Quality Evaluation of Kithul (*Caryota urens*) Treacle Available in the Market with Respect to Chemical and Sensory Parameters and Identification of Volatile Flavor Compounds

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Declaration

The research work described in this thesis was carried out by me at the Agro and Food Technology Division, Industrial Technology Institute and Faculty of Applied Science under the supervision of Mrs.D.R.Rajapaksha and Mr. R.M.U.S.K. Rathnayaka. A report on this has not been submitted to any other university for another degree.

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DEDICATED TO ALL MY TEACHERS.

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Abstract

Caryota urens is a native tree in tropical Asian continent and it is grown in wet and intermediate wet zone of Sri Lanka. This palm is capable of storing large volumes of Carbohydrates, which is converted to sugars. Tapping the inflorescence enables the collection of the exudate sap from which the main product treacle is manufactured. According to the Sri Lanka standard specifications for treacle the total sugar content should be more than 65%. The kithul sap has about 8-9% sugar. During the process of preparation of kithul treacle there is a tendency for the manufactures to add cane sugar to obtain higher yield. Due to this quality of product is affected and the original kithul flavor is reduced. The objective of the study was to evaluate some market samples of treacle to identify the amount of adulteration. According to the work done by Industrial Technology Institute, the ratio of reducing sugars to total sugars was found to be a useful parameter to detect whether kithul treacle samples are adulterated with cane sugar. In addition an attempt was made to identify the flavor profile of some kithul treacle samples to see weather there are definite volatile flavor compounds responsible for the kithul flavor.

Five market samples of kithul treacle were randomly collected and they were tested for chemical parameters such as brix value, pH value, acidity, amount of reducing sugar, total sugars and the sensory parameters such as color, consistency, taste & flavor and overall acceptability. These parameters were compared with those of pure kithul treacle. According to the results brix value of samples varied between 64.4 – 72.0 and pH value varied between 4.09 to 4.95, acidity was between 0.17 %- 0.21% and amount of reducing sugars varied between 5.93% - 24.03%. Amount of total sugars varied between 64.02%-71.15 %. There was a relationship between chemical and sensory parameters, and according to the results 50% of samples were not within the required ratio (0.1 – 0.2) of reducing sugars to total sugars. Those samples may have been adulterated with cane sugar. According to the results of sensory evaluation there was no difference in color at 5% significance level. However taste & flavor, overall acceptability and consistency were different in samples.

During preliminary studies of volatile flavor components in kithul treacle using Gas Chromatography-Mass Spectrometry, it was observed that Benzene acetaldehyde and 2-Furancarboxaldehyde were common in all Gas Chromatography-Mass Spectrometry chromatograms. Further studies are necessary to conclude volatile flavor profile of kithul treacle.

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Chapter 01

INTRODUCTION

1.1 Introduction

Kithul palm (*Caryota urens)* is a native tree in tropical Asian continent. This tree grows in wet and intermediate zone of Sri Lanka.Although there is no commercial cultivation, this tree is ideally a home garden tree in villages. Kithul tree is considered as a multi purpose tree. The palm is capable of storing large volumes of carbohydrate. This is converted into sugars and trans located to the inflorescence where tapping will enable to collect the exudates sap. This sap is commonly known as "Teldiya" or "Telijja". Unfermented sap is used for the preparation of treacle or jaggery. Both products have a high demand as a substitute for sugars as well as an indigenous medicine.

According to the Sri Lanka standard specifications for treacle the total sugar content should be more than sixty five percent. The kithul sap has about 8-9% sugar. An average 6-9 liters of sap is required to make one liter of treacle. Therefore during the process of preparation of kithul treacle there is a tendency for the manufacturers to add cane sugar to obtain a higher yield. This is a major malpractice in the production of kithul treacle. Therefore quality of product is affected and it reduces the original kithul flavor.

Although there are standards for treacle and jaggery there is no specific standard for kithul treacle and jaggery. There is no standard procedure to check the adulteration of kithul products. Therefore it is very difficult to identify pure kithul treacle and jaggery in the local market. Due to this it is difficult to get good quality product with natural kithul flavor.

However kithul treacle is very popular in local and export market and most of the people like the natural kithul treacle flavor. Therefore quality evaluation of kithul treacle available in the market with respect to chemical and sensory parameters was undertaken in this study.

Reducing sugars, non-reducing sugars and other amino acids like non-volatiles mainly contribute to the kithul treacle flavor. However not only those non volatiles, other volatile components are also given major contribution to its flavor. Volatiles give its native flavor characters and these volatiles can be identified by extracting

them from kithul treacle and this study attempt to use gas chromatographic and mass spectrometer analysis to identify them.

1.2 Objectives

This study was carried out with the objectives of,

i. Quality evaluation of Kithul treacle available in the market with respect to chemical and sensory parameters.
ii. Identification of the flavor profile of kithul treacle to see whether there are definite volatile flavor components responsible for the natural kithul flavor.

1.2.1 Specific Objectives

i. Evaluation of some chemical and sensory parameters of treacle samples available in the market with genuine kithul treacle.
ii. Detect whether kithul treacle samples available in the market are adulterated with cane sugar according to the method developed by industrial technology institute.
iii. Identification of volatile flavor components of natural kithul treacle using gas chromatography-mass spectrometer (GC-MS).

Chapter 02

Review of literature

2.1 Kithul – (*Cariyota urens)*

2.1.1 Introduction

Kithul Palm (*Cariyota urens*) is a native tree in tropical Asian continents. This tree is a multipurpose tree of the family palmae and found widely distributed in home gardens as well as in natural forests through wet and intermediate zones of Sri Lanka such as Galle, Kandy, Rathnapura, Matale, Kegalle, Monaragala, and Badulla districts (Fernando, 2003). There is no commercial cultivation but the Kithul palm is ideally a home garden tree in the villages. Not only that this tree is naturally grown in the forests and many people in the rural villages earn their daily living from this tree (Farhad, 2004).

2.1.2 Classification

Kingdom	– Plantae
Phylum	– Magnoliophyta
Class	–Liliopsida
Sub Class	- Arecidae
Order	–Arecales
Family	– Arecaceae/ palmae
Sub family	-Arecaideae
Tribe	-Caryoteae
Genus	– Caryota
Species	– *Caryota urens*

2.1.3 Vernacular name

English	– Fish tail palm, Elephant's palm, Wine palm, Toddy palm.
Sinhala	– Kithul
Tamil	– Tippilipna, Kuntalpanai, Kuntarpanai
Hindi	– Mari, Bankhajor
Malay	– Cuntappana, Anappano, Trampana (Sharma et al, 2003)

2.1.4 Varieties

In Sri Lanka there are two types of kithul trees. They are local large type that produces more sap and dwarf one that produces very low sap (Farhad, 2004).

In local large type the lifetime is about 20-25 years. Production of sap is about 10 bottles per day. Dwarf type is very small and the maximum height of the plant is 4-5m. The size of inflorescence is smaller then the local one and the production of sap per day are about 2 bottles (Gunathilaka, 2004).

2.1.5 Distribution

Caryota urens occurs in tropical Asia. Centre of origin is hotter moisture places of India, Sri Lanka, Burma and Thailand (Rajapaksha, 1998). It is common in the mid and low country moist regions up to 300ft, altitude in Sri Lanka (Jayaweera, 1982).

2.1.6 Description

2.1.6.1 Trunk

Kithul is a single stemmed monocarpic monoccious palm (Rajapaksha, 1998). A lofty handsome palm with a smooth, cylindrical, shiny, annulated trunk bearing a crown of large leaves (Sharma et al, 2003). The trunk is about 13 – 20 m high and 0.3 m diameter. The trunk is very strong and it bears 10-20 leaves (Wealth of India, 1957).

