim), β-vicianoside (Vic), and β-acuminoside of these alcoholic aglycon aroma compounds have been identified from fresh tea leaves (*4*−*11*). Ogawa et al. (*12*) and Ijima et al. (*13*) have previously purified primeverosidase from fresh tea leaves and suggested this to be strongly involved in the formation of the aroma of oolong tea and black tea. We have recently synthesized several naturally occurring mono- and disaccharide glycosides that are present in tea leaves and have established a method to analyze these glycosides directly in tea leaves by GC-MS via trifluoroacetylation (*14*, *15*) according to the method of Voirin et al. (*16*). We have also reported the analysis of these glycosides during the black tea manufacturing process and showed that all were substantially hydrolyzed during black tea manufacture and contributed greatly to the formation of the black tea aroma (*17*). In this study, we investigated the quantitative changes in these glycosides during the oolong tea manufacturing process and attempted to clarify the role of these glycosides in the formation of the oolong tea aroma by comparing the changes in contents of the free aroma compounds.

* Author to whom correspondence should be addressed (telephone/fax +81-3-5978-5759; e-mail kubota@cc.ocha.ac.jp).
[†] Ochanomizu University.
[§] Present address: Department of Pharmacy, School of Life Science, Zhongshan University, 135 Xingang Road Western, Guangzhou, Guangdong Province 510275, China.
[‡] Taiwan Tea Experiment Station.

10.1021/jf010235+ CCC: $20.00
Published on Web 10/02/2001

Table 1. Change in the Glycoside Contents of Tea Leaves during the Manufacturing Process for Chin-shin-oolong

glycoside[a]	RRT[e] synthetic[f]	RRT[e] natural	content (mg/100 g of dried leaves) dried fresh leaves	solar-withered	indoor-withered	oolong tea
(*Z*)-3-hexenyl-Glc	0.896	0.894	1.5 ± 0.2	3.1 ± 0.4	5.5 ± 0.3	5.5 ± 0.5
benzyl-Glc	1.126	1.130	11.3 ± 0.8	13.5 ± 0.9	12.8 ± 0.6	12.1 ± 1.3
2-phenylethyl-Glc	1.272	1.279	1.2 ± 0.3	1.8 ± 0.2	2.4 ± 0.2	2.0 ± 0.1
methyl salicylate-Glc	1.455	1.457	2.1 ± 0.3	2.4 ± 0.3	4.8 ± 0.4	5.4 ± 1.8
(3*S*,6*S*)-LO I-Glc[b]	1.055	1.053	0.9 ± 0.0	0.9 ± 0.1	1.8 ± 0.1	1.6 ± 0.3
(3*S*,6*R*)-LO II-Glc[b]	1.074	1.073	1.4 ± 0.2	1.8 ± 0.2	2.3 ± 0.2	1.6 ± 0.0
(3*S*)-LO III and IV-Glc[b]	1.188	1.187	0.6 ± 0.2	0.9 ± 0.2	1.6 ± 0.2	1.4 ± 0.1
geranyl-Glc[b]	1.227	1.220	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
total glucosides			19.8 ± 2.0	25.3 ± 2.4	32.1 ± 2.2	30.6 ± 4.2
(*Z*)-3-hexenyl-Prim	1.685	1.684	0.9 ± 0.1	1.2 ± 0.2	4.8 ± 0.4	4.5 ± 0.1
benzyl-Prim	1.877	1.881	10.7 ± 0.8	11.8 ± 0.9	13.4 ± 0.8	12.3 ± 0.5
2-phenylethyl-Prim	1.978	1.984	11.9 ± 1.5	12.7 ± 1.6	11.9 ± 0.7	15.5 ± 0.3
methyl salicylate-Prim[c]		2.008	9.3 ± 0.9	10.6 ± 1.1	13.9 ± 0.6	17.0 ± 0.3
LO I and (3*R*,6*S*)-LO II-Prim[b,d]	1.650	1.658	2.4 ± 0.1	2.5 ± 0.1	2.2 ± 0.0	2.5 ± 0.1
(3*S*,6*R*)-LO II-Prim[b]	1.665	1.673	5.6 ± 0.5	6.4 ± 0.9	5.6 ± 1.8	5.2 ± 0.6
geranyl-Prim	2.039	2.045	12.0 ± 2.3	14.7 ± 1.9	9.7 ± 1.0	16.4 ± 0.6
geranyl-Vic	2.061	2.064	1.9 ± 0.2	2.1 ± 0.2	1.8 ± 0.1	2.0 ± 0.1
total disaccharide glycosides			54.6 ± 6.4	62.0 ± 6.9	63.2 ± 5.3	75.5 ± 2.6
total glycosides			74.4 ± 8.4	87.4 ± 9.2	95.3 ± 7.5	106.1 ± 6.7

