DEVELOPMENT OF APPLE FRAGRANCE PERFUME FOR HAIR CREAM

BY

ENEKE, SYLVIA IJEOMA
(M.ENG/EC/02/472)

DECEMBER, 2009

DEVELOPMENT OF APPLE FRAGRANCE PERFUME FOR HAIR CREAM

A THESIS

Presented to

The School of Post Graduate Studies
Federal University of Technology,
Yola

By

ENEKE, Sylvia Ijeoma
(M.ENG/EC/02/472)

In Partial Fulfilment of the Requirement for the
Degree of Masters of Engineering
(Chemical Engineering)

Department of Chemical Engineering,
School of Engineering and Engineering Technology,
Federal University of Technology, Yola,

December, 2009.

DECLARATION

I, Sylvia Ijeoma Eneke, declare that this thesis is solely the result of my work and has never been submitted anywhere for any degree. Any literature cited has been duly acknowledged in the references.

……

……

Sign Date

APPROVAL PAGE

This thesis entitled Development of Apple Perfume for Hair Cream, by Sylvia Ijeoma Eneke, meets the regulations governing the award of the degree of Masters of Engineering (Chemical Engineering) of Federal University of Technology, Yola, and is approved for its contributions to knowledge and literal presentations.

Mr T.M. Chiroma **(Head of Department)**	**Date**
Prof.Ir.P.B. Onaji **(Project Supervisor)**	**Date**
Dr. Barminas, J.T **(Internal Examiner)**	**Date**
Prof. F. Aberuagba **(External Examiner)**	**Date**
Prof. A. Nur **Dean, School of post** **Graduate studies**	**Date**

DEDICATION

This piece of work is dedicated to my late Father, Evangelist Christian Chidi Eneke, who wished I made it to this level.

ACKNOWLEDGEMENT

My gratitude goes to my God who put this thought of studies in my heart in the first place and saw me go through this work. I wish to thank my husband Arch. K.C Okoye for putting a crown on my head at the end of this program. My heart felt thanks go to my dear mother for supporting me immensely, financially and otherwise through out this work. I also sincerely thank my supervisor, Prof Ir P.B Onaji, for his intelligent and useful contributions during this work.

I will like to thank my siblings, and friends who endured all my whole stories through out this work. I wish to thank the lighthouse, Professor Iyiola Tella and Pastor Bolou for all their efforts.

This section will be incomplete if I fail to mention the likes of Kasom, Ade, Victor, Silas Manji, Sister Rose Abu, Brother Sunday Abu, Brother Elijah Olakunle, Mr lawrence (Abti Academy), Mr Salawu, Mr Sunday Osemeahon, Mr Joseph Manu, Mr Jovian, Aunty Magda, Mr Sunday Coleman, Members Celestial Church of Christ Parish 3, lecturers and, all my postgraduate colleagues.

ABSTRACT

In this project, apple perfume concentrate was developed from locally sourced raw materials (orange oil, pear oil and paraffin oil) and some synthetic materials (Isoamyl valerate apple essence and isoamyl acetate banana oil). This perfume is being developed with a view to substituting the totally imported commercial perfume. A total of eighty one formulations of the perfume were prepared from the raw materials and subjected to a sensory analysis. The best fragrance composition was further analysed by gas chromatography, infrared spectroscopy, and other physico–chemical analysis and compared to that of the commercial apple perfume. Samples $S_{O1, B2, A2, P1}$ (0% orange oil, 20% banana oil, 40% apple oil, 0% pear oil, 40% paraffin oil), $S_{O1, B2, A2, P2,}$ (0% orange oil, 20% banana oil, 40% apple oil, 1% pear oil,39% paraffin oil) and $S_{O3,B1,A3,P1}$ (5% orange oil, 10% banana oil, 60% apple oil, 5% pear oil, 20% paraffin oil) gave the highest fragrance values of 4.25, 4.00 and 4.00 respectively as compared to 5.00 for the commercial apple perfume. Sample $S_{O1,B2,A2,P1}$ is the best formulation that gives the fragrance closest to the commercial apple fragrance with a fragrance value of 4.25. However, the fragrance was different from the commercial apple perfume in being slightly less fruity. Isoamyl valerate was found to be a major ester in the formulation of the commercial apple perfume. Some key esters were present in the commercial apple perfume which were absent in the best developed perfume. These may be responsible for the slightly less fruitiness of the fragrance developed. The developed apple perfume was very similar to the commercial apple perfume in most of the other physical-chemical properties such as the refractive index, viscosity, ester value and peroxide value. The fractional distillation of the commercial apple perfume shows that the carrier (paraffin oil and other fixed oils) is about 50% of the total perfume. This means that a substantial improvement in local content of over 50% by volume can be achieved with local production. The total price of the best developed apple perfume of ₦15,349.00 was higher than the price of the commercial product in the market ₦10,000.00. This was due to the fact that the apple essence was purchased at a retail price. However, carrying out a sensitivity analysis using half the price of the raw materials (due to a bulk purchase of the apple essence) shows that the cost of a litre reduces to ₦5, 638, and at 50% profit margin the retail price drops to ₦8,457 which is lower than that of the commercial apple perfume.

TABLE OF CONTENTS

		Page
Title Page		ii
Declaration		iii
Approval Page		iv
Dedication		v
Acknowledgement		vi
Abstract		vii
Table of Content		viii
CHAPTER ONE		
1.0	Introduction	1
CHAPTER TWO		
2.0	Survey of Literatures	2
2.1	History of Perfumes	2
2.2	Basic Constituents of a Perfume	2
2.3	Fragrance Notes	4
2.3.1	Top Note	4
2.3.2	Middle Note	4
2.3.3	Base Note	4
2.3.4	The Vehicle	5
2.3.5	The Fixative	5
2.3.6	The Odiferous Element	5
2.3.6.1	Plant sources	5
2.3.6.2	Animal Sources	6
2.3.6.3.	Synthetic Sources	7
2.4	Selected Fruits Characteristics	7
2.4.1	Avocado	7
2.4.2	Orange	7
2.4.3	Apple Essence	8

2.4.4 Banana Essence 8

2.5 The Separation of Natural Odorants 9
2..5.1 Extraction with Cold Fat 10
2.5.2 Extraction with Hot Fat 10
2.5.3 Extraction with Volatile Solvent 10
2.5.4 Soaking Method 11
2.5.5 Extraction with liquefied Gases 11
2.5.6 Expression 11
2.5.5.1 The Sponge Method 12
2.5.5.2 The Ecuelle Method 12
2.5.5.3 Machine Process 12
2.6 Distillation 12
2.6.1 Water Distillation 12
2.6.2 Steam and Water Distillation 12
2.6.3 Cohobation 13
2.6.4 Steam Distillation 13
2.6.5 Oil Isolation Fundamentals 14
2.7 Synthetic Production of Essential Oil 16
2.8 Oil Based Perfumes formulations 17
2.9.1 Jojoba Oil as Base 17
2.9.2 Synthetic Apple Oil 17
2.9 Quality Assessment Techniques 18
2.9.1 Sensory Evaluations 18
2.9.2 Physical Tests 18
2.9.2.1 Moisture Content 18
2.9.2.2 Specific Gravity 19
2.9.2.3 Viscosity 19
2.9.2.4 Refractive Index 19
2.9.3 Chemical Tests 20
2.9.3.1 Peroxide value 20
2.9.3.1 Acid Value 20
2.9.3.2 Ester Value 20
2.10 Instrumental techniques 21

2.10.1 Gas Chromatograph 21
2.10.2 Fractional Distillation 22
2.10.3 Infrared Spectrophotometer 22
2.10.4 Purpose of fractional distillation 22
2.10.5 Infrared Spectrphotometer 23
2.11 Factorial Design 24

CHAPTER THREE

3.0 Equipment and Instrumentation 25

3.1 Steam Distillation Unit 25
3.2 Soxhlet Apparatus Unit 26
3.3 Fractional Distillation Unit 27

CHAPTER FOUR

4.0 Experiment Procedures 28
4.1 Extraction Using Solvent 28
4.2 Soaking Method 28
4.3 Steam Distillation 28
4.4 Maceration 29
4.5 Perfume Formulation 29
4.5.1 Selection of Component for Formulation 29
4.5.2 Experimental Design (Factorial Design) 30
4.5.3 Making the Hair Cream 31
4.5.4 Statistical Analysis 31
4.6 Fractional Distillation 31
4.7 Infrared Spectroscopy 31
4.8 Gas Chromatograph 32
4.9 Determination of Moisture Content 32
4.10 Determination of Specific Gravity 32
4.11 Determination of Refractive Index 33
4.12 Determination of Viscosity 33
4.13 Determination of Peroxide 33
4.14 Determination of Acid Value 33
4.15 Determination of Ester Content 34

CHAPTER FIVE

5.0	Results and Discussion	35
5.1	Extraction	35
5.2	Steam Distillation	36
5.3	Fragrance of Perfume Formulations	36
5.4	Physico - chemical Characteristics	39
5.4.1	Fractional Distillation	39
5.4.2	Gas Chromatography	40
5.4.3	Infrared Spectroscopy	45
5.4.4	Other physico-chemical Characteristics	51
5.5	Selling Price Calculation	52

CHAPTER SIX

6.0	CONCLUSION	54

CHAPTER SEVEN

7.0	RECOMMENDATION	56
REFERENCES		57
APPENDIX		58

LIST OF TABLES

Table		Page
1	Perfume Composition	3
2	Properties of Synthetic Apple essence	8
3	Properties of Synthetic Banana essence	8
4	Hair Cream Components	18
5	Levels of Blending	30
6	Analysis of Variance Result	31
7	Mean of Fragrance Value for each Level of Composition	36
8	Percentages of Components in the Chromatograms	37
9	Major Peaks Produced from IR Spectra of both Perfumes	39
10	Other Physico-chemical Characteristics of the best Apple perfume and the commercial apple Perfume	40
11	Selling Price Calculation for the best Apple Perfume	46

LIST OF FIGURES

Figure		Page
1	Block diagram of a Gas chromatograph	22
2	Steam Distillation Set-up	25
3	Soxhlet Apparatus Set-up	26
4	Fractional Distillation Set-up	27
5	Yield of Pear Oil as a function of Time Using Soxhlet Apparatus	34
6	Yield of Pear Oil as a function of Time using Soaking Method	34
7	Yield of Orange Oil as a function of Time Using Steam Distillation	35
8	Volume of each Distillate at various Temperatures	39
9	Gas Chromatogram of Pure isoamyl Valerate	40
10	Gas Chromatogram of Ester present in Commercial Apple Perfume	40
11	Gas Chromatogram showing Hydrocarbon Content of best Apple Fragrance.	41 41
12	Gas Chromatogram showing Hydrocarbon Content of Commercial Apple Fragrance	42 42
13	Gas Chromatogram of Paraffin Oil	43
14	IR Spectrum of Commercial Apple Perfume	44
15	IR Spectrum of the best Apple Perfume	44
16	IR Spectrum of first Distillate of Commercial Apple Perfume	45
17	IR Spectrum of second Distillate of Commercial Apple Perfume	45

18	IR Spectrum of third Distillate of Commercial Apple Perfume	46
19	IR Spectrum of fourth Distillate of Commercial Apple Perfume	47
20	IR Spectrum of fifth Distillate of Commercial Apple Perfume	48
21	IR Spectrum of Paraffin Oil.	48
22	Pie Chart Showing the Breakdown of the Total manufacturing Cost/annum of the best Apple Perfume	50

CHAPTER ONE

1.0 INTRODUCTION

Commercial apple perfume is a fragrance concentrate with an apple-like fragrance. It is the most successful fragrance used in most Nigerian cosmetic industry for the manufacture of hair creams such as Emily millionaire, soul mate and apple products.

Apple perfume is currently imported into the country from countries like Germany and India and sold at very exorbitant rates. There is no local production. Fragrance concentrates are made from a mixture of natural and synthetic esters that are contained in a carrier. The raw materials for the production of these concentrate can be obtained from fruits such as orange fruit (essential oil from the peels), pear fruit, date palm fruit, lime fruit, quince fruit and the pineapple fruit. The carrier of the apple fragrance which can be sourced locally can be vegetable oils, Jojoba oil, mineral oil and paraffin oil. Hence, there is a need to develop this product locally for import substitution. The resultant local manufacture of the product will also provide gainful employment and develop the Nigerian economy.

So far, no work is known to have been done on the formulation of the fragrance concentrate in this country. There is also no available information in the open literature on the apple fragrance concentrate in spite of the fact that the product is being manufactured commercially in foreign countries. Many of the materials that can be used for formulating the concentrates such as essential oils and fixed oils have been well studied (Wells and Marcel, 1994). There is considerable literature on the formulation of alcohol based perfumes (Shreves, 1984) which can serve as a guide for the formulation of the oil based hair cream fragrance.

The main objectives of the project are to extract natural ester from the pear fruit and orange fruit to formulate and characterize the fragrance concentrate from natural esters and imported synthetic esters with a view to developing a commercially competitive fragrance concentrate.

Different methods of extraction will be used to produce the natural esters. Imported synthetic esters, local additives and the natural ester will be used to formulate and develop the best fragrance concentrate. The physico-chemical analysis of the best apple perfume will be carried out and compared to that of the commercial product.

CHAPTER TWO

2.0 SURVEY OF LITERATURE

2.1 History of Perfumes

If it were possible to delve into the past at a sufficiently remote period, it will probably be found that the romance of perfumery has its beginning with the Atlantians who flourished at period conjectured to antedate the Christian era by about 23,000 years. The Chinese have been the forerunners of western civilization, and although they are believed to have conquered the aboriginal tribes that inhabited that part of Asia sometime during the third millennium B,C., little is known about their history before 800BC. It is therefore necessary to turn to Egypt for the earlier records of perfumery (Poucher, 1974).

The manufacture of perfumes, colognes and toilet waters collectively known as fragrances has undergone drastic changes in the past quarter century. Perfume takes its name from the Latin word 'perfumer' (to fill with smoke); since in its original form it was incensed and burned in Egyptian temples (Shreves, 1984).

The precise formulas of commercial perfumes are kept secret. Even if they were widely published, they would be dominated by such complex chemical procedures and ingredients that they would be of little use in providing a useful description of the experience of a scent, Nonetheless, connoisseurs of perfume can become extremely skilful at identifying components and origins of scents in the same manner as wine experts (Burr, 2003). The most practical way to start describing a perfume is according to its concentration level, the family it belongs to, and the notes of the scent, which all affect the overall impression of a perfume from first application to the last lingering hint of scent.

2.2 Basic Constituents of a Perfume

A perfume may be defined as any mixture of pleasantly odorous substances incorporated in a vehicle. Formerly, practically all the products used in perfumery were of natural origin. Even though when humans started synthesizing materials for use in this field, they endeavoured to duplicate the finest in nature (Shreves, 1984). The finest modern perfumes are neither wholly synthetic nor completely natural. The best product of the art is a judicious blend of the two in order to enhance the natural perfume and to reduce the price. An actual example of a compounded perfume similar

to widely sold products is given in Table 1. The formulation odors are from eugenol, methyllinone, and bergamot oil (Shreves, 1984).

Table 1 Perfume Composition.

Components	Quantity
Essential oil:	
Sandalwood oil	10
Bergamot oil1	17.5
Yland-Yland oil	40
Petitgrain oil	10
Rose otto	15
Jasmine absolute	20
Isolate:	
Eugenol (from clove oil)	90
Santanol (from sandalwood oil)	15
Semi Synthesis:	
Isoeugenol (from eugenol)	110
Heliotropin (from satrole)	15
Methyl ionone (from citral)	237.5
Synthesis:	
Coumarin	27.5
Vanillin (from gaiacol)	20
Benzyl acetate	30
Gloeresin, opapanax	2.5
Balsam (rosmoid):	
Tolu	5
Peru	7
Benzion	70
Animal Fixative Castor Tinture	12.2
Synthetic fixatives:	
Musk ketone	32.5
Musk ambrette	12.5
Vehicle, ethyl alcohol	450kg

This perfume formulation serves as a guide for any perfumer. However, the carrier used was alcohol. This research will use an oily carrier as its vehicle.

