EFFECTS OF SOME AGRONOMIC PRACTICES ON THE FATTY ACID CONTENT AND COMPOSITION OF TEA LEAVES.

BY

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This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my family and my parents

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ABSTRACT

The young tender shoots of tea Camellia sinensis (L.) O. Kuntze were found to contain appreciable amounts of fatty acids (FA). Linolenic acid ($C_{18:3}$) constituted between 58 and 63% of the total FA content, linoleic acid ($C_{18:2}$) between 14 and 18% of the total FA, palmitic acid ($C_{16:0}$) between 9 and 10%, stearic acid ($C_{18:0}$) between 8 and 9%, oleic acid ($C_{18:1}$) between 4 and 6% and palmitoleic acid ($C_{16:1}$) between 0 and 1%.

For clones 54/40 and 7/14 sampled from all the tea growing areas of Kenya, there were larger variations in the FA content of shoots East of the Rift Valley as compared to West of the Rift Valley. Similar variations had earlier been noted in the Group I volatile flavour compounds. There were changes in the FA content of the two clones with geographical area of production. These changes however, did not follow any pattern, were clonally dependent and were not affected by altitude.

There was no major difference in the mean content and composition of the FA in the leaves East and West of the Rift Valley although clones grown West of the Rift Valley appear to have higher amounts of the unsaturated FA. For all the sampling sites, there were no appreciable changes in the FA composition due to period from last prune. This was attributed to the many micro-climatic and ecological changes occurring in the areas of production. Even within a radius of about 10 kilometers the FA content changed with

site of growth. The FA levels only marginally increased with decrease in altitude and the relationship was insignificant. These variations emphasised that clones be tested in the areas of intended release before being made available to farmers.

For clone S15/10 sampled from one site there was a decrease in the FA levels with increased time from last pruning.

A very significant increase in the polyunsaturated FA with increasing rates of nitrogenous fertilizer application was observed. The two sources of nitrogen, fertilizers NPKS 25:5:5:5 and NPK 20:10:10 affected the FA composition in the same way.

The content of linolenic and linoleic acids increased with coarse plucking standards. The earlier noted quality deterioration arising from coarse plucking standards was partly attributed to this increase.

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CHAPTER ONE

INTRODUCTION

1.0 THE TEA INDUSTRY IN KENYA:

Kenya is primarily an agricultural country with agriculture still the major backbone of the economy. The tea industry is one of the biggest growth industry in Kenya. It is a major contributor to the Nation's economy, ranking third only to tourism and coffee as an earner of foreign exchange[1].

Kenya is the fourth leading world tea producer after India, China and Sri Lanka (Table 1). Kenya is also the fourth leading world tea exporter after India, Sri Lanka and China (Table 2).

Table 1: World production of tea (10^3 MT)[2]

Country	Calendar Year							
	1980	1981	1982	1983	1984	1985	1986	1987
India	570	560	561	582	640	656	621	674
China	304	343	397	401	414	432	461	508
Sri Lanka	191	210	189	180	209	215	213	215
Kenya	90	91	96	120	116	147	143	156
Indonesia	99	109	90	112	126	132	130	128

Table 2: World exports of tea (10^3 MT)[2]

Country	Calendar Year							
	1980	1981	1982	1983	1984	1985	1986	1987
India	224	241	190	209	217	214	203	204
Sri Lanka	185	183	181	158	204	198	208	201
China	108	92	106	125	145	137	172	174
Kenya	75	76	80	101	91	126	117	135
Indonesia	68	71	64	69	86	90	79	90

Kenya is however closing the gap of tea production due to her unmatched rate of growth in tea production[3]. This is evident from the fact that Kenya's tea production in 1988 was 164 thousand metric tonnes. This was the highest crop ever to be recorded in the history of Kenya's tea industry[4]. Kenya is now producing and exporting more than 50% of Africa's tea.

Most of Kenya's tea is sold at the Mombasa international tea auction centre where between 1976 and 1987 it had the highest average price per kilogram. At the London tea auctions, where Kenya's tea competes with teas from major producers, Kenya's tea between 1974 and 1987 also commanded the highest average price per kilogram[2]. Thus Kenyan tea is of very high quality.

The total tea exports during the calendar year 1985 earned the country about KShs. 3.8 billion, while the earnings for 1986, 1987 and 1988 were KShs. 3.5 billion,

KShs. 3.3 billion[5], and KShs. 3.7 billion[6] respectively. These earnings provide a livelihood for more than one million Kenyans and contribute revenue in form of duty and licenses.

1.1 THE IMPORTANCE OF QUALITY IN TEA:

The beverage tea is non-alcoholic and its refreshing and mild stimulating effects makes it the most widely drunk beverage in the world. As such, the quality of the liquor in the cup is of prime importance. The beverage is manufactured from the young tender shoots of Camellia sinensis (L) O. Kuntze. Kenya's black tea is included in the small group of teas having the finest quality and this is reflected in the consistently high prices they fetch on international markets[2]. The buyers of Kenya's tea are primarily interested in the quality of the tea on sale and not upon the quantity. The survival of the Kenyan tea as a viable entity depends upon maintenance of the present quality status or production of even higher quality tea. It is therefore of atmost importance to understand the chemical factors responsible for the high quality and to determine how agronomic practices can be optimised to maintain or improve the present quality level.

The Tea Research Foundation of Kenya (TRFK) is the technical executing agency of the Tea Board of Kenya charged with the responsibility of carrying out research and providing technical support based on research findings to the Kenyan tea industry. The TRFK has been engaged in tea research programmes which have resulted in increased tea yields, reduced

cost of tea growing and processing and, most important, improved quality of tea. This has been with a view to ensuring that the farmer gets the best possible price from his crop, while the reputation of Kenya's tea remains high in the tea quality - conscious, discriminating world tea markets. It then follows that any factor related to tea quality, no matter how trivial, stands a good chance of justifying some research effort. Factors which affect quality usually have an effect upon the economics of tea production.

1.2 ASSESSMENT OF TEA QUALITY:

Values and/or prices of black tea are determined organoleptically. This method of valuation has, however, often been criticised as being subjective and influenced by consumers, market demands and/or individual taster's personal preferences[7]. After fermentation and firing the tea is graded according to the size of the particle of dried leaf. This is also called sorting. The sorted tea is then assessed for quality.

Black teas are classified as flavoury or plain. Plain teas do not have flavour and are sold mainly on the basis of briskness, brightness, thickness, strength and colour[7,8]. Flavoury black teas, on the other hand, are bought for their special aromas and flavours[9,10]. Kenyan black teas are classified as medium flavoury to plain[9]. The subjective nature of the tasters' assessment explains how it is possible for different tasters to assign different classifications to the same sample of tea. There could be wide divergencies of

opinion among liquorers regarding the characteristics of identical tea samples as was observed by Biswas[11-14]. There is therefore need for a more precise, reproducible, and objective method of assessing tea quality.

1.2.1 Assessment of quality by chemical analysis.

Research has been directed to the determination of measurable chemicals or groups of chemicals inherently found in black tea. This has been with a view to understand the chemistry of tea and particularly where specific chemical compounds have been shown to influence the quality of tea. Work has been done to relate these essential chemical constituents found in black tea to the various classifications of tea with the eventual aim of arriving at a scientific assessment of the quality of black tea. This, to a large extent has been achieved.

The positive contribution of Theaflavins (TF) and Thearubigins (TR) to black tea quality was first demonstrated in 1958 by Roberts[15,16]. Since then, several studies have shown both the components to be important in valuation of teas. Because the structures of TR are not precisely known, TF have received more attention as a quality parameter[17-29].

Results from TF studies have shown that strength, brightness and briskness of black tea are related to the TF content of the liquor and that there is a direct linear relationship between the TF content and tea valuations and/or prices.

Consequently, in some countries TF content has been adopted as the main quality parameter of black teas. Kenya's tea has been noted to have very high amounts of TF when compared to teas from other parts of the world[7]. This partly explains why Kenya's tea is classified as plain in the tea trade. For Kenyan black teas, the correlation coefficients between TF and the tasters' evaluations have been shown to be positive but statistically non-significant[7,30]. The positive correlations imply that TF content is a real quality parameter but lack of significant correlations with prices/ valuations implies that TF alone is not enough to describe quality of Kenyan teas.

The exact contribution of TR to tea quality is still elusive but it is known that TR are necessary for the total colour and thickness of black tea and that very high amounts of TR in tea is deleterious to quality as it makes the tea flat and muddy[8]. Linear regression analysis between the tasters' evaluations and TR content were shown to be positive[30]. This showed that TR has positive contribution towards quality and is thus a quality parameter.

Caffeine has been known to contribute to the quality of tea[31]. Bhatia[32] and Mullin[14,33] asserted that caffeine contributes towards the briskness (astringency) of black tea. The mild stimulating effects of the beverage tea is mainly due to the presence of caffeine[34-36]. Linear regression analysis between the tasters' evaluations and caffeine content of Kenyan

black teas were positive showing that caffeine content is a good quality parameter. Generally TF, TR and caffeine are the quality parameters which have been extensively studied for Kenyan teas. Although these chemical components are necessary for black tea quality, no individual component has significant direct relationship with the organoleptic evaluations.

Ash, total water soluble solids and crude fibre contents of tea are also important to tea quality[37]. A comparison of these attributes from the main black tea producing parts of the world show that the compositions of these parameters are comparable. Their variations were minimal. The ash, water soluble solids and crude fibre of all the teas used met the requirements of the International Standard Organisation (ISO) 3720[38]. The set levels of the ISO 3720 are as follows:- water soluble solids minimum 32%, total ash 4-8%, and crude fibre maximum of 16.5% [39,40]. High quality teas normally have high total water soluble solids and low ash and crude fibre contents.

The high quality of the Kenyan teas is due to the general favourable growing conditions, improved methods of growing and husbandry and manufacture which have resulted in favourable balance of the chemicals responsible for quality.

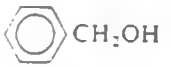
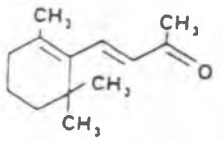
1.3 THE IMPORTANCE OF FLAVOUR TO OVERALL TEA QUALITY:

Work on the flavour of Kenyan teas was very recently started. Owuor et al[9,41] have shown that the composition of volatile flavour compounds of commercial Kenyan teas is comparable

to that of teas from areas famous for the production of teas with high flavour qualities. The flavour index (F.I.)[42] of the world famous flavoury teas were noted to be slightly higher or even equal to those of the Kenyan clones used in the study.[41] Therefore the general notion that Kenyan black teas are plain is not true. In these studies it was also shown that flavour of Kenyan black tea is one of the criteria determining overall quality and selling price, and hence flavour is an important quality parameter.

The compounds contributing to the flavour of black tea have been identified and studied in a number of detailed investigations. These investigations have shown that black tea contains over 200 volatile compounds. These compounds, especially those which occur in appreciable quantities impart flavour to black tea. In these studies only the major compounds were reported. The volatile flavour compounds (VFC) were classified into two groups[9]. The compounds in Group I - hexanal, 1-penten-3-ol, (3Z)-hexenal, (2E)-hexenal, (2Z)-pentenol, (3Z)-hexenol, (2E)-hexenol[40,43], pentanol, hexanol and 2,4-heptadienal[40,44](1) - although important for characteristic black tea flavour, give tea an inferior flavour at high concentrations. These compounds are products of lipid degradation during black tea manufacture[10]. Benzaldehyde[40,42](2), phenylacetaldehyde[45](3), methylsalicylate[46,47](4), geraniol (5), geranic acid (6), linalool (7) and linalool oxide[40,42,48], benzylalcohol[48](8) and β-ionone[49](9) have been shown to impart a sweet flowery aroma to black teas. These compounds

Table 3: Structures of some Group I and II VFC

	Common Name	I.U.P.A.C. Name	Structure
(1)		2,4-heptadienal	$C_2H_5CH=CHCH=CHCHO$
(2)	Benzaldehyde	Phenylmethanal	C_6H_5CHO
(3)	Phenylacetaldehyde	Phenylethanal	$C_6H_5CH_2CHO$
(4)	Methylsalicylate	Methyl 2-hydroxybenzoate	$2\text{-}(OH)C_6H_4COOCH_3$
(5)	Geraniol	3,7-dimethyl-2,6-octadien-1-ol	$(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCH_2OH$
(6)	Geranic acid	3,7-dimethyl-2,6-octadienoic acid	$(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCOOH$
(7)	Linalool	3,7-dimethyl-1,6-octadien-3-ol	$(CH_3)_2C=CHCH_2CH_2C(CH_3)(OH)CH=CH_2$
(8)	Benzylalcohol	Phenylmethanol	
(9)	β-ionone	4-(2,6,6-trimethyl-1-cyclo-hexenyl)-3-buten-2-one	

are classified as Group II. The classification of the rest of the compounds into Group I or II is based on the method of Yaminishi et al[42] and Wickremasinghe et al[44].

By this method, compounds with GC retention times less than linalool are placed in Group I while those with higher retention times are placed in Group II. The ratio of Group II to Group I VFC is called the flavour index (F.I.) and can be used to classify the tea qualitatively in order of flavour quality[42,44]. The qualitative nature of FI is stressed since it is known that the olfactory perception limits of the compounds are different. Some compounds may therefore exist as VFC in only small amounts but affect the flavour greatly and vice versa[47].

It is known that the higher the FI the better the flavour quality of black tea[9,50]. For the Kenyan teas there were significant positive correlations between flavour index and the tasters' evaluations of the tea. This demonstrated that FI is a measure of the flavour quality and thus an important quality parameter for Kenyan black teas[50]. Of all the quality parameters studied for Kenyan teas, the Group I VFC concentration correlated negatively with the tasters' evaluation. This confirmed the notion that high concentration of these compounds is deleterious to tea quality.

