INFLUENCE OF AREA OF PRODUCTION, NITROGENOUS FERTILIZER RATES AND PLUCKING INTERVALS ON THE PRODUCTION OF FATTY ACIDS IN CLONAL TEA (*Camellia sinensis* (L.O) Kuntze) LEAVES

BY

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ABSTRACT

Tea, *Camellia sinensis,* is widely grown in the highlands of Kenya for manufacture of mainly black tea. The most costly inputs in tea cultivation are nitrogenous fertiliser and plucking which are key determinants of yield and quality. The black teas are classified in the tea trade either as plain or flavoury. Flavoury teas are sold for their special aroma normally caused by volatile flavour compounds (VFC). Unsaturated fatty acids break down during tea processing to produce volatile flavour compounds responsible for green grass smell leading to low aroma quality. Previous studies on fatty acid levels and composition were conducted at single locations and results used to draw general agronomic recommendations. Consequently, planting materials have been assumed to replicate their chemical composition in different areas even though factors affecting growth and quality change with environment. Blanket agronomic input, may be producing tea leaves of varying levels of fatty acids especially unsaturated fatty acids resulting in quality differences in different locations. This study aimed at establishing the variation in fatty acid levels of a single tea clone grown under same agronomic inputs and management in different locations. Trials were conducted in plots in five different tea growing locations in Kenya using clone BBK 35 cultivar. Each plot was treated to varying nitrogenous fertiliser rates (0, 75, 150, 225, 300 Kg N/ha/year) and plucking rounds (7, 14, 21 days). The experiments were in a factorial two design laid out in a randomised complete block. From each location two leaves and a bud were plucked when plucking intervals coincided. The lipids were extracted using chloroform/methanol (2:1v/v) mixture, converted into fatty acid methyl esters and quantified through GC-FID analysis and confirmed by analysis of fatty acid authen tic standa rds under similar conditions. The levels of saturated fatty acids, unsaturated fatty acids, total unsaturated fatty acid and total fatty acid varied significantly ($P \leq 0.05$) with locations and significantly increased ($P \leq 0.05$) with increasing nitrogenous fertiliser rates and longer plucking intervals. These variations did not follow particular pattern hence significant ($P \leq 0.05$) interaction effects implying similar agronomic inputs and management in different locations result in different fatty acid levels and may lead to different aroma quality of processed tea. It is necessary to develop region specific nitrogen fertilizer rates and plucking intervals to maximise production of leaves with low levels of unsaturated fatty acids leading to high quality black teas.

CHAPTER ONE: INTRODUCTION

1.1: Background information

Tea, *Camellia sinensis* L. O. Kuntze, is widely grown in the highlands of Kenya and is mainly used to process black tea. It is grown on the foothills of Aberdares and Kenya mountains in the East of the Rift Valley and on the foothills of Mau ranges, Nandi, Kisii and Kakamega Hills in the West of the Rift Valley (Anon, 2004). Tea is an important player in the economy of the producing countries as it is a major economic activity creating employment to a vast majority of the population both skilled and unskilled. In Kenya, agriculture is the backbone of the economy. The tea industry is one of the largest contributors to the economy, ranking second after tourism as a foreign exchange earner. Kenya is the third leading tea producer after China and India accounting for 9.1% world tea production and was the second largest (23.4%) exporter of tea globally in 2008 (Anon 2009). The tea industry and its allied activities employ directly over 500,000 families, each on the average supporting 6 members (Ogola and Kibiku, 2004). It is estimated that tea and allied industries/activities support over 3 million Kenyans, most of them smallholder families living in rural areas where economic activities are low. Tea therefore contributes to poverty reduction, promotes infrastructural development in the rural areas and earns the government foreign exchange and revenue. Over time the prices of Kenya's tea has stagnated or declined despite the increase in costs of production. It is necessary to develop methods that can enhance tea quality and hence prices. This makes it necessary to develop agronomic and cultural practices that will improve the yields and quality of tea. Most tea growing agronomic recommendations in Kenya were developed in Kericho for seedling tea and recommended for use in the whole country for all cultivars (Othieno, 1988; Anon, 2002). However variations in yields (Wachira *et al.*, 2002; Owuor *et al.*, 2009), plain quality parameters (Owuor *et al.*, 1987c, 2009, 2010) and black tea aroma quality (Owuor *et al.*, 1988) have been recorded even in the same cultivar grown in different parts of Kenya. Thus the use of recommended agronomic practices has not given identical tea yields and plain quality, in different locations of Kenya. However, there has been no research to investigate the variations of aroma quality or precursors to aroma quality parameters with location. There is urgent need to

assess the appropriateness of the agronomic recommendations to establish the optimal requirements for high yields and quality of tea in different localities.

The favourable conditions for tea cultivation include suitable temperature (15-25°C), high relative humidity (80-90%), high and well distributed annual rainfall (1200-2000 mm) and acidic soils pH (4.0-5.6) (Othieno, 1988; Anon, 2002) conditions that prevail in the Kenya highlands. Such lands have high potential and should be subjected to maximum economic production for faster industrial development and poverty reduction. Over the years, the land under tea production has increased (Anon, 2007, 2009) (Table 1), but breeding programmes have remained at centralised. It has been assumed the cultivars will replicate yields and quality at the selection sites contrary to recent research results (Wachira *et al.*, 2002).

Table 1: Area under tea and tea production in Kenya

Year	Area under tea (Ha)	Tea production (metric tons)
1997	110,222	209,422
1998	114,458	294,165
1999	117,437	248,818
2000	117,350	236,286
2001	118,650	294,631
2002	124,201	287,102
2003	126,203	293,670
2004	131,581	324,609
2005	139,976	328,584
2006	131,419	310,607
2007	149,196	369,606
2008	157,723	345,816

Source: Anon, 2007, 2009

1.2: Influence of fatty acids on flavour quality of tea

Tea is a non-alcoholic beverage consumed for its refreshing and stimulating effects. It has been claimed to be the second most widely consumed fluid after water (Gardner *et al.*, 2007). Tea beverage is manufactured from tender shoots of *Camellia sinensis* plant. The world tea production has risen fast without a commensurate rise in consumption (Anon, 2009) while the cost of production continues to rise (Herath and Weersink, 2007). In Kenya in particular, tea production has increased over the years as the land acreage under tea also increase (Table 1). As

a result of increased global production, the world prices of black tea have been stagnating or declining. But in the competitive market, only producers of premium quality can survive the competition. In the tea trade, the quality of black tea is determined organoleptically. This method has been criticized as being subjective and influenced by the taster's personal preferences (Owuor *et al.*, 1986; Biswas and Biswas 1971a, 1971b; Biswas *et al.*, 1973). Moreover, this method only determines the quality of tea after processing, so there is no way predicting quality at the green leaf stage. Extensive research has been done to asses the quality of tea in more qualitative and reproducible manner using black tea chemical constituents. In Kenya for example, the theaflavins (Owuor *et al.*, 1986, 1987b), especially individual theaflavins (Owuor and Obanda, 1995, Owuor *et al.*, 2006, 2007) have been shown to influence tea prices. Other studies have shown that agronomic practice (Owuor, 1996; Odhiambo *et al.*, 1988) and processing technologies (Baptista *et al.*, 1998, 1999; Owuor *et al.*, 2008) affect black tea quality. It is necessary to understand how quality of same cultivar of tea varies with agronomic inputs due to location of production.

The black teas are classified in the tea trade either as plain or flavoury. Plain teas are valued for their taste, briskness and colour characteristics. The plain teas are mainly obtained from seedling teas and their quality is mainly influenced by non-volatile (theaflavin and thearubigin) components. Recently, plain tea quality was reported to vary with location even in the same genotype under similar agronomic inputs (Owuor *et al,* 2010). On the other hand, flavoury teas are obtained from high altitude grown teas, mostly clonal material (Owuor *et al.*, 1988). At present, 80% of Kenyan tea is from clonal material. The flavoury teas are valued for their special aroma and flavour (Yamanishi *et al.*, 1966; Bondarovitch *et al.*, 1967; Reynolds *et al.*, 1974; Asiaka *et al.*, 1978; Takeo, 1983; Takeo and Mahanta, 1983; Mick *et al.*, 1984; Horita and Owuor, 1987; Cloughley *et al.*, 1982; Robinson and Owuor, 1992). The aroma quality of black tea is attributed to volatile flavour compounds (VFC) mainly derived from lipids in the leaves (Hatanaka *et al.*, 1978; Yamanishi *et al.,* 1978; Robinson and Owuor, 1992). A significant relationship has been demonstrated between aroma and sensory evaluation of black tea (Owuor *et al.*, 1988; Owuor, 1992; Robinson and Owuor, 1992).

The aroma compounds in tea can be classified into primary and secondary products (Sanderson and Graham, 1973). The primary aroma compounds are biosynthesized by the plant and are

present in the fresh tea leaf, while the secondary products are produced during black tea manufacture via enzymic, redox or pyrrolytic reactions from carotenes, amino acids, unsaturated fatty acids and other lipids (Sanderson and Graham, 1973; Yamanishi, 1981; Yamanishi *et al.*, 1978) and terpene glycosides (Skobeleva *et al.*, 1987). The volatile flavour compounds (VFC) contributing to the aroma quality of black tea have been identified and studied in a number of detailed investigations (Hatanaka *et al.*, 1978; Hatanaka, 1993; Hatanaka *et al.*, 1995; Robinson and Owuor, 1992, Yamanishi *et al.*, 1978). The VFC are further classified into two groups; group 1 VFC that impart undesirable grassy aroma and group II VFC that impart sweet flowery aroma to black tea (Robinson and Owuor, 1992; Yamanishi *et al.*, 1978). The group I VFC is of much interest in tea production as they impart undesirable aroma to black tea hence reduce tea quality. The group I VFC is composed mostly of C_6 aldehydes, alcohols and acids, which are products of unsaturated fatty acids breakdown during black tea processing (Galliard, 1975; Robinson and Owuor, 1992). Group I VFC levels in black tea correlate negatively with tasters' evaluation confirming that high concentration of group I VFC is deleterious to tea quality (Owuor, 1992). The levels of group I VFC increase with increase in amounts of unsaturated fatty acids in green tea leaves (Owuor *et al.*, 1990a, 1990b, 1990c) leading to poor aroma quality of black tea (Mahanta *et al.*, 1988; Owuor *et al.*, 1987c, 1987d; Owuor and Langat, 1988). The amounts of unsaturated fatty acids in green tea leaves should be kept low to enhance desirable aroma. Although plain tea quality varies with location (Owuor *et al.*, 2010, Jondiko, 2009), aroma quality parameters or their precursors of a single clone of tea under similar agronomic inputs and management in different locations have not been investigated.

1.3: Nitrogenous fertilizer rates and fatty acid levels

Fertilizer application is the second most expensive agronomic input in tea production (Othieno, 1980; Ellis and Grice, 1981; Ruto *et al.*, 1994) after harvesting. Tea production is a long time monoculture and without applied fertilizer, the supplies of nutrients available in the soil become exhausted leading to mineral deficiencies and degraded soils. The loss of nutrients through harvesting, leaching and fixation implies that the plants require replenishment of the lost nutrients through the addition of fertilizer. Nitrogen is important for plant growth and is a constituent of plant biochemicals like chlorophyll, nucleotide, proteins, amino acids hormones, protoplasm, vitamins and enzyme. Nitrogen is important in creating plant dry matter as well as

many energy-rich compounds that regulate photosynthesis and plant production. Nitrogen application influences the yield through the variations in the rate of shoot extension, individual shoot weight and density (Odhiambo, 1989; Owuor *et al.*, 1997). Appropriate use of nitrogenous fertilizers lead to increase in tea production (Wanyoko, 1983; Willson, 1975; Owuor and Wanyoko, 1996; Bonheure and Willson, 1992) but the high rates of fertilizer application reduce black tea quality (Owuor *et al.*, 1990a, 2000) especially black tea aroma/flavour (Owuor *et al.*, 1987a, 1987b, 1991, 1997, 2000; Owuor and Othieno, 1996; Owuor and Wanyoko, 1996). This decline in aroma quality was attributed to increase in unsaturated fatty acid levels with increase in nitrogenous fertilizer rates (Owuor *et al.*, 1990a; Muritu, 1989) since the unsaturated fatty acids breakdown during black tea processing to form group I VFC (Sekiya *et al.*, 1984).

Studies relating the fatty acids responses to nitrogen rates have been conducted at single sites or using different cultivars (Owuor *et al.*, 1990a, 1990c, Muritu 1989). There has been no study in different locations using single genotype hence it was hard to isolate the variations due to location from previous studies. Presently, uniform nitrogenous fertilizer rates are recommended across the country at 100 to 250 Kg/Ha/Year as NPKS 25:5:5:5 or NPK 20:10:10 (Othieno, 1988; Anon, 2002) the actual rate being dependent on the level of production. It is not known if similar nitrogenous fertilizer application rate would result in the similar amounts of arom a precursor compounds (especially fatty acids) in different locations. An evaluation of the fertilizer application rate in different locations is necessary so as to provide baseline information for the establishment of region specific requirements that would minimise losses by maximizing fertilizer requirements since the costs of fertilizer is high.

1.4: Plucking intervals and fatty acid levels

Plucking is an important practice in tea production that removes young tender shoots for processing into various tea beverages (Willson, 1992). In the processing of black tea, the recommended plucking standard is two leaves and a bud that gives desirable good quality teas (Othieno, 1988; Owuor *et al.,* 1987a; Willson, 1992). Coarse plucking standard of more than two leaves and a bud increases the group I VFC especially C_6 aldehydes alcohols and carboxylic acids while decreasing the group II VFC (Mahanta *et al.*, 1988; Owuor *et al.*, 1990c). At single site, the unsaturated fatty acids levels increase with maturity of the shoot (Bhuyan *et al.*, 1989; Mahanta *et al.*, 1995; Owuor, *et al.*, 1990c) implying a tendency to produce poor flavoury tea with coarse plucking standard. Plucking two leaves and a bud is considered the compromise between yields, plucker productivity and quality of black tea in all cultivar (Othieno, 1988; Owuor *et al.*, 1987a; Willson, 1992). In practice, it is difficult to achieve exclusively the two leaves and a bud (Odhiambo *et al.*, 1988). One way to achieving two leaves and a bud is by optimising the harvesting intervals such that plucking is done when most leaves are in the two leaves and a bud level. Plucking interval influences the quality of black tea especially aroma quality (Owuor *et al.*, 1990c, 1997, 2000; Owuor and Odhiambo, 1994). Indeed the sum of group I VFC decrease with shortening of plucking interval and group II VFC decrease with lengthening of plucking interval. Thus, shorter plucking intervals improve the aroma quality of black tea (Baruah *et al.*, 1986; Owuor *et al.*, 1997; Owuor and Odhiambo, 1994).