2.1.6.2 Leaves

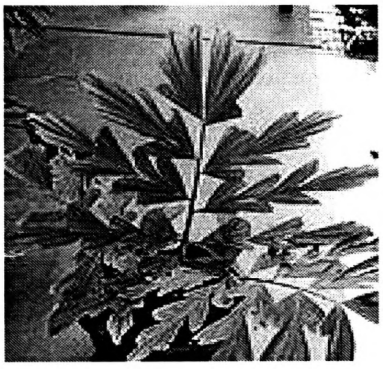

Figure 2.1 leaves of *Caryota urens*

Kithul leaves are very large and bipinnated. Size of the leaves is about 6 -7 m long and 3 – 5 m broad, primary divisions 1.6 – 2 m arched and dropping, leaflets 10 – 20 cm long, fascicled or alternate, cuneiform, sessile, obliquely truncate, irregularly serrate –

toothed on the truncate margin, upper margin, produced beyond the leaflets in to a tail. These leaflets shaped like the tail of a fish. Therefore called "fish tail palm" also. This is quite glamour and bright green in color and shining margins (Jayaweera, 1982). There is no distinct midrib, but several prominent veins, tips broadly toothed (Rajapaksha, 1998). Spathes 3 – 5, incomplete, tubular, spadix interfolior, shortly peduncled, 3 – 4 m long, the branches simple, are forming a dense tassel drooping from the very stout short peduncle. Spathes closely embracing the peduncle of the spadix (Jayaweera, 1982).

2.1.6.3 Inflorescence (Flower)

It begins at the top of the stem when the palm is fifteen years old and downwards for several years, during which time the leaves die and break off. Flowers are arranged in-group of three with single female between two males. The latter opening first and filling, male flower switch numerous stamens (Rajapaksha, 1998).

Flowers are crowded, males solitary or binate with an interposed female, cylindrical when closed, 1.2 cm long, sepals 3, orbicular imbricate pekals 3, linear-oblong, coriaceous concave, connate at base, valvate, stamens many filaments short, on there acuminate with no pistillodes, female flower subglobose, sepals 3, short, staminodes 3 or 6, ovary superior, obovoid, trigonous, 3-locular, stigma 3 lobed, ovule erect (Jayaweera, 1982).

The tree is very much different from other palm trees because the tree produces the inflorescence only after it reaches the maturity. It produces around 8-15 inflorescence during lifetime (Farhad, 2004).

The first inflorescence emerge from the apical meristem may consists of 2 to 7 main branches. Subsequent inflorescences are unbranched and arise from the axillary buds. Each inflorescence consists of long bent rachis, where secondary inflorescence could be observed at the distal part of the main rachis. A large number of sets of flowers could be observed spirally arranged on rachillae and each set is composed of two staminate flowers on either side of pistil late individual staminate flower remains open for 16-20 days and flower opening of a single inflorescence commune for about 6 weeks. The pistil late flowers open about 2-3 weeks after all the staminate flowers have bloomed and remain receptive for 3-13 days. The ovary is trilocular, usually with two fertile locules (Rathnayaka et al, 1990).

Figure 2.2 Inflorescence of *Caryota urens*

The tapers have classified inflorescence according to the shape, length, smoothness like characters. According to that long inflorescence is called "Verun mal", short inflorescence is called "Bakmunu mal", thin long one is called "An-mal", smooth tissues are called "Ala mal" and more fibrous are called "Kohu mal".

According to the length of inflorescence the no of "Puruk" also change. Near to the trunk it is "Kohu puruka" next one is "smooth puruka" /La puruka". Not only has that named but also such as "Diya puruka" peni puruka, gal puruka etc.... are given depending on the place.

There are 3-7 branches in main inflorescence and there are no branches in others. The place that branches occurs in the flower called "Ithi gataya or Balal pahura". The middle one is called "Mudun iththa" and the stem of flower is called trunk or "Mal leeya". There is a belief that there is a higher yield of sap near the "ithi gataya" and the sap from "mal leya" is salty taste that is not sweet. When tapping some days, the yield of sap is high and then the yield is reduced (Senevirathene, 2004).

The shape of the inflorescence can be categorized into two major types. Those are elongated and compact. The compact type produced more sap yield than the elongate type (Kithsiri and Pathirana, 1995).

2.1.6.4 Fruit

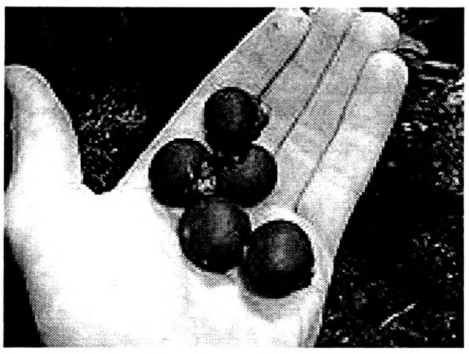

Figure 2.3 Fruits of *Caryota urens*

One inflorescence may produce 17500 – 22500 fruits. Shape of fruits are globular, about 2 cm in diameter and color is red or yellow. 1-2 seeded pericarps acrid and full of rapider. Fruit development takes place from 32 to 38 weeks (Rajapaksha, 1998).

Each fruit contain two large hemispherical seeds with ruminate endosperm and this is covered with fleshy mesocarp and smooth epicarp. Mesocarp contain an irritant, which may cause skin is directly touched, and probably this chemical is a growth inhibitor green unripened fruits become yellow or red in maturity and finally turn into dark brown color (Gunarathene et al, 2003).

2.1.7 Tapping

The inflorescence or flower is prepared for tapping before it reaches the maturity stage. A mixture of ingredients such as pepper, chillies, onion, lime, garlic are introduced into a cavity (¼") made of the base of the inflorescence. Then this cavity is properly sealed and the tip of the inflorescence is severed with a sharp stainless steal knife. The sap flows in small quantities and gradually increases within two weeks and continues to yield up to about 3-4 months. Flow of sap from the treated inflorescence is very high and it is collected daily in the morning and evening. The cut edge of the inflorescence is shaved daily to keep the edge fresh and enable the sap to flow without a block. It is also a method of maintaining the hygiene of the open edge of the inflorescence. The kithul palm yields an average of 11.25 liters/day (Farhad, 2004).

Figure 2.4 tapping of kithul inflorescence

2.1.7.1Composition of sap

The most important primary product of the kithul palm is the sap. The sap is commonly known as "Telijja" or "Telidiya" (Gunarathne et al, 2003) .The exudate that is flowing from the phloem and xylem of the trunk is identified as the sap.

The sap when collected fresh is an acidic, cloudy juice with sweet and sour taste. The pH value is 4.4 – 6.3 and the specific gravity is 1.033 – 1.0369 g/l.

The composition of sap	Contents gm/100gm of sap
Moisture	90.00 – 87.00
Non reducing sugar	8.30 – 9.00
Reducing sugar	0.10 – 0.16
Nitrogen	0.18 – 0.18
Ash	0.20 – 0.21
Pectin & Gums	0.90 – 1.10

(Farhad, 2004)

2.1.8 Food uses of kithul plant

Sap is the main raw material of the kithul tree. In India and Sri Lanka this sap is used to prepare toddy, vinegar treacle and jaggery. If the sap is fermented it is known as toddy. Before fermentation sap is taken for preparation of treacle and jaggery by heating. The main economic advantage of kithul is home-based preparation of treacle and jaggery for local market.

Trunk is ground into flour, which is used to prepare porridge, talapa, pudding and other snack foods. Matured trees yield 30-40 kg of flour (Rajapaksha, 1998).

