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VARIATIONS IN FATTY ACID COMPOSITION OF TEA LEAVES (*CAMELLIA SINENSIS*) DUE TO PLUCKING TIME AND CULTIVARS

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ABSTRACT

Fatty acid in green tea leaves is important flavour precursor which has a negative effect on aroma quality of black teas. In this study, fatty acid composition of three cultivars including Shan Chat Tien, Trung Du and PH11 plucked at different time was analyzed by GC-MS/MS. The results showed that C16:0, C18:2, C18:1, C18:0 and C18:3 were the most abundant fatty acids indentified in tea leaves. In particular, total saturated fatty acid in the tea leaves plucked in June and September was higher than total unsaturated fatty acid and vice versa for sample plucked in March. Principal component analysis (PCA) showed that fatty acid compositions in samples plucked in June of three cultivars were similar to each other and they were characterized by C18:0, C22:0 and C20:0. The sample plucked in March and in September were characterized by C18:1, C18:2 and C18:3, andC18:1, C16:1, C18:0, C14:1 and C14:0; respectively. The sample plucked in March had the highest total fat content followed by sample plucked in September and June. The results showed that plucking time and cultivars significantly influenced the fatty acid composition of tea leaves.

Keywords: fatty acids, teas.

1. INTRODUCTION

Black tea as well as green tea is a perennial evergreen shrub belonging to the Camellia genus of the *Theaceae* family. It is manufactured by a variety of processes, producing a range of beverages from green, non-fermented tea through to black, fermented tea [1]. The manufacture of black tea involves complex biological processes which comprise withering, rolling or crushing, fermentation, drying and sorting [1, 2]. These each step of the Orthodox black tea processing has major influence on the final Orthodox black tea quality due to chemical and physiscal changes. . The consumer acceptability of these beverages is mainly dependent on the aroma and taste of the finished product [3]. Black tea aroma, which is composed of the volatile flavour compounds (VFCs) generated during tea processing, is an important quality parameter, determining the price of made black tea. 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 flavour aroma to black tea, are mainly derived from 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 VFC index [4]. 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 [5]. 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 [6].

Thus, lipid emerges as an important area for research and quality control. The major fatty acids, such as linolenic acid, are mostly found in the galactolipid fraction, while oleic and linoleic acids are derived from neutral and phospholipid components. The palmitic acid content is found to be highest in the phospholipid fraction and the minor fatty acids, such as lauric, myristic and stearic acids are higher in the neutral lipid fraction. The precursors of negative flavours in tea are a subject of great interest to the tea industry, and it is therefore necessary to determine their distribution in the tea leaves due to plucking time and cultivars. The present investigation reports the fatty acid composition of various lipid fractions in different cultivars including Shan Chat Tien, Phu Ho 11 and Trung Du which is ranged from higher to lower quality, respectively and its changes at different plucking times.

2. MATERIAL AND METHOD

2.1. Materials

Tea leaves of cultivars Trung Du (TD), Shan Chat Tien (SH) and Phu Ho 11 (PH11), representing the genetically diverse Northern Vietnam cultivars, harvested from Phu Tho, Vietnam, in March, June, and September, were used for analyzing chemical composition.

2.2. Fatty acid analysis

Lipids were extracted from green tea leaves by using chloroform-methanol 2:1 (v/v) and centrifuged at 3000 rpm for 10 min. Fatty acids were converted to fatty acid methyl ester (FAME) by dissolving in hexane and 0.5 N methanolic NaOH. The fatty acid composition was identified in triplicate by separating the FAME on a GC-MS/MS (model GC 7890A/GCMS 7000 triple quad-Agilent, USA) equipment with DB1 column (30 m × 0.25 mm × 0.25 µm). The temperature program was 0,5 min at 50 ^{o}C and then it was increased to 200 ^{o}C with 15 ^{o}C/min, and then increased to 250 ^{o}C with 3 ^{o}C/min, held for 5 min. The injection temperature was 250 ^{o}C; interface temp. was 280 ^{o}C; split flow was 1 (ml/m); and the split ration was 10:1 [5].

