Food Chemistry 38 (1990) 211–219

Changes in Fatty Acid Levels of Young Shoots of Tea (*Camellia sinensis* L.) due to Nitrogenous Fertilizers

P. O. Owuor

Tea Research Foundation, PO Box 820, Kericho, Kenya

&

R. M. Munavu & J. W. Muritu

Chemistry Department, College of Biological
and Physical Sciences, University of Nairobi, PO Box 30197, Nairobi, Kenya

(Received 6 October 1989; revised version received
and accepted 12 December 1989)

ABSTRACT

The unsaturated fatty acids (FA), linolenic acid (C18:3) and linoleic acid (C18:2), responsible for the production of undesirable volatile flavour compounds in black tea, dominate FA composition of young shoots of clonal tea. These FA levels increase with increase in nitrogenous fertilizer rates whether NPKS 25:5:5:5 or NPK 20:10:10 fertilizer is used. Results explain the general flavour quality deterioration with increase in nitrogenous fertilizer rates.

INTRODUCTION

The unsaturated fatty acids (FA) in the young tender shoots of tea (*Camellia sinensis* L.) break down through lipoxygenase-initiated oxidation during manufacture of black tea to form volatile flavour compounds (VFC) (Hatanaka *et al.*, 1977, 1979, 1987). Through this process linoleic acid ((*Z*, *Z*)-9,12-hexadecadienoic acid (C18:2)) breaks down to form hexanal which reduces to 1-hexanol (Saijo & Takeo, 1972; Hatanaka & Harada, 1973; Hatanaka *et al.*, 1987). The VFC produced by FA breakdown normally impart inferior green flavour to black tea (Yamanishi *et al.*, 1968; Wickremasinghe *et al.*, 1973; Yamanishi, 1978; Fernando & Roberts, 1984),

Food Chemistry 0308-8146/90/$03·50

and are classified as Group I VFC (Owuor *et al.*, 1987*a*,*b*). It has been demonstrated that high rates of nitrogenous fertilizers lower quality of black tea (Owuor *et al.*, 1987*a*). However, the changes in the levels of the precursor to the compounds responsible for quality due to changes in nitrogenous fertilizers have not been quantified. Thus, it is not known whether it is the precursor compounds or the enzymes responsible for their transformation to the flavour quality compounds which change due to nitrogenous fertilizers.

Although nitrogenous fertilizers are known to lower quality, their application to tea is a prerequisite, as several studies have shown yield benefits due to their application (Wanyoko, 1981, 1983). In East Africa, no significant tea yield response has been recorded from phosphatic (Anon., 1965) or potash (Chennery, 1963) fertilizer application. However, both these nutrients are known to be beneficial to tea as potassium helps tea to recover from pruning while phosphorus helps tea to build strong frames and roots. Since appreciable amounts of nitrogen (3–3·4%), phosphorus (0·20–0·33%) and potassium (0·51–1·80%) are lost in normal harvestable crop (Wanyoko, 1983), it is mandatory that these nutrients be added annually. Additionally, large amounts of these nutrients are lost due to leaching and fixation. Othieno (1980) recently speculated that the efficiency of nutrients uptake by the tea bush in Kenya is less than 50%. Thus large amounts of NPK are lost through fixation and leaching.

Clone S15/10 is very high-yielding in Kenya and up to 9000 kg made black tea ha^{-1} $year^{-1}$ has been attained by this clone in years with good cropping weather. Although the recommended rate of fertilizer in Kenya is 150–200 kg N ha^{-1} $year^{-1}$ as compound fertilizer (NPKS 25:5:5:5 or NPK 20:10:10), these rates may be inadequate for such a high-yielding clone. An experiment was therefore initiated on this clone where it is yielding up to 9000 kg black tea ha^{-1} $year^{-1}$, using high rates of nitrogen to determine its appropriate nutritional requirement under such situations and to determine how the high rates of nitrogen affect quality of made black teas.

The results of studies to determine the effects of the high rates and different sources (NPKS 25:5:5:5 or NPK 20:10:10) of nitrogenous fertilizers on the fatty acids composition of Clone S 15/10 in Kenya are reported.

MATERIALS AND METHODS

Tea samples

The tea leaves used in this experiment were obtained from Clone S15/10, grown at Kaproret Estate of the African Highlands Produce Co. in Kericho.

The experimental fertilizer application was made on 25 March 1988. Leaf samples for FA analysis were taken on 23 April 1988, i.e. 1 month after the first fertilizer application.

