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:

AMOS WERE OKAL

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DECLARATIONS

I certify that this thesis has not been previously presented for a degree in Maseno University or in any other University. The work reported herein is my original work and all sources of information have been supported by relevant references.

Sign:.. Date: ....

Amos Were Okal

PG/MSC/031/2007

Supervisors:

This thesis has been submitted for examination with our approval as the University supervisors.

1st Supervisor:

Sign: .... Date: ....

Prof P. Okinda Owuor

Department of Chemistry

Maseno University

P.O. Box 333, Maseno

Kenya.

2nd supervisor:

Sign: .... Date: ....

Prof Lawrence A. O. Mang'uro

Department of Chemistry

Maseno University

P.O. Box 333, Maseno

Kenya.

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DEDICATION

To the late Ken Okal and my late Mum.

You encouraged me to start

But you never lived to see me to this end.

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 authentic standards 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.

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LIST OF ABREVIATIONS

BBK 35	Cultivar/Clone BBK 35
FA	Fatty acid
FAMES	Fatty acid methyl esters
FID	Flame Ionisation Detector
GC	Gas chromatography
N	Nitrogen
LNA	Linolenic acid
USFA	Unsaturated fatty acid
SFA	Saturated fatty acids
TFA	Total fatty acid
TRFK	Tea Research Foundation of Kenya
TUFA	Total unsaturated fatty acid
VFC	Volatile flavour compounds
C12:0	dodecanoic acid
C14:0	tetradecanoic acid
C16:0	hexadecanoic acid
C16:1	9Z-hexadecaenoic acid
C18:0	octadecanoic acid
C18:1	9Z-octadecenoic acid
C18:2	9Z, 12Z-octadecadienoic acid
C18:3	9Z, 12Z, 15Z-octadecatrienoic acid
CV%	Co-efficient of Variation
LSD	Least Significant Difference

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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). This method only determines the quality of tea after processing, so there is no way predicting quality at the green leaf stage. However, 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

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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 is 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 is 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 aroma 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 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

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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) shows 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). Increase in the amounts of unsaturated fatty acids in the green tea leaves leads 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 (C16:0) acid is present in phosphatidyl inositol (PI) **[VII]** and sulphoquinovosyldiglyceride (SQDG) **[VI]**, while steroacylmonoglucoside (SMG) **[IX]** is dominated by stearic (C18:0) acid.

[I]=Neutral lipid

R₁ ≠ R₂ ≠ R₃ =Fatty acid residues (mainly saturated fatty acids; C12:0, C14:0, C18:0)
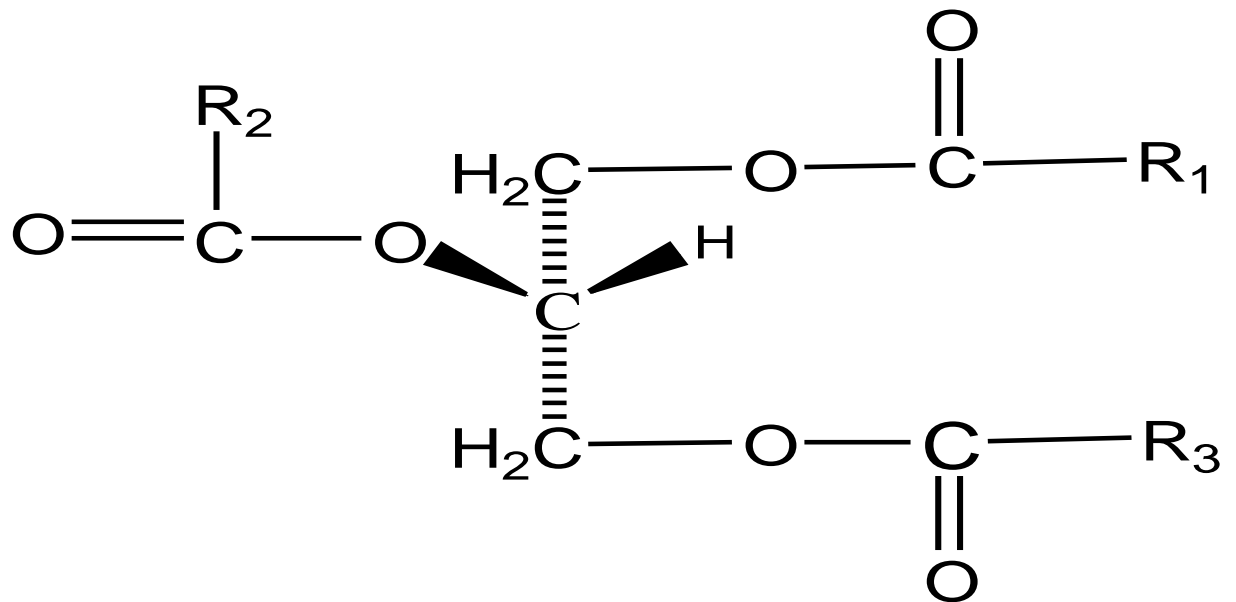
$R_1 \neq R_2 \neq R_3$ =Fatty acid residues (mainly saturated fatty acids; C12:0, C14:0, C18:0)

[II]=Monogalactosyldiglyceride (MGDG)
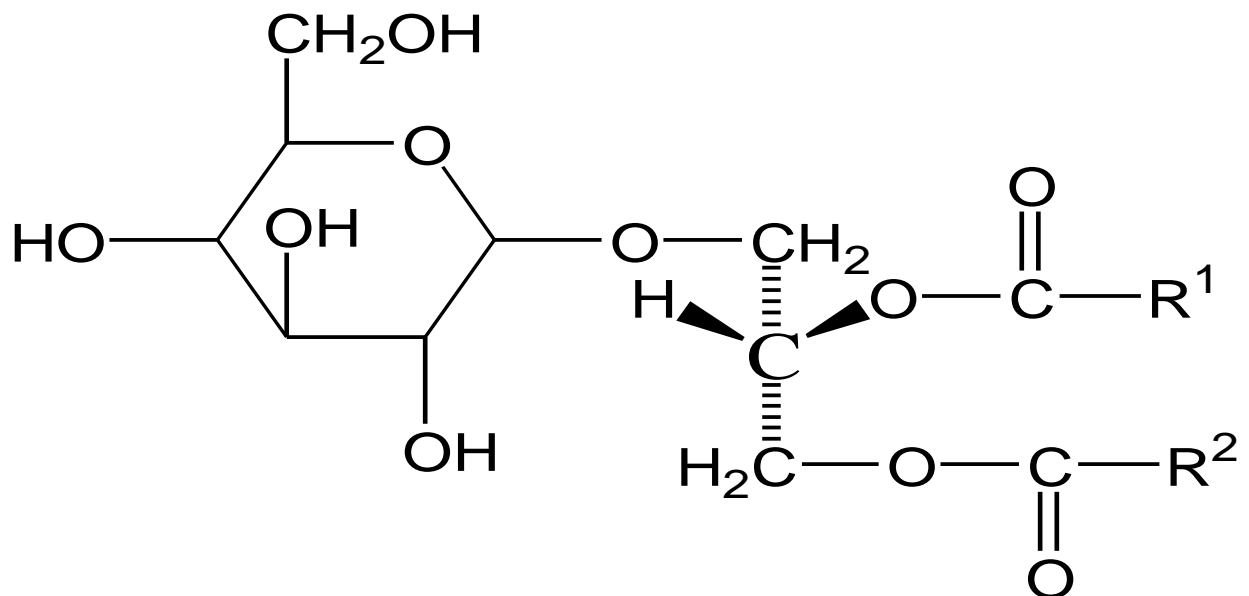
$R^1 \neq R^2$=Fatty acid residues (mainly, C18:3)

[III]=digalactosyldiglyceride (DGDG)
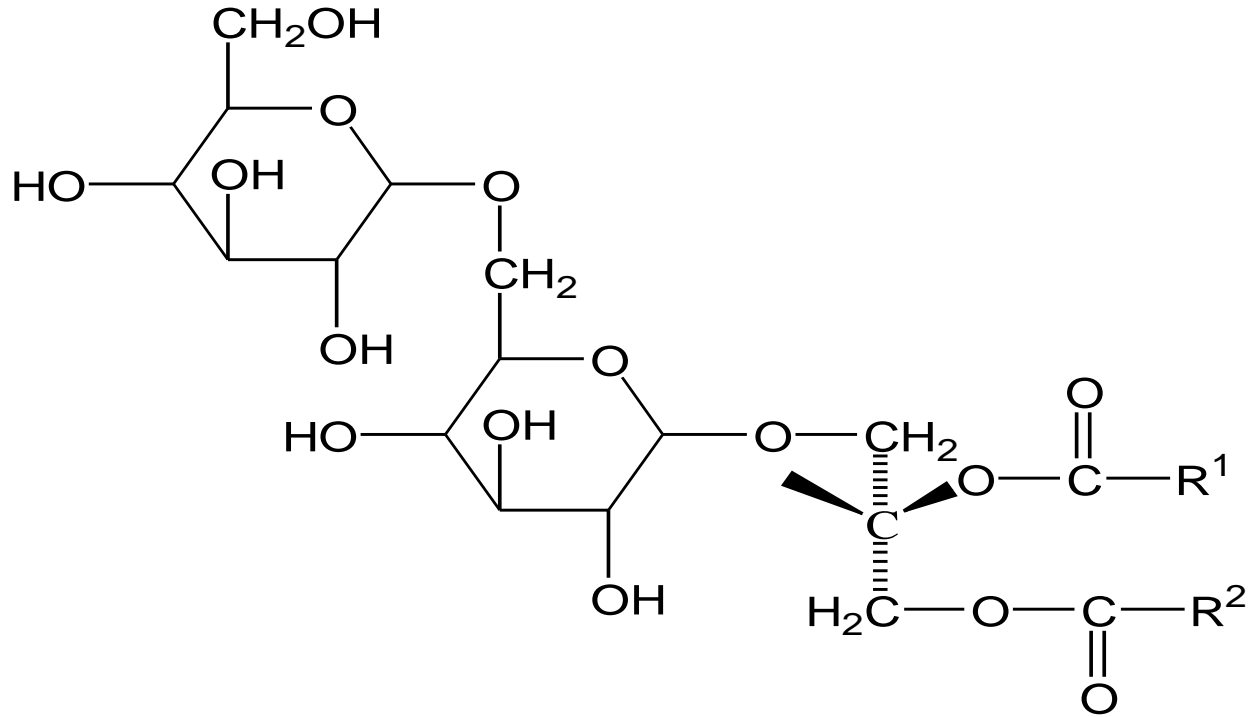
$R^1 \neq R^2$=Fatty acid residues (mainly, C18:3)

[IV]=Phosphatidylcholine (PC)
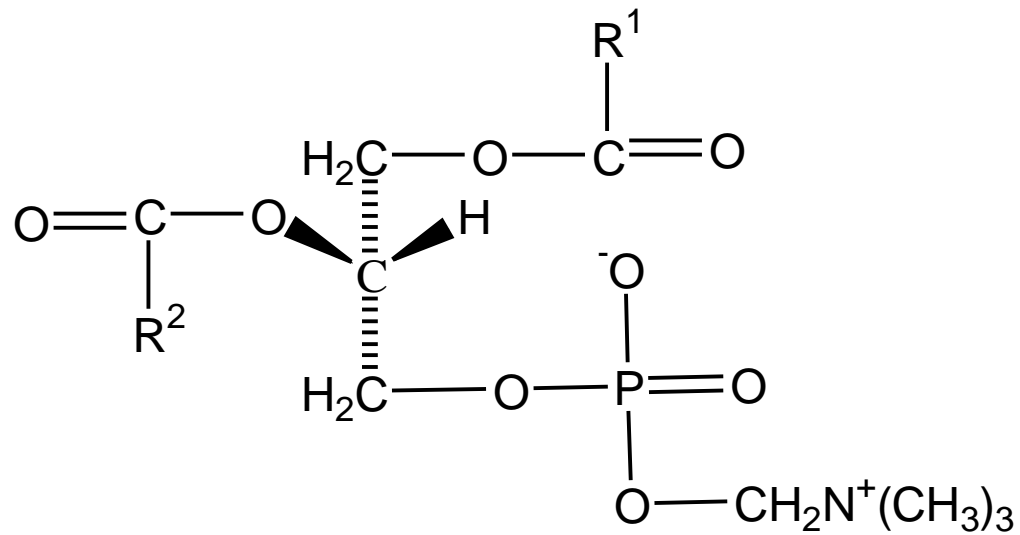
$R^1 \neq R^2$=Fatty acid residues (mainly, C18:1, C18:2)

[V]=Phosphatidylethanolamine (PE)

$R^1{\neq}R^2$ =Fatty acid residues (mainly, C18:1, C18:2)
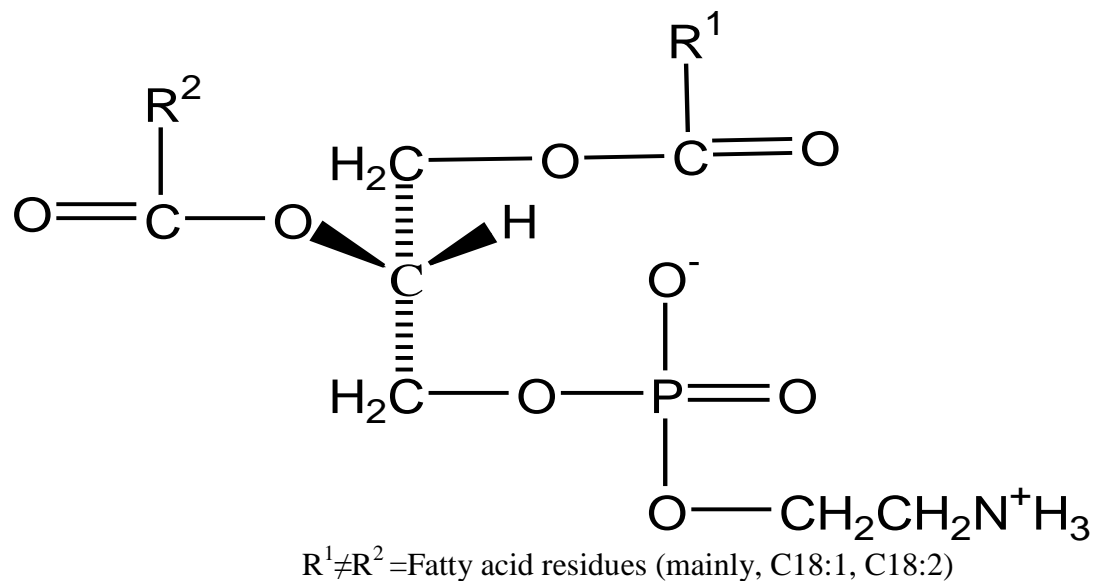
[VI]=Sulphoquinovosyldiglyceride (SQDG)
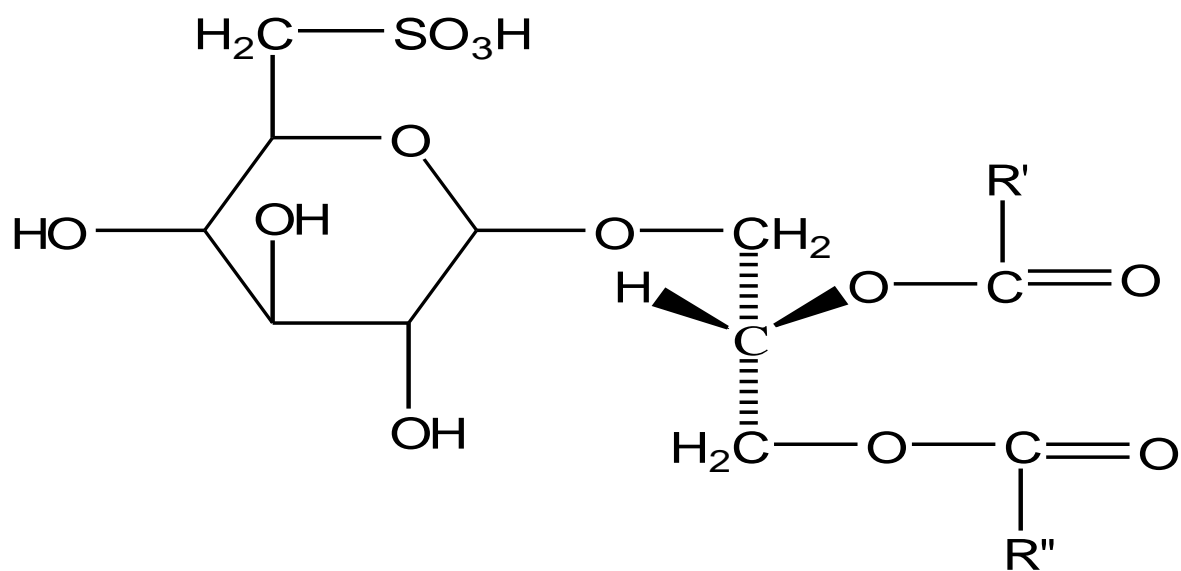
R'≠R''=Fatty acid residues (mainly, C16:0)