[a] Positive identification except for methyl salicylate-Prim (tentative identification). [b] Quantification based on the GC-MS analysis in an HP-50+ column or otherwise in a DB-5 column. [c] Calibration curve for methyl salicylate-Glc used for quantification. [d] Overlapped with (*S*)-linalyl-Prim. [e] RRT, relative retention time ratio to phenyl β-D-glucoside. [f] Data quoted from Wang et al. (*15*).

MATERIALS AND METHODS

Reagents and Reference Samples. Analytical reagent grade solvents were used. All of the glycosides and other chemicals were purchased or synthesized in our laboratory as previously described in Part 1 of this study (*15*).

Materials and the Manufacturing Process for Oolong Tea. Fresh leaves of *Camellia sinensis* var. *sinensis* cv. Chin-Shin-Oolong and cv. Chinhsuan-Oolong (TTES No. 12), two dominant cultivars of oolong tea in Taiwan, were plucked at the Taiwan Tea Experiment Station on April 28 and May 4, 1998, respectively.

Oolong tea was manufactured from these fresh leaves at this same station. After plucking, the fresh leaves were spread in bamboo trays outside to expose them to sunlight for 20 min for solar withering. Then the leaves was moved indoors and withered at a temperature of 25−27 °C and at a relative humidity of 75−85% for 4 h, accompanied by two turn-over treatments. The obtained indoor-withered leaves were parched at 230 °C for 4−5 min, followed by rolling for 5 min. Then the leaves are packed in a piece of cloth to be mass-rolled about 10 times to impart a curl in the leaves. After drying at 93−98 °C for ∼1.5 h, the oolong tea products were obtained. Samples of fresh leaves, solar-withered leaves, and indoor-withered leaves were collected after each manufacturing stage and then immediately dried by heating at 93−98 °C. The three dried samples obtained and the final oolong tea product from each cultivar were subsequently subjected to analysis of the glycosides. The dried fresh leaves and oolong tea product were also analyzed for their free aroma constituents.

Preparation of Glycosidic Fractions. The glycosidic fractions were prepared on XAD-2 resin from four samples of each cultivar according to the method previously described in Part 1 (*15*), using phenyl β-D-glucopyranoside as an internal standard. The experiments were run in triplicates on each sample.

Trifluoroacetyl (TFA) Derivatization of Glycosidic Fractions. The method used was the same as that described in Part 2 using *N*-methylbis(trifluoroacetamide) as the trifluoroacetylated reagent (*17*).

Preparation of Free Aroma Concentrates. The free aroma concentrates were prepared from dried fresh leaves and oolong tea product of each cultivar by using the brewed extraction method reported by Kawakami et al. (*18*). To 50 g of a powdered sample was added 1 mL of an ethyl decanoate/diethyl ether solution (1.23 mg/mL) as the internal standard (IS) for quantification of the free aroma components. After brewing in 350 mL of boiling water for 10 min, the hot water extract was filtered through a nylon mesh. The filtrate was cooled to room temperature, saturated with sodium chloride, and then extracted with 200 mL of a mixture of pentane/diethyl ether (1:1). The resulting extract was dried with anhydrous sodium sulfate for 12 h and subsequently concentrated at 39.5 °C to yield the free aroma concentrate of each sample for GC and GC-MS analyses. The experiments were run in triplicates on each sample.

GC and GC-MS Analyses. Hewlett-Packard 5890 series II gas chromatographs equipped with an FID or with a Hewlett-Packard 5972 series mass selective detector were respectively used for GC and GC-MS analyses.

