

CHANGES IN VOLATILE COMPOUNDS DURING ORTHODOX BLACK TEA (*Camellia sinensis*) MANUFACTURING

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ABSTRACT

The content of volatile compounds in tea leaves at different stages of OTD black tea manufacture was measured. Aroma concentrate was prepared by Brewed Extraction Method (BEM) and analyzed by GC/MS. The results showed that volatile compounds were products of lipid breakdown (group I) and derivatives from terpenoids, carotenoids and amino acids (group II). During processing the changes of group I and group II were significantly different from each others and effect to the flavour index. A gradual increase of percentage relative content from 16.72 to 18.09 in the group I volatile compounds was noticed during withering. While during fermentation, the sum of group II compounds content was higher than that of group I compounds therefore the value of flavour index, and the value of FI is higher than 1.

Keywords: black tea, volatile compounds.

1. INTRODUCTION

Black tea (*Camellia sinensis*) is the most widely consumed beverage in the world. The high acceptability of black tea due to many factors, one of the most contributory factors is its flavour. During the manufacture of OTD black tea from fresh tea leaves of *Camellia sinensis*, compounds conferring flavour are generated by enzymic oxidation of the peptidase, lipoxygenase, carotenase etc and subsequent reactions, including coupled chemical oxidations, of the active intermediates generated. When the desired quality has been reached, drying is used to halt the reactions. Chemical change is not instantly arrested, but is initially accelerated by the increase in temperature until the enzymes are inactivated by loss of water or denatured by heat. Enzyme destruction is essential to stabilize the product during storage.

The volatile compounds of black tea have been investigated extensively by many researchers, and more than 600 volatile compounds have been reported [1 – 3]. Vietnamese black tea, produced by OTD was also investigated, and the results showed that about 40 volatile compounds were identified [4]. The amounts of volatile components after drying processing of both the OTD and CTC black tea were analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS) [5]. As was identified by many previous studies, in black tea, volatile

flavour compounds (VFC) are present in very low quantities, i.e. about 0.01% to 0.02% of the total dry weight, but they have high impact on the flavour of the products due to their low threshold value and resulting high odour units [3, 5]. Moreover, the olfaction is better developed in humans for flavour perception compared to gestation. These VFCs can be divided into two groups. The Group I compounds are mainly the products of lipid breakdown, which imparts an undesirable grassy odour. However, the Group II compounds, which impart a sweet flavoury aroma to black tea, are mainly derived from terpenoids, carotenoids and amino acids. The flavour of made tea depends on the ratio of the sum of VFC Group II to that of VFC Group I, which is the flavour index or volatile flavour compounds (VFC) index [6].

Volatile compounds play an important role in determining the aroma character of black tea [2, 7], but detailed studies of quantitative changes in these classes of compounds which occur at various stages of orthodox black tea processing including withering, rolling, fermenting and on firing have received insufficient attention especially orthodox black tea was produced from the tea clones which was cultivated in Vietnam. Therefore, this paper presents the results of the systematic study of progressive change in the volatile content during each stage of manufacture.

2. MATERIALS AND METHODS

2.1. Materials

Tea leaves of cultivars PH11, harvested from Phu Tho and representing the genetically diverse Northern Vietnam cultivars, were used for analyzing chemical composition. Ten kilograms of young shoots, comprising about 70 % two leaves and a bud, plus minor amounts of three leaves and a bud and loose leaf were plucked. The plucked leaf was allowed to wither under ambient conditions for 16 h and then miniature rolling–dhoos. The dhoos were fermented for 180 min at 30 °C. The fermentation was terminated by drying the dhoos to a moisture content of about 3 % using a miniature dryer set at 120 °C inlet and 80 °C exhaust air temperature [2].