[VII]=Phosphatidylinositol (PI)
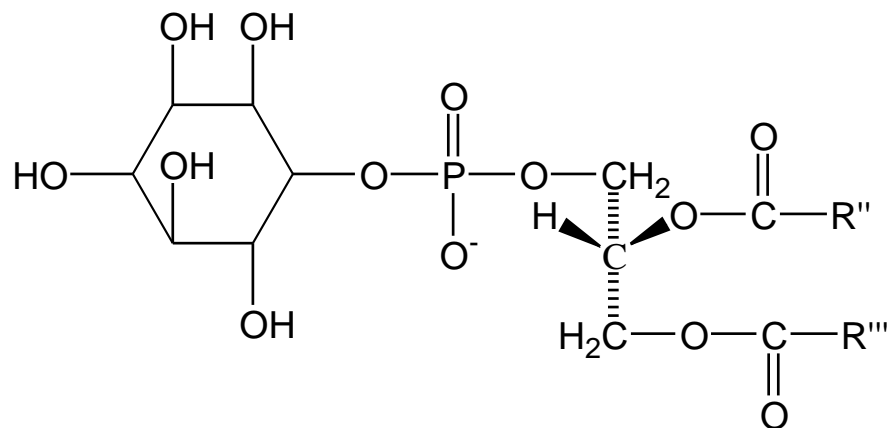
R"≠R'''=Fatty acid residues (mainly, C16:0)

[VIII]=Phosphatidylglycerol (PG)
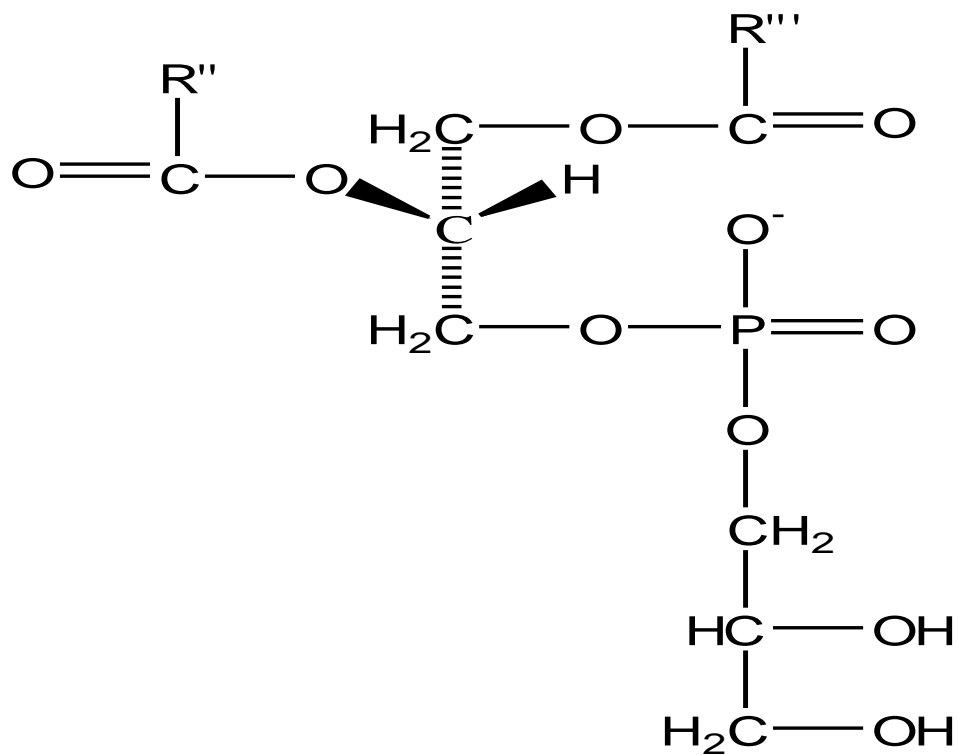
R"≠R'''=Fatty acid residues (mainly, C18:1, C18:2)

[IX]=Steroacylmonoglucoside (SMG)
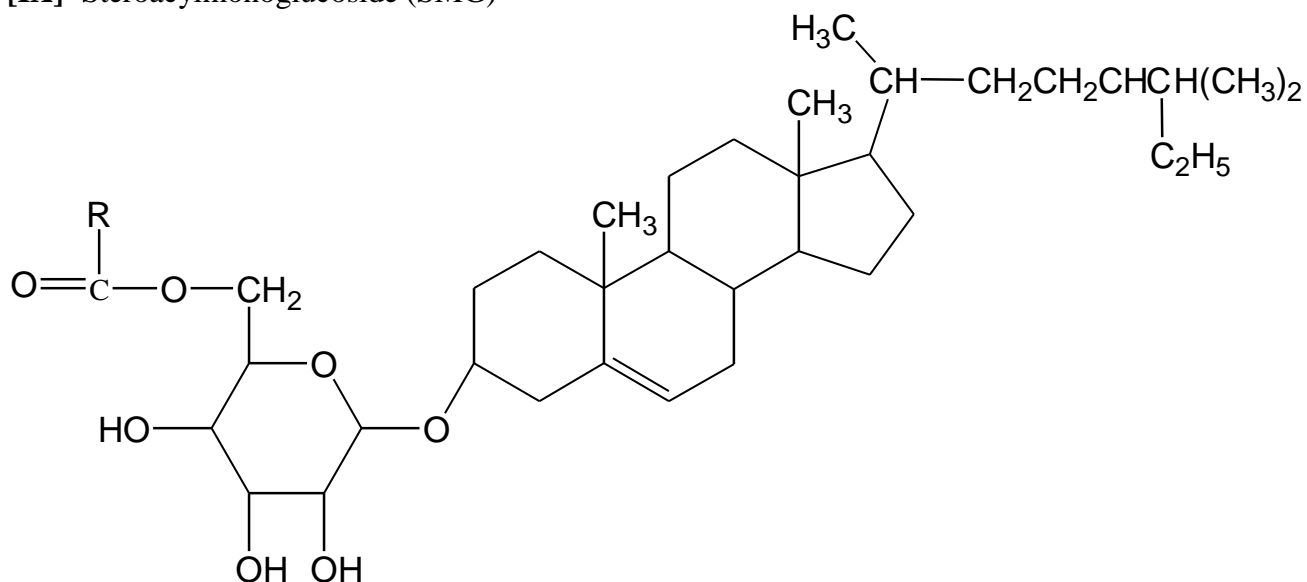
R=Fatty acid residue (C18:0)

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	$C16:1^{\Delta9}$	
Stearic acid	octadecanoic acid	C18:0	
Oleic acid	9Z- octadecenoic acid	$C18:1^{\Delta9}$	
Linoleic acid	9Z, 12Z-octadecadienoic acid	$C18:2^{\Delta9,\ 12}$	
Linolenic acid	9Z, 12Z, 15Z-octadecatrienoic acid	$C18:3^{\Delta9,12,15}$	

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,

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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) enzyme 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).

Figure 1: Scheme for the formation of VFC from linoleic and linolenic acid.
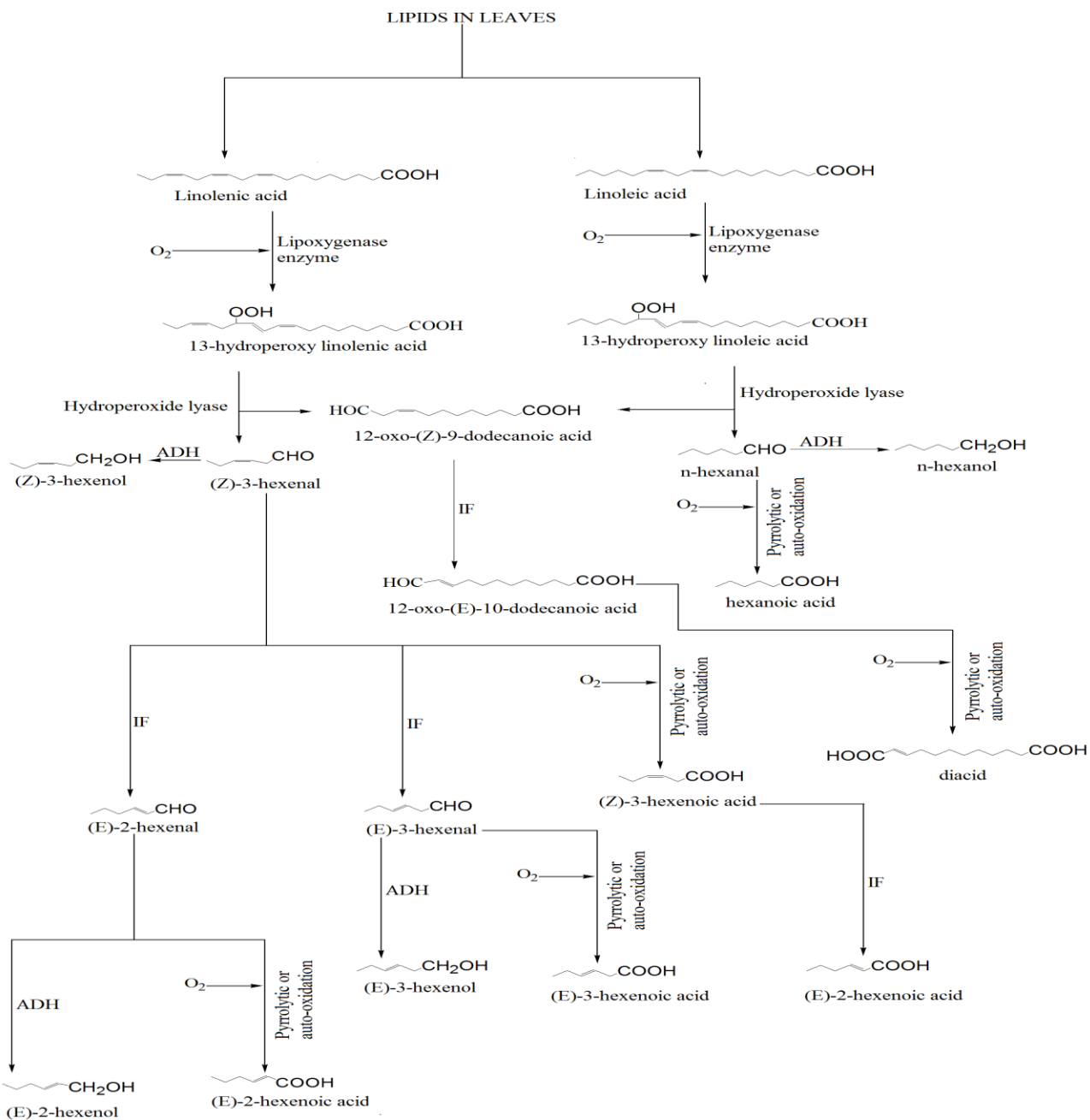
ADH=Alcohol dehydrogenase
IF=Isomerization factor

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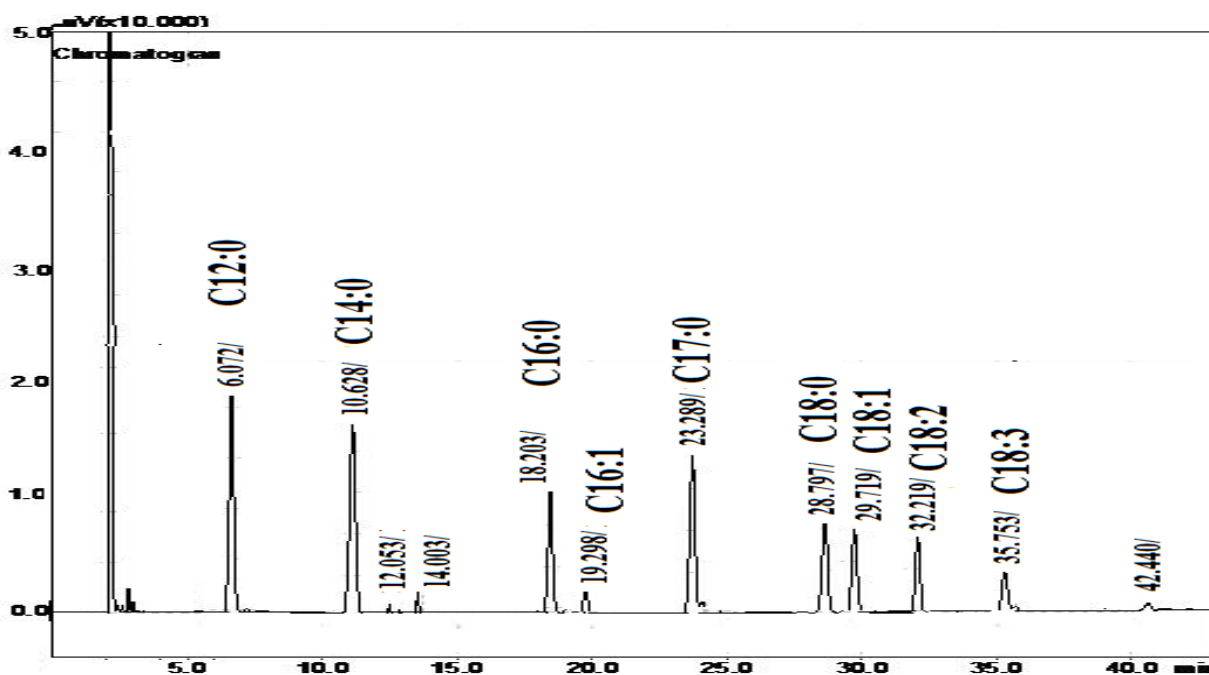
Peak#	Ret.Time	Area	Height
1	6.072	751700	146648
2	10.628	1016150	125174

