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Comparison of Volatile Compounds of Iranian Black Teas During Process

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Volatile free compounds in fresh, withered, fermented and dried leaves in two samples of Iranian tea were analyzed by gas chromatography and gas chromatography mass spectrometry during black tea manufacture. In sample I, linalool decreased during withering and fermentation and increased in the drying stage but never reached the highest level in fresh leaves and α-farensene decreased during all stages, especially during withering but at the end of drying, the amounts of linalool and farensene were equal. In sample II, linalool decreased sharply during manufacture but it is noticeable that α-farensene increased greatly during all stages, so it can be taken as the main fraction in black tea aroma in this sample. Amount of geraniol is not noticeable in both the samples except in the withering stage.

Key Words: Black tea, α-Farensene, Geraniol, Linalool, Volatile free compounds.

INTRODUCTION

Various kinds of tea are widely consumed all over the world. But most are produced from the same plant species, *Camellia sinensis* L.

According to the manufacturing process, most teas are classified into three major categories: unfermented green tea, semi-fermented oolong tea and fully fermented black tea. Each category of tea, according to the manufacturing process, has its own characteristics[1]. The high acceptability of black tea is due to many factors, one of the most contributory factors being its flavour[2]. Monoterpene alcohols such as linalool and geraniol are known to contribute to the floral aroma[3]. The process for the manufacture of black tea involves four stages: withering, rolling, fermenting and drying[4]. Withering time, rolling condition, degrees of fermentation and even different kinds of drying stages are identified, the changes in aromatic compounds which may be used as indicators in quality and production control of tea[1]. The aroma which is composed of the volatile flavour compounds (VFCs) generated during tea processing specially in fermentation was recently demonstrated to be an important quality parameter determining the price of made tea. These VFCs can be divided into 2 groups. The group I compounds are products of lipid breakdown mainly of the unsaturated fatty acids; these products of fatty acid degradation during black tea manufacture make up over 90% of group I, which imparts an undesirable grassy odour. However, the group II compounds, which impart a sweet flowery aroma to black tea are mainly produced from glycosides of terpenoid related compounds; they comprise terpenoids, derived from amino acids and sugars though the Strecker degradation and the Maillard reaction[5]. Flavour index is the ratio of the sum of VFC group II to that of VFC group I whose compositions vary depending on the processing techniques[6]. However, monoterpene alcohols (linalool, geraniol, etc.) and aromatic alcohols (benzyl alcohol, 2-phenyl ethanol, etc.) are known to contribute to the floral aroma of semi-fermented tea[3]. The alcoholic aroma compounds possessing a floral fruity aroma are known to be the main aroma components of black tea. They are mainly present as glycosides in fresh leaves and are released during the