(a) Analyses of TFA Derivatives of the Glycosides. To satisfactorily separate each glycoside studied, we employed two types of columns with different polar stationary phases, a DB-5 [(5% phenyl)-methylpolysiloxane, J&W Scientific, Folsom, CA] and an HP-50+ [(50% phenyl)-methylpolysiloxane, Hewlett-Packard], under the experimental conditions previously described in detail in Part 1 of this study (*15*).

(b) Analyses of Aroma Concentrates. A DB-Wax column (polyethylene glycol, J&W Scientific) was used to perform the GC and GC-MS analyses on the aroma concentrates, under the experimental conditions previously described in detail in Part 2 of this study (*17*).

RESULTS AND DISCUSSION

Change in Glycoside Content during Oolong Tea Manufacturing Process. The same methods as those previously described in Parts 1 and 2 of this study (*15*, *17*) were used to prepare the glycosidic fractions by XAD-2 column chromatography from samples taken at each manufacturing stage of Chin-shin-oolong and Chinhsuan-oolong teas. After TFA derivatization and a subsequent GC-MS analysis, the glycosides in each sample were identified by comparing their retention indices and mass spectra with those of authentic synthesized glycosides. The content of each glycoside was determined from the peak area on the total ion chromatogram from GC-MS by making a regression based on the calibration curve equations that had been previously established (*15*).

Table 2. Change in the Glycoside Contents of Tea Leaves during the Manufacturing Process for Chinhsuan-oolong

glycoside[a]	RRT[e] synthetic[f]	RRT[e] natural	content (mg/100 g of dried leaves) dried fresh leaves	solar-withered	indoor-withered	oolong tea
(*Z*)-3-hexenyl-Glc	0.896	0.895	1.4 ± 0.2	2.3 ± 0.2	5.5 ± 0.2	6.9 ± 0.8
benzyl-Glc	1.126	1.130	13.2 ± 0.4	13.8 ± 0.9	17.7 ± 0.9	17.8 ± 1.3
2-phenylethyl-Glc	1.272	1.278	4.9 ± 0.0	5.3 ± 0.4	6.6 ± 0.5	7.4 ± 0.9
methyl salicylate-Glc	1.455	1.459	3.7 ± 0.3	3.7 ± 0.1	4.5 ± 0.3	5.4 ± 0.5
(3*S*,6*S*)-LO I-Glc[b]	1.055	1.055	2.5 ± 0.2	2.1 ± 0.1	3.2 ± 0.2	3.2 ± 0.3
(3*S*,6*R*)-LO II-Glc[b]	1.074	1.076	3.9 ± 0.4	3.6 ± 0.2	4.0 ± 0.3	4.1 ± 0.2
(3*S*)-LO III and IV-Glc[b]	1.188	1.192	8.4 ± 0.4	7.2 ± 1.4	7.4 ± 0.8	9.1 ± 0.6
geranyl-Glc[b]	1.227	1.222	1.5 ± 0.1	1.3 ± 0.1	1.5 ± 0.0	1.4 ± 0.1
total glucosides			39.5 ± 2.0	39.3 ± 3.3	50.3 ± 3.3	55.2 ± 4.7
(*Z*)-3-hexenyl-Prim	1.685	1.685	0.4 ± 0.0	0.6 ± 0.1	1.6 ± 0.1	2.3 ± 0.2
benzyl-Prim	1.877	1.879	3.2 ± 0.0	3.0 ± 0.0	3.1 ± 0.0	4.0 ± 0.3
2-phenylethyl-Prim	1.978	1.983	4.8 ± 0.1	4.5 ± 0.1	4.7 ± 0.2	6.9 ± 0.8
methyl salicylate-Prim[c]		2.007	4.4 ± 0.2	3.9 ± 0.3	4.3 ± 0.2	6.8 ± 0.8
LO I and (3*R*,6*S*)-LO II-Prim[b,d]	1.650	1.654	3.1 ± 0.1	2.7 ± 0.1	3.1 ± 0.1	3.6 ± 0.1
(3*S*,6*R*)-LO II-Prim[b]	1.665	1.671	5.6 ± 0.3	4.8 ± 0.4	5.6 ± 0.3	6.3 ± 0.5
geranyl-Prim	2.039	2.046	7.2 ± 0.5	7.1 ± 0.6	7.9 ± 0.5	12.4 ± 1.7
geranyl-Vic	2.061	2.066	1.5 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
total disaccharide glycosides			30.2 ± 1.3	28.1 ± 1.8	31.9 ± 1.6	44.1 ± 4.5
total glycosides			69.8 ± 3.3	67.4 ± 5.0	82.2 ± 5.0	99.3 ± 9.2