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
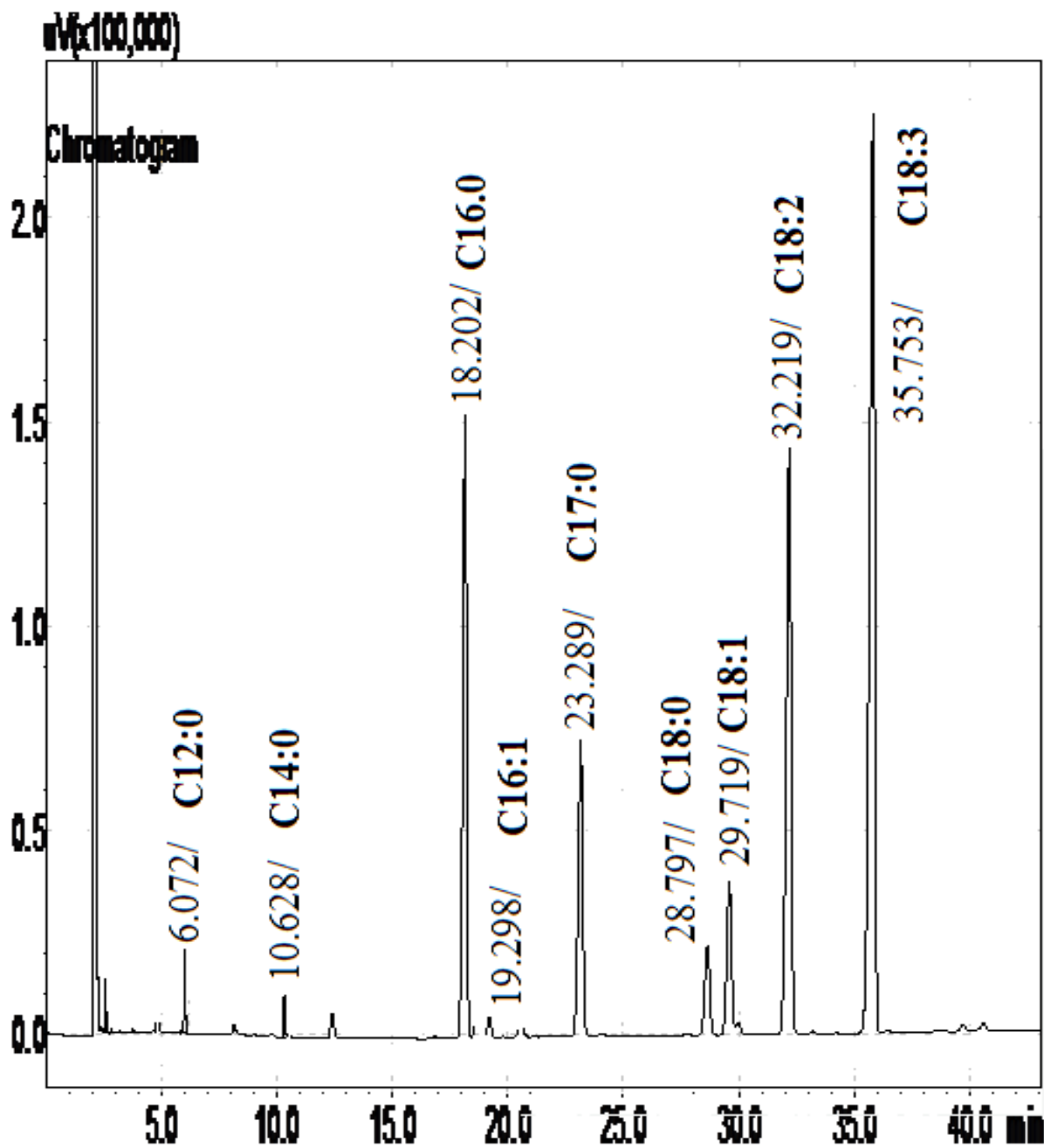
Figure 3: Chromatogram of a typical sample analyte

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 rate of fertilizer (Tables 6 7 and 8) do not produce similar effects in all locations. Thus 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	0	75	150	225	300	Mean location
				Nitrogen rates (kg N/ha/year)			
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 ≤ 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 ≤ 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 ≤ 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 (C18:1) acid the order was Changoi > Timbilil >Kipkebe > Karirana > Sotik Highlands. And for palmitoleic (C16:1) acid 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 ≤ 0.05			0.090			0.090
	Interaction, P ≤ 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 ≤ 0.05			0.263			0.263
	Interaction, P ≤ 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 ≤ 0.05			0.601			0.601
	Interaction P ≤ 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 ≤ 0.05			0.804			0.804
	Interaction, P ≤ 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 nitrogen 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 nitrogen at any location (Tables 6 7 and 8), reinforcing the earlier observations made in earlier studies (Muritu,

1989; Owuor *et al*., 1990a) that increase of fertilizer 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, 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 is the same in all locations i.e. increase of fatty acids with nitrogen rates, the rate of this response is not the constant for all locations (Tables 6, 7 and 8). Indeed a constant increase in fertilizer does not produce equal responses in all locations. This implies that other environmental factors influence the formation of fatty acids besides fertilizer. Therefore, it is not possible to produce tea leaves with same levels of fatty acids in different locations under similar fertilizer rate. To produce tea with similar levels of fatty acids in different locations would require different optimal 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 adopt use of fertilizer rates for a clone that is a compromise between yields and quality for a particular area, hence, 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

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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

				Locations			
Item	Plucking frequency (days)	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, $P \leq 0.05$			0.254			0.3049
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			0.192			0.229
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			0.435			0.5227
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			0.289			0.347
	Interaction, $P \leq 0.05$			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 variation observed here is 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 is 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 level 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 acid levels 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	Karirana	Timbilil	Changoi	Sotik	Kipkebe	Mean plucking
				locations			
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 ≤ 0.05			2.063			2.477
	Interaction, P ≤ 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 quantity.

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)					Mean plucking
		0	75	150	225	300	
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, $P \leq 0.05$			0.090			0.108
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			0.264			0.316
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			0.601			0.722
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			0.804			0.966
	Interaction, $P \leq 0.05$			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, $P \leq 0.05$			1.092			1.311
	Interaction, $P \leq 0.05$			1.572			

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

Item	Plucking frequency (days)	Nitrogen rates (Kg N/ha/year)					Mean plucking
		0	75	150	225	300	
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 may 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 may be subjecting 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 long plucking intervals irrespective of location, implying that long plucking intervals and high nitrogenous fertilizer rates increase the level of aroma precursor compounds hence may reduce aroma quality of black tea. The extent and level vary with locations meaning it may not be possible to make black teas of same aroma quality from different regions.

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 acid that would be deleterious to aroma quality of tea.
3. Further work need to be done to establish fertilizer-plucking interval combination that would optimize aroma quality of black tea.

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APPENDICES

7.1: Appendix 1: Assigned treatments to plots

Plot treatment allocation on randomisation (for all the 5 locations)

TREATMENTS	PLOT NO
$N_{300}Pf_7$	Plot 1, Plot 24, Plot 39
$N_{300}Pf_{14}$	Plot 8, Plot 22, Plot 38
$N_{300}Pf_{21}$	Plot 2, Plot 10, Plot 25
$N_{225}Pf_7$	Plot 11, Plot 21, Plot 35
$N_{225}Pf_{14}$	Plot 4, Plot 19, Plot 31
$N_{225}Pf_{21}$	Plot 7, Plot 20, Plot 33
$N_{150}Pf_7$	Plot 9, Plot 26, Plot 42
$N_{150}Pf_{14}$	Plot 12, Plot 23, Plot 37
$N_{150}Pf_{21}$	Plot 18, Plot 3, Plot 34
$N_{75}Pf_7$	Plot 15, Plot 27, Plot 40
$N_{75}Pf_{14}$	Plot 6, Plot 17, Plot 32
$N_{75}Pf_{21}$	Plot 13, Plot 44, Plot 30
N_0Pf_7	Plot 5, Plot 28, Plot 43
N_0Pf_{14}	Plot 14, Plot 29, Plot 45
N_0Pf_{21}	Plot 16, Plot 36, Plot 41

Source: Kamau *et al.*, 1997.

* NxPfy = Nitrogen rates and corresponding plucking frequencies

Nx = Nitrogen fertilizer rates = $0, 75, 150, 225, 300 \frac{Kg}{Ha}$/year

Pfy = Plucking frequency = $7, 14, 21$ days

7.2: Appendix 2: Table of statistical results for all the variables

```
Data file: FATTYACIDS
    Title:      Location, nitrogen rates, plucking intervals on fatty acids
    Function: FACTOR
    Experiment Model Number 10:
        Three Factor Randomized Complete Block Design

    Data case no. 1 to 225.
    Factorial ANOVA for the factors:
        Replication (Var 1: Replication) with values from 1 to 3
        Factor A (Var 2: Location (1=Kari, 2=Timb, 3=Chang, 4=Sotik H,
5=Kipkebe)) with values from 1 to 5
        Factor B (Var 3: N Rates (1=0, 2=75, 3=150, 4=225,
5=300kgN/ha/year)) with values from 1 to 5
        Factor C (Var 4: Plucking intervals (1=7, 2=14, 3=21days)) with
values from 1 to 3


    Variable 5: C12:0
    Grand Mean = 1.909   Grand Sum = 429.584   Total Count = 225
                 T A B L E   O F   M E A N S
     1   2   3   4             5            Total
    -------------------------------------------------------------
     1   *   *   *           2.031          152.346
     2   *   *   *           1.864          139.825
     3   *   *   *           1.832          137.413
    -------------------------------------------------------------
     *   1   *   *           1.625           73.126
     *   2   *   *           0.846           38.052
     *   3   *   *           2.333          104.970
     *   4   *   *           1.962           88.311
     *   5   *   *           2.781          125.126
    -------------------------------------------------------------
     *   *   1   *           0.240           10.807
     *   *   2   *           0.511           22.982
     *   *   3   *           1.153           51.893
     *   *   4   *           2.330          104.860
     *   *   5   *           5.312          239.042
    -------------------------------------------------------------
     *   1   1   *           0.146            1.315
     *   1   2   *           0.395            3.555
     *   1   3   *           0.905            8.141
     *   1   4   *           2.052           18.467
     *   1   5   *           4.627           41.647
     *   2   1   *           0.219            1.972
     *   2   2   *           0.388            3.495
     *   2   3   *           0.503            4.530
     *   2   4   *           0.670            6.026
     *   2   5   *           2.448           22.030
     *   3   1   *           0.558            5.025
     *   3   2   *           1.041            9.369
     *   3   3   *           2.185           19.662
     *   3   4   *           3.355           30.198
     *   3   5   *           4.524           40.716
```

```
*   4   1   *        0.234            2.105
*   4   2   *        0.650            5.851
*   4   3   *        1.313           11.813
*   4   4   *        2.463           22.169
*   4   5   *        5.153           46.373
*   5   1   *        0.043            0.391
*   5   2   *        0.079            0.712
*   5   3   *        0.861            7.747
*   5   4   *        3.111           27.999
*   5   5   *        9.809           88.277
----------------------------------------------------------------
*   *   *   1        1.378          103.358
*   *   *   2        1.843          138.224
*   *   *   3        2.507          188.002
----------------------------------------------------------------
*   1   *   1        1.170           17.548
*   1   *   2        1.637           24.552
*   1   *   3        2.068           31.027
*   2   *   1        0.470            7.050
*   2   *   2        0.777           11.656
*   2   *   3        1.290           19.345
*   3   *   1        1.960           29.393
*   3   *   2        2.339           35.087
*   3   *   3        2.699           40.489
*   4   *   1        1.383           20.745
*   4   *   2        1.902           28.526
*   4   *   3        2.603           39.039
*   5   *   1        1.908           28.622
*   5   *   2        2.560           38.403
*   5   *   3        3.873           58.101
----------------------------------------------------------------
*   *   1   1        0.121            1.817
*   *   1   2        0.262            3.926
*   *   1   3        0.338            5.064
*   *   2   1        0.393            5.900
*   *   2   2        0.541            8.110
*   *   2   3        0.598            8.971
*   *   3   1        0.799           11.986
*   *   3   2        1.076           16.134
*   *   3   3        1.585           23.773
*   *   4   1        1.942           29.125
*   *   4   2        2.243           33.640
*   *   4   3        2.806           42.095
*   *   5   1        3.635           54.529
*   *   5   2        5.094           76.414
*   *   5   3        7.207          108.099
----------------------------------------------------------------
*   1   1   1        0.114            0.341
*   1   1   2        0.150            0.451
*   1   1   3        0.174            0.523
*   1   2   1        0.242            0.726
*   1   2   2        0.456            1.368
*   1   2   3        0.487            1.462
*   1   3   1        0.607            1.821
*   1   3   2        0.683            2.050
*   1   3   3        1.423            4.270
*   1   4   1        1.769            5.307
```

*	1	4	2	2.010	6.031
*	1	4	3	2.376	7.129
*	1	5	1	3.118	9.353
*	1	5	2	4.884	14.651
*	1	5	3	5.881	17.643
*	2	1	1	0.151	0.452
*	2	1	2	0.237	0.710
*	2	1	3	0.270	0.810
*	2	2	1	0.318	0.955
*	2	2	2	0.393	1.178
*	2	2	3	0.454	1.361
*	2	3	1	0.471	1.412
*	2	3	2	0.481	1.443
*	2	3	3	0.558	1.674
*	2	4	1	0.626	1.879
*	2	4	2	0.663	1.990
*	2	4	3	0.719	2.157
*	2	5	1	0.784	2.352
*	2	5	2	2.112	6.335
*	2	5	3	4.448	13.343
*	3	1	1	0.170	0.509
*	3	1	2	0.661	1.984
*	3	1	3	0.844	2.531
*	3	2	1	0.928	2.784
*	3	2	2	1.042	3.127
*	3	2	3	1.153	3.459
*	3	3	1	1.740	5.221
*	3	3	2	2.112	6.337
*	3	3	3	2.701	8.104
*	3	4	1	2.894	8.681
*	3	4	2	3.301	9.904
*	3	4	3	3.871	11.613
*	3	5	1	4.066	12.198
*	3	5	2	4.579	13.736
*	3	5	3	4.927	14.782
*	4	1	1	0.147	0.440
*	4	1	2	0.216	0.649
*	4	1	3	0.339	1.016
*	4	2	1	0.415	1.245
*	4	2	2	0.739	2.217
*	4	2	3	0.796	2.389
*	4	3	1	1.023	3.070
*	4	3	2	1.286	3.857
*	4	3	3	1.629	4.886
*	4	4	1	2.099	6.298
*	4	4	2	2.429	7.286
*	4	4	3	2.862	8.585
*	4	5	1	3.231	9.693
*	4	5	2	4.839	14.517
*	4	5	3	7.388	22.163
*	5	1	1	0.025	0.076
*	5	1	2	0.044	0.132
*	5	1	3	0.061	0.183
*	5	2	1	0.063	0.190
*	5	2	2	0.073	0.220
*	5	2	3	0.100	0.301
*	5	3	1	0.154	0.463