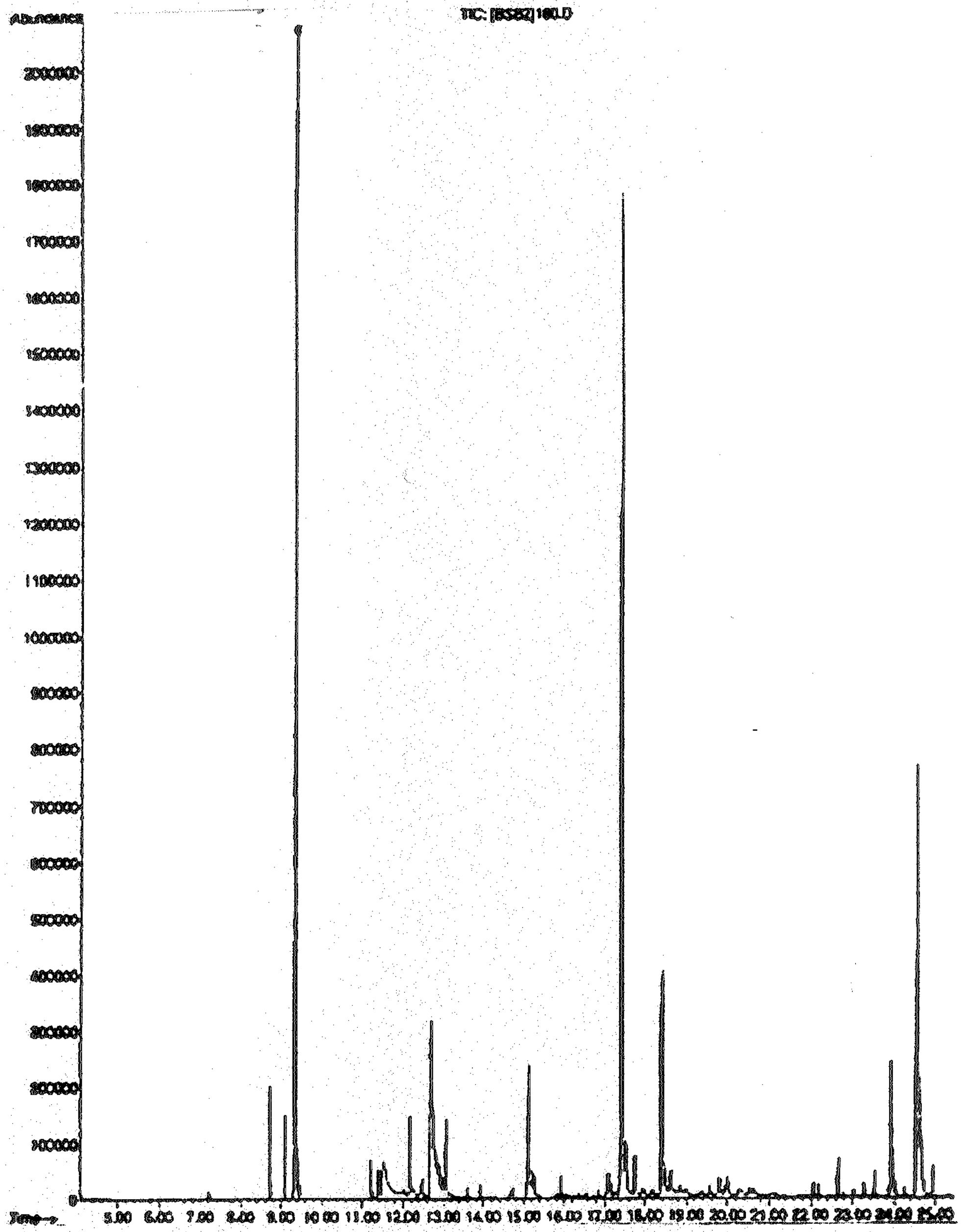

Fig. 1a. Gas chromatograms of the aroma concentrates of sample I prepared using Clevenger method (distillation by water): fresh leaves

manufacturing process of withering, rolling and fermentation[7, 8]. These results suggest that a lot of aroma precursors are present in the fresh leaves that can make many aromatic fractions during each manufacture stage.

EXPERIMENTAL

Two batches of fresh leaves of *Camellia sinensis* var. *Sinensis* were plucked from the research institute of tea farm (Lahijan, Iran) as sample I and from nemoone factory farm (Leiserood, Iran) as sample II at the same time (April 25, 2004). Black tea was manufactured from these two kinds of fresh leaves in both the factories (withering, rolling, fermenting and drying) with their special conditions (temperature, humidity, etc.). Samples of fresh, withered, fermented

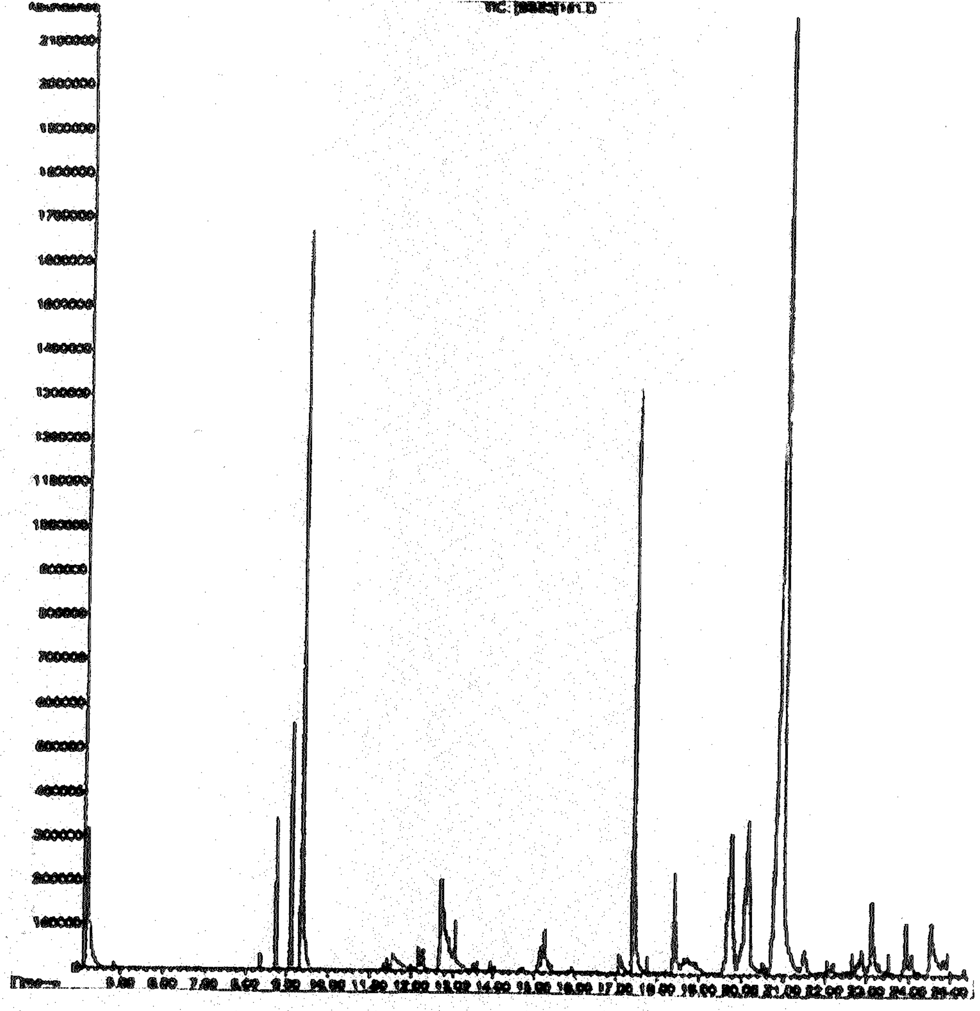

Fig. 1b. Gas chromatograms of the aroma concentrates of sample I prepared using Clevenger method (distillation by water): withered leaves

and dried leaves (black tea) were collected and were subsequently subjected to analysis of the aromatic fraction[5, 9].