2.3 Fragrance Notes

Perfume is also described generally in a musical metaphor as having fragrance ‘notes’, making the harmonious chord of the scent. The notes unfold over time, with the immediate impression of the top note leading to the deeper middle notes, and the base notes gradually appearing as the final stage. These notes are created carefully with knowledge of the evaporating process of the perfume (Burr, 2003).

2.3.1 Top Notes

The scents are perceived immediately on application of a perfume. Top notes consist of small, light molecules that evaporate quickly; they are the initial impression of a perfume and are thus very important in the selling of a perfume. The scents of this note class are usually described as ‘fresh’, ‘assertive’ or ‘sharp’. The compounds that contribute to top notes are strong in scent, very volatile and evaporate quickly. Citrus and ginger scents are common top notes. They are also called head notes (Burr, 2003).

2.3.2 Middle Notes

These are the perfume that emerges after the top notes dissipate. The middle note compounds form the ‘heart’ or main body of a perfume and act to mask the often unpleasant initial expression of base notes, which become more pleasant with time. Not surprisingly, the scent of the middle note compounds is usually more mellow and ‘rounded’. Lavender and rose scents are typical middle notes or the heart notes (Burr, 2003).

2.3.3 Base Notes

The scent of a perfume that appears after the departure of the middle notes are called base notes. The base and the middle notes together are the main theme of the perfume. Compounds of this class are often the fixative used to hold and boost the strength of the lighter notes. Consisting of large heavy molecules that evaporate slowly, base notes, particularly the sources are detectable in excess of 24h after application (Burr, 2003).

The major constituents of perfumes can be classified under the following:

1. The vehicle
2. The fixative

3. The odiferous elements

2.3.4 The Vehicle

Perfume oil is necessarily diluted with a solvent because undiluted oils (natural or synthetic) contain high concentrations of volatile components that will likely result in allergic reactions and possibly injury when applied directly to the skin or clothing.

By far the most common solvent for perfume oil is ethanol or a mixture of ethanol and water. Perfume oil can also be diluted by means of neutral smelling lipids such as jojoba, fractionated coconut oil or wax (Burr, 2003).

The vehicle of the perfume helps it to project its scent. The vehicle is most times deodorised if any slight odour is perceived in it. Until recently the vehicle used as bases for blended perfumes in the 17^{th} century and 18^{th} century was in the classes of oils, pomades and powder (Wells and Marcel, 1994). The modern vehicle now being alcohol is predominantly used in the perfume industry**.**

2.3.5 The Fixative

In ordinary solution of perfume substances in alcohol the more volatile materials evaporate. Fixatives retard evaporation of the odorous constituents. The types of fixatives available are; animal secretions, resinous products, essential oils and synthetic chemical (Shreves, 1984)

2.3.6 The Odiferous Elements

2.3.6.1 Plant Sources

Plants have long been used in perfumery as a source of essential oils and aroma compounds. These aromatics are usually secondary metabolites produced by plants as protection against herbivores, infections, as well as to attract pollinators. Plants are by far the largest source of fragrance used in perfumery. The sources of these compounds may be derived from various parts of the plant. A plant can offer more than one source of aromatics, for instance the aerial portion of seeds of coriander have remarkably different odours from each other. Orange leaves, blossoms, and fruit zest are the respective sources of petit grain, neroli, and orange oils (Fleisher, 1993). The following plant parts are sources of odiferous elements .

i. Bark: commonly used barks include cinnamon and cascarilla. The fragrant oil in sassafras root bar is also used either directly or purified for its main constituent, safrole, which is used in the synthesis of other fragrant compounds such as helional.

ii. Flowers and blossoms: These are undoubtedly the largest source of aromatics. These include the flower of several spices of rose and jasmine, as well as osmanthus, mimosa, and the blossoms of citrus ylang-ylang trees.

iii. Fruits: Fresh fruits such as apples, strawberries, and cherries unfortunately do not yield the expected odours when extracted; if such fragrance notes are found in perfumes, they are synthetic. Notable exceptions include litsea cubeba, vanilla, and juniper berry. The most commonly used fruits yield their aromatic sources from the rind; they include citrus such as oranges, lemons, limes and grape fruits.

iv. Leaves and twigs: Those commonly used for perfumery are lavender , patchouli, sage , violets, rosemary and citrus leaves. Some times leaves are valued for the green smell they give to perfumes.

v. Resins: Resins are valued since antiquity and have been widely used in incense and perfumery. Highly fragrant and anti-septic resins containing perfumes have been used by many cultures as medicines for a large variety of ailments. Commonly used resins in perfumery include labdanum, frankincense/libanum, myrrh, Peru balsam and gum benzion. Pine and fir resins are a particularly valued source of terpenes used in the organic synthesis of many naturally occurring aromatic compounds (Burr, 2003).

2.3.6.2 Animal Sources

Animal sources of odiferous elements include;

i. Ambergris: Lumps of oxidized fatty compounds, whose precursors were secreted and expelled by the sperm whale. Ambergris is commonly referred to as ‘amber’ in perfumery.

ii. Castoreum: Obtained from odorous sacs of the North American beaver

iii. Civet: Also called civet musk, this is obtained from odorous sacs of civets, animals in the family viverridae.

iv. Honeycomb: Distilled from the honeycomb of the honeybee.

v. Musk: Originally derived from the musk sacs from the Asian musk deer; it has now been replaced by the use of synthetic musk (Burr, 2003).

2.3.6.3 Synthetic Sources

Modern perfumes are almost exclusively made from synthetic odorants that are commonly synthesized from petroleum distillates, pine resins or relatively cheap organic feedstock. Synthetics can provide fragrances which are not found in nature. For instance, calone, a synthetic compound imparts fresh ozonous metallic marine scent that is widely used in contemporary perfumes. Synthetic aromatics are often used as an alternate source of compounds that are not easily obtained from natural sources. For example, linalool and coumarin are both naturally occurring compounds that can be cheaply synthesized from terpenes. Orchid scents (typically salicylates) are usually not obtained directly from the plant itself but are instead synthetically created to match the fragrant compounds found in various orchids.The majority of the world's synthetic aromatics are created by relatively few companies. They include; International Flavour and Fragrances (IFF), Firmenich (Switzerland), Takasago (Japan) , Symrise (Singapore), Mane SA (France).

2.4 Characteristics of Some Selected Fruits

2.4.1 Avocado

The avocado *(persea americana)* belongs to the family lauraceae, a family of mainly tropical trees and shrubs. Other well-known members are laurel, cinnamon, sassafras and greenheart. Avocado is a native of tropical America. It is a shallow-rooted evergreen tree that grows up to 20 m tall. Its leaves are simple, ovate and spirally arranged. The avocado fruit is generally pear-shaped with a large, round to egg shaped central seed. The flesh is buttery in texture and contains a high percentage of oil (18-42 %) and protein and has a high calorific value. Avocado is common through the tropics and subtropics (Martin and Cambell, 1987). The edible pulp of the avocado, which surrounds the seed, contains about 30% of non-drying oil. The oil is separated by dehydrating the pulp, then pressing or extracting with solvents. The oil is used in cosmetics (Martin and Cambell, 1987).

2.4.2 Sweet Orange

Sweet Orange *(Citrus sinenesis)* originated from Southern China to Portugal, about 450 years ago. The main constituent, quantitatively, of expressed sweet orange is limonene, which occurs to the extent of 90 %. The decyl aldehyde content ranges from 1 to 2.5 %. Orange oil is obtained by expression or distillation of the peel of the fruit in yields of 0.3%. The oil is used in making soaps, air fresheners, drinks, flavourings, cosmetics, lotions, and perfumes (Wells and Marcel, 1994).

2.4.3 Apple Essence

Fresh fruits such as apple, strawberries and cherries unfortunately do not yield the expected odours when extracted; if such fragrance notes are found in perfume, they are synthetic. Synthetic apple essence also known as isoamyl valerate is produced by the esterification reaction of isoamyl alcohol and valeric (pentanioc) acid. Its synonyms are isoamyl 3-methyl butanoate, isoamyl 3-methyl butyrate , isoamyl 3-methyl pentanoate e.t.c. Its molecular weight is 172, with formula $C_{10}H_{20}O_2$ and structure $(CH_3)_2CHCH_2CO_2CH_2CH_2CH(CH_3)$. Synthetic apple essence readily blends well with paraffin oil, alcohol, and fixed oils (Emil, 1991). The properties of Isoamyl valerate are shown in Table 2.

Table 2 Properties of Isoamylvalerate

Odor type :	Fruity
Odor strength:	At 100% sweet fruity green ripe apple.
Substantivity:	<3 hours at 100%
Appearance:	colourless clear liquid
Assay:	98.00-100%
Specific gravity :	0.85100-0.85700 @ 25.00 °C
Refractive index:	41100-1.41400 at 20.00 °C
Boiling point:	191.00-193.00 °C at 760.00 mm

2.4.4 Bannana Essence

Isoamyl acetate, also known as isopentyl acetate, is an organic compound that is the ester formed from isoamyl alcohol and acetic acid. It is a clear colorless liquid that is only slightly soluble in water, but very soluble in most organic solvents. Isoamyl acetate has a strong odor (similar to juicy fruit) which is also described as similar to banana. Banana oil is a term that is applied either to pure isoamyl acetate or

to flavorings that are mixtures of isoamyl acetate, amyl acetate, nitrocellulose and other flavors (Emil, 1991). The properties of Isoamyl acetate are given in Table 3.

Table 3 Properties of Isoamylacetate

IUPAC name	3-methy-1-butyl acetate
Molecular formula	$C_7H_{14}O_2$
Molar mass	130.19 g/mol
Melting point	-78 °C
Density	0.876 g/cm^3
Boiling point	142 °C

2.5 The Separation of Natural Odorous Materials

Natural odorous plants can be separated using the following processes:

- Distillation (rose, lavender, neroli etc)
- Expression (citrus fruits)
- Extraction by means of (a) enfluerage, (b) maceration, (c) volatile solvents.

2.5.1 Extraction With Cold Fat

Certain plants continue physiological activities of developing and giving off perfumes even after picking. Fat possesses a high power of absorbtion and if brought in contact with fragrant flowers, readily absorbs the perfume emitted. Application of this process is called Effleurage (Naves and Mazuyer, 1974). A high mixture of highly purified tallow and lard is used for this purpose. Fat should be odourless and of proper consistency. Benziom and alum are added to preserve the fat from becoming rancid. A rectangular wooden frame called chassis is fitted with a glass plate upon both sides of which the fat is applied. When piled one above the other, the chassis form air tight compartment. Freshly picked flowers are strewn over the surface of fat and left there for twenty four hours, then replaced by fresh flowers. The fat is not renewed during the process which lasts for about 70 days, and becomes quite saturated with flower oil. The oil saturated fat, called pomade, is extracted with high proof ethyl alcohol. The extract is kept in a refrigerator below freezing point, preferably at -15 ^{0}C. The dissolved fat separates out and is removed by filtration.

Ethyl alcohol is removed completely by distillation at reduced pressure. The residue is called ‘absolute’ (Wells and Marcel, 1981).

2.5.2 Extraction With Hot Fat (Maceration)

The essence in plants can be extracted using this method. The plant is immersed in hot oil and strained. The hot oil is treated systematically with several batches of fresh plant materials until the oil becomes saturated with its perfume. This is also known as pomade. Alcohol extracts and absolute are prepared from pomade in the similar manner as in cold process (Wells and Marcel, 1981).

2.5.3 Extraction With Volatile Solvents

Fresh plant materials are extracted with purified solvent, which dissolves perfume along with some quantity of waxes, and albuminuos and colouring matters. The extract is concentrated in reduced pressure to yield plant oil or concrete. Examples of oils that are extracted using this method are: pear fruit oil, melon seed oil and almost all seeds containing oils (Adhikary and Amatya, 1989). The desired properties of a solvent for such extraction are as follows:

- non-reacting towards essential oil;
- low-boiling point;
- high volatility;
- high selectivity for the desired product;
- reasonably cheap and pure;
- immiscible with water;

None of the solvents available fulfil these criteria completely. Commonly used solvents are petroleum ether, hexane dichloromethane, benzene, acetone and ethyl alcohol (Wells and Marcel, 1981).

2.5.4 Soaking Method

This is a scientific method where the plant material is soaked for a long time in the solvent and later separated and recovered. This process takes a lot of time but it has the advantage of producing oil in a larger quantity**.** The solvent used for this method must satisfy the general characteristics of solvents (Bryon et al, 1960).

2.5.5 Extraction With Liquefied Gases (Super Critical Fluids)

Extraction of natural flavours and essential oils from plant materials using gases like carbon-dioxide as solvent is a recent development of great importance. Presence of solvent residues in the final product has been a problem with the conventional solvent extraction process. Liquid carbon dioxide near its critical point (9731 x 10^4 N/m^2 pressure and 31 ^0C) has been found to be an efficient solvent for extracting a number of natural products (Adhikary and Amatya, 1989). Being inert and non-toxic, carbon dioxide poses no problem of objectionable residues. The possibility of fire hazard with use of volatile solvents is totally eliminated when carbon dioxide is used.

2.5.6 Expression

There are three main processes for the separation of so-called citrus oils from peels of lemon, orange, bergamot, and lime. These methods may include the following;

(a) Sponge process.
(b) Ecuelle method.
(c) Machine process.

2.5.5.1 The sponge process

The oil cells of the rind of the above fruits are easily broken by turning a piece of lemon peel backwards. This process on a large scale, therefore does not offer any serious difficulty, nor does it require very heavy pressure for the extraction of the oil (Esau, 1977).

2.5.5.2 The Ecuelle method

The Ecuelle method is practised more in Italy, and consists of rolling the fruits about in hollow vessels, the walls of which are covered with spikes. The oil cells are punctured, and the liquid flows to the bottom, being collected in a receptacle situated in the handle of the vessel. The product is then clarified (Esau, 1977).

2.5.5.3 Machine method

Another hand machine consists of two channels between which the fruit is rolled; the skin is lacerated by means of spikes, and the mixture of the oil and juice is collected and subsequently separated and clarified as mentioned above (Denny, 1991).

2.6 DISTILLATION

2.6.1 Water Distillation

The principle of water distillation is to boil a suspension of an aromatic plant material and water so that its vapour can be condensed. The oil, which is immiscible with water, is then separated. Most water distillations are performed in areas where no access to steam boiler is possible, or if a satellite boiler is available it complicates the process of oil production. In water distillation the plant material is always in direct contact with water. An extremely important factor is that in stills where the water is boiled by direct contact with fire, the water present in the still must always be more than enough to last through out the distillation; otherwise the plant material can be over-heated and char. When this happens, depending on the plant material charge, off-notes are formed from Maillard reactions, strecker degradation and eventually the pyrolitic degradation of the material. As a result, the oil can assume a variety of off-notes, generally known as still notes (Denny, 1991).

2.6.2 Steam and Water Distillation

Steam and water distillation is a widely used process in rural areas, as it does not require a great deal more capital expenditure than water distillation. Also the design of the equipment that is used is generally very similar to that used in water distillation. Only the plant material is supported above the boiling water on a perforated grid. In fact it has become the traditional progression from water distillation to water and steam distillation.

2.6.3 Cohobation

Cohobation is a procedure that can only be used in water distillation or steam and water distillation. It uses the practise of returning the distillate water to the still after the oil has been separated from it so that it can be re-boiled. The principle behind it is to minimize the losses of oxygenated components, particularly phenols which will dissolve to some extent in the distillate water (Denny, 1991).

2.6.4 Steam Distillation

As the name suggests, steam distillation is the process of distilling plant material with steam generated outside the still in a satellite steam generator generally referred to as a boiler. As in steam and water distillation, the plant material is supported on a perforated grid above the steam inlet. A real advantage in the satellite steam generation is that the amount of steam can be readily controlled. Because steam is generated in a satellite boiler, the heat contact of the plant material will be no higher than 100 °C. Consequently the amount of heat which the plant material will come in contact is acceptable and should not cause any thermal degradation of it. The process of steam distillation is the most widely accepted process for the production of essential oils on a large scale (Kahol, 1982).