Nutritional values of kithul flour (100g)

Moisture	13.1 g
Energy	339.0 K cal
Protein	2.4 g
Fats	0.3 g
Carbohydrates	81.7 g
Calcium	130.0 mg
Phosphorus	60.0 mg
Iron	20.0 mg

(Jayaweera, 1982)

2.1.8.1 Kithul treacle

Figure 2.5 Bottle of Kithul treacle

Sap extracted from inflorescence of the kithul palm boiled down to syrup is called treacle. This shall be made from clean, filtered unfermented sap of the plant. The color of treacle shall be uniform through out and may vary from light brown to dark brown. It shall be free from any plant tissue and dead insects, fragment or any other extraneous matter. Kithul treacle shall have a pleasant characteristics odour and flavor characteristic of the particular type and free of any objectionable odour and flavors.

Specific standards for treacle according to Sri Lankan standards as follows.

Moisture % by mass maximum	30.00
Total sugar % by mass minimum	65.00
Acidity (expressed as acidity acid % by mass maximum	0.50

Total ash content on dry basis % by mass maximum	1.50
Acid insoluble as dry basis % by mass maximum	0.15
Matter insoluble in water on dry basis % by mass maximum	0.50

(SLS No772, 1987)

The color development is governed by the interaction of reducing sugars and nitrogenous matter. This form of browning reaction is referred to as non enzymatic browning or Mailard reaction and the factor that governs the color development are, temperature of heating, heating time, moisture content, and developed acidity in a multi component system. Higher the heating temperature, higher the tendency for browning, and longer the heating time, higher the tendency for browning, Further, lower the moisture in the system, higher the tendency for browning. Flavor and odour development needs, careful control of temperature, and preventing over heating especially at the final stage of evaporation. It is important to decide the correct end point in the evaporation operation, and experienced operators decide this primarily based on the sticky consistency of the treacle, and the nature of the appearance of air bubbles in the evaporator. A flame temperature of 670 ^{0}C with L.P. gas and a 102 to 106 ^{0}C on the liquid / evaporator interphase was noted (Siriwardane, 2004).

2.1.8.2 Kithul jaggery

It shall be the crystallized product manufactured from the sap exudates collected from the inflorescence of the kithul palm. Jaggery should be made from clean filtered unfermented sap and should be free from foreign matter and from any objectionable flavor (SLS 521, 1981).

The sap from the palm is collected in pots exposed to smoke or coated with lime, bark of *Vateria acuminate* or leaves of *Acronychia laurifolia, Cuminosna pedunculata* are sometimes added to check fermentation. The juice is strained into open clay or copper pots and boiled over a slow wood fire. It is continuously stirred and the froth periodically removed. The boiling is continued with stirring till graining occurs, when it is allowed to cool for a few minutes and poured into moulds to set (Wealth of India, 1957).

Percentage composition of kithul Jaggery as follows.

Moisture	9.16%
Protein	2.28%
Fat	0.11%

Total Carbohydrates	84.89%
Sucrose	84.31%
Glucose & fructose	0.53%
Ash	3.66%
Calcium	1.35%
Phosphorus	0.37%

(Wealth of India, 1957)

Standards for Jaggery according to the Sri Lankan standards as follows.

	% By mass
Moisture maximum	10.0
Total ash maximum	3.5
Acid insoluble ash maximum	0.5
Matter insoluble in water maximum	2.0
Reducing sugar maximum	13.0
Sugars non reducing minimum	70.0

(SLS 521, 1981)

2.1.9 Non food uses

There are lots of non-food uses. The kithul timber, mature inflorescence, stem of the leaf, root bunch are used to manufacture different types of household items, furniture fishing rods, decorative articles, gift item etc... (Rajapaksha, 1998).

The root bark and cabbage of this palm are used for treatment of rheumatic swellings and snakebite poisoning.

The leaves produce the kithul fiber of commerce. The cabbage of this palm before flowering is a favorite food of elephants. It is used medicinally for gastric ulcers. The root is employed for tooth ailment, the bark and seed on boils and the tender flowers for promoting growth of hair (Jayaweera, 1982).

2.2 Flavor Evaluation

The type of foods and its processing affects flavoring efficiency and they must be tasted in the food it self for the evaluation of flavor materials. Therefore there has been a lack of standardization of testing techniques, a committee on sensory evaluation (Chang, 1966).

2.2.1 Flavors

Flavor is a very complex sensation composed primarily of aroma and taste, but also complemented by tactile and temperature responses (Reineccius, 1997).

Definition of Flavor is as follows. "Flavor is that property of a substance commonly a food which causes a simultaneous reaction or sensation of taste on the tongue and odour in the olfactory center in the nose". Therefore taste and odour are descriptive of a sensation.

"Flavor" is a word derived from "Savor", which is defined as "the power to excite the sense of smell or taste. Therefore power to arouse interest or zest. As a verb "Savor" has a synonym, "smack," which is perhaps most descriptive of the physical process of enjoyment of food. Not only is that flavor commonly accepted as a pleasure giving property (Guenther and Hamann, 1966).

Very simply, flavor is the combination of taste and odour. It may be influenced by sensations of pain, heat, and cold and by tactile sensations (Belz and Maarse, 1985).

2.2.1.1Taste

Some basic principals are involved in the physiology of flavor perception. There are four basic tastes. Taste means the sensation perceived via the taste buds resulting from the presence of certain soluble substances. The four basic tastes are sweet, salty, sour and bitter. Metallic has been proposed as a fifth taste. The four basic tastes are perceived by certain sense organs, taste buds are chemically stimulated and the reaction can be measured by electrical impulses of the nerve fibers so stimulated. There is some evidence to say that the stimulation may be physical. When several tastes qualities are perceived partially simultaneously the overall sensation depends upon the temporal and spatial pattern of the total activity (Chang, 1966).

2.2.1.2 Odour

Odour means sensation perceived via the olfactory organ from certain volatile substances (Belz and Maarse, 1985). The physiology of odour, which is the determinative characteristic of flavor, is more complex and less known than that of taste. A vast number of odours are distinguishable but we do not know how this is accomplished. Olfactory response is only observed when the substance contains the olfactory membrane. This membrane composes a small surface area above the nasal passages. It is generally accepted that in order to have an odour a substance must be volatile and of relatively low molecular weight so that by inspiration it may make contact in the nasal passage. That a substance has odour is most logically attributed to its molecular structure, and as in taste it is perception is a chemical reaction with a receptor cell, which excites a nerve center giving a sensation. The identification of odour may not depend upon a single sensory reaction but upon several analysis occurring almost simultaneously (Chang, 1966).

2.2.2 Sensory evaluation

2.2.2.1 Introduction

Sensory evaluation is a child of industry. It was spawned in the late 40's by the rapid growth of the consumer product companies; mainly food companies. This sensory evaluation field has grown rapidly in the second half of the 20^{th} century, along with the expansion of the processed food and consumer products industries. This comprises a set of techniques for accurate measurement of human response to foods and other information influences on consumer perception. As such, it attempts to isolate the sensory properties of foods themselves and provides important and useful information to product developers, food scientists and manager about the sensory characteristics of their products.

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and heaving (Harry and Hildegorde, 1999).

In other words, sensory evaluation is made by the sense of taste, smell, touch and heaving when food is eaten. The complex sensation that results from the interaction of our sense is used to measure food quality in programs for quality control and new product development. This evaluation may be carried out by one person or by several hundred.

Sensory evaluation panels can be grouped in to three types. They are highly trained experts, laboratory panels and large consumer panels. Highly trained experts evaluation quality and large consumer panels are used to determine consumer reaction to a product (Elizabeth, 1977).

2.2.2.2 Types of test

There are several different sensory evaluation methods have been developed. The most practical and efficient method should be selected for each situation. There are three fundamental types of sensory tests.