Preparation of free aroma concentration

Free aroma concentrations were prepared from the four stages (fresh, withered, fermented and dried leaves) of each cultivar sample by using Clevenger extraction method (distillation by water).

To 400 g of each powdered sample was added 400 cc deionized hot water in Clevenger balloon; the balloon was heated for 4 h and during boiling, volatile free compounds (VFC) were separated by vaporized boiling water. All the VFCs were cooled by Clevenger condenser; the extract was dried over anhydrous sodium sulfate and concentrated to about 100 µL. The resulting aroma concentrate of each cultivar sample was subjected to GC and GC-MS analyses, respectively to study their individual VFC contribution.

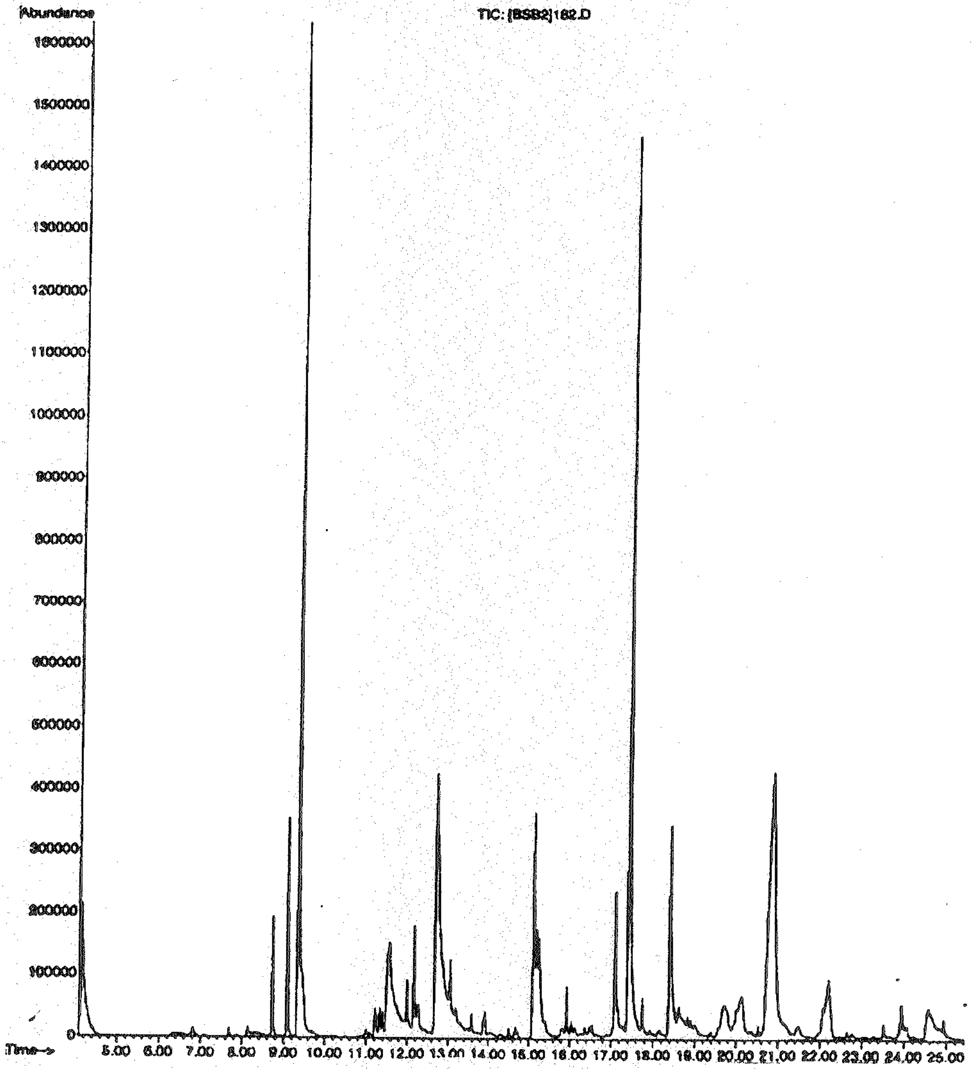

Fig. 1c. Gas chromatograms of the aroma concentrates of sample 1 prepared using Clevenger method (distillation by water): fermented leaves

GC and GC-MS analyses

An HP-6800 gas chromatograph coupled to an HP-5973 mass spectrometer was used. The column was a 60 m × 0/25 mm i.d. DB-wax fused silica capillary column with film thickness of 0.25 µm. The column temperature was programmed from 60–220 c at the rate of 5 c/min in all runs. The flow rate of the helium carrier gas was 1 mL/min and the split ratio was 1 : 50. The mass spectrometer was used under the following conditions[5]: ionization voltage 70 eV, ion source temperature 150 c.