It is apparent, therefore that to improve the flavour quality of tea, the concentration of these Group I VFC must be reduced. Investigations into the contribution of lipids, especially fatty acids, to the aroma and flavour volatiles during

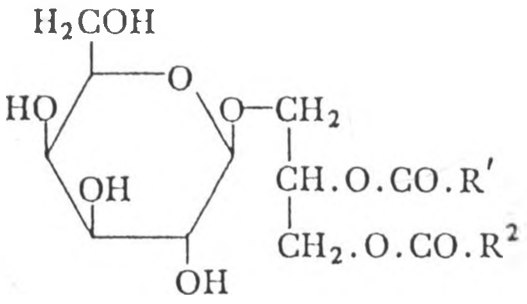
tea processing is one subject with this underlying view.

1.4 ASPECTS OF THE CHEMISTRY OF LIPIDS AND FATTY ACIDS OF TEA LEAVES:

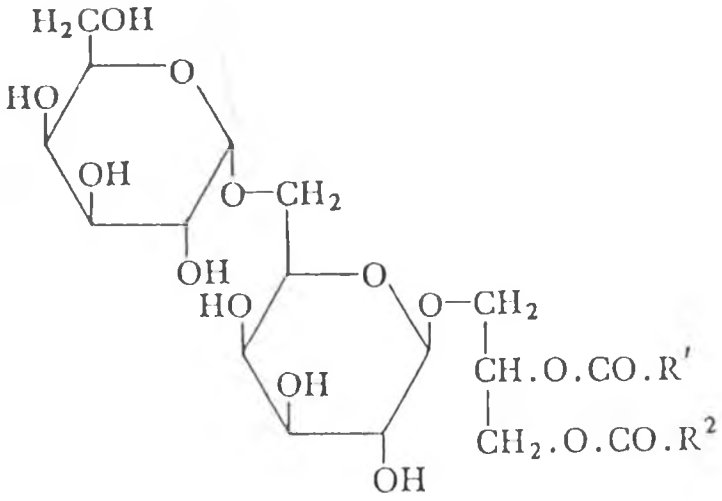
1.4.1 Occurrence

The development of improved analytical techniques permitted Anan and Nakagawa[51] and Wright and Fishwick[52] to carry out quantitative analyses of lipids and fatty acids of tea shoots. They made it clear that tea leaves contain mainly monogalactosyl diglyceride (10), digalactosyl diglyceride (11), phosphatidyl choline (12), phosphatidyl ethanolamine (13), sulphoglycolipids such as sulphoquinovosyl diglyceride (14), sterolacyl monoglucoside (15), phosphatidyl glycerol (16), phosphatidyl inositol (17), cerebrosides (18) and neutral lipids.

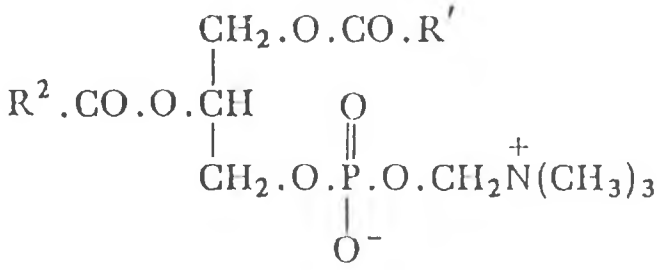
The component fatty acids were found to be palmitic (hexadecanoic), palmitoleic (cis-9-hexadecenoic), stearic (octadecanoic), oleic (cis-9-octadecenoic), linoleic (cis-9, cis-12-octadecadienoic) and linolenic (cis-9, cis-12, cis-15-octadecatrienoic). The structures are given in Table 4 (Page 14).



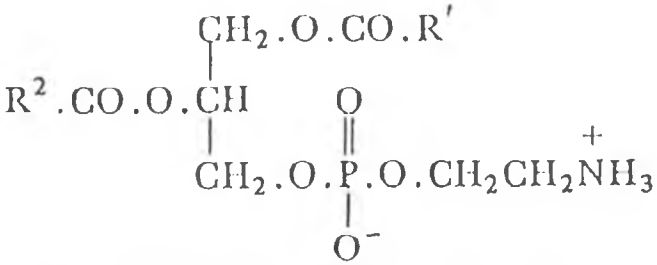
(10) Monogalactosyl diglyceride (MGDG)



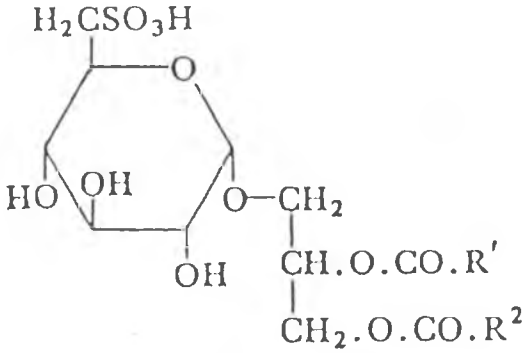
(11) Digalactosyl diglyceride (DGDG)



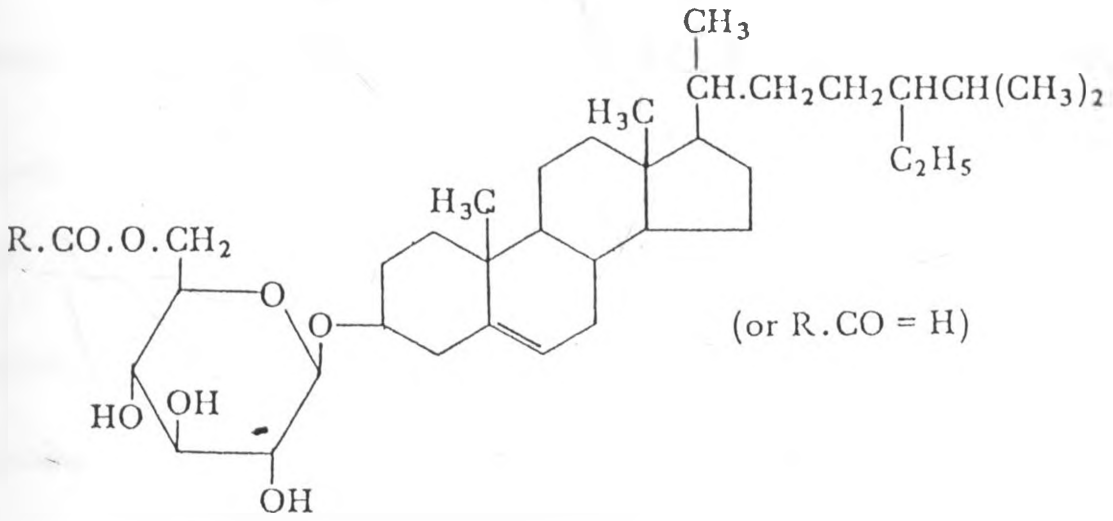
(12) Phosphatidyl choline (PC)



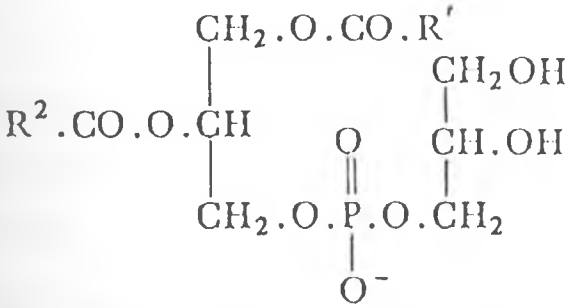
(13) Phosphatidyl ethanolamine (PE)



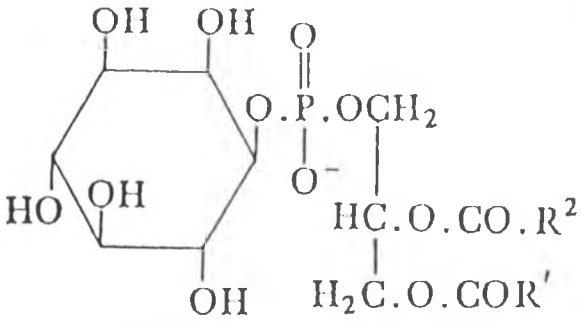
(14) Sulphoquinovosyl diglyceride (SQDG)



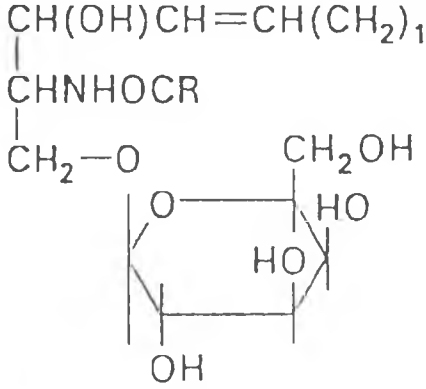
(15) Sterolacyl monoglucoside



(16) Phosphatidyl glycerol (PG)



(17) Phosphatidyl inositol (PI)



(18) Cerebrosides

Table 4: The major fatty acids found in tea leaves.

Common Name	Symbol	Structure
Palmitic	$C_{16:0}$	$CH_3(CH_2)_{14}\overset{O}{\overset{\|}{C}}-OH$
Palmitoleic	$C_{16:1}$	$CH_3(CH_2)_5CH=CH(CH_2)_7\overset{O}{\overset{\|}{C}}-OH$
Stearic	$C_{18:0}$	$CH_3(CH_2)_{16}\overset{O}{\overset{\|}{C}}-OH$
Oleic	$C_{18:1}$	$CH_3(CH_2)_7CH=CH(CH_2)_7\overset{O}{\overset{\|}{C}}-OH$
Linoleic	$C_{18:2}$	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7\overset{O}{\overset{\|}{C}}-OH$
Linolenic	$C_{18:3}$	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7-\overset{O}{\overset{\|}{C}}-OH$

These are the principal fatty acids present in tea leaves which account for over 98% of the fatty acid composition. Considering the fatty acid composition of individual lipids, linolenic acid is derived from mono- and digalactosyl diglycerides, linoleic acid from phosphatidylcholine and phosphatidylethanolamine, oleic acid from phosphatidylglycerol, stearic acid from sterolacylmonoglucoside and neutral lipids, and palmitic acid from phosphatidylinositol and sulphoquinovosyldiglyceride[51-54]

The lipids constitute less than ten per cent of the total dry weight of leaves and most of the lipids are located in the

chloroplasts. Lipids comprise about 30% of the dry weight of the chloroplast, and 50% of the chloroplast lamellae.

1.4.2 Production of volatiles by degradation of lipids.

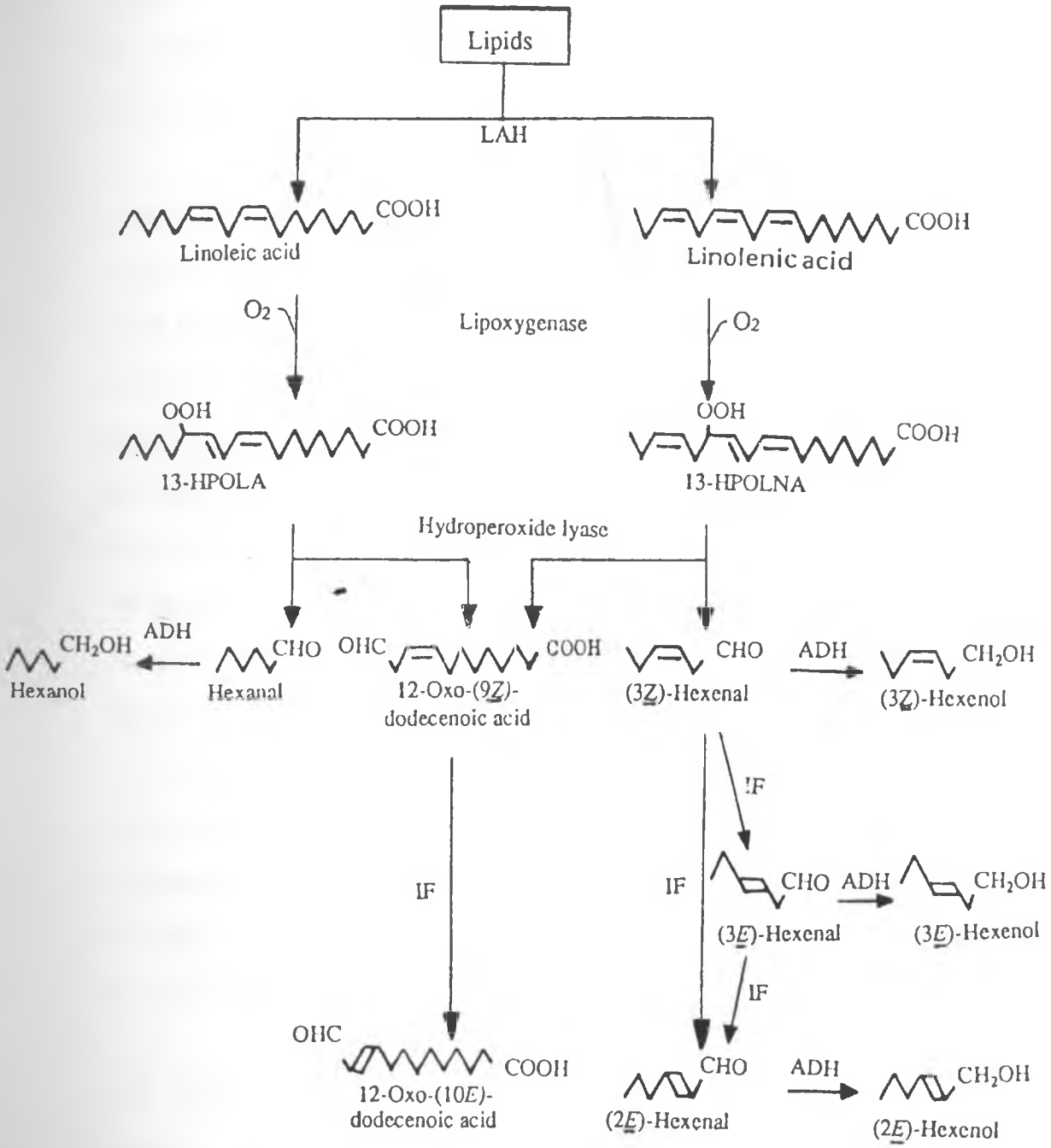
Sanderson[55] and Saijyo[56] have shown that during manufacture of tea there is widespread damage to membrane structures. This releases lipid-degrading enzymes which attack lipoprotein and lipocarbohydrate membrane structures to release fatty acids[57]. The fatty acids released undergo further degradation to produce the volatile constituents with characteristic flavour properties. These volatile constituents are mainly the Group I VFC[9,10,43-48].