An obvious drawback to steam distillation is the much higher capital expenditure needed to build such a facility. In some situations such as large scale production of low cost oils such as rosemary, Chinese cedar wood, lemongrass spike lavender, eucalyptus, citronella oils, etc, the world market prices of the oils are barely high enough to allow them to be produced by steam distillation without amortizing the capital expenditure required to build the facility over a period of 10 years or more. To further discuss steam distillation, it is prudent to examine the process by describing the distillery design (Denny, 1991). Examples of essential oils produced by steam distillation include,

1. The allspice (pimenta berry) allspice oil: the yield of oil is 3.3-4.3%; its main constituent is eugenol. This oil is used to flavour pickles and spicy table sauces.
2. Anise oil: This oil is obtained by steam distillation using the dried ripe fruit, its yield is 1.9-3.1% and contains 90% of anethole.
3. Ginger oil: This is produced from the steam distillation of the dried rhizome with a yield of 1.5-3.0%. Ginger oil is used primarily as a flavour in bakery goods, cakes, ginger snaps, and soft drinks of the ginger-ale type and in condiment mixtures.
4. Rose oil: This oil is also produced by steam distillation of its fresh flowers (Rosa cent folia l.). The yield of the oil is between 0.02-0.03%. The oil is used primarily as a high grade fragrance in cosmetics, toilet waters, and perfumes. It is used also in the flavouring of tobacco (Harold and Clarke, 1979).

2.6.5 Oil Isolation Fundamentals

For the oil phase to change from the liquid phase to the vapour phase, it must receive latent heat such as, within a field still, could only come from condensing steam. Consequently, the temperature of the steam within the still must be higher than the temperature at which the oil boils in the presence of water on the surface of the charge or plant material, otherwise there would be a temperature gradient to take the latent heat from the condensing steam to vaporise the oil droplet (2^{nd} law of thermodynamics).

This follows Fourier's law that states that heat is conducted from one surface to another surface at a rate proportional to their contact area. This difference in temperature is the temperature gradient noted above. Obviously the thermal conductivity of a material is a critical factor in the heat transfer (Denny, 1991).

On the surface of the charge, the concentration of the vapour of the oil at its evaporation point, and the proportion of the total ambient pressure it exerts will be at its minimum, which is appropriate to saturation at the prevailing temperature. The boiling temperature of two immiscible liquids (one being water) will be lower than the boiling point of the two liquids individually. In fact it will be the lowest that can produce a boiling liquid (mixture of oil and water) under the prevailing pressure.

During distillation within a still the oil vapour is dispersed throughout the steam vapour as a result, the concentration of oil vapour/ water vapour is reduced so that the proportion of the total vapour pressure that the oil exerts is much less because in this situation it is present in a less than saturated condition. Consequently the share of pressure that the steam must exert within the still is even greater than that which it exerted at the surface of the charge .The temperature gradient within the vapour space in the still and the evaporation point on the charge is the principle of the distillation process (Denny, 1991).

The magnitude of the transference of latent heat from the condensing steam to the evaporating oil governs the rate at which the oil will evaporate from the charge surface. In practise, to isolate oil by steam distillation under controlled conditions, there is a point above which the oil to water vapour concentration cannot be surpassed (Kahol, 1982).

Any further enrichment of the mix in oil vapour would result in a reduction in temperature and a reduction of the gradient, i.e. less heat would get to the oil , the rate of evaporation would be reduced until a new balance is reached, after which the

optimized rate of oil isolation would be restored. This is referred to in automatic control system as feedback. Furthermore, several factors could have an effect on the temperature gradient and correspondingly the ratio of oil to water in the condensate (Denny, 1991).

The factors that affect temperature gradient at which feedback controls the oil/ water ratio in the condensate are:

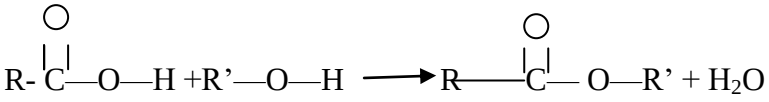
1. The amount of steam required to pass through a charge depends on the amount of oil to be isolated (the oil yield) not on the mass of the charge.
2. The varied action of different charge surfaces with the wetness action carried out by saturated steam (the ability of the charge to remove the saturation from the steam)
3. Steam cloud particles (in saturated steam) can roll up coatings of oxygenated compounds when they impinge on the surface of the charge by hydrophilic bonding.
4. The steam can preferentially solubilize to a limited extent the more polar oxygenated compounds.

2. 7 SYNTHETIC PRODUCTION OF ESSENTIAL OILS

Essential oil could be produced synthetically by combining a variety of different alcohols and carboxylic acids. An ester is a chemical compound that is formed when an organic acid reacts with an alcohol. Esters are derivatives of carboxylic acids and are mainly prepared by one of the four methods (Emill, 1991).

- ❖ Direct esterification of carboxylic acid with an alcohol.
- ❖ Alcoholysis of acid chlorides, anhydrides, nitriles.
- ❖ Reaction of carboxylic acid salt with an alkyl halide or sulphate.
- ❖ Via the trans-esterification reaction.

Esters frequently have distinctive odours, and are found in the flavourings of many fruits and plants. The reaction between an organic acid and an alcohol is:



(1)

In the diagram, R and R' represents organic groups such as hydrocarbons. The –H group from the acid combines with the –OH from the alcohol producing water molecules. The R'-O- group from the alcohol then attaches to carbon on the acid

forming the ester. Adding some concentrated sulphuric acid, H_2SO_4, catalyses the reaction. Concentrated sulphuric acid is a strong dehydrating agent, and helps the reaction by removing the water molecules formed. If acetic acid and methanol are reacted, the product is called methylacetate.The systematic name for acetic acid is called ethanoic acid and the product is also known as methylethanoate.

$$CH_3\text{-}COOH + CH_3OH \longrightarrow CH_3\text{-}COOCH_3 + H_2O \qquad (2)$$

If acetic acid and isopentylalcohol are reacted, the product is called isopentylacetate (Banana oil). Reacting acetic acid and 1-propanol, the product is called propylacetate (pear oil). Reacting acetic acid and 1-octanol the product is called octylacetate (orange oil). Reacting propionioc acid and isobutyl alcohol the product is called isobutylpropionate (rum oil) (Emil, 1991).

2.8 OIL BASE FORMULATIONS

2.8.1 Jojoba Oil as Base Oil

Thorough studies have shown that Jojoba oil is non-irritating to the eyes, skin and hair. The skin rapidly absorbs the Jojoba oil, thus pores and hair follicles remain open and can function freely. Another remarkable property is that Jojoba oil is non-greasy and leaves no oily after-feel.

The following perfumes were formulated using Jojoba oil as its base.

1. Kenzo**:** The summer meadows filled with violets, vanilla and rose;
2. Paris: the essence mimosa, orange flower, rose, moss, sandalwood and amber;
3. Opium: An oriental fragrance of rose, carnation sandalwood, lilly of the valley and clove;
4. Eternity: This is a perfume and is refreshing , made of white lily, marigold mandarin, sandalwood fragrance;
5. Amber romance**:** Warm alluring blends of black cherry, creme anglaise and sandalwood;

   Love Spell: A citrus fragrance with grape fruit and clementines, also apple of peach and blackcurrent;
6. Angel**:** A refreshing, oriental, woody fragrance with a blend of vanilla, sandalwood and patchouli;.
7. Jade Rose**:** Notes of fresh citrus, melons, peaches and plums;

8. Witching Hour**:** Warm alluring blends of black cherry, creme anglaise and sandalwood.□

Sequim lavender Farms also produced a hair cream fragrance made up of lavender and orange essence.

2.8.2 Synthetic apple oil

Synthetic apple oil was invented using the constituents in Table 4. (Federick and Chestnut, 1996).

Table 4 Constituents of synthetic apple oil

Constituents	Parts
Isoamyl ester of formic acid	10
Isoamyl ester of acetic acid	10
Isoamyl ester of normal caprioc acid	5
Acetaldehyde	2
Geraniol	1
Geranyl ester of formic acid	1
Geranyl ester of acetic acid	1

The above mentioned constituents are given in parts per volume and they should be employed in the purest possible state (Federick and Chestnut, 1996).

2.9 QUALITY ASSESSMENT TECHNIQUES

Quality assessment of essential oils can be done by the following analysis; sensory evaluations, physical tests, chemical tests and instrumental techniques. Quality judgement of an essential oil should be based on the combined data obtained by the above analysis (Baser, 1992).

2.9.1 Sensory Evaluations

This can be carried out only by expert noses. Such ability can be gained after years- long tedious but systematic olfactive training. To an experienced perfumer, the evaporation pattern of an essential oil smeared on a smelling strip, over a period of time, gives information about its source, age, main components and even its authenticity. Smelling must be carried out at intervals immediately after dipping for, 2 and 6 h, and after standing over night for a period not less than 18 h. Comparison

with the authentic sample of acceptable quality will help the assessor to make a correct judgment. As obvious, sensory evaluations are subjective and may vary from person to person. Therefore, such assessments are, in general, realized by a panel of experts and in all cases their assessments should be verified and documented by experimental proof (Adhikary and Amatya, 1989).

2.9.2 Physical Tests

The following physical tests are conducted: moisture content, specific gravity, viscosity and refractive index

2.9.2.1 Moisture content

The determination of moisture content in an essential oil can be done by karl-Fischer titration, gas chromatographic, spectroscopic or electrometric methods. Drying of an essential oil is accomplished by the addition of or the filtering through desiccating agents such as anhydrous sodium sulphate or copper anyhdride. A simple way to check the presence of moisture in an essential oil is carried out by mixing 0.5 ml essential oil with 1 ml carbon disulphide. A clear solution indicates the absence of moisture (Adhikary and Amatya, 1989).

2.9.2.2 Specific gravity

The specific gravity of an oil is the weight of a given volume of the oil at a specified temperature compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air. A specific gravity bottle is used for this determination (Adhikary and Amatya, 1989).

2.9.2.3 Viscosity

Although all real fluids resist any force tending to cause one layer to move over another, the resistance is offered only while the movement is taking place. Thus when the external force is removed, the flow subsides because of the resisting forces, but when the flow stops the particles of fluid stay in the position they have and have no tendency to revert to their original positions. This resistance to the movement of one layer of fluid over an adjoining one is ascribed to the viscosity of the fluid. Since relative motion between layers requires shearing forces, that is, forces parallel to the surfaces over which the resisting forces must be in the exactly opposite directions and

so they also are parallel to the surfaces. It is a matter of common experience that, under particular conditions, one fluid offers greater resistance to flow than another. Such liquids as tar, treacle and glycerine that can not readily be poured or easily stirred, are commonly spoken of as 'thick'; on the other hand , so-called 'thin' liquids such as water, petrol and paraffin flow much more readily. Viscosity of fluid cannot be measured directly, but its value can be calculated from some equation relating it to quantities that are directly measurable. A piece of apparatus especially suitable for the necessary measurements is known as a viscometer e.g. the Ostwald viscometer invented by Wilhem Ostwald. When the fluid under test is a liquid the volume rate of flow, Q, may be determined simply by collecting and measuring the quantity passing through the tube in a certain time. For gases, however a special arrangement must be made for measuring the flow (Bryon et al, 1960).

2.9.2.4 Refractive index

The refractive index (n_D^{20}) of oil with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the oil. It varies with the wavelength of the light used in its measurement. Abbe type refractometers are widely used for the determination of the refractive index. The refractive index of perfumes falls within the range of 1.4550-1.479 (Bryon et al, 1960). The refractive index value gives the percentage purity of the sample.

2.9.3 Chemical Tests

Chemical tests includes: peroxide value, acid value and ester content.

2.9.3.1 Peroxide value

Peroxide value is the milliequivalent of iodine liberated per kilogram of oil. The peroxide value is used to indicate the extent or degree to which fats or oils have been oxidised when they are stored, Oxygen may be absorbed, leading to the formation of peroxides with prolonged storage and oxidative rancidity results (Kahol, 1982). Highly unsaturated oil can be oxidized to give peroxide linkages and such linkages can lead to massive polymeric frame work responsible for the tough protective coatings that remain after paint is dried.

2.9.3.2 Acid value

This is a numerical value equivalent to the number of milligrams of potassium hydroxide required to neutralize the free acids present in 1 g of the oil.

$$(RCOOH + KOH \longrightarrow RCOOK + H_2O) \quad (3)$$

This determination is of importance because the perfume is applied to the skin, which may be damaged by the acid.

2.9.3.3 Ester value

This is the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of the esters present in 1 g of the oil.

$$RCOOH^1 + KOH \longrightarrow RCOOK + H_2O \quad (4)$$

The determination of the ester value is of great importance in the evaluation of essential oils. It is so because most of the esters determine the pleasant odour of the oil (Adhikary and Amatya, 1989).

2.10 INSTRUMENTAL TECHNIQUES

Instrumental techniques include, gas chromatography, fractional distillation, and infrared spectrophotometer. They are described in this section.

2.10.1 Gas Chromatography

Chromatography is a molecular separation process in which a mixture is separated into its constituents by passing it through a fixed bed of adsorbent. In gas chromatography the sample is volatilized into an inert carrier gas stream which is sweeping through a column of sorbent. The sample components are detected and may also be collected as they emerge from the column as a series of discrete bands having varying degrees of separation. The process must be carried out at a temperature sufficient to maintain the sample in the vapour state. This is also appropriate to maintain a favourable distribution between phases. The sample components must be stable at this temperature (John and Williams, 1979).

2.10.2 Equipment for Gas Chromatography.

The process is carried out in a gas chromatograph consisting of three principal elements: a sample volatilization- injection system, a separation column, and a detection system with a means of readout that is capable of producing a

chromatogram (Figure 1). Each subsystem of the chromatograph may have its own temperature control system, permitting each to be operated at a different temperature.

The principal function of the sample introduction system is to introduce the sample as a discrete plug of gas into the carrier gas stream which is under several atmospheres of pressure. Gases are usually introduced into the system by means of a multiport sample valve which is equipped with a sample loop of predetermined volume. The loop may be switched out of the pressurized carrier loadings and the sample injected into it for injection (John and Williams, 1979).

The separation column consists of a stainless steel or a glass tube 6.35×10^{-3} m to 3.18×10^{-3}m in diameter, filled with a sorbtive packing consisting of small uniform sized particles. The packing can be either a high surface area adsorbent or an inert support coated with a non-volatile liquid adsorbent. Column length is chosen to provide the separation required. However, 1.87 m length is commonly used for typical applications. For difficult separations requiring very long columns, the liquid phase is distributed on the wall of small internal diameter open tubular column. This practice permits columns of great length (93.5 m) to be used without the need for excessive column head pressures. Under these conditions, sample size and injector size must be matched to the reduced volume of the stationary phase. This is accomplished by injectors which split the sample and direct only small fraction through the capillary column (Adhikary and Amatya, 1989).

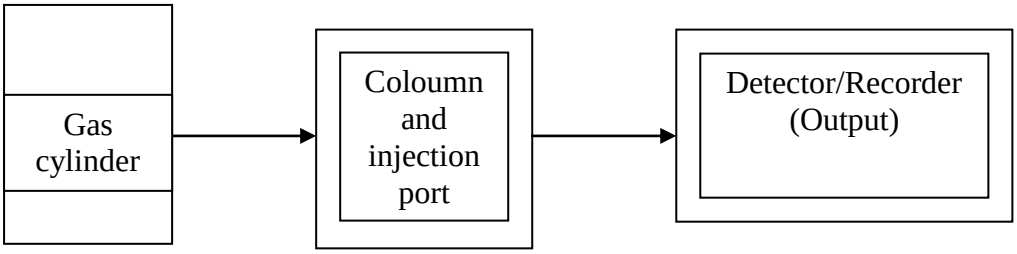


Figure1 Block diagram of a gas liquid chromatograph

2.10.3 Fractional Distillation

Fractional distillation is applied extensively in the field of essential oils both in the laboratory as well as in the industry (Adhikary and Amatya, 1989). One of the purposes of fractional distillation in the industry is to remove some of the components which have offensive odour and thus improve the smell of the product. Essential oils are a mixture of mono and sesquiterpenes with small quantities of solid residues

comprising of waxes and paraffin. Among the terpeniods some are hydrocarbons whereas others are oxygenated compounds having a variety of functional groups such as alcohols, phenols, esters, aldehydes, ketones, esters, lactones, etc. The hydrocarbon portion which is known in commerce as 'terpene' is the one which is mostly removed because it is the oxygenated fraction which has the desired smell. Once the terpene fractions is removed the 'terpeneless' fraction becomes more soluble in alcohol thus becoming more handy to be used in perfume formulations. Moreover preparing terpenless fraction has an added advantage of yielding a less bulky material for handling and transport. This is also known as the 'concentration'. The degree of concentration can be as high as fifty times such as in case of orange oil which has 2% oxygenated compounds and 98% 'terpenes' or can be quite low as in the case of proposed eucalypts camaldulensis oil cultivated in Sagarrnath forest in Nepal, where the percentage of cineole has to be raised from 60 to 70 % . Because of the presence of double bonds, terpenes are prone to oxidation and polymerisation; hence their removal also helps in improving the keeping quality of essential oils.