Identification of components

The identification of the components was made by comparison of their kovats GC retention indices and mass spectral fragmentation[9] (8-peak).

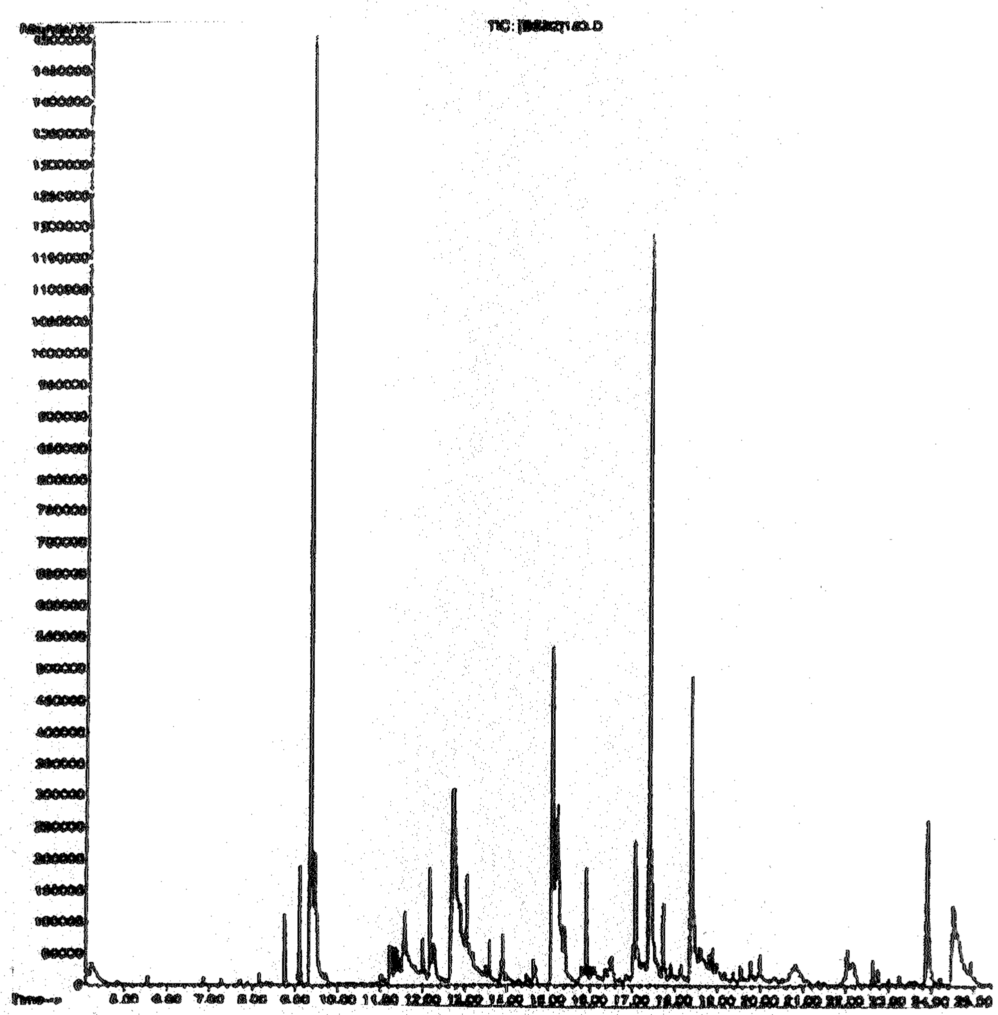

Fig. 1d. Gas chromatograms of the aroma concentrates of sample I prepared using Clevenger method (distillation by water): dried leaves

RESULTS AND DISCUSSION

Gas chromatograms of sample I during the process are shown in Figs. 1a–d and the gas chromatograms of sample II during the process are shown in Figs. 2a–d. The identified compounds and relative quantities of each compound as caculated by peak area are listed in Tables 1 and 2.

