S. L. J. Tea Sci. 57 (2), 46-49, 1988, Printed in Sri Lanka.

ANALYSIS OF MAJOR FATTY ACIDS IN TEA

A.C. Liyanage, M. J. de Silva and A. Ekanayake*
(*Tea Research Institute of Sri Lanka, Talawakele, Sri Lanka*)

Lipids were extracted from tea leaves using $CHCl_3$: MeOH (2:1) mixture from ten tea clones and a seedling during the flavour season in order to examine possible differences in their fatty acid composition. No significant relationship was observed between the fatty acid distribution and the quality of the clone.

INTRODUCTION

In black tea, trans-2-hexenal and hexenal has been shown to arise from the oxidative enzymic breakdown of linolenic and linoleic acids respectively (Bajaj and Devchandhury, 1984). In the manufacture of black tea, during fermentation linolenic and linoleic acids decrease and trans-2-hexenal and hexenal increase (Hatanaka and Harada, 1973). Although excess of hexenal and trans -2-hexenal is detrimental to tea flavour, several of their esters such as cis-3-hexenyl trans-2-hexenate and hexyl butyrate, etc have been found to enhance tea flavour (Kawamura, Nogao and Yamazaki, 1985). However there is no data available on the role of fatty acids on the quality of tea while only a few papers have been published on fatty acid analysis in tea leaves (Saijo, 1973; Yamanishi, 1981).

In the present study, an attempt was made to quantify some of the major fatty acids present in different clones and a seedling during the Dimbulla flavour season.

EXPERIMENTAL

Sample preparation

The clones used in this study were TRI 777, TRI 2142, TRI 2023, TRI 62/9, TRI 62/1, TRI 2025, TRI 2043, DT1, CY9, DN and a seedling. One hundred g of flush taken from each of the clones and the seedling was steamed for 5 min and dried in an oven using hot air flow at 60° C. Ten g of dried flush was macerated in a Waring Blendor with 100 ml $CHCl_3$:MeOH (2:1) mixture for 5 min. The suspension was stirred on a magnetic stirrer for 3h at room temperature and the extract was subjected to vacuum filtration. The residue on the filter was washed with the $CHCl_3$:MeOH mixture and re-macerated with a further 60 ml of the solvent mixture for 5 min, and stirred on a magnetic stirrer for ½h at room temperature and filtered. The combined extracts were dried with anhy-

* Present address: Department of Biochemistry, University of Peradeniya, Peradeniya.

drous Na_2SO_4, filtered and evaporated to dryness on a rotary vacuum evaporator and the dry residue dissolved in abs. E.OH (30 ml). The ethanolic solution was saponified under nitrogen for 18 h at room temperature with 60% (w/v) KOH (8 ml). After saponification, water (90 ml) was added, and the non-saponifiable substances and carotenoids removed by extraction with peroxide free Et_2O (4 x 50 ml). The aqueous layer contains saponified substances. The Et_2O extracts were washed with water and the aqueous layers were pooled, acidified with conc. HCl (5-6 ml) and extracted with $CHCl_3$ (2 x 50 ml). The aqueous layer was discarded and the $CHCl_3$ layer washed with water (2x50 ml) to remove HCl and dried with anhydrous Na_2SO_4, filtered and evaporated to dryness *in vacuo* by rotary evaporation at 40° C. The dried extract was transferred quantitatively to a 10 ml volumetric flask with a small quantity of $CHCl_3$. The volume was made up to 10 ml and analysed by gas chromatography. The experiment was done in duplicate for each clone and seedling and the mean value taken.

Analysis of fatty acids by gas chromatography

The analysis was performed using a Tracor 60 model chromatographic apparatus connected with Varian data system. The analytical conditions were as follows:

Column:	Glass column 1 m x 2.0 mm (i.d.) packed with GP 5% DEGS-PS,A
Column temp:	200° C isothermal
Injection temp:	200° C
Detector:	FID, 250° C
Carrier gas:	Ar 20 ml min^{-1}
Sample size:	2.0 μl

Preparation of standard mixture of fatty acids

0.1g of each fatty acid was dissolved in 10 ml $CHCl_3$. This original solution was diluted 2,4 and 8 times for a calibration curve.