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```
           *    5    3    2              0.815            2.446
           *    5    3    3              1.613            4.838
           *    5    4    1              2.320            6.960
           *    5    4    2              2.809            8.428
           *    5    4    3              4.204           12.611
           *    5    5    1              6.978           20.933
           *    5    5    2              9.059           27.176
           *    5    5    3             13.389           40.168
          ----------------------------------------------------------
           A N A L Y S I S   O F   V A R I A N C E   T A B L E
     K                    Degrees of   Sum of        Mean            F
   Value      Source       Freedom     Squares       Square        Value       Prob
          ----------------------------------------------------------
     1     Replication       2          1.714        0.857         4.5472     0.0121
     2     Factor A          4         96.906       24.227       128.5667     0.0000
     4     Factor B          4        768.137      192.034      1019.0994     0.0000
     6     AB               16        230.470       14.404        76.4420     0.0000
     8     Factor C          2         48.259       24.129       128.0506     0.0000
    10     AC                8          8.351        1.044         5.5396     0.0000
    12     BC                8         59.708        7.463        39.6076     0.0000
    14     ABC              32         24.062        0.752         3.9904     0.0000
   -15     Error           148         27.888        0.188
          ----------------------------------------------------------
           Total           224       1265.495
          ----------------------------------------------------------
     Coefficient of Variation: 22.74%
     s_  for means group 1:     0.0501      Number of Observations: 75
      y
     s_  for means group 2:     0.0647      Number of Observations: 45
      y
     s_  for means group 4:     0.0647      Number of Observations: 45
      y
     s_  for means group 6:     0.1447      Number of Observations: 9
      y
     s_  for means group 8:     0.0501      Number of Observations: 75
      y
     s_  for means group 10:    0.1121      Number of Observations: 15
      y
     s_  for means group 12:    0.1121      Number of Observations: 15
      y
     s_  for means group 14:    0.2506      Number of Observations: 3
      y
   =======================================================================
     Variable 6: C14:0
     Grand Mean = 2.506   Grand Sum = 563.903   Total Count = 225
                  T A B L E   O F   M E A N S
      1   2   3   4             6              Total
          ----------------------------------------------------------
      1   *   *   *           2.627           197.020
      2   *   *   *           2.481           186.100
      3   *   *   *           2.410           180.783
          ----------------------------------------------------------
      *   1   *   *           2.679           120.575
      *   2   *   *           0.937            42.150
      *   3   *   *           3.485           156.803
      *   4   *   *           2.130            95.846
      *   5   *   *           3.301           148.530
```

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------------------------------------------------------------------
    *      *      1      *           0.287              12.900
    *      *      2      *           0.924              41.577
    *      *      3      *           1.797              80.874
    *      *      4      *           3.504             157.658
    *      *      5      *           6.020             270.894
------------------------------------------------------------------
    *      1      1      *           0.300               2.698
    *      1      2      *           0.993               8.935
    *      1      3      *           1.923              17.311
    *      1      4      *           3.793              34.133
    *      1      5      *           6.389              57.497
    *      2      1      *           0.288               2.593
    *      2      2      *           0.523               4.709
    *      2      3      *           0.696               6.263
    *      2      4      *           1.154              10.386
    *      2      5      *           2.022              18.199
    *      3      1      *           0.256               2.307
    *      3      2      *           0.947               8.519
    *      3      3      *           2.165              19.488
    *      3      4      *           4.897              44.070
    *      3      5      *           9.158              82.419
    *      4      1      *           0.352               3.169
    *      4      2      *           0.868               7.811
    *      4      3      *           1.381              12.433
    *      4      4      *           2.696              24.260
    *      4      5      *           5.353              48.173
    *      5      1      *           0.237               2.133
    *      5      2      *           1.289              11.604
    *      5      3      *           2.820              25.379
    *      5      4      *           4.979              44.810
    *      5      5      *           7.178              64.605
------------------------------------------------------------------
    *      *      *      1           1.972             147.870
    *      *      *      2           2.481             186.069
    *      *      *      3           3.066             229.965
------------------------------------------------------------------
    *      1      *      1           2.025              30.372
    *      1      *      2           2.738              41.071
    *      1      *      3           3.275              49.132
    *      2      *      1           0.773              11.589
    *      2      *      2           0.957              14.360
    *      2      *      3           1.080              16.201
    *      3      *      1           2.650              39.757
    *      3      *      2           3.509              52.637
    *      3      *      3           4.294              64.408
    *      4      *      1           1.583              23.745
    *      4      *      2           1.924              28.859
    *      4      *      3           2.883              43.241
    *      5      *      1           2.827              42.406
    *      5      *      2           3.276              49.141
    *      5      *      3           3.799              56.982
------------------------------------------------------------------
    *      *      1      1           0.111               1.662
    *      *      1      2           0.229               3.432
    *      *      1      3           0.520               7.806
    *      *      2      1           0.715              10.729
```

*	*	2	2	0.887	13.312
*	*	2	3	1.169	17.536
*	*	3	1	1.471	22.064
*	*	3	2	1.779	26.692
*	*	3	3	2.141	32.118
*	*	4	1	2.782	41.734
*	*	4	2	3.581	53.710
*	*	4	3	4.148	62.215
*	*	5	1	4.779	71.681
*	*	5	2	5.928	88.923
*	*	5	3	7.353	110.290

---

*	1	1	1	0.065	0.194
*	1	1	2	0.156	0.468
*	1	1	3	0.679	2.036
*	1	2	1	0.848	2.543
*	1	2	2	1.012	3.035
*	1	2	3	1.119	3.356
*	1	3	1	1.496	4.488
*	1	3	2	1.998	5.994
*	1	3	3	2.276	6.829
*	1	4	1	2.873	8.619
*	1	4	2	3.914	11.743
*	1	4	3	4.590	13.771
*	1	5	1	4.842	14.526
*	1	5	2	6.610	19.831
*	1	5	3	7.713	23.140
*	2	1	1	0.177	0.532
*	2	1	2	0.292	0.877
*	2	1	3	0.395	1.184
*	2	2	1	0.440	1.319
*	2	2	2	0.524	1.573
*	2	2	3	0.606	1.817
*	2	3	1	0.628	1.883
*	2	3	2	0.641	1.922
*	2	3	3	0.819	2.458
*	2	4	1	1.066	3.197
*	2	4	2	1.149	3.446
*	2	4	3	1.248	3.743
*	2	5	1	1.553	4.658
*	2	5	2	2.181	6.542
*	2	5	3	2.333	6.999
*	3	1	1	0.069	0.208
*	3	1	2	0.220	0.661
*	3	1	3	0.479	1.438
*	3	2	1	0.598	1.793
*	3	2	2	1.086	3.258
*	3	2	3	1.156	3.468
*	3	3	1	1.621	4.862
*	3	3	2	2.287	6.860
*	3	3	3	2.589	7.766
*	3	4	1	3.533	10.598
*	3	4	2	4.979	14.937
*	3	4	3	6.178	18.535
*	3	5	1	7.432	22.296
*	3	5	2	8.974	26.922
*	3	5	3	11.067	33.202

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```
*   4   1   1              0.124                 0.371
*   4   1   2              0.336                 1.009
*   4   1   3              0.596                 1.789
*   4   2   1              0.745                 2.236
*   4   2   2              0.821                 2.464
*   4   2   3              1.037                 3.110
*   4   3   1              1.173                 3.520
*   4   3   2              1.335                 4.004
*   4   3   3              1.636                 4.909
*   4   4   1              2.229                 6.688
*   4   4   2              2.729                 8.187
*   4   4   3              3.128                 9.384
*   4   5   1              3.643                10.929
*   4   5   2              4.398                13.194
*   4   5   3              8.016                24.049
*   5   1   1              0.119                 0.357
*   5   1   2              0.139                 0.417
*   5   1   3              0.453                 1.359
*   5   2   1              0.946                 2.837
*   5   2   2              0.994                 2.982
*   5   2   3              1.928                 5.785
*   5   3   1              2.437                 7.310
*   5   3   2              2.638                 7.913
*   5   3   3              3.385                10.156
*   5   4   1              4.210                12.631
*   5   4   2              5.132                15.397
*   5   4   3              5.594                16.782
*   5   5   1              6.424                19.271
*   5   5   2              7.478                22.434
*   5   5   3              7.633                22.900
```

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	1.828	0.914	8.6077	0.0003
2	Factor A	4	190.049	47.512	447.5664	0.0000
4	Factor B	4	957.288	239.322	2254.4241	0.0000
6	AB	16	179.116	11.195	105.4551	0.0000
8	Factor C	2	45.003	22.501	211.9632	0.0000
10	AC	8	8.514	1.064	10.0248	0.0000
12	BC	8	25.276	3.160	29.7631	0.0000
14	ABC	32	17.973	0.562	5.2910	0.0000
-15	Error	148	15.711	0.106		
	Total	224	1440.758			

```
Coefficient of Variation: 13.00%
s_  for means group 1:     0.0376      Number of Observations: 75
 y
s_  for means group 2:     0.0486      Number of Observations: 45
 y
s_  for means group 4:     0.0486      Number of Observations: 45
 y
s_  for means group 6:     0.1086      Number of Observations: 9
 y
s_  for means group 8:     0.0376      Number of Observations: 75
```

```
 y
s_ for means group 10:      0.0841      Number of Observations: 15
 y
s_ for means group 12:      0.0841      Number of Observations: 15
 y
s_ for means group 14:      0.1881      Number of Observations: 3
 y
==============================================================================

    Variable 7: C16:0
    Grand Mean = 17.077   Grand Sum = 3842.431   Total Count = 225
                 T A B L E   O F   M E A N S
     1   2   3   4              7            Total
    -----------------------------------------------------------------
     1   *   *   *           17.399         1304.932
     2   *   *   *           16.888         1266.632
     3   *   *   *           16.945         1270.866
    -----------------------------------------------------------------
     *   1   *   *           17.941          807.358
     *   2   *   *           18.509          832.883
     *   3   *   *           19.929          896.789
     *   4   *   *           12.988          584.464
     *   5   *   *           16.021          720.937
    -----------------------------------------------------------------
     *   *   1   *            8.130          365.831
     *   *   2   *           12.070          543.153
     *   *   3   *           15.413          693.581
     *   *   4   *           21.196          953.819
     *   *   5   *           28.579         1286.046
    -----------------------------------------------------------------
     *   1   1   *            9.936           89.426
     *   1   2   *           12.625          113.625
     *   1   3   *           15.617          140.552
     *   1   4   *           21.186          190.675
     *   1   5   *           30.342          273.080
     *   2   1   *            7.910           71.192
     *   2   2   *           14.788          133.093
     *   2   3   *           17.991          161.916
     *   2   4   *           24.318          218.865
     *   2   5   *           27.535          247.817
     *   3   1   *            6.271           56.443
     *   3   2   *            9.242           83.179
     *   3   3   *           14.689          132.197
     *   3   4   *           26.278          236.502
     *   3   5   *           43.163          388.467
     *   4   1   *            4.243           38.184
     *   4   2   *            9.676           87.082
     *   4   3   *           13.147          118.322
     *   4   4   *           16.792          151.132
     *   4   5   *           21.083          189.744
     *   5   1   *           12.287          110.586
     *   5   2   *           14.019          126.174
     *   5   3   *           15.622          140.594
     *   5   4   *           17.405          156.646
     *   5   5   *           20.771          186.938
    -----------------------------------------------------------------
     *   *   *   1           15.222         1141.656
```

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```
*     *     *     2          16.853              1264.007
*     *     *     3          19.157              1436.767
-----------------------------------------------------------------
*     1     *     1          16.026               240.391
*     1     *     2          18.026               270.395
*     1     *     3          19.771               296.572
*     2     *     1          16.812               252.176
*     2     *     2          18.170               272.543
*     2     *     3          20.544               308.164
*     3     *     1          16.652               249.787
*     3     *     2          18.977               284.654
*     3     *     3          24.157               362.348
*     4     *     1          11.307               169.599
*     4     *     2          13.172               197.586
*     4     *     3          14.485               217.279
*     5     *     1          15.314               229.704
*     5     *     2          15.922               238.828
*     5     *     3          16.827               252.405
-----------------------------------------------------------------
*     *     1     1           5.936                89.045
*     *     1     2           8.098               121.469
*     *     1     3          10.354               155.316
*     *     2     1          11.055               165.826
*     *     2     2          11.999               179.984
*     *     2     3          13.156               197.343
*     *     3     1          14.205               213.081
*     *     3     2          14.926               223.897
*     *     3     3          17.107               256.603
*     *     4     1          19.324               289.866
*     *     4     2          21.159               317.378
*     *     4     3          23.105               346.576
*     *     5     1          25.589               383.838
*     *     5     2          28.085               421.278
*     *     5     3          32.062               480.930
-----------------------------------------------------------------
*     1     1     1           7.227                21.682
*     1     1     2          10.406                31.217
*     1     1     3          12.176                36.527
*     1     2     1          12.305                36.914
*     1     2     2          12.602                37.805
*     1     2     3          12.969                38.906
*     1     3     1          14.086                42.259
*     1     3     2          15.149                45.448
*     1     3     3          17.615                52.845
*     1     4     1          19.545                58.636
*     1     4     2          21.740                65.220
*     1     4     3          22.273                66.819
*     1     5     1          26.966                80.899
*     1     5     2          30.235                90.705
*     1     5     3          33.825               101.475
*     2     1     1           3.695                11.084
*     2     1     2           7.396                22.189
*     2     1     3          12.640                37.920
*     2     2     1          14.047                42.141
*     2     2     2          14.253                42.758
*     2     2     3          16.065                48.194
*     2     3     1          16.214                48.642
```