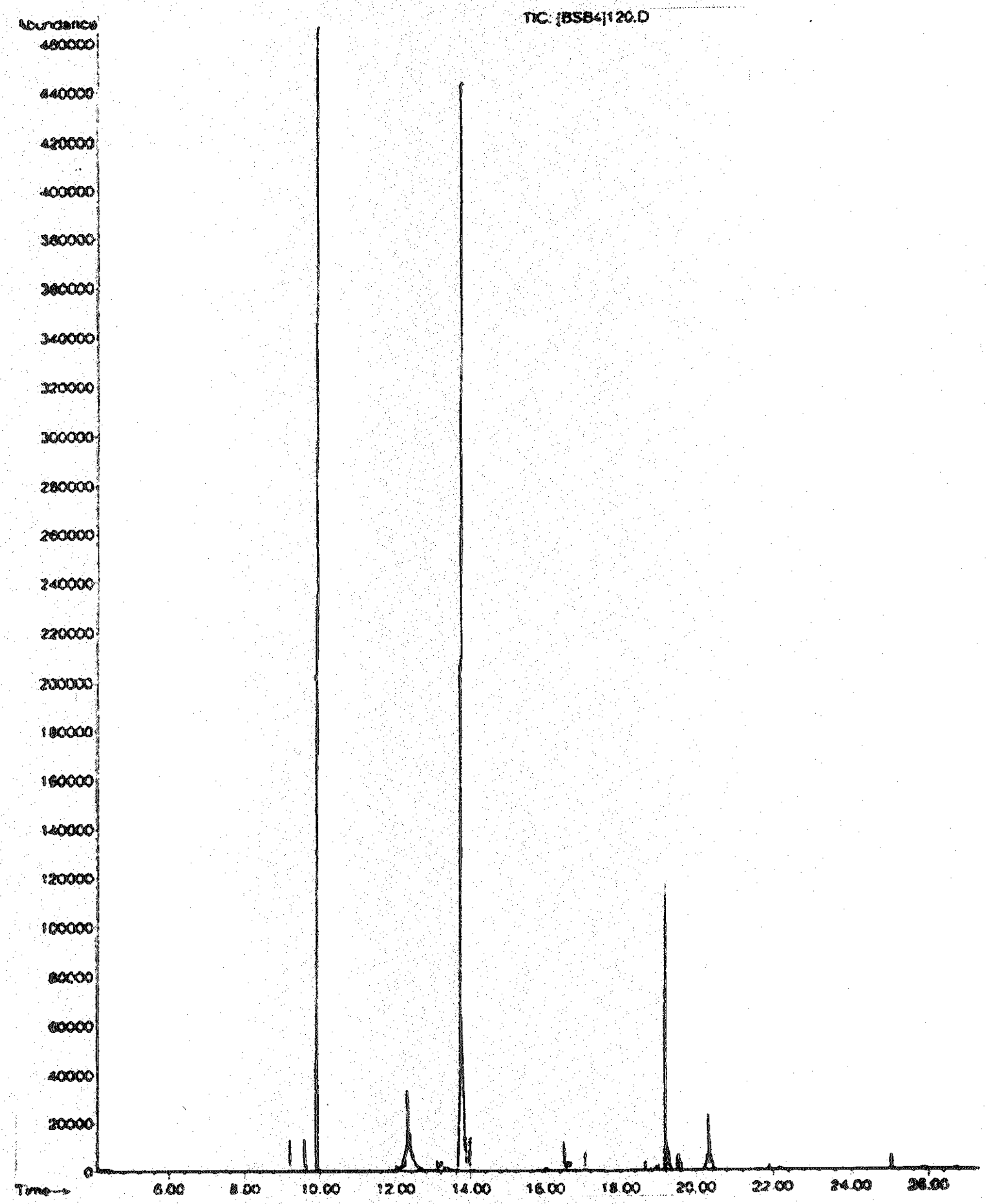

Fig. 2a. Gas chromatograms of the aroma concentrates of sample II prepared using Clevenger method (distillation by water): fresh leaves

TABLE-1
COMPOSITION OF AROMA EXTRACTS OF FRESH, WITHERED, FERMENTED AND DRIED LEAVES (SAMPLE I)

Sample I		GC peak area %		
Compound	Fresh	Withered	Fermented	Dried
Linalool oxide (*cis*)	2/01	1/68	1/42	1/18
Linalool oxide (*trans*)	1/77	2/64	2/73	1/82
Linalool	26/41	10/12	14/77	17/74
3-Hexenyl isobutyrate	0/81	–	0/42	–
Methyl salicylate	2/59	0/81	6/15	2/44
3-Hexenyl-2-methyl butanoate	1/49	–	1/32	1/61
Geraniol	7/21	4/84	9/92	4/28
3-Hexenyl hexanoate	3/22	–	3/74	–
α-Farensene	23/04	0/26	16/67	17/89
Butyl hydroxy toluene	1/64	–	–	–
Δ-Cadinene	1/02	8/67	0/58	1/48
β-Bisabolane	1/02	8/68	–	–
Neophytadiene	0/65	6/68	–	–
Hexadecanoic acid methyl ester	3/62	1/05	0/5	4/75
Hexadecanoic acid	17/29	–	1/26	–
Hexanol	–	3/73	3/21	–
Hexenyl-3-hexanoic acid	–	1/06	–	6/81
Hexyl-hexanoic acid	–	0/34	1/71	3/12
Hexenyl-2-hexanoic acid	–	0/34	2/30	4/71
β-Ionone	–	0/92	1/41	3/57
Nerolidol	–	0/26	–	–
Methyl octa-decanoic acid ester	–	6/3	–	–
Methyl estearate	–	4/5	–	–
Phytol	–	0/75	–	–
Ethyl linaloate	–	0/24	–	–
Ethyl linolenate	–	–	1/81	–
Hexadecane	–	–	0/37	–
Butyl phthalate	–	–	1/9	–
Heptadecane	–	–	0/28	–
β-Cyclocitral	–	–	0/87	–
β-Cariophylene	–	–	0/62	1/85
Methyl linoleate	–	–	1/46	–

TABLE-2
COMPOSITION OF AROMA EXTRACTS OF FRESH, WITHERED, FERMENTED AND DRIED LEAVES (SAMPLE II)