[a] Positive identification except for methyl salicylate-Prim (tentative identification). [b] Quantification based on the GC-MS analysis in an HP-50+ column or otherwise in a DB-5 column. [c] Calibration curve for methyl salicylate-Glu used for quantification. [d] Overlapped with (*S*)-linalyl-Prim. [e] RRT, relative retention time ratio to phenyl β-D-glucoside. [f] Data quoted from Wang et al. (*15*).

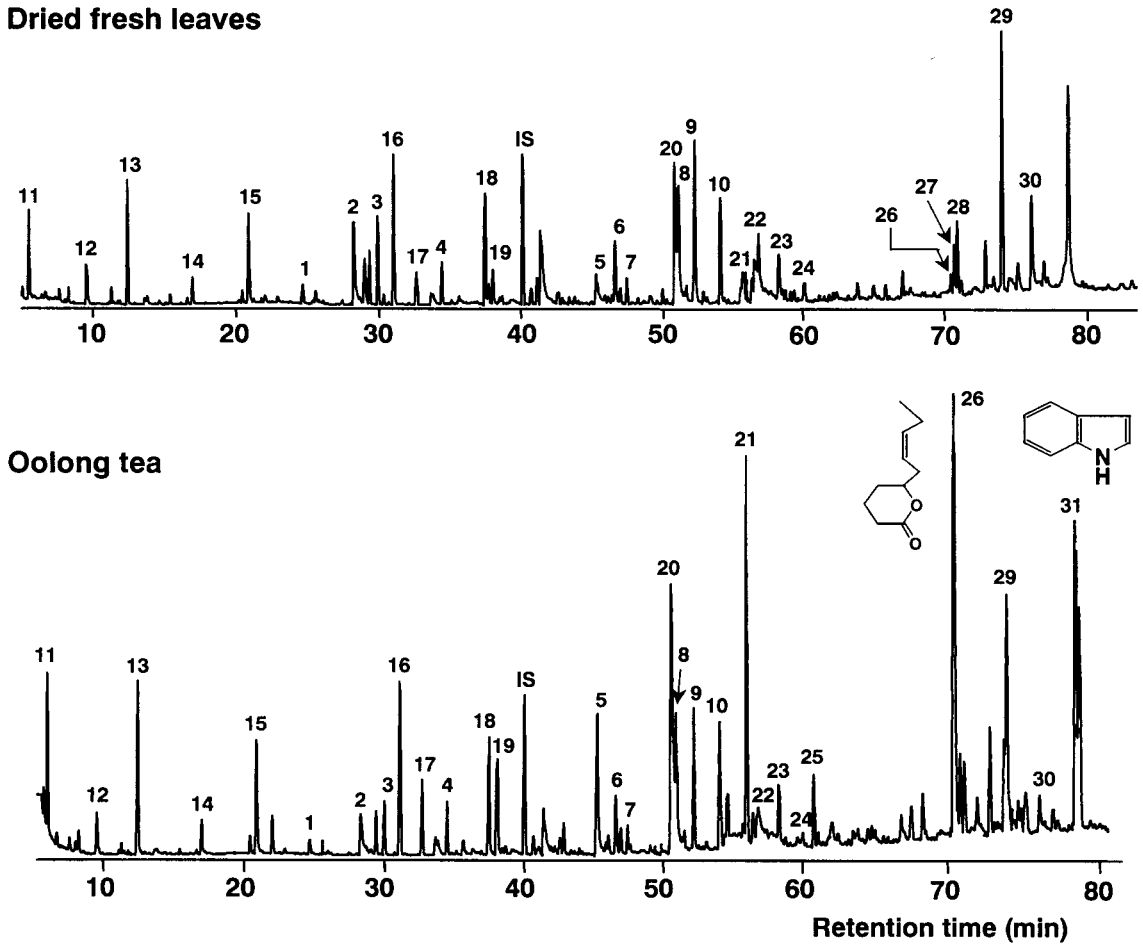

Figure 1. Gas chromatograms of the aroma concentrates prepared using the brewed extraction method from dried fresh leaves and oolong tea of cv. Chin-shin-oolong. (Peak numbers correspond to the numbers in Table 3. IS is the internal standard of ethyl decanoate.)