Another purpose for which fractional distillation is employed in the industry is the separation of important components for commercial use. Examples are eugenol from cloe oil, citronella from citronella oil, geraniol from palmrosa oil, citral from lemongrass oil, pinenas, careen and longifolene from turpentine. Many of these isolates are further converted to ionones and vitamin A, Pinenes and careen are used for perfumery compounds and terpene polymers are used in rubber and adhesive formulation. Fractional distillation can be used also for the analysing of perfumes using the differences in their boiling points and the different fractionates can then be individually characterised using the gas chromatograh, and the infra red spectroscopy. Thus, it can be said that fractional distillation is the backbone of the essential oil industry (Tuley de Silva, 1995).

2.10.5 Infrared Spectrophotometer

The energy of most molecular vibrations corresponds to that of the infrared region of the electromagnetic spectrum. Molecular vibrations may be detected and measured in an infrared spectrum. The most useful vibrations, from a point of view of the organic chemist, occur in the narrower range of 2.5-16 µm = (1µm=10^{-1} cm) which most infrared spectrometers covers. The position of an absorption band in the spectrum may be expressed in microns (µm). Or very commonly in terms of the

reciprocal of the wavelength, cm^{-1}. The usual range of an infrared spectrum is, therefore between 4000 cm^{-1} at the high frequency end and 625 cm^{-1} at the low frequency end (Barrow, 1962).

Functional groups have vibration frequencies characteristic of that functional group, within well- defined regions of this range; the fact that many functional groups can be identified by their characteristics vibration frequencies makes the infrared spectrum the simplest, most rapid and often most reliable means for assigning a compound to its class (Criddle and Ellis, 1976).

The spectrometer consists of a source of light emitting radiation throughout the whole frequency range of the instrument. The light is split into two beams of equal intensity, and one beam is arranged to pass through the sample to be examined. If the frequency of a vibration of the sample molecule falls within the range of the instrument, the molecule may absorb energy of this frequency from the light. The spectrum is therefore, scanned by comparing the intensity of the two beams after one has passed through the sample to be examined. The wavelength range over which the comparison is made is spread out in the usual way with grating or a prism. The whole operation is done in such a way that the usual finished spectrum consists of a chart showing downward peaks corresponding to absorption, plotted against wave length or frequency (Barrow, 1962).

Compounds may be examined in the vapour phase, as pure liquids, in solutions and in the solid state. In the vapour phase; the vapour is introduced into a special cell, usually about 10 cm long, which can then be placed directly in the path of one of the infrared beams. The end walls of the cell are usually made of sodium chloride, which is transparent to infrared.

2.11 FACTORIAL DESIGN

Factorial design is a statistical approach, which allows for simultaneous variation of process variables and more importantly all possible combinations of these variables at chosen levels of investigation. (Scheffe, 1989).

There are a number of statistical approaches that could be used depending on the number of possible combinations; examples of such are the Box Wilson experimental design, Bayesian experimental design, quasi experiment, Taguchi method, e.t.c. But the factorial experiment is suitable for these design because they are experiments whose designs consist of two or more factors, each with discrete possible values or “levels”. A full factorial design allows the studying of the effect of

each factor on the response variable, as well as the effects of interactions between factors on the response variable. For vast majority of factorial experiments, each factor has only two levels. The number of combinations,'N' of these process variables is given by $N = n^k$, Where 'n' stands for the number of possible levels of each variable and 'k' for the number of independent process variables that are to be considered for any particular experiment. The possible combinations are usually chosen by using the unifactor approach that is by varying one factor at a time while the other factors are fixed. The level that gives the best range of performance is chosen.

CHAPTER THREE

3.0 EQUIPMENT AND INSTRUMENTION

In this section the equipment for steam distillation, soxhlet extraction and fractional distillation are described.

3.1 STEAM DISTILLATION UNIT.

The steam distillation set-up is shown in Figure 2. An Utsav laboratory pressure cooker was used as the steam generator. It was connected via a delivery tube to a round bottomed flask containing the sample to be extracted. A manometer was attached to the delivery tube to control the pressure from the steam generator. The round bottomed flask was then connected to a condenser which condenses the hot vapours coming from the flask. The cooled vapour, now liquid, was collected in a beaker.

Figure 2 Steam Distillation set-up

3.2 SOXHLET APPARATUS UNIT.

The soxhlet apparatus set-up is shown in Figure 3. A round bottomed flask containing hexane was placed on a heating mantle. The column containing the sample was fitted into the flask. The top of this column was then attached to a condenser which cools the vapours in the column;

Figure 3 Soxhlet Apparatus

3.3 FRACTIONAL DISTILLATION UNIT.

The fractional distillation set-up is shown in Figure 4. A round bottomed flask containing the mixture was placed on a heating mantle. A packed column was fitted into this flask. Attached to this packed column was a thermometer which reads the temperature of the fractionate and a condenser which condenses the hot vapour. The cooled vapour was collected using flask.

Figure 4 Fractional Distilltion Set-up

CHAPTER FOUR

4.0 EXPERIMENTAL PROCEDURE

This section presents in details the experimental procedure carried out viz, soxhlet extraction, maceration, soaking method, steam distillation, material selection for apple perfume formulation, physico-chemical characteristics, gas chromatograph, and the infrared spectroscopy.

4.1 SOXHLET EXTRACTION

The pear fruit was obtained from Yola market. The subcutaneous layer was cut out with a knife and cut into about 1 cm^3 pieces. The pieces were dried in ambient air for 48 h. For each extraction 10 g of the dried pear fruit was weighed with an Ohaus electronic digital weighing balance model B500-00uk and placed in the thimble of the soxhlet extractor and the sample extracted with 100 ml analar grade hexane.

After extraction the solvent was distilled off and the oil in the flask was dried using a water bath and the flask with the oil was weighed. The extraction time for this experiment varied from 15 min to 1 h 15 min. This process was repeated until the liquid in the column siphoned back to the flask.

4.2 SOAKING METHOD

The pear fruit was obtained from Yola market. The subcutaneous layer was cut out with a knife and cut into about 1 cm^3 pieces. The pieces were dried in ambient air for 48 h. For each extraction 20 g of the dried pear fruit was weighed with an Ohaus electronic digital weighing balance model B500-00uk and soaked in a beaker with 100 ml of hexane (analar grade). After extraction the solvent was distilled off and the oil in the flask was dried using a water bath and the flask with the oil was weighed. The extraction time for this experiment was varied from 12 h to 72 h.

4.3 STEAM DISTILLATION

The following fruits were extracted using steam distillation. The orange fruit peels, apple fruit peels and the subcutaneous layer of the pineapple fruits were obtained from Yola market. The parts to be used for the extraction were cut out into about 1 cm^3 pieces with a knife. Only the apple fruit and the pineapple pieces were

dried at room temperature for 12 h. For each distillation, 100 g of the fruit was weighed with an Ohaus electronic digital weighing balance model B500-00uk and distilled using steam. The set-up of the steam distillation equipment is given in Figure 1 in the preceding chapter. The steam passed through the flask and extracted the essential oil in the fruit. The distillate was collected and mixed with 50 ml of hexane (analar grade). The mixture which now had two layers was separated with the aid of a separator. The essential oil contained in the solvent (hexane) was separated from the solvent by distilling off the solvent at a low temperature and drying the oil in a water bath. Then the flask and its content was weighed. The distillation time for these experiments varied from 15 min to 1 h, 15 min.

4.4 MACERATION

The following fruits were extracted using maceration: The banana fruit (peels), apple fruit peels and the subcutaneous layer of pineapple fruit. These fruits were obtained from Yola market and the parts to be used for the extraction purpose were cut out with a knife into about 1 cm^3 pieces. The apple fruit, banana fruit and the pineapple fruit were dried at room temperature for 12 h. Fifty grams of the fruit was weighed with an Ohaus electronic digital weighing balance model B500-00uk. For maceration 150 g of the oil (Bernuili vegetable oil obtained from Yola supermarket) was weighed and warmed to a temperature of 70 ºC in a beaker. The weighed fruit was immersed into the hot oil and the whole mixture was stirred and maintained at that temperature for 15 min after which the oil was separated from the sample and another fresh sample was introduced into the hot oil. This process was repeated until saturation of the oil was reached with the essence from each of the different fruits.

4.5 PERFUME FORMULATION

4.5.1 Selection of Component for Formulation

Based on the existing literature , the orange oil and the banana oil were used as the top note of the apple fragrance, the apple oil was used as the middle note (heart note) of the fragrance, while the pear oil and the carrier (paraffin oil) were used as the base note of the fragrance. A true perfume however should contain more materials but these few have been selected in order to reduce the large number of experiments. Results obtained from the extractions carried out showed that some fruits gave no significant yield of oil. These fruits include the banana fruit and the apple fruit.

Therefore the synthetic esters of these fruit fragrance were obtained to substitute their natural oils. These synthetic oils were products from the International Flavour and Fragrance Company in Germany.

4.5.2 Experimental Design (Factorial design)

For this design the number of compositional, variables C, were five. If four of the variables are varying, the 5^{th} one is therefore dependent. The number of independent variables (k) then reduce to C-1 = 4.

These variables were taken at three levels.

Therefore if $N = n^k$ (5)

Where k = Number of independent compositional variables.

n = Number of levels

N = Number of experiments

Substituting the number of compositional variables and the number of levels into Equation (1) gives $N = 3^4 = 81$ experiments. (6)

Therefore, in this research, 81 experiments were carried out.

Each bottle of fragrance was tested on a hair cream to make it easier to perceive the odour and to see its effect on the final product. Three levels of blending was used for each of the esters. Table 4 shows the different levels of each ester in percentage.

The table showing the total number of perfumes blended using different levels and different compositions is shown Table C.1 in appendix C.

.

Table 5 Levels of blending

Orange oil (o)%	**Banana oil(b) %**	**Apple oil(a) %**	**Pear oil (p) %**	**Levels**
0	10	20	0	$(O, B, A,P)_1$
2	20	40	1	$(O, B, A,P)_2$
5	30	60	3	$(O, B, A,P)_3$

4.5.3 Making the Hair Cream

A beaker of size 250 ml was put on a heating mantle, then the following were added: paraffin oil, very small quantity of colour to taste and then petroleum jelly in

that sequence. The mixture was stirred and allowed to heat to about 60 ºC. It was poured into a plastic container and allowed to cool to about
35 °C, then 1 ml of each of the 81 fragrances was added and the mixture was put aside and allowed to solidify.

Table 6 Hair Cream Components

Components	Quantity
Paraffin oil	3g
Petroleum jelly	7g
Colour	to taste
Best apple Fragrance	1ml

4.5.4 Statistical Analysis

The fragrance values obtained from each of the different compositions of perfume (Appendix C.1) were analysed using the ANOVA tool to test for its significance statistically. A post hoc result was obtained for each blend of the 81 bottles of perfume.

4.6 FRACTIONAL DISTILLATION

The commercial apple fragrance was obtained from the old Panteka Road in Kaduna State. A volume of 100 ml of this fragrance was measured using a measuring cylinder (100 ml) and distilled using a fractional distillation column. The set-up of the fractional distillatory is given in Figure 3. Each distillate was collected at different ranges of temperature. The 1st distillate was collected between 90-100 ºC, 2nd distillate between 100-140 ºC, 3rd distillate between 140- 219 ºC, 4th distillate between 219-252 ºC, and the 5th distillate between 252-300 ºC.

4.7 INFRARED SPECTROSCOPY

An infrared examination was carried out on the following fragrances a) the commercial apple fragrance, b) the best apple fragrance sample, c) liquid paraffin oil, d) fractionates of the commercial apple fragrance. For each oil, a drop was squeezed between flat plates of sodium chloride (transparent throughout the 4000 to 625 cm^{-1} region). The whole operation was done in such a way that the usual finished spectrum consisted of a chart showing downward peaks corresponding to absorption, plotted against the wave length.

4.8 GAS CHROMATOGRAPHY

One milligram of each perfume was injected into the Chrompak gas chromatograph by means of a multipart sample valve which was equipped with a sample loop of predetermined volume into the separation column. The column system was housed in an oven in which the temperature was held constant (programmed) during the elution. The flame ionization detector was used in this case. The GC operation was carried out under the following conditions.

Carrier gas	Nitrogen
Injection Volume	0.5 µl
Carrier pressure flow	10psi
Detector type	FID
Column type	Capillary HP column copper coat GL 5cb 50m * 0.31m
Elution order	lighter component to heavier component.

This analysis was carried out for the commercial apple fragrance and the best apple fragrance sample.

4.9 DETECTION OF MOISTURE CONTENT

A weight of 3 g of the perfume oil was poured into a beaker. One gramme of anhydrous copper sulphate was added to the oil, and the mixture was stirred and observed for any change in colour. A colour change from white to blue indicated the presence of water in the perfume.

4.10 DETERMINATION OF SPECIFIC GRAVITY

The density of oil was determined using a density bottle. The stoppered bottle was weighed (W_1). The bottle was then filled with distilled water, stopered and weighed (W_2). The same bottle was dried and filled with the perfume covered with the stopper and weighed (W_3). The specific gravity of the substance is given by.

$$S.G = (W_3 - W_1)/(W_2 - W_1) \quad (7)$$

4.11 DETERMINATION OF THE REFRACTIVE INDEX

The prism was cleaned with ethyl alcohol and water was circulated through an Abbe refractometer at 28 °C. Two or three drops of oil was placed on the surface of the prism. The prism was closed and the mirror was adjusted until the reading was

sharp. The instrument was allowed to stand for few minutes. After measurement, the prism was cleaned and dried, so that no contaminating material was carried over to any subsequent measurements. These procedures were repeated for the three samples and their corresponding refractive indices taken and recorded.

4.12 DETERMINATION OF VISCOSITY

The Oswald viscometer was washed and dried. The instrument was mounted vertically using a retort stand; 20 ml of the oil was poured into it and drawn up into the upper bulb and beyond the mark painted red. It was then allowed to flow back and the passage of the liquid level between two red marks M_1 and M_2 was timed. The surface tension effects were assumed to be negligible. The viscosity of the fluid was obtained by multiplying the time with the specific gravity of the fluid.

4.13 DETERMINATION OF PEROXIDE VALUE

One milligram of oil was weighed into a conical flask and 300 ml of acetic acid, chloroform (3-2v/v) solution and 1 g of potassium iodide were added and swirled. Thirty millilitre of distilled water was added, the mixture was boiled for 1 min and the hot solution was transferred into a flask containing 20 ml of 5% potassium iodide. Some drops of 0.5% starch solution was added and the mixture was titrated with Na_2SO_3. A blank solution was also prepared.

4.14 DETERMINATION OF ACID VALUE

One milligram of oil sample was added to 100 ml of neutral ethyl alcohol, the mixture was gently heated to boiling. It was then titrated with 0.1M KOH using 2 drops of phenolphthalein indicator. The titration was stopped when a permanent pink colour was noticed.

4.15 DETERMINATION OF ESTER CONTENT

Two gramms of oil sample was mixed with alcohol (ethanol) and gently heated to boiling. It was then titrated with 0.1M ethanolic KOH solution using two drops of indicator. Ethanolic potassium hydroxide 25 ml was added to the neutralised solution and the mixture boiled under a reflux condenser for thirty minutes. The solution was cooled, and 20 ml of water was added and the solution was immediately titrated with

0.5M hydrochloric acid using an additional 0.5 ml of phenolphthalein indicator. A blank determination was carried out using the same procedure but omitting the oil.