1. Discrimination test and
2. Descriptive test.
3. Preference / acceptance tests. / Affective test

Table 2.1 Classification of test methods in sensory evaluation.

Class	Question of Interest	Type of test	Panelist characteristics
Discrimination	Are products different in any way?	"Analytic"	Screened for sensory acuity, oriented to test method, sometimes trained.
Descriptive	How do products differ in specific sensory characteristics?	"Analytic"	Screened for sensory acuity and motivation, trained or highly trained
Affective	How well are products liked or which; products are preferred?	"Hedonic"	Screened for product use, untrained

(Harry and Hildegorde, 1999)

2.2.2.3 Preference test

Preference tests are affective tests based on a measure of preference or a measure from which relative preference can be determined (Elizabeth, 1977). In this is to offer people a choice among alternative products. Then see if there is a clear preference from the majority of respondents (Harry and Hildegorde, 1999).

The most commonly used scale for preference testing is the nine point hedonic scale. The term "hedonic" is defined as "having to do with pleasure" It should only be used in connection with scales in which the panelist expresses his degree of liking or disliking according to the following scale (Elizabeth, 1977).

Table 2.2 The 9 point hedonic scale used to assess liking and disliking.

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

This scale has achieved wide popularity since it was first invented in the 1940's. This is very simple to use and easy to implement. In this the most straight forward approach to this problem is to offer people a choice among alternative products and then see if there is a clear preference from the majority of respondents (Harry and Hildegorde, 1999).

2.3 Flavor characterization

Food volatiles, although usually present in small amounts, represent an important part of the total composition. They are the major factor in the taste, smell and consequently acceptability of a food product. Therefore chemical analysis of the volatile components in foods some of which may contribute to flavor has recently become one of the most important areas of flavor chemistry. A complete analysis of this type includes the isolation of the volatile compounds, and the determination of odour characteristics and chemical identities of the pure fractions. These type of analysis has been applied both to the desirable flavors of foods and to the undesirable off flavors. There are some objectives in knowing what compounds are responsible for a certain flavor in a certain food.

They are,

1. To understand the mechanisms of the formalization of the flavor compounds in order to develop methods to accelerate or to increase their

formation if the flavor is desirable and to prevent the formation if the flavor is undesirable.

2. To manufacture the flavor of a food by blending chemical compounds and through the use of these resultant synthetic flavors to improve the flavor of food products, to restore, the fresh flavor to processed food, and to create new food items with specific flavors.

3. To assist geneticists to grow and breed food raw materials with improved or intensified flavor compounds or precursors of such compounds.

4. To supply a basis that could be used as quality control for the flavor of food products.

2.3.1 Isolation of food flavors

The volatile flavor compounds amount to no more than ppm to ppb of the food. Due to the different physical and chemical nature of different foods, there are various techniques for the isolation of volatile compounds that may contribute to flavors (Chang, 1966).

Usually more than one method can be applied to isolate volatiles from a food product. The most appropriate one should be carefully chosen. Because if any losses of important compounds caused by improper selectivity or low efficiency of the method (Belz and Maarse, 1985).

2.3.1.1 Distillation

Distillation is one of the oldest methods for flavor isolation from foods. In this method it takes advantage of the volatility of flavor compounds and non-volatility of the major food constituents.

In distillation that includes techniques such as steam distillation and high vacuum distillation, molecular distillation etc... In steam distillation typically involves a source of steam the sample vessel and series of cold traps. Steam enters sample vessel so that the food sample is continuously agitated. Volatile compounds steam distills from the food and is condensed in the cold traps. The initial trap is generally cooled with ice water, then trap cooled with dry ice / acetone and final traps with liquid N_2. The distillate is a very

dilute solution of volatile flavor and water. The flavor must then be isolated from aqueous solution. Extracting the distillation with an organic solvent like pentane, diethyl ether or dichloromethane generally does this. A simpler method of isolating flavors via distillation is to use a simultaneous distillation extraction method (Reineccius, 1997).

2.3.1.1.1 Simultaneous steam distillation / extraction

An ingenious device designed by LIKENS and NICKERSON is frequently used to isolate food volatiles.

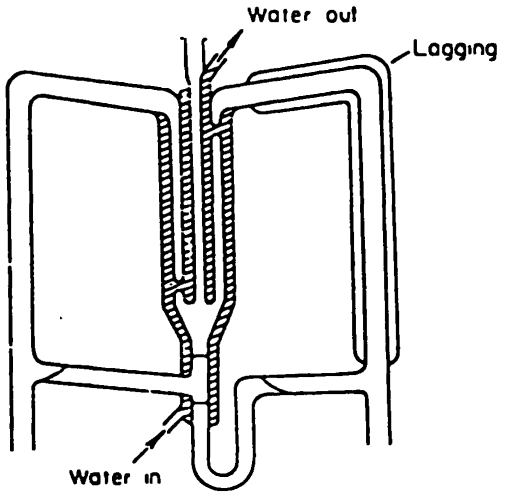

Figure 2.6 LIKENS and NICKERSON apparatus used in flavor isolation.

Aqueous slurry of the product is put in a flask attached to the right side of the apparatus, while the flask connected to the other side holds the solvent. The flasks are heated separately, and steam and solvent vapors are condensed at the same time in the cooled central section. Subsequently, the water and the solvent phases, which are immiscible, are separated in the "U" section of the aerators and flow back to their respective flasks. This result in continues procedure during which distillation and extraction are carried out simultaneously and which requires only a small volume of solvent to extract large quantities of food (Belz and Maarse, 1985).

This equipment provides for very efficient flavor extraction, but this is not suitable for lipid-based samples.

The advantage of distillation over extraction is that in aroma isolates and concentrates obtained by distillation no non-volatile matter is present that might interfere with the ensuing chromatographic analysis. A draw back is that by distillation dilute

aqueous solutions are obtained from which the volatiles have to be isolated, which means an extra step in the analysis (Belz and Maarse, 1985).

2.3.1.1.2 High vacuum distillation

Volatiles can be isolated from a food product also by high vacuum distillation. When water is present a high vacuum distillation can be carried out because of the water vapors. Then at as an "entrainment" gas, for the volatile molecules released from the product. This is used not only for the isolation of volatiles from foods, but also to remove co-extracted non-volatile compounds from odour extract and concentrates (Belz and Maarse, 1985).

2.3.1.1.3 Molecular distillation

This involves the direct transfer of a volatile from a food sample to a cold condenser. This method often used for the isolation of flavors from lipid based food because the vacuum requirement limits this method to the isolation of volatiles from pure fat and oils (Reineccius, 1997).

2.3.1.2 Extraction.

Extraction methods are used to isolate volatiles directly from foods or to recover them from dilute aqueous solution obtained via distillative isolation. The methods are based on favorable distillation coefficient of the volatile between a solvent phase and a food, or the aqueous distillate. When selecting a solvent it should not be selective if we need to study all volatile compounds (Belz and Maarse, 1985).

Solvent extraction is generally practiced only on fat free foods. Various devices are available for the extraction of foods. But the method used for this is determined by the physical state of food (Solid or liquid), quantity of food, and extracting solvent. The simplest method of solvent extraction is batch extraction using separatory funnels or a mixxor. But disadvantage of this method is limited extraction capacity to 10ml. Other problem is emulsion formation in the separatory funnels (Reineccius, 1997).

2.3.1.3 Headspace Method

Direct analysis of the headspace vapors above a food product seems to be the ideal method of flavor isolation. This is a simple method, rapid, reproducible. The primary problem with direct headspace analysis is that too little sample is available for

instrumental analysis. Direct headspace injections are generally limited to 10ml or less, only those volatiles present in the headspace at concentration exceeding 10^{-2} g/L will be detected by gas chromatography and only those exceeding 10^{-2} g/L will be adequate for mass spectrometry. From this direct headspace sampling analyzed only the most abundant volatiles above a food. But this is still not adequate for the analysis of trace volatiles in foods.

