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Free and glycosidically bound monoterpene alcohols in Qimen black tea

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An extraction and analysis method to determine the free and bound monoterpene alcohols in Qimen black tea was developed, and the varietal and seasonal changes in both free and bound monoterpene alcohols were investigated. The contents of the flavour components released from the bound forms increased greatly after addition of the crude enzyme, which was extracted from fresh tea leaves, and incubated at 37°C for 10 h. Most of the b/f values of monoterpene alcohols, the ratios of bound to free forms, were greater than 1, indicating that the bound forms were in higher concentrations than the corresponding aglycones in fresh tea leaves. Geraniol was the highest among the monoterpene alcohols both in the amount released from the bound form and the b/f value. This indicated that the crude enzyme used in this experiment was very effective for the release of geraniol. The sequence of the contents of both bound and free flavours in different seasons was, generally: spring > summer≥autumn. Both forms of monoterpene alcohols exhibited a marked decrease in concentration in summer and autumn compared with those in spring. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

It is well known that some components of tea flavour are present either in the free state or bound to sugars in the form of glycosides. Many studies have been carried out on free flavours in different kinds of tea (Yamanishi, 1991; Takeo *et al.*, 1985; Wang & Li, 1989). Recently, the importance of glycosidically bound volatile constituents and their contribution to the aroma is receiving increasing attention by many researchers (Seung & Janice, 1991; Yano *et al.*, 1990). In addition to the free odour-producing forms of monoterpene alcohols, the presence of glycosidically bound monoterpene alcohols in tea was first suggested by Takeo (1981). Yano *et al.* (1990) also found liberation of some aroma constituents including monoterpene alcohols when non-volatile materials from tea leaves were treated with a crude enzyme preparation from tea leaves.

Qimen (Keemun) black tea is famous throughout the world for its unique style of flavour. It was reported in our previous paper that geraniol, a kind of monoterpene alcohol, was considered to be responsible for the characteristic aroma of Qimen black tea, and it might be released from the glycosidically bound form through hydrolysis (Wang *et al.*, 1993). To enhance the flavour quality of tea, it is extremely important to investigate how to convert the glycosidically bound monoterpene alcohols into the free forms to some extent. In this paper, an extraction and analysis method to determine the free and bound monoterpene alcohols in Qimen black tea was developed, and the varietal and seasonal changes in both free and bound monoterpene alcohols were also studied.

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MATERIALS AND METHODS

Sample preparation

Fresh leaves of tea cultured at Qimen country of Anhui Province were plucked at the Qimen Tea Research Institute, Anhui Academy of Agricultural Sciences. Plucked leaves were steamed and then fired up directly at 105°C. The dried tea leaves were subjected to analysis.

Preparation of crude enzyme

The crude enzyme was prepared from the corresponding fresh leaves. The fresh tea leaves were crushed in cooled acetone (−15°C). After homogenizing for 3 min in a dispersing mixer, the acetone layer was removed by

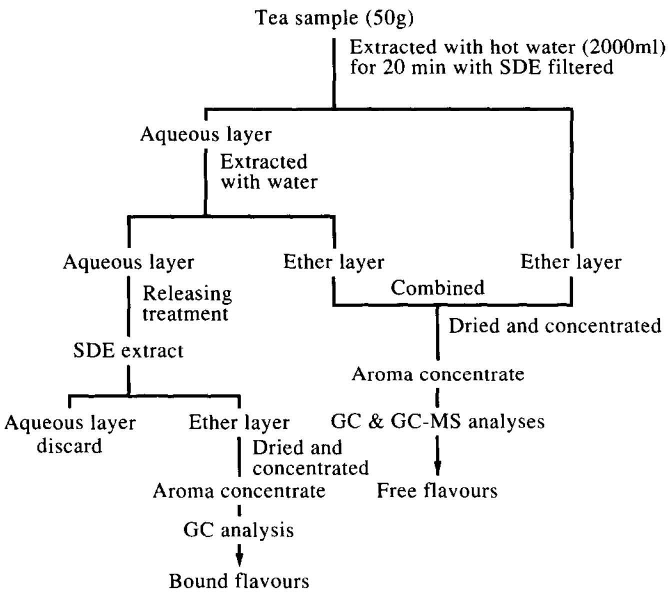

Fig. 1. Preparation of the tea aroma concentrate.

filtration. The debris was washed with cold acetone until it became colourless and then dried at room temperature.