CHAPTER FIVE

5.0 RESULTS AND DISCUSSION

In this section the results obtained in extraction and steam distillation are presented. The fragrance of the various perfume formulations are presented and analysed statistically. Finally, the properties of the best fragrance formulated and the commercial perfume are discussed.

5.1 EXTRACTION

The raw data for the extraction of the pear oil as a function of time using the soxhlet apparatus is presented in Table A.1 in Appendix A. The yield of pear oil is plotted against time in Figure 5.

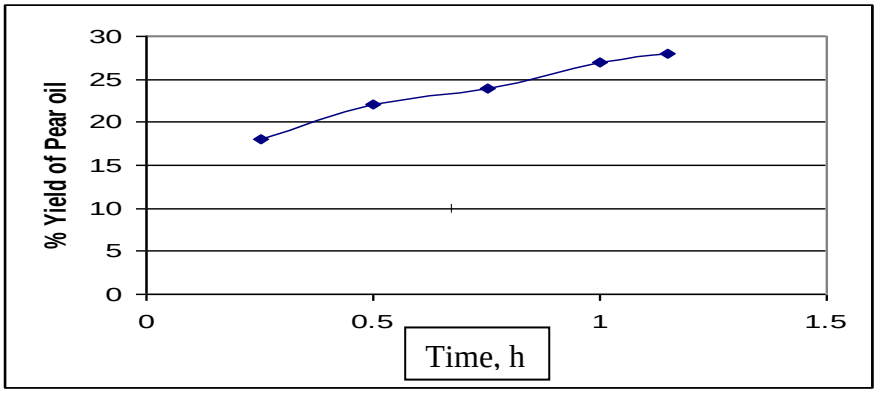


Figure 5 Yield of Pear Oil as a Function of Time using Soxhlet Apparatus.

The pear oil yield using the soxhlet extractor increased from 18% to 28% as time increased from 0.25 h to 1.25 h. The yield appears to be levelling up at about 1 h. The maximum yield of about 28% agrees with the range of maximum yield given in literature (Martins et al, 1987) which is between 18%-42%.

The raw data for the extraction of the pear oil as a function of time using the soaking method is presented in Table A.2 in Appendix A. The yield of pear oil is plotted against time in Figure 6.

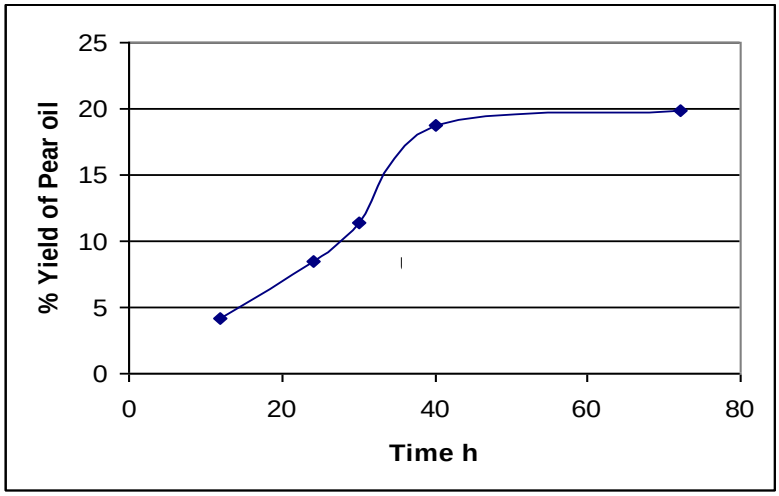


Figure 6 Yield of Pear Oil as a Function of Time Using the Soaking method.

The pear oil yield using the soaking method increased from 4.1% to a maximum of a 19.9% as the time increased from 12 to 72 h. The maximum yield was obtained after about 45 h. The maximum yield obtained agrees with the range of maximum yield given in literature which is between 18%-42%.

5.2 STEAM DISTILLATION

The raw data for the extraction of the orange oil as a function of time using steam distillation is presented in Table B.1 in Appendix B. Figure 7 gives the yield of orange oil as a function of time.

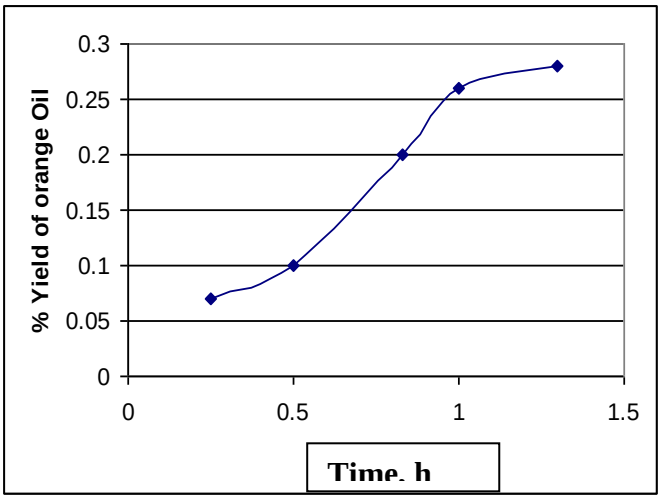


Figure 7 Yield of Orange Oil as a Function of Time Using Steam Distillation.

The yield of orange using the steam distillation method increased from 0.07% to 0.28% as the time increased from 0.25 h to 1.30 h. The maximum yield obtained agrees with the value given in literature (Fleisher, 1993) which is about 0.3%.

5.3 FRAGRANCES OF PERFUME FORMULATIONS

The raw data for the composition of the 81 fragrance concentrates and their fragrances are presented in Table C.1 in Appendix C. The results of the four way analysis of variance (ANOVA) for the fragrance data are presented in Table 7.

Table 7. Analysis of Variance for fragrance Data

Source of variation	**D.F**	**S.S**	**M.S**	**V.R**	**F.Pr**
Rep Stratum	3	41.540	13.847	10.39	
Orange Oil	2	8.988	4.494	3.37	**0.036**
Banana Oil	2	0.599	0.299	0.22	0.799
Apple Oil	2	2.969	1.485	1.11	0.330
Pear Oil	2	4.469	2.235	1.68	0.189
Orange Oil.Banana Oil	4	20.383	5.096	3.83	**0.005**
Orange Oil.Apple Oil	4	9.123	2.281	1.71	0.148
Banana Oil.Apple Oil	4	19.235	4.809	3.61	**0.007**
Orange Oil.Pear Oil	4	1.235	0.309	0.23	0.920
Apple Oil. Pear Oil	4	4.864	1.216	0.91	0.457
Orange,Banana,Apple Oils	8	40.562	5.070	3.81	**0.001**
Orange,Banana,Pear Oils.	8	9.284	1.160	0.87	0.541
Orange,Apple,Pear Oils	8	8.377	1.047	0.79	0.615
Banana,Apple,Pear Oils	8	5.821	0.728	0.55	0.821
Orange,Banana,Apple,Pear Oils.	16	27.883	1.743	1.31	0.193
Residual	240	319.710	1.332		

Legend: D.F =Degree of freedom, S.S = Sum of squares M.S = Mean of squares, F.pr = Fischers probability, V.R = variance ratio

The result from this analysis shows that the individual independent variables did not have any significant effect on the fragrance formulations at 0.05 significance level, except for the orange oil which has a Fischer probability of 0.036. The interactions between orange oil and banana oil which had a Fischer probability of 0.005, the interactions between apple and banana which has a Fischer probability of

0.007 and the interactions between orange banana and apple oils which has a Fischer probability of 0.001, had a significant effect on the formulations.

Mean values of the fragrances obtained from the post hoc analysis of variance are presented in Table 7. The values indicate that samples $S_{O1, B2, A2, P1}$ (0% orange oil, 20% banana oil, 40% apple oil, 0% pear oil) and $S_{O1, B2, A2, P2,}$ (0% orange oil, 20% banana oil, 40% apple oil, 1% pear oil), $S_{O3,B1,A3,P1}$ (5% orange oil, 10% banana oil, 60% apple oil, 5% pear oil) have the highest mean values of 4.250, 4.000 and 4.000 respectively. The other formulations have mean values < 3.750. Therefore, sample $S_{O1,B2,A2,P1}$ is the best formulation that gives the fragrance closest to the commercial apple fragrance with a fragrance value of 4.25, Samples $S_{O1,B2,A2,P2,}$ and Samples $S_{O3,B1,A3,P1}$ are the next best formulations. However, the three fragrances were essentially indistinguishable. They were different from the commercial apple perfume in being slightly less fruity.

5.4 PHYSICO-CHEMICAL CHARACTERISTICS

5.4.1 Fractional Distillation

The raw data for the fractional distillation of the commercial apple fragrance is presented in Table D.1 in Appendix D. The volumes of distillate obtained at different temperatures are plotted in Figure 8.

The first two distillates boiling at 90-100 °C and 100-140 °C are likely to be the top notes of the fragrance. Together, they constitute about 10% of the volume of the fragrance. The fraction boiling between 252-300 °C constitutes about 50% of the total volume. Since the boiling point of liquid paraffin and other fixed oils are within this range, the carrier used for the commercial apple perfume is likely to be some form of liquid paraffin and other fixed oils which can be sourced locally, it means that a substantial improvement in local content of over 50% by volume can be achieved with local production.

Table 7. Means of Fragrance Values for each Level of Composition.

LEVELS OF OILS					
Orange Oil	**Banana Oil**	**Apple Oil**	**Pear Oil**		
			0.0	1.0	3.0
			Mean Fragance Values		
0.0	10.0	20.0	3.000	3.750	3.250
		40.0	1.750	1.500	1.500
		60.0	2.500	1.750	1.500
	20.0	20.0	1.500	2.000	1.750
		40.0	**4.250**	**4.000**	2.250
		60.0	1.750	2.750	2.500
	30.0	20.0	2.500	2.250	1.500
		40.0	2.250	2.000	2.250
		60.0	2.000	2.500	2.250
2.0	10.0	20.0	1.500	2.250	2.000
		40.0	2.500	1.750	1.500
		60.0	2.750	2.750	2.750
	20.0	20.0	1.000	2.500	1.500
		40.0	1.250	1.500	2.000
		60.0	2.250	1.750	1.250
	30.0	20.0	3.250	2.750	3.750
		40.0	2.250	2.750	1.250
		60.0	1.750	2.000	2.250
5.0	10.0	20.0	1.250	1.000	1.500
		40.0	1.250	1.750	1.000
		60.0	2.250	**4.000**	1.250
	20.0	20.0	1.750	1.750	3.000
		40.0	1.750	2.250	2.250
		60.0	3.000	2.500	2.250
	30.0	20.0	2.500	1.750	1.750
		40.0	2.250	1.250	1.250
		60.0	2.250	1.500	1.500

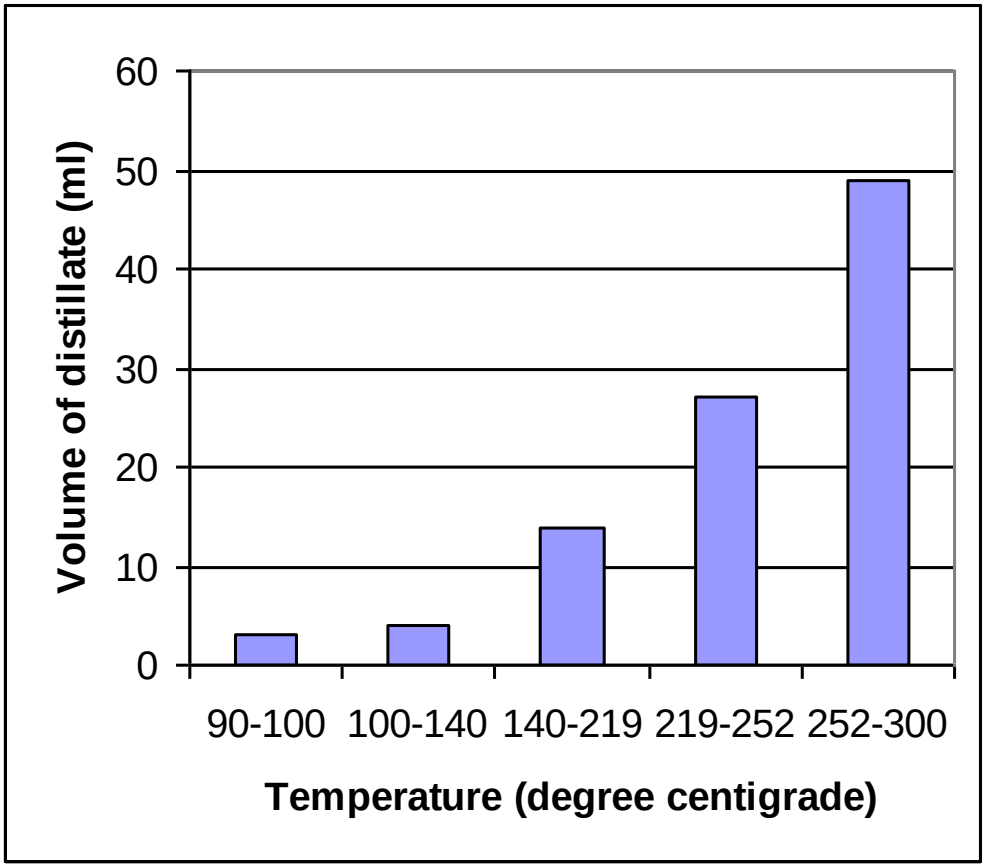


Figure 8 Volume of each Distillate at Various Temperatures.

5.4.2 Gas Chromatography

Presented in this section is a chromatogram (Figure 9) showing the peak of pure isoamyl valerate, the chief ester in the apple fruit. This peak was compared to the peaks of esters in the commercial apple fragrance (Figure 10) to see if they match.

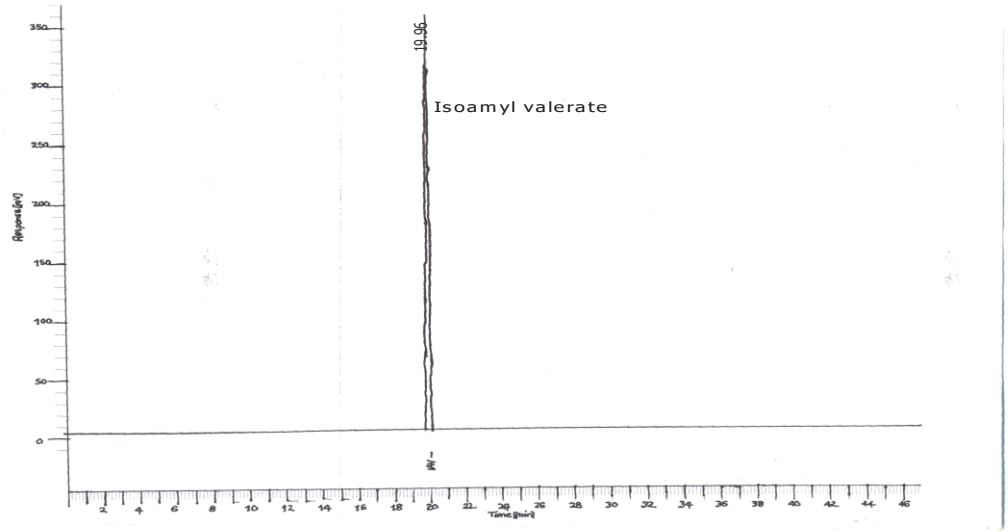


Figure 9 Gas Chromatogram of Pure Isoamyl Valerate.