The majority of these Group I VFC are C_6 alcohols and aldehydes. Biosynthetic studies on homogenates of Camellia sinensis leaves have shown that the C_{18} polyunsaturated fatty acids are the precursors of the C_6 alcohols and aldehydes[58-62]. Linolenic and linoleic acids were identified as the actual precursors of the aldehydes (3Z)-hexenal and hexanal respectively and that C_6 alcohols are reduction products of these aldehydes. The C_6 aldehydes are biosynthesised as shown in Scheme 1[63].

The reactions involved in the major biosynthetic pathway for C_6 aldehydes consist of five sequential steps; acylhydrolysis of lipids, hydroperoxidation of linoleic and linolenic acids, cleavage of the fatty acid hydroperoxides, isomerization of (3Z)-hexenal to (2E)-hexenal and conversion of the C_6 aldehydes into alcohols.

The four enzymes and one non-enzymic factor involved in the pathway are lipolytic acyl hydrolase (LAH), lipoxygenase,

Scheme 1: Biosynthetic pathway of C_6-aldehydes and C_6-alcohols from linoleic acid and linolenic acid in plant tissues.



LAH, lipolytic acyl hydrolase. IF, isomerization factor.
ADH, alcohol dehydrogenase.

fatty acid hydroperoxide lyase (hydroperoxide lyase), alcohol dehydrogenase (ADH) and an isomerization factor (IF). Among these enzymes lipoxygenase and hydroperoxide lyase are the most important since these enzymes catalyse formation of C_6-aldehydes.

The lipolytic acyl hydrolase (LAH) hydrolyses the lipids and releases the fatty acids. The free fatty acids, linoleic acid (LA) and linolenic acid (LNA) are oxidised at the C-13 position. This is catalysed by lipoxygenase. This enzyme catalyses the aerobic oxidation of LA and LNA since they contain a cis-1 and cis-4-pentadiene system[62,64,65]. The oxidation products are 13-hydroperoxide of LA, 13-HPOLA (13-L-hydroperoxy-cis-9,trans-11-octadecadienoic acid) and 13-hydroperoxide of LNA, 13-HPOLNA (13-L-hydroperoxy-cis--9,trans-11,cis-15-octadecatrienoic acid). The aldehydes (3Z)-hexenal and hexanal and C_{12}-oxo-acid, or 12-oxo-cis-9-dodecenoic acid are produced through an oxygenative cleavage at the double bond between C-12 and C-13. This cleavage is catalysed by hydroperoxide lyase[64,66]. The formed (3Z)-hexenal is isomerized to (2E)-hexenal and (3E)-hexenal by the isomerization factor (IF).

The corresponding alcohols, (3Z)-hexenol, (2E)-hexenol and (3E)-hexenol are formed by reduction with alcohol dehydrogenase (ADH). Hexanal is reduced to hexanol by ADH. Also during tea processing, oleic acid can break down to form nonanal and n-nonanol while palmitoleic acid can form heptanal and n-heptanol[58,61]. The C_6 compounds dominate the Group I

VFC and contribute to the characteristic flavour properties of black tea. They are the compounds which impart an inferior, green and grassy flavour to tea when present in relatively high amounts[10,30]. Indeed the total amounts of these compounds were noted to correlate negatively with the tasters' evaluations of Kenyan black teas[30]. The formation of these important C_6 aldehydes and alcohols from degradation of linoleic and linolenic acids have served to emphasize the importance of FA in flavour development.

Formation of flavour compounds is not confined to tea only. The C_6 aldehydes and alcohols are widely distributed in fresh foliage, vegetables and fruits. Major et al[67,68], for example, reported that fresh leaves of Gikgo biloba (L.) produced (2E)-hexenal when they were ground in the presence of air, and that linolenic acid was converted into (2E)-hexenal in the leaves. Drawert et al[69] reported that hexanal and (2E)-hexenal were produced enzymatically with participation of atmospheric oxygen, from linoleic acid and linolenic acid respectively in apples and grapes. A number of volatile carbonyl compounds saturated or unsaturated have been isolated from reverted or oxidised soybean oils, and the volatile substances isolated from autoxidised C_{18} unsaturated fatty acids have been shown to be mainly carbonyl compounds[70].

It is therefore not questionable that the current tea quality problems in Kenya would require further work to relate the volatile flavour compounds to their chemical precursors,

namely, linoleic acid, linolenic acid, oleic acid and palmitoleic acids in the green tea leaves. Information regarding the chemical compounds which determine overall quality would further enable farmers to be correctly and precisely advised as to what is actually necessary agronomically or otherwise to enhance the quality of their processed product.

1.5 SOME FACTORS AFFECTING THE CHEMICAL COMPOSITION AND QUALITY OF CTC BLACK TEA :

1.5.1 Geographical area of production.

The areas producing tea in the world have large variations of climatic conditions from equatorial climates to sub-tropical climates and at altitudes from sea leavel to beyond 2,600 meters above absolute mean sea level. Due to these variations in the geographical and climatic conditions of growth, there are normally chemical concentration changes and hence quality differences[9,40,41,47,71-74]. Owuor et al[9], for example, compared the chemical compositions of black teas from the main black tea producing parts of the world and found that all the teas had large differences in the contents of TF, TR and flavour compounds. Yamanishi et al[40] and Horita and Owuor[41] also compared the flavour of tea from different parts of the world while Cloughley et al[74] compared the flavour of teas during spring, summer and autumn in Malawi. The climatic and geographical conditions in all these areas are diverse.

In Kenya, tea is grown in different locations at high altitudes between 1500 and 2700m above sea level. Compared

to the tea growing regions of the world, the geographical and climatic changes are minimal. A study by Owuor et al[50,75] to determine any changes in the chemical composition of black tea occurring due to the minimal geographical and climatic changes was done. This study showed that TF varied widely with geographical areas of production for all the clones tested. TR contents also changed with geographical locations. There were no significant changes in TR contents with altitude for all clones. In all clones different geographical areas of growing tea produced varying amounts of caffeine. No significant relationships were shown between caffeine content and altitude. There were highly significant differences in the VFC content with locations. Generally, there was no significant relationship between Group I or II compounds and altitude. The magnitude and order of the variations changed according to the location where the tea was grown. All these differences in chemical composition and hence quality partly explain why farmers from different regions do not always get the same payment for their produce. The differing chemical composition and hence quality of tea can be attributed to the teas's chemical/biochemical composition reacting differently to varying geographical and climatic areas of production.

1.5.2 Clonal variations.

When different varieties of tea (clones) are grown under similar environmental conditions, they do not react the same way in terms of growth and hence their chemical/

biochemical compositions are different. A study by Owuor et al[30] on the variations in the chemical composition of some Kenyan clonal teas showed that clones of tea produce different amounts of TF, TR, caffeine and the VFC in varying patterns. Clone 6/8 had the highest TF, the second highest TR, and the best flavour index. This clone is used as a quality standard in organoleptic valuations. The FI of the clones studied were in the order 6/8 > 31/8 > Ejulu > 31/11 > STC 5-3 > TN 14-3 > S 15/10. It was therefore suggested that clones' chemical composition be used in conjuction with organoleptic evaluations in the regions of intended release before they are made available to farmers.

1.5.3 Nitrogenous fertilizers.

The application of fertilizers to tea is a normal agronomic practice which is essential for continuous economic production of tea. Studies on the effects of fertilizers on the chemical composition of tea have been intensified. Bajaj et al[76], demonstrated that in North and East India, the application of phosphatic fertilizer did not seem to have any significant impact on the total quantity of fatty acids in fresh tea leaves.

In Kenya, nitrogenous fertilizers have been shown to affect the chemical composition of tea leaves. Owuor et al[77,78] investigated the variations in the content of TF, TR and caffeine with different sources and rates of nitrogenous fertilizer. The general observation was that the TF and caffeine levels

increased with increase in fertilizer rates, while TR levels decreased. Nitrogenous fertilizers affected the composition of the volatile flavour compounds[77,78]. VFC in both Groups I and II increased with increasing rates of fertilizer application but flavour index showed an inverse trend. The VFC of Group I showed the largest variations due to the varying rates of fertilizer application. The increase in Group I VFC composition and decrease in the value of FI can partly explain the general observation by tea growers that the quality of black tea deteriorates with increasing nitrogenous fertilizer application rates.

1.5.4 Plucking standards.

Plucking standards is one agronomic practice which adversely affects the quality of tea. In most black tea producing countries, the recommended plucking standard is tender shoots of two leaves and a bud, but occasionally some producers are known to have used less tender shoots of more than two leaves and a bud. The argument for use of coarse plucking is the extra biomass obtained in a plucking round. However, coarse plucking standard reduces the plucking frequency as extra time must be allowed for production of more shoots. Over an extended period there is not any significant advantage in biomass production gained by coarse plucking as fine plucking leads to more frequent pluckings.

In Kenya, Owuor et al[79] have shown that fine plucking of upto two leaves and a bud, produced black teas with high

contents of the required caffeine and TF, and low contents of TR. The highest TF content was recorded in two leaves and a bud. Coarse plucking produced teas with very high TR contents. It was also demonstrated that the VFC especially the C_6 alcohols and aldehydes in Group I VFC increased with coarse plucking standard while Group II VFC decreased with coarse plucking, thus FI also decreased with coarse plucking standards. The increase in Group I VFC and decrease in the value of the FI with coarse plucking standards partly explains the poor quality normally obtained when coarse plucking standards are used[65].

1.5.5 Pruning.

Pruning is also an essential agronomic practice in the production of tea since it maintains the tea bushes under manageable conditions for plucking. Data presented by Owuor and Langat[80] showed that both TF and caffeine contents of tea improve with time from pruning. Longer time from pruning lowered the TR contents of tea. Thus measured by TF, TR and caffeine contents which are essential quality parameters for Kenyan black tea, increase in time from pruning improved tea quality.

In the same study, it was observed that increasing time from pruning lowered the sum of Group I VFC but enhanced both sum of Group II VFC and FI. Thus even measured by flavour characteristics, increasing time from pruning, tended to improve tea quality.

1.6 OBJECTIVE OF THIS STUDY :

In view of the discussion above, it is important to find ways and means of lowering the content and composition of the Group I VFC. This can first be done by determining the factors which inhibit or accelerate the formation of the precursors of these Group I VFC. Presently, there is no report available on the effects of agronomic practices on the fatty acids of tea leaves.

It therefore was the main objective of this study to determine how different agronomic practices affect the content and composition of FA of tea leaves. By relating the FA to the Group I VFC it was possible where appropriate, to suggest ways and means in which these agronomic practices can be manipulated so as to improve tea quality. Specifically, the following agronomic practices were targeted for assessment.

a) Geographical area of production.

In Kenya the amount of the Group I VFC was observed to vary with the actual geographical location where the tea was grown[50]. Thus some clones produce higher amounts of C_6 alcohols and aldehydes at some sites and not at others. This study proposed to find out if the fatty acids in the fresh leaves of clones 7/14 and 54/50 would also vary with the site where the tea is grown. The study also undertook to quantify the changes in fatty acid content of clones obtained within Kericho tea estates where climatic changes within a radius of about 10 kilometers was assumed to be minimal, but the sampling sites are in different altitudes.

b) Nitrogenous fertilizers.

In Kenya, the Group I VFC content was observed to increase with increasing rates of nitrogenous fertilizer application[78]. Changes in lipid levels due to nitrogenous fertilizer were therefore speculated but not proved. This study proposed to quantify any changes in the fatty acids which may be brought about by variations in nitrogenous fertilizer application rates.

c) Plucking standard or harvesting policy.

Studies[79] have demonstrated that the C_6 alcohols and aldehydes in Group I VFC increased with coarse plucking standard. It was thus speculated that the FA content of green tea leaves would also vary with plucking standards. This study was aimed at finding any changes in FA content of tea leaves with coarse plucking standards. This would help to explain why C_6 alcohols and aldehydes increase with coarse plucking treatments.

d) Pruning.

Increasing time from pruning lowered the sum of Group I VFC[80]. This study proposed to identify any changes in the FA content of green tea leaves with increasing time from pruning.

CHAPTER TWO

RESULTS AND DISCUSSION

2.0 IDENTIFICATION OF THE FATTY ACIDS OF TEA LEAVES:

Shortly after introducing a sample of the fatty acid methyl ester mixture into the injection port of the GC there was a small air peak followed by a peak for the solvent (hexane) in which the sample was dissolved. The base line soon stabilised and peaks for the various components emerged. Figures 1 and 2 are the GC traces for the two columns showing peaks for the various fatty acids present in tea leaves.

Thus both columns gave excellent separations of the fatty acid methyl esters. The separation was mainly on the basis of chain-length and degree of unsaturation. By comparing the retention times or more reliably, the relative retention times (Table 5) of the isolated fatty acids with those of known fatty acids under the same conditions, sample fatty acids were identified. The identifications were confirmed by carrying out their GC-MS analysis (Appendix).