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*	2	3	2	16.591	49.773
*	2	3	3	21.167	63.501
*	2	4	1	23.966	71.897
*	2	4	2	24.412	73.235
*	2	4	3	24.577	73.732
*	2	5	1	26.137	78.412
*	2	5	2	28.196	84.588
*	2	5	3	28.272	84.817
*	3	1	1	5.274	15.822
*	3	1	2	6.283	18.848
*	3	1	3	7.258	21.773
*	3	2	1	7.635	22.905
*	3	2	2	9.033	27.098
*	3	2	3	11.059	33.177
*	3	3	1	13.226	39.677
*	3	3	2	13.983	41.950
*	3	3	3	16.857	50.571
*	3	4	1	20.952	62.856
*	3	4	2	25.635	76.904
*	3	4	3	32.247	96.741
*	3	5	1	36.176	108.527
*	3	5	2	39.951	119.854
*	3	5	3	53.362	160.086
*	4	1	1	1.778	5.333
*	4	1	2	4.499	13.497
*	4	1	3	6.451	19.353
*	4	2	1	7.929	23.787
*	4	2	2	10.092	30.276
*	4	2	3	11.006	33.018
*	4	3	1	12.146	36.439
*	4	3	2	13.296	39.888
*	4	3	3	13.998	41.994
*	4	4	1	15.580	46.741
*	4	4	2	16.630	49.891
*	4	4	3	18.167	54.501
*	4	5	1	19.099	57.297
*	4	5	2	21.344	64.033
*	4	5	3	22.804	68.413
*	5	1	1	11.708	35.124
*	5	1	2	11.906	35.718
*	5	1	3	13.248	39.743
*	5	2	1	13.359	40.078
*	5	2	2	14.016	42.047
*	5	2	3	14.683	44.048
*	5	3	1	15.354	46.063
*	5	3	2	15.613	46.839
*	5	3	3	15.897	47.692
*	5	4	1	16.579	49.736
*	5	4	2	17.376	52.127
*	5	4	3	18.261	54.783
*	5	5	1	19.567	58.702
*	5	5	2	20.699	62.098
*	5	5	3	22.046	66.138

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```
                ------------------------------------------------------------
                    A N A L Y S I S   O F   V A R I A N C E   T A B L E
     K                        Degrees of   Sum of          Mean          F
  Value       Source          Freedom      Squares        Square       Value      Prob
                ------------------------------------------------------------
     1     Replication            2        11.757          5.879       10.6280    0.0000
     2     Factor A               4      1294.328        323.582      585.0050    0.0000
     4     Factor B               4     11571.867       2892.967     5230.2065    0.0000
     6     AB                    16      3027.163        189.198      342.0513    0.0000
     8     Factor C               2       586.250        293.125      529.9422    0.0000
    10     AC                     8       162.849         20.356       36.8020    0.0000
    12     BC                     8        88.794         11.099       20.0663    0.0000
    14     ABC                   32       320.437         10.014       18.1037    0.0000
   -15     Error                148        81.863          0.553
                ------------------------------------------------------------
           Total                224     17145.309
                ------------------------------------------------------------
       Coefficient of Variation: 4.36%
       s_ for means group 1:      0.0859      Number of Observations: 75
         y
       s_ for means group 2:      0.1109      Number of Observations: 45
         y
       s_ for means group 4:      0.1109      Number of Observations: 45
         y
       s_ for means group 6:      0.2479      Number of Observations: 9
         y
       s_ for means group 8:      0.0859      Number of Observations: 75
         y
       s_ for means group 10:     0.1920      Number of Observations: 15
         y
       s_ for means group 12:     0.1920      Number of Observations: 15
         y
       s_ for means group 14:     0.4294      Number of Observations: 3
         y
===============================================================================

       Variable 8: C16:1
       Grand Mean = 0.647   Grand Sum = 145.531   Total Count = 225
                   T A B L E   O F   M E A N S
        1   2   3   4               8              Total
       ------------------------------------------------------------
        1   *   *   *             0.684            51.315
        2   *   *   *             0.613            45.991
        3   *   *   *             0.643            48.225
       ------------------------------------------------------------
        *   1   *   *             1.033            46.491
        *   2   *   *             0.385            17.343
        *   3   *   *             0.515            23.197
        *   4   *   *             0.844            37.994
        *   5   *   *             0.456            20.505
       ------------------------------------------------------------
        *   *   1   *             0.117             5.261
        *   *   2   *             0.278            12.519
        *   *   3   *             0.441            19.834
        *   *   4   *             0.648            29.149
```

```
   *    *    5    *              1.750          78.769
----------------------------------------------------------------
   *    1    1    *              0.210           1.893
   *    1    2    *              0.327           2.941
   *    1    3    *              0.531           4.775
   *    1    4    *              0.990           8.914
   *    1    5    *              3.107          27.967
   *    2    1    *              0.108           0.975
   *    2    2    *              0.233           2.096
   *    2    3    *              0.311           2.799
   *    2    4    *              0.458           4.118
   *    2    5    *              0.817           7.354
   *    3    1    *              0.098           0.878
   *    3    2    *              0.283           2.545
   *    3    3    *              0.432           3.886
   *    3    4    *              0.551           4.956
   *    3    5    *              1.215          10.932
   *    4    1    *              0.089           0.798
   *    4    2    *              0.379           3.409
   *    4    3    *              0.616           5.545
   *    4    4    *              0.762           6.860
   *    4    5    *              2.376          21.382
   *    5    1    *              0.080           0.716
   *    5    2    *              0.170           1.528
   *    5    3    *              0.314           2.828
   *    5    4    *              0.478           4.301
   *    5    5    *              1.237          11.133
----------------------------------------------------------------
   *    *    *    1              0.442          33.182
   *    *    *    2              0.654          49.031
   *    *    *    3              0.844          63.318
----------------------------------------------------------------
   *    1    *    1              0.673          10.099
   *    1    *    2              1.032          15.476
   *    1    *    3              1.394          20.916
   *    2    *    1              0.311           4.671
   *    2    *    2              0.383           5.744
   *    2    *    3              0.462           6.928
   *    3    *    1              0.370           5.543
   *    3    *    2              0.497           7.448
   *    3    *    3              0.680          10.207
   *    4    *    1              0.528           7.927
   *    4    *    2              0.913          13.694
   *    4    *    3              1.092          16.374
   *    5    *    1              0.330           4.943
   *    5    *    2              0.445           6.669
   *    5    *    3              0.593           8.894
----------------------------------------------------------------
   *    *    1    1              0.071           1.071
   *    *    1    2              0.119           1.782
   *    *    1    3              0.161           2.408
   *    *    2    1              0.217           3.256
   *    *    2    2              0.274           4.110
   *    *    2    3              0.344           5.153
   *    *    3    1              0.392           5.887
   *    *    3    2              0.441           6.608
   *    *    3    3              0.489           7.338
```

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*   *   4   1              0.553              8.291
*   *   4   2              0.643              9.641
*   *   4   3              0.748             11.217
*   *   5   1              0.978             14.676
*   *   5   2              1.793             26.890
*   *   5   3              2.480             37.202
-----------------------------------------------------------------
*   1   1   1              0.127              0.382
*   1   1   2              0.231              0.692
*   1   1   3              0.273              0.820
*   1   2   1              0.288              0.863
*   1   2   2              0.319              0.956
*   1   2   3              0.374              1.122
*   1   3   1              0.437              1.310
*   1   3   2              0.526              1.579
*   1   3   3              0.629              1.886
*   1   4   1              0.786              2.358
*   1   4   2              0.984              2.952
*   1   4   3              1.201              3.604
*   1   5   1              1.729              5.186
*   1   5   2              3.099              9.297
*   1   5   3              4.495             13.484
*   2   1   1              0.072              0.216
*   2   1   2              0.111              0.332
*   2   1   3              0.142              0.426
*   2   2   1              0.182              0.547
*   2   2   2              0.234              0.701
*   2   2   3              0.283              0.848
*   2   3   1              0.295              0.886
*   2   3   2              0.305              0.915
*   2   3   3              0.333              0.998
*   2   4   1              0.395              1.185
*   2   4   2              0.463              1.389
*   2   4   3              0.515              1.544
*   2   5   1              0.612              1.836
*   2   5   2              0.802              2.407
*   2   5   3              1.037              3.112
*   3   1   1              0.048              0.145
*   3   1   2              0.092              0.275
*   3   1   3              0.153              0.458
*   3   2   1              0.219              0.656
*   3   2   2              0.278              0.835
*   3   2   3              0.352              1.055
*   3   3   1              0.400              1.200
*   3   3   2              0.443              1.328
*   3   3   3              0.453              1.358
*   3   4   1              0.468              1.405
*   3   4   2              0.561              1.683
*   3   4   3              0.623              1.868
*   3   5   1              0.713              2.138
*   3   5   2              1.109              3.327
*   3   5   3              1.823              5.468
*   4   1   1              0.049              0.148
*   4   1   2              0.085              0.255
*   4   1   3              0.132              0.396
*   4   2   1              0.266              0.799
*   4   2   2              0.368              1.104
```

```
*    4    2    3                    0.502                  1.506
*    4    3    1                    0.571                  1.713
*    4    3    2                    0.610                  1.830
*    4    3    3                    0.667                  2.002
*    4    4    1                    0.727                  2.180
*    4    4    2                    0.761                  2.283
*    4    4    3                    0.799                  2.396
*    4    5    1                    1.029                  3.087
*    4    5    2                    2.741                  8.222
*    4    5    3                    3.358                 10.074
*    5    1    1                    0.060                  0.180
*    5    1    2                    0.076                  0.228
*    5    1    3                    0.103                  0.308
*    5    2    1                    0.130                  0.391
*    5    2    2                    0.171                  0.514
*    5    2    3                    0.208                  0.623
*    5    3    1                    0.259                  0.778
*    5    3    2                    0.318                  0.955
*    5    3    3                    0.365                  1.094
*    5    4    1                    0.388                  1.164
*    5    4    2                    0.444                  1.333
*    5    4    3                    0.601                  1.804
*    5    5    1                    0.810                  2.430
*    5    5    2                    1.213                  3.638
*    5    5    3                    1.688                  5.065
```

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	0.191	0.095	4.0492	0.0194
2	Factor A	4	13.967	3.492	148.3653	0.0000
4	Factor B	4	75.469	18.867	801.6764	0.0000
6	AB	16	21.752	1.360	57.7658	0.0000
8	Factor C	2	6.060	3.030	128.7444	0.0000
10	AC	8	1.751	0.219	9.2998	0.0000
12	BC	8	11.430	1.429	60.7092	0.0000
14	ABC	32	5.009	0.157	6.6505	0.0000
-15	Error	148	3.483	0.024		

| | Total | 224 | 139.112 | | | |

```
Coefficient of Variation: 23.72%
s_ for means group 1:     0.0177     Number of Observations: 75
 y
s_ for means group 2:     0.0229     Number of Observations: 45
 y
s_ for means group 4:     0.0229     Number of Observations: 45
 y
s_ for means group 6:     0.0511     Number of Observations: 9
 y
s_ for means group 8:     0.0177     Number of Observations: 75
 y
s_ for means group 10:    0.0396     Number of Observations: 15
 y
s_ for means group 12:    0.0396     Number of Observations: 15
 y
```

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```
     Variable 9: C18:0
     Grand Mean = 8.230   Grand Sum = 1851.642   Total Count = 225
                     T A B L E   O F   M E A N S
       1   2   3   4            9              Total
     ------------------------------------------------------------
       1   *   *   *          8.447           633.516
       2   *   *   *          8.115           608.623
       3   *   *   *          8.127           609.503
     ------------------------------------------------------------
       *   1   *   *         10.650           479.265
       *   2   *   *          8.470           381.153
       *   3   *   *         10.864           488.876
       *   4   *   *          5.785           260.346
       *   5   *   *          5.378           242.002
     ------------------------------------------------------------
       *   *   1   *          2.800           126.019
       *   *   2   *          4.267           192.025
       *   *   3   *          6.529           293.814
       *   *   4   *          9.984           449.293
       *   *   5   *         17.566           790.491
     ------------------------------------------------------------
       *   1   1   *          3.412            30.704
       *   1   2   *          4.546            40.916
       *   1   3   *          8.179            73.611
       *   1   4   *         14.563           131.067
       *   1   5   *         22.552           202.966
       *   2   1   *          2.738            24.643
       *   2   2   *          4.330            38.971
       *   2   3   *          5.472            49.245
       *   2   4   *          9.415            84.733
       *   2   5   *         20.396           183.560
       *   3   1   *          2.987            26.885
       *   3   2   *          5.084            45.752
       *   3   3   *          9.516            85.642
       *   3   4   *         12.890           116.009
       *   3   5   *         23.843           214.589
       *   4   1   *          1.711            15.401
       *   4   2   *          3.214            28.929
       *   4   3   *          4.647            41.821
       *   4   4   *          6.921            62.286
       *   4   5   *         12.434           111.909
       *   5   1   *          3.154            28.385
       *   5   2   *          4.162            37.457
       *   5   3   *          4.833            43.495
       *   5   4   *          6.133            55.198
       *   5   5   *          8.607            77.467
     ------------------------------------------------------------
       *   *   *   1          6.869           515.196
       *   *   *   2          8.323           624.243
       *   *   *   3          9.496           712.202
     ------------------------------------------------------------
       *   1   *   1          8.869           133.037
       *   1   *   2         10.713           160.689
```