(Sample II)		GC peak area %		
Compound	Fresh	Withered	Fermented	Dried
Linalool oxide (*cis*)	1/78	1/68	0/64	–
Linalool oxide (*trans*)	1/83	1/97	1/55	0/77
Linalool	35/07	23/09	8/6	3/71
3-Hexenyl isobutyrate	2/71	–	0/73	0/74
Methyl salicylate	4/49	4/10	15/23	3/21
Hexenyl-3-hexanoic acid	3/13	–	–	7/19
α-Farensene	9/47	16/95	18/43	33/95
2-Hexenyl butanoate	–	0/76	–	–
Dodecane	–	0/56	–	–
Geraniol	–	23/45	–	15/23
3-Hexanyl caproate	–	0/67	3/73	–
Hexenyl-2-hexanoic acid	–	0/79	–	0/95
Bergamotene	–	0/3	0/61	–
Δ-Cadinene	–	0/47	0/49	–
Farensole	–	1/81	–	–
Hexadecanoic acid	–	1/02	3/66	–
Neophytidiene	–	18/83	2/050	0/77
Benzaldehyde	–	–	0/65	–
β-Ocimene	–	–	1/81	1/61
β-Cyclocitral	–	–	0/37	–
Geranil acetate	–	–	17/47	–
Cyclohexyl hexanoate	–	–	1/52	–
β-Cariophylene	–	–	0/32	–
α-Ionone	–	–	0/33	0/51
Germacrane	–	–	0/28	–
β-Ionone	–	–	2/16	3/46
β-Cyclo germacerane	–	–	0/4	–
Nerolidol	–	–	11/95	–
3-Hexenyl benzoate	–	–	3/10	2/28
Amyl benzoate	–	–	0/61	–
Methyl hexadecanoic acid ester	–	–	0/46	–
Hexenyl-3-hexanoic acid	–	–	7/19	–
β-Damascenone	–	–	–	2/55
β-Farensene	–	–	–	0/85
β-Bisabolane	–	–	–	17/17
Hexanoic acid	–	–	–	2/55

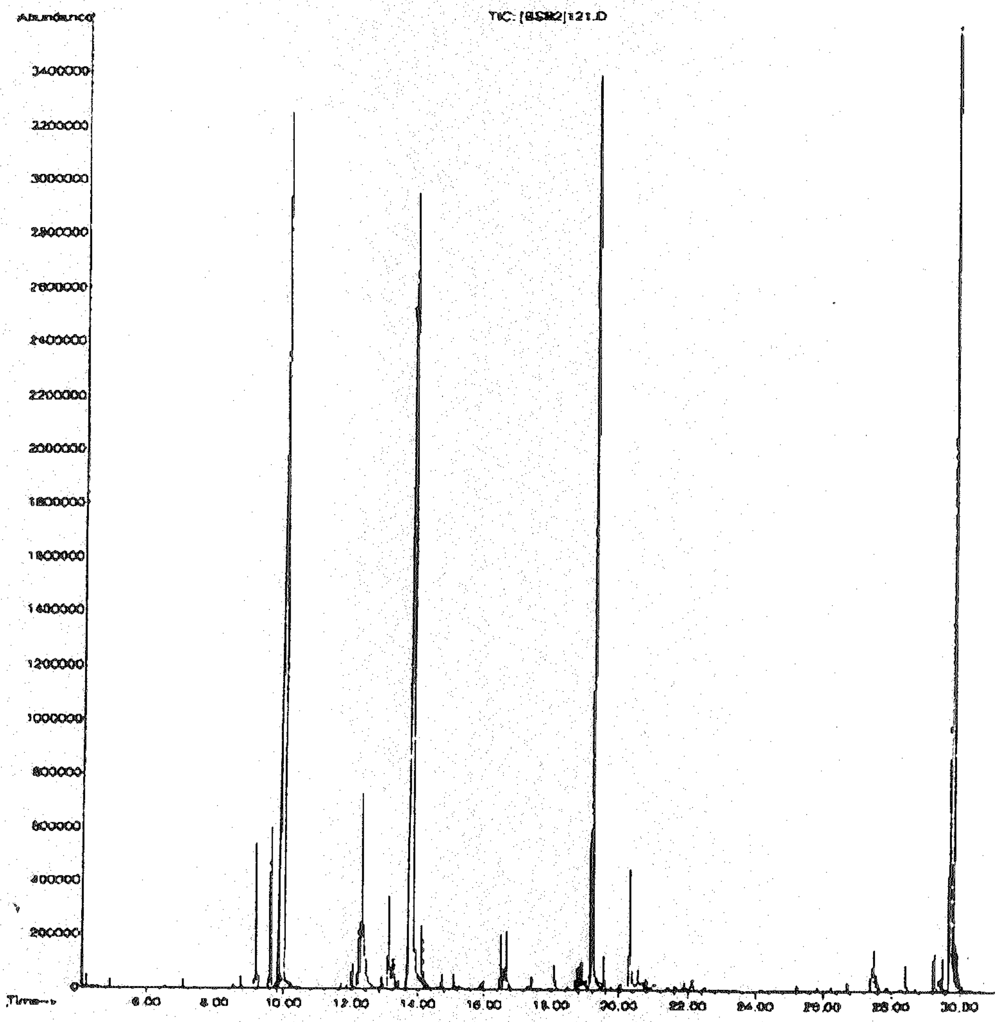

Fig. 2b. Gas chromatograms of the aroma concentrates of sample II prepared using Clevenger method (distillation by water): withered leaves

Comparison of sample I VFCs during black tea production

In sample I, 15 compounds were identified from fresh leaves and α-farensene (23%), hexadecane acid (17%) and some terpene alcohols such as linalool (26%) and geraniol (7%) were the major components. Floral and sweet odours were made of linalool and linalool oxide[10].