Preparation of the tea aroma

Preparation of the tea aroma extracts and the separations and analyses procedures are summarized in Fig. 1. Using a Liens–Nickerson Simultaneous Steam Distillation Continuous Extraction (SDE) with ether as solvent, the samples were boiled for 20 min at 100°C. Before SDE extraction, 50 μg of ethyl decanoate was added to the tea infusion as an internal standard for determining the relative amount of each compound.

Identification and determination of the tea aroma compounds

GC analysis
A Shimadzu GC-9A gas chromatograph equipped with a flame ionization detector was used. The column was a PEG-20 M fused-silica capillary column (50 m×0.20 mm ID). The temperature was programmed from 50 to 190°C at 2°C/min, and then held at 190°C for 20 min. A C-R2AX data processor was used to calculate the peak area and the retention index. The quantification was performed using ethyl decanoate as an internal standard.

GC–MS analysis
A Hewlett-Packard 5890A gas chromatograph coupled with a Hewlett-Packard 5970 BMSD mass spectrometer was used. The GC conditions were the same as those above. The ionization energy was 70 eV. Peak identification was achieved by interpretation of mass spectra and by coincidence of retention times with authentic samples.

RESULTS AND DISCUSSION

Free monoterpene alcohols released from glycosidically bound form

The volatile components released by the releasing treatment were analysed by GC and GC–MS. Compared to the chromatogram of aroma concentrate of the tea (free form, Fig. 2a), the contents of the flavours released from the bound forms were increased greatly after the crude enzyme was added (Fig. 2b). In addition, other major flavours of tea, such as (*Z*)-3-hexen-1-ol, methyl salicylate and 2-phenyl ethanol, had the same tendency.

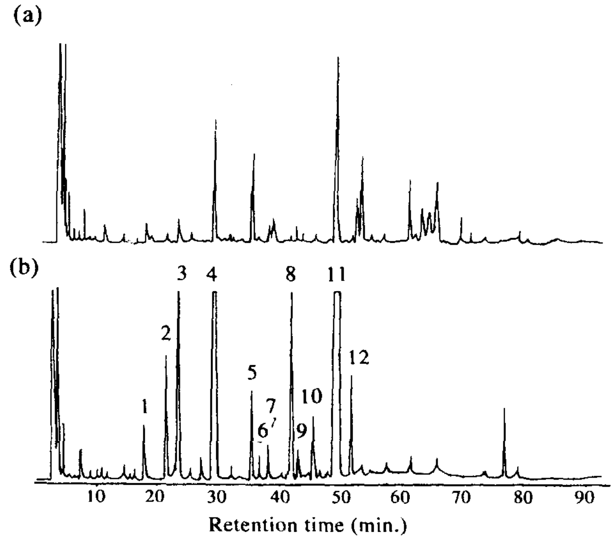

Fig. 2. Gas chromatograms of the aroma concentrates from free and bound forms: 1, (*Z*)-3-hexen-1-ol; 2, linalool oxide (1); 3, linalool oxide (1); 4, linalool; 5, ethyl deconoate; 6, α-terpineol; 7, linalool oxide (3); 8, methyl salicylate; 9, linalool oxide (4); 10, nerol; 11, geraniol; 12, 2-phenylethanol.