The identified glycosides and their contents in the sample from each manufacturing stage of Chin-shin-oolong and Chinhsuan-oolong are summarized in Tables 1 and 2, respectively.

Table 3. Change in the Contents of Volatile Compounds in Dried Fresh Leaves and Oolong Tea Prepared from Chin-shin-oolong and Chinhsuan-oolong

		content (mg/100 g of dried leaves)			
		Chin-shin-oolong		Chinhsuan-oolong	
peak	compound	dried fresh leaves	oolong tea	dried fresh leaves	oolong tea
	Group I				
1	(*Z*)-3-hexenol	0.46 ± 0.02	0.33 ± 0.03	0.60 ± 0.07	0.09 ± 0.00
2	LO I	0.92 ± 0.06	0.90 ± 0.07	2.62 ± 0.17	1.15 ± 0.04
3	LO II	1.86 ± 0.09	1.21 ± 0.04	2.90 ± 0.23	1.41 ± 0.05
4	linalool	0.84 ± 0.06	1.05 ± 0.02	0.82 ± 0.04	0.58 ± 0.01
5	LO III	0.52 ± 0.04	3.35 ± 0.29	1.78 ± 0.17	1.72 ± 0.03
6	LO IV	1.88 ± 0.13	1.66 ± 0.15	3.84 ± 0.37	1.50 ± 0.03
7	methyl salicylate	0.40 ± 0.03	0.57 ± 0.01	trace	0.21 ± 0.02
8	geraniol	5.07 ± 0.64	4.74 ± 0.70	0.61 ± 0.04	0.33 ± 0.03
9	benzyl alcohol	4.80 ± 0.11	3.45 ± 0.21	1.55 ± 0.07	1.65 ± 0.04
10	2-phenylethanol	3.24 ± 0.23	3.68 ± 0.26	1.06 ± 0.08	0.93 ± 0.03
	total group I compounds	19.97 ± 1.42	20.93 ± 1.78	15.78 ± 1.24	9.56 ± 0.28
	Group II				
11	2-butanone	3.42 ± 0.55	4.58 ± 0.12	3.22 ± 0.52	2.27 ± 0.14
12	hexanal	0.68 ± 0.04	0.59 ± 0.05	0.57 ± 0.01	0.46 ± 0.04
13	1-penten-3-ol	2.02 ± 0.08	2.51 ± 0.04	3.23 ± 0.43	2.15 ± 0.13
14	pentanol	0.56 ± 0.05	0.69 ± 0.09	0.88 ± 0.12	0.62 ± 0.05
15	(*Z*)-2-pentenol	1.77 ± 0.17	2.09 ± 0.16	2.66 ± 0.40	1.91 ± 0.11
16	(*E*,*E*)-2,4-heptadienal	3.10 ± 0.21	2.83 ± 0.13	3.98 ± 0.09	2.35 ± 0.15
17	(*E*,*E*)-3,5-octadien-2-one	0.85 ± 0.06	1.58 ± 0.16	1.14 ± 0.10	1.86 ± 0.12
18	2,6,6-trimethyl-2-hydroxycyclohexanone	2.54 ± 0.30	2.44 ± 0.47	3.38 ± 0.30	2.58 ± 0.18
19	hotrienol	0.69 ± 0.04	2.78 ± 0.61	3.13 ± 0.15	4.55 ± 0.20
20	hexanoic acid	5.25 ± 0.82	9.87 ± 1.61	4.91 ± 2.68	6.96 ± 0.80
21	3,7-dimethyl-1,5-octadiene-3,7-diol	0.69 ± 0.07	12.79 ± 2.16	3.95 ± 0.97	3.06 ± 0.52
22	2-acetylpyrrole	0.22 ± 0.29	2.45 ± 0.84	6.04 ± 0.36	0.76 ± 0.08
23	5,6-epoxy-β-ionone	1.62 ± 0.50	1.39 ± 0.41	1.94 ± 0.68	1.37 ± 0.01
24	furaneol	0.86 ± 0.14	0.55 ± 0.15	2.06 ± 0.20	0.16 ± 0.01
25	*trans*-nerolidol	trace	1.40 ± 0.12	trace	0.90 ± 0.03
26	jasmine lactone	0.56 ± 0.03	31.55 ± 3.84	0.60 ± 0.18	23.66 ± 0.31
27	4-hydroxymaltol	1.31 ± 0.19	0.80 ± 0.23	2.08 ± 0.09	0.00 ± 0.00
28	3-ethyl-4-methyl-1*H*-pyrrole-2,5-dione	1.94 ± 0.33	1.67 ± 0.38	2.73 ± 0.03	1.85 ± 0.06
29	dihydroactinidiolide	8.17 ± 0.80	8.25 ± 0.96	9.50 ± 0.32	7.07 ± 0.12
30	2,3-dihydrobenzofuran	3.37 ± 0.66	1.62 ± 0.72	5.24 ± 1.47	1.42 ± 0.01
31	indole	trace	12.52 ± 0.72	0.49 ± 0.08	7.38 ± 0.13
	total group II compounds	39.62 ± 5.34	104.94 ± 13.98	61.72 ± 9.17	73.32 ± 3.18
	overall total	59.60 ± 6.75	125.87 ± 15.75	77.50 ± 10.41	82.88 ± 3.46