2.2. Volatile analysis

Brewed Extraction Method: Twenty grams of black tea sample were brewed by 140 ml of deionized boiling water for 10 min. After filtration, the filtrate was saturated with sodium chloride and was extracted by using 100 ml of dichloromethane. The extract was dried over anhydrous sodium sulfate for 1 h. After sodium sulfate was filtrated out, the solvent was removed carefully by using an evaporative concentrator. The extraction was carried out in duplicate for each sample [1].

GC-MS analysis: The Thermo trace GC Ultra gas chromatograph coupled with the DSQ II mass spectrometer was used to perform the aroma analysis. An HP-5 capillary column (30 m×0.25 mm × 0.25 µm) was equipped, with purified helium as the carrier gas, at a constant flow rate of 1 ml/min. After extraction, the fibre was desorbed in the injector port of the GC at 220 °C for 5 min. The oven temperature was held at 50 °C for 3 min and then increased to 190 °C at a rate of 5 °C min⁻¹ and held at 190 °C for 1 min, and then increased to 240 °C at a rate 20 °C min⁻¹, held at this temperature for 3 min. Ion source temperature was 200 °C and spectra were performed in the electron impact (EI) mode at 70 eV. The mass spectrometer was operated in the full scan, and the peak area was determined by Xcalibur software (Thermo Technologies). Volatile compounds were identified by retention time, electron impact mass spectrum and similarly match index.

2.3. Statistical analysis

Statistical comparisons of the mean values for each experiment were performed by one-way analysis of variance (ANOVA), followed by the general linear model with repeated measured defined factors using SPSS 22 for Windows software. Significance was declared at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Although the lipid content of tea is low, about 1 - 3 % dry basis, lipid metabolism appears to be important and processing techniques could play a significant role in the biogenesis of the flavour found in finished black teas. Apart from lipid oxidation, non-enzymic browning reaction is a characteristic feature of traditional tea processing [8]. During black tea processing, the long-chain unsaturated fatty acids are liberated from mechanically disrupted leaves which further undergo oxidative degradation by the LOX, producing different carbonyl compounds, which in turn is required for the formation of aroma compounds [9]. During withering, in general, the volatile compounds belong to the group I, (*E*)-2-pentenal, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal and decanal, were decreased while 3-hexen-1-ol, (*E*)-2-hexen-1-ol, hexanal, nonanal and hexanol were identified to be increased. The results showed that a gradual increase of percentage relative content from 16.72 to 18.09 in the group I volatile compounds was noticed during withering in response to degradation of polyunsaturated fatty acids present in tea leaves while increase in the group volatile compounds and their relation with Flavour Index were also substantiated by the degradation of carotenoids and amino acid [6]. The relative contents of some of group I compounds such as (*E*)-2-hexen-1-ol, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal and nonanal were continuously increasing during fermentation while hexanal, hexanol, 2-penten-1-ol, decanal and (*E*)-2-pentenal. were observed an decreasing trend from the beginning of fermentation step..

Carotenoids included β -carotene and zeaxanthin accounted for approximately 85 % of total carotenoid content in leaves and they have been identified as precursors for many tea flavours and play key roles in deciding the quality of tea [9]. Majority of volatile compounds belonging to the group II, which are degradation products of carotenoids were found to be increased after rolling step such as β -ionone, α -ionone, β -damascenone and β -cyclocitral. The Table 1 showed that the amounts of major volatile compounds belonging to group II such as β -linalool, benzyl alcohol, *trans*-linaloloxide, *cis*-linaloloxide, β -cyclocitral, phenyl ethanol and *trans*-geraniol was found to be increased during withering, in consequence of which the value of flavour index expanded from 0.74 to 1.09. Afterwithering step, the amount of hexanol, hexanal, 3-hexen-1-ol and (*E*)-2-hexenal in withered leaves were determined highly accounted for 5.77 %; 2.62 %; 2.33 %, respectively. The carotenoids degradation products (β -damascenone, β -ionone, β -ionol and α -ionone) and glycosides derivatives (β -linalool, *trans*-linaloloxide, *trans*-geraniol and *cis*-linaloloxide) were continuously increasing, but this was not valid for intermediate degradation products like 3-hydroxy- β -damascone.. In general, during fermentation, the sum of group II compounds content was higher than that of group I compounds therefore the value of flavour index was higher than 1.