Despite the amounts of VFCs that were found to have increased in withered leaves (25 compounds), important terpene alcohols such as linalool (10%) and geraniol (4%) in fresh leaves, that have the main role in fresh leaf aroma, were found to have decreased sharply in withered leaves. In addition, α-farensene which has been reported previously as the main flavour, were found only in small amounts in withered leaves (0.26%). During fermentation the identified 20 VFCs, which form the contents of most main aromatic compound ssuch as linalool and α-farensene, increased, but never reached the highest level in fresh leaves.After fermentation, it

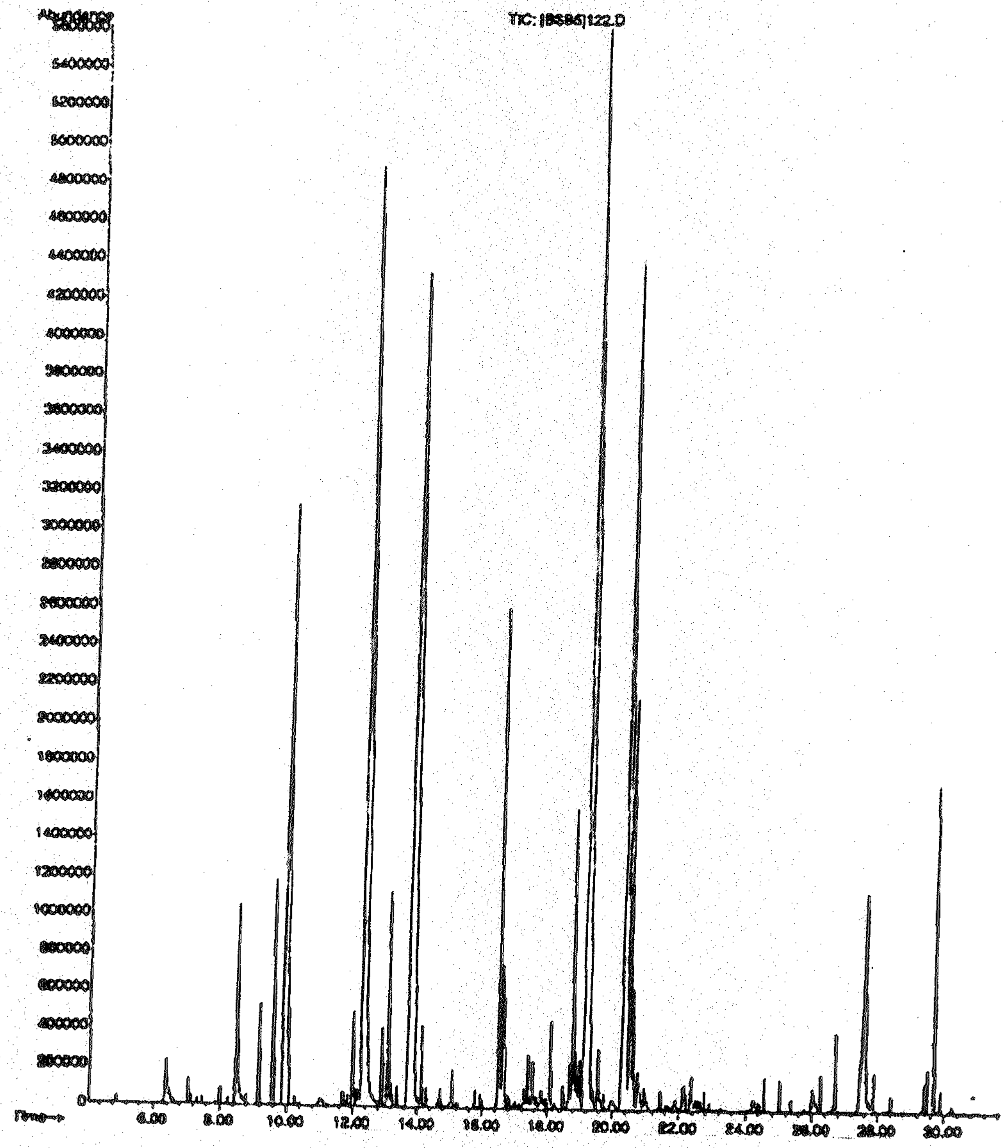

Fig. 2-c. Gas chromatograms of the aroma concentrates of sample II prepared using Clevenger method (distillation by water): fermented leaves

is notable that the amount of neither linalool and oxides, nor even α-farensene decreased during drying and all of them remained the same during fermentation and drying. These findings show that most of the VFCs decreased greatly during withering and some aromatic compounds such as nerolidol, α and β-ionon, mehtyl salicylate were not found noticeable in sample I.