2.3 Lipid content

The lipid content of the samples were determined by AOAC (2004) method [7].

2.4. Statistical analysis

Principal component analysis (PCA) was performed by SPAD 5.5 software.

3. RESULTS AND DISCUSSION

The fatty acid compounds contribute to the flavour of tea leaves and tea products. There have been many efforts to establish genotypical, plucking standard, process variables in lipid and fatty acid composition of plants [1, 5, 6]. Variation in fatty acids could play an important role in the selection of cultivars with improved tea-making potential. According to literature searched, there is no study on fatty content of tea leaves in these different cultivars in Vietnam. Thus, these results may be the first study to provide data that the tea leaves possess fatty acids. The results showed that tea leaves studied contained five major compounds including linoleic acid, linolenic acid, palmitic acid, oleic acid and stearic acid (Table 1), while the content of fatty acids which has number carbon from C8:0 to C12:0 as well as C20:0 to C24:0 is detected low in all samples. Table 1 shows that plucking time changes in total lipid content along with climatic conditions. The lipid content was found to be highest in March followed by September, and lowest in June. Thus, it is clear that climatic factors have an impact on lipid synthesis. It is also noteworthy that the variations of fatty acids in all the three cultivars studied are not similar. Marked variations in proportions of unsaturated and saturated fatty acids due to plucking time and cultivars were observed. In general, a high content of polyunsaturated fatty acid in samples was plucked in March and vice versa for June and September's samples (Fig. 1). The high content of polyunsaturated fatty acid is known as the `Pacha Taint' problem which causes off-flavour in black tea on storage, and is dominant in South tea industries, is reported to be due to poor lipid metabolism [1].

Table 1. Variation in the fatty acid composition (relative content) with plucking time and cultivars.

Fatty acids	**March**			**June**			**September**		
	TD	**SH**	**PH11**	**TD**	**SH**	**PH11**	**TD**	**SH**	**PH11**
	Percentage of total fatty acid methyl ester, %								
C8:0	nd	0.53	0.07	nd	nd	nd	0.81	2.57	1.06
C10:0	nd	0.58	nd	0.34	nd	0.09	0.96	2.95	0.86
C11:0	nd	nd	nd	nd	nd	11.9	nd	nd	0.38
C12:0	0.38	4.53	0.1	2.29	nd	nd	1.18	5.21	7.64
C14:1	nd	nd	nd	nd	1.68	nd	2.47	6.26	nd
C14:0	2.91	3.19	0.51	3.92	5.21	1.06	18.42	9.67	3.28
C16:1c	1.65	0.99	0.6	0.97	nd	1.29	nd	1.54	0.84
C16:0	22.64	29.8	15.72	15.3	34.98	16.39	24.3	25.9	32.41
C18:2n6	16.42	12.14	39.04	14.37	nd	6.8	15.75	10.52	13.53
C18:3n3	22.58	13.34	19.22	nd	nd	nd	nd	nd	nd
C18:1c	17.07	23.3	15.03	nd	nd	16.47	25.25	21.9	29.9
C18:0	10.83	7.46	6.59	49.8	23.05	41.12	7.35	9.52	7.27
C18:1t	2.45	0.52	nd	nd	nd	nd	0.41	nd	nd
C20:0	nd	nd	nd	9.2	33.08	2.23	1.23	0.55	nd
C22:0	0.55	0.62	nd	1.54	nd	1.25	0.86	0.56	nd
C24:0	nd	nd	nd	nd	nd	nd	nd	nd	0.84
Total saturated FA	***37.31***	***46.71***	***22.99***	***82.39***	***96.32***	***74.04***	***55.11***	***56.93***	***53.74***

Total unsaturated FA	***60.17***	***50.29***	***73.89***	***15.34***	***1.68***	***24.56***	***43.88***	***40.22***	***44.27***
Total lipid*	***2.68***	***1.99***	***2.31***	***2.18***	***1.91***	***1.93***	***2.36***	***1.94***	***2.04***
MAT, ^{o}C		*20*			*28.9*			*25*	
THS, *h*		*15*			*161*			*106*	
MAP, mm		*102.5*			*398.0*			*244.8*	