However, most studies have related plucking rounds to the VFC and not their precursors despite the observation that unsaturated fatty acids degenerate to VFC during tea manufacture (Saijo and Takeo, 1972, Hatanaka *et al.*, 1987). Furthermore, most of the studies relating plucking rounds with fatty acids were done at single sites despite differences in growth rates at different locations (Obaga *et al.,* 1988, 1989; Squire, *et al.,* 1993; Squire, 1979; Ng'etich and Stephens, 2001a, 2001b; Ng'etich *et al.,* 2001) leading to achievement of the recommended two leaves and a bud (Othieno, 1988) after different time lengths in different locations (Mwakha, 1985a, 1985b), suggesting that suitable plucking intervals may vary with locality. No study has been conducted to investigate how the precursors to group I VFC vary with location at constant plucking interval.

1.5: Locality and fatty acid levels

The region of growth is one of the factors determining the potential productivity of tea cultivars (Ng'etich and Stephens, 2001a, 2001b; Ng'etich *et al.*, 2001). Variations in yields and plain tea quality have been recorded due to area of production in the same cultivar under the same agronomic practices (Babu *et al.*, 2007; Owuor *et al.*, 2009, 2010, Jondiko, 2009). Besides, the VFC levels also change with region of production (Owuor *et al.*, 1988). Indeed, variations in VFC have been witnessed in tea from same cultivar under similar processing conditions in Kenya and Malawi (Owuor *et al.*, 2008). However it is not known if there are variations in fatty acid levels to account for the variations in VFC so far witnessed. Presently, studies on fatty acids have only been conducted on different clones at single sites (Owuor *et al.*, 1990a, 1990c) except when same cultivars were used within a radius of 10 km and changes in environmental conditions were minimal (Owuor *et al.*, 1990b). Changes in fatty acid levels in single cultivar under wide environmental variations have not been investigated. Possibly, the levels of the precursors of aroma quality parameters especially fatty acids vary with location under similar plucking management. Currently, cultivars that were bred in Kericho are grown in the entire tea growing locations in Kenya and many parts of Africa. Most farmers believe these cultivars maintain the same chemical composition and quality potential as assessed in the region it was developed and that these cultivars respond uniformly to the agronomic practices everywhere it is grown. It is not known if the cultivars maintain the composition of fatty acids in the new habitats which differ widely in environmental conditions such as total rainfall and distribution, temperature and edaphic factors including drought, cold, frost, solar radiation and soil characteristics. There is need to evaluate the changes in precursors of aroma quality in the same cultivar under similar plucking management with areas of production to help determine the suitability of using uniform fertilizer rates and plucking intervals. This will help in predicting how aroma quality will be affected by agronomic inputs at specific locations of growth.

1.6: Statement of the problem

Making the correct choice of agronomic inputs remains a big drawback among farmers. It is not known if nitrogenous fertiliser rate and plucking interval leading to production of high quality at one site are suitable in other tea growing locations. As a result the current blanket agronomic

recommendations in all locations may not be ideal and may lead to farmers' failure to achieve the desired quality, hence low incomes. There is need to assess the variations of the biochemical composition of the green leaf that influence the resultant black tea quality. The unsaturated fatty acids break down during black tea processing to produce group I VFC that lower tea quality. At present it is not known whether the fatty acid composition and levels in the leaf vary or remain the same in all the tea growing areas when the blanket agronomic practices are applied to one cultivar.

1.7: Research objectives

1.7.1: Broad objective

To determine the variations of precursors of aroma quality of black tea in relation to locality, nitrogen fertilizer rates and plucking interval hence predict quality potential of single cultivar in different localities in Kenya.

1.7.2: Specific objectives

1. To quantify the variations of fatty acids especially the unsaturated fatty acid contents of single tea cultivar with localities under similar nitrogenous fertilizer application rates.
2. To quantify the variations of fatty acids especially unsaturated fatty acids contents of single tea cultivar with localities under different plucking intervals.
3. To determine the changes in fatty acids especially unsaturated fatty acids content for a single tea cultivar due to nitrogenous fertilizer rates and plucking intervals.

1.8: Hypothesis

Stable performance of a crop cultivar over a wide range of locations is regarded as desirable. It is hypothesized that the fatty acid amounts and composition of a single tea cultivar planted in different locations of the country will not vary due to nitrogenous fertiliser rates and plucking intervals in the same levels and patterns.

1.9: Research questions

1. Will amounts and composition of fatty acid especially the unsaturated ones in a single cultivar under similar nitrogen fertilizer rates remain constant with locality?
2. Will the levels of fatty acids especially the unsaturated ones in single cultivar under similar plucking interval remain constant in all locations?
3. Will the fatty acid levels of a single cultivar grown in different localities vary with nitrogenous fertilizer rates and plucking intervals?

1.10: Justification

Tea prices are stagnant or declining due to world overproduction and new production technologies. Only producers of high or premium quality tea will survive the competition. In Kenya, agriculture and tea production in particular remain the major pillar for economic growth and the attainment of millennium development goals thus production of high quality tea is of great importance. Nitrogenous fertiliser application and harvesting are the most expensive field agronomic inputs in tea production (Anon, 2002; Othieno, 1988). Inappropriate fertiliser use leads to farming losses through low yields and poor quality. Recommended plucking frequencies and fertilizer rates in tea growing locations of Kenya are the same (Anon, 2002; Othieno, 1988). However, yields (Wachira *et al.,* 2002) and quality (Owuor *et al.,* 1987c, 1988, 2010) vary with geographical area of production. It is necessary to establish how same agronomic practices influence the distribution of precursors of aroma quality chemicals in the plant in different tea growing areas in Kenya. This would enable the prediction of quality potential due agronomic inputs and management practices in different tea growing areas at the green leaf level to minimise losses even before tea is processed.

1.11: Significance of the study

This study targets fatty acids which are precursor compounds for black tea aroma quality. Monitoring these precursor compounds at the green leaf level will enable the prediction of quality potential due to agronomic practices in different regions hence optimizing quality even before tea is processed. Moreover, the information provides a basis for appropriate agronomic practices in various localities.

CHAPTER TWO: LITERATURE REVIEW

2.1: Background information

The manufacture of black tea is a complex biological process, where the quality of the final product depends on many factors including the chemical composition of the leaf, the extent to which the tea shoots are dehydrated and broken down by mechanical means and enzymic action during processing (Hampton, 1992). Certain marked chemical changes take place during black tea manufacture and are largely responsible for the development of colour and flavour (Sanderson, 1972) hence quality of black tea.

2.2: Tea quality assessment

In the tea trade, the quality is determined organoleptically. This method has however been criticised as being subjective and influenced by consumers', market demands or the tasters personal preferences (Biswas *et al.*,1973; Biswas and Biswas 1971a, 1971b). The subjective nature of the organoleptic evaluation explains how it is possible for different tasters to assign different classifications to the same sample of tea. Research efforts have been directed to the determination of the biochemical compounds or class of compounds responsible for quality of black tea (Ellis and Cloughley, 1981; Hilton and Palmers-Jones, 1973, 1975; Davies, 1983; Cloughley, 1980, 1981, 1983; Hilton and Ellis, 1972; Hilton *et al.*, 1973; Owuor *et al.*, 1986, 2006; Wright *et al.*, 2002). This has been with the view of understanding and objectively determining the relationships between particular classes of compounds with sensory evaluation.

Black teas are classified either as flavoury or plain. Plain black teas are predominantly made from seedling teas and are sold mainly based on briskness, strength and colour (Biswas and Biswas 1971a, 1971b; Biswas *et al.*, 1973; Owuor *et al.*, 1986). These attributes are due to the levels of theaflavins and thearubigins in black tea. Indeed, significant relationship has been demonstrated between total theaflavins (TF) and sensory evaluation of Central African black teas (Hilton and Ellis, 1972; Hilton *et al.*, 1973; Wright *et al.*, 2002). Although positive relationships have been reported for black teas, the regressions were not significant (Owuor *et al.*, 1986). Consequently the total theaflavins were not considered to be critical quality parameters for black

teas (Owuor *et al.*, 1987b). However, the individual theaflavins have different astringencies (Sanderson *et al.*, 1976) and therefore contribute differently to the quality of and taste of black tea (Sanderson *et al.*, 1976; Thanaranj and Seshadri, 1990). Theaflavin-3, 3'-digallate (TF-3, 3'-dig) is 6.4 times while theaflavin-3-gallate (TF-3-g) and theaflavin-3'-gallate (TF-3'-g) are 2.22 times as stringent as theaflavins (TF) (Sanderson *et al.*, 1976). Thus the total theaflavin *per se* may not be a critical factor in quality determination. In recent studies, when the contributions of individual theaflavins were normalized, there was a good relationship between the normalized factor (theaflavin digallate equivalent) and sensory evaluation (Owuor and McDowell, 1994; Owuor and Obanda, 1995). Indeed using this factor, there was better relationship with sensory evaluation for both Kenyan and Central African black teas (Owuor *et al.*, 2006) demonstrating the importance of theaflavins to tea quality. Although the contributions of theaflavins and thearubigins (TR) to quality are acknowledged (Wood and Roberts, 1964), the relationship between thearubigin levels and quality remains obscure. Caffeine has also been known to contribute to the quality of black tea (Owuor and Chavanji, 1986; Bhatia, 1968; Deb and Ullah, 1968; Millin *et al.*, 1969) as it contributes towards briskness (astringency) of black tea. Caffeine complexes with polyphenols in tea (Roberts and Smith, 1963; Collier, 1972) and the complex formed modifies the taste characteristics of both caffeine and theaflavins (Millin *et al.*, 1969; Sanderson *et al.*, 1976). A positive linear regression analysis between the tasters' evaluation and caffeine content of has been established (Obanda and Owuor, 1997). More recently, the plain black tea quality (Babu *et al.*, 2007, Owuor *et al.*, 2008, 2009, 2010) has been reported to vary even in the same cultivar under similar agronomic practices in different localities.

Plain black teas are predominantly from seedling teas in western Kenya. However, at present, 80% of Kenyan tea is from the clonal material. These clonal teas are however, more flavoury, thus aroma is an important quality parameter (Robinson and Owuor, 1992). A significant relationship has been demonstrated between aroma and sensory evaluation in Kenyan black tea (Owuor *et al.*, 1988; Owuor, 1992). The composition of the volatile flavour compounds of commercial Kenyan teas is comparable to that of teas from other areas known to produce teas with high flavour qualities (Owuor *et al.*, 1986; Horita and Owuor, 1987). The flavoury teas are valued for their special aroma and flavour (Yamanishi *et al.*, 1966; Bondarovitch *et al.*, 1967; Reynold *et al.*, 1974; Asiaka *et al.*, 1978; Takeo, 1983; Takeo and Mahanta, 1983; Mick *et al.*, 1984; Horita and Owuor, 1987; Cloughley *et al.*, 1982). Over 600 volatile flavour compounds

have been identified in black tea (Robinson and Owuor, 1992). There are two types of volatile flavour compounds (VFC); those that impart sweet flowery aroma (group II VFC) and those that impart green grassy undesirable aroma (group I VFC) (Yamanishi *et al.*, 1968; Wickremashinghe *et al.*, 1973; Gianturco *et al.*, 1974; Owuor *et al.*, 1986; Robinson and Owuor, 1992; Owuor, 1992). The classification of aroma compounds into group I and group II compounds has been based on either the odour characteristics (Owuor *et al.*, 1987a, b, c, d) or the retention times of the aroma compounds during gas chromatographic analysis (Yamanishi *et al.*, 1968; Wickremashinghe *et al.*, 1973). The ratio of group II to group I aroma compounds has been used to classify teas in order of flavour quality (Yamanishi *et al.*, 1968; Wickremashinghe *et al.*, 1973; Owuor *et al.*, 1987a, b, c, d, 1990a, b, c; Owuor and Lang'at, 1988). Flavour index (as a ratio of gas chromatographic peak areas of group II VFC to those of group I VFC) showed good relationships between sensory evaluations and quality of flavoury black teas (Owuor, 1992; Owuor *et al.*, 1988). However, the olfactory perception limits of the aroma compounds differ widely thus the ratio must be used with caution.

The group I VFC is composed mostly of C_6 aldehydes, alcohols and acids, which are products of unsaturated fatty acids breakdown during black tea processing (Galliard, 1975). Some flavour compounds are susceptible to chemical changes of various kinds (Flath *et al*,. 1981) for example aldehydes are easily oxidised to acids, thus the degree of oxidation during black tea manufacture determines the VFC formed hence quality of black tea. Group I VFC levels in black tea correlate negatively with tasters' evaluation confirming that that high concentration of group I VFC is deleterious to tea quality (Owuor, 1992). The levels of group I VFC increase with increase in amounts of unsaturated fatty acids in green tea leaves (Owuor *et al.*, 1990a, b, c). High amounts of unsaturated fatty acids in the green tea leaves have been reported to lead to poor aroma quality of processed tea (Mahanta *et al.*, 1988; Owuor *et al.*, 1987c, 1987d; Owuor and Lang'at, 1988). The amounts of unsaturated fatty acids in green tea leaves should be kept low to enhance desirable aroma. The fatty acids thus emerge as an important area of research and quality control. It is not known how the unsaturated fatty acids levels which are the flavour precursor compounds change due to agronomic inputs in different locations.

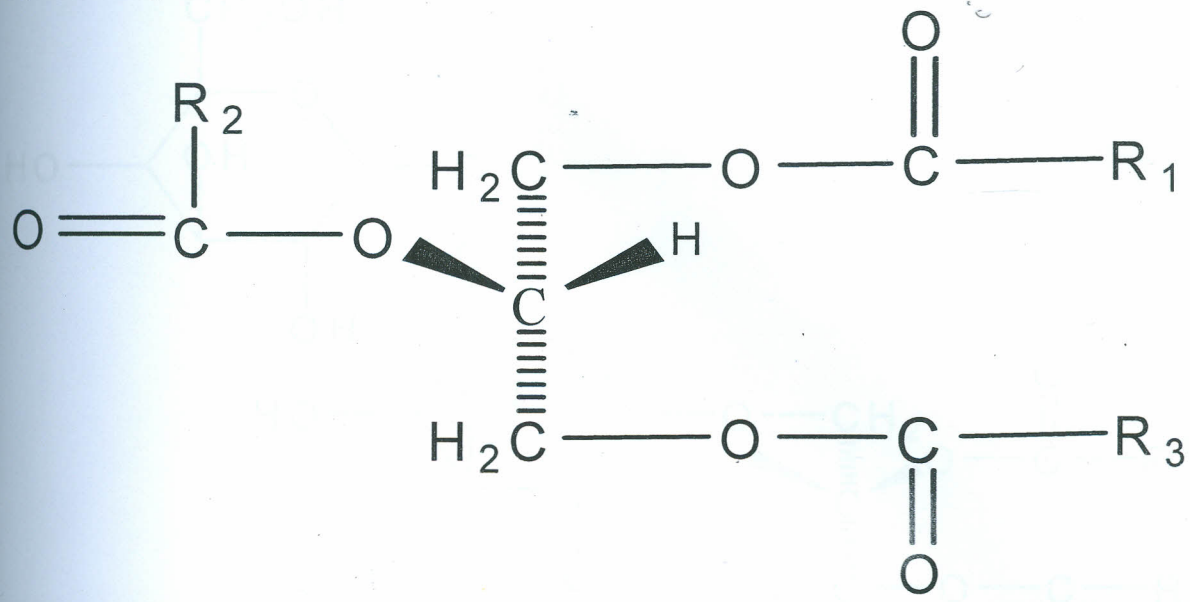
2.3: Chemistry of lipids and fatty acids in tea leaves

2.3.1: Lipids and fatty acids in tea leaves

Due to improved analytical techniques, (Anan and Nakagawa, 1977; Anan, 1983) quantitative analysis of lipids and fatty acids of tea shoots have been possible. Most of the plant lipids are located in the chloroplast. Several Fatty acids have been identified in tea shoots including lauric [dodecanoic] acid (C12:0), myristic [tetradecanoic] acid (C14:0), palmitic [hexadecanoic] acid (C16:0), palmitoleic [9Z-hexadecaenoic] acid ($16:1^{\Delta 9}$), stearic [octadecanoic] acid(18:0), oleic [9Z-octadecaenoic] acid ($18:1^{\Delta 9}$), linoleic [9Z,12Z-octadecadienoic] acid ($18:2^{\Delta 9,12}$) and linolenic [9Z,12Z,15Z-octadecatrienoic] acid ($18:3^{\Delta 9,12,15}$) (Bhuyan *et al.*, 1991; Ramaswamy and Ramaswamy, 2000, Muritu, 1989).