Trace analysis of food volatile may be accomplished via headspace concentration technique (Reineccius, 1997).

2.3.2 Concentration of volatiles

Distillative isolation of volatile compounds, from food, often results in dilute aqueous solutions. These solutions typically must be concentrated to permit gas chromatographic detection. The volatiles they contain have to be concentrated by one or more of the methods. Difference in boiling point, freezing point or polarity between the solvent and flavor compounds typically from the bases for concentration. The method of choice depends upon the type of flavor involved and solvent used. But the major problems encountered in the concentration process include the loss of volatile flavor compounds and the introduction of artifacts.

2.3.2.1 Evaporation

This technique takes advantage of the difference in boiling point between the flavor compounds and solvent. Evaporative techniques are typically used for flavor isolates in organic solvents such as pentane, dichloromethane dactyl ether and iso-pentane. The solvent must be low in boiling point, inert, thermally stable and of extreme purity.

A disadvantage of this is that volatiles may be lost by co-distillation. The loss of different compounds may not be the same nor predictable. Therefore quantitative results may be in error even when multiple internal standards are employed (Reineccius, 1997).

2.3.2.2 Freeze concentration

This is used to aqueous solutions of volatiles, such as steam distillate. Partial concentration of the aqueous system before to solvent extraction and it has the advantage of reducing solvent volume for extraction. Freeze concentration is based on the property

that water will freeze out of solution as a pure crystal. Therefore as the water freeze, solutes become concentrated in the unfrozen matrix (Reineccius, 1997).

2.3.2.3 Adsorption

Another attractive means for isolating aroma volatiles free of large volumes of water or organic solvent is adsorption. This technique used for the concentration of flavors in aqueous solutions. In this may utilize charcoal, silica gel, alumina, porous polymers or reverse phase pack as absorbents. Charcoal is most often used for this purpose because it is not deactivated by water and it has a large adsorptive capacity. Typically, an aqueous distillate is passed through a column of adsorbent. The absorbent is rinsed and then process accomplishes both concentration and transfer of the flavor components to a non-polar solvent for analysis (Reineccius, 1997).

2.3.2.4 Zone melting

This method has been reported in very few instances. Like freeze concentration it minimizes loss of volatiles and thermal degradation. It too results in concentration of organic materials because their distribution coefficient favors the liquid rather than the frozen phase. Though this is very use full, it appears not to be widely applicable due to the complexicity of the required apparatus, the restriction to small sample size, the need to use solvents that have already been purified by zone melting, and the time required to accomplish the overall concentration (Teranishi et.al, 1972).

2.4 Identification of volatile flavor components

2.4.1 Chromatography

The term chromatography applies to all separation processors of components based on their distribution between a stationary and mobile phase. The mass transfer between the two phases is effected either by distribution of the molecular of a mixture between the two phases, or adsorption-desorption on the surface of the stationary phase. According to the stationary and mobile phase we can classify chromatography as follows.

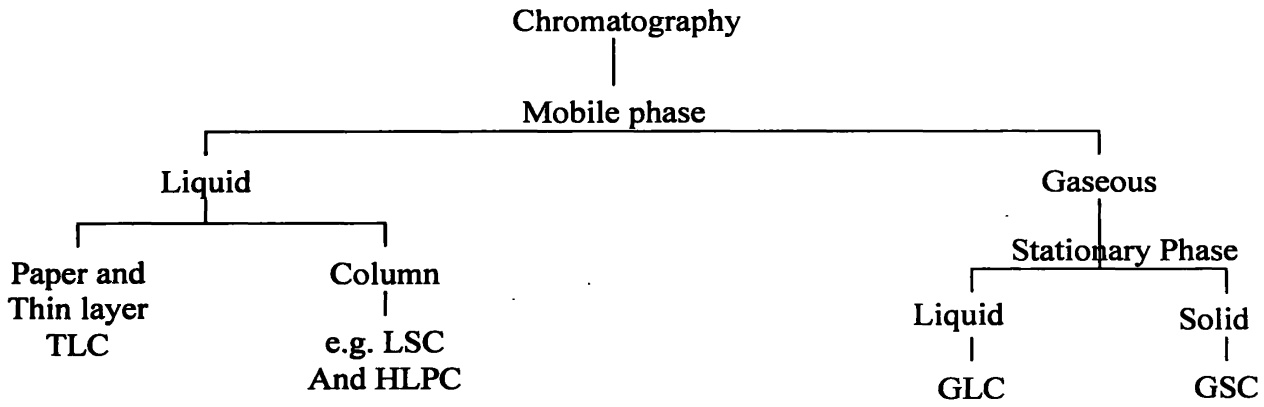

Figure 2.7 Scheme of chromatographic technique

The chromatographic behavior of a solute can be described by the retention volume (V_R), the retention time (t_R) or the retardation factor (R value) of the compound (Belz and Maarse, 1985).

Gas chromatography is ideally suitable to flavor studies because it has excellent separation powers and extreme sensitivity. These resolution and sensitivity are essential for analysis of complex flavor ((Reineccius, 1997).

2.4.2 Gas chromatography

2.4.2.1 History

Gas chromatography is a unique and very versatile technique. In the first stages of development it was applied to the analysis of gases and of vapors from very volatile components. Gas chromatography can be used for the direct separation and analysis of gaseous samples, liquid solutions and volatile solids. The techniques of derivatization or pyrolysis gas chromatography can be utilized to analysis of nonvolatile samples (Grob, 1985).

2.4.2.2 Introduction

Gas chromatography is a method for separating components of mixtures of volatile compounds. It is one of many modes of chromatography. It has become extremely popular in 1952 because of the rapidity and ease with which complex mixture

can be analyzed, because of the very small sample required and because of the flexibility, reliability and low cost of the instrumentation required (Guiochon and Guillenin, 1988).

2.4.2.3 Definition

Chromatography is a separation process which utilizes the difference between the equilibrium coefficients of the components of the mixture to be separated between a stationary phase of large specific surface area and a moving fluid which percolated across it (Guiochon and Guillenin, 1988).

2.4.2.4 Basic apparatus of gas chromatography

The instrumentation is very simply compared to many other analytical techniques.

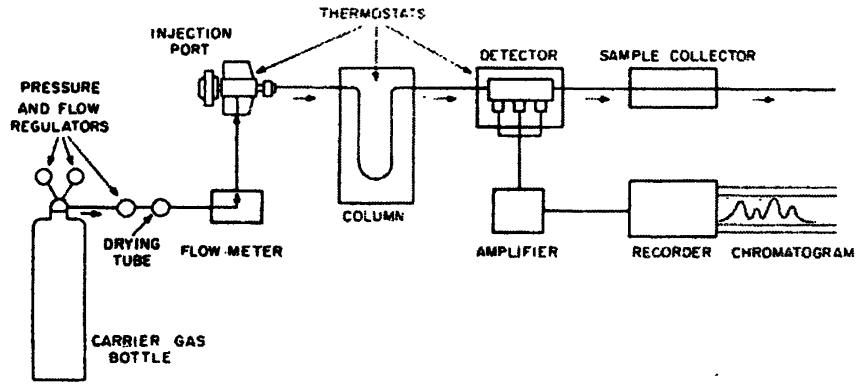

Figure 2.8 Schematic drawing of apparatus for gas chromatography

Carrier gas from the tank of compressed gas first passes to a controller, the usual purpose of which is to maintain a constant flow of gas. The flow meter is used to measure the rate of flow of gas.