With respect to the total contents of glucosides and disaccharide glycosides in the dried fresh leaves of each cultivar, Chin-shin-oolong contained a much greater disaccharide glycoside than glucoside content due to the high amount of primeverosides (Table 1). On the other hand, in Chinhsuan-oolong, the content of disaccharide glycosides was less than or almost equal to that of the glucosides (Table 2). The compositions of these glycosides between the two cultivars were also significantly different. For example, dried fresh leaves of Chin-shin-oolong contained fewer glucosides of four isomers of linalool oxide (LO I, *trans*-furanoid; LO II, *cis*-furanoid; LO III, *trans*-pyranoid; and LO IV, *cis*-pyranoid) and many more primeverosides of phenyl aroma compounds such as benzyl alcohol, 2-phenylethanol, and methyl salicylate than Chinhsuan-oolong.

During the manufacturing process of Chin-shin-oolong tea, as shown in Table 1, the contents of most of the glucosides and primeverosides increased from the solar-withering stage and reached the highest values at the final stage of the preparation of oolong tea. In particular, the contents of (*Z*)-3-hexenyl-β-D-glucoside and (*Z*)-3-hexenyl-β-primeveroside in oolong tea increased to 3.7- and 5.0-fold the levels in dried fresh leaves, respectively. The contents of the glucoside and primeveroside of methyl salicylate in oolong tea also significantly increased to 2.6- and 1.8-fold the levels in dried fresh leaves. It is notable that the amounts of neither glycosides nor even primeverosides decreased during the whole manufacturing process. The same result was also obtained for Chinhsuan-oolong tea (Table 2). These findings are quite different from those for black tea, in which almost all of the glycosides, and especially the primeverosides, decreased greatly during the manufacturing process (*17*).

Change in the Content of Free Aroma Compounds during Oolong Tea Manufacturing Process. To avoid isomerization of the aroma compounds and hydrolysis of the glycosides by excessive heating, free aroma concentrates were prepared according to the brewed extraction method (*18*) from dried fresh leaves and oolong tea of each cultivar and were subsequently subjected to GC and GC-MS analyses. Figure 1 shows gas chromatograms of the aroma concentrates prepared from the dried fresh leaves and oolong tea of Chin-shin-oolong. Thirty-one principal aroma compounds were identified and are listed in Table 3. Compounds **1**−**10** (group I) correspond to the aglycon moieties of the glycosides identified in tea leaves, whereas compounds **11**−**31** (group II) are considered not to be directly concerned with the glycosides in their formation during the oolong tea manufacturing process.

The contents of these aroma compounds in dried fresh leaves and oolong tea of both cultivars were determined

from the peak area ratios to that of the internal standard (IS, ethyl decanoate) and are summarized in Table 3.