Table 1. Free and bound monoterpene alcohols from tea leaves of different varieties in different seasons[a]

	Geraniol		Linalool		α-Terpenol		Nerol	
	b	f	b	f	b	f	b	f
Anhui 7								
Spring	2.58	1.69	13.11	1.22	0.22	0.25	0.25	0.07
Summer	1.32	0.39	11.0	1.02	0.28	0.29	0.22	0.09
Autumn	1.85	0.31	10.41	0.84	0.23	0.29	0.26	0.03
Cutivar 163								
Spring	53.5	2.80	7.88	1.22	0.33	0.20	0.58	0.12
Summer	18.7	0.54	9.58	0.58	0.45	0.20	0.39	0.07
Autumn	18.7	0.37	9.59	1.04	0.45	0.27	0.39	0.05
Zhuye population								
Spring	47.5	2.82	14.24	1.38	0.38	0.27	0.72	0.14
Summer	26.6	0.51	11.11	0.83	0.30	0.30	0.46	0.03
Autumn	13.3	0.36	11.08	0.96	0.39	0.32	0.34	0.02

[a]The values show the ratio of the area of each component to that of the internal standard on the gas chromatogram.b, bound form; f, free form.

Table 1 shows the free and glycosidically bound monoterpene alcohols from fresh tea leaves of different varieties in different seasons. The corresponding b/f values, the ratios of the bound to free forms, are listed in Table 2. As shown in the two tables, the bound forms are found in higher concentrations than the corresponding aglycones in fresh tea leaves. This indicates that monoterpene alcohols are present in the fresh leaves mainly in the glycosidically bound forms before they are manufactured to make tea. In our previous report, we found that the b/f value can be used as a measurement of the release ability from bound flavour to flavour free (You *et al.*, 1993). As shown in Table 2, the b/f values of the monoterpene alcohols were mostly greater than 1. It was noticeable that geraniol was the highest among the monoterpene alcohols both in the amount released from bound flavour and the b/f value. This indicated that the preparation of the crude enzyme used in this experiment was the most effective for the release of geraniol. The result shows that the flavour release treatment is promising for enhancing the flavour quality of Qimen black tea because geraniol is regarded as the most important component of the characteristic aroma of Qimen black tea (Wang *et al.*, 1993).

Table 2. The b/f values in tea leaves of different varieties in different seasons

	Geraniol	Linalool	α-Terpenol	Nerol
Anhui 7				
Spring	1.53	10.8	0.88	3.57
Summer	3.38	10.8	0.90	2.44
Autumn	5.19	12.4	0.79	8.67
Cutivar 163				
Spring	19.1	6.46	1.65	4.83
Summer	34.6	17.1	2.25	5.57
Autumn	50.5	9.22	1.67	6.50
Zhuye population				
Spring	16.8	10.3	1.41	5.14
Summer	52.1	13.3	1.00	15.33
Autumn	37.0	11.5	1.22	17.00

Varietal and seasonal changes in both free and bound flavours

As shown in Table 1, the general sequence of the contents of both bound and free monoterpene alcohols in different seasons is: spring > summer≥autumn. This suggests that accumulation of bound monoterpene alcohols in tea leaves reaches the highest level in spring. Both forms of monoterpene alcohols, especially for geraniol, exhibited a marked decrease in concentration in summer and autumn compared with those in spring. Seung & Janice (1991) found a similar phenomenon in their study on grapes. They deduced that the decrease in concentration of geraniol and linalool could be a response to high temperatures. It was found that the bound form of geraniol in tea leaves dominated the total content of monoterpene alcohols except for Anhui 7. In contrast, for Anhui 7, the most abundant monoterpene alcohol was linalool, showing its different varietal characteristics to the other two varieties. As shown in Table 2, the b/f values sequenced as autumn > summer≥spring. Experience, however, shows that summer and autumn teas are short of flavour. It is interesting that the relatively higher b/f values of tea leaves in summer and autumn are beneficial for enhancing the flavour quality of summer and autumn teas.

From Tables 1 and 2 it can be seen that three major varieties cultured in the Qimen area expressed evident varietal characteristics. In Anhui 7, for example, the content of free form of geraniol was not lower than that of any other varieties, but its b/f value was much lower than that of other varieties. So geraniol in the made tea was also found in low concentration and probably, because of this, Anhui 7 is not regarded as suitable for making good Qimen black tea. On the other hand, Cultivar 163 was found suitable for the manufacture of green tea (Tea Research Institute, 1982). However, according to this experiment, the b/f values of monoterpene alcohols, such as geraniol, were relatively higher, so it was considered that by using flavour release treatment, the Cultivar 163 might be suitable for making excellent Qimen black tea.

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