```
*    1    *    3         12.369        185.538
*    2    *    1          6.207         93.111
*    2    *    2          8.966        134.489
*    2    *    3         10.237        153.553
*    3    *    1          9.437        141.558
*    3    *    2         10.702        160.528
*    3    *    3         12.453        186.791
*    4    *    1          5.031         75.458
*    4    *    2          5.830         87.443
*    4    *    3          6.496         97.445
*    5    *    1          4.802         72.033
*    5    *    2          5.406         81.094
*    5    *    3          5.925         88.875
---------------------------------------------------------------
*    *    1    1          2.089         31.331
*    *    1    2          2.859         42.879
*    *    1    3          3.454         51.808
*    *    2    1          3.826         57.386
*    *    2    2          4.212         63.175
*    *    2    3          4.764         71.465
*    *    3    1          5.765         86.473
*    *    3    2          6.451         96.768
*    *    3    3          7.372        110.573
*    *    4    1          8.704        130.559
*    *    4    2          9.761        146.412
*    *    4    3         11.488        172.322
*    *    5    1         13.963        209.448
*    *    5    2         18.334        275.009
*    *    5    3         20.402        306.034
---------------------------------------------------------------
*    1    1    1          2.912          8.735
*    1    1    2          3.238          9.715
*    1    1    3          4.085         12.254
*    1    2    1          4.326         12.979
*    1    2    2          4.345         13.034
*    1    2    3          4.968         14.904
*    1    3    1          6.280         18.839
*    1    3    2          8.191         24.572
*    1    3    3         10.067         30.201
*    1    4    1         12.737         38.211
*    1    4    2         14.985         44.954
*    1    4    3         15.968         47.903
*    1    5    1         18.091         54.274
*    1    5    2         22.805         68.414
*    1    5    3         26.759         80.277
*    2    1    1          1.892          5.677
*    2    1    2          2.950          8.851
*    2    1    3          3.372         10.116
*    2    2    1          4.039         12.118
*    2    2    2          4.288         12.865
*    2    2    3          4.662         13.987
*    2    3    1          4.966         14.898
*    2    3    2          5.273         15.820
*    2    3    3          6.176         18.528
*    2    4    1          8.120         24.360
*    2    4    2          8.958         26.874
*    2    4    3         11.166         33.499
```

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       *    2    5    1              12.019               36.058
       *    2    5    2              23.360               70.079
       *    2    5    3              25.808               77.423
       *    3    1    1               2.253                6.759
       *    3    1    2               2.983                8.950
       *    3    1    3               3.725               11.176
       *    3    2    1               4.334               13.002
       *    3    2    2               5.053               15.159
       *    3    2    3               5.864               17.591
       *    3    3    1               8.777               26.330
       *    3    3    2               9.297               27.891
       *    3    3    3              10.474               31.421
       *    3    4    1              11.192               33.576
       *    3    4    2              12.014               36.043
       *    3    4    3              15.463               46.390
       *    3    5    1              20.630               61.890
       *    3    5    2              24.162               72.486
       *    3    5    3              26.737               80.212
       *    4    1    1               1.131                3.394
       *    4    1    2               1.817                5.452
       *    4    1    3               2.185                6.556
       *    4    2    1               2.388                7.164
       *    4    2    2               3.220                9.661
       *    4    2    3               4.035               12.104
       *    4    3    1               4.319               12.957
       *    4    3    2               4.665               13.996
       *    4    3    3               4.956               14.868
       *    4    4    1               5.912               17.735
       *    4    4    2               6.826               20.479
       *    4    4    3               8.024               24.072
       *    4    5    1              11.403               34.208
       *    4    5    2              12.618               37.855
       *    4    5    3              13.282               39.845
       *    5    1    1               2.255                6.766
       *    5    1    2               3.304                9.912
       *    5    1    3               3.902               11.707
       *    5    2    1               4.041               12.122
       *    5    2    2               4.152               12.457
       *    5    2    3               4.293               12.878
       *    5    3    1               4.483               13.449
       *    5    3    2               4.830               14.490
       *    5    3    3               5.185               15.556
       *    5    4    1               5.559               16.677
       *    5    4    2               6.021               18.063
       *    5    4    3               6.819               20.458
       *    5    5    1               7.673               23.018
       *    5    5    2               8.724               26.173
       *    5    5    3               9.425               28.276
       -----------------------------------------------------------
          A N A L Y S I S   O F   V A R I A N C E   T A B L E
     K                    Degrees of   Sum of         Mean            F
   Value      Source       Freedom     Squares        Square        Value      Prob
   -------------------------------------------------------------------------------
      1     Replication        2         5.320         2.660       10.9198    0.0000
      2     Factor A           4      1213.368       303.342     1245.2368    0.0000
      4     Factor B           4      6224.580      1556.145     6388.0684    0.0000
      6     AB                16      1086.843        67.928      278.8473    0.0000
```

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  8     Factor C          2      259.731      129.865    533.1053   0.0000
 10     AC                8       53.966        6.746     27.6916   0.0000
 12     BC                8      163.972       20.496     84.1392   0.0000
 14     ABC              32      151.529        4.735     19.4386   0.0000
-15     Error           148       36.053        0.244
        -------------------------------------------------------------------
        Total           224     9195.361
        -------------------------------------------------------------------
        Coefficient of Variation: 6.00%
        s_ for means group 1:     0.0570     Number of Observations: 75
         y
        s_ for means group 2:     0.0736     Number of Observations: 45
         y
        s_ for means group 4:     0.0736     Number of Observations: 45
         y
        s_ for means group 6:     0.1645     Number of Observations: 9
         y
        s_ for means group 8:     0.0570     Number of Observations: 75
         y
        s_ for means group 10:    0.1274     Number of Observations: 15
         y
        s_ for means group 12:    0.1274     Number of Observations: 15
         y
        s_ for means group 14:    0.2850     Number of Observations: 3
         y
========================================================================

        Variable 10: C18:1
        Grand Mean = 4.990   Grand Sum = 1122.814   Total Count = 225
                   T A B L E   O F   M E A N S
           1   2   3   4           10              Total
        ----------------------------------------------------------------
           1   *   *   *           5.176           388.204
           2   *   *   *           4.914           368.516
           3   *   *   *           4.881           366.094
        ----------------------------------------------------------------
           *   1   *   *           4.422           199.009
           *   2   *   *           5.324           239.558
           *   3   *   *           6.745           303.521
           *   4   *   *           3.509           157.922
           *   5   *   *           4.951           222.805
        ----------------------------------------------------------------
           *   *   1   *           1.692            76.155
           *   *   2   *           3.161           142.250
           *   *   3   *           4.308           193.882
           *   *   4   *           6.131           275.887
           *   *   5   *           9.659           434.640
        ----------------------------------------------------------------
           *   1   1   *           0.893             8.040
           *   1   2   *           2.616            23.547
           *   1   3   *           3.950            35.554
           *   1   4   *           6.341            57.066
           *   1   5   *           8.311            74.802
           *   2   1   *           1.809            16.277
           *   2   2   *           3.189            28.698
           *   2   3   *           4.013            36.116
           *   2   4   *           5.848            52.633
```

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*	2	5	*	11.759	105.833
*	3	1	*	1.570	14.131
*	3	2	*	3.181	28.631
*	3	3	*	5.200	46.797
*	3	4	*	8.336	75.021
*	3	5	*	15.438	138.941
*	4	1	*	0.933	8.399
*	4	2	*	2.502	22.519
*	4	3	*	3.326	29.930
*	4	4	*	4.460	40.139
*	4	5	*	6.326	56.934
*	5	1	*	3.256	29.308
*	5	2	*	4.317	38.855
*	5	3	*	5.054	45.486
*	5	4	*	5.670	51.027
*	5	5	*	6.459	58.129
---	---	---	---	---	---
*	*	*	1	4.213	315.952
*	*	*	2	5.013	375.955
*	*	*	3	5.745	430.907
---	---	---	---	---	---
*	1	*	1	3.824	57.355
*	1	*	2	4.277	64.154
*	1	*	3	5.167	77.499
*	2	*	1	4.351	65.265
*	2	*	2	5.302	79.534
*	2	*	3	6.317	94.758
*	3	*	1	5.338	80.076
*	3	*	2	6.984	104.754
*	3	*	3	7.913	118.691
*	4	*	1	3.001	45.012
*	4	*	2	3.464	51.954
*	4	*	3	4.064	60.956
*	5	*	1	4.549	68.242
*	5	*	2	5.037	75.559
*	5	*	3	5.267	79.003
---	---	---	---	---	---
*	*	1	1	1.027	15.404
*	*	1	2	1.839	27.579
*	*	1	3	2.211	33.172
*	*	2	1	2.681	40.219
*	*	2	2	3.137	47.054
*	*	2	3	3.665	54.976
*	*	3	1	4.065	60.968
*	*	3	2	4.271	64.064
*	*	3	3	4.590	68.849
*	*	4	1	5.306	79.587
*	*	4	2	6.139	92.083
*	*	4	3	6.948	104.216
*	*	5	1	7.985	119.772
*	*	5	2	9.678	145.174
*	*	5	3	11.313	169.694
---	---	---	---	---	---
*	1	1	1	0.749	2.248
*	1	1	2	0.938	2.815
*	1	1	3	0.992	2.977
*	1	2	1	1.714	5.141

*	1	2	2	2.634	7.902
*	1	2	3	3.501	10.504
*	1	3	1	3.581	10.742
*	1	3	2	3.888	11.664
*	1	3	3	4.382	13.147
*	1	4	1	5.899	17.696
*	1	4	2	6.417	19.252
*	1	4	3	6.706	20.118
*	1	5	1	7.176	21.527
*	1	5	2	7.507	22.521
*	1	5	3	10.251	30.754
*	2	1	1	1.541	4.623
*	2	1	2	1.815	5.445
*	2	1	3	2.070	6.209
*	2	2	1	2.732	8.196
*	2	2	2	3.163	9.488
*	2	2	3	3.671	11.014
*	2	3	1	3.791	11.372
*	2	3	2	3.929	11.786
*	2	3	3	4.319	12.958
*	2	4	1	5.007	15.021
*	2	4	2	5.779	17.338
*	2	4	3	6.758	20.275
*	2	5	1	8.685	26.054
*	2	5	2	11.826	35.477
*	2	5	3	14.767	44.302
*	3	1	1	0.377	1.132
*	3	1	2	2.010	6.030
*	3	1	3	2.323	6.969
*	3	2	1	2.794	8.383
*	3	2	2	2.969	8.907
*	3	2	3	3.780	11.341
*	3	3	1	4.981	14.942
*	3	3	2	5.189	15.567
*	3	3	3	5.429	16.288
*	3	4	1	6.321	18.962
*	3	4	2	8.348	25.045
*	3	4	3	10.338	31.015
*	3	5	1	12.219	36.658
*	3	5	2	16.402	49.205
*	3	5	3	17.692	53.077
*	4	1	1	0.356	1.069
*	4	1	2	0.806	2.419
*	4	1	3	1.637	4.911
*	4	2	1	2.076	6.228
*	4	2	2	2.605	7.815
*	4	2	3	2.826	8.477
*	4	3	1	3.134	9.401
*	4	3	2	3.289	9.866
*	4	3	3	3.554	10.663
*	4	4	1	3.833	11.500
*	4	4	2	4.518	13.554
*	4	4	3	5.028	15.085
*	4	5	1	5.605	16.814
*	4	5	2	6.100	18.299
*	4	5	3	7.274	21.821
*	5	1	1	2.111	6.332

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         *   5   1   2                3.623              10.870
         *   5   1   3                4.035              12.106
         *   5   2   1                4.091              12.272
         *   5   2   2                4.314              12.942
         *   5   2   3                4.547              13.641
         *   5   3   1                4.837              14.512
         *   5   3   2                5.060              15.181
         *   5   3   3                5.264              15.793
         *   5   4   1                5.469              16.408
         *   5   4   2                5.632              16.895
         *   5   4   3                5.908              17.724
         *   5   5   1                6.240              18.719
         *   5   5   2                6.557              19.672
         *   5   5   3                6.580              19.739
         -----------------------------------------------------------
              A N A L Y S I S   O F   V A R I A N C E   T A B L E
    K                    Degrees of   Sum of        Mean            F
  Value     Source        Freedom    Squares      Square       Value      Prob
         -----------------------------------------------------------
     1    Replication        2        3.922        1.961       9.6884    0.0001
     2    Factor A           4      256.808       64.202     317.2170    0.0000
     4    Factor B           4     1700.176      425.044    2100.1100    0.0000
     6    AB                16      438.466       27.404     135.4017    0.0000
     8    Factor C           2       88.156       44.078     217.7854    0.0000
    10    AC                 8       18.383        2.298      11.3533    0.0000
    12    BC                 8       35.524        4.440      21.9402    0.0000
    14    ABC               32       43.617        1.363       6.7346    0.0000
   -15    Error            148       29.954        0.202
         -----------------------------------------------------------
          Total            224     2615.004
         -----------------------------------------------------------
     Coefficient of Variation: 9.02%
     s_ for means group 1:     0.0519      Number of Observations: 75
      y
     s_ for means group 2:     0.0671      Number of Observations: 45
      y
     s_ for means group 4:     0.0671      Number of Observations: 45
      y
     s_ for means group 6:     0.1500      Number of Observations: 9
      y
     s_ for means group 8:     0.0519      Number of Observations: 75
      y
     s_ for means group 10:    0.1162      Number of Observations: 15
      y
     s_ for means group 12:    0.1162      Number of Observations: 15
      y
     s_ for means group 14:    0.2597      Number of Observations: 3
      y
===============================================================================

     Variable 11: C18:2
     Grand Mean = 18.625   Grand Sum = 4190.727   Total Count = 225
                T A B L E   O F   M E A N S
      1   2   3   4              11              Total
         -----------------------------------------------------------
      1   *   *   *             19.000          1425.012
      2   *   *   *             18.410          1380.742
```

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3	*	*	*	18.466	1384.973
---	---	---	---	---	---
*	1	*	*	21.943	987.415
*	2	*	*	20.036	901.603
*	3	*	*	19.453	875.391
*	4	*	*	16.769	754.583
*	5	*	*	14.927	671.734
---	---	---	---	---	---
*	*	1	*	10.079	453.563
*	*	2	*	13.622	612.969
*	*	3	*	17.301	778.531
*	*	4	*	22.891	1030.079
*	*	5	*	29.235	1315.584
---	---	---	---	---	---
*	1	1	*	11.960	107.644
*	1	2	*	15.215	136.931
*	1	3	*	18.616	167.542
*	1	4	*	28.850	259.647
*	1	5	*	35.072	315.651
*	2	1	*	12.380	111.424
*	2	2	*	15.598	140.384
*	2	3	*	19.612	176.508
*	2	4	*	23.659	212.934
*	2	5	*	28.928	260.353
*	3	1	*	7.745	69.707
*	3	2	*	11.190	100.708
*	3	3	*	15.798	142.185
*	3	4	*	25.117	226.056
*	3	5	*	37.415	336.734
*	4	1	*	8.927	80.339
*	4	2	*	13.868	124.808
*	4	3	*	17.665	158.985
*	4	4	*	19.818	178.360
*	4	5	*	23.566	212.092
*	5	1	*	9.383	84.449
*	5	2	*	12.237	110.137
*	5	3	*	14.812	133.311
*	5	4	*	17.009	153.083
*	5	5	*	21.195	190.754
---	---	---	---	---	---
*	*	*	1	17.006	1275.440
*	*	*	2	18.628	1397.077
*	*	*	3	20.243	1518.210
---	---	---	---	---	---
*	1	*	1	19.687	295.308
*	1	*	2	22.519	337.788
*	1	*	3	23.621	354.319
*	2	*	1	18.923	283.846
*	2	*	2	19.682	295.232
*	2	*	3	21.502	322.525
*	3	*	1	17.441	261.612
*	3	*	2	19.352	290.281
*	3	*	3	21.567	323.498
*	4	*	1	15.228	228.423
*	4	*	2	16.573	248.593
*	4	*	3	18.505	277.568
*	5	*	1	13.750	206.252

*	5	*	2	15.012	225.182
*	5	*	3	16.020	240.300

---

*	*	1	1	8.312	124.686
*	*	1	2	10.207	153.103
*	*	1	3	11.718	175.774
*	*	2	1	12.643	189.640
*	*	2	2	13.563	203.442
*	*	2	3	14.659	219.887
*	*	3	1	15.403	231.048
*	*	3	2	16.781	251.722
*	*	3	3	19.717	295.761
*	*	4	1	21.255	318.821
*	*	4	2	23.134	347.017
*	*	4	3	24.283	364.241
*	*	5	1	27.416	411.245
*	*	5	2	29.453	441.793
*	*	5	3	30.836	462.547