MAT: Monthly Average Temperatures; THS: Total number of hours of sunshine;
MAP:Monthly Average Precipitation; TD: Trung Du cultivar; SH: Shan Chat Tien cultivars; PH11; FA: fatty acid
nd: not detective
** Total lipid (g/100g)*

In total 12 fatty acids identified in Trung Du cultivar which plucked in March, C16:0 was determined as the most abundant fatty acid; its concentration was 22.64 %, followed by C18:3 (22.58 %), and next is C18:1 and C18:2 (17.07 % and 16.42 %, respectively). The lowest fatty acid content was identified that are C8:0 (0.29) and C12:0 (0.38 %), while total 14 fatty acid was found in Shan Chat Tien cultivar, C16:0 was also the most abundant fatty acid (29.8 %), next is C18:1 (23.30 %), and the lowest is fatty acids such as C20:1 (0.52 %), C10:0 (0.58 %) and C16:1t (0.47 %). The highest fatty acid in total 10 fatty acid that was found in PH11 cultivar is C18:2 (39.04 %), followed by C18:3, C18:1 and C16:0 (19.22 %, 15.72 % and 15.03 %, respectively).

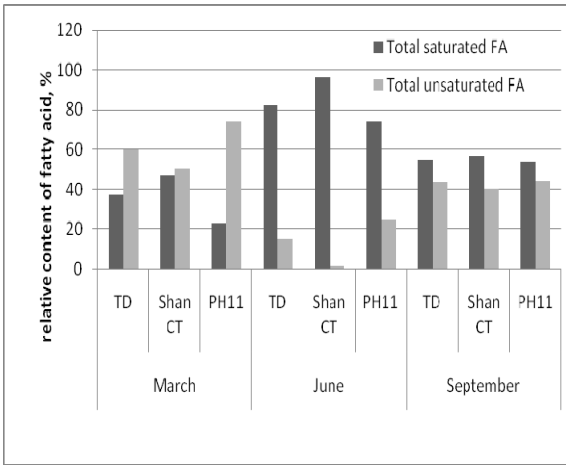

Figure 1. Polyunsaturated and saturated fatty acid content in tea leaves at different plucking time and cultivars.

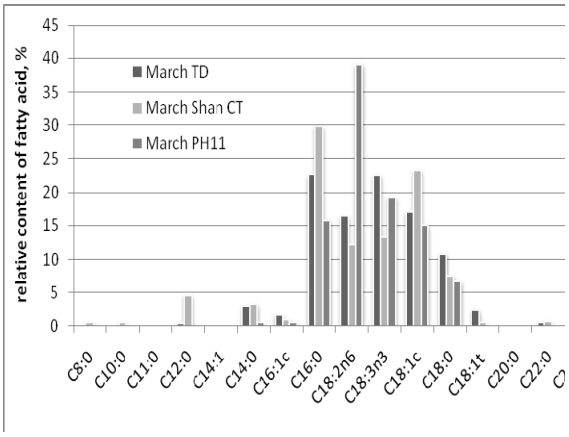

Figure 2. Fatty acid content in tea cultivars plucked in March.

Analysis fatty acids in three cultivars were plucked in June. The results showed that the content of saturated is increased in comparison with samples plucked in March (Fig 3). Only 9 fatty acids were identified, and the C18:0 was determined as the highest content (49.8 %), followed by C16:0 (15.3 %) and C18:2 (14.37 %). Meanwhile, just only 5 fatty acids was identified in Shan Chat Tien cultivar. In that, C16:0 (34.98 %), C20:0 (33.08 %), C18:0 (23.05 %) were the most abundant. Eleven fatty acids was found in tea leaves of PH11 cultivar with the highest content is C18:0 (41.12 %), C18:1 (16.47 %) and C16:0 (16.39 %). The results also showed that just only C14:0, C18:1 and C11:0 were identified in PH11 cultivar, which was plucked in June.