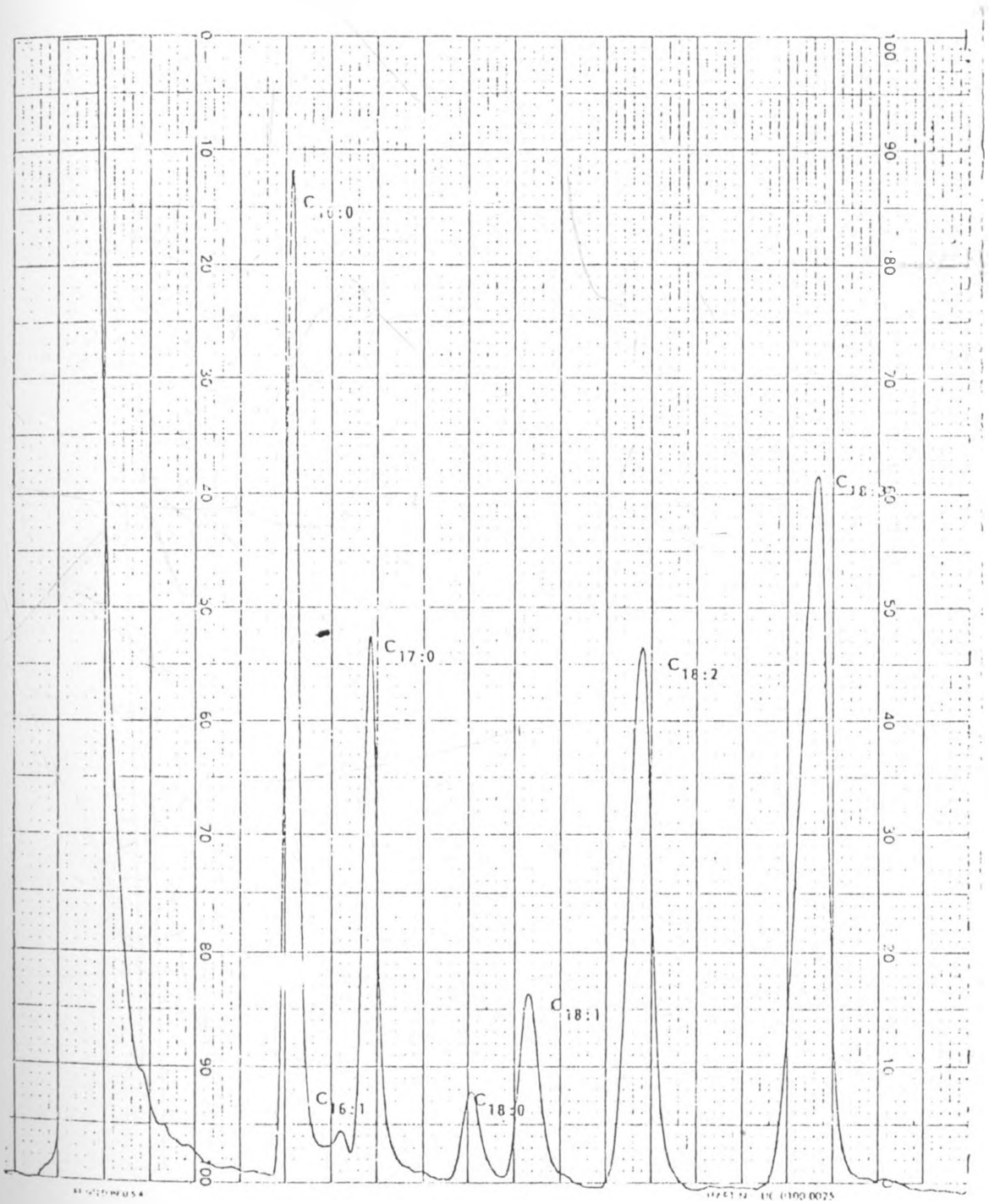


Figure 1: Separation of the major fatty acids (as methyl esters) of tea leaves by GLC on a 15% DEGS on chromosorb W (80/100 mesh) column.
Column temperature 180 ± 1°C
Injector-Detector temperature 220 ± 1°C
Nitrogen gas flow 40 ml/min.

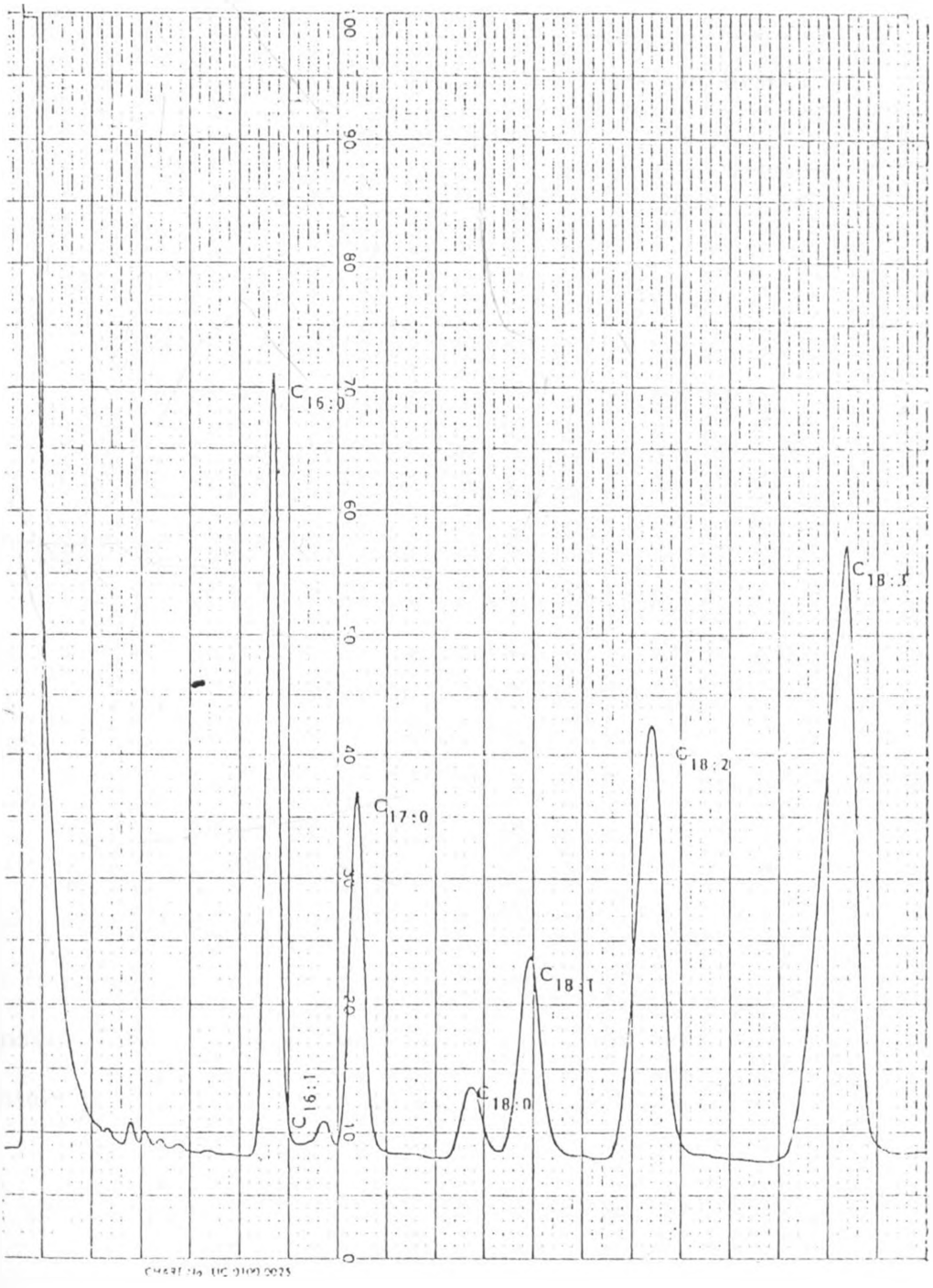


Figure 2: Separation of the major fatty acids (as methyl esters) of tea leaves by GLC on a 10% PEGA on chromosorb W (80/100 mesh) column.
Column temperature 180 ± 1°C
Injector-Detector temperature 220 ± 1°C
Nitrogen gas flow 40 ml/min.

Table 5: Retention times (minutes) and relative retention times* of tea leaves fatty acid methyl esters.

Methyl ester	Retention time		Relative retention time	
	DEGS	PEGA	DEGS	PEGA
$C_{16:0}$	5.8	4.4	0.59	0.57
$C_{16:1}$	7	5.3	0.71	0.69
$C^{**}_{17:0}$	7.6	5.8	0.78	0.75
$C_{18:0}$	9.8	7.7	1	1
$C_{18:1}$	11.1	8.6	1.13	1.12
$C_{18:2}$	13.6	10.4	1.39	1.35
$C_{18:3}$	17.6	13.2	1.80	1.72

*Relative retention time = $\frac{\text{Retention time of ester}}{\text{Retention time of } C_{18:0} \text{ ester}}$

**the added internal standard

Thus it is apparent that increasing chain length and/or degree of unsaturation increases the retention time and relative retention time of fatty acids on the GC columns used.

2.1 CLONAL DIFFERENCES AND GEOGRAPHICAL AREA OF PRODUCTION.

The areas producing tea in Kenya can be divided into two geographical locations, namely East and West of the Rift Valley. In both clones, at all the sites from which sampling was done, the fatty acid (FA) composition was found to be as shown in Table 6.

Table 6: Composition of fatty acids in the young tender leaves of tea.

Fatty acid	Percentage composition of the total FA content
$C_{16:0}$	9-10
$C_{16:1}$	0-1
$C_{18:0}$	8-9
$C_{18:1}$	4-6
$C_{18:2}$	14-18
$C_{18:3}$	58-63

Thus the order of occurrence of the FA is $C_{18:3} > C_{18:2} > C_{16:0} > C_{18:0} > C_{18:1} > C_{16:1}$. Hence green tea leaves are generally very rich in linolenic acid, rich in linoleic acid, palmitic acid, stearic acid and have minor constituents of

oleic acid and very low amounts of palmitoleic acid. Only trace amounts of lauric ($C_{12:0}$) and myristic ($C_{14:0}$) acids were detected. This composition pattern of the FA in the tea leaves is similar to that observed in Sri Lanka by Wright and Fishwick[52] and is different from that observed in Japan by Anan[54], Anan and Nakagawa[51], in India by Bajaj et al[76] and Bhuyan and Mahanta[53].

The distribution of the fatty acids in the young tender shoots of clones 54/40 and 7/14 are presented in Table 7a and 7b for teas from the East of the Rift Valley and Table 8a and 8b for teas from the West of the Rift Valley.

Disregarding clones and production site, and only considering the FA it can be seen that changes (as percentage coefficient of variation) of between 14 and 31% were noted in the individual FA composition East of the Rift Valley and changes of between 6 and 21% in the individual FA composition West of the Rift Valley. Thus larger variations were recorded East of the Rift as compared to the West of the Rift Valley. Similar variations were noted in the Group I volatile flavour compounds (VFC)[50] and this can be attributed to the similar distribution in the FA content East of the Rift Valley as compared to the West of the Rift Valley.

The unsaturated FA are of economic importance in fresh tea leaves due to their degradation to Group I VFC during tea manufacture. The saturated FA do not degrade to the VFC[52]. Mechanisms of the enzyme activated degradation of linolenic and linoleic acid to VFC is as presented in Scheme 1.[63]

Table 7a:Fatty acid distribution (mg/100g dry leaf) in young shoots of tea East of Rift Valley

Source	District	Altitude (m) above sea level	Clone 54/40							
			C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[a]	Total
Kaaga	Kiambu	2190	17.39	0.67	16.37	8.44	26.46	99.75	135.38	169.08
Makamboki	Murang'a	2100	25.51	0.65	30.85	19.40	44.93	146.50	211.48	267.84
Kionyo	Meru	2090	21.95	0.55	19.96	10.92	36.45	140.65	188.57	230.48
Kiarutera	Meru	2070	25.57	0.65	23.82	17.61	45.82	159.72	223.80	273.19
Kangaita	Kirinyaga	2050	32.04	1.17	26.75	20.33	54.41	210.77	286.68	345.47
Gatundu	Kiambu	2030	19.18	0.76	17.67	9.48	30.61	113.45	154.30	191.15
Mahiga	Nyeri	2000	22.22	1.18	22.24	11.80	26.88	92.48	132.70	177.16
Iria ini	Nyeri	1990	22.02	0.87	20.56	13.84	36.46	144.66	195.83	238.41
Kiriti	Murang'a	1970	26.08	1.15	20.18	14.58	39.44	133.17	188.34	234.60
Uruku	Meru	1970	21.61	0.92	18.52	9.85	27.58	123.65	162.00	202.13
Mugui	Embu	1900	26.46	0.66	20.10	14.42	39.11	130.51	184.70	231.26
Othaya Lower	Nyeri	1870	21.53	0.97	23.62	12.71	36.63	161.22	211.53	256.68
Kiaratha	Murang'a	1840	24.22	0.70	22.74	13.13	39.42	159.79	213.04	260.00
Kiambagathi	Embu	1730	23.92	0.93	25.24	15.14	35.35	129.33	180.75	229.91
Manyata	Embu	1730	22.83	0.38	21.04	13.83	37.88	142.36	194.45	238.35
Maua	Meru	1640	17.63	0.52	16.55	9.30	29.16	100.92	139.90	174.08
Nithi	Meru	1510	25.95	0.46	22.23	17.34	39.00	160.83	217.63	265.81
	Mean		23.30	0.78	21.67	13.65	36.80	138.24	189.47	234.45
	C.V.%		14.98	30.77	16.71	25.20	19.46	20.22	19.71	18.29
	r^b		0.01	0.13	0.06	0.13	0.12	-0.02	-0.02	0.10

Table 7b: Fatty acid distribution (mg/100g dry leaf) in young shoots of tea East of Rift Valley.

Source	District	Altitude (m) above sea level	Clone 7/14							
			C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.	Total
Kaaga	Kiambu	2190	17.37	0.85	19.99	12.12	43.82	140.21	197.00	239.36
Makamboki	Murang'a	2100	30.02	0.92	32.28	17.51	55.23	155.85	229.51	291.81
Kionyo	Meru	2090	34.81	0.60	21.74	12.77	52.82	163.90	230.09	286.64
Kiarutera	Meru	2070	29.55	0.77	29.71	18.80	59.31	197.29	305.88	335.43
Kangaita	Kirinyaga	2050	36.37	1.34	26.51	17.34	55.83	161.16	235.67	298.55
Gatundu	Kiambu	2030	15.55	0.68	16.90	7.14	25.26	82.58	115.66	148.11
Mahiga	Nyeri	2000	21.31	0.67	23.11	13.66	39.50	152.23	206.06	250.48
Iria ini	Nyeri	1990	22.78	0.47	22.11	14.26	42.46	136.48	193.67	238.56
Kiriti	Murang'a	1970	23.45	0.84	20.38	14.56	47.42	148.85	211.67	255.50
Uruku	Meru	1970	20.19	0.75	21.72	12.68	34.99	181.91	230.33	272.24
Mugui	Embu	1900	24.50	0.68	22.26	13.59	46.53	132.85	193.65	240.41
Othaya Lower	Nyeri	1870	26.40	0.78	26.56	14.24	45.53	153.67	214.22	267.18
Kiaratha	Murang'a	1840	25.59	0.74	20.03	15.06	45.69	148.80	210.29	255.91
Kiambagathi	Embu	1730	23.64	0.77	21.38	13.58	44.79	148.70	207.07	252.86
Manyata	Embu	1730	21.56	0.62	21.90	12.72	42.45	152.09	207.88	251.34
Maua	Meru	1640	22.09	0.70	19.09	11.77	41.17	148.82	202.46	243.64
Nithi	Meru	1510	29.35	0.70	25.46	19.01	50.20	177.99	247.90	302.71
	Mean		25.27	0.76	23.01	14.14	45.47	151.95	214.06	260.63
	C.V.%		20.46	23.68	16.60	19.66	17.44	15.54	16.77	14.78
	r[b]		0.01	0.14	0.05	0.10	0.12	0.06	0.02	0.05

[a]Total unsaturated = C16:1 + C18:1 + C18:2 + C18:3

[b]Correlation coefficient of linear regression analysis between altitude and fatty acids.