The gas then passes to the beginning of the column, at the inlet to which is sample injector through which the sample to be analyzed can be introduced. The carrier gas then elutes the components of the mixture through the column. End of this device there is a detector .The purpose of which is to detect the separate components of the mixture as they emerge one by one. The detector uses some physical or chemical property of the vapors by which they can be indicated. Amplifier amplifies the signals from the detector and it is

fed to a recorder where the chromatogram is drawn on a strip chart (Zweig and Sherma, 1972).

2.4.2.5 Chromatographic data

Chromatogram is a diagram, which obtains analytically useful information. This is use to identify the individual components of a mixture qualitatively. From the data recorded during a chromatographic analysis, five parameters can be measured for each peak such as retention time, the gas hold up time, the peak width, the peak height and the peak area. Most important parameters are retention time and area.

2.4.2.5.1 Retention time (t_R)

This is the time between injection of the sample and appearance at the column's exit of the maximum concentration of the band of the corresponding compound.

2.4.2.5.2 The peak area (A)

The peak area is the area under the signal. The peak area is proportional to the sample size. The peak size is proportional to the amount of compound contained in the injected sample.

2.4.3 Gas chromatograph –Mass spectrometer (GC-MS)

Retention data are totally insufficient to permit the identification of an unknown compound. Therefore development of GC-MS is very important to identify unknown compound by a combination of selective reactions and the use of retention data. In GC-MS, the first technique separates the components of the mixture and delivers pure substances to the mass spectrometer. This obtained contain a large amount of information and are generally sufficient to characterize an unknown one (Guiochon and Guillenin, 1988).

2.4.3.1 Principle of Mass Spectrometry

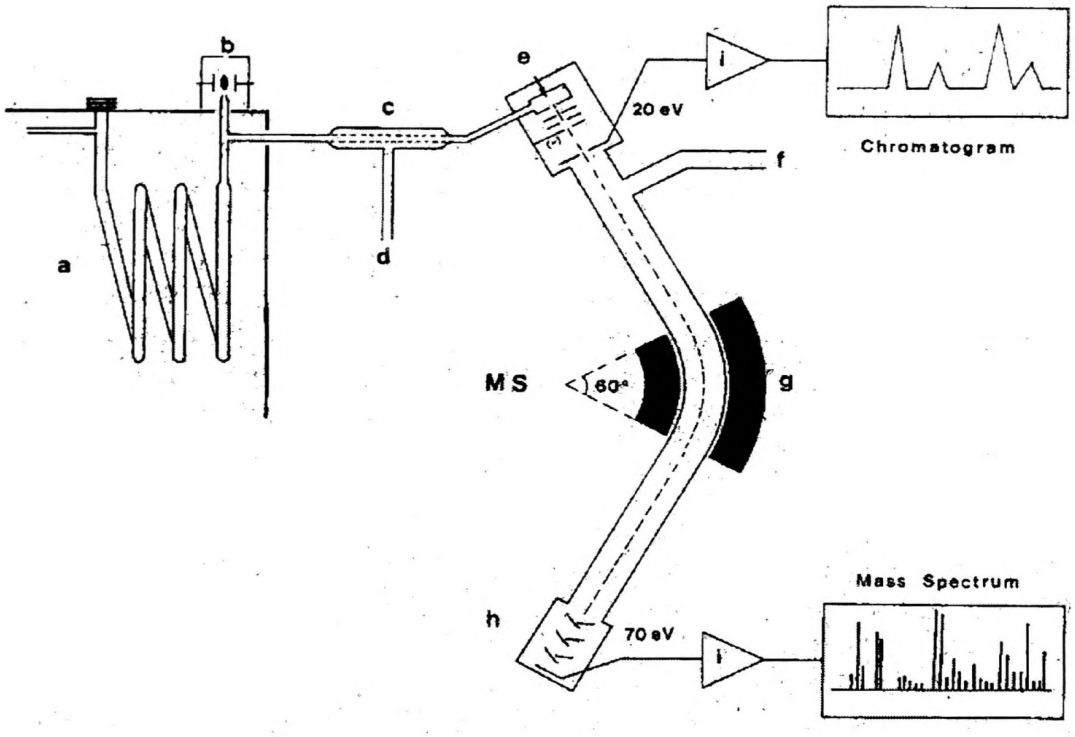

Figure 2.9 Schematic of a Gas Chromatograph coupled to a Mass Spectrometer

a-Chromatographic column

b-Flame ionization detector

c-Interface

d-Vacuum pump

e-Ionization chamber of the mass spectrometer

f-Vacuum pump

g -Magnet

h-Ion collector and electron multiplier

i-Amplifier

This includes tree main parts, the ion source, the ion analyzer and the ion detector. The ion source generates ions from the sample. Ions of different masses are analysed by a magnetic sector. Then it gives the relevant mass spectrum to the compound and according to that it can be identify the unknown compound with use of the GC-MS library (Guiochon and Guillenin, 1988).

Chapter 03

Materials and Methods

3.1 Sampling

Five samples of kithul treacle with popular brand names in the market were selected randomly (Sample volume 325.0ml and container type glass bottles). Reference sample was prepared at the institute.

3.2 Chemical analysis

3.2.1 Equipments

- Refractometer (ATAGO N-3Ebrir 58%-90%)
- pH Meter (744 pH Meter)
- Volumetric Flasks (100.0ml,250.0ml)
- Conical flasks (250.0ml)
- Pipettes (5.0ml)
- Burettes
- Burette stands
- Water Bath
- Hot plate with a magnetic stirrer
- Analytical balance (PB303-5$ - Mettler Toledo)
- Beakers (500.0ml)
- Measuring cylinder
- Spillers
- Droppers
- Watch glasses
- Funnels

3.2.2 Reagents

- Fehling's solution (A & B)
- Standard glucose
- Treacle samples
- Methylen blue indicator

- Phenolphthalein indicator
- 0.1M sodium hydroxide solution

3.2.3 Methods

3.2.3.1 pH value

20.0 ml of treacle sample was taken into a 25.0 ml beaker and then pH value was taken and the temperature of the sample was recorded for each treacle sample.

3.2.3.2 Brix Value

Brix value was measured in each sample using a hand refractometer.

3.2.3.3 Acidity

Weighed (Nearest mg) approximately 5g of sample into a 100.0 ml conical flask and recently boiled and cooled distill water was added. Then 1 ml of phenolphthalein was added and titrated against standard sodium hydroxide (0.1M) and observed the color change. End point reading was recorded. It was repeated three times for each sample.

Calculation

$$\text{Acidity (as acetic acid) \% by mass} = \frac{6.4 * VC}{M}$$

V – Volume in standard sodium hydroxide required for titration

C – Concentration of sodium hydroxide standard

M – Mass in g of sample taken for the test.

3.2.3.4 Sugar

3.2.3.4.1. Preparation of solutions

Fehlin's A

Dissolved 34.63 g of copper sulphate ($CuSO_4$. $5H_2O$) in water, added 0.5 ml of concentrated sulphuric acid of relative density 1.84 and diluted to 500 ml in graduated flask. Filtered the solution through prepared asbestos.

Fehling's B

Dissolved 173 g of Rochelie salt (Potassium sodium tartarate-$KNaC_4H_4O_6 .4H_2O$) and 50 g of sodium hydroxide analytical reagent in water, diluted to 500 ml in graduated flask and allow the solution to stand for two days. Filtered the solution through prepared asbestos.

Methylene blue indicator

Dissolved 0.2g of Methylen blue in water and diluted to 100.0 ml.

3.2.3.4.2 Reducing sugars

Sample preparation

Approximately 5 g of treacle sample was weighed and transferred to 100.0 ml volumetric flask and made up to mark with distilled water.