The experiment was laid out in a split plot design (rates vs fertilizers) and was replicated three times. The fertilizer rates were 0, 100, 150, 300, 450 and 600 kg of N as NPKS 25:5:5:5 and NPK 20:10:10 put in a single application. Tea (two leaves and a bud shoot) was plucked from different treatments. Within 1 h of sampling, the leaves were steamed for 1 min to deactivate the enzymes responsible for degradation of the fatty acids. The leaves were oven-dried for 4 h at 96°C. The dried samples were then ground to fine powder using a coffee grinder. The powder was weighed (10 g) plus 0·15 g heptadecanoic acid (C17:0) (internal standard), then extracted twice with 2:1 (v/v) chloroform and methanol mixture for 3 h with continuous stirring at room temperature. The mixture was shaken with 20 ml dilute potassium chloride solution. The organic layer was separated and solvent removed using rotatory evaporator.

The lipids were then transesterified or esterified to their methyl esters as follows: to the lipids mixture, in a round bottom flask fitted with a condenser, was added 10 ml of 0·5N methanolic sodium hydroxide solution. A small volume of tetrahydrofuran was added to effect solubility of the lipids. The mixture was then refluxed for 10 min. About 10 ml of boron trifluoride–methanol complex (about 14% BF_3) was added and the mixture was refluxed for a further 2 min. The solution was cooled to room temperature followed by addition of 5 ml hexane. The mixture was boiled again for 2 min.

A saturated sodium chloride solution was added and the hexane layer was separated into a container with anhydrous sodium sulphate. The extraction was done twice. To the hexane layer was added activated silica gel with continuous stirring until all the chlorophyll was removed from the solution. The silica gel was then filtered off and hexane removed under reduced pressure on the rotatory evaporator. The fatty acid methyl esters (FAMES) in a small amount of hexane were stored in a sample bottle.

GC analysis was done under the conditions of Munavu (1983). Samples were identified by corroborative retention times on the GC of authentic samples.

RESULTS AND DISCUSSION

The results of the determinations are presented in Tables 1 and 2. *n*-Decanoic (lauric) acid (C12:0) and *n*-tetradecanoic (myristic) acid (C14:0) were not detected in the leaves of clone S15/10. The order of occurrence of

TABLE 1
Changes in Fatty Acid levels[a] (mg/100 g dry wt) with Rates and Sources of Nitrogen

Fatty acid	*Source of nitrogen*	*r*[b]	*Rate of nitrogen (Kg N ha^{-1} year^{-1})*						*Mean nitrogen source*			
			0	*100*	*150*	*300*	*450*	*600*				
C16:0										CV% Rate	(*a*) =	1·81
	NPKS 25:5:5:5	0·79*	13·78	12·40	16·39	15·61	16·47	17·38	15·34	Source	(*b*) =	3·98
										LSD (*P* =	0·05	0·01)
	NPK 20:10:10	0·94*	14·94	15·40	14·62	16·49	17·52	17·74	16·12	Rate (*a*)	0·73	1·04
										Source (*b*)	0·45	0·64
	Mean rate	0·95*	14·36	13·90	15·51	16·05	17·00	17·56		Rate × source (*a* × *b*)	1·11	1·56
C16:1										CV% Rate	(*a*) =	36·49
	NPKS 25:5:5:5	−0·14	Trace[c]	0·85	1·28	1·06	0·92	Trace	0·69	Source	(*b*) =	58·03
										LSD (*P* =	0·05	(0·01)
	NPK 20:10:10	−0·88*	0·93	1·02	1·07	0·59	0·68	Trace	0·54	*a*	0·58	NS
										b	NS	NS
	Mean rate	−0·46	0·47	0·94	0·64	0·83	0·82	Trace		(*a* × *b*)	1·11	1·56
C18:0									11·95	CV% Rate	(*a*) =	6·80
	NPKS 25:5:5:5	0·21	12·17	10·40	12·76	12·60	10·41	13·35		Source	(*b*) =	10·41
										LSD (*P* =	0·05	0·01)
	NPK 20:10:10	0·70	11·59	11·45	5·41	13·61	15·64	16·48	12·37	*a*	2·13	3·02
										b	NS	NS
	Mean rate	0·76*	11·88	10·93	9·09	13·11	13·03	14·91		(*a* × *b*)	2·25	3·16