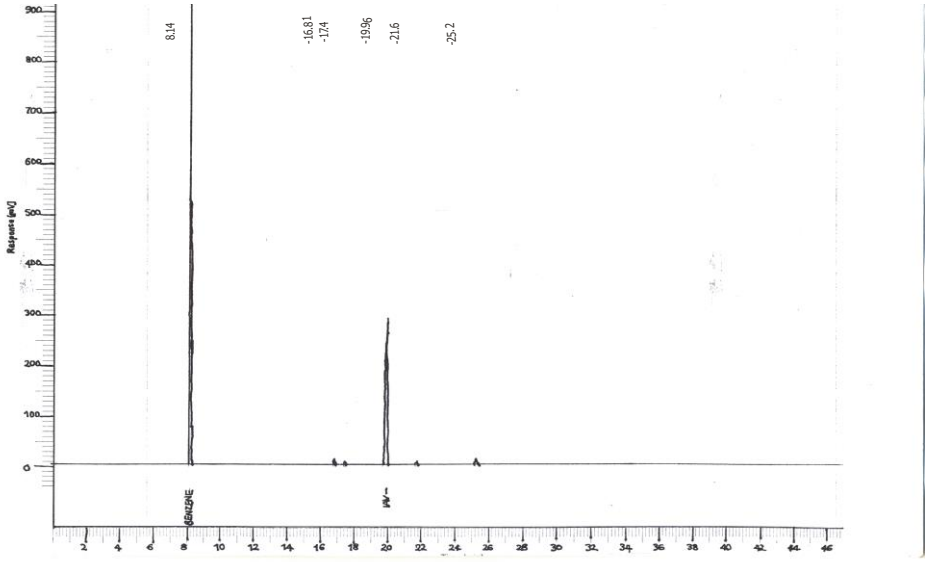


Figure 10, Gas Chromatogram of Esters Present in Commercial Apple Perfume.

In both Figures 8 and 9 there is a peak with the elution time for the pure isoamyl valerate of 19.96 min, hence isoamyl valerate is clearly present in the commercial apple perfume. It was the major peak in the chromatogram of the commercial perfume which proves that isoamyl valerate is a major component in the formulation of the hair cream apple fragrance. The gas chromatogram of the hydrocarbon content of the best apple fragrance, commercial apple fragrance and paraffin oil are shown in Figures11, 12 and 13 respectively. In other to have a more detailed comparism, the compositions are presented in in Table 9.

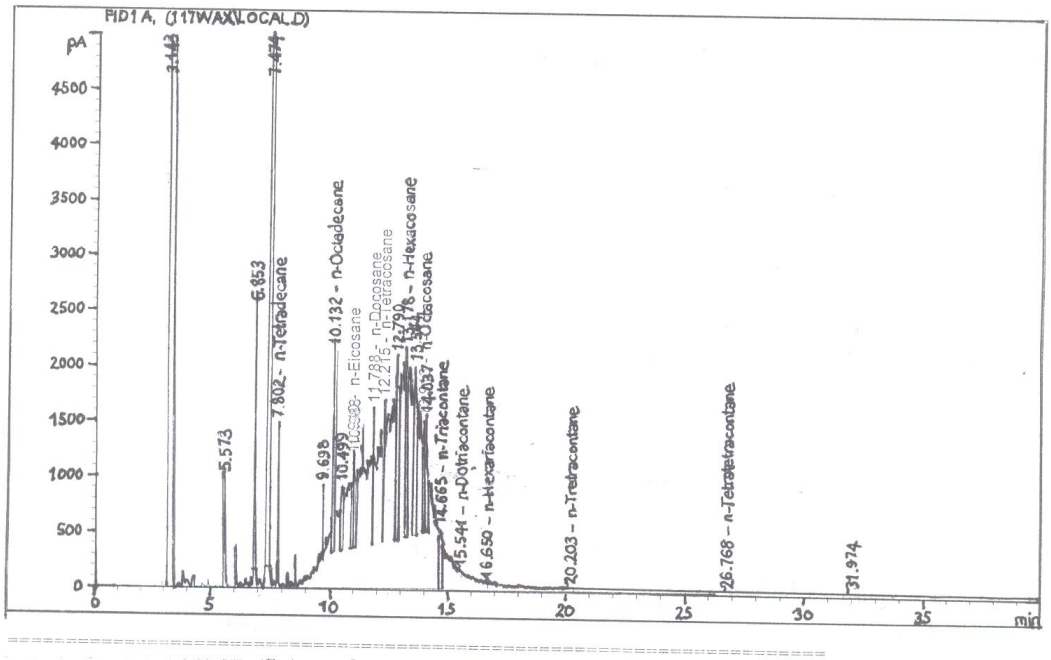


Figure 11 Gas Chromatogram Showing Hydrocarbon Content of Best Apple Fragrance.

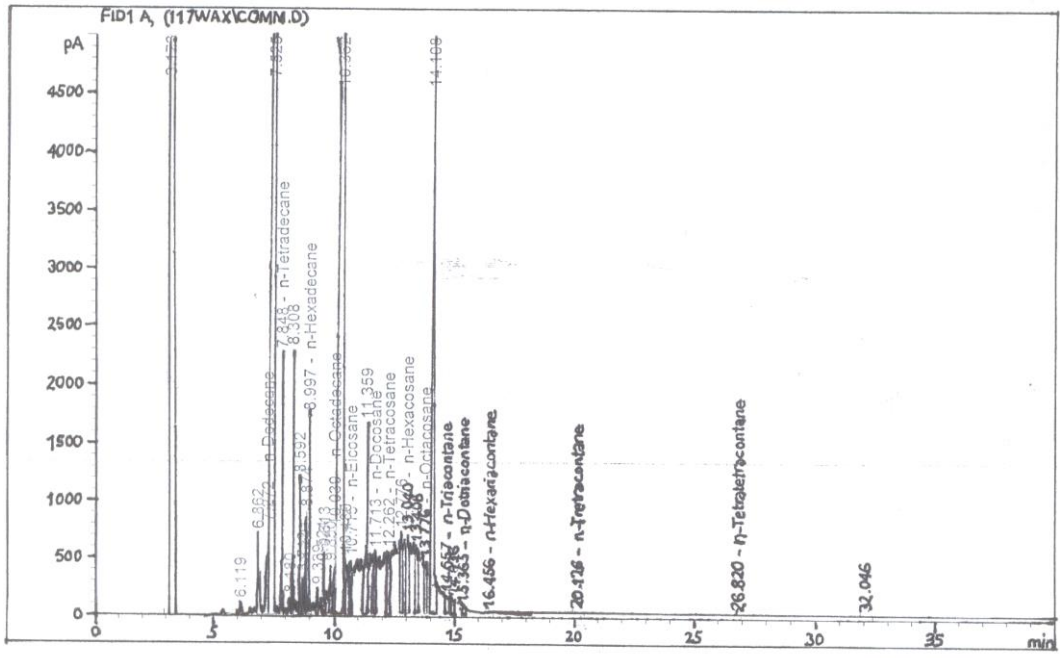


Figure 12 Gas Chromatogram Showing Hydrocarbon Content of the Commercial Apple Fragrance.

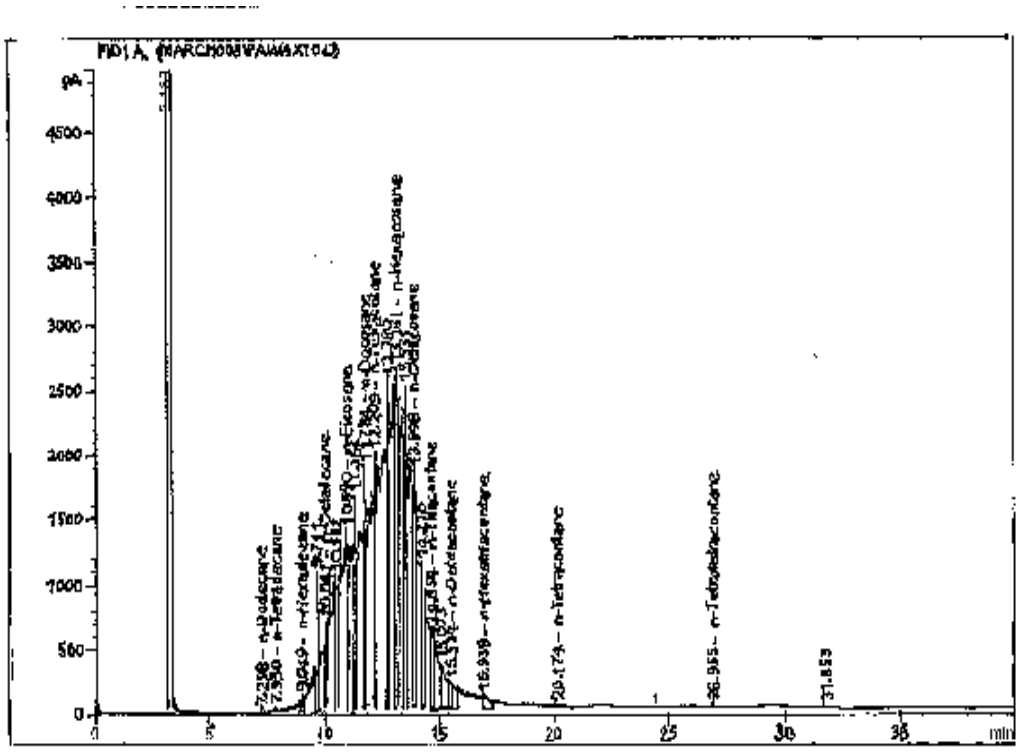


Figure 13 Gas Chromatogram of Paraffin Oil

Table 9 Percentages of Components in the Chromatogram of Commercial Apple Perfume,the Best Developed Apple Perfume and Paraffin Oil.

	PERCENTAGES OF COMPONENTS		
Hydrocarbon Components	**Commercial perfume**	**Best Developed apple perfume**	**Paraffin Oil**
n-Dodecane	58	-	7.08
n- Tetradecane	26	53.39	1.63
n- Hexadecane	9	-	3.61
n- Octadecane	2	17.03	11.17
n- Eicosane	0.847	3.96	12.70
n- Docosane	0.907	5.604	15.69
n- Tetracosane	0.807	4.92	14.27
n- Hexacosane	0.888	5.96	16.96
n- Octacosane	0.588	2.89	10.48
n- Triacontane	0.288	1.17	3.52
n- Dotriacontane	0.211	4.95	1.28
n- Hexatriacontane	0.160	0.042	0.75
n- tetracontane	0.152	0.028	0.29
n-tetratetracontane	0.152	0.056	0.57

The peaks of the three chromatogram are quite similar occurring at elution times between 3 and 17 min. Essentially similar hydrocarbons from n- dodecane (C_{12}) to n-tetratetradecane (C_{44}) are present in all the three samples. The commercial fragrance contains more of the lighter hydrocarbons especially n-dodecane (58%), n-tetradecane(26%) and n-octadecane (9%). The paraffin contains more of the heavier hydrocarbons from C_{18}-$C_{44.}$ The best apple perfume does not contain dodecane and hexadecane which is unexpected since it was formulated from paraffin oil which contains both components. The peaks may have not appeared due to possible experimental errors.

There appears to be paraffin oil in the commercial fragrance but clearly, the lighter hydrocarbons must have been contributed by other fixed oils because of their high content. This is also observed in the best developed fragrance, it contains paraffin oil but the n- tetradecane is unexpectedly high and may have been contributed from pear oil. The fixed oil in the commercial fragrance should be paraffin oil and other fixed oils.

5.4.3 Infra-Red Spectroscopy

The Infra-red spectra of the commercial apple perfume and best developed apple perfume are shown in Figures 14 and 15.

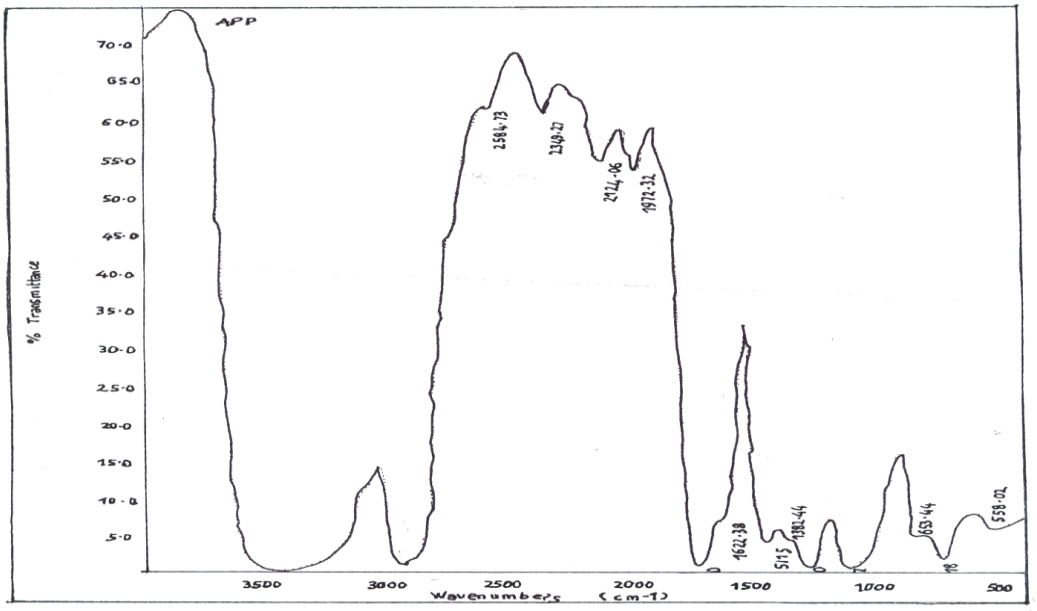


Figure14 IR Spectrum of Commercial Apple Perfume.

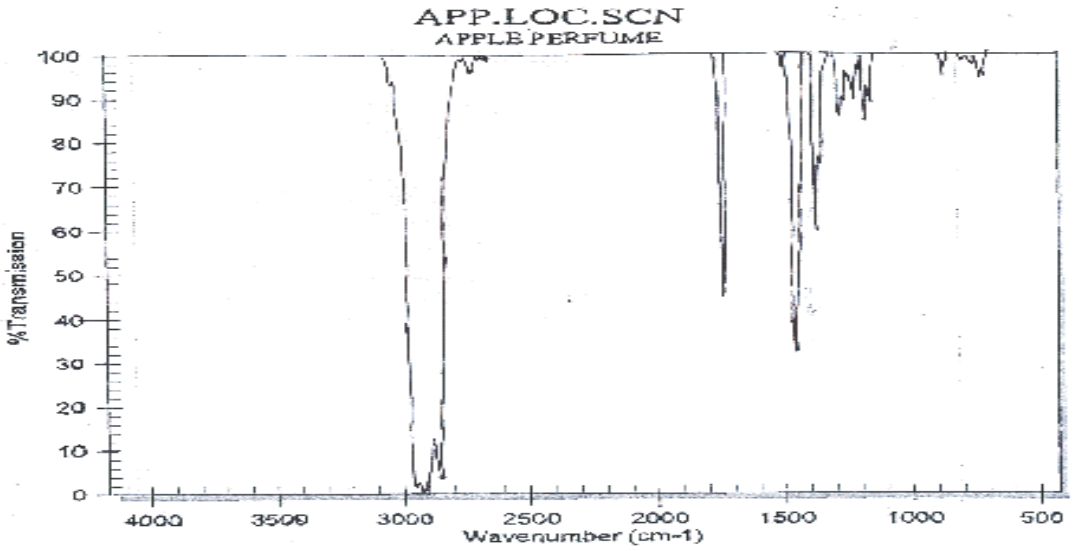


Figure 15 Infrared Spectrum of the Best Apple Perfume Sample.

The major peaks produced by both spectra are shown in Table 10. The spectra are quite similar but have quite some differences. To further compare the two spectra a list of all the peaks in the spectra and their functional group is given in Table10. Peak number 2924 cm^{-1} for functional group C-H, 1750 cm^{-1} for functional group –C-O and 650 cm^{-1} for functional group C-H are essentially identical in both spectra. However, peak number 3494 cm^{-1} for functional group C-O, 1040 cm^{-1} for functional group C-O and 700 cm^{-1} for functional group 5-adjacent aromatic esters are present in the commercial apple perfume and absent in the best developed apple perfume. These peaks are for esters and clearly indicate the missing esters in the developed apple perfume. This confirms the results obtained from the gas chromatographs.

Table10 Major Peaks Produced from the IR Spectra of Commercial Apple Perfume and the Best Developed Apple Perfume.

Wavelength Number of peak	**Functional Group.**	**Commercial Apple Perfume**	**Best Developed Apple Perfume.**
3494 cm^{-1}	-C-O	Present	Absent
2924 cm^{-1}	C-H	Present	Present
1750 cm^{-1}	C-O	Present	Present
1390 cm^{-1}	$-C(CH)_3$	Absent	Present
1400 cm^{-1}	C-H	Absent	Present
1040 cm^{-1}	-C-O	Present	Absent
700 cm^{-1}	5-adjacent aromatic esters.	Present	Absent

The infra-red spectra of the five distillates obtained from the fractional distillation of the commercial apple perfume are shown in Figures 16- 21.