In some previous studies showed that the α -amino acids present in tea leaves material will undergo Strecker degradation to form corresponding aldehydes in the presence of, and only in presence of, oxidizing tea flavanols. In principle, all free amino acids should have their corresponding Strecker aldehydes. However, only amino acids such as glycine, alanine, valine, leucine, phenylalanine, isoleucine and methionine have their Strecker aldehydes and their

corresponding Strecker aldehydes are formaldehyde, acetaldehyde, isobutyraldehyde, isovaleraldehyde, phenylacetaldehyde, 2-methylbutanal and methional, respectively. One reason is that non-volatile products are generated instead of volatile aldehydes. The other possibility is some Strecker aldehydes are so unstable that they readily decompose into other volatiles by cyclization, coupling, or dehydration [10, 11].

Table 1. Volatile compounds during processing of PH11 cultivar .

Volatile compounds*	Fresh leaf	Withered leaf			Rolled	Fermented leaf			Dried
		At 6h	At 12h	At 16h		At 60m	At 120m	At 180m	
Group I									
2-penten-1-ol	0.00	0.00	0.00	0.05	0.61	0.55	0.44	0.34	0.00
(E)-2-pentenal	2.89	1.26	1.19	1.02	0.88	0.61	0.28	0.15	0.00
3-hexen-1-ol	1.56	2.14	2.32	2.62	2.77	2.95	3.84	4.20	2.70
hexanal	1.31	1.42	1.89	2.62	2.74	3.01	2.85	2.69	2.42
(E)-2-hexen-1-ol	1.58	1.87	1.56	1.26	2.30	3.57	5.37	5.76	2.77
(E)-2-hexenal	2.61	3.23	2.78	2.33	3.48	3.68	4.46	5.59	3.61
hexanol	3.55	4.48	5.08	5.77	4.78	4.71	3.80	3.54	2.17
nonanal	1.14	1.22	1.29	1.42	1.68	1.94	2.35	3.77	1.95
decanal	2.09	1.59	1.09	1.00	0.98	0.68	0.46	0.12	0.02
2-nonanol	nd	nd	nd	nd	nd	nd	nd	0.23	0.11
Group II									
<i>trans</i> -linaloloxide	0.56	1.19	1.29	1.43	1.02	1.11	0.99	0.8	1.82
β -linalool	3.91	4.79	5.79	6.03	5.09	4.46	4.03	3.33	1.53
benzyl alcohol	2.06	2.19	2.27	2.49	2.39	2.40	2.97	3.78	1.53
benzeneacetaldehyde	0.22	0.40	0.60	1.01	1.22	1.28	1.95	2.13	1.01
isobutyraldehyde	nd	nd	nd	nd	0.75	0.81	0.90	0.98	0.00
phenyl ethanol	1.01	1.19	1.02	1.51	2.31	2.40	2.88	3.46	0.89
phenylethylaldehyde	nd	nd	nd	nd	0.44	0.65	0.79	0.88	0.23
<i>cis</i> -linaloloxide	0.42	0.52	0.57	0.61	0.67	0.49	0.37	0.16	0.00
2-methylbutanal	nd	nd	nd	nd	0.35	0.47	0.55	0.92	0.00
isovaleraldehyde	nd	nd	nd	nd	0.55	0.67	0.83	0.96	0.00
naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	0.97
N-ethyl-succinimide	nd	nd	nd	nd	nd	nd	nd	nd	2.68
β -cyclocitral	0.27	0.32	0.52	0.55	0.88	2.28	2.77	3.61	2.47
acetaldehyde	nd	nd	nd	nd	0.46	0.60	0.77	0.94	0.00
<i>trans</i> -geraniol	0.09	0.11	0.15	0.22	0.27	0.30	0.32	0.39	0.06
nerolidol isobutyrate	nd	nd	nd	nd	nd	nd	nd	nd	0.77
5,6-epoxy-6-methyl-2-heptanone	nd	nd	nd	nd	nd	nd	nd	nd	0.78
8-hydroxylinalool	1.02	0.78	0.52	0.47	0.39	0.27	0.22	0.12	0.09
β -damascenone	0.16	0.22	0.32	0.49	1.04	1.24	1.96	3.37	2.68
benzaldehyde, 4-hydroxy-3-methoxy-	0.84	0.67	0.51	0.42	0.60	0.62	0.74	1.50	0.93
benzeneethanol, 4-hydroxy-	nd	nd	nd	nd	0.38	0.88	1.17	2.44	1.85
β -ionone	0.55	0.78	0.85	1.10	1.39	2.46	3.24	4.55	2.68
α -ionone	0.67	0.72	0.88	0.95	1.28	2.68	3.57	4.01	2.87
ethyl linalool	0.12	0.32	0.39	0.62	0.85	0.92	0.69	0.23	0.11
3-hydroxy-.beta.-damascone	0.32	0.57	0.75	0.98	1.27	1.07	0.52	0.21	0.06
β -ionol	0.22	0.3	0.57	0.84	0.95	1.13	1.21	1.29	0.77
Sum of group I	16.72	17.20	17.19	18.09	20.22	21.70	23.85	26.39	15.75
Sum of group II	12.43	15.07	17.01	19.73	24.66	29.42	33.88	40.56	26.77
Flavour Index	0.74	0.88	0.99	1.09	1.21	1.35	1.40	1.52	1.69