Procedure

10.0 ml of Fehling's (5.0 ml for each A & B solutions) was pipetted into a 300.0 ml conical flask. Then 15.0 ml of prepared sample was added from burette and it was boiled using the hot plate with a magnetic stirrer for about 15 seconds. 1 ml of Methylene blue indicator was added and continued boiling 1-2 minutes, then small quantities (1ml or less at time) of prepared sample was added and it was allowing boiling for about 10 seconds between successive additions till it appeared brick red color. This was repeated three times for each sample. End point reading was taken.

Calculation

$$\text{Reducing sugar \%} = \frac{\text{S mg} * 100}{\text{V} * \text{W mg}} * 100\%$$

S– mg of sugars in 10.0ml of Fehling's solution (from standardization the solutions).

V–Volume of the prepared treacle sample required to titration (end point reading).

W– Weight of the treacle sample in milligrams.

3.2.3.4.3 Total sugars

25.0 ml of prepared sample was taken into a flask and 2.5 ml concentrated hydrochloric acid and 10ml of distill water were added to it. Then it was boiled in a water

bath maintained at 60^0C for 10 minutes for conversion of non – reducing sugars to reducing sugars. Sample was immediately cooled and neutralized with sodium hydroxide. Then it was transferred to 250.0 ml volumetric flask and volume made up to the mark. The same Fehling's test was repeated for total sugars.

Calculation

$$\text{Total sugar \%} = \frac{S \text{ mg} * 250.0 \text{ ml} * 100.0 \text{ ml} * 100\%}{V_1 * 25.0\text{ml} * W \text{ mg}}$$

S – mg of sugars in 10.0ml 0f Fehling's solution.

V_1– Volume of the prepared treacle sample required to titration (end point reading).

W – Weight of the treacle sample in milligrams.

3.2.3.4.4 Adulteration with sugars.

Adulteration was checked by using the ratio between reducing sugars to total sugars.

$$\text{Ratio} = \frac{\text{Reducing sugars}}{\text{Total sugars}}$$

If ratio is between 0.1<Ratio<0.2, sample is considered not adulterated according to method of Industrial Technology Institute.

3.3 Sensory analysis

3.3.1 Materials and Equipments

- Trays
- Spoons
- 50.0ml beakers
- Glasses
- Cups
- Treacle

3.3.2 Method

The six samples were evaluated as two batches by giving only three samples at once. It was conducted at good ventilated and clean sensory room and well-trained 20 panelists were used.

Samples were kept in clear glass containers with spoons and samples were coded using random numbers with a three-digit code. Then the samples were evaluated according to a nine point Hedonic scale (see App 01). Four parameters color, consistency, taste and overall acceptability were evaluated.

3.4 Gas Chromatography-Mass Spectroscopy analysis for volatile flavors

3.4.1 Materials

- Likens and Nickerson Apparatus
- Round Bottom flasks (100.0 ml, 250.0 ml)
- Cooler
- Water bath
- Measuring cylinder
- Heating mantel
- Adapters
- Clamps
- Separation funnel
- Small test tube
- GC-MS equipment
- n-pentane
- Distill water
- Treacle

3.4.2 Method

One of the best market sample of kithul treacle without adulteration and the authentic sample were used for the analysis. Samples were distillated separately. 300.0 ml of treacle was measured, 200.0 ml of distill water was added to it and it was transferred to a one liter round bottom flask. 100.0 ml of normal pentane was transferred to another 250.0 ml round bottom flask and the distillation kit was set as bellow.

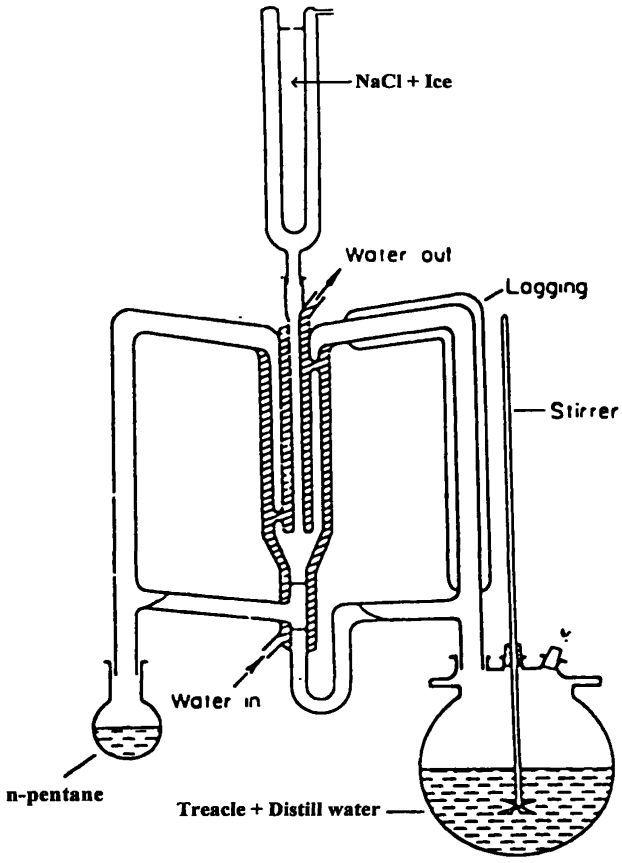

Figure 3.1 Distillation unit

Flask containing treacle was kept in a heating mantel and flask with n-pentane was kept in a water bath. The distillation was carried out for 2 ½ hours passing cold water through out the condenser and then collected pentane layer to "U" shape area in condenser was separated using separating funnel. It was evaporated to concentrate the sample to 1.0 ml. Finally, Gas Chromatography-Mass Spectrometer analysis was carried out. Following conditions were used in the Gas Chromatography-Mass Spectrometer.

Hewlett – Packard 6890 column HP 5MS * 30 M* 0.32 mm i.d df = 0.25 μm carrier gas flow 0.71 ml He/min, inlet pressure 12.9 Psi, injected volume 1 μl, split ratio 1:27 injector temperature 250 ^{0}C

Temperature program

4 min 50^{0}C – 1 min hold

4 min 200^{0}C – 1 min hold

2 min 244^0 C – 1min hold

10 min 275 ^{0}C– 1min hold

Detection Hewlett – Packard mass sensitive detector (MSP model 5973) working in the scan mode for 50 to 500 amu at 1.68 scan/s ionization by El at 70 eV and 0.8 mA.

Same procedure was carried out to other samples. In addition to that the blank distillation was carried out (300.0 ml distilled water to instead of treacle) and Gas Chromatography-Mass Spectrometry was carried out for pure pentane.

Chapter 04

Results and Discussion

4.1 Chemical analysis of kithul treacle

Table 4.1 Chemical Analysis results of treacle

Sample No / Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Brix value	72.0	69.0	68.2	67.2	67.0	64.4
pH Value	4.09 at 25.9 ^{0}C	4.21 at 26.3 ^{0}C	4.53 at 25.9 ^{0}C	4.95 at 28.9 ^{0}C	4.65 at 26.7 ^{0}C	4.75 at 27.1 ^{0}C
Acidity %	0.21	0.2	0.19	0.17	0.18	0.18
Reducing Sugars %	24.034	23.29	13.04	6.85	8.05	5.93
Total sugars %	71.15	68.43	68.05	67.97	66.78	64.02
$^{RS}/_{TS}$	0.33	0.34	0.19	0.1	0.12	0.09

According to the results, brix values of the samples are within the Sri Lanka standards (>65) except one sample. Sample no 6 is very close to the standard value. pH values of all the samples are between 4.00 to 5.00.According to these values samples are slightly acidic. But acidity of the samples is less than 0.5%. That is also within the Sri Lanka standards. Percentages of reducing sugars in samples vary between 5.0% to 24%. Total sugars of the samples are greater than the 65% except for one sample. Five samples are within the limits of Sri Lanka standards. According to the chemical analysis five samples are of good quality.