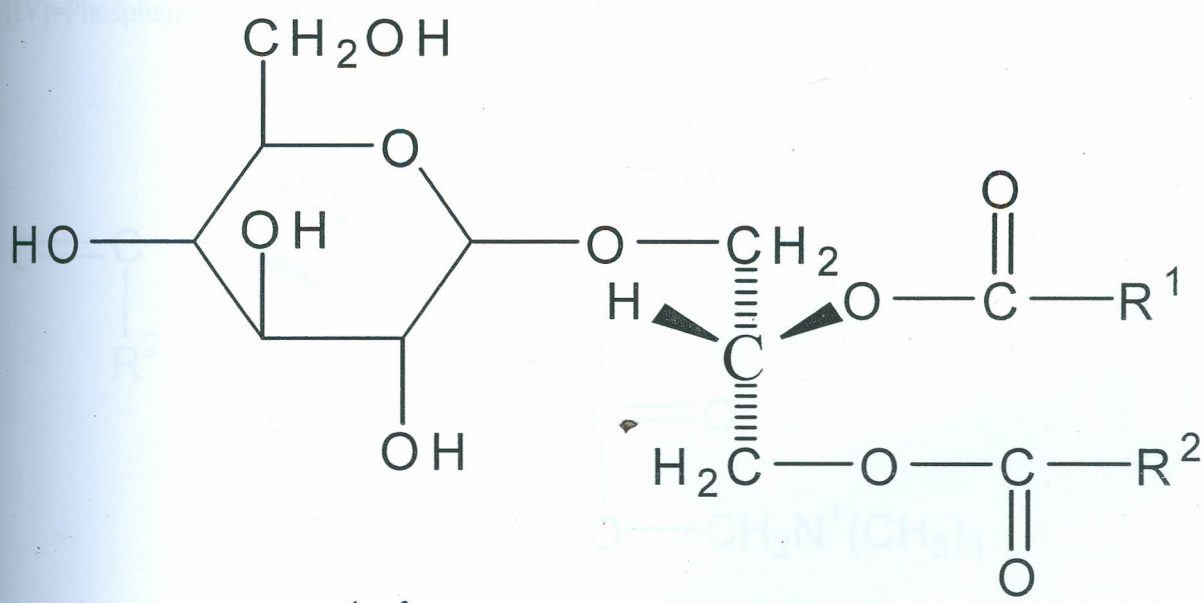
The fatty acids in tea leaves are contained in three fractions of lipids including neutral lipids, glycolipids and phospholipids (Bhuyan *et al.*, 1991; Ramaswamy and Ramaswamy, 2000). Indeed, as observed in many plants, the content of lipids in tea is in the order; glycolipid > neutral lipid > phospholipid (Bhuyan *et al.*, 1991). The fatty acid composition of individual fractions of lipid is mixed and contains appreciable amounts of the fatty acids (Bhuyan *et al.*, 1991; Ramaswamy and Ramaswamy, 2000). Nevertheless, the proportions of the fatty acids vary in all lipid fractions (Ramaswamy and Ramaswamy, 2000). Most saturated fatty acids such as lauric (12:0), myristic (14:0) and stearic (C18:0) acids are higher in the neutral lipid fraction **[I]** (Bhuyan *et al.*, 1991; Ramaswamy and Ramaswamy, 2000). The proportion of linolenic acid ($18:3^{\Delta 9,\ 12,\ 15}$) is highest in glycolipids including monogalactosyldiglyceride (MGDG) **[II]** and digalactosyldiglyceride (DGDG) **[III]** (Bhuyan *et al.*, 1991; Ramaswamy and Ramaswamy, 2000; Anan, 1983; Mahanta *et al.*, 1985). Other acids, oleic ($C18:1^{\Delta 9}$) and linoleic ($C18:2^{\Delta 9,\ 12}$) acids are derived from neutral lipid and the phospholipid fractions including phosphatidylcholine (PC) **[IV]**, phosphatidylethanolamine (PE) **[V]**, phosphatidylinositol (PI) **[VII]** and phosphatidylglycerol (PG) **[VIII]**. Palmitic acid (C16:0) is present in phosphatidyl inositol (PI) **[VII]** and sulphoquinovosyldiglyceride (SQDG) **[VI]**, while steroacylmonoglucoside (SMG) **[IX]** is dominated by stearic acid (C18:0).

[I]=Neutral lipid



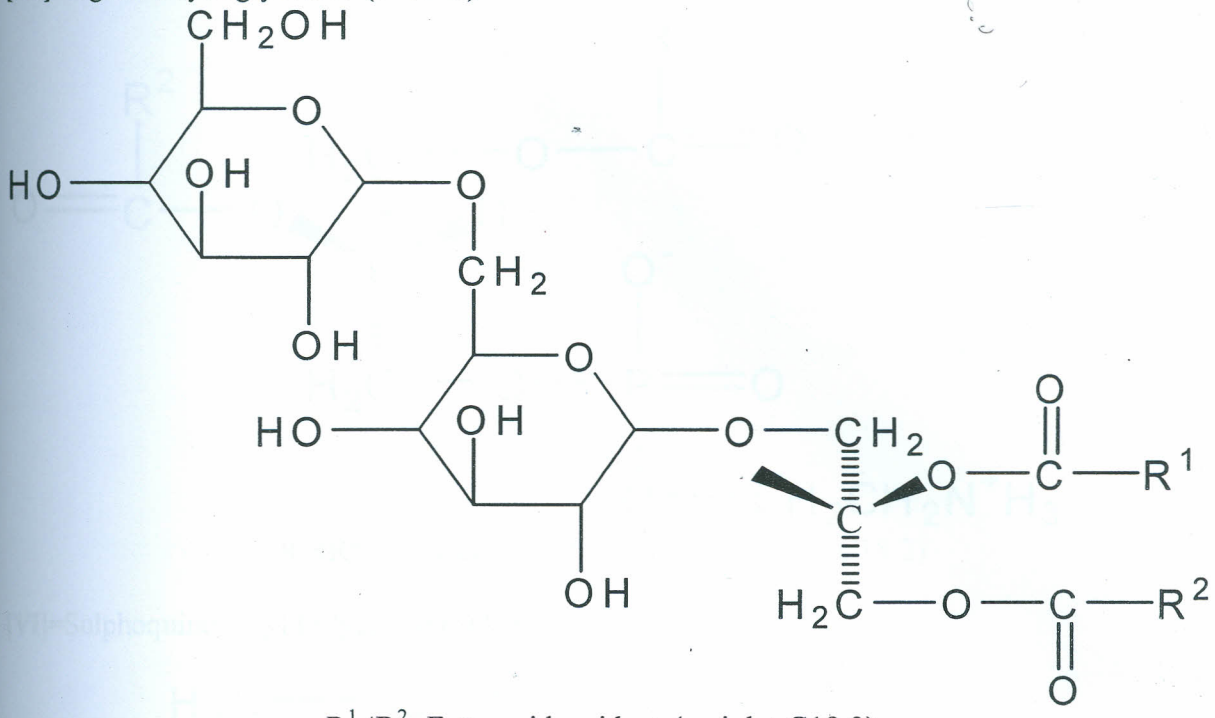
$R_1 \neq R_2 \neq R_3$ =Fatty acid residues (mainly saturated fatty acids; C12:0, C14:0, C18:0)

[II]=Monogalactosyldiglyceride (MGDG)



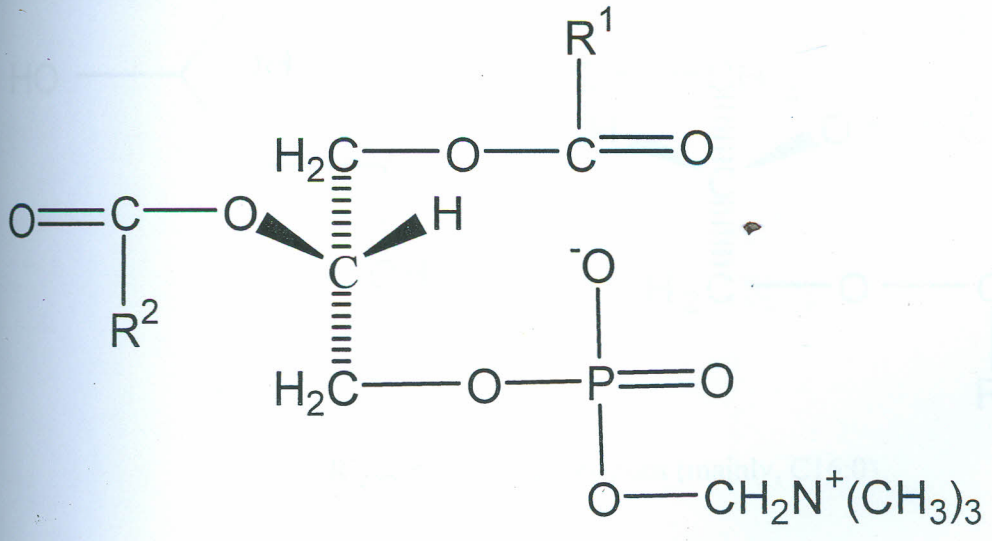
$R^1 \neq R^2$=Fatty acid residues (mainly, C18:3)

[III]=digalactosyldiglyceride (DGDG)



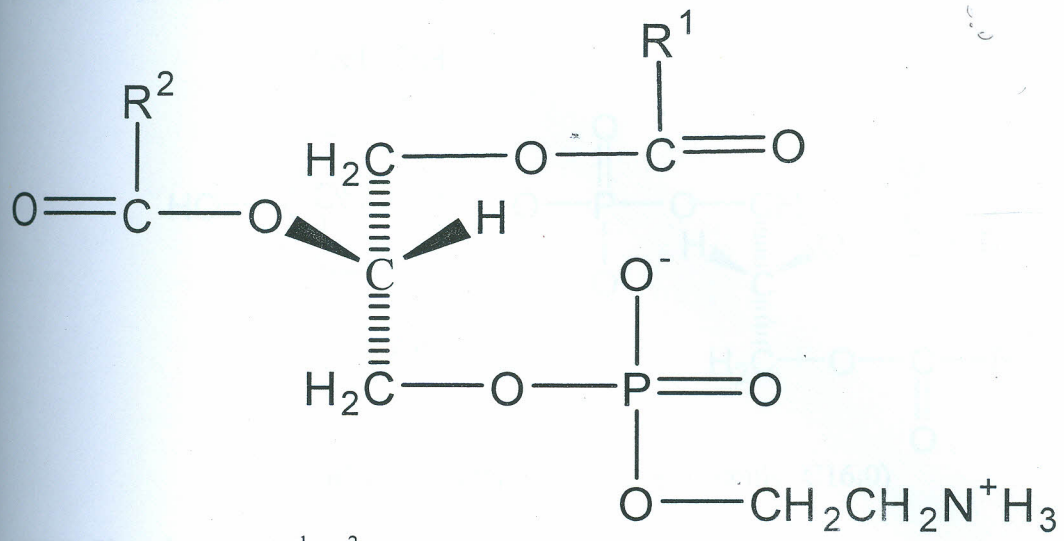
$R^1 \neq R^2$=Fatty acid residues (mainly, C18:3)

[IV]=Phosphatidylcholine (PC)



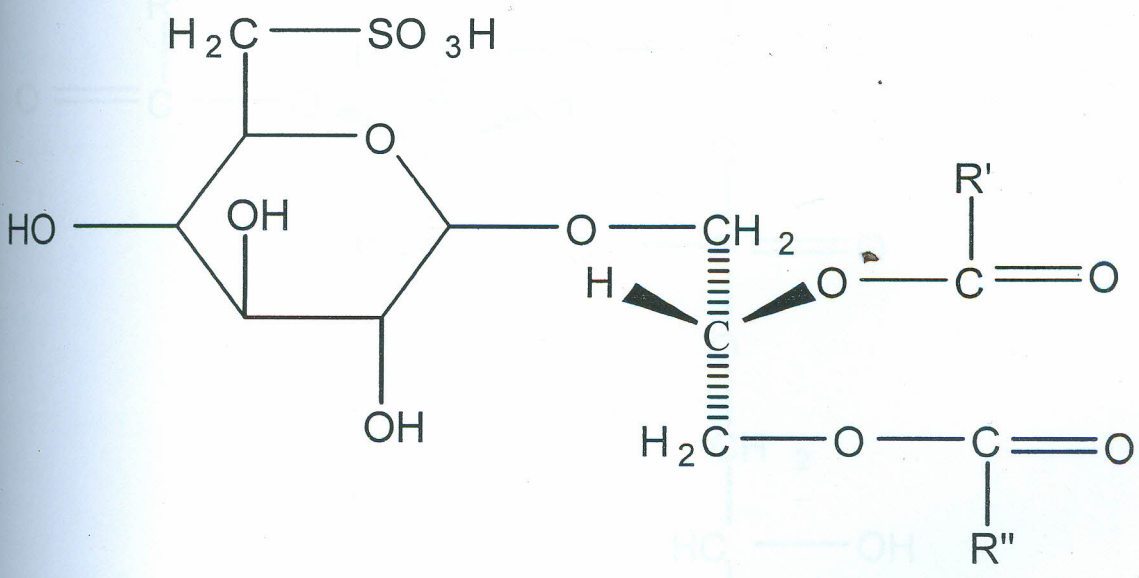
$R^1 \neq R^2$=Fatty acid residues (mainly, C18:1, C18:2)

[V]=Phosphatidylethanolamine (PE)