In cv. Chin-shin-oolong, there was no significant increase in content of the alcohols of group I (except for linalool and LO III) during the manufacturing process. The total content of group I compounds in oolong tea constituted only ~17% of the total amount of aroma compounds. However, a large increase in the aroma compounds of group II was apparent due to the substantial formation of jasmine lactone and indole during the manufacturing process. Jasmine lactone, which possesses a jasmine-like floral and fruity odor, is well-known to be one of the most important compounds to oolong tea aroma (*19*), and its content increased from 0.56 to 31.55 mg in 100 g of oolong tea during the manufacturing process. The content of indole also increased from the trace content in dried fresh leaves to 12.52 mg/100 g of oolong tea. The combined amount of these two compounds constituted ~35% of the oolong tea aroma compounds.

Similar characteristics were also shown by cv. Chinhsuan-oolong, in which the content of group I compounds slightly decreased, whereas the contents of jasmine lactone and indole increased greatly during the oolong tea manufacturing process. The latter two compounds together constituted ~38% of the total aroma compounds, whereas the group I compounds accounted for only 12%. Tokitomo et al. (*2*) and Kobayashi et al. (*3*) have also observed the same characteristics during the manufacture of pouchong tea.

It is well-known that Chin-shin-oolong and Chinhsuan-oolong are the best cultivars for making oolong tea (containing pouchong tea) due to their elegant flavor. The different patterns of the contents of glycosides in dried fresh tea leaves between the two cultivars seemed not to significantly affect the aroma quality of oolong tea product. Furthermore, the above results for the formation of oolong tea aroma during the manufacturing process also suggested that compounds in group II might be more important than the alcohols of group I. Consequently, one or more biosynthetic pathways, such as the formation of jasmine lactone and indole, should be considered to take priority over the hydrolysis of glycosides. This is quite different from the formation of black tea aroma, in which the hydrolysis of glycosides plays the main role as we described in a previous study (*17*). The mechanism for the formation of the two compounds has not yet been clarified.

The oolong tea samples of cv. Chin-shin-oolong and cv. Chinhsuan-oolong used are relatively lightly fermented tea among oolong teas. It is processed while the tea leaves are intact and alive, even though they are under water-stress conditions. This is in contrast to the processing of black tea, whereby the structure of the leaf cells is disrupted during the rolling process and the contents of the cells are completely mixed. Therefore, hydrolysis of the glycosides should be dominant in the black tea manufacturing process. However, in the manufacture of oolong tea, newly biosynthesized glycosides seem to have appeared because of the increase in the amount of glycosides, although further study remains to be done to elucidate this.

LITERATURE CITED

(1) Hara, Y.; Luo, S.-J.; Wickremasinghe, R. L.; Yamanishi, T. Processing of Tea. *Food Rev. Int.* **1995**, *11*, 409−434.

(2) Tokitomo, Y.; Ikegami, M.; Yamanishi, T., Juan, I.-M.; Chiu, W. T.-F. Effects of withering and mass-rolling processes on the formation of aroma components in Pouchong type semi-fermented tea. *Agric. Biol. Chem.* **1984**, *48*, 87−91.

(3) Kobayashi, A.; Tachiyama, K.; Kawakami, M.; Yamanishi, T.; Juan, I.-M.; Chiu, W. T.-F. Effects of solar-withering and turn over treatment during indoor-withering on the formation of Pouchong tea aroma. *Agric. Biol. Chem.* **1985**, *49*, 1655−1660.

(4) Yano, M.; Joki, Y.; Mutoh, H.; Kubota, K.; Kobayashi, A. Benzyl glucoside from tea leaves. *Agric. Biol. Chem.* **1991**, *55*, 1205−1206.

(5) Kobayashi, A.; Kubota, K.; Joki, Y.; Wada, E.; Wakabayashi, M. (*Z*)-3-hexenyl β-D-glucopyranoside in fresh tea leaves as a precursor of green odor. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 592−593.

(6) Guo, W.; Sakata, K.; Watanabe, N.; Nakajima, R.; Yagi, A.; Ina, K.; Luo, S. Geranyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside isolated as an aroma precursor from tea leaves for oolong tea. *Phytochemistry* **1993**, *33*, 1373−1375.