*: Volatile compounds were calculated in % (peak area)
 nd- not detected; h-hours; m-minutes

The results showed that Strecker aldehydes products which was identified during OTD manufactures included acetaldehyde, isobutyraldehyde, isovaleraldehyde, phenylalacetaldehyde, and 2-methylbutanal appeared and showed an increasing trend during fermentation.

4. CONCLUSION

The results showed that during processing the changes of volatile compounds belonging to both groups I and II were significantly different from each others and effect to the flavour index. A gradual increase of percentage relative content from 16.72 to 18.09 in the group I and from 12.43 to 19.97 in the group II compounds was noticed during withering. While during fermentation, the sum of group II compounds content was higher in comparison to the sum of group I content, and that bring the flavour index higher than 1. To the best of our knowledge, this is the first report about the changes of flavour precursors during OTD black tea manufacture at Vietnam.

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TÓM TẮT

SỰ BIẾN ĐỔI THÀNH PHẦN BAY HƠI TRONG QUÁ TRÌNH SẢN XUẤT CHÈ ĐEN ORTHODOX (*Camellia sinensis*)

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Thành phần các cấu tử bay hơi trong lá chè ở các giai đoạn khác nhau trong quá trình sản xuất chè đen OTD được phân tích. Thành phần bay hơi được thu nhận bằng phương pháp chiết nước-dung môi và được phân tích bằng GC/MS. Kết quả cho thấy trong quá trình sản xuất thành phần bay hơi là sản phẩm chuyển hóa từ nhóm tiền chất là lipid, carotenoids, acid amin có sự khác biệt ở các giai đoạn khác nhau của quá trình sản xuất và nó ảnh hưởng đến chỉ số chất lượng hương thơm của chè. Kết quả nghiên cứu cho thấy thành phần bay hơi thuộc nhóm chất I tăng từ 16,72 lên 18,09 ở giai đoạn héo. Trong khi đó, trong giai đoạn lên men tổng thành phần bay hơi thuộc nhóm II có hàm lượng cao hơn so với nhóm I, điều này giải thích cho việc tăng lên của chỉ số chất thơm và chỉ số này có giá trị lớn hơn 1.

Từ khóa: chè đen, thành phần bay hơi.