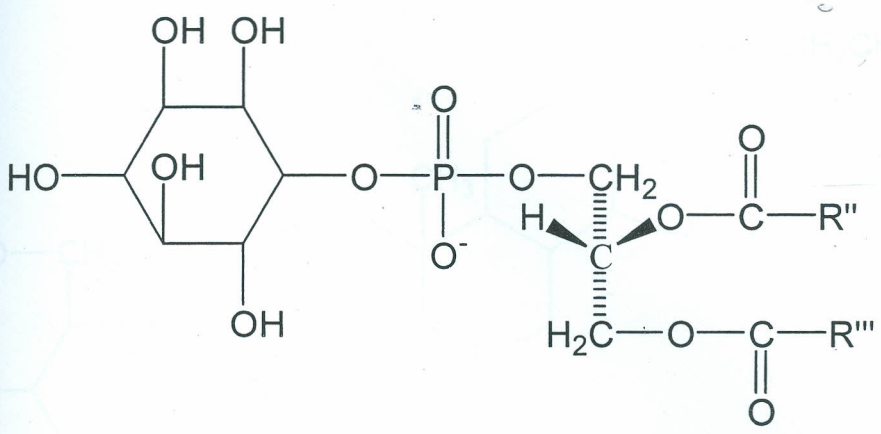
$R^1 \neq R^2$ =Fatty acid residues (mainly, C18:1, C18:2)

[VI]=Sulphoquinovosyldiglyceride (SQDG)



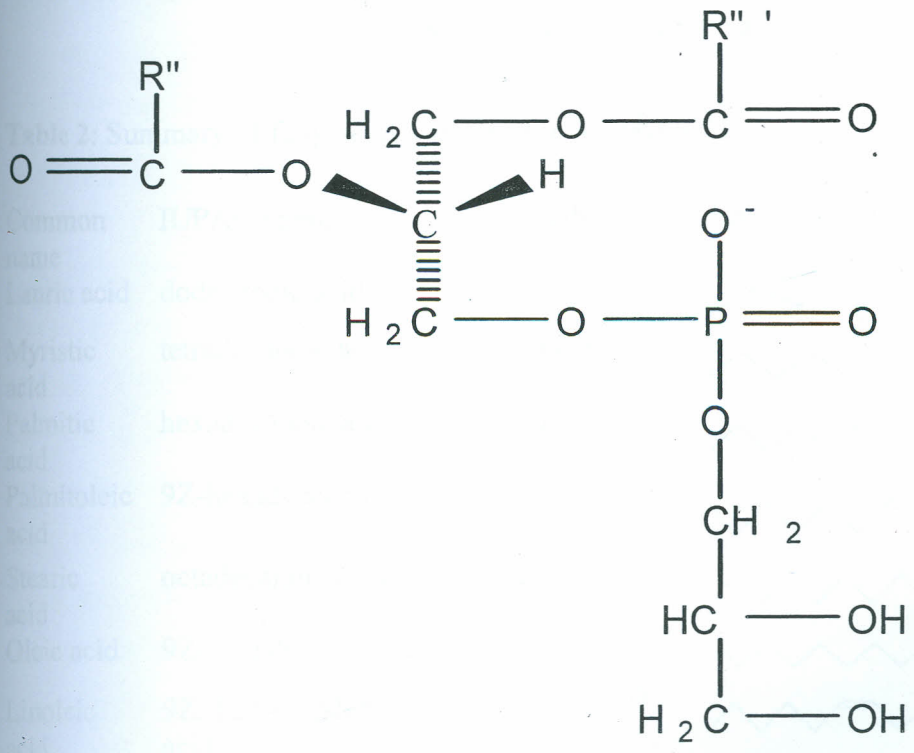
R'≠R"=Fatty acid residues (mainly, C16:0)

[VII]=Phosphatidylinositol (PI)



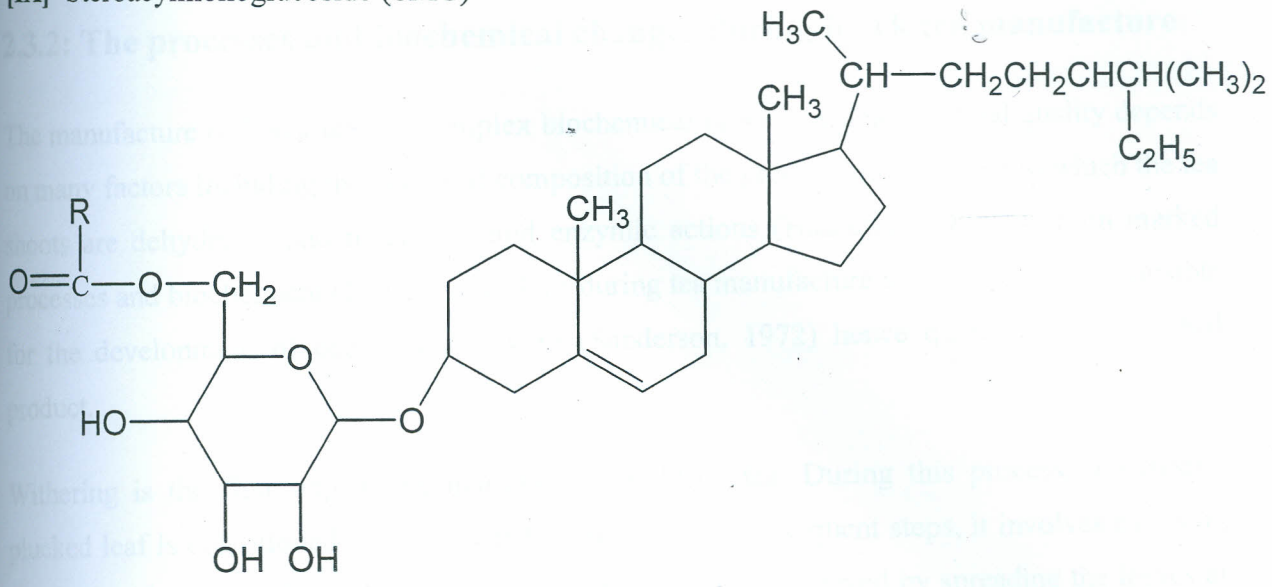
R"≠R'"=Fatty acid residues (mainly, C16:0)

[VIII]=Phosphatidylglycerol (PG)



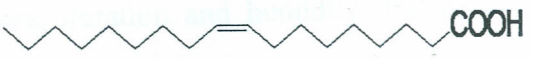
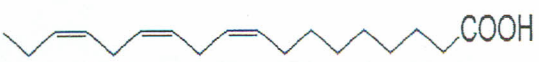
R"≠R'"=Fatty acid residues (mainly, C18:1, C18:2)

[IX]=Steroacylmonoglucoside (SMG)



R=Fatty acid residue (C18:0)

Table 2: Summary of fatty acids contained in tea leaf lipids

Common name	IUPAC name	Symbol	Structure
Lauric acid	dodecanoic acid	C12:0	
Myristic acid	tetradecanoic acid	C14:0	
Palmitic acid	hexadecanoic aid	C16:0	
Palmitoleic acid	9Z-hexadecaenoic acid	$C16:1^{\Delta 9}$	
Stearic acid	octadecanoic acid	C18:0	
Oleic acid	9Z- octadecenoic acid	$C18:1^{\Delta 9}$	
Linoleic acid	9Z, 12Z-octadecadienoic acid	$C18:2^{\Delta 9, 12}$	
Linolenic acid	9Z, 12Z, 15Z-octadecatrienoic acid	$C18:3^{\Delta 9,12,15}$	

Source: Ramaswamy and Ramaswamy, 2000.

2.3.2: The processes and biochemical changes during black tea manufacture

The manufacture of black tea is a complex biochemical process, where the final quality depends on many factors including the chemical composition of the green leaf, the extent to which the tea shoots are dehydrated and macerated and enzymic actions (Hampton, 1992). Certain marked processes and biochemical changes take place during tea manufacture and are largely responsible for the development of colour and flavour (Sanderson, 1972) hence quality of the finished product.

Withering is the first step in the manufacture of black tea. During this process, the freshly plucked leaf is conditioned physically and chemically for subsequent steps, it involves exposing the plucked leaves uniformly to atmospheric oxygen. This is achieved by spreading the leaves at a given uniform thickness and blowing air evenly to achieve uniform wither. Once the leaf is plucked, the anabolic reactions practically cease and catabolic reactions start (Takeo, 1980, 1984). In this direction, large molecules like chlorophylls, proteins, cellulose and lignin, which are responsible for the rigidness of the shoots, degrade to make the shoots more flaccid increasing the cell permeability. The carbohydrates which are also large molecules degrade to produce sugars that burn in the presence of atmospheric oxygen and produce energy to run the various biochemical changes, which are enzymic and temperature dependent. Lipids also degrade to produce free fatty acids which eventually degrade to aroma compounds (VFC) this is due to increased activity of lipoxygenase enzyme with withering (Ramaswamy and Ramaswamy, 1998) leading to higher degradation of lipids (Ramaswamy and Ramaswamy, 2000). These chemical processes are all intrinsic of the biochemical structure of the leaf, but the range and extent depends on physical parameters like temperature, duration and humidity. Indeed low temperature favours the development of quality while high temperature may develop colour at the expense of quality.

The fully withered leaf is then taken through maceration process. The principal objective of leaf maceration is to undertake cell rupture carried out in a rolling machine where disintegration of cellular organelles takes place. The shoots with different degree of tenderness is subjected to considerable deformation during rolling, and, during the process of gradual rapture of the leaf,

the epidermis is torn up in pieces, cells are crumpled, the cuticle wrinkled and the intercellular space increased. The process results in the exposure of the cell sap leading to the mixing of chemical constituents and enzymes in the presence of atmospheric oxygen to form the important compounds responsible for characteristics of tea. The degradation of lipids to VFC is highest at this stage of manufacture (Ramaswamy and Ramaswamy, 2000). Indeed lipoxygenase enzyme activity is enhanced upon mechanical injury during maceration (Ramaswamy and Ramaswamy, 1998). Lipoxygenase in tea is an important enzyme in the formation of aroma compounds as it catalyses the peroxidation of 1, 4-diene unsaturated fatty acids leading to formation of carbonyl compounds responsible for the characteristic odour (Hatanaka *et al.*, 1987; Vick and Zimmerman, 1987; Mahanta *et al.*, 1995).

Once maceration of the leaf starts, the 'fermentation', which is primarily an oxidation process, begins. This process principally, gives the difference between black tea and other forms of teas like green tea and oolong tea. During this process, the green leaf catechins are oxidised by polyphenol oxidase (PPO) enzymes to form larger condensed catechins called theaflavins (TF) (Takino *et al.*, 1964) responsible for the astringency (briskness) and brightness of plain black tea (Biswas and Biswas 1971a, 1973; Biswas & Biswas, 1971b). Thearubigins are formed by the action of peroxidise (PO) enzyme on the same catechins (Takino *et al.*, 1964) in presence of oxygen. The thearubigins contribute to the colour and thickness (mouth feel) (Biswas & Biswas, 1971a, 1973). However, appreciably lower degradation of the lipids continues in this stage as the activity of lipoxygenase enzyme reduces at the fermentation stage. (Ramaswamy and Ramaswamy, 1998). The enzymic reactions are arrested by drying the product that is sorted and packed.

2.3.3: Production of volatile flavour compounds

For flavour, one of the most important reactions is the lipid degeneration initiated by enzymes which are released upon leaf maceration. Although the lipid content in tea leaves is low, lipid metabolism is important and processing techniques plays an important role in the biogenesis of flavour of the finished product (Bondarovitch *et al.*, 1967; Mahanta *et al.*, 1993). Lipids make up between 4 and 9% dry weight of fresh tea leaf (Wright and Fishwick, 1979; Mahanta *et al.*, 1985) and are composed mainly of free fatty acids and fatty acid esters. During tea manufacture, there is widespread damage of the membrane structures resulting into the release of lipolytic

acylhydrolase (LAH) en zyme that hydrolyses the lipids to release the fatty acids (Galliard, 1975). The contribution of the saturated fatty acids to tea has not been known; however, during tea manufacture, unsaturated fatty acids especially linolenic and linoleic acids undergo further degradation to form the group I flavour compounds (Robinson and Owuor, 1992). Common group I flavour compounds from lipid degradation in black tea include the C_6 aldehydes, alcohols and acids (Table 3).

Table 3: Common volatile flavour compounds from fatty acid degeneration

Group I VFC
n-Hexanal
(3Z)-Hexenal
(2E)-Hexenal
n-Hexenoic acid
(3E)-Hexenoic acid
n-Hexenol
(3Z)-Hexenol
(2E)-Hexenol
(3Z)-Hexenoic acid
(2E)-Hexenoic acid

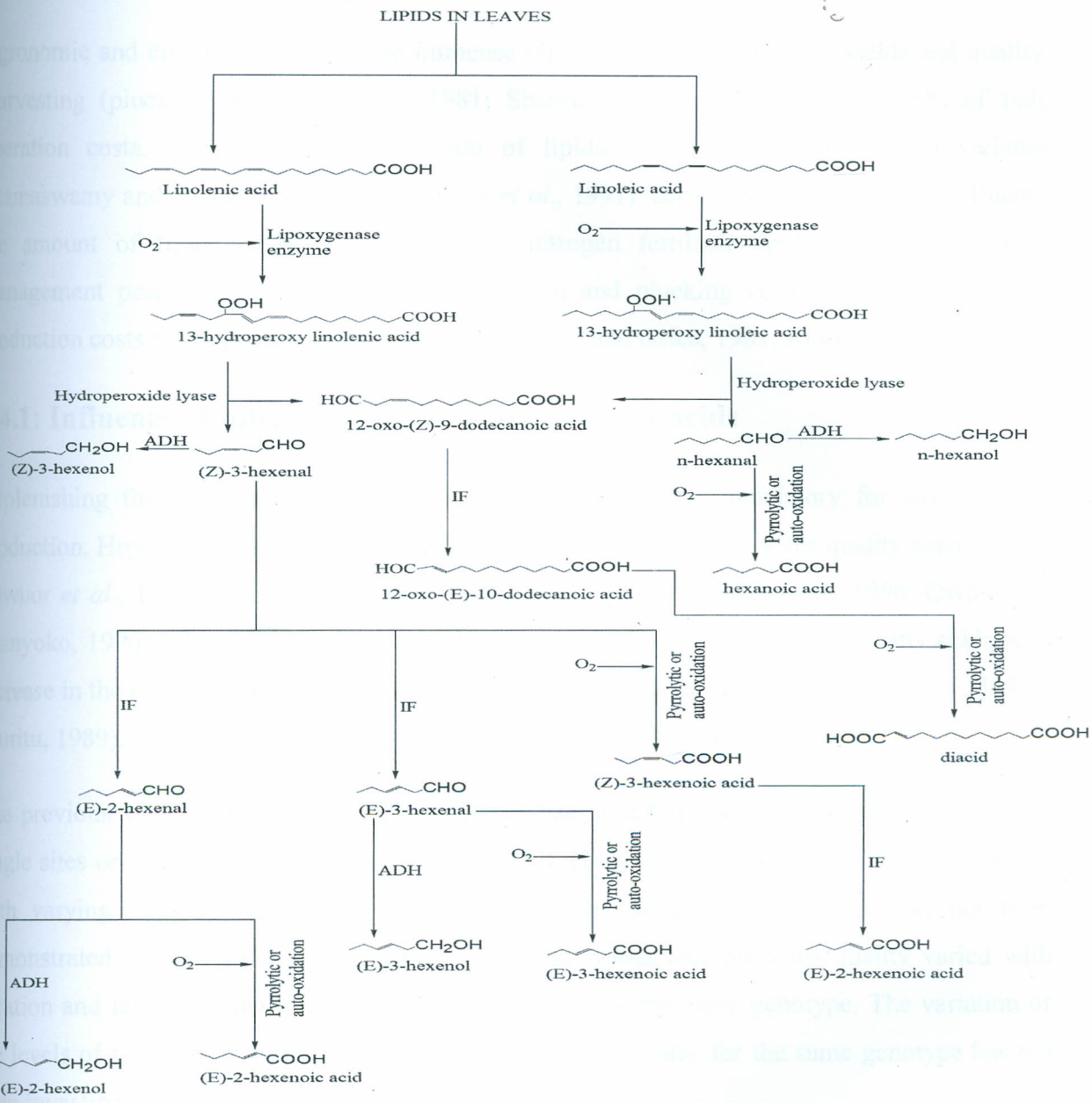
Source: (Horita and Owuor, 1987; Yamanishi *et al.*, 1978; Asiaka *et al.*, 1978; Fernando and Robert, 1984; Howard, 1979, Robinson and Owuor 1992).