(7) Guo, W.; Hosoi, R.; Sakata, K.; Watanabe, N.; Yagi, A.; Ina, K.; Luo, S. (*S*)-linalyl, 2-phenylethyl, and benzyl disaccharide glycosides isolated as aroma precursors from oolong tea leaves. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1532−1534.

(8) Moon, J.-K.; Watanabe, N.; Sakata, K.; Yagi, A.; Ina, K.; Luo, S. *trans*- and *cis*-Linalool 3,6-oxide 6-*O*-β-D-xylopyranosyl-β-D-glucopyranosides isolated as aroma precursors from leaves for oolong tea. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1742−1744.

(9) Moon, J.-K.; Watanabe, N.; Ijima, Y.; Yagi, A.; Sakata, K. *cis*- and *trans*-Linalool 3,7-oxides and methyl salicylate glycosides and (*Z*)-3-hexenyl β-D-glucopyranoside as aroma precursors from tea leaves for oolong tea. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 1815−1819.

(10) Nishikitani, M.; Kubota, K.; Kobayashi, A.; Sugawara, F. Geranyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside isolated as an aroma precursor from leaves of a green tea cultivar. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 929−931.

(11) Nishikitani, M.; Wang, D.; Kubota, K.; Kobayashi, A.; Sugawara, F. (*Z*)-3-Hexenyl and *trans*-linalool 3,7-oxide β-primeverosides isolated as aroma precursors from leaves of a green tea cultivar. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 1631−1633.

(12) Ogawa, K.; Ijima, Y.; Guo, W.; Watanabe, N.; Usui, T.; Dong, S.; Tong, Q.; Sakata, K. Purification of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves (cv. Shuixian) to be processed to oolong tea. *J. Agric. Food Chem.* **1997**, *45*, 877−882.

(13) Ijima, Y.; Ogawa, K.; Watanabe, N.; Usui, T.; Ohnishi-Kameyama, M.; Nagata, T.; Sakata, K. Characterization of β-primeverosidase, being concerned with alcoholic aroma formation in tea leaves to be processed into black tea, and preliminary observations on its substrate specificity. *J. Agric. Food Chem.* **1998**, *46*, 1712−1718.

(14) Matsumura, S.; Takahashi, S.; Nishikitani, M.; Kubota, K.; Kobayashi, A. The role of diglycosides as tea aroma precursors: Synthesis of tea diglycosides and specificity of glycosidases in tea leaves. *J. Agric. Food Chem.* **1997**, *45*, 2674−2678.

(15) Wang, D.; Yoshimura, T.; Kubota, K.; Kobayashi, A. Analysis of glycosidically bound aroma precursor in tea leaves. 1. Qualitative and quantitative analyses of glycosides with aglycone as aroma compounds. *J. Agric. Food Chem.* **2000**, *48*, 5411−5418.

(16) Voirin, S. G.; Baumes, R. L.; Günata, Z. Y.; Bitteur, S. M.; Bayonove, C. L. Analytical methods for monoterpene glycosides in grape and wine. I. XAD-2 extraction and gas chromatographic-mass spectrometric determination of synthetic glycosides. *J. Chromatogr.* **1992**, *590*, 313−328.

(17) Wang, D.; Kurasawa, E.; Yamaguchi, Y.; Kubota, K.; Kobayashi, A. Analysis of glycosidically bound aroma precursors in tea leaves. 2. Changes in glycoside contents and glycosidase activities in tea leaves during the black tea manufacturing process. *J. Agric. Food Chem*. **2001**, *49*, 1900−1903.

(18) Kawakami, M.; Ganguly, S. N.; Banerjee, J.; Kobayashi, A. Aroma composition of oolong tea and black tea by brewed extraction method and characterizing compounds of Darjeeling tea aroma. *J. Agric. Food Chem*. **1995**, *43*, 200−207.

(19) Yamanishi, T.; Kosuge, M.; Tokitomo, Y.; Maeda, R. Flavor constituents of pouchong tea and a comparison of the aroma pattern with jasmine tea. *Agric. Biol. Chem*. **1980**, *44*, 2139−2142.

Received for review February 21, 2001. Revised manuscript received July 20, 2001. Accepted July 23, 2001.

JF010235+