C18:1									9·79	CV% Rate	(*a*) = 2·71
	NPKS 25:5:5:5	0·68	8·68	10·09	9·98	9·06	10·09	10·83		Source	(*b*) = 5·24
										LSD (*P* =	0·05 0·01)
	NPK 20:10:10	0·997*	8·43	8·78	9·05	9·78	10·74	11·54	9·72	*a*	0·68 0·97
										b	NS NS
	Mean rate	0·95*	8·56	9·43	9·52	9·42	10·42	11·19		(*a* × *b*)	0·91 NS
C18:2									22·37	CV% Rate	(*a*) = 2·29
	NPKS 25:5:5:5	0·90*	19·03	20·57	23·27	22·37	23·54	25·47		Source	(*b*) = 4·36
										LSD (*P* =	0·05 0·01)
	NPK 20:10:10	0·95*	16·52	20·32	20·63	23·51	25·59	25·84	22·07	*a*	1·31 1·86
										b	NS NS
	Mean rate	0·96*	17·78	20·44	21·95	22·93	24·56	25·66		(*a* × *b*)	1·73 2·42
C18:3									77·67	CV% Rate	(*a*) = 1·59
	NPKS 25:5:5:5	0·87*	58·85	65·54	82·64	81·35	88·17	89·46		Source	(*b*) = 2·33
										LSD (*P* =	0·05 0·01)
	N 20:10:10	0·97*	60·47	66·64	68·61	85·05	88·90	93·99	77·28	*a*	3·18 4·52
										b	NS NS
	Mean rate	0·96*	59·66	66·09	75·63	83·20	88·54	91·73		(*a* × *b*)	3·22 4·52

[a] Fatty acid methyl esters (FAMES).
[b] Correlation coefficient of linear regression analysis between FAMES levels and rate of nitrogen.
[c] Where only trace levels of the FAMES were obtained, a figure of 0·001 was used in the statistical analysis.
* Significant at $P = 0·05$.
a, Rate of nitrogen; *b*, source of nitrogen.
CV%, coefficient of variation.

TABLE 2

Changes in Total Fatty Acids, Total Unsaturated Fatty Acids and Sum of C18:2 plus C18:3 levels[a] (mg/100 g dry wt) due to Rates and Sources of Nitrogen

Fatty acid	*Source of nitrogen*	*r*[b]	*Rate of nitrogen (Kg N ha^{-1} year^{-1})*						*Mean nitrogen source*		
			0	*100*	*150*	*300*	*450*	*600*			
Total	NPKS 25:5:5:5	0·86*	112·5	119·9	146·7	142·4	149·7	156·5	137·9	CV% Rate	(*a*) = 0·88
										Source	(*b*) = 2·54
	NPK 20:10:10	0·97*	112·7	123·6	118·7	149·0	159·1	165·6	138·2	LSD (*P* =	0·05 / 0·01)
										a	3·13 / 4·45
	Mean rate	0·98*	112·7	121·7	132·7	145·7	154·4	161·0		*b*	NS / NS
										(*a* × *b*)	6·22 / 8·73
Total unsaturated acids	NPKS 25:5:5:5	0·87*	86·56	97·05	117·2	114·2	122·8	125·8	110·6	CV% Rate	(*a*) = 0·98
										Source	(*b*) = 2·54
	NPK 20:10:10	0·98*	86·35	96·76	98·62	118·9	125·9	131·4	109·6	LSD (*P* =	0·05 / 0·01)
										a	2·76 / 3·93
	Mean rate	0·96*	86·48	96·91	107·9	116·6	124·3	128·6		*b*	NS / NS
										(*a* × *b*)	4·20 / 5·89
C18:2 plus C18:3	NPKS 25:5:5:5	0·88*	78·05	86·11	105·9	103·7	111·7	114·9	100·6	CV% Rate	(*a*) = 1·08
										% Source	(*b*) = 2·25
	NPK 20:10:10	0·97*	76·99	86·96	89·24	108·6	114·5	119·8	109·6	LSD (*P* =	0·05 / 0·01)
										a	2·78 / 3·96
	Mean rate	0·96*	77·52	86·54	97·58	106·1	113.1	117·4		*b*	NS / NS
										(*a* × *b*)	3·98 / 5·58

[a] Fatty acid methyl esters (FAMES).

[b] Correlation coefficient of linear regression analysis between FAMES levels and rate of nitrogen.

* Significant at $P = 0{\cdot}05$.

a, Rate of nitrogen; *b*, source of nitrogen.