RESULTS AND DISCUSSION

A gas chromatogram of the standard mixture of fatty acids along with that of a typical gas chromatogram of fatty acid extract from tea is shown in Figures 1 and 2.

Quantitative determination was obtained by calculations based on the peak area percentage and the recorder response of individual fatty acids (Table 1).

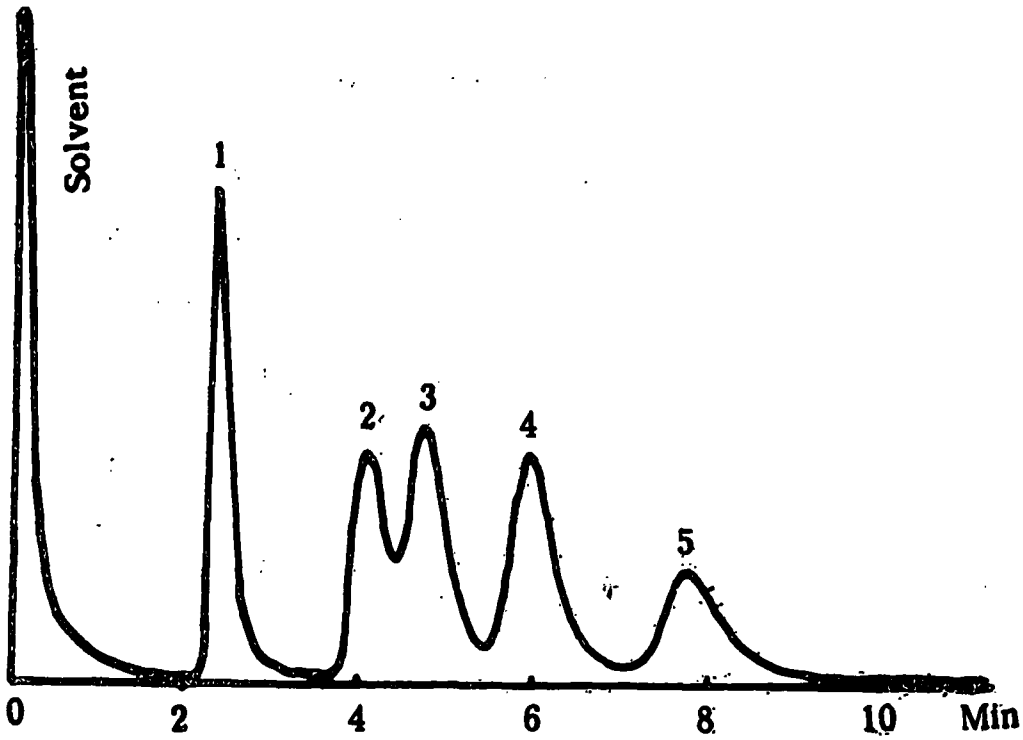

Fig. 1 — Gas chromatogram of the standard fatty acid mixture
Peak 1 — Palmitic acid. Peak 2 — Stearic acid Peak 3 — Oleic acid.
Peak 4 — Linoleic acid, Peak 5 — Linolenic acid.