With respect to ratios between reducing sugars to total sugars only three samples are between 0.1 to 0.2.One sample is below that limit and other two are above the limits. Therefore only three samples considered to be pure kithul treacle. These are not adulterated with cane sugar. When we consider sample no 1 and sample 2 the amounts of reducing sugars are high and the acidity is also higher than the other samples. Therefore there is a possibility to convert ion of non-reducing sugars to reducing sugars in acidic medium. Due to that ratio can be over the limits. According to this except for one sample other five samples are of good quality without adulteration with cane sugar.

4.2 Sensory evaluation of kithul treacle

Table 4.2 Mean values of ranks from the sensory data

Sample No Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Color	7.30	7.40	7.10	7.20	7.40	6.95
Consistency	6.00	6.30	6.65	7.30	7.15	5.60
Taste & Flavor	4.60	7.05	7.25	7.10	6.75	5.15
Overall acceptability	5.10	7.02	7.15	7.15	6.95	5.30

4.2.1 Color of kithul treacle

According to the above results, there is no clear difference in mean values of color. It varied between 6.95-7.40.The sensory data for color (see App.02) was analyzed using SAS systems. According to that,

H_0: There is no color difference in samples

H_1: There is a color difference in samples

$P = 0.7254$

So $P > 0.05$

Do not reject H_0 at 5% significance level. Therefore there is no color difference in all samples (see App.03).

4.2.2 Consistency of kithul treacle

According to the mean value variation in consistency (Table 4.2), there is a great difference in the six samples and it varied between 5.60-7.30.According to results sample 4 is the most preferred sample. The sensory data were analyzed using SAS systems. Due to that,

H_0: There is no consistency difference in samples

H_1: There is a consistency difference in samples

$P = 0.0081$

So $P < 0.05$

Reject H_0 at 5% significance level. Therefore there is a difference in all samples according to the consistency.

According to the mean separation of samples, 2, 3, 4 and 5 samples are not very much different from each other but sample 1 is different from 4 and 5 samples and 6th is different from 3,4 and 5 samples (see App.04).

4.2.3 Taste and flavor of kithul treacle

Mean values of the preference to taste and flavor had a large variation. That is between 4.60-7.25.According to mean values least preference is the sample 1 and most preferred one is sample 3.According to chemical analysis data, sample 1 has low pH and that can be acidic taste. That may be the reason for the least preference to sample 1. But there is no greater difference on sample 2, 3 and 4. The sensory data for taste and flavor were analyzed using SAS systems. According to that,

H_0: There is no taste and flavor difference in samples

H_1: At least one sample is difference from others with respect to taste and flavor

$P = 0.001$

So $P < 0.05$

Reject H_0 at 5% significance level. Therefore there is a difference in samples according to the taste and flavor.

According to the mean separation samples, no 1 and 6 are different from the others (see App.05).

4.2.4 Overall acceptance to the kithul treacle

According to the mean values variation in overall acceptability, there is a great difference in the six samples and it varied between 5.10-7.15.But there is no clear difference in sample 2, 3, 4 and 5 and sample 1and 6 are different from others. The results from sensory evaluation were analyzed using SAS system. According to that,

H_0: There is no difference in overall acceptability in the samples

H_1: There is a difference in overall acceptability in the samples

$P = 0.001$

So $P < 0.05$

Reject H_0 at 5% significance level. Therefore there is a difference in samples according to the overall acceptability.

According to the mean separation of samples 1 and 6 are different from the others (see App.06).

4.3 Volatile Flavor components analysis

4.3.1 Gas Chromatography-Mass Spectrometer analysis for selected market sample (Sample 3)

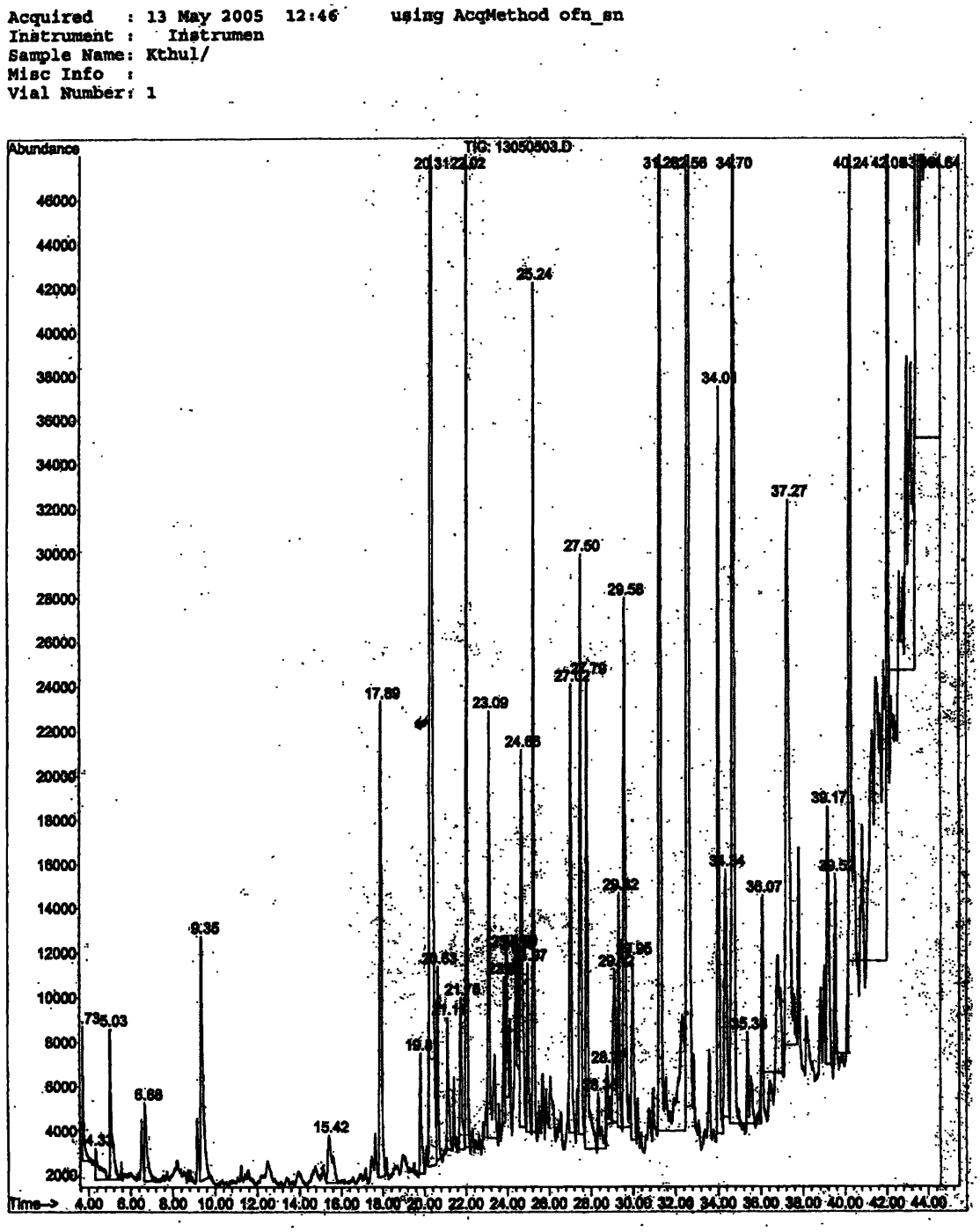

Figure 4.1 Chromatogram of the selected market sample

4.3.1.1 Chemical parameters of selected market sample

pH value 4.