The unsaturated fatty acids especially linolenic acid (LNA) and linoleic (LA) acid are oxidised by lipoxygenase enzyme. Lipoxygenase catalyses the peroxidation process of the unsaturated fatty acids and is highly regiospecific (Takeo and Tsushida, 1980; Hatanaka *et al.*, 1982) for Δ-13 position for both linolenic and linoleic acids yielding Δ-13 fatty acid hydroperoxides; 13-L-hydroperoxy linolenic acid (13-L-HPOLNA) [13-hydroperoxy (9Z, 11E, 15Z) octadecatrienoic acid] and 13-L-hydroperoxy linoleic acid (13-L-HOPLA) [13-hydroxy (9Z, 11E) octadecadienoic acid]. The enzyme is more active with linolenic acid than linoleic acid (Sekiya *et al.*, 1984). The higher lipoxygenase activity for linolenic acid than linoleic acid supports the biogenesis of more of the six-carbon unsaturated volatile compounds (Figure 1), which are the

major constituents of black tea aroma (Selvendran *et al.*, 1978; Hatanaka *et al.*, 1976, 1974; Saijo and Tokeo, 1972, 1970, Saijo, 1967, 1977; Hatanaka and Harada, 1972). Indeed, the study of tea leaves hydroperoxide lyase substrate-inhibition (Matsui *et al.*, 1991 and 1992) reveals that the enzyme is irreversibly inhibited by 13-hydroperoxide of linoleic acid.

The 13-hydroperoxides of linolenic and linoleic acids are further acted upon by hydroperoxide lyase (Hatanaka *et al.*, 1979; Kajiwara *et al.*, 1982). Hydroperoxide lyases can be classified into two categories based on their substrate specificity: the 9-hydroperoxide specific enzyme which cleaves exclusively the 9-isomer and the 13-specific one which uses the 13-isomer as substrate. The 13-specific one is major in tea leaves (Hatanaka *et al.* 1982). Hydroperoxide lyase in photosynthetic organs usually shows high activity with linolenic acid hydroperoxide (Sekiya *et al.*, 1984) and is substrate-specific (Hatanaka *et al.*, 1982; Hatanaka, 1993). Indeed the hydroperoxide lyase extracted from tea leaves is more reactive with 13-hydroperoxy (9Z, 11E, 15Z) octadecatrienoic than 13-hydroperoxy (6Z, 9Z, 11E) octadecatrienoic acid (Hatanaka *et al.*, 1992; Hatanaka, 1993). This enzyme catalyzes the cleavage of linoleic and linolenic acid hydroperoxides into C_6 aldehydes and ω-oxo-acids (Hatanaka *et al.*, 1982, 1983). The action of the enzyme is enantioselective (Kajiwara *et al.*, 1982) and the break takes place between the carbon which contains the hydroperoxide group and the proximate ethylenic carbon (Figure 1).

The aldehydes from 13-L-hydroperoxy linolenic acid particularly, (3Z)-hexenal undergoes three different changes to produce more VFC; it is reduced by alcohol dehydrogenase (ADH) to (3Z)-hexenol, on pyrrolytic oxidation it produces (3Z)-hexenoic acid which is isomerised to (2E)-hexenoic acid. (3Z)-hexenal is isomerised to (E)-3-hexenal which on pyrrolytic oxidation produces (3E)-hexenoic acid and on reduction by ADH forms (3E)-hexenol. Isomerisation of (3E)-hexenal produces (2E)-hexenal which on pyrrolytic oxidation produces (2E)-hexenoic acid and (E)-2-hexenol on reduction by ADH. Hexanal, hexanol and hexanoic acid are formed from linoleic acid (Figure 1). Low levels of palmitoleic and oleic acids have been detected in fresh tea leaves with oleic acid breaking down to form nonanal and nonanal while palmitoleic acid can form heptanal and heptanol (Anan and Nakagawa, 1977; Selvendran, *et al.*, 1978; Hatanaka *et al.*, 1976; Hatanaka and Harada, 1972; Saijo and Takeo, 1972, Horita and Owuor, 1987).



ADH=Alcohol dehydrogenase
IF=Isomerization factor

Figure 1: Scheme for the formation of VFC from linoleic and linolenic acid.

Reconstructed from; Hatanaka *et al.*, 1979; Kajiwara *et al.*, 1982, Robinson and Owuor 1992; Hatanaka *et al.*, 1992; Hatanaka, 1993; Croft *et al.*, 1993; Grechkin, 1998.

2.4: Influence agronomic and cultural practices on fatty acids

Agronomic and cultural practices have immense effects on tea productivity, yields and quality. Harvesting (plucking) (Sharma *et al.*, 1981; Sharma, 1987) constitute up to 70% of field operation costs. Although the composition of lipids in tea leaves varies with varieties (Ramaswamy and Ramaswamy, 2000, Bhuyan *et al.*, 1991), certain agronomic factors influence the amount of lipids in tea plant including nitrogen fertilizer application and plucking management practices. Indeed fertilizer application and plucking constitute most expensive production costs in tea production (Othieno, 1980; Ellis and Grice, 1981; Ruto *et al.*, 1994).

2.4.1: Influence of nitrogen fertilizer rates on fatty acids

Replenishing the soil nutrients through fertilizer application is mandatory for sustained tea production. However, high amount of nitrogenous fertilizers cause black tea quality deterioration (Owuor *et al.*, 1987a, 1987b, 1991, 1997, 2000, 2010; Owuor and Othieno, 1996; Owuor and Wanyoko, 1996). This is partly due to the increase in the levels of unsaturated fatty acids with increase in the rates of nitrogenous fertilizers leading to high levels of VFC (Owuor *et al.*, 1990a, Muritu, 1989).

The previous studies on rates of nitrogenous fertilizer and fatty acid levels were conducted at single sites or using different clones (Muritu, 1989). However, the variation of fatty acid levels with varying rates of fertilizers in different localities using same genotype has not been demonstrated. Recent studies have demonstrated that yields and plain tea quality varied with location and fertilizer rates (Owuor *et al.*, 2009, 2010) for the same genotype. The variation of the levels of fatty acids with locations under same fertilizer rates for the same genotype has not been investigated.

2.4.2: Influence of plucking/harvesting on fatty acids

The recommended plucking standard is two leaves and a bud (Othieno, 1988; Anon, 2002) as this produces high quality black tea (Owuor *et al.*, 1987c). During this operation, young tender shoots are removed for processing into various tea beverages (Willson, 1992). When there is an

extra growth, tea planters tend to harvest more than two leaves. However, this produces black tea of inferior quality (Mahanta *et al.*, 1988; Owuor *et al.*, 1987c; Owuor and Odhiambo, 1993). Coarse plucking of more than two leaves and a bud increases the group I VFC especially C_6 aldehydes and alcohols while decreasing the group II VFC (Mahanta *et al.*, 1988; Owuor *et al.*, 1987c).

The unsaturated fatty acids levels increase with maturity of the shoot (Bhuyan *et al.*, 1989; Mahanta *et al.*, 1995; Owuor *et al.*, 1990c) implying a tendency to produce poor flavoury teas by harvesting more mature leaves. However, plucking two leaves and a bud is still considered a compromise between yields, plucker productivity and quality of processed tea in all cultivars. In practice, it is difficult to achieve exclusively two leaves and a bud (Odhiambo *et al.*, 1988). One way of achieving two leaves a bud is to optimize harvesting intervals so that plucking is done when most leaves are in the two leaves and a bud stage. At single site, long plucking interval reduced black tea quality (Baruah *et al.*, 1986; Owuor, 1992; Owuor *et al.*, 1990d, 1997, 2000; Owuor and Odhiambo, 1994). In the earlier studies, the sum of group I VFC levels increased while that of group II decreased with increasing plucking intervals thus decreasing the flavour index. Shorter plucking intervals thus improve the aroma quality of tea (Baruah *et al.*, 1986; Owuor *et al.*, 1990d; Owuor and Odhiambo 1994). Recently, the optimal plucking rounds for the production of high yields and high quality plain teas were demonstrated to vary with locality (Owuor *et al.*, 2009, 2010). However, it has not been established how plucking intervals affect the levels of green leaf unsaturated fatty acids which contribute to black tea aroma in different locations.

2.4.3: Effects of area of production of biochemical composition of tea leaf

Due to large demand, commercial production of *Camellia sinensis* has been reported from as far north as 49^0N, outer Carpathians, in the former Soviet union as far South as 33^0 S, Natal South Africa (Shoubo, 1989) and from sea level to altitudes as high as 2,700m above sea level in Kenya and Rwanda (Owuor *et al.*, 2007). The plant is adaptable to environments with large variations in climatic conditions. These variations in environment and growing conditions cause variations in growth (Ng'etich and Stephens, 2001a, 2001b; Ng'etich *et al.*, 2001; Obaga *et al.*, 1988,1989; Squire *et al.*, 1993) leading to changes in yields (Ng'etich and Stephens, 2001b;

Ng'etich *et al.*, 2001; Wachira *et al.*, 2002) and plain tea quality (Owuor *et al.*, 1987a, 1988; Owuor *et al.*, 2009, 2010). The theaflavins and thearubigins (Owuor *et al.*, 1987a, 2006, 2008) and black tea aroma (Owuor *et al.*, 1988, 2008) vary widely with geographical area of production for some clonal tea. 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. Where environmental conditions are large such variations are also large as was observed in the biochemical parameters of same cultivars grown in Kenya and Malawi (Owuor *et al.*, 2008). These changes due to locations are partly due to differences in growth rate (Obaga *et al.*, 1988, 1993; Squire *et al.*, 1993). The growth rate differences are such that in Kenya highlands shoot regeneration takes from 80 to 120 days (Mwakha, 1985a, 1985b; Odhiambo *et al.*, 1993; Squire *et al.*, 1993) unlike further away from the equator in Malawi where shoot regeneration can be as short as 42 days during the favourable growing seasons (Smith *et al.*, 1993; Tanton, 1981 1982). Indeed, yield (Wachira *et al.*, 2002) and fermentation rates (Owuor *et al.*, 2008) change with geographical area of production. Indeed, variations in yields and plain tea quality have been recorded due geographical area of production even in the same cultivar under similar agronomic inputs and management (Owuor *et al.*, 2009, 2010). Where the VFC were examined, the total VFC, the sum of group I and group II were higher for tea grown in Kenya than Malawi (Owuor *et al.*, 2008) or the VFC changed with region of production (Owuor *et al.*, 1988). It is thus possible that the levels of precursor chemicals for black tea aroma quality especially fatty acids also vary with regions. However, recommended tea production technologies were developed at single sites and the results extrapolated to other areas. It is likely that optimal agronomic inputs vary with geographical area of production for a given clone due to changes in growth rate (Ng'etich and Stephens, 2001a, 2001b) and possibly nutrient uptake. Furthermore, in most of the aroma quality studies conducted, only VFC were analysed, thus it is not known whether the variations in the VFC levels were due to the variations in the precursor biochemicals. It is necessary to quantify the possible changes in precursors of the black tea aroma quality parameters with geographical areas of production to help in predicting how quality will be affected by agronomic practices at specific locations.

CHAPTER THREE: MATERIALS AND METHODS

3.1: Site selection and cultivar

The trials were set on selected mature clone BBK 35 plantations. This clone is a popular clone in the east African tea growing areas and is classified as high yielding and of good quality. The plantations were of the same age after pruning that had been uniformly managed and with known past cultivation history in different geographical areas, namely Karirana tea Estate in Limuru, Timbilil tea Estate in upper Kericho, Changoi tea Estate in lower Kericho, Sotik highlands tea Estate in Sotik and Kipkebe tea Estate also in Sotik. The altitude, latitude/longitude, year of planting, age of plantation and last date of pruning are summarised in Table 4.

Table 4: Site locality and history

Site	Karirana	Timbilil	Changoi	Sotik Highlands	Kipkebe
Locality/history					
Altitude (m)	2260	2180	1860	1800	1800
Latitude	1^0 6'S	0^0 22'S	0^0 29'S	0^0 35'S	0^0 41'S
Longitude	36^0 39'E	35^0 21'E	35^0 14'E	35^0 5'E	35^0 5'E
Year planted	1991	1986	1989	1974	1978
Plantation age*	17	22	19	34	30
Last prune date	2005	2005	2005	2005	2005

* As at year 2008.

Source: (Kamau *et al.*, 2005)

3.2: Design of experiments and treatments

At each site, the experiments were a factorial two arrangement laid out in a randomised complete block design with five fertiliser rates (0, 75, 150, 225 and 300 Kg N/ha/year) NPKS and three plucking frequencies (7, 14 and 21 day rounds) replicated three times (Appendix 1). Each effective plot comprised 60 plants surrounded by a line of tea bushes that served as guard rows. Fertilizer application was done in November every year. First fertilizer application was done in the year 1998. The plots were uniformly managed and were pruned every four years. Prior to the experiments, all the plots were receiving 150 Kg N/ha/year NPKS. The standard plucking of two leaves and a bud was done in all the plots and at all plucking frequency.

3.3: Sample collection and preparation

Samples were collected by plucking 100gms of two leaves and a bud from each plot, the sampling was done on three occasions when all the plucking intervals coincided. The leaves were then steamed within an hour of plucking for two minutes to deactivate the enzyme responsible for fatty acid breakdown (Philips and Privett, 1979). The leaves were then cooled to room temperature and then dried in oven at 80^0C for 24 hours or till a constant weight is reached. The dried samples were ground using a coffee grinder into fine powder ready for extraction. The samples were kept in a freezer at 0^0C before extraction.