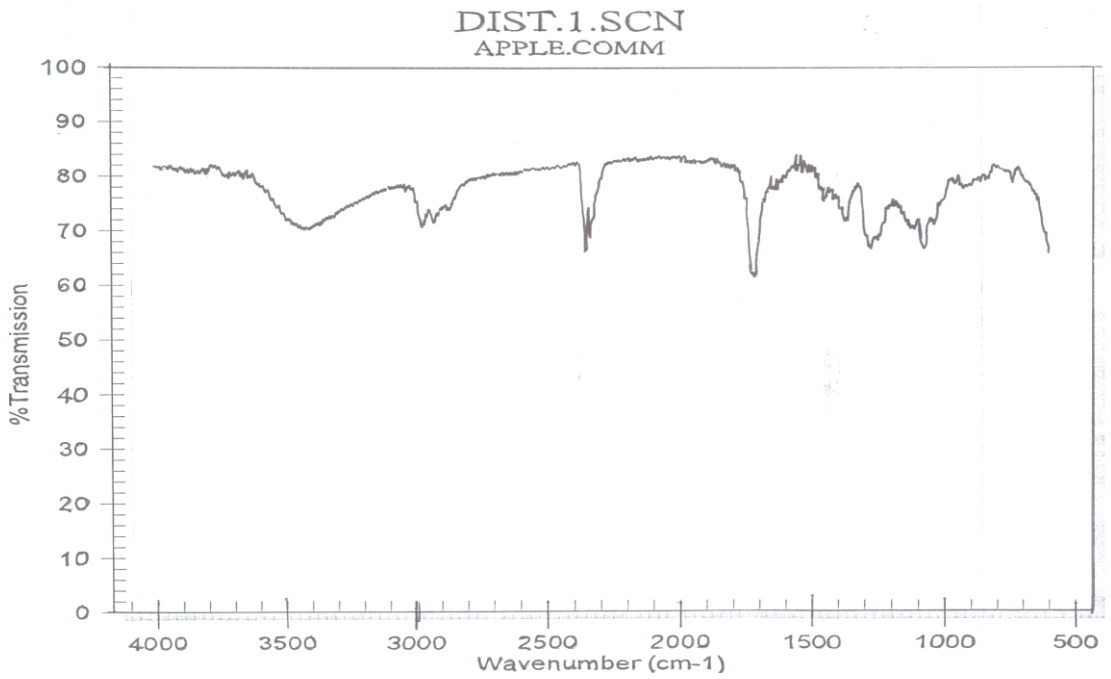


Figure 16 IR Spectrum of 1st Distillate of the Commercial Apple Perfume.

Figure 17 IR Spectrum of the 2nd Distillate of the Commercial Apple Perfume.

Figure18 IR Spectrum of the 3r[d] Distillate of the Commercial Apple Perfume.

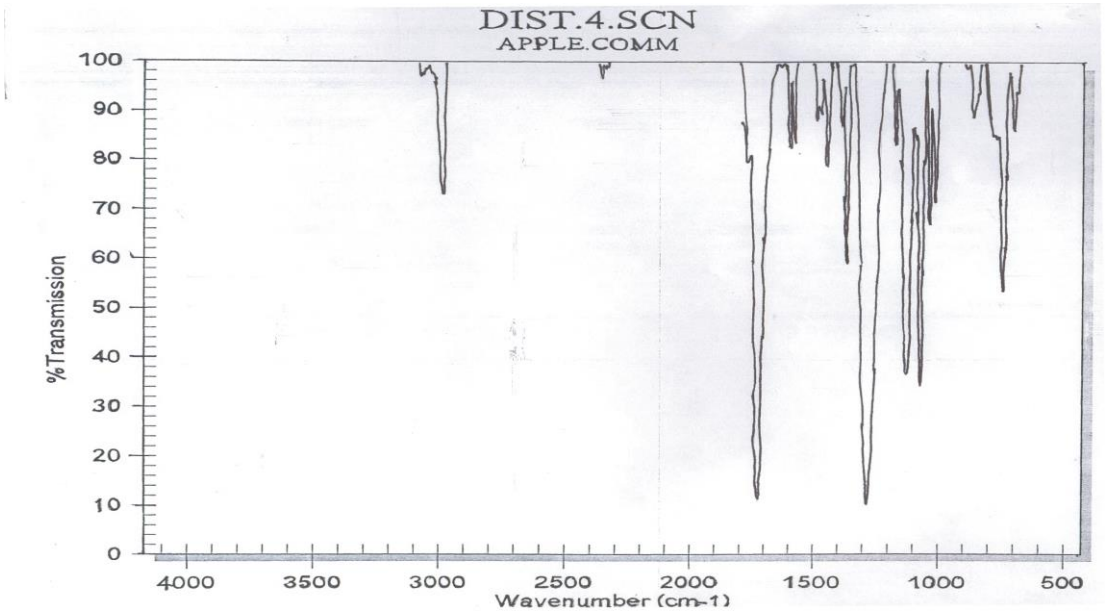


Figure 19 Showing IR Spectrum of the 4[th] Distillate of Commercial Apple Perfume.

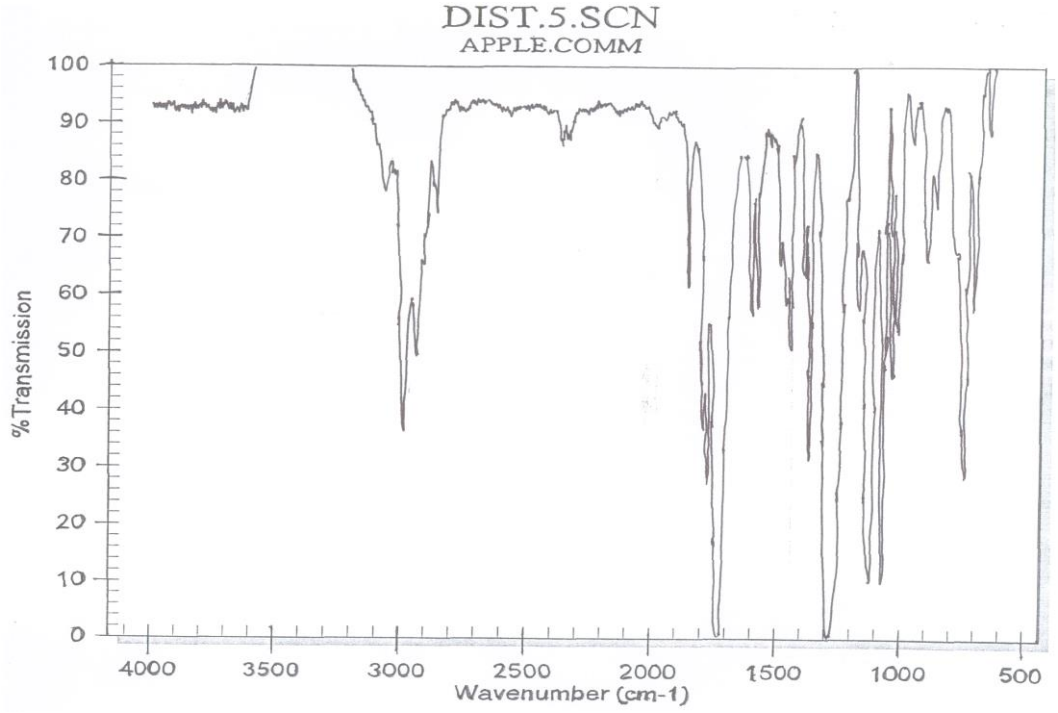


Figure 20 Showing IR Spectrum of the 5th Distillate of Commercial Apple Perfume.

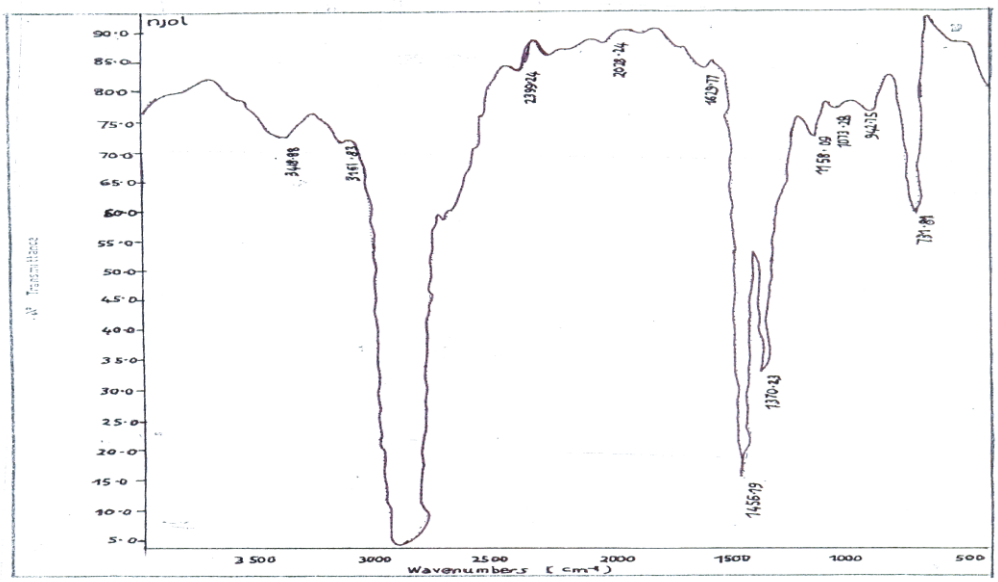


Figure 21 IR Spectrum of Paraffin Oil

The three spectra in Figures16,17 and 18 are quite similar with peak number 3494 cm^{-1} for functional group –C-O appearing in the three spectra. This ester appears in a larger quantity in the second distillate, suggesting that the second distillate contains more of the middle note ester (the heart note).

5.4.4 Other Physico-Chemical Characteristics

The other important physico-chemical properties of the best developed and commercial apple fragrance are presented in Table 12.

Table 12 Physico-Chemical Characteristics of the Best Apple Fragrance Sample and the Commercial Apple Perfume.

Characteristics	**Best apple fragrance sample**	**Commercial Apple Perfume.**
Specific Gravity	0.8674	0.9455
Density g/ml	0.8347	0.9098
Peroxide value mEq/kg	0.24	0.16
Refractive Index at 28°C	1.4635	1.4595
Viscosity Cp at 30°C	31.75	34.133
Acid Value	0.2805	0.561
Ester value	91.163	131.835
Moisture Content	0.00	0.00

The refractive index of the oil samples were measured at 28°C and was found to be 1.4595 and 1.4635 which agrees with the range of refractive index for perfumes given in literatures (Poucher,1974). No moisture was found in both perfumes because it is an oil based perfume and not alcoholic based. The acid value for the commercial perfume is higher than that of the local perfume. The ester value for the local apple perfume and the commercial apple perfume is 91.163mg KOH/kg which agrees with the range given in literatures (Poucher, 1974) for ester values for the quantity of oil used.

5.5 Selling Price Calculation

The selling price of the best developed apple fragrance was calculated based on the following assumptions.

Production capacity = 250 L/day, 75, 000L/ annum

Operating Labour / support personnel = N 212,000

Rent for ware house = N 48,000

Rent for service house = N 24,000

1,000 distribution cans 100 L, 50 L = N 50,000.

Cost of Apple Essence150 L (imported) = N 2,454,000

Cost of Banana Essence 25 L = N 25,000

Cost of Paraffin Oil 50 L = N18,750

The cost of purchasing the imported apple essence was very high because it was sold at the retail price, this increased the total cost of the raw materials. However this price could be reduced if the apple essence was purchased in bulk.

The raw data for the selling price of the best developed apple perfume is presented in Table G.1.in Appendix G.

Table 9 Selling Price Calculation for the Best Developed Apple Perfume.

Items	**Naira**
Raw materials	749,325,000
Operating Labour/support personnel C_{OL}	180,000
Supervisory $0.18C_{OL}$	32,000
Total	749,537,000
Fixed Manufacturing Costs/annum (FMC)	
Depreciation 0.1FCI	12,940,680
Rent	72,000
Plant overhead costs ($0.708C_{OL}$ +0.036 FCI)	4,913,524
Total	17,926,204
General Expenses (GE)/annum	
Product distribution	50,000
Total	50,000

Total cost of manufacturing/annum	=	DMC+ FMC +GE
	=	797,513,524/annum
Volume of best developed apple perfume produced/annum	=	75,000 L
Costs for one litre of perfume	=	10, 233
At 50% profit margin, retail price	=	15, 349 per litre

The price of the best developed apple perfume (N 15,349) is higher than the price of the commercial perfume sold in the market (N10, 000). The cost of importing the apple essence at a retail price could be a major factor affecting this price.

Figure 22 shows a pie chart of the different percentages that make up the total manufacturing costs for the Best developed Apple Perfume.

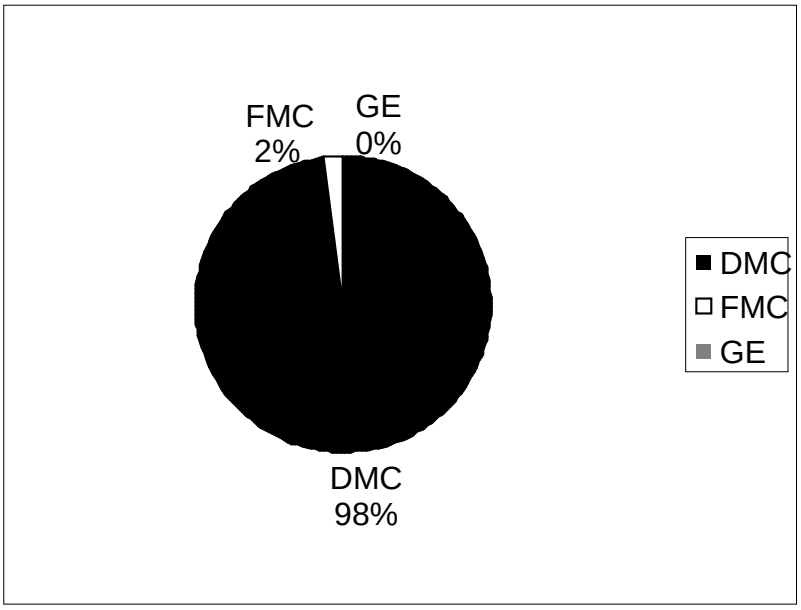


Figure 22 Pie Chart Showing Total Manufacturing Costs for the Best Developed Apple Perfume.

The pie chart shows that the direct manufacturing costs (DMC) is 98%, the fixed manufacturing costs 2%, while general expenses is at 0% of the whole production costs. The raw materials were purchased at a retail price which made the (DMC) to have a high percentage.

However, carrying out a sensitivity analysis using half the price of the raw materials (due to a bulk purchase of the apple essence) shows that the cost of a litre

reduces to N5, 638, and at 50% profit margin the retail price drops to N 8,457 as compared to N 10, 000.00 market price of the commercial apple perfume.

CHAPTER SIX

6.0 CONCLUSIONS

1. The maximum yield of pear oil were 28% using soxhlet extractor and 19.9% using the soaking method.
2. The maximum yield of orange oil using the steam distillation method was 0.28%.
3. The apple fruit gave no significant yield of oil and the odour of the extract was not near apple, it was unpleasant, and not so useful for perfuming.
4. The mean values of the fragrance indicate samples $S_{O1, B2, A2, P1}$ (0% orange oil, 20% banana oil, 40% apple oil, 0% pear oil, 40% paraffin oil), $S_{O1, B2, A2, P2,}$ (0% orange oil, 20% banana oil, 40% apple oil, 1% pear oil,39% paraffin oil) and $S_{O3,B1,A3,P1}$ (5% orange oil, 10% banana oil, 60% apple oil, 5% pear oil,20% paraffin oil) have the highest mean values of 4.25,4.00 and 4.00 respectively. Sample $S_{O1,B2,A2,P1}$ is the best formulation that gives the fragrance closest to the commercial apple fragrance with a fragrance value of 4.25. However, the fragrance was different from the commercial apple perfume in being slightly less fruity.
5. The fraction boiling between 252-300 ^{0}C constitutes about 50% of the volume. Since the boiling point of liquid paraffin and other fixed oils are within this range, the carrier used for the commercial apple perfume is likely to be some form of liquid paraffin and other fixed oils which can be sourced locally. This means that a substantial improvement in local content of over 50% by volume can be achieved with local production.
6. Isoamyl valerate is clearly present in the commercial apple perfume and it is a major component in the formulation of the hair cream apple fragrance.
7. There are some key esters which are present in the commercial apple perfume but absent in the developed perfume.
8. The total price of the best developed apple perfume of N15,349.00 was higher than the price of the commercial product in the market N10,000.00. This was due to the fact that the apple essence was purchased at a retail price. However, carrying out a sensitivity analysis using half the price of the raw materials (due to a bulk purchase of the apple essence) shows that the cost of a litre of the developed perfume can be reduced to N 8,457.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

1. More analysis should be carried out on the commercial apple perfume to find out the ester that gives the very sweet fruity odour that seems to be absent in the new formulation.