Twelve fatty acids were identified in Trung Du cultivar which was plucked in September, in that the fatty acids with high content are C18:1c (25.25 %), C14:0 (18.42 %) and C16:0 (24.3

%). Meanwhile, in 13 fatty acids was found in Shan Chat Tien cultivar with the same time plucking, C16:0 (25.90 %), C18:1c (21.90 %) was the most abundant, and next is C18:2 (10.5 %), C14:0 (9.67 %) and C18:0 (9.5 %); 11 fatty acids were identified in PH11 cultivar, in that C16:0 (32.41 %), C18:1c (29.90 %) are two fatty acids to have a high content, while C20:0 and C22:0 is very low (0.55 %), 0.56 %, respectively). After identification and qualification of fatty acids in tea leaves, the data matrix of relative contents was preprocessed through generalized log transformation and pareto scaling. In exploratory data analysis, principal component analysis (PCA) was employed to screen cluster of sampling and fatty acid distribution in three groups. From the sample score plot obtained from PCA in Figure 5, nine tea leaves samples from three cultivars and plucking times were clearly classified in to three groups in that samples which were plucked in the same month are close to each others and may be combined as a group.

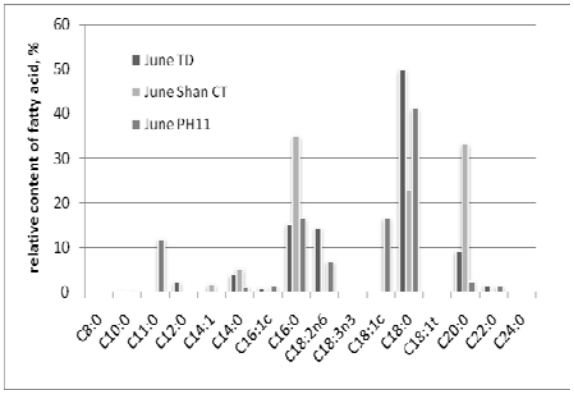

Figure 3. Fatty acid content in tea cultivars plucked in June.

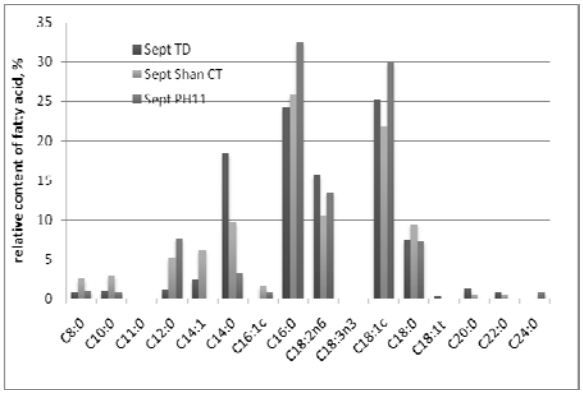

Figure 4. Fatty acid content in tea cultivars plucked in September.

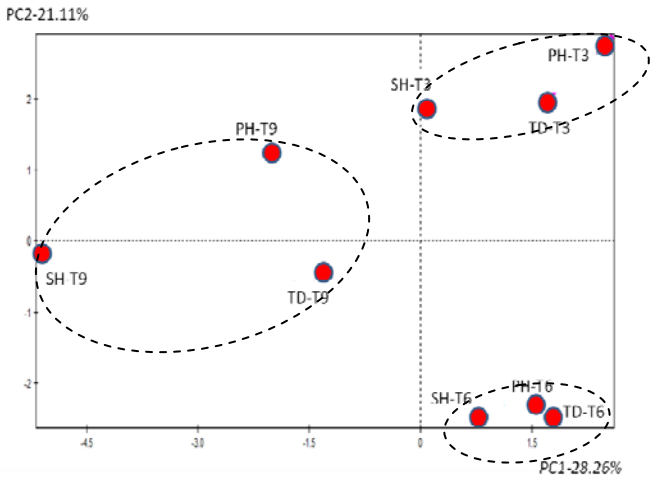

Figure 5. Score plot between the first 2 PCs.