CV%, coefficient of variation.

the fatty acids was (*Z*, *Z*, *Z*)-9, 12, 15-octadecatrienoic (linolenic) acid (C18:3), (*Z*, *Z*)-9,12-octadecadienoic (linoleic) acid (C18:2), *n*-hexadecanoic (palmitic) acid (C16:0), *n*-octadecanoic (stearic) acid (C18:0), (*Z*)-9-octadecaenoic (oleic) acid (C18:1), (*Z*)-9-hexadecaenoic (palmitoleic) acid (C16:1) at all rates and irrespective of the source of fertilizers. Similar occurrence of the fatty acids but in slightly different ratios had been noted in Japan (Anan & Nakagawa, 1977) and Assam, India (Bajaj & Dev Choudhury, 1984).

The slight variations noted in this study and the studies in Japan and India can be attributed to variations in climatic conditions of growth and probably to different varieties used in the study. However, in a recent study, Bhuyan and Mahanta (1989) demonstrated absence of stearic acid in Assam, India tea.

The saturated FA, C16:0 and C18:0 do not contribute to the VFC as they do not break down to more volatile compounds during black tea manufacture. However, the unsaturated FA break down to more volatile compounds contributing negatively towards the flavour of tea. Thus (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (C18:3) forms *Z*-3-hexenal which partly either reduces to *Z*-3-hexenol or isomerises to *E*-2-hexanal also reducing to *E*-2-hexenol (Hatanaka & Harada, 1973; Hatanaka *et al.*, 1977, 1987). Similarly (*Z*, *Z*)-9,12-octadecadienoic acid (C18:2) forms hexanal which partially reduces to *n*-hexanol (Hatanaka & Harada, 1973; Hatanaka *et al.*, 1977, 1987). Via the same mechanism (*Z*)-9-octadecanoic acid (C18:1) forms nonanal which reduces to *n*-nonanol, and (*Z*)-9-hexadecaenoic acid (C16:1) breaks down to heptanal reducing to *n*-heptanol. All these volatile compounds have been detected in Kenya teas (Horita & Owuor, 1987) and the Group I VFC of Kenyan teas are normally over 80% hexenals and hexenols. This is followed by hexanal and *n*-hexanol. The dominance of the FA, by C18:3 followed by C18:2, explains this observation. Indeed, C18:3 and C18:2 made up more than 80% of the total FA in the shoots of Clone S15/10 (Table 2).

The C16:1 occurred at the lowest level compared to the other FA. There was a general variation in the C16:1 levels with changes in nitrogenous fertilizer rates but the order was not significant. Indeed, only C16:1 showed an inverse relationship between rates of nitrogen and levels of occurrence. This is attributed to occurrence at very low levels causing inaccuracy in the analysis.

For the rest of the FA, there was an increase in the FA levels with increase in the nitrogenous fertilizer rates. This indicates that high levels of nitrogenous fertilizer would cause production of high amounts of Group I VFC and hence reduce the flavour quality of tea. Such an observation was recorded in our earlier studies on made black tea (Owuor *et al.*, 1987*a*). Indeed, both the total FA, total unsaturated FA or total C18:2 plus C18:3 showed the same trend.

One factor causing quality of tea deterioration with increase in rates of nitrogen fertilizer (Cloughley, 1983; Owuor *et al.*, 1987*a*) is thus demonstrated to be the rise in the levels of the FA. However, for economic production of black tea, fertilizer application is a prerequisite. In deciding on the correct rates of nitrogenous fertilizer, quality considerations should also be a factor, so that the amounts used compromise both yield and quality.

It was thought that higher amounts of phosphorus in NPK 20:10:10 might help form more FA in the tea leaves than NPKS 25:5:5:5. But there were no significant differences in the FA levels due to the source of nitrogenous fertilizers. Thus, use of NPKS 25:5:5:5 or NPK 20:10:10 produced similar effects. Bajaj and Dev Choudhury (1984) had recorded no response in FA levels to phosphatic fertilizers. The quality of tea cannot therefore be enhanced by using either form of fertilizer. However, there were significant interactions in the FA levels due to rates and sources of nitrogenous fertilizers. For the major FA (C18:2 and C18:3, total FA, or total unsaturated FA), NPKS 25:5:5:5 produced lower levels of FA at high nitrogen rates while NPK 20:10:10 produced higher amounts of the FA at low nitrogen rates. Such information is useful in deciding the appropriate fertilizer to use at the different levels of nitrogen.

ACKNOWLEDGEMENT

Data presented in part of the MSc thesis of J. W. M. This work was supported by a grant from the National Science Council of Kenya. We thank the Director, Tea Research Foundation of Kenya for permission to publish this paper.