3.4: Extraction procedure

Analytical grade solvents obtained from Sigma Aldrich were used in the extraction. For each dry sample, 10 g was weighed together with 0.015g heptadecanoic acid (as internal standard) and placed into a round bottomed flask and extracted twice with 200ml of 2:1 V/V chloroform/methanol mixture for four hours with continuous stirring at room temperature. The mixture was then filtered and a further 200 mls of chloroform-methanol mixture (2:1 v/v) was added to the residue and extraction done for another four hours. The two filtrates were combined and used in subsequent steps (Tunlid *et al.*, 1989; Muritu., 1989; Mishra *et al.*, 1998).

3.5: Purification of lipid extract

The extract mixture prepared above was mixed with 20 ml dilute potassium chloride solution and the mixture thoroughly shaken before being allowed to settle. This was done to remove the non-lipid contaminants (Folch, *et al.*, 1957, Ways and Hannah, 1964). The lower organic layer was separated and the solvent removed using a rotary evaporator at 35^0 C. This gave the total lipid extracts.

3.6: Preparation of fatty acid methyl esters (FAMES)

The lipid extracts were then trans-esterified while the free fatty acids were esterified to their methyl esters. About 1.0g of the lipid mixture and 10ml of 0.5N methanolic sodium hydroxide solution was placed in 100ml round-bottomed flask fitted with a condenser. The mixture was refluxed for 10 minutes until the lipids dissolved. About 0.5ml of Tetrahydrofuran was added to affect solubility of the lipids. A volume of 10 ml of Borontrifluoride-methanol complex (about 14% w/w BF_3) was added to the mixture, which was then refluxed further for two minutes (Morrison and Smith 1964, Vorbeck *et al.*, 1961). The solution was cooled to room temperature, and 5ml of hexane added before the mixture was boiled again for 2 min. The mixture was then cooled to room temperature. A saturated solution of sodium chloride was added and the hexane (organic) layer separated into a vessel containing anhydrous Sodium sulphate. The hexane layer was treated with activated silica gel added with continuous stirring until all the chlorophyll is removed from the solution. The silica gel was filtered off and hexane removed under reduced pressure on the rotary evaporator. The fatty acid methyl esters (FAMES) were then stored in small amount of hexane in sample bottle before injection into the GC (Ayhan, 2008: Christie 1993; IUPAC, 1979).

3.7: Analysis, identification and quantification of FAMES

3.7.1: Analysis of FAMES

The GC analysis was carried out using Shimadzu GC 2010 model. The GC was fitted with fused 50 m silica gel capillary columns of film thickness of 0.2µm and internal diameter of 0.25mm coated carbowax 20 m and flame ionization detector (F.I.D) (Philips *et al.*, 1992). The column was first conditioned by passing nitrogen gas at 180^0 C for about 5 hours. The column temperature was isothermal throughout the column length at 180 ± 1^0 C. The injector temperature was 220 ± 1^0 C, nitrogen gas flow was 40 ml/min. The total run time was 43.15 min (Appendix 3).

3.7.2: Identification of FAMES

A sample of known amounts of standard samples of the fatty acids including the internal standard was prepared. A constant volume of the standard FAMES was injected into the GC under similar conditions as the analyte samples. The FAMES in the analyte samples were identified by the corroborative retention times of the authentic samples (James and Martin, 1956). The fatty acid standards were obtained from Sigma-Aldrich U.S.-Bellefonte, United States.

3.7.3: Quantification of FAMES

To quantify the amount of the FAMES in the analyte sample, a sample of known amounts of standard samples of the fatty acids including the internal standard was prepared and injected into the GC in equal volume as the analyte. The response factor of each of the FAMES was calculated using the formula below;

$$\text{Internal response factor} = \frac{\text{Area(IS)} \times \text{Amount(SF)}}{\text{Amount(IS)} \times \text{Area(SF)}}$$

IS = Internal Standard

SF = Specific fatty acid methyl ester

The peak area of the sample component was then corrected by the multiplying factor and divided by the peak area of the internal standard in the analyte sample to give the amount of particular compound(Metcalfe *et al.*, 1966) using the formula;

$$\text{Weight (SF)} = \frac{\text{Peak area (SF)} \times \text{Internal response factor (SF)} \times \text{Weight(IS) in sample}}{\text{Peak area Internal standard}} .$$

3.8: Statistical analyses

The results were analysed using a 5×5×3 factorial design with locations as main treatments, nitrogen NPKS 25:5:5:5 fertiliser rates as sub treatments and harvesting intervals days as sub-sub treatments using (MSTAT-C, 1993) programme for ANOVA (Appendix 2).

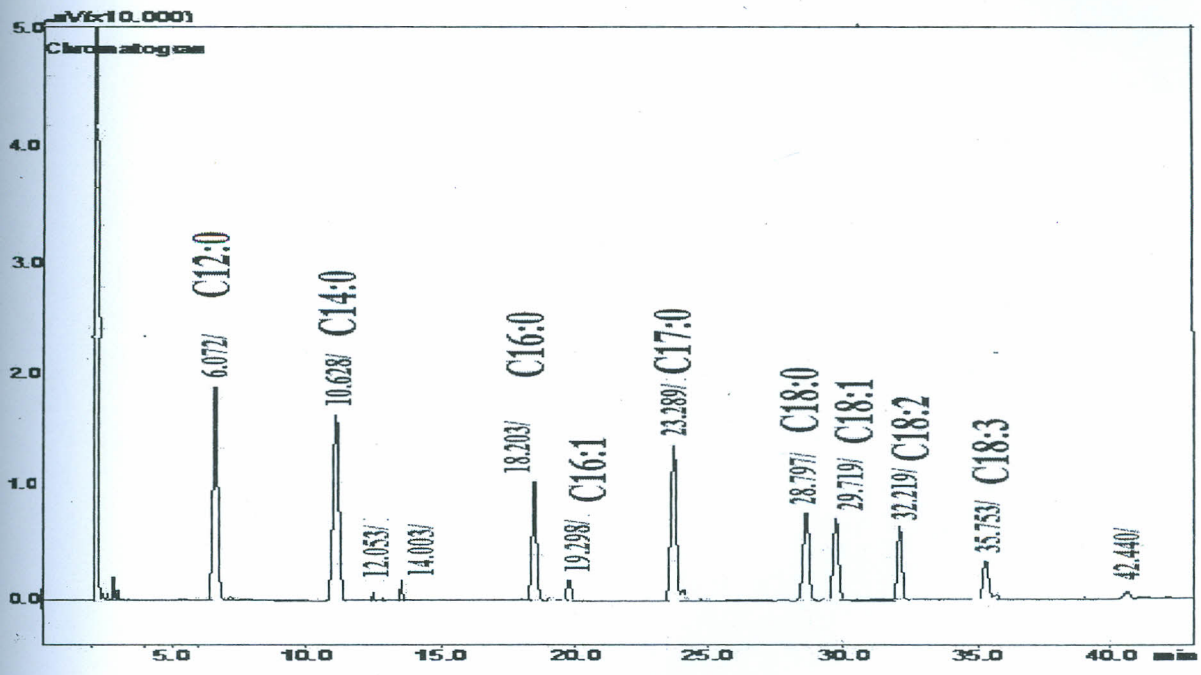
CHAPTER FOUR: RESULTS AND DISCUSSION

4.2: Fatty acids identification and quantification

The separation of the fatty acid methyl esters was mainly on the basis of chain length and degree of unsaturation. The retention times of the fatty acids varied in the order of C18:3 > C18:2 > C18:1 > C18:0 > C17:0 (Internal standard) > C16:1 > C16:0 > C14:0 > 12.0 (Table 5, Figures 2 & 3).

Table 5: Retention times of fatty acid methyl esters

FA	C12:0	C14:0	C16:0	C16;1	C17:0	C18:0	C18:1	C18:2	C18:3
Ret. time (min)	6.072	10.628	18.203	19.298	23.289	28.797	29.719	32.219	35.753



Peak#	Ret.Time	Area	Height
1	6.072	751700	146648
2	10.628	1016150	125174

3	18.202	923452	76899
4	19.298	122732	8991
5	23.289	1593242	108491
6	28.797	1188461	75591
7	29.719	945398	63935
8	32.219	857332	57986
9	35.753	660216	45382

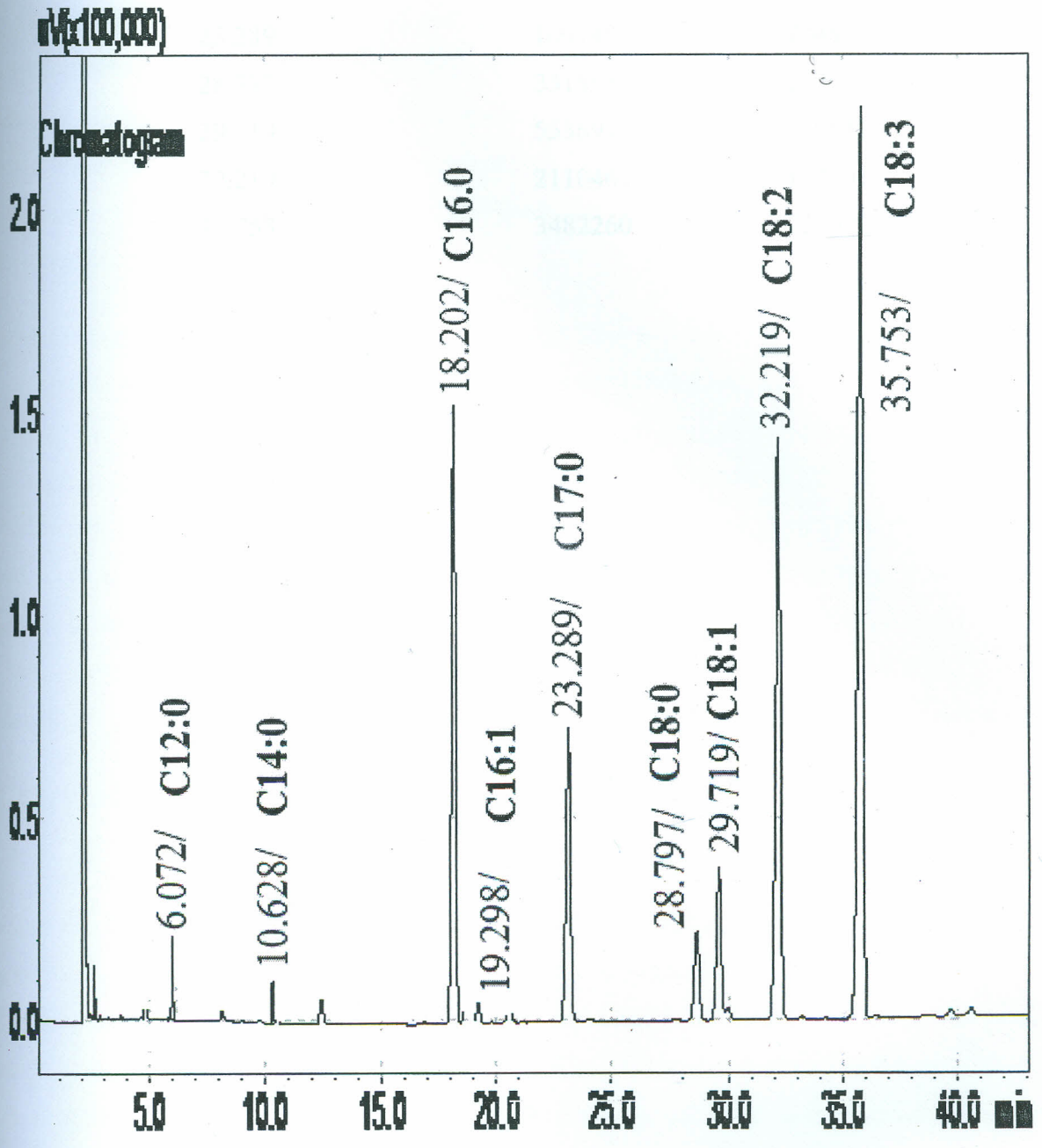


Figure 3: Chromatogram of a typical sample analyte

Peak#	Ret.Time	Area	Height
1	6.072	2752	565
2	10.628	12190	1620
3	18.203	1754397	152536

4	19.298	17864	1471
5	23.289	1001499	72467
6	28.797	331895	22206
7	29.719	555697	37964
8	32.219	2110463	143601
9	35.753	3482260	225002

Increasing chain length and/or degree of unsaturation increased the retention times of the fatty acid methyl esters on the column used. Certain peaks at 12.058 min, 14.003 min and 42.440 min obtained in all the cases are attributable to contaminants contained in the solvent since they are detected when the solvent is run alone. The standard fatty acids chromatograms were used for identification of the individual fatty acids methyl esters present in the samples by the comparison of the retention times (Figures 2 and 3).

For gas chromatographs equipped with a flame ionisation detector (FID), the peak areas are proportional to the amount of the component in the sample (James and Martin, 1956). Since the analyte sample had a known amount of the internal standard, the relative response of the detector to the analyte and the standard remains constant over a wide range of conditions. Incorporation of the internal standard (C17:0) during work up compensates for any losses that occur during analyte sample preparation prior to analysis, as the ratio of internal standard to the analyte remains constant because the same fraction of each is lost in any operation. The use of C17:0 as an internal standard is ideal because it behaves similarly to the analytes of interest, but is unlikely to be present in natural lipid samples since it is not formed via the acetyl CoA pathway.

4.2: Variation of fatty acids with location and nitrogen rates

It has been thought that high quality yielding tea cultivars would produce similar quality attributes in all areas of growth under similar fertilizer and plucking application hence uniform agronomic practices currently practised in most tea growing areas. But the results presented here demonstrate that the levels of saturated, unsaturated, and total fatty acids vary significantly due locations, indicating that similar nitrogenous fertilizer application rates do not produce similar effects in all tea growing locations (Tables 6, 7 and 8) implying each region of production has an optimal fertilizer application rate.