Table 8a: Fatty acid distribution (mg/100g dry weight) in young shoots of tea West of Rift Valley.

Source	District	Altitude (m) above sea level	Clone 54/40							
			C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[a]	Total
Kepchorop	Keiyo/ Marakwet	2160	19.84	0.94	18.26	11.16	31.17	121.02	164.29	202.39
Lessos	Nandi	2000	21.81	1.24	18.39	9.64	31.57	150.13	192.58	232.78
Isukha	Kakamega	2000	26.87	1.00	23.96	13.57	35.07	164.40	214.04	264.87
Sirongo	Kisii	1990	22.95	0.80	20.38	12.15	36.09	164.30	213.34	256.67
Baraton	Nandi	1970	22.78	0.73	19.68	8.71	32.74	164.10	206.28	248.74
Matuthe Scheme	Kisii	1920	23.83	1.14	18.66	11.92	37.53	155.93	206.52	249.01
Kapsara/ Cherangani	Trans Nzoia	1900	24.26	0.72	22.82	14.44	37.90	141.27	194.33	241.41
Buret	Kericho	1840	23.75	0.88	20.39	15.39	35.35	158.41	210.03	254.15
Matongo	Kisii	1710	24.10	1.06	20.20	9.75	34.46	151.16	196.43	240.70
	Mean		23.35	0.95	20.30	11.86	34.65	152.30	199.76	243.41
	C.V.%		7.59	17.89	9.11	18.21	6.58	8.71	7.33	7.00
	r[b]		0.04	0.13	0.07	0.01	0.21	0.17	0.08	0.01

Table 8b: Fatty acid distribution (mg/100g dry weight) in young shoots of tea West of Rift Valley.

Source	District	Altitude (m) above sea level	Clone 7/14							
			C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.	Total
Kepchorop	Keiyo/ Marakwet	2160	21.97	0.71	19.18	13.64	41.81	137.76	193.92	235.09
Lessos	Nandi	2000	21.12	0.80	17.43	8.70	35.06	169.32	213.88	252.43
Isukha	Kakamega	2000	27.33	0.85	25.01	13.99	48.81	179.32	242.97	295.31
Sirongo	Kisii	1990	24.24	0.59	16.45	10.59	44.69	173.07	245.39	269.63
Baraton	Nandi	1970	20.79	0.80	16.45	8.71	35.47	146.36	191.34	228.55
Matuthe Scheme	Kisii	1920	24.31	0.88	21.81	12.81	50.15	184.69	248.53	294.66
Kapsara/ Cherangani	Trans Nzoia	1900	21.97	0.58	23.12	13.80	43.16	145.49	203.03	248.12
Buret	Kericho	1840	25.45	0.83	21.94	16.45	49.88	175.86	243.02	290.30
Matongo	Kisii	1710	22.12	0.84	20.41	10.06	34.42	136.45	181.77	224.30
	Mean		23.26	0.76	20.20	12.08	42.61	160.92	223.66	259.78
	C.V.%		8.90	14.47	13.91	20.94	14.18	11.21	11.08	10.38
	r^b		-0.02	0.03	0.17	0.13	0.07	-0.16	0.09	0.05

[a]Total unsaturated.

[b]Correlation coefficient of linear regression analysis between altitude and fatty acids.

In Kenyan black teas, the Group I VFC are dominated by the unsaturated C_6 alcohols and aldehydes which are products of linolenic acid degradation. These are followed by hexanal and hexanol which are products of linoleic acid degradation.[9,41,50,80] The saturated C_6 constituents are followed by very low levels of nonanal and n-nonanol which are products of oleic acid degradation. These are followed by heptanal and n-heptanol which are products of palmitoleic acid degradation. The order of occurrence of these Group I VFC follows the order of occurrence of their precursor fatty acid content in the fresh tea leaves (Table 6). This order of occurrence is only an approximation due to the VFC losses during tea manufacture. The lower boiling volatile flavour compounds will volatilize more and proportionately higher amounts of the compounds will be lost during tea manufacture and processing.

Analyses of the correlation coefficients, r (Table 7 and 8) of the linear regression between the FA content and altitude showed that the r values are very small and close to zero. These values are not significant. This, basically, implies that there is no association between FA and altitude under the wide variations of ecological and climatic conditions.

Owuor et al[50] also observed that there was generally no significant relationship between the Group I VFC and altitude for teas obtained East and West of the Rift Valley. Thus this observation by Owuor et al[50] can partly be attributed to lack of a correlation between the FA and altitude. This further indicates that other factors determine the FA content

and the flavour quality aspect of Kenyan teas whether the tea is grown East or West of the Rift Valley.

Tables 7 and 8 show that the FA content of a clone changes with any change in the locational area of production. For example, the total FA content of clone 54/40 in the East of the Rift Valley changed from 169.08 mg/100g dry leaf (Kaaga, Kiambu) to 345.47 mg/100g dry leaf (Kangaita, Kirinyaga). The total FA content of clone 7/14 varied from 148.11 mg/100g dry leaf (Gatundu, Kiambu) to 335.43 mg/100g dry leaf (Kiarutara, Meru). These were very large differences especially when one considers that the various areas of production are not more than 200 kilometers apart and have only an altitude difference of 680 meters. In the West of the Rift Valley clone 54/40 had a total FA content varying from 202.39 mg/100g dry leaf (Kapcherop, Keiyo, Marakwet) to 264.87 mg/100g dry leaf (Isukha, Kakamega) while clone 7/14 had a total FA content varying from 224.30 (Matongo, Kisii) to 295.31 mg/100g dry leaf (Isukha, Kakamega). These are also significant differences within an area of radius 100 kilometers and an altitude difference of only 450 meters. Even within the same district it was observed that the FA content of a clone changed when the site of growth changed. The variations had no pattern, were independent of altitudes and were clonally dependent.

Similar observations were made by Owuor et al[50] on the content and composition of the Group I VFC. The content of these off-flavour volatile compounds, like the content of

their precursor FA, was observed to vary with the locational area where the tea was grown. The variations did not follow any pattern and were clonally dependent. Thus the observed concentration changes in Group I VFC with locational area of production can be explained as due to similar variations in the FA content of green tea leaves.

A further comparison of the teas from East and West of the Rift Valley showed that the content of the total unsaturated FA, especially the polyunsaturated, linolenic acid $C_{18:3}$, was higher in clones West of the Rift Valley as compared to clones East of the Rift Valley. Thus the quality differences that have been noted in the past, where teas East of the Rift Valley were rated better quality can partly be attributed to teas East of the Rift Valley having lower FA contents.

A comparison of the two clones used in this study, at any one site, showed marked species variations in the amounts of the FA. In fact, in the East of the Rift Valley the differences in the total FA content of the two clones 54/40 and 7/14 which are grown at the same site, ranged from 0.15 (Iriaini, Nyeri) to 73.32 mg/100g dry leaf (Mahiga, Nyeri). In the West of the Rift Valley, the differences in the total FA content ranged from 6.71 (Cherangani, Nzoia) to 45.65 mg/100g dry leaf (Matutu Scheme, Kisii). Thus clonal differences were larger East of the Rift Valley compared to West. The observation made by Owuor et al[50] regarding the Group I VFC where clonal differences East of the Rift Valley were more

than West of the Rift Valley can be attributed to the observed clonal differences in the FA content.

An interesting observation, however, about clonal variations was that for nearly all sampling sites in Kenya clone 7/14 had a higher FA content than clone 54/40. Consequently clone 7/14 would have a higher content of Group I VFC than clone 54/40 and thus this clone is expected to have a lower flavour quality.

The noted variations in the FA of clonal teas grown at the same site and receiving same agronomic and cultural practices may be attributed to the basic genetic differences or may reflect the response of the particular clone to the immediate environment.

The differing FA composition of the tea leaves due to geographical location of production can be attributed to the tea chemical/biochemical composition reacting differently to a variety of environmental conditions that are found in the areas where the tea is grown. This differing chemical/biochemical composition of tea results in quality variations and is brought about by different growth rates.[81,82] Factors that are known to cause variations in tea growth rate and hence quality include average air temperature,[83] distribution and total amounts of rainfall,[47,84] sunshine hours and cloud cover[85], fog and type of soil[47]. Although rainfall distributions in East and West of the Rift Valley in Kenya are not very different, not all areas where the sampling was done receive equal amounts of rainfall in any one season. Also, in all these tea growing areas, there are differences in the average air temperature,

sunshine hours and cloud cover (hence light intensities), fog and soil types. The difference in all these factors with localities help to explain the observed significant variations in tea FA content. The magnitude of the variations in different geographical locations for different clones indicate that not all clones behave in the same manner under different geographical conditions.

In Kenya, time from last prune has been shown to affect the chemical composition and hence quality of black tea[80]. For example, teas with longer periods since last pruning had lower Group I VFC and thus teas with longer periods since last pruning had higher values of flavour index and were of better flavour quality. The teas used in this study were in different periods from last pruning. The teas were grouped on the basis of year of pruning and Table 9 shows the mean FA compositions. From the table it is apparent there were no appreciable changes in the FA composition due to periods from last pruning for the two clones studied. Lack of appreciable changes in the FA content due to periods from last pruning can be attributed to the many climatic and environmental changes occurring due to the differing locational area of production.

The many climatic and environmental differences occurring in the areas producing tea in Kenya cause variations in the growth rate hence chemical composition and quality of the teas from these areas.[9,50,71,73,75,80,86] Mwakha[81] has shown that the growth rates of clonal teas vary with altitude such that as altitude increases growth rate decreases. Thus

Table 9: Effect of pruning period on the mean distribution (mg/100g dry weight) of lipids in young shoots

Clone	54/40					7/14				
Year of prune	1987	1986	1985	1984	1982	1987	1986	1985	1984	1982
Number of sites	9	9	7	1	1	9	8	7	1	1
$C_{16:0}$	23.36 (19.56)	23.13 (8.47)	23.54 (6.75)	23.75 -	24.10 -	25.39 (22.65)	24.44 (10.19)	20.07 (7.62)	25.45 -	22.12 -
$C_{16:1}$	0.85 (32.94)	0.81 (27.16)	0.86 (20.93)	0.88 -	1.06 -	0.86 (22.09)	0.69 (15.94)	0.71 (9.85)	0.83 -	0.84 -
$C_{18:0}$	21.27 (22.38)	21.33 (8.39)	21.43 (10.50)	20.34 -	20.20 -	22.72 (20.82)	22.04 (17.97)	21.34 (10.68)	21.94 -	20.41 -
$C_{18:1}$	13.27 (33.01)	13.63 (13.28)	12.39 (19.85)	15.39 -	9.75 -	13.38 (28.40)	14.26 (15.15)	12.67 (14.29)	16.45 -	10.06 -
$C_{18:2}$	36.53 (22.64)	37.93 (9.89)	33.26 (14.16)	35.35 -	34.46 -	45.02 (20.88)	46.17 (11.46)	40.74 (10.90)	49.88 -	34.42 -
$C_{18:3}$	144.74 (23.13)	148.28 (9.85)	130.28 (16.32)	158.41 -	151.16 -	155.55 (18.86)	156.00 (11.87)	151.26 (9.89)	175.86 -	136.45 -
Total	240.01 (22.83)	245.04 (8.29)	223.01 (10.32)	254.12 -	240.70 -	262.38 (17.91)	263.58 (11.16)	254.19 (7.10)	290.41 -	224.30 -

Figures in brackets are standard deviations.

high altitude teas have been considered of better quality. To minimise as much as possible the effects of environmental and climatic differences on the FA content and composition of tea leaves, a study was carried out on the FA of clonal teas obtained in Kericho tea estates. Samples were obtained from four growing sites all within a radius of about 10 kilometers and apart from altitude and location of the production site, climatic changes within such a short radius were assuemd to be minimal. Clones 6/8, TN14-3 and S15/10 were used.

The results of this study are given in Table 10a, b and c. The table also shows the correlation coefficient values (r) of the linear regression analysis between altitude and FA. Palmitic acid of all the three clones generally showed significant correlations with altitude. Palmitic acid levels increased with decreasing altitude except for clone TN 14-3 where a reverse trend was observed. Stearic acid content for all clones decreased with increasing altitude but the decrease was quite significant for clone 6/8. Oleic acid content of the clones 6/8 and TN 14-3 showed some increase with altitude while for clone S15/10 oleic acid decreased with altitude. The changes were not significant. Linoleic acid also changed with area of sampling. For clone 6/8 linoleic acid increased with increasing altitude. The correlation coefficient for linoleic acid and altitude was significant at $P<0.05$. However, for clones TN 14-3 and S15/10 linoleic acid showed a decrease with increase in altitude but this relationship was not significant. Linolenic acid of clone 6/8 and TN 14-3 showed negative correlations with altitude while it had

Table 10a:Effects of altitude on the fatty acid content[b] of green tea leaves obtained within Kericho tea estates

Source	Altitude (m) above sea level	Clone 6/8							
		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[d]	Total
Timbilil	2180	12.58	0.32	8.54	5.81	22.25	71.19	99.57	120.67
Chepgoiben	2120	12.67	ND[c]	12.13	5.60	21.15	67.68	93.83	118.63
Cheptabes	1940	13.14	0.19	13.12	5.42	20.89	69.72	95.82	122.08
Kaproret	1860	13.56	ND	13.46	5.04	19.74	70.43	95.21	122.26
	r[a]	-0.98*	-	-0.85	0.66	0.95*	-0.55	0.44	-0.81
	C.V.%	3.03	-	16.52	6.56	4.25	1.87	2.09	1.18

a Correlation coefficient of linear regression analysis between altitude and fatty acids.