2. Laboratory production of the esters required for the formulation of apple perfume should be investigated with a view to producing them locally.

3. More search for literature containing the formulation of the commercial apple perfume should be carried out.

4. Necessary equipment should be provided by the institution such as a gas chromatograph machine, mass Spectrophotometer e.t.c which help to give very important results in the perfumery field.

REFERENCES

Adhikary ,S.R, Amatya, K.R. & Thapa B.B., National Workshop on Chemical Investigation and Processing of Aromatic Plants., Pg 23-36 UNESCO and AIDAB.. 1989.

Barrow G.R., Introduction to Molecular Spectroscopy, Pg 45-79 McGraw-Hill. New York,. 1962.

Baser, K.H.C., "Turkish Rose Oil". Perfum. Flav., 17(3), 45- 52 (1992)

Bryon R. Bird, Warren E. Stewart, Edwin N.Lightfoot., Transport Phenomena Pg 3-30 John Wiley &Sons .Inc, New York 1960

Criddle, W.J and Ellis G.P.,. Spectral and Chemical Characterization of Organic compounds. , Pg 30-70, A Laboratory Handbook. 3rd edition, 1976.

Esau,K., Secretory Structures anatomy of Seed Plants. 2nd Edition, Pg 50-51 John Wiley & Sons , New York.. 1977.

Edward Sagarin, The Science and Art of Perfumery. Pg. 76-105 McGraw- Hill Book Company New York. . 1992.,

Kahol,A.P., .Distillation Technology. Practical Manual , The Essential Oil Industry . UNIDO, Vienna. (1982).

Harold, F. V and Clarke, B.J .,Liquid CO_2 Hop Extraction – Commercial Reality. Pg. 45-58 The Brewers Digest,. 1979.

Fleisher , A., "Citrus Hydrocarbon-free Essential Oils" Perfum (1), 11-15. 1993.

Denny, E.F.K., Field Distillation for Herbaceous Oils 2nd Edition., Pg. 45-60. Denny Mckenzie Association., Lilydale, Tasmania. 1991..

John., C. M., & William , A.C., Encyclopaedia of Chemical processing and Design. Pg 167. Library Congress Cataloguing in publication data, Printed in U.S.A. 1979.,

Emil Fischer,. An Ester from a Carboxylic Acid & Alcohol. Chem 306 Lab Manual. 2-6. 1991.

Naves Y. R. and Mazuyer,G., Natural Perfume Materials. Reinhold publishers corporation, Pg 12-27 New York,. 1974.

Poucher W.A., Perfumes ,Cosmetics and Soaps. Pg 85-111 Published by Chapman and Hall ltd. 11 New Felter Lane, London.. 1974.

Robert H. Perry ,Don W. Green Chemical Engineers Handbook. Pg 3-69 to 3-89 Mcgraw-Hill New York. Seventh Edition. 1997..

Sahai, O.,. Plant Tissue Culture . In: Bioprocess Production of Flavor, Fragrance and Color Ingredients. John Wiley &Sons .Inc, New York. 1994.

Scheffe,H., “The Simplex Lattice Design Experiment with Mixtures”. Journal of the Royal Statiscal Society B, 25(14): pp 235- 263. 1989

Shreves , M.R. Chemical Process Industries 5th Edition. Pg 70-72 to 93-121 John Wiley &Sons .Inc, New York.. 1984,

Tulley de Silva, K,. “A Manual on the Essential Oil Industry” Turkey, Pg 4-49 to 5-76. 1995

Udeh P.K., Petroleum Processing and Technology Lecture Note. Pg 213 PTI Warri . Unpublished. 1999

United States Patent inventors Federick B. Power and Chestnut, Assignee Givaudan –Roure Coporation, Clifton N.J. Num -5,585,34 Dec, 17, 1996

Wells F.V. &, Marcel Billot, 1981 Perfumery Technology,. 2nd Edition. Pg. 20 – 108 John Wiley &Sons, Inc, New York. 1994.

APPENDIX A

Table A.1 Extraction of Pear Oil using the Soxhlet Aparratus

Ωειγητ οφ Σαμπλε (γ)	Ωειγητ οφ Φλασκ + οιλ (γ)	Τιμε, (η)	Ψιελδ (γ)	ςολυμε οφ σολωεντ υσεδ (μλ)	Περχενταγε οφ οιλ (%)
10	141.9	15	1.80	100	0.018
10	112.5	30	2.20	100	0.022
10	142.5	45	2.40	100	0.024
10	113.0	1.00	2.70	100	0.027
10	121.9	1.15	2.80	100	0.028

Table A.2,Extraction of Pear Oil Using Soaking Method

Weight of sample (g)	**Time (h)**	**Weight of flask +oil (g)**	**Yield (g)**	**Volume of solvent (ml)**	**Percentage Yield of Oil.**
20	12	141.924	0.824	100	0.00824
20	24	142.796	1.696	100	0.01696
20	30	143.387	2.287	100	0.02287
20	40	144.853	3.753	100	0.03753
20	72	145.076	3.976	100	0.03976

APPENDIX B

Table B.1 Steam Distillation of Orange oil

Weight of sample (g)	Distillation time.(h)	Weight of oil extracted (g)	% of oil extracted	Volume of solvent used (ml)
100	1.30	0.28	0.0028	100
100	1.00	0.26	0.0026	100
100	0.83	0.20	0.0020	100
100	0.50	0.10	0.0010	100
100	0.25	0.07	0.0007	100

Table B.2 Extraction of Orange Oil for make up.

Weight of sample (g)	Distillation time.(h)	Weight of oil extracted (g)	% of oil extracted	Volume of solvent used (ml)
100	1.30	0.286	0.286%	100
100	1.30	0.275	0.275%	100
100	1.30	0.281	0.281%	100
100	1.30	0.282	0.282%	100
100	1.30	0.269	0.269%	100

APPENDIX C

Table C.1 Fragrance of various perfume formulations

S/ N	Orange oil	Banana Oil	Apple oil	Pear oil	Carrier	FV	FV	FV	FV
1	5	10	60	0	25	4	3	2	2
2	5	10	40	0	45	2	1	2	2
3	5	10	20	0	65	1	2	2	2
4	5	10	60	1	24	5	3	5	5
5	5	10	40	1	44	3	1	2	3
6	5	10	20	1	64	1	1	2	2
7	5	10	60	3	22	1	2	2	2
8	5	10	40	3	42	1	1	2	2
9	5	10	20	3	72	3	1	2	2
10	5	20	60	0	15	5	3	1	3
11	5	20	40	0	35	2	1	3	1
12	5	20	20	0	55	1	3	2	1
13	5	20	60	1	14	5	2	1	2
14	5	20	40	1	36	2	2	2	3
15	5	20	20	1	54	1	2	3	1
16	5	20	60	3	12	5	1	2	1
17	5	20	40	3	32	3	1	1	4
18	5	20	20	3	52	3	3	5	1
19	5	30	60	0	5	4	1	3	1
20	5	30	40	0	25	3	4	1	1
21	5	30	20	0	45	4	4	1	1
22	5	30	60	1	4	1	3	1	1
23	5	30	40	1	26	1	1	1	2
24	5	30	20	1	44	1	2	2	2
25	5	30	60	3	2	3	1	1	1
26	5	30	40	3	28	1	1	1	2
27	5	30	20	3	42	1	1	1	4
28	2	10	60	0	28	3	1	2	5
29	2	10	40	0	48	1	3	5	1
30	2	10	20	0	68	2	2	1	1
31	2	10	60	1	27	1	4	4	2
32	2	10	40	1	47	2	3	1	1
33	2	10	20	1	67	2	2	1	4
34	2	10	60	3	25	4	1	1	5

35	2	10	40	3	45	2	1	2	1
36	2	10	20	3	65	2	4	1	1
37	2	20	60	0	18	1	2	2	4
38	2	20	40	0	38	1	1	2	1
39	2	20	20	0	58	1	1	1	1
40	2	20	60	1	17	1	1	1	4
41	2	20	40	1	37	1	2	1	2
42	2	20	20	1	57	4	1	1	4
43	2	20	60	3	15	1	2	1	1
44	2	20	40	3	35	4	2	1	1
45	2	20	20	3	55	3	1	1	1
46	2	30	60	0	8	2	2	2	1
47	2	30	40	0	28	3	1	1	4
48	2	30	20	0	48	4	4	4	1
49	2	30	60	1	7	4	1	1	2
50	2	30	40	1	27	4	2	1	4
51	2	30	20	1	47	4	3	1	3
52	2	30	60	3	5	4	3	1	1
53	2	30	40	3	25	1	1	2	1
54	2	30	20	3	45	5	4	5	1
55	0	10	60	0	30	3	3	3	1
56	0	10	40	0	50	2	2	2	1
57	0	10	20	0	70	5	1	5	1
58	0	10	60	1	29	3	2	1	1
59	0	10	40	1	49	1	2	1	2
60	0	10	20	1	69	4	4	5	2
61	0	10	60	3	27	2	2	1	1
62	0	10	40	3	47	2	2	2	1
63	0	10	20	3	67	5	2	5	1
64	0	20	60	0	20	3	1	2	1
65	0	20	40	0	40	5	3	4	5
66	0	20	20	0	60	2	2	1	1
67	0	20	60	1	19	3	4	2	2
68	0	20	40	1	39	4	3	4	5
69	0	20	20	1	59	5	1	1	1
70	0	20	60	3	17	4	3	1	2
71	0	20	40	3	37	2	1	3	3
72	0	20	20	3	57	4	1	1	1

73	0	30	60	0	10	3	2	2	1
74	0	30	40	0	30	4	2	1	2
75	0	30	20	0	50	4	3	1	2
76	0	30	60	1	9	2	3	4	1
77	0	30	40	1	29	2	4	1	1
78	0	30	20	1	49	4	2	1	2
79	0	30	60	3	7	3	3	2	1
80	0	30	40	3	27	3	4	1	1
81	0	30	20	3	47	0	1	1	0

Legend: Fv = Fragrance values

APPENDIX D

Table D.1 Fractional Distillation of 100 ml Commercial Apple Perfume

Distillate No.	**Temperature Range. (°C)**	**Volume of each Distillate obtained. (ml)**	**Percent composition of each distillate (%)**
1st	90-100	3	0.03
2nd	100-140	4	0.04
3rd	140-219	14	0.14
4th	219-252	27	0.27
5th	252-300	49	0.49

APPENDIX E

Calculation of Various Physical Properties

Specific Gravity:

Specific Gravity = Weight of Oil/Weight of equal volume of water

Weight of empty bottle = 20.36g

Weight of bottle + Oil = 45.33g

Therefore weigth of Oil = 45.33-20.36 = 21.66g

Weight of Local Apple Perfume using density bottle = 21.66g

Weight of equal volume of water using density bottle = 24.97g

Therefore S.G of L.A.P 21.66/ 24.97 = 0.8674.

Weight of empty bottle = 20.36g

Weight of bottle + Oil = 43.97g

Therefore weight of Oil = 43.97-20.36 = 23.61g

Therefore S.G of C.A.P 23.61/ 24.97 = 0.9455.

Density:

Density of L.A.P = Mass of sample / Volume of Sample

Weight of Sample/ Volume of Sample

21.66/25.95 = 0.8347 g/ml .

Density of C.A.P = Mass of sample / Volume of Sample

Weight of Sample/ Volume of Sample

23.61/25.95 = 0.9098g/ml

Viscosity:

Viscosity = Time (for the emptying of sample through bulb) * Specific Gravity.

Viscosity of L.A.P = 36.60mins * 0.8674

= 31.75 cp at 30°C

Viscosity of C.A.P = 36.1mins * 0.9455

Peroxide Value:

Table E.1 Titration Results

Sample	Mass in grammes of sample used.(g)(M)	Molarity of Thiosulfate used (T)	Titre Value (V)
L.A.P	2.0	0.002	2.40
C.A.P	2.0	0.002	1.60

Peroxide Value = V* T* 100/ M

P.V of L.A.P = 2.4 * 0.002 * 100/ 2 = 0.24.

P.V of C.A.P = 1.6 * 0.002 * 100/ 2 = 0.16.

Acid Value:

Acid Value = 56.1 * T * V/ M

Table E.2 Titration Results

Sample	Mass in grammes of oil used. (M)	Molarity of ethanolic KOH used. (T)	Titre value ()
L.A.P	4	0.1	0.2
C.A.P	4	0.1	0.4

Substitutng these values in the equation above we have,

A.V for L.A.P = 56.1* 0.1 * 0.2/ 4 = 0.2805

A.V for C.A.P = 56.1* 0.1 * 0.4/ 4 = 0.561

Ester Value:

Ester Value = 28.05* (B-V)/W

Table E.3 Titration Results

Sample	Mass in grammes of oil used. (W)	Volume of HCL used for the blank. B (cm^3)	Volume of HCL used for the excess alkali + oil. V cm^3)
L.A.P	2	17.6	11.1
C.A.P	2	17.6	8.2

Substituting these values in the equation above we have,

E.V for L.A.P = 28.05* (17.6 - 11.1)/2 = 91.163 mgkoh/g.

E.V for C.A.P = 28.05*(17.6 – 8.2)/ 2 = 131.84mgkoh/g.

APPENDIX F

Table F.1 Showing components in both peaks and their amounts

Hydrocarbon peaks	**Paraffin Oil mg/l**	**Commercial apple perfume mg/l**	**Best developed apple perfume mg/l**
n-Dodecane	1.48e5	6140.67147	-
n-Tetradecane	6.84e4	1418.72411	3.89e4
n-Hexadecane	2.37e4	3133.29442	-
n- Octadecane	5113.89	9697.87053	1.27e4
n- Eicosane	1869.72	1.10108e4	3294.99
n- Docosane	1944.56	1.3597e4	4471.12
n- Tetracosane	1684.60	1.23749e4	3978.82
n- Hexacosane	1886.35	1.47015e4	4733.20
n- Octacosane	1112.09	9093.87599	2523.08
n- Tricontane	369.19	3046.03005	1284.68
n- Dotriacontane	158.17	1106.40833	43.70
n-Hexatriacontane	26.10	651.14722	32.69
n- Tetracontane	10.65	252.20504	19.37
n-Tetratetracontane	12.34	485.40547	38.38

APPENDIX G

Selling Price Calculation for the best Apple perfume.

Direct manufacturing Cost

Production Capacity	=	250L /day, 75000 L /annum
Cost of Apple Essence150 L	=	N 2,454,000
Cost of Banana Essence 25 L	=	N 25,000
Cost of Paraffin Oil 50 L	=	N18,750
Total cost of RMC / annum	=	N 749,325,000
Operating Labour / support personnel	=	N 212,000

Fixed Manufacturing Costs

Fixed Capital Investment (Raw materials storage and product storage)

	=	N 129,406,800
Depreciation 0.1FCI	=	N 12,940,680
Rent for ware house	=	N 48,000
Rent for service house	=	N 24,000
Plant overhead costs	=	N 4,913,524
Total FMC	=	N 17,9262204

General Expenses

1,000 distribution cans 100 L, 50 L	=	N 50,000.
Total Manufacturing Costs / annum	=	N 797,513,204
For 1L of apple perfume	=	797,513,204/75,000
	=	N 10,233.
At 50% profit margin = 0.5 x 10,233	=	N5,116
	=	N15, 349

15% for factory, 15% for wholesale and 15% for retailers