Comparison of sample II VFCs during black tea production

In sample II, 7 compounds were identified from fresh leaves of which linalool (30%) was the highest. At the end of withering, not only more VFCs were found

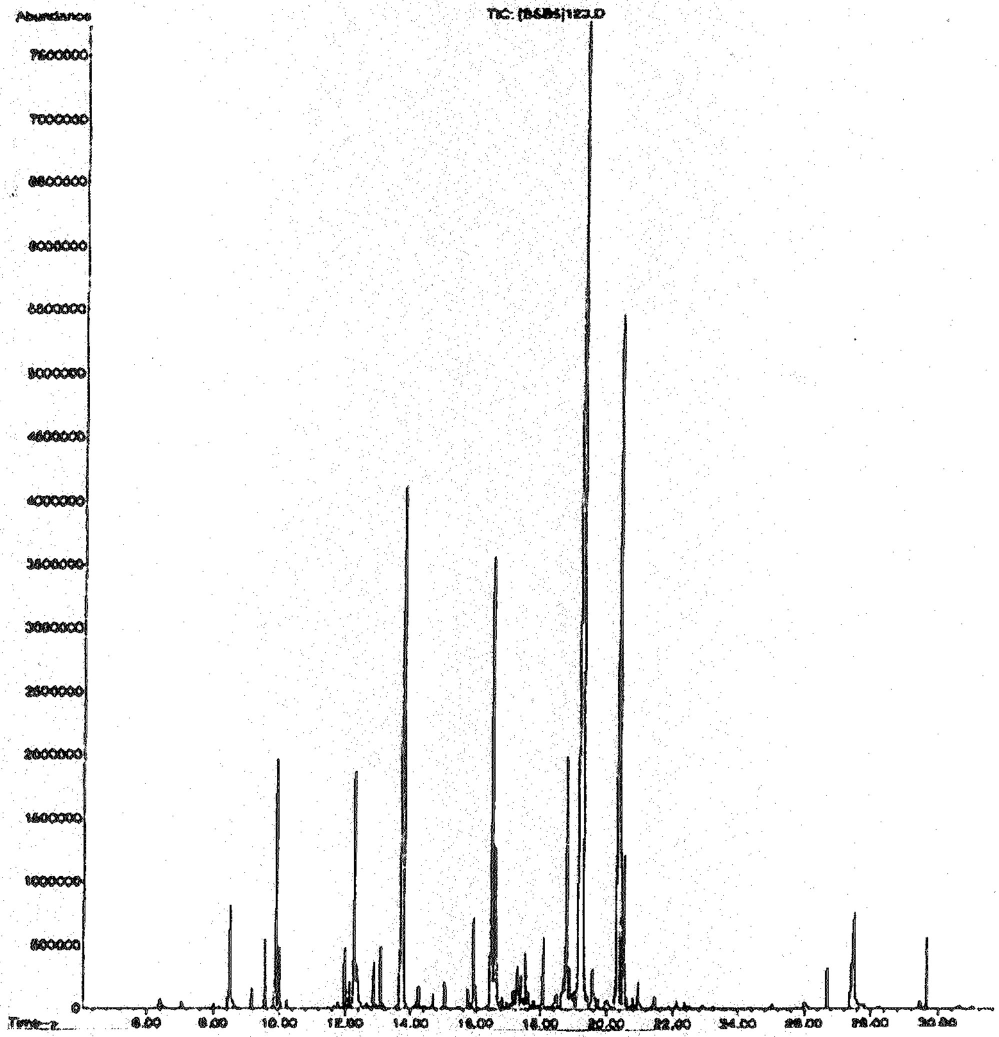

Fig. 2d. Gas chromatograms of the aroma concentrates of sample II prepared using Clevenger method (distillation by water): dried leaves

(15 compounds) but geraniol (23%) and α-farensene (16%) increased sharply and this increasing continued during all the processes also and it is notable that during withering some neophytadiene (18%) was found; also linalool decreased slowly.

After the third stage (fermentation), although 25 compounds were identified, but linalool, which has the main role in aroma, decreased greatly and geraniol was shown as geraniol acetate. In addition, methyl salicylate increased sharply in this stage; at the end of drying the content of main aromatic compounds such as linalool and geraniol decreased.

Comparison of same stages of samples I and II

Sample I includes lower amount of linalool than sample II in fresh leaves, but α-farensene, hexadecanoic acid and geraniol were higher in sample I.

Then, when withered, sample II contains higher α-farensene, geraniol, linalool and nephytadiene than sample I and we conclude that the mild flavour of withered leaves is dependent on the fruity odour of hexenyl esters[10]. After fermentation in sample II were found higher α-farensene, geraniol and methyl salicylate than sample I.

At the end of drying, sample II consists of higher geraniol and α-farensene than sample I, but sample I contains higher linalool and linalool oxides.

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