* Significant at $P < 0.05$

b Mg/100g dry leaf

c Not detectable

d Total unsaturated

Table 10b: Effects of altitude on the fatty acid content[b] of green tea leaves obtained within Kericho tea estates.

Source	Altitude (m) above sea level	Clone TN 14-3							
		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[d]	Total
Timbilil	2180	13.30	ND[c]	8.11	6.27	21.31	69.79	97.37	118.78
Chepgoiben	2120	12.33	0.5	4.76	4.35	17.75	58.02	80.62	97.71
Cheptabes	1940	12.69	ND	7.31	6.02	19.45	64.09	89.56	109.56
Kaproret	1860	15.32	ND	7.25	5.76	23.01	68.42	97.19	119.76
	r[a]	0.62	-	-0.17	0.50	-0.45	-0.34	-0.26	-0.30
	C.V.%	8.62	-	18.34	13.28	9.68	7.05	7.75	8.15

a Correlation coefficient of linear regression analysis between altitude and fatty acids

* Significant at $P < 0.05$

b Mg/100g dry leaf

c Not detectable

d Total unsaturated

Table 10c: Effects of altitude on the fatty acid content[b] of green tea leaves obtained within Kericho tea estates

Source	Altitude (m) above sea level	Clone S 15/10							
		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[d]	Total
Timbilil	2180	11.07	0.18	9.02	6.93	18.21	70.67	95.99	116.08
Chepgoiben	2120	12.28	ND[c]	5.40	5.86	16.52	60.60	85.98	100.66
Cheptabes	1940	13.55	ND	6.35	7.47	18.64	62.66	88.77	108.67
Kaproret	1860	13.55	0.32	9.27	7.28	17.43	53.92	80.95	101.77
	r[a]	-0.95*	-	-0.18	-0.64	-0.42	0.59	0.36	0.57
	C.V.%	8.17	-	22.25	9.04	5.85	9.48	6.23	4.69

a Correlation coefficient of linear regression analysis between altitude and fatty acids

* Significant at $P < 0.05$

b Mg/100g dry leaf

c Not detectable

d Total unsaturated

positive correlations for clone S15/10. However, all these correlations were not significant. The general observation was that the FA have a marginal increase with a decrease in altitude. Similar observations were made by Owuor et al[87] on the behaviour of Group I VFC.

Even for clones growing at the same site big variations in the FA content were observed. The magnitude and order of the variations depend on the location. The areas from which the tea samples were collected, namely, Timbilil, Chepgoiben, Cheptabes and Kaproret have an altitude difference ranging between 0 and 320 meters. Climatic and environmental changes are obviously minimal but data presented here show that even with such small microecological factors or geographical changes, the lipid and fatty acid of the tea would change.

It has been recognised that improved quality tea can only be obtained from plants with high quality potential, provided other agronomic and manufacturing processes are optimal. It is for this reason that efforts to produce high quality black tea from clonal materials have been intensified. At present, however, most plant improvement work in Kenya is centralised in the Kericho region. This should not be the case.

Data presented here and in previous work[50,75,87] have demonstrated that for black tea production there are variations in the chemical composition and hence quality of the tea due to the locality of growth, in addition to clonal variations and these may change with even marginal changes

in environmental factors. Similar variations have been observed in the growth of clonal teas planted in different locations.[88] This suggests that clones chemical composition be used in conjunction with organoleptic evaluations and that clonal field evaluations be done in the areas of intended release before the clones are made available to farmers. This would ensure that clones are only planted in the areas they are likely to have the best chemical composition required to produce the best quality black tea. This is very important if quality tea production is to be intensified in Kenya.

2.2 EFFECTS OF NITROGENOUS FERTILIZERS:

The effects of nitrogenous fertilizers on the fatty acid content of the young tender shoots of clone S15/10 are as shown in Table 11a and b for the nitrogen sources NPKS 25:5:5:5 and NPK 20:10:10.

There were very significant increases in the FA (total fatty acids, total unsaturated fatty acids, or individual fatty acids) content with increasing rates of nitrogenous fertilizer applications. The higher the application rates the higher the FA contents. Except for palmitic acid, the variations (as percentage coefficient of variation) in the FA for NPK 20:10:10 was always higher than for NPKS 25:5:5:5. Owuor et al[78] similarly observed that the variation of Group I VFC when NPK 20:10:10 was applied was higher than when NPKS 25:5:5:5 was applied to clone S15/10. This observation for NPK 20:10:10 is due to the observed variation in the FA

Table 11a: Effects of nitrogenous fertilizers on the fatty acid content[c] of clone S15/10

Rate Kg N/ha/yr	NPKS 25:5:5:5							
	Fatty Acids							
	C:16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[a]	Total
0	13.78	ND[d]	12.17	8.68	19.03	58.84	86.55	112.50
100	12.40	0.82	10.40	10.09	20.57	65.53	97.01	119.81
150	16.72	1.36	12.76	9.98	23.27	82.63	117.24	146.72
300	15.61	1.06	12.60	9.39	22.35	81.34	114.14	142.35
450	16.47	0.97	10.41	10.09	23.53	88.17	122.76	149.64
600	17.38	ND	13.38	10.83	25.47	89.49	125.79	156.55
r[b]	0.75*	-0.14	0.25	0.73	0.90*	0.87*	0.87*	0.86*
C.V.%	11.40	18.73	9.63	6.78	9.33	14.76	12.77	11.67

* Significant at $P \leqslant 0.05$
a Total unsaturated
b Correlation coefficient of linear regression analysis between fatty acid content and fertilizer application rates
c Mg/100g dry leaf
d Not detectable

Table 11b: Effects of nitrogenous fertilizers on the fatty acid content[c] of clone S15/10

Rate Kg N/ha/yr	NPK 20:10:10							
	FATTY ACIDS							
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[a]	Total
0	14.94	1.80	11.09	8.42	16.51	60.47	87.20	113.23
100	15.41	1.02	11.45	8.78	20.32	66.68	96.80	123.66
150	14.63	ND[d]	8.81	9.38	20.63	68.58	98.59	122.03
300	16.49	1.12	13.61	9.78	23.51	85.11	119.52	149.62
450	17.52	1.35	15.67	10.74	25.58	88.90	126.57	159.76
600	17.74	ND	16.48	11.54	25.84	93.99	131.37	165.59
r[b]	0.93*	-0.48	0.88*	0.99*	0.95*	0.97*	0.97*	0.97*
C.V.%	7.53	22.72	20.85	11.07	14.88	16.24	15.05	14.50

* Significant at $P \leq 0.05$

a Total unsaturated

b Correlation coefficient of linear regression analysis between fatty acid content and fertilizer application rates

c Mg/100g dry leaf

d Not detectable

when this fertilizer is applied.

The correlation coefficients, r, of the linear regression between the FA and nitrogenous fertilizer application rates are as shown in Table 11. All correlation coefficients (except for palmitoleic acid which was insignificant and negative) were positive, statistically confirming the increase of FA content with increasing nitrogen levels. A rather very interesting observation was the very high values of r that were obtained. Most of these r values were significant at $P<0.05$. This implies that the increase in the FA content with increasing nitrogen application rates was linear. The r values for NPK 20:10:10 are generally higher than for NPKS 25:5:5:5, implying that there exist a more linear relationship between NPK 20:10:10 fertilizer rates and FA levels than there is for NPKS 25:5:5:5 and FA levels. Owuor et al[78] similarly observed that r values between Group I VFC and rates of nitrogen fertilizer applications for NPK 20:10:10 were higher than r values for NPKS 25:5:5:5. This observation can be attributed to the observed effects of the two fertilizers on the FA composition.

For the high rates of nitrogen application NPK 20:10:10 appeared to influence higher formation of FA than NPKS 25:5:5:5. However, for lower rates the effects of the two fertilizers on FA was the same. It is thought that higher amounts of phosphorus in NPK 20:10:10 helps more formation of FA in tea leaves than NPKS 25:5:5:5. Owuor et al[78] observed that the quality parameter for Kenya's tea that is affected most by

variations in fertilizer application rates is flavour. The Group I VFC significantly ($P < 0.05$) increased with increasing rates of fertilizer application while Group II VFC and flavour index decreased. The significant increase in Group I VFC with increasing nitrogen levels can be attributed to the significant increases in the level of the FA especially the precursor polyunsaturated FA with increasing rates of fertilizer application.

The increase in FA levels and hence Group I VFC, the decrease in Group II VFC and the resulting decrease in FI with increasing rates of nitrogenous fertilizer application partly explains the general observation by tea growers that the quality of black tea deteriorates with increasing nitrogenous fertilizer application rates. This also serves to emphasize the importance of observing the recommended rate of fertilizer application in Kenya which is 150 to 200 kg N/ha/year either as compound fertilizer NPKS 25:5:5:5 or NPK 20:1010. This rate of fertilizer application optimizes both quality and quantity for good economic returns. As can be observed in Table 11, the impact of nitrogenous fertilizer application rates on the FA was more noticeable at rates between 100 and 300 kg N/ha/year. This bracket includes the recommended rates.

The major biochemical effect of nitrogen levels in a plant such as tea is interference with protein synthesis and hence growth rate of the plant. When nitrogen levels in the soil increase (by for example fertilizer application) there occurs

increased photosynthesis. This causes the tea plant to have not only the required amino acids but there also occurs an enhancement of the machinery for synthesis of lipids containing fatty acids. Thus the overall synthesis of FA especially the unsaturated FA increases with increased nitrogen levels in the soil.

2.3 EFFECTS OF PLUCKING STANDARDS AND THE DISTRIBUTION OF FATTY ACIDS IN TEA LEAVES

The distribution of the FA in the different portions of the tea shoots is presented in Table 12. There were significant changes in the FA in the different parts of the shoot. The stem had the lowest accumulation of FA. This implies that the stem does not greatly influence the content and composition of the Group I VFC. The bud contained the highest amounts of palmitic acid. However, there was no major difference in the palmitic acid content of the 1st leaf to 4th leaf. Reasonable quantities of palmitoleic acid were detected in the 2nd to 4th leaf but the bud and 1st leaf had only trace amounts of the acid. Stearic acid levels increased from the bud to 3rd leaf while oleic acid and linoleic acid levels decreased as the leaf matured. The linolenic acid, total FA and total unsaturated FA levels, however, increased with increased maturity of the leaf. The increase in total unsaturated FA especially linolenic acid implies that the good flavour potential will decrease with leaf maturity.

Similar observations regarding the distribution of FA in tea shoots were made by Mahanta et al[89], Selvendran[58]

Table 12: Distribution of fatty acids (mg/100g dry leaf) in different parts of the tea shoot.

Leaf	Fatty Acid							
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[a]	Total
Bud	18.68	ND[b]	10.08	5.58	20.75	53.49	79.83	108.60
1st leaf	11.52	ND	11.51	5.53	18.74	62.64	86.91	109.93
2nd leaf	11.31	0.84	14.36	5.72	18.74	72.83	98.13	123.79
3rd leaf	10.23	0.81	14.64	4.82	15.90	81.76	103.28	128.15
4th leaf	10.17	0.84	13.47	3.75	12.38	91.13	108.09	131.74
Stem	6.96	ND	1.27	1.33	10.16	25.29	36.78	45.02
C.V.%	25.81	1.70	13.67	14.46	16.79	18.45	10.96	7.86

a Total unsaturated FA

b Not detectable

and Anan and Nakagawa[51]. They observed that the FA content of young tea leaves increased from 1st leaf, 2nd leaf, 3rd leaf and 4th leaf. Thus the initial growth of tea leaves is accompanied by increased FA levels.

The effects of different plucking standards on the FA levels of clones 31/8 and 6/8 are presented in Table 13a and b. The tables shows that the concentration of linoleic and linolenic acids increased with coarse plucking treatments. The increase was more significant and pronounced for linolenic acid. Linoleic and linolenic acid are of major interest in this study since they comprise more than 90% of the unsaturated FA which undergo enzymic and chemical oxidations to produce the Group I VFC. The overall pattern of variation of the FA with plucking standards for the two clones was different. This further indicates the importance of clonal differences.

The amount of palmitic acid was observed to gradually decline with coarse plucking standards for the two clones while there was not any trend in the level of oleic acid. There was not any significant change in the amount of stearic acid with plucking standards as is evidenced by the low C.V.% values.

Owuor et al[79] demonstrated that the amount of Group I VFC increased with coarse plucking standards while Group II VFC and the FI decreased. Thus coarse pluckings produce tea with lower flavour quality than finely plucked tea. The increase of Group I VFC especially the C_6 compounds with coarse plucking standards can be attributed to similar increase

Table 13a: Effects of plucking standard (harvesting policy) on the fatty acid content[a] of green tea leaves

Plucking Standard	Clone 31/8							
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[c]	Total
Bud	22.15	ND[b]	14.09	5.25	34.03	118.02	157.30	193.54
One leaf and a bud	18.78	0.92	14.61	8.23	31.02	123.37	163.54	196.93
Two leaves and a bud	18.10	0.88	14.21	9.32	31.46	125.90	167.56	199.87
Three leaves and a bud	16.93	1.31	14.35	8.46	33.12	129.06	171.95	203.23
Four leaves and a bud	15.29	1.33	14.93	6.66	34.79	130.43	173.21	203.43
C.V.%	12.5	18.97	2.08	19.10	4.40	3.52	3.49	1.89

a mg/100g dry weight

b Not detectable

c total unsaturated

Table 13b: Effects of plucking standard (harvesting policy) on the fatty acid content[a] of green tea leaves.