---

*	1	1	1	7.462	22.386
*	1	1	2	13.892	41.676
*	1	1	3	14.527	43.582
*	1	2	1	14.989	44.967
*	1	2	2	15.115	45.345
*	1	2	3	15.540	46.619
*	1	3	1	16.530	49.590
*	1	3	2	17.958	53.874
*	1	3	3	21.359	64.078
*	1	4	1	26.022	78.067
*	1	4	2	30.160	90.481
*	1	4	3	30.366	91.099
*	1	5	1	33.432	100.297
*	1	5	2	35.471	106.412
*	1	5	3	36.314	108.941
*	2	1	1	11.939	35.816
*	2	1	2	11.676	35.027
*	2	1	3	13.527	40.582
*	2	2	1	14.047	42.141
*	2	2	2	15.295	45.885
*	2	2	3	17.453	52.358
*	2	3	1	17.871	53.612
*	2	3	2	19.306	57.918
*	2	3	3	21.659	64.978
*	2	4	1	22.365	67.095
*	2	4	2	23.132	69.395
*	2	4	3	25.481	76.444
*	2	5	1	28.394	85.182
*	2	5	2	29.003	87.008
*	2	5	3	29.388	88.163
*	3	1	1	7.242	21.726
*	3	1	2	7.558	22.673
*	3	1	3	8.436	25.309
*	3	2	1	10.121	30.363
*	3	2	2	11.058	33.173
*	3	2	3	12.391	37.173
*	3	3	1	12.709	38.126
*	3	3	2	15.218	45.653

```
    *   3   3   3             19.469              58.407
    *   3   4   1             23.256              69.767
    *   3   4   2             25.101              75.304
    *   3   4   3             26.995              80.984
    *   3   5   1             33.877             101.630
    *   3   5   2             37.826             113.478
    *   3   5   3             40.542             121.625
    *   4   1   1              7.123              21.368
    *   4   1   2              8.415              25.246
    *   4   1   3             11.241              33.724
    *   4   2   1             12.729              38.186
    *   4   2   2             14.079              42.236
    *   4   2   3             14.795              44.385
    *   4   3   1             15.829              47.488
    *   4   3   2             16.578              49.734
    *   4   3   3             20.588              61.763
    *   4   4   1             18.558              55.674
    *   4   4   2             20.258              60.773
    *   4   4   3             20.638              61.913
    *   4   5   1             21.902              65.707
    *   4   5   2             23.535              70.604
    *   4   5   3             25.260              75.781
    *   5   1   1              7.797              23.390
    *   5   1   2              9.494              28.482
    *   5   1   3             10.859              32.577
    *   5   2   1             11.327              33.982
    *   5   2   2             12.268              36.803
    *   5   2   3             13.117              39.352
    *   5   3   1             14.078              42.233
    *   5   3   2             14.848              44.543
    *   5   3   3             15.512              46.535
    *   5   4   1             16.073              48.218
    *   5   4   2             17.022              51.065
    *   5   4   3             17.934              53.801
    *   5   5   1             19.476              58.429
    *   5   5   2             21.430              64.290
    *   5   5   3             22.679              68.036
    ---------------------------------------------------------------
       A N A L Y S I S   O F   V A R I A N C E   T A B L E
    K                 Degrees of  Sum of         Mean           F
  Value    Source     Freedom    Squares        Square        Value      Prob
    ---------------------------------------------------------------------------
    1    Replication      2         15.915          7.957       7.5377    0.0008
    2    Factor A         4       1386.018        346.505     328.2263    0.0000
    4    Factor B         4      10376.619       2594.155    2457.3126    0.0000
    6    AB              16       1575.314         98.457      93.2635    0.0000
    8    Factor C         2        392.915        196.458     186.0944    0.0000
   10    AC               8         31.392          3.924       3.7170    0.0005
   12    BC               8         29.581          3.698       3.5026    0.0010
   14    ABC             32         95.707          2.991       2.8331    0.0000
  -15    Error          148        156.242          1.056
    ---------------------------------------------------------------------------
         Total          224      14059.702
    ---------------------------------------------------------------------------
       Coefficient of Variation: 5.52%
       s_ for means group 1:     0.1186        Number of Observations: 75
        y
```

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```
s_ for means group 2:      0.1532      Number of Observations: 45
 y
s_ for means group 4:      0.1532      Number of Observations: 45
 y
s_ for means group 6:      0.3425      Number of Observations: 9
 y
s_ for means group 8:      0.1186      Number of Observations: 75
 y
s_ for means group 10:      0.2653      Number of Observations: 15
 y
s_ for means group 12:      0.2653      Number of Observations: 15
 y
s_ for means group 14:      0.5932      Number of Observations: 3
 y
====================================================================
    Variable 12: C18:3
    Grand Mean = 24.336   Grand Sum = 5475.579   Total Count = 225
                  T A B L E   O F   M E A N S
     1   2   3   4              12              Total
    ----------------------------------------------------------------
     1   *   *   *             25.119          1883.947
     2   *   *   *             23.905          1792.868
     3   *   *   *             23.984          1798.764
    ----------------------------------------------------------------
     *   1   *   *             32.760          1474.180
     *   2   *   *             22.925          1031.614
     *   3   *   *             22.955          1032.959
     *   4   *   *             21.598           971.930
     *   5   *   *             21.442           964.895
    ----------------------------------------------------------------
     *   *   1   *             11.733           528.006
     *   *   2   *             16.806           756.274
     *   *   3   *             22.962          1033.285
     *   *   4   *             29.570          1330.635
     *   *   5   *             40.608          1827.379
    ----------------------------------------------------------------
     *   1   1   *             13.740           123.663
     *   1   2   *             21.744           195.692
     *   1   3   *             32.928           296.351
     *   1   4   *             40.974           368.767
     *   1   5   *             54.412           489.707
     *   2   1   *             13.872           124.848
     *   2   2   *             18.180           163.624
     *   2   3   *             21.576           194.184
     *   2   4   *             25.325           227.922
     *   2   5   *             35.671           321.036
     *   3   1   *              9.326            83.932
     *   3   2   *             14.232           128.090
     *   3   3   *             20.217           181.952
     *   3   4   *             29.782           268.034
     *   3   5   *             41.217           370.952
     *   4   1   *             13.016           117.142
     *   4   2   *             16.314           146.825
     *   4   3   *             20.488           184.394
     *   4   4   *             25.342           228.078
     *   4   5   *             32.832           295.491
     *   5   1   *              8.713            78.421
```

*	5	2	*	13.560	122.044
*	5	3	*	19.600	176.403
*	5	4	*	26.426	237.835
*	5	5	*	38.910	350.192
--					
*	*	*	1	21.951	1646.320
*	*	*	2	24.332	1824.881
*	*	*	3	26.725	2004.378
--					
*	1	*	1	29.463	441.952
*	1	*	2	33.105	496.577
*	1	*	3	35.710	535.651
*	2	*	1	20.975	314.623
*	2	*	2	22.986	344.783
*	2	*	3	24.814	372.208
*	3	*	1	20.638	309.563
*	3	*	2	22.447	336.712
*	3	*	3	25.779	386.684
*	4	*	1	19.870	298.051
*	4	*	2	21.713	325.691
*	4	*	3	23.213	348.188
*	5	*	1	18.809	282.131
*	5	*	2	21.408	321.118
*	5	*	3	24.110	361.647
--					
*	*	1	1	9.786	146.787
*	*	1	2	11.698	175.468
*	*	1	3	13.717	205.751
*	*	2	1	15.071	226.067
*	*	2	2	16.991	254.872
*	*	2	3	18.356	275.335
*	*	3	1	20.499	307.478
*	*	3	2	22.689	340.330
*	*	3	3	25.699	385.478
*	*	4	1	26.760	401.396
*	*	4	2	29.132	436.979
*	*	4	3	32.817	492.259
*	*	5	1	37.639	564.592
*	*	5	2	41.149	617.232
*	*	5	3	43.037	645.555
--					
*	1	1	1	12.814	38.442
*	1	1	2	13.245	39.734
*	1	1	3	15.163	45.488
*	1	2	1	17.722	53.166
*	1	2	2	22.653	67.960
*	1	2	3	24.855	74.565
*	1	3	1	28.184	84.551
*	1	3	2	32.935	98.804
*	1	3	3	37.665	112.996
*	1	4	1	38.961	116.882
*	1	4	2	40.492	121.475
*	1	4	3	43.470	130.411
*	1	5	1	49.637	148.911
*	1	5	2	56.201	168.604
*	1	5	3	57.397	172.192
*	2	1	1	10.683	32.049

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*	2	1	2	14.709	44.127
*	2	1	3	16.224	48.672
*	2	2	1	17.425	52.275
*	2	2	2	18.252	54.756
*	2	2	3	18.864	56.592
*	2	3	1	20.231	60.693
*	2	3	2	21.420	64.261
*	2	3	3	23.077	69.230
*	2	4	1	24.261	72.784
*	2	4	2	24.884	74.653
*	2	4	3	26.828	80.485
*	2	5	1	32.274	96.822
*	2	5	2	35.662	106.985
*	2	5	3	39.076	117.229
*	3	1	1	7.459	22.378
*	3	1	2	9.362	28.086
*	3	1	3	11.156	33.467
*	3	2	1	12.319	36.956
*	3	2	2	14.365	43.095
*	3	2	3	16.013	48.038
*	3	3	1	17.337	52.012
*	3	3	2	19.195	57.586
*	3	3	3	24.118	72.354
*	3	4	1	25.509	76.527
*	3	4	2	27.985	83.956
*	3	4	3	35.850	107.551
*	3	5	1	40.563	121.690
*	3	5	2	41.329	123.988
*	3	5	3	41.758	125.273
*	4	1	1	11.679	35.038
*	4	1	2	12.731	38.194
*	4	1	3	14.637	43.910
*	4	2	1	15.504	46.513
*	4	2	2	16.014	48.042
*	4	2	3	17.423	52.270
*	4	3	1	19.588	58.764
*	4	3	2	20.139	60.418
*	4	3	3	21.737	65.211
*	4	4	1	22.248	66.744
*	4	4	2	26.388	79.165
*	4	4	3	27.389	82.168
*	4	5	1	30.331	90.992
*	4	5	2	33.290	99.870
*	4	5	3	34.876	104.629
*	5	1	1	6.293	18.880
*	5	1	2	8.442	25.327
*	5	1	3	11.405	34.214
*	5	2	1	12.385	37.156
*	5	2	2	13.673	41.018
*	5	2	3	14.623	43.869
*	5	3	1	17.153	51.459
*	5	3	2	19.753	59.259
*	5	3	3	21.895	65.686
*	5	4	1	22.820	68.460
*	5	4	2	25.910	77.730
*	5	4	3	30.548	91.645
*	5	5	1	35.392	106.176

```
      *   5   5   2              39.261          117.784
      *   5   5   3              42.078          126.233
     ---------------------------------------------------------------
            A N A L Y S I S   O F   V A R I A N C E   T A B L E
     K                   Degrees of   Sum of       Mean         F
   Value     Source      Freedom      Squares     Square      Value      Prob
     ---------------------------------------------------------------
     1     Replication      2          69.271      34.636     18.3403   0.0000
     2     Factor A         4        4082.615    1020.654    540.4574   0.0000
     4     Factor B         4       22931.754    5732.939   3035.7104   0.0000
     6     AB              16        1763.310     110.207     58.3568   0.0000
     8     Factor C         2         854.706     427.353    226.2923   0.0000
    10     AC               8          50.164       6.270      3.3203   0.0016
    12     BC               8          51.962       6.495      3.4394   0.0012
    14     ABC             32         183.052       5.720      3.0291   0.0000
   -15     Error          148         279.498       1.888
     ---------------------------------------------------------------
           Total          224       30266.333
     ---------------------------------------------------------------
     Coefficient of Variation: 5.65%
     s_  for means group 1:     0.1587      Number of Observations: 75
      y
     s_  for means group 2:     0.2049      Number of Observations: 45
      y
     s_  for means group 4:     0.2049      Number of Observations: 45
      y
     s_  for means group 6:     0.4581      Number of Observations: 9
      y
     s_  for means group 8:     0.1587      Number of Observations: 75
      y
     s_  for means group 10:    0.3548      Number of Observations: 15
      y
     s_  for means group 12:    0.3548      Number of Observations: 15
      y
     s_  for means group 14:    0.7934      Number of Observations: 3
      y
   ===============================================================

     Variable 13: Total Unsaturated
     Grand Mean = 48.598   Grand Sum = 10934.470   Total Count = 225
                T A B L E   O F   M E A N S
      1   2   3   4            13              Total
     ---------------------------------------------------------------
      1   *   *   *            50.008          3750.573
      2   *   *   *            47.902          3592.660
      3   *   *   *            47.883          3591.238
     ---------------------------------------------------------------
      *   1   *   *            60.179          2708.045
      *   2   *   *            48.669          2190.114
      *   3   *   *            49.743          2238.442
      *   4   *   *            42.484          1911.767
      *   5   *   *            41.913          1886.102
     ---------------------------------------------------------------
      *   *   1   *            23.608          1062.369
      *   *   2   *            33.844          1523.002
      *   *   3   *            44.925          2021.629
      *   *   4   *            59.330          2669.849
```