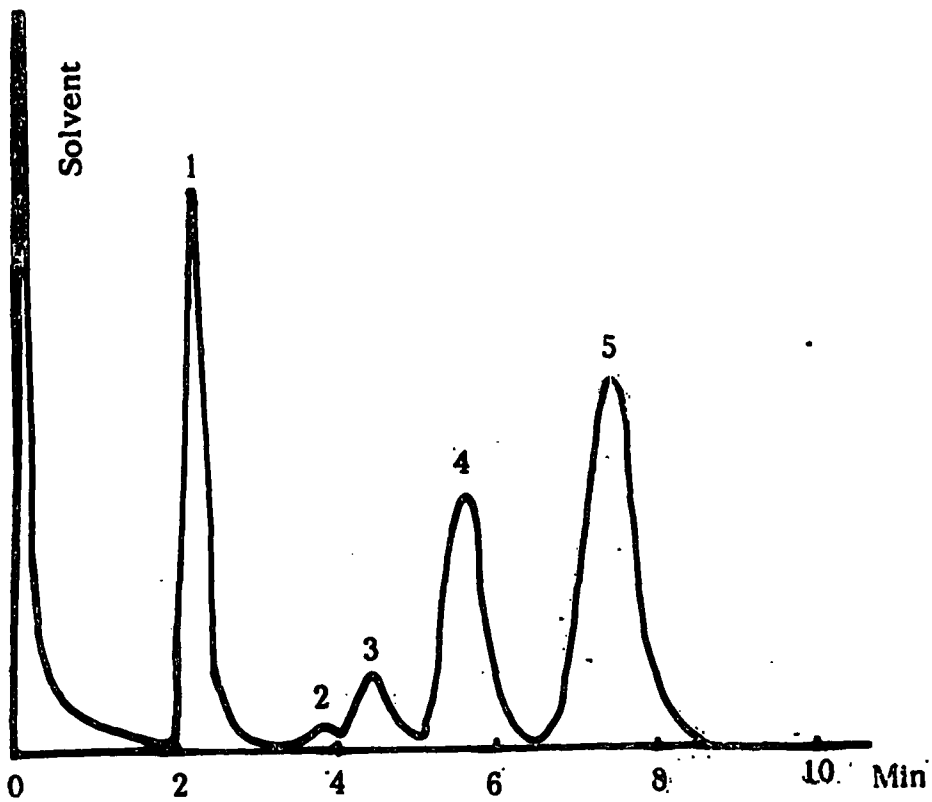

Fig. 2 — Typical gas chromatogram of fatty acids from tea leaves
Peak 1 — Palmitic acid. Peak 2 — Stearic acid. Peak 3 — Oleic acid.
Peak 4 — Linoleic acid. Peak 5 — Linolenic acid.

TABLE I — *Composition of major fatty acids in tea leaves*

Clone	*Palmitic acid P 16:0 mg/100g tea leaves* *	*Oleic acid O 18: 1 mg/ 100g tea leaves* *	*Linoleic acid L18: 2 mg/ 100g tea leaves* *	*Linolenic acid L18:3 mg/ 100g tea leaves* *	*Total fatty acids*
TRI 777	545	240	535	930	2250
DTI	340	165	325	565	1395
TRI 2142	315	225	365	565	1470
TRI 2023	395	190	400	690	1675
TRI 62/9	335	120	320	575	1350
TRI 62/1	370	125	325	515	1335
TRI 2025	440	175	455	875	1945
CY 9	445	205	460	905	2015
DN	510	200	485	930	2125
TRI 2043	505	135	455	810	1905
Seedling	375	170	365	745	1655

Note *dry weight basis

No significant relationship with the quality of clone or seedling was shown. In all instances linolenic acid was the most abundant while linoleic acid was about half the concentration of linolenic acid. These two acids are well known precursors of C_6 alcohols, their esters and aldehydes which are very important flavour compounds in tea (Kawamura *et al*, 1985).

Studies will be continued during the non flavour season to monitor any changes in the fatty acid composition.

ACKNOWLEDGEMENTS

We thank Prof. Tei Yamanishi of Ochanomizu University, Tokyo, for her valuable suggestions and help in preparing this manuscript.

REFERENCES

BAJAJ, K.L. and DEVCHANDHURY M.N. (1984). Effect of Phosphrous on Fatty Acids of Tea leaves and on the quality of teas. *J. F. Sci, Tech*. 21, 392 - 394.

HATANAKA, A, and HARADA, T. (1973). Formation of Cis -3-hexenal; trans-2 hexenal and cis-hexenal in macerated *Thea sinensis* leaves. *Phytochem* 12, 2341 - 2346.

KAWAMURA S, NOGAO A, and YAMAZAKI, M. (1985). Lipids of Tencha. Nippon Shokuhim Kogyo Gakkaishi (Journal of Food Industry). Japanese with English summary 32, 870 - 875.

SAIJO, R. (1973). Biochemical studies on the formation of volatile compounds in black tea, *Bull Nat Res. Inst. Tea*. 48, 171.

YAMANISHI, T. (1981). In Flavour Research: Recent Advances, eds R. Teramishi, R.A.Flatt and Sugisawa, Marcel Dekker, New York.