Plucking Standard	Clone 6/8							
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[c]	Total
Bud	20.17	ND[b]	15.45	5.33	35.09	116.10	156.52	192.14
One leaf and a bud	22.90	ND	14.62	5.29	34.23	127.64	167.16	204.68
Two leaves and a bud	16.81	1.02	13.85	7.52	34.87	130.69	174.10	204.76
Three leaves and a bud	13.74	0.78	14.09	6.33	35.81	134.43	177.35	205.18
Four leaves and a bud	13.48	0.73	14.00	6.73	36.02	135.37	178.85	206.33
C.V.%	21.10	-	4.06	13.63	1.84	5.39	4.80	2.60

a Mg/100g dry weight

b Not detectable

c Total unsaturated

in their precursor fatty acids in green tea leaves with coarse plucking standards.

This data shows that the low quality tea associated with poor pluckings of more than two leaves and a bud is partly due to high levels in the content of the polyunsaturated FA which degrade to Group I VFC. Thus the recommended plucking policy of two leaves and a bud in Kenya should be observed if quality tea is to be produced. Plucking two leaves and a bud has also been proved to give good economic yield returns.

2.4 EFFECTS OF PRUNING ON THE FATTY ACID CONTENT OF TEA LEAVES

Table 9 showed that there were no appreciable changes in the FA composition of the clones with periods from last prune. Lack of a pattern in the changes of the FA content with period from last prune was attributed to many microecological and microclimatic changes occurring due to the geographical areas of production or the varying cultural practices to which the clones were subjected.

Further study on the effect of period from last prune on the FA composition of clone S15/10 was done. The shoots were plucked from parent plants grown on the same plot, receiving identical agronomic practices, and growing under identical climatic and environmental conditions. The only difference was that the tea blocks from which the shoots were obtained were in different periods from last prune.

Table 14 shows the effects of periods from last prune on the FA content of clone S15/10. Palmitic acid was observed to significantly increase with decreasing periods since last prune. Stearic acid did not change with time from previous pruning. Oleic acid appeared to increase with increasing periods since last prune although the increase was insignificant. The total FA content of shoots plucked from parent plants that were recently pruned was higher than from plants that were pruned earlier. The total unsaturated FA were also observed to be higher in the shoots obtained from plants that had short periods from pruning than from plants with longer periods since last pruning. Thus increasing time from the last pruning lowered the contents of the polyunsaturated linoleic and linolenic acids.

It has been shown that leaf manufactured from teas with longest time from pruning were low in Group I VFC, but Group II VFC were not significantly affected by time from pruning[80]. The observed increase of Group I VFC content of black tea with decreasing periods from last prune can be explained as due to the increase in the FA content with decreasing periods since pruning. Teas made from leaves plucked from plants closest to their next pruning therefore are of better flavour quality than those manufactured from plants which have just been pruned.

It is known that quality of tea is affected by growth rate and improves as growth rate decrease[81,82]. Grice[90] and Tubbs[91] demonstrated that shoot growth rate of tea is

Table 14: Effects of time since last prune on fatty acid composition (mg/100g dry leaf) of clone S15/10

Year of pruning	Period since last prune (months)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	T.U.[c]	Total
1985	43	12.40	ND[a]	7.01	7.27	18.29	63.82	89.38	108.72
1986	31	12.59	ND	6.52	6.54	18.44	67.39	92.34	111.48
1987	19	14.03	ND	6.90	5.80	17.76	71.46	95.01	115.04
1988	7	14.87	ND	6.50	7.71	21.48	68.74	97.93	120.29
C.V.%	-	6.42	-	26.94	13.45	3.75	4.98	3.60	3.73
r[b]	-	-0.97	-	0.64	-0.09	-0.68	-0.85	-1.0*	-0.99*

a Not detectable

b Correlation coefficient in linear regression analysis between pruning time and fatty acid levels

c Total unsaturated

* Significant at p = 0.05

slower when time from last pruning is long. Thus the observed variation of FA content with time from last pruning can be explained on the basis of growth rate. Teas with short periods from last pruning have high growth rate, the high growth rate results in increased protein and chlorophyll synthesis. This is accompanied by increased synthesis of membrane systems of which lipids form a big percentage composition. Increased lipid synthesis is concomitant with increased synthesis of the constituent fatty acids especially the polyunsaturated fatty acids.

It must be emphasised that pruning of tea must be done to keep the bushes under manageable levels. This study has only shown that teas with long periods since last pruning have lower FA contents and are thus better in terms of flavour quality.

CHAPTER THREE

EXPERIMENTAL SECTION

3.0 GENERAL REVIEW:

Chemical changes in tea leaves start as soon as the tender shoots are plucked from the parent plants. Tea, particularly contains lipolytic enzymes bound to the cellular structure of the leaves. Soon after the leaves are plucked these lipolytic enzymes are released. These highly active enzymes cause appreciable hydrolysis of acyl lipids to release fatty acids[57]. The released fatty acids especially the polyunsaturated ones undergo chemical oxidations and degradations as shown in Scheme 1[63].

These enzymic alterations in lipid structure result in loss of specific components or result in production of artefacts. Hence for total lipid extraction and analysis the tissue must be subjected to some treatments to deactivate these enzymes prior to the initial stages of lipid extraction. Among the procedures for deactivation which have proved satisfactory with leaves are; direct maceration of the leaves in cold iso-propanol[92-94], plunging the leaves into boiling water for very brief periods[95], or boiling the leaves in ethanol or dilute acetic acid[96].

Most of the lipids of leaf tissues occur within membraneous structures where they are associated with proteins or carbohydrates by a combination of hydrogen bonds, Van der Waals and electrostatic forces as components of

lipoprotein or lipocarbohydrate complexes. Thus the ideal solvent or solvent mixture for extracting lipids from tissues should be sufficiently polar to remove all lipids from their association with cell membranes or with lipoprotein or lipocarbohydrates but should not react chemically with those lipids. The solvent should not be so polar that non-polar simple lipids do not dissolve and are left adhering to the tissues.

The main features of lipids which affect their solubility in organic solvents are the non-polar hydrocarbon chains of the fatty acid or other aliphatic moieties and any functional groups such as phosphates or sugar residues[97]. Lipids which contain no markedly polar groups eg. cholesterol esters are highly soluble in non-polar solvents or slightly more polar solvents such as chloroform or diethyl ether. They are insoluble in polar solvents such as methanol. Polar lipids, on the other hand dissolve readily in more polar solvents such as methanol, ethanol or chloroform.

Hence quantitative extraction of lipids is achieved by employing polar solvents such as ethanol, methanol or acetone which not only break the lipid-protein or lipid-carbohydrate linkages, but also dissolve all of the polar lipids available in the tissue. These solvents are used in combination with non-polar ones such as chloroform, benzene, petroleum ether, or diethyl ether.

The use of elevated temperatures (above 35°C) for long periods can cause detrimental effects on some of the lipid components, e.g. can accelerate the oxidation of the polyunsaturated

fatty acids, while use of temperatures below 20°C limits solubility of many of the lipids. Room temperature or at most 30°C is best for the most effective lipid extractions.

Most polar organic solvents used to extract lipids from tissues also extract significant amounts of non-lipid contaminants such as sugars, urea, amino acids and salts. Methods which have been used to remove all potential contaminants of lipid extracts are; adsorption and cellulose column chromatography[98], electrodialysis and electrophoresis, or even pre-extraction of tissues with 0.25% acetic acid[96].

A generally satisfactory method of removing non-lipid contaminants is the procedure given by Folsh, Lees and Stanley[99] as modified by Ways and Hanahan[100]. This procedure recovers 95-99% of the lipids. It was the method used in this study and is generally called the "Folsh" wash.

Many simple and complex lipids are either too polar or of too high a molecular weight to be subjected to some chromatographic procedure. So it is necessary to convert them to derivatives for further analysis. The fatty acid composition of lipids is determined by gas-liquid chromatography (GLC). This is the technique that is chosen in most circumstances since it was introduced in 1956 by James and Martin[101].

Before the fatty acid composition of the lipids is carried out by GLC they are converted to the methyl esters. Conversion of the fatty acids to their methyl ester derivatives increases the volatility of the fatty acids (which otherwise have too high boiling points), reduces the adsorption of the fatty acids on the support and column surface and improves separation. The

volatility of methyl esters is sufficiently high to permit the determination of compounds ranging from C_{10}-C_{24} (or even higher fatty acids) in the same sample[102].

A method of preparing fatty acid methyl esters (FAMES) for GLC analysis should ideally be simple, rapid, quantitative and should give rise to no unwanted structural changes or side reactions. There are several methods for the preparation of FAMES. The most widely used are those which employ diazomethane[103,104] and methanolic solution of boron trifluoride[105-107]. Also frequently used are reactions with hydrochloric acid and methanol and sulphuric acid and methanol[108].

3.1 MATERIALS:

For all the study cases except plucking standards, plucking of plant materials from parent plants conformed to the normal commercial practice i.e. 90% good leaf, mainly two leaves and a bud with minor amounts of three leaves and a bud. Materials for all study cases were obtained as follows.

3.1.1 Clonal differences and geographical area of production.

Clone 7/14 and 54/40 were sampled from the Kenya Tea Development Authority (KTDA) clonal observational field trials set up in all the tea growing districts of Kenya in 1981[88]. Sampling was done between 9th and 21st November, 1987. There were 17 sampling sites East of the Rift Valley and 9 sampling sites West of the Rift Valley.

Clone 6/8, S 15/10 and TN 14-3 were sampled from Kericho tea estates within a radius of about 10 kilometers from plots at Kaproret, Cheptabes and Chepgoiben estates (at 1960, 1940 and 2120 meters above mean sea level, respectively) of the African Highlands Produce Company, Kericho. The same clones were also sampled from the Clonal Field Trials (CFT) at the Timbilil estate of the TRFK 2180 m above mean sea level. Sampling was done on 15th December, 1988.

3.1.2 Effects of nitrogenous fertilizers.

Clone S15/10 was used. This is a high-yielding clone and yields of up to 8,000 kg of black tea per hectare per year have been recorded. The recommended rate, of fertilizer application in Kenya is 150 to 200 kg of nitrogen per hectare per year. These rates may be inadequate for such a high yielding clone. To correct for possible inadequacy, higher rates of fertilizers on this clone was used. The fertilizer rates used were 0, 100, 150, 300, 450 and 600 kg N/ha/year. This was put as NPKS 25:5:5:5 and NPK 20:10:10 in a single application.

The experimental fertilizer was applied on 11th March 1989, and leaf samples were taken on 12th April, 1989 i.e. one month after the fertilizer application. The experiment was laid out on the same block of tea.

3.1.3 Effects of plucking standards.

The tea shoots used to determine the distribution of the FA in the different parts of young shoots were obtained from mother bushes of clone 6/8 at the TRFK. The shoots were then

divided into bud, 1st leaf, 2nd leaf, 3rd leaf, 4th leaf and stem (between bud and 4th leaf). The parent bushes had received 200 kg N/ha/year as NPKS 25:5:5:5 fertilizer. Sampling was done in triplicate. Clones 6/8 and 31/8 were coarsely plucked from TRFK clonal field trials, set up in 1982 to determine effect of plucking standards on FA levels of tea shoots. The plucked shoots were divided into bud, one leaf and a bud, two leaves and a bud, three leaves and a bud and four leaves and a bud. The teas were receiving 150 kg N/ha/year as NPKS 25:5:5:5. Sampling was done in triplicate.

3.1.4 Effects of pruning.

Shoots were plucked from randomized but non-replicated fields from blocks pruned at different times within the Applied Research Department (Chepgoiben Estate) of the African Highlands Produce Co. Ltd., Kericho, Kenya, at an altitude of 2120 m above mean sea level. Plucking was done in December 1988. The teas had been pruned 43, 31, 19 and 7 months prior to sampling. The high yielding clone S15/10 was used.

3.2 LEAF TREATMENT AND CHEMICAL ANALYSIS:

As soon as the tea leaves were plucked from the parent plants, they were immediately steamed on a water bath for one minute[95]. The teas were cooled to room temperature and placed in well-aerated bags. The samples were air dried in an oven at 60°C for 6 hours. The dried samples were then ground to a fine powder using a coffee grinder and stored in a deep-freezer.

3.2.1 Extraction procedure.

To remove all potential lipid contaminants present in the solvents, the solvents were distilled twice before any extraction was started.

Ten grams of the powdered leaves was weighed out and extracted with 200 mls of chloroform and methanol mixture (2:1 v/v) for four hours with continous stirring at room temperature. The mixture was then filtered through a sintered glass funnel with the aid of sunction pump. A further 200 mls of chloroform-methanol mixture (2:1 v/v) was added to the residue and extraction done for a further 4 hours. The two filtrates which represented the "total lipids" were combined and the volume determined. 0.015g of heptadecanoic acid (the internal standard) was added and the contents thoroughly stirred.

3.2.2 Purification of the lipid extracts.

100 mls of 0.88 per cent potassium chloride solution in water was added to the total lipid extracts and the mixture thoroughly shaken before being allowed to settle. The solvents partitioned into a lower layer and an upper layer. The lower layer contained the purified lipids and the upper phase contained the non-lipid contaminants. The purified lipid layer was filtered before the solvent was removed at 35°C on a rotary evaporator. The weight of the lipid recovered was determined.

3.2.3 Preparation of fatty acid methyl esters (FAMES).

Base-catalysed transesterification was used in conjunction with acid-catalysed esterification and transesterification[109].

1.0g of the lipid mixture and 10 ml of 0.5N methanolic NaOH was placed in a 100 ml round bottomed flask fitted with a condenser. The mixture was refluxed for 10 minutes until the lipids dissolved. 12 ml of borontrifluoride-methanol complex (about 14% w/w BF_3) was added through the top of the condenser and refluxing done for a further two minutes. The solution was cooled, 5 ml hexane added and the mixture boiled once more for two minutes. A solution of saturated sodium chloride was then added, the organic layer separated and dried with anhydrous sodium sulphate. Under the conditions described no isomerization of double bonds in polyunsaturated fatty acids occurs.