```
 *     *     5     *          81.280          3657.620
---------------------------------------------------------------
 *     1     1     *          26.816           241.341
 *     1     2     *          39.790           358.111
 *     1     3     *          56.230           506.072
 *     1     4     *          77.155           694.394
 *     1     5     *         100.903           908.127
 *     2     1     *          28.058           252.524
 *     2     2     *          37.311           335.799
 *     2     3     *          45.512           409.607
 *     2     4     *          55.290           497.607
 *     2     5     *          77.175           694.577
 *     3     1     *          18.761           168.848
 *     3     2     *          28.775           258.971
 *     3     3     *          41.647           374.821
 *     3     4     *          64.230           578.066
 *     3     5     *          95.304           857.735
 *     4     1     *          22.963           206.668
 *     4     2     *          33.062           297.562
 *     4     3     *          40.900           368.101
 *     4     4     *          50.393           453.537
 *     4     5     *          65.100           585.900
 *     5     1     *          21.443           192.988
 *     5     2     *          30.284           272.560
 *     5     3     *          40.336           363.028
 *     5     4     *          49.583           446.246
 *     5     5     *          67.920           611.281
---------------------------------------------------------------
 *     *     *     1          43.681          3276.087
 *     *     *     2          48.660          3649.529
 *     *     *     3          53.451          4008.855
---------------------------------------------------------------
 *     1     *     1          53.654           804.814
 *     1     *     2          60.856           912.846
 *     1     *     3          66.026           990.386
 *     2     *     1          44.560           668.401
 *     2     *     2          48.486           727.293
 *     2     *     3          52.961           794.419
 *     3     *     1          43.786           656.794
 *     3     *     2          49.425           741.371
 *     3     *     3          56.018           840.276
 *     4     *     1          38.628           579.413
 *     4     *     2          42.628           639.419
 *     4     *     3          46.196           692.935
 *     5     *     1          37.778           566.664
 *     5     *     2          41.907           628.600
 *     5     *     3          46.056           690.838
---------------------------------------------------------------
 *     *     1     1          19.210           288.148
 *     *     1     2          23.928           358.921
 *     *     1     3          27.687           415.300
 *     *     2     1          30.612           459.176
 *     *     2     2          33.965           509.478
 *     *     2     3          36.957           554.349
 *     *     3     1          40.692           610.381
 *     *     3     2          44.138           662.073
 *     *     3     3          49.945           749.176
```

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*	*	4	1	53.873	808.096
*	*	4	2	59.181	887.720
*	*	4	3	64.936	974.033
*	*	5	1	74.019	1110.286
*	*	5	2	82.089	1231.336
*	*	5	3	87.733	1315.998

----

*	1	1	1	21.186	63.558
*	1	1	2	28.305	84.916
*	1	1	3	30.956	92.867
*	1	2	1	34.712	104.137
*	1	2	2	40.388	121.164
*	1	2	3	44.270	132.810
*	1	3	1	48.731	146.194
*	1	3	2	55.257	165.772
*	1	3	3	64.702	194.107
*	1	4	1	71.667	215.002
*	1	4	2	78.053	234.160
*	1	4	3	81.744	245.231
*	1	5	1	91.974	275.923
*	1	5	2	102.278	306.834
*	1	5	3	108.457	325.371
*	2	1	1	24.235	72.704
*	2	1	2	28.644	85.931
*	2	1	3	31.296	93.889
*	2	2	1	34.385	103.156
*	2	2	2	37.277	111.830
*	2	2	3	40.271	120.812
*	2	3	1	42.187	126.562
*	2	3	2	44.960	134.880
*	2	3	3	49.388	148.165
*	2	4	1	52.028	156.084
*	2	4	2	54.258	162.775
*	2	4	3	59.583	178.748
*	2	5	1	69.965	209.894
*	2	5	2	77.292	231.877
*	2	5	3	84.269	252.806
*	3	1	1	15.127	45.381
*	3	1	2	19.021	57.064
*	3	1	3	22.135	66.404
*	3	2	1	25.453	76.358
*	3	2	2	28.670	86.010
*	3	2	3	32.201	96.604
*	3	3	1	35.426	106.279
*	3	3	2	40.045	120.135
*	3	3	3	49.469	148.407
*	3	4	1	55.553	166.660
*	3	4	2	62.663	187.988
*	3	4	3	74.473	223.418
*	3	5	1	87.372	262.116
*	3	5	2	96.725	290.175
*	3	5	3	101.815	305.444
*	4	1	1	19.208	57.624
*	4	1	2	22.035	66.104
*	4	1	3	27.647	82.940
*	4	2	1	30.575	91.726
*	4	2	2	33.066	99.197

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```
        *    4    2    3              35.546              106.638
        *    4    3    1              39.122              117.365
        *    4    3    2              40.449              121.347
        *    4    3    3              43.130              129.389
        *    4    4    1              45.366              136.099
        *    4    4    2              51.925              155.775
        *    4    4    3              53.888              161.663
        *    4    5    1              58.867              176.600
        *    4    5    2              65.665              196.995
        *    4    5    3              70.768              212.305
        *    5    1    1              16.294               48.882
        *    5    1    2              21.635               64.906
        *    5    1    3              26.400               79.200
        *    5    2    1              27.933               83.798
        *    5    2    2              30.426               91.277
        *    5    2    3              32.495               97.485
        *    5    3    1              37.994              113.982
        *    5    3    2              39.979              119.938
        *    5    3    3              43.036              129.108
        *    5    4    1              44.750              134.250
        *    5    4    2              49.007              147.022
        *    5    4    3              54.991              164.973
        *    5    5    1              61.918              185.753
        *    5    5    2              68.485              205.456
        *    5    5    3              73.357              220.072
```

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	223.671	111.836	32.1385	0.0000
2	Factor A	4	9787.468	2446.867	703.1627	0.0000
4	Factor B	4	91753.395	22938.349	6591.8544	0.0000
6	AB	16	7249.518	453.095	130.2071	0.0000
8	Factor C	2	3580.100	1790.050	514.4115	0.0000
10	AC	8	176.718	22.090	6.3480	0.0000
12	BC	8	263.063	32.883	9.4496	0.0000
14	ABC	32	241.238	7.539	2.1664	0.0010
-15	Error	148	515.011	3.480		

| | Total | 224 | 113790.182 | | | |

Coefficient of Variation: 3.84%

$s_{\bar{y}}$ for means group 1:   0.2154   Number of Observations: 75

$s_{\bar{y}}$ for means group 2:   0.2781   Number of Observations: 45

$s_{\bar{y}}$ for means group 4:   0.2781   Number of Observations: 45

$s_{\bar{y}}$ for means group 6:   0.6218   Number of Observations: 9

$s_{\bar{y}}$ for means group 8:   0.2154   Number of Observations: 75

$s_{\bar{y}}$ for means group 10:   0.4817   Number of Observations: 15

$s_{\bar{y}}$ for means group 12:   0.4817   Number of Observations: 15

```
      s_ for means group 14:       1.0770         Number of Observations: 3
       y
===============================================================================

      Variable 14: Total fatty acids
      Grand Mean = 78.225   Grand Sum = 17600.620   Total Count = 225
                     T A B L E   O F   M E A N S
       1   2   3   4           14                 Total
      -----------------------------------------------------------------
       1   *   *   *          80.496              6037.186
       2   *   *   *          77.287              5796.524
       3   *   *   *          76.892              5766.910
      -----------------------------------------------------------------
       *   1   *   *          93.295              4198.275
       *   2   *   *          76.670              3450.152
       *   3   *   *          86.417              3888.760
       *   4   *   *          65.354              2940.937
       *   5   *   *          69.389              3122.497
      -----------------------------------------------------------------
       *   *   1   *          35.093              1579.189
       *   *   2   *          51.604              2322.169
       *   *   3   *          69.906              3145.787
       *   *   4   *          95.675              4305.380
       *   *   5   *         138.847              6248.094
      -----------------------------------------------------------------
       *   1   1   *          40.599               365.394
       *   1   2   *          58.349               525.142
       *   1   3   *          83.743               753.684
       *   1   4   *         118.749              1068.737
       *   1   5   *         165.035              1485.318
       *   2   1   *          39.180               352.624
       *   2   2   *          57.241               515.166
       *   2   3   *          70.173               631.561
       *   2   4   *          87.180               784.617
       *   2   5   *         129.576              1166.183
       *   3   1   *          29.040               261.358
       *   3   2   *          45.091               405.821
       *   3   3   *          70.312               632.810
       *   3   4   *         111.649              1004.844
       *   3   5   *         175.992              1583.927
       *   4   1   *          29.503               265.531
       *   4   2   *          47.615               428.534
       *   4   3   *          61.388               552.490
       *   4   4   *          79.365               714.284
       *   4   5   *         108.900               980.098
       *   5   1   *          37.142               334.282
       *   5   2   *          49.723               447.506
       *   5   3   *          63.916               575.242
       *   5   4   *          81.433               732.899
       *   5   5   *         114.730              1032.568
      -----------------------------------------------------------------
       *   *   *   1          69.086              5181.422
       *   *   *   2          77.760              5831.978
       *   *   *   3          87.830              6587.220
      -----------------------------------------------------------------
       *   1   *   1          81.744              1226.166
       *   1   *   2          93.964              1409.455
```

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```
*   1   *   3        104.177         1562.654
*   2   *   1         68.829         1032.428
*   2   *   2         75.089         1126.341
*   2   *   3         86.092         1291.383
*   3   *   1         74.556         1118.340
*   3   *   2         85.085         1276.278
*   3   *   3         99.609         1494.142
*   4   *   1         57.951          869.260
*   4   *   2         65.389          980.838
*   4   *   3         72.723         1090.840
*   5   *   1         62.349          935.228
*   5   *   2         69.271         1039.067
*   5   *   3         76.547         1148.201
------------------------------------------------------------
*   *   1   1         27.524          412.858
*   *   1   2         35.436          531.537
*   *   1   3         42.320          634.794
*   *   2   1         46.628          699.417
*   *   2   2         51.604          774.059
*   *   2   3         56.580          848.693
*   *   3   1         62.599          938.985
*   *   3   2         68.437         1026.560
*   *   3   3         78.683         1180.242
*   *   4   1         86.625         1299.379
*   *   4   2         93.724         1405.861
*   *   4   3        106.676         1600.140
*   *   5   1        122.052         1830.783
*   *   5   2        139.597         2093.961
*   *   5   3        154.890         2323.351
------------------------------------------------------------
*   1   1   1         31.505           94.514
*   1   1   2         42.224          126.673
*   1   1   3         48.069          144.207
*   1   2   1         52.433          157.300
*   1   2   2         58.802          176.405
*   1   2   3         63.812          191.437
*   1   3   1         71.200          213.600
*   1   3   2         81.277          243.832
*   1   3   3         98.751          296.252
*   1   4   1        108.592          325.776
*   1   4   2        120.703          362.108
*   1   4   3        126.951          380.852
*   1   5   1        144.992          434.976
*   1   5   2        166.812          500.436
*   1   5   3        183.302          549.907
*   2   1   1         30.150           90.449
*   2   1   2         39.519          118.558
*   2   1   3         47.873          143.618
*   2   2   1         53.264          159.791
*   2   2   2         56.401          169.204
*   2   2   3         62.057          186.172
*   2   3   1         64.466          193.398
*   2   3   2         67.946          203.837
*   2   3   3         78.109          234.326
*   2   4   1         85.806          257.417
*   2   4   2         78.440          235.321
*   2   4   3         97.293          291.879
```

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```
              *    2    5    1              110.458              331.374
              *    2    5    2              133.140              399.421
              *    2    5    3              145.130              435.389
              *    3    1    1               23.243               69.729
              *    3    1    2               29.502               88.506
              *    3    1    3               34.374              103.122
              *    3    2    1               38.947              116.842
              *    3    2    2               44.884              134.651
              *    3    2    3               51.443              154.328
              *    3    3    1               60.790              182.369
              *    3    3    2               68.057              204.171
              *    3    3    3               82.090              246.269
              *    3    4    1               94.124              282.371
              *    3    4    2              108.592              325.776
              *    3    4    3              132.232              396.697
              *    3    5    1              155.676              467.028
              *    3    5    2              174.391              523.173
              *    3    5    3              197.909              593.726
              *    4    1    1               22.387               67.161
              *    4    1    2               28.905               86.716
              *    4    1    3               37.218              111.654
              *    4    2    1               42.153              126.459
              *    4    2    2               48.272              144.816
              *    4    2    3               52.420              157.260
              *    4    3    1               57.784              173.352
              *    4    3    2               61.031              183.093
              *    4    3    3               65.348              196.045
              *    4    4    1               71.187              213.561
              *    4    4    2               80.540              241.619
              *    4    4    3               86.368              259.105
              *    4    5    1               96.243              288.728
              *    4    5    2              108.198              324.595
              *    4    5    3              122.259              366.776
              *    5    1    1               30.335               91.005
              *    5    1    2               37.028              111.084
              *    5    1    3               44.064              132.192
              *    5    2    1               46.342              139.025
              *    5    2    2               49.661              148.983
              *    5    2    3               53.166              159.498
              *    5    3    1               58.756              176.267
              *    5    3    2               63.875              191.626
              *    5    3    3               69.117              207.350
              *    5    4    1               73.418              220.254
              *    5    4    2               80.346              241.037
              *    5    4    3               90.536              271.608
              *    5    5    1              102.893              308.678
              *    5    5    2              115.446              346.337
              *    5    5    3              125.851              377.554
        ------------------------------------------------------------
             A N A L Y S I S   O F   V A R I A N C E   T A B L E
       K                    Degrees of   Sum of          Mean              F
     Value      Source       Freedom     Squares        Square          Value      Prob
       ------------------------------------------------------------------------
       1     Replication        2        585.977        292.988        23.5707    0.0000
       2     Factor A           4      24316.471       6079.118       489.0606    0.0000
       4     Factor B           4     297797.775      74449.444      5989.4036    0.0000
       6     AB                16      25336.211       1583.513       127.3925    0.0000
```

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```
   8    Factor C        2     13199.473     6599.736    530.9440   0.0000
  10    AC              8       772.524       96.565      7.7686   0.0000
  12    BC              8      2377.538      297.192     23.9089   0.0000
  14    ABC            32      1198.689       37.459      3.0136   0.0000
 -15    Error         148      1839.669       12.430
-------------------------------------------------------------------------
        Total         224    367424.325
-------------------------------------------------------------------------
        Coefficient of Variation: 4.51%
        s_ for means group 1:     0.4071      Number of Observations: 75
          y
        s_ for means group 2:     0.5256      Number of Observations: 45
          y
        s_ for means group 4:     0.5256      Number of Observations: 45
          y
        s_ for means group 6:     1.1752      Number of Observations: 9
          y
        s_ for means group 8:     0.4071      Number of Observations: 75
          y
        s_ for means group 10:    0.9103      Number of Observations: 15
          y
        s_ for means group 12:    0.9103      Number of Observations: 15
          y
        s_ for means group 14:    2.0355      Number of Observations: 3
          y
```

7.3: Appendix 3: GC Conditions

[GC Information]

GC MODEL	: Shimadzu GC 2010

[Injection Port]

Temperature	: 180°C
Carrier Gas	: N_2/Air
Flow Control Mode	: Pressure
Pressure	: 42.7 psi
Total Flow	: 73.7 mL/min
Column Flow	: 2.43 mL/min
Linear Flow	: 43.9 cm/sec
Purge Flow	: 3.0 mL/min
Split Ratio	:28.1
High Pressure Injection	: Auto
Pressure	: 14.5 psi

[Column Oven]

Initial Temperature	: 160°C
Equillibration Time	: 1.0 min

=Column Oven Temperature Program=

Total Program Time	:43.75 min

Rate (°C/min)	Temperature(°C)	Hold Time(min)
....	160.0	4.0
0.6	170.0	9.0
3.5	200.0	4.0

[Column Information]

Column Name	: WCOT
Serial Number	: 7723
Film Thickness	: 0.20 μm
Column Length	: 50 m
Inner Diameter	: 0.25 mm ID

Column Max Temp.   : 250.0°C

[Detector Channel FID]

Detector Type   : FID

Temperature   : 230.0°C

Sampling Rate   : 40 msec

Stop Time   : 43.75 min

Makeup Gas   : N_2/Air

Makeup Flow   : 30.0 mL/ min

H_2 Flow   : 30.0 mL/min

Air Flow   : 400.0 mL/min