Table: 6: Variations of saturated fatty acids (in mg/10g of dry leaf) due to location and nitrogenous fertilizer rates

FA	Locations	Nitrogen rates (kg N/ha/year) 0	75	150	225	300	Mean location
C12:0	Karirana	0.146	0.395	0.905	2.052	4.627	1.625
	Timbilil	0.219	0.388	0.503	0.670	2.448	0.846
	Changoi	0.558	1.041	2.185	3.355	4.524	2.333
	Sotik Highlands	0.234	0.650	1.313	2.463	5.153	1.962
	Kipkebe	0.043	0.079	0.861	3.111	9.809	2.781
	Mean rate	0.240	0.511	1.153	2.330	5.312	
	CV (%)			22.74			
	LSD, $P \leq 0.05$			0.254			0.254
	Interaction, $P \leq 0.05$			0.434			
C14:0	Karirana	0.300	0.993	1.923	3.793	6.389	2.679
	Timbilil	0.288	0.523	0.696	1.154	2.022	0.937
	Changoi	0.256	0.947	2.165	4.897	9.158	3.485
	Sotik Highlands	0.352	0.868	1.381	2.696	5.353	2.130
	Kipkebe	0.237	1.289	2.820	4.979	7.178	3.301
	Mean rate	0.287	0.924	1.797	3.504	6.020	
	CV (%)			13.0			
	LSD, $P \leq 0.05$			0.191			0.191
	Interaction, $P \leq 0.05$			0.326			
C16:0	Karirana	9.936	12.625	15.617	21.186	30.342	17.941
	Timbilil	7.910	14.788	17.991	24.318	27.535	18.509
	Changoi	6.271	9.242	14.689	26.278	43.163	19.929
	Sotik Highlands	4.243	9.676	13.147	16.792	21.083	12.988
	Kipkebe	12.287	14.019	15.622	17.405	20.771	16.021
	Mean rate	8.130	12.070	15.413	21.196	28.579	
	CV (%)			4.6			
	LSD, $P \leq 0.05$			0.435			0.435
	Interaction, $P \leq 0.05$			0.743			
C18:0	Karirana	3.412	4.546	8.179	14.563	22.552	10.650
	Timbilil	2.738	4.330	5.472	9.415	20.396	8.470
	Changoi	2.987	5.084	9.516	12.890	23.843	10.864
	Sotik Highlands	1.711	3.214	4.647	6.921	12.434	5.785
	Kipkebe	3.154	4.162	4.833	6.133	8.607	5.378
	Mean rate	2.800	4.267	6.529	9.984	17.566	
	CV (%)			6.000			
	LSD, $P \leq 0.05$			0.289			0.289
	Interaction, $P \leq 0.05$			0.483			

The influence of the saturated fatty acids tea black tea quality is not known. However, they are present in appreciable amounts. Although, in earlier studies, lauric (C12:0) and myristic (C14:0) (Muritu, 1989; Owuor *et al.*, 1990a, b, c) were not detected in some cultivars in Kenya and stearic acid in Assam, India tea (Bhuyan and Mahanta, 1989), in this study the acids were present in detectable amounts in the leaves of clone BBK35. The occurrence of the two acids had been reported in earlier studies (Ramaswamy and Ramaswamy, 2000). The order of occurrence of the saturated fatty acids n-hexadecanoic (palmitic) acid (C16:0) > n-octadecanoic (stearic) acid (C18:0) > n-tetradecanoic (myristic) acid (C14:0) > n-dodecanoic (lauric) acid (C12:0) in all locations at any given fertilizer rates. Similar occurrence of the fatty acids had been reported (Ramaswamy and Ramaswamy, 2000; Anan and Nakagawa, 1977; Wright and Fishwick, 1979).

Disregarding location, there were significant increases ($P \leq 0.05$) in the levels of the saturated fatty acids with increase in nitrogen fertilizer rate such that high amounts of fertilizer rates result in high levels of fatty acid in all locations (Table 6). However, uniform fertilizer application in all the locations produces significantly ($P \leq 0.05$) varying levels of fatty acids. Similar fertilizer applications in all locations thus result in different saturated fatty acid amounts in the tea leaves. The significant variations in fatty acid levels can be attributed to variation in micro-environmental condition in the area of growth like humidity, sunshine, temperature changes and soil type and fertility gradient.

The unsaturated fatty acids which degenerate into group I VFC varied significantly with location. The proportions of the acids was in the order linolenic (octadecatrienoic) acid (C18:3) > linoleic (octadecadienoic) acid (C18:2) > oleic (octadecaenoic) acid (C18:1) > palmitoleic (hexadecaenoic) acid (C16:1) at all locations irrespective of fertilizer rate. Similar pattern of occurrence of the fatty acids but in slightly different ratios had been noted in different varieties in Kenya (Owuor *et al.*, 1990a) and in India (Bhuyan *et al.*, 1991). The levels of these acids significantly varied ($P \leq 0.05$) with location. The variations of linolenic acid (C18:3) was in the order; Karirana > Changoi > Timbilil > Sotik Highlands > Kipkebe, however, insignificant variations were observed between Timbilil and Changoi and between Sotik and Kipkebe (Table 7). Linoleic acid (C18:2) varied in the order; Karirana > Timbilil >Changoi > Sotik Highlands >

Kipkebe. For oleic acid (C18:1) the order was Changoi > Timbilil >Kipkebe > Karirana > Sotik Highlands. And for palmitoleic acid (C16:1) the order was Karirana > Sotik Highlands > Changoi > Kipkebe > Timbilil.

Table 7: Variations of unsaturated fatty acids (mg/10g of dry tea leaves) due to location and nitrogen fertilizer rates

		Nitrogen rates (kg N/ha/year)					
FA	Locations	0	75	150	225	300	Mean location
C16:1	Karirana	0.210	0.327	0.531	0.990	3.107	1.033
	Timbilil	0.108	0.233	0.311	0.458	0.817	0.385
	Changoi	0.098	0.283	0.432	0.551	1.215	0.515
	Sotik Highlands	0.089	0.379	0.616	0.762	2.376	0.844
	Kipkebe	0.080	0.170	0.314	0.478	1.237	0.456
	Mean rate	0.117	0.278	0.441	0.648	1.750	
	CV (%)			23.72			
	LSD, $P \leq 0.05$			0.090			0.090
	Interaction, $P \leq 0.05$			0.153			
C18:1	Karirana	0.893	2.616	3.950	6.341	8.311	4.422
	Timbilil	1.809	3.189	4.013	5.848	11.759	5.324
	Changoi	1.570	3.181	5.200	8.336	15.438	6.745
	Sotik Highlands	0.993	2.502	3.326	4.460	6.326	3.509
	Kipkebe	3.256	4.317	5.054	5.670	6.459	4.951
	Mean rate	1.692	3.161	4.308	6.131	9.659	
	CV (%)			9.02			
	LSD, $P \leq 0.05$			0.263			0.263
	Interaction, $P \leq 0.05$			0.450			
C18:2	Karirana	11.960	15.215	18.616	28.850	35.072	21.943
	Timbilil	12.380	15.598	19.612	23.659	28.928	20.036
	Changoi	7.745	11.190	15.798	25.117	37.415	19.453
	Sotik Highlands	8.927	13.868	17.665	19.818	23.566	16.769
	Kipkebe	9.383	12.237	14.812	17.009	21.195	14.927
	Mean rate	10.079	13.622	17.301	22.891	29.235	
	CV (%)			5.52			
	LSD, $P \leq 0.05$			0.601			0.601
	Interaction $P \leq 0.05$,			1.027			
C18:3	Karirana	13.740	21.744	32.928	40.974	54.412	32.760
	Timbilil	13.872	18.180	21.576	25.325	35.671	22.925
	Changoi	9.326	14.232	20.217	29.782	41.217	22.955
	Sotik Highlands	13.016	16.314	20.488	25.342	32.832	21.598
	Kipkebe	8.713	13.560	19.600	26.426	38.910	21.442
	Mean rate	11.733	16.806	22.962	29.570	40.608	
	CV (%)			5.65			
	LSD, $P \leq 0.05$			0.804			0.804
	Interaction, $P \leq 0.05$			1.373			

Table 8: Variations of total fatty acids (mg/10g of dry tea leaves) due to location and nitrogen application rates

		Nitrogen rates (kg N/ha/year)					
Items	Locations	0	75	150	225	300	Mean location
TFA	Karirana	40.599	58.349	83.743	118.749	165.035	93.295
	Timbilil	39.180	57.241	70.173	87.180	129.576	76.670
	Changoi	29.040	45.091	70.312	111.649	175.992	86.417
	Sotik Highlands	29.503	47.615	61.388	79.365	108.900	65.354
	Kipkebe	37.142	49.723	63.916	81.433	114.730	69.389
	Mean rate	35.093	51.604	69.906	95.675	138.847	
	CV (%)			4.51			
	LSD, $P \leq 0.05$			2.063			2.063
	Interaction, $P \leq 0.05$			3.523			

Previously, studies on fatty acids had been conducted at single sites or using different clones in different locations, hence the variation due to location could not be established. This has resulted into the current uniform fertilizer inputs in all tea growing areas. However, this study for the first time has established that same cultivar of tea under similar fertilizer rate does not produce the same levels of aroma precursors (unsaturated fatty acids). The variation in the levels of unsaturated fatty acids with regions could explain the previously observed variation of aroma quality with location (Owuor *et al.*, 1988, 2008). These variations in the unsaturated fatty acids can be attributed to the different responses of the plant biochemicals to different micro-ecological factors like rainfall (amount and intensity), sunshine (exposure and intensity), humidity, temperature, soil type and fertility gradient. These factors expose the plants to different stress levels in different locations leading to different activities of enzymes involved in the fatty acid synthesis in the plants. Even though these factors were not investigated in this study, it is apparent that they were not constant in all areas of study.

The lipid composition of tea is species/genotype dependent (Ramaswamy and Ramaswamy, 2000), however, the levels of nitrogenous fertilizer applied to the plant influence the fatty acid levels in the leaf. This is evident from the significantly low levels of fatty acids at the control, 0 Kg N/ha/year and the significant increases ($P \leq 0.05$) of fatty acid levels with nitrogenous fertilizer rates (Tables 6,7 and 8), reinforcing observations made in earlier studies (Muritu, 1989;

Owuor *et al.*, 1990a) that increase of fertilizer rate at single site raises the levels of fatty acids. This can also partly explain the earlier observation that high nitrogenous fertilizer rate produce more VFC hence reduce the flavour quality of black tea (Owuor; 1989; Cloughley, 1980, 1981, 1983; Owuor *et al.*, 1987a, 1990d, 2000). However, for economic production of tea, nitrogenous fertilizer application is a prerequisite with yield being the target to most farmers. The major biochemical effect of nitrogen levels in plants is enhancement of protein synthesis and hence growth rate of the plants. When nitrogen levels in the soil increases for example by fertilizer application, there occurs increased photosynthesis. This causes the plant to have not only the required amino acids but also enhanced machinery for the synthesis of lipids containing fatty acids.

Significant interaction effects ($P \leq 0.05$) were observed in this study implying that, although the pattern of response of fatty acids to fertilizer application rate is the same in all locations i.e. increase of fatty acids with nitrogen rates, the rate of this response is not constant for all locations (Tables 6, 7 and 8). Indeed, a constant increase in fertilizer does not produce equal responses in all locations. This could be due to other environmental factors that influence the formation of fatty acids besides fertilizer. It is therefore, not possible to produce tea leaves with same levels of fatty acids in different locations under similar fertilizer rate. The significant interaction effects observed in this study also imply that it is only possible to produce tea with similar levels of fatty acids in different locations at different optimal nitrogenous fertilizer application rates. Thus, the current blanket fertilizer application rates practised in all locations would lead to teas of different quality. Different regions therefore need to domesticate fertilizer rates for a clone that is a compromise between yields and quality for a particular area. The optimal nitrogenous fertilizer requirements need to be evaluated in areas of intended release before large scale production, and that when deciding the correct amount of nitrogenous fertilizer, the clones biochemical composition of the leaves be used in conjunction with yield evaluations in areas of intended release so that optimal nitrogen rates in different regions are the best balance between quality and yields.

The differing chemical/biochemical composition of tea leaves result in quality variations and is caused by different growth rates (Mwakha, 1985a, 1985b) caused by certain factors including average air temperature (Squire, *et al.*, 1993), distribution and total amounts of rainfall (Carr,

1977), Sunshine hours and cloud cover (Portsmouth and Rajiah, 1957) and type of soil/fertility gradient. Though these factors were not examined in this study, it is apparent that the regions under study had different conditions hence the observed variation.

4.3: Variation of fatty acids due to locations and plucking intervals

The distribution of fatty acids due to plucking intervals in different locations is represented in Tables 9-12. There were significant variations in the levels of saturated fatty acids, unsaturated fatty acids, total unsaturated fatty acids and the total fatty acids in the different locations of growth. The levels of saturated fatty acids in the leaf was found to vary in the order of n-hexadecanoic (palmitic) acid (C16:0) > n-octadecanoic (stearic) acid (C 18:0) > n-tetradecanoic (myristic) acid (C14:0) > n-dodecanoic acid (lauric) (C12:0) in all the locations and at all plucking intervals (Table 9). Longer plucking intervals resulted into high levels of the fatty acids i.e. 21 days > 14 days > 7 days. The saturated fatty acids are of less economic value in tea production as they do not degenerate to the quality compounds.

The levels of the individual saturated fatty acids significantly ($P \leq 0.05$) varied with location. The average variations in the levels of these acids were not uniform to all locations. Indeed, for n-dodecanoic (lauric) acid (C12:0) the order was Kipkebe > Changoi > Sotik Highlands >Karirana > Timbilil, n-tetradecanoic (myristic) acid (C14:0) the order was Changoi > Kipkebe > Karirana > Sotik > Timbilil. The level of n-hexadecanoic (palmitic) acid (C16:0) was; Changoi > Timbilil > Karirana > Kipkebe > Sotik Highlands and the order for n-octadecanoic (stearic) acid was Changoi > Karirana > Timbilil > Sotik Highlands > Kipkebe. Although there was recorded increase in the levels of the saturated fatty acids with plucking intervals, the rate of increase was not constant in all the locations hence the observed interaction effects (Table 9). The non-uniform variation in the levels of these acids can be attributable to different climatic conditions prevailing in the different regions subjecting the plants to different types and levels of stress.

Table 9: Variations of saturated fatty acids (mg/10g of dry tea leaves) due to location and plucking intervals

Item	Plucking frequency (days)	Locations: Karirana	Timbilil	Changoi	Sotik Highlands	Kipkebe	Mean plucking round
C12:0	7	1.170	0.470	1.960	1.383	1.908	1.378
	14	1.637	0.777	2.339	1.902	2.560	1.843
	21	2.068	1.290	2.699	2.603	3.873	2.507
	Mean location	1.625	0.846	2.333	1.962	2.781	
	CV (%)			22.74			
	LSD,P ≤ 0.05			0.254			0.3049
	Interaction, P ≤ 0.05			0.366			
C14:0	7	2.025	0.773	2.650	1.583	2.827	1.972
	14	2.738	0.957	3.509	1.924	3.276	2.481
	21	3.275	1.080	4.294	2.883	3.799	3.066
	Mean location	2.679	0.937	3.485	2.130	3.301	
	CV (%)			13.00			
	LSD, P ≤ 0.05			0.192			0.229
	Interaction, P ≤ 0.05			0.134			
C16:0	7	16.026	16.812	16.652	11.307	15.314	15.222
	14	18.026	18.170	18.977	13.172	15.922	16.853
	21	19.771	20.544	24.157	14.485	15.314	19.157
	Mean location	17.941	18.509	19.929	12.988	16.021	
	CV (%)			4.36			
	LSD, P ≤ 0.05			0.435			0.5227
	Interaction, P ≤ 0.05			0.626			
C18:0	7	8.869	6.207	9.437	5.031	4.802	6.869
	14	10.713	8.966	10.702	5.830	5.406	8.323
	21	12.369	10.237	12.453	6.496	5.925	9.496
	Mean location	10.650	8.470	10.864	5.785	5.378	
	CV (%)			6.000			
	LSD, P ≤ 0.05			0.289			0.347
	Interaction, P ≤ 0.05			0.416			

Considering plucking frequencies with disregard to location, the levels of the unsaturated fatty acids which are precursors to most group I VFC increased significantly ($P \leq 0.05$) in the order 21 days > 14 days > 7 days. Similar observation is made for total unsaturation and total fatty acid levels (Tables 10 and 11). Similar increases with plucking intervals had been made in earlier studies (Muritu 1989, Owuor *et al.*, 1990c) for teas grown at single sites. Longer plucking intervals results into higher amounts of precursors of compounds that influence the aroma quality of black tea, this would partly explain the earlier observations that group I VFC in black tea increased with long plucking intervals resulting into decline of aroma quality of black tea with longer plucking interval (Baruah *et al.*, 1986; Owuor *et al.*, 1997; Owuor and Odhiambo, 1994).