3.2.4 Analysis of the FAMES by GLC.

Two standard types of chromatographic stationary phases were used for this purpose. Both were polyester phases, namely polyethylene glycol adipate (PEGA) and diethylene glycol succinate (DEGS). These polyester column materials resolve fatty acids esters according to both chain length and degree of unsaturation. Two stainless steel columns (2M by 3.2 mm Od) were used. One column was supplied already packed with 10% (w/w) PEGA on acid-washed chromosorb w, A/W mesh size 80/100. The second column was packed in the laboratory with 15% DEGS on acid-washed chromosorb w, A/W mesh 80/100. The two columns were first conditioned by passing nitrogen gas through them at 180°C for 5 hours. The columns were then fitted on a GOW-MAC gas chromatograph equipped with a flame ionisation detector (F.I.D.), column temperature was isothermal

throughout the column length at 180 ± 1°C. Injector-detector temperature was 220 ± 1°C, nitrogen gas flow rate was 40 ml/min. A sample of the FAMES in hexane solution was injected into the GC using a microlitre syringe. There was no need to control the amount of the solution actually injected as long as peaks of reliably measurable size were obtained.

3.2.5 Identification and quantification of the FAMES.

Seven major peaks were observed on the GC chart paper. Thus there were seven major fatty acids in the injected sample. Provisional identification of the fatty acids was done by direct comparison of the retention times of their methyl esters with those of some known standard methyl esters (Sigma Chemicals, U.S.A.) on the same two columns under identical conditions. The identity of the FAMES was confirmed by carrying out their gas chromatograph-mass spectrometry analyses.

For gas chromatographs equipped with a flame ionisation detector the areas under the peaks on the GC chart traces are linearly proportional to the amount of material eluting from the columns[101]. For the purpose of quantitative fatty acid composition analysis it was assumed that all the sample together with the internal standard were injected and that the area response recorded for the internal standard represented its weight. The weights of the other peaks were then directly calculated from the weight of the internal standard. The peak areas of the component FAMES were measured manually by multiplying the height of the peak by the width at half height[102].

The peak area of the sample components were then corrected by a multiplying factor and divided by the peak area of the internal standard to give the concentration of the particular component. This can be expressed in the form of an equation as follows[105],

$$\frac{\text{Peak area of component x multiplying factor x weight of internal standard}}{\text{peak area of internal standard}} = \text{Weight of the component}$$

The multiplying factor was obtained by performing GC of a standard solution of representative fatty acids prepared on an equal weight basis. The multiplying factors or response ratios in terms of the internal standard was then calculated by dividing the peak area of the internal standard by the peak area of each component[108]. Weight of the internal standard is the amount (0.015g) of internal standard added to the lipid extracts.

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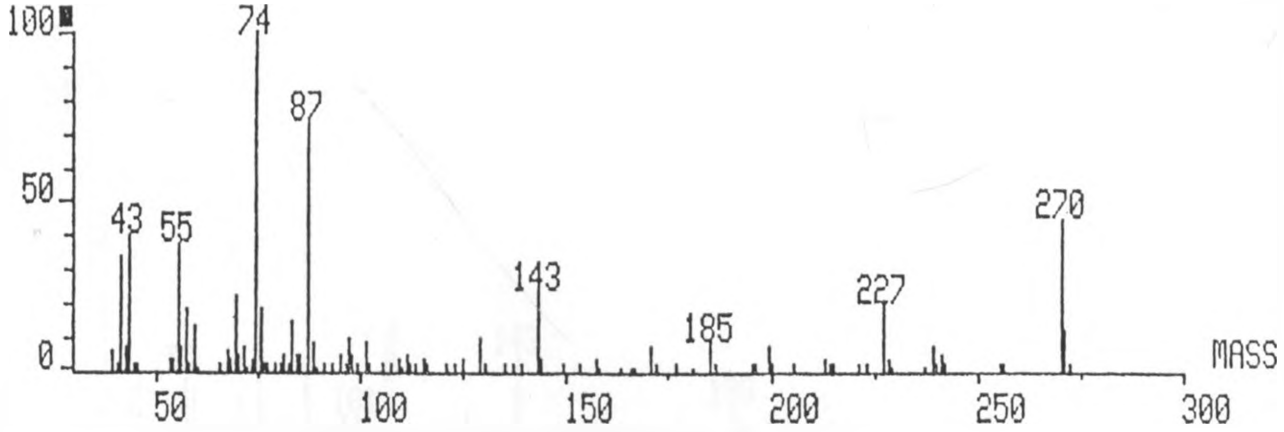
A P P E N D I X

Mass spectra of the fatty acids of tea leaves.

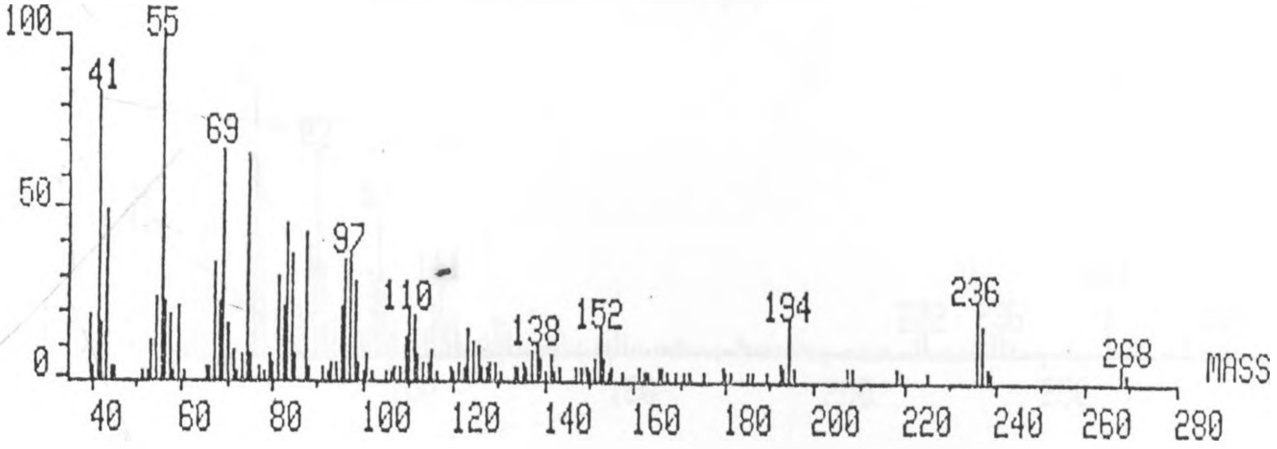
METHYL HEXADECANOATE

$CH_3(CH_2)_{14}C(=O)-O-CH_3$



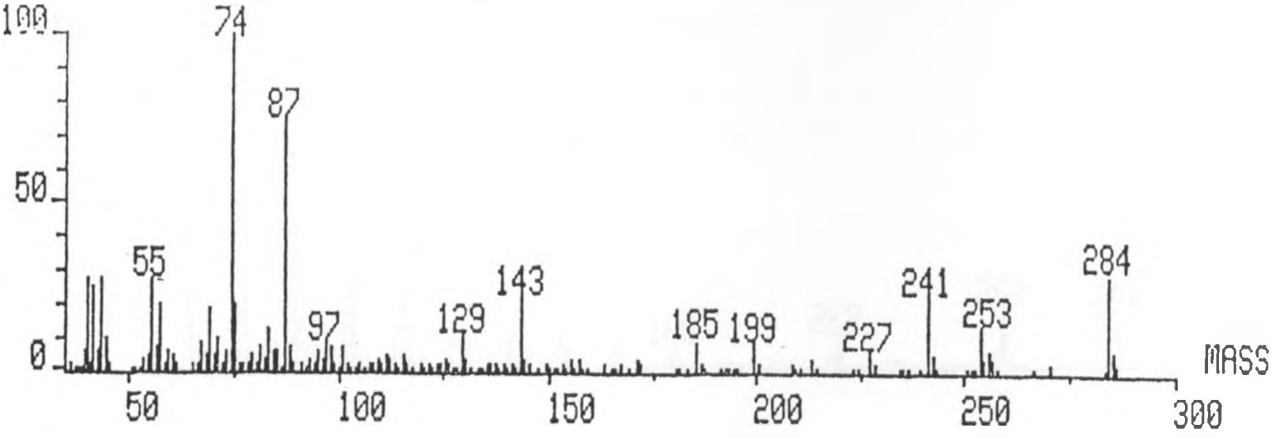
METHYL PALMITOLEATE

$CH_3(CH_2)_5CH=CH(CH_2)_7C(=O)-O-CH_3$



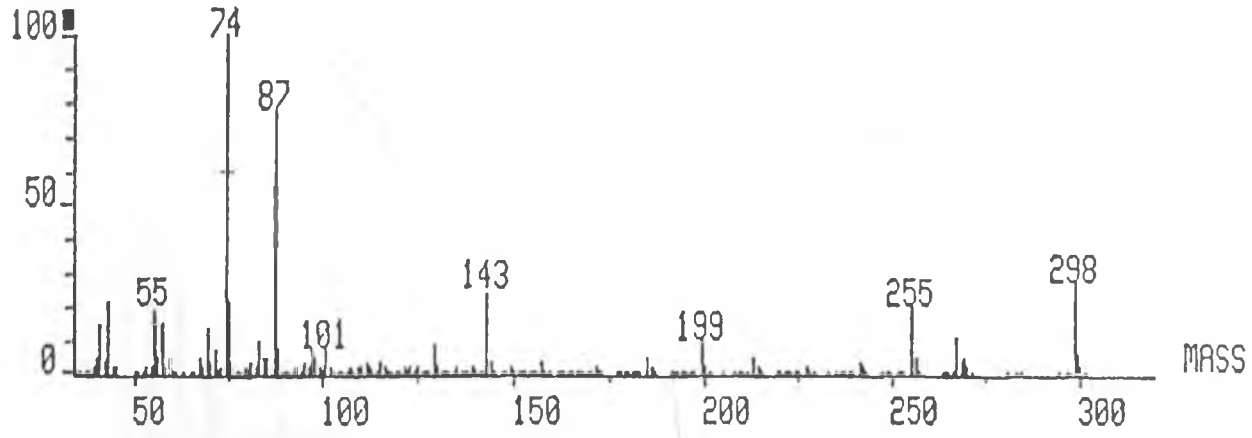
METHYL HEPTADECANOATE

$CH_3(CH_2)_{15}C(=O)-O-CH_3$



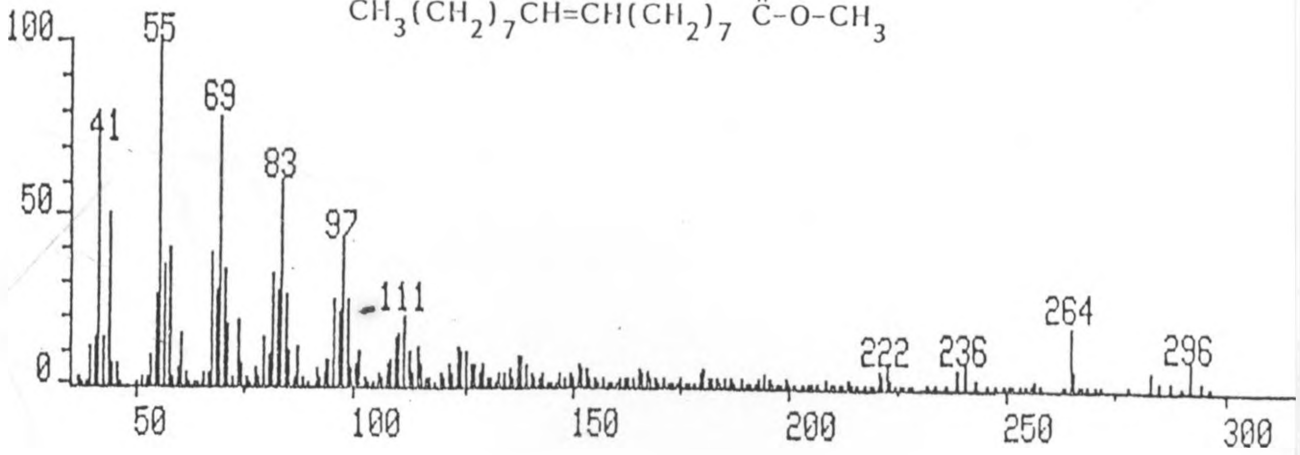
METHYL STEARATE

$$CH_3(CH_2)_{16}\overset{O}{\overset{\|}{C}}-O-CH_3$$



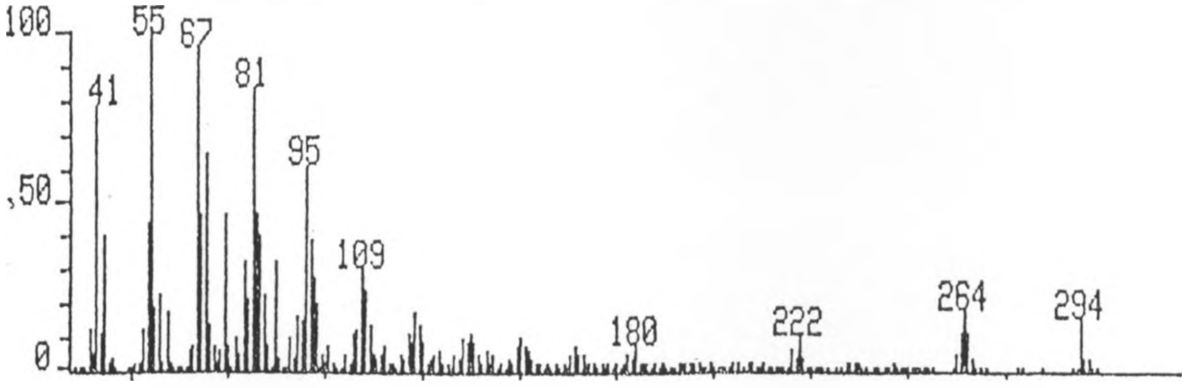
METHYL OLEATE

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7\overset{O}{\overset{\|}{C}}-O-CH_3$$



METHYL LINOLEATE

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7\overset{O}{\overset{\|}{C}}-OCH_3$$



METHYL LINOLENATE