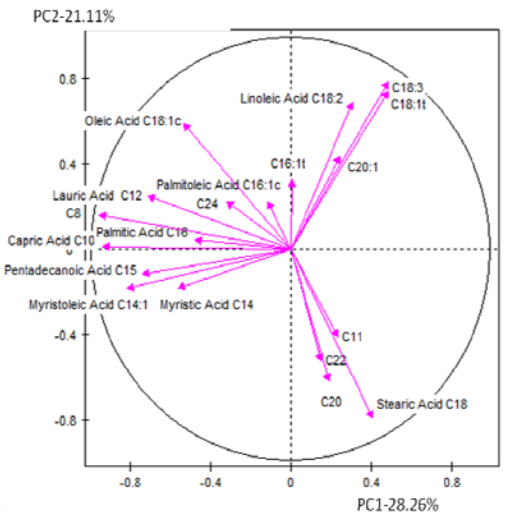

Figure 6. Variables plot between the first 2 PCs.

As shown in the Figure 6, the variables plot between the first 2 PCs. More importantly, the results showed that the fatty acids distribution in three groups from this figure as follows: (*a*) the tea leaves plucked in March, relatively high content of C18:2, C18:1t and C18:3; (*b*) the tea

leaves plucked in June, relatively high content of C20:0, C22:0, C18:0 and C11:0; (*c*) the tea leaves plucked in September, relatively high content of C18:1c, C16:1c, C8:0 and C10:0.

4. CONCLUSION

This study showed that fatty acids composition of tea leaves varies depending on the cultivars and plucking times. The most abundant fatty acids in all samples were C18:1, C18:2 and C18:3. Total lipid in March's samples is higher than June and September's samples. This result demostrated that the lipid content tend to be higher in dry season and decreased in rainy season. The high content of polyunsaturated fatty acid is known as the `Pacha Taint' problem that causes off- flavour in black tea on storage, which is reported to be due to poor lipid metabolism.

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TÓM TẮT

SỰ THAY ĐỔI HÀM LƯỢNG ACID BÉO TRONG LÁ CHÈ (*Camellia sinensis*)
THEO THỜI GIAN THU HÁI VÀ GIỐNG CHÈ

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Các axít béo trong lá chè đóng vai trò quan trọng đối với chất lượng cảm quan mùi của sản phẩm chè đen. Trong nghiên cứu này, thành phần axít béo trong lá chè của 3 giống chè bao gồm Shan Chất Tiền, Trung Du và PH11 ở các thời điểm thu hái khác nhau được phân tích bằng kĩ thuật GC-MS/MS. Kết quả nghiên cứu cho thấy trong tất cả các mẫu phân tích thành phần axít béo chiếm chủ yếu là các axít béo C16:0, C18:2, C18:1, C18:0 và C18:3. Trong đó, mẫu tháng 6 và tháng 9 có hàm lượng axít béo no cao hơn so với acid không no, tỉ lệ này ngược lại đối với mẫu tháng 3. Kết quả phân tích thành phần chính (PCA) cho thấy, thành phần axít béo trong mẫu chè thu hái vào tháng 6 có xu hướng gần nhau và được đặc trưng bởi các axít béo như C18:0, C22:0 và C20:0. Mẫu thu hái vào thời điểm tháng 3 được đặc trưng bởi các axít béo như C18:1, C18:2 và C18:3. Trong khi đó, mẫu thu hái vào tháng 9 được đặc trưng bởi các axít béo như C18:1, C16:1, C18:0, C14:1 và C14:0. Tổng hàm lượng axít béo trong mẫu tháng 3 cao nhất, tiếp theo là mẫu tháng 9 và cuối cùng là mẫu tháng 6. Kết quả nghiên cứu cho thấy thành phần axít béo trong lá chè bị ảnh hưởng bởi thời điểm thu hái và giống chè.

Từ khóa: axít béo, chè.