The increase of these fatty acid levels due to plucking interval have been attributed to the tendency to pluck more mature leaves at longer intervals. However, in this study, the plucking was selective and only two leaves and a bud were plucked and the influence of coarse plucking was minimal hence the variations observed here were due to plucking intervals The increase observed here can be attributed to the fact that lipids as structural components of plant tissues accumulate in the tissues with time.

Earlier studies on the variations of fatty acid levels were conducted at single sites or using different genotypes grown in different areas thus it was difficult to isolate the response due to location. Indeed in most tea growing countries, tea breeding and clonal selection programmes are usually concentrated at single site within a country and the results assumed to be the same in other areas. A superior genotype at the selection site is expected to replicate the attributes within all the tea growing areas especially within one country where variations are considered minimal. The data presented here demonstrate for the first time that within Kenya, there are significant ($P \leq 0.05$) variations in unsaturated fatty acid and total saturated fatty acid levels in one genotype subjected to the similar plucking management in different regions.

There were significant differences ($P \leq 0.05$) in the levels of fatty acids with location at constant plucking intervals such that (9Z, 12Z, 15Z)-octadecatrienoic (linolenic) acid ($C18:3^{\Delta 9,\ 12,\ 15}$) varied in the order Karirana > Changoi, Timbilil > Sotik Highlands > Kipkebe. The variation in (9Z, 12Z)-octadecadienoic (linoleic) acid ($18:2^{\Delta 9,\ 12}$) was significant ($P \leq 0.05$) in all location such that Karirana > Timbilil > Changoi >Sotik > Kipkebe, (9Z)-octadecaenoic (oleic) acid

(18:1$^{\Delta 9}$) varied in the order Changoi > Timbilil > Kipkebe >Karirana > Sotik Highlands and (9Z)-hexadecaenoic (palmitoleic) acid (C16:1$^{\Delta 9}$) varied in the order Karirana > Sotik Highlands > Changoi > Kipkebe > Timbilil (Table 10). Total unsaturated fatty acid and total fatty acid levels varied significantly with location in the order Karirana > Changoi > Timbilil > Sotik Highlands > Kipkebe (Table 11). This means that similar plucking intervals would not lead to the production of similar levels of precursors of aroma quality in all areas and this can partly explain the differences observed in differences in VFC in black teas grown in Kenya and Malawi (Owuor *et al.*, 2007).

Although there were significant ($P \leq 0.05$) increases in fatty acids le vels with increase in plucking intervals, the rate of the increase was not constant in all the locations hence the observed significant interactions effects ($P \leq 0.05$) (Table 10 and 11). This implies that similar plucking intervals would lead to different levels of precursors of aroma quality parameters in different regions hence differences in quality. This is attributable to different growth rates in different locations (Obaga *et al.*, 1988, 1989; Ng'etich and Stephens, 2001a, 2001b, Ng'etich *et al.*, 2001) leading to achievement of the recommended two leaves and a bud (Othieno, 1988) after different time lengths in different locations. Therefore the uniform blanket recommended plucking interval throughout Kenya (Othieno, 1988) may be subjecting some areas to low quality production. Indeed, studies on plain quality parameters on (Owuor *et al.*, 2010; Jondiko, 2009) varied with location and plucking frequency. This implies that each location has an optimal plucking management practice.

Table 10: Variations of unsaturated fatty acids (mg/10g dry leaf) due to location and plucking rounds

FA	Plucking frequency	locations					
		Kariran a	Timbilil	Changoi	Sotik	Kipkebe	Mean plucking
C16:1	7 days	0.673	0.311	0.370	0.528	0.330	0.442
	14 days	1.032	0.383	0.497	0.913	0.445	0.654
	21 days	1.394	0.462	0.680	1.092	0.593	0.844
	Mean location	1.033	0.385	0.515	0.844	0.456	
	CV (%)			23.72			
	LSD, P ≤ 0.05			0.089			0.108
	Interaction, P ≤ 0.05			0.129			
C18:1	7 days	3.824	4.351	5.338	3.001	4.549	4.213
	14 days	4.277	5.302	6.984	3.464	5.037	5.013
	21 days	5.167	6.317	7.913	4.064	5.267	5.745
	Mean location	4.422	5.324	6.745	3.509	4.951	
	CV (%)			9.02			
	LSD, P ≤ 0.05			0.263			0.316
	Interaction, P ≤ 0.05			0.379			
C18:2	7 days	19.687	18.923	17.441	15.228	13.750	17.006
	14 days	22.519	19.682	19.352	16.573	15.012	18.628
	21 days	23.621	21.502	21.567	18.505	16.020	20.243
	Mean location	21.943	20.036	19.453	16.769	14.927	
	CV (%)			5.52			
	LSD, P ≤ 0.05			0.601			0.722
	Interaction, P ≤ 0.05			0.865			
C18:3	7 days	29.463	20.975	20.638	19.870	18.809	21.951
	14 days	33.105	22.986	22.447	21.713	21.408	24.332
	21 days	35.710	24.814	25.779	23.213	24.110	26.725
	Mean location	32.760	22.925	22.955	21.598	21.442	
	CV (%)			5.65			
	LSD, P ≤ 0.05			0.804			0.966
	Interaction, P ≤ 0.05			1.157			
TUFA	7	53.654	44.560	43.786	38.628	37.778	43.681
	14	60.856	48.486	49.425	42.628	41.907	48.660
	21	66.026	52.961	56.018	46.196	46.056	53.451
	Mean location	60.179	48.669	49.743	42.484	41.913	
	CV (%)			3.84			
	LSD, P ≤ 0.05			1.092			1.312
	Interaction, P ≤ 0.05			1.571			

Table 11: Variations in levels of total fatty acid (mg/10g dry leaf) due to location and plucking rounds

		locations					
Item	Plucking frequency in days	Karirana	Timbilil	Changoi	Sotik	Kipkebe	Mean plucking round
TFA	7	81.744	68.829	74.556	57.951	62.349	69.086
	14	93.964	75.0589	85.085	65.389	69.271	77.760
	21	104.177	86.092	99.609	72.723	76.547	87.830
	Mean location	93.295	76.670	86.417	65.354	69.389	
	CV (%)			4.51			
	LSD, $P \leq 0.05$			2.063			2.477
	Interaction, $P \leq 0.05$			2.969			

4.3: Variation of fatty acid levels due to fertilizer rates and plucking intervals

The effects of the nitrogenous fertiliser rates and plucking intervals on levels of saturated fatty acid, unsaturated fatty acids, total unsaturation and total fatty acids are presented in (Tables12, 13, 14). All the quality fatty acid level significantly ($P \leq 0.05$) increased with plucking intervals and nitrogenous fertiliser rates. There were significant interactions suggesting non-uniform response patterns for all the fatty acids.

The unsaturated fatty acids increased with increasing fertilizer rates and plucking intervals at all locations. These results indicate that indeed, high rates of nitrogenous fertilizer and longer plucking intervals increase in the fatty acid levels as had been observed in earlier studies (Muritu, 1989; Owuor *et al.*, 1990a) leading to higher production of VFC hence lower flavour/aroma quality (Cloughley, 1980, 1981, 1983; Owuor *et al.*, 1987a, 1990d, 2000). However, for economic production of tea, fertilizer application is a prerequisite with yield being the target to most farmers. These results indicate that when deciding the correct amount of nitrogenous fertilizer, quality considerations should be a factor, so that the amount used does not compromise quality and yields.

Table 12: Variation in the levels of saturated fatty acids (mg/10g dry leaf) due to nitrogen rates and plucking intervals

FA	Plucking frequency (days)	Nitrogen rates (Kg/ha/year) 0	75	150	225	300	Mean plucking
C12:0	7	0.121	0.393	0.799	1.942	3.635	1.378
	14	0.262	0.541	1.076	2.243	5.094	1.843
	21	0.338	0.598	1.585	2.806	7.207	2.507
	Mean rate	0.240	0.511	1.153	2.330	5.312	
	CV (%)			22.74			
	LSD,P ≤ 0.05			0.254			0.305
	Interaction, P ≤ 0.05			0.366			
C14:0	7	0.111	0.715	1.471	2.782	4.779	1.972
	14	0.229	0.887	1.779	3.581	5.928	2.481
	21	0.520	1.169	2.141	4.148	7.353	3.066
	Mean rate	0.287	0.924	1.797	3.504	6.020	
	CV (%)			13.000			
	LSD, P ≤ 0.05			0.191			0.229
	Interaction, P ≤ 0.05			0.274			
C16:0	7	5.936	11.055	14.205	19.324	25.589	15.222
	14	8.098	11.999	14.926	21.159	28.085	16.853
	21	10.354	13.156	17.107	23.105	32.062	19.157
	Mean rate	8.130	12.070	15.413	21.196	28.579	
	CV (%)			4.36			
	LSD, P ≤ 0.05			0.435			0.523
	Interaction, P ≤ 0.05			0.626			
C18:0	7	2.089	3.826	5.765	8.704	13.963	6.869
	14	2.859	4.212	6.451	9.761	18.334	8.323
	21	3.454	4.764	7.372	11.488	20.402	9.496
	Mean rate	2.800	4.267	6.529	9.984	17.566	
	CV (%)			6.000			
	LSD, P ≤ 0.05			0.289			0.347
	Interaction, P ≤ 0.05			0.416			

Table 13: Variation of unsaturated fatty acid content (mg/10g dry leaf) due to nitrogen fertilizer rates and plucking intervals

FA	Plucking frequency in days	Nitrogen rates (Kg N/ha/year) 0	75	150	225	300	Mean plucking
C16:1	7 days	0.071	0.217	0.392	0.553	0.978	0.442
	14 days	0.119	0.274	0.441	0.643	1.793	0.654
	21 days	0.161	0.344	0.489	0.748	2.480	0.844
	Mean rate	0.117	0.278	0.441	0.648	1.750	
	CV (%)			23.72			
	LSD, P ≤ 0.05			0.090			0.108
	Interaction, P ≤ 0.05			0.129			
C18:1	7 days	1.027	2.681	4.065	5.306	7.985	4.213
	14 days	1.839	3.137	4.271	6.139	9.678	5.013
	21 days	2.211	3.665	4.590	6.948	11.313	5.745
	Mean rate	1.692	3.161	4.308	6.131	9.659	
	CV (%)			9.02			
	LSD, P ≤ 0.05			0.264			0.316
	Interaction, P ≤ 0.05			0.379			
C18.2	7 days	8.312	12.643	15.403	21.255	27.416	17.006
	14 days	10.207	13.563	16.781	23.134	29.453	18.628
	21 days	11.718	14.659	19.717	24.283	30.836	20.243
	Mean rate	10.079	13.622	17.301	22.891	29.235	
	CV (%)			5.52			
	LSD, P ≤ 0.05			0.601			0.722
	Interaction, P ≤ 0.05			0.865			
C18:3	7 days	9.786	15.071	20.499	26.760	37.639	21.951
	14 days	11.698	16.991	22.689	29.132	41.149	24.332
	21 days	13.717	18.356	25.699	32.817	43.037	26.725
	Mean rate	11.733	16.806	22.962	29.570	40.608	
	CV (%)			5.65			
	LSD, P ≤ 0.05			0.804			0.966
	Interaction, P ≤ 0.05			1.157			
TUFA	7 days	19.210	30.612	40.692	53.873	74.019	43.681
	14 days	23.928	33.965	44.138	59.181	82.089	48.660
	21 days	27.687	36.957	49.945	64.936	87.733	53.451
	Mean rate	23.608	33.844	44.925	59.3330	81.280	
	CV (%)			3.84			
	LSD, P ≤ 0.05			1.092			1.311
	Interaction, P ≤ 0.05			1.572			

Table 14: Changes in levels of TFA (mg/10g dry leaf) due to fertilizer rates and plucking rounds

		Nitrogen rates (Kg N/ha/year)					
Item	Plucking frequency (days)	0	75	150	225	300	Mean plucking
TFA	7	27.524	46.628	62.599	86.625	122.052	69.086
	14	35.436	51.604	68.437	93.724	139.597	77.760
	21	42.320	56.580	78.683	106.676	154.890	87.830
	Mean rate	35.093	51.604	69.906	95.675	138.847	
	CV (%)			4.51			
	LSD, $P \leq 0.05$			2.063			2.477
	Interaction, $P \leq 0.05$			2.969			

CHAPTER FIVE: SUMMARY CONCLUSIONS, RECOMMENDATIONS AND SUGGESTION FOR FUTURE STUDIES

5.1: Summary

1. Fatty acid levels significantly vary with regions in a single cultivar under similar nitrogenous fertilizer rates and plucking intervals. The rates of this variation is not uniform hence the significant interaction effects observed.
2. Increase in nitrogenous fertilizer rates results into significant increases in fatty acid levels at all of locations.
3. Increase in plucking intervals results into significant increase in fatty acid levels at all locations.

5.2: Conclusions

1. The fatty acid levels in one cultivar under similar nitrogenous fertilizer rates vary from region to region. Therefore, similar rates of nitrogenous fertilizer do not produce teas of the same quality potential in all regions. The extent and levels of these variations differ from region to region.
2. Fatty acids levels in one cultivar under similar plucking intervals vary from region to region. Therefore, uniform plucking intervals subject the different tea growing areas to different quality potentials. The extent and level of variation vary with locations.
3. Fatty acid levels increased with increase in nitrogenous rates and plucking intervals irrespective of location, implying that long plucking intervals and high nitrogenous fertilizer rates increase the level of aroma precursor compounds which would reduce aroma quality of black tea. The extent and levels vary with locations implying that each region has optimal agronomic and management practices to produce similar aroma quality potentials.

5.3: Recommendations

1. Different regions need to adopt optimal use of fertilizer rates that ensures a compromise between quality and yields for more economic returns.
2. Different regions should adopt region specific plucking intervals to enhance tea quality.
3. To produce black tea of high aroma quality in all locations, lower rates of nitrogenous fertilizer and shorter plucking intervals should be practised.

5:4: Suggestion for future studies

1. Further work should be done to establish a relationship between the fatty acid variations with organoleptic evaluation of black tea in these regions.
2. Further work need to be done to establish the threshold levels or ranges of fatty acids that would be deleterious to aroma quality of tea.
3. Further work need to be done to establish fertilizer rate-plucking interval combinations that would optimize aroma quality of black tea.

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