REFERENCES

Anan, T. & Nakagawa, M. (1977). Studies in the lipids of tea leaves. Part I. Changes in lipid content and fatty acid composition of tea leaves during growing period of first and second flushes. *Nippon Shokuhin Kogyo Gakkaishi*, **24**, 305–10 (in Japanese with English summary).

Anon. (1965). Annual Report, Tea Research Institute of East Africa.

Bajaj, K. L. & Dev Choudhury, M. N. (1984). Effect of phosphorus on fatty acids of tea leaves and on quality of teas. *J. Food Sci. Tech.*, **35**, 160–64.

Bhuyan, L. P. & Mahanta, P. K. (1989). Studies on fatty acid composition of tea *Camellia sinensis*. *J. Sci. Food Agric.*, **46**, 325–30.

Chennery, E. W. (1963). Does phosphatic fertilizer pay? *Tea in East Africa*, **4**, 13.

Cloughley, J. B. (1983). The effect of harvesting policy and nitrogen application rates on production of tea in Central Africa II. Quality and total value of the crop. *Expl. Agric.*, **19**, 42–54.

Fernando, V. & Roberts, G. R. (1984). The effect of process parameters on seasonal development of flavour in black tea. *J. Sci. Food Agric.*, **35**, 71–6.

Hatanaka, A. & Harada, T. (1973). Formation of *Cis*-3-hexenal, *trans*-2-hexenal and *Cis*-3-hexenol in macerated *Thea sinensis* leaves. *Phytochem.*, **12**, 2341–6.

Hatanaka, A., Kajiwara, T., Sekiya, J. & Kido, Y. (1977). Formation of 12-Oxo-trans-10-dodecanoic acid from chloroplasts of *Thea sinensis* leaves. *Phytochem.*, **16**, 1828–1929.

Hatanaka, A., Kajiwara, T. & Sekiya, J. (1987). Enzymic oxygenative-cleavage reaction of linolenic acid in leaves-chloroplastic lipoxygenase and fatty acid hydroperoxide lyase in tea leaves. In *The Metabolism, Structure and Function of Plant Lipids*, ed. P. K. Stumpf, J. B. Mudd & W. D. Nes. Plenum Publishing Corporation, New York, pp. 391–8.

Horita, H. & Owuor, P. O. (1987). Comparison and characterization of volatile components of Kenyan clonal black teas and various black teas from other producing parts of the world. *Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea (Japan)*, **9**(B), 55–65 (in Japanese with English summary).

Munavu, R. M. (1983). *Croton megalocarpus*. Hutch seed kernel oil: A potentially rich source of octadeca-9,12-dienoic acid. *Kenya J. Sci. Tech.*, **4**(A), 77–81.

Othieno, C. O. (1980). Nutrient requirement of the tea plant. *Tea*, **1**, 11–20.

Owuor, P. O., Othieno, C. O., Horita, H., Tsushida, T. & Murai, T. (1987*a*). Effects of nitrogenous fertilizers on chemical composition of black tea. *Agric. Biol. Chem.*, **51**, 2665–70.

Owuor, P. O., Obanda, A. M., Othieno, C. O., Horita, H., Tsushida, T. & Murai, T. (1987*b*). Changes in chemical composition and quality of black tea due to plucking standards. *Agric. Biol. Chem.*, **51**, 3383–4.

Saijo, R. & Takeo, T. (1972). The importance of linoleic acid and linolenic acid as precursors of hexanal and trans-2-hexenal in black tea. *Plant and Cell Physiol.*, **13**, 991–8.

Wanyoko, J. K. (1981). Nutrients contents in the maintenance foliage of the tea plant. *Tea*, **2**, 14–18.

Wanyoko, J. K. (1983). Fertilizers on tea: Nitrogen—A review. *Tea*, **4**, 28–35.

Wickremasinghe, R. L., Wick, E. L. & Yamanishi, T. (1973). Gas chromatographic-mass spectrophotometric analysis of flavoury and non-flavoury Ceylon black aroma concentrates prepared by different methods. *J. Chromat.*, **79**, 75–80.

Yamanishi, T. (1978). The aroma of various teas. In *Flavour of Foods and Beverages*, ed. G. Charalamobuns & G. E. Inglett. Academic Press, New York, pp. 305–28.

Yamanishi, T., Kobayashi, A., Nakamura, H., Uchida, A., Mori, S., Ohsawa, K. & Sasakura, J. (1968). Comparison of aroma of various types of black tea. *Agric. Biol. Chem.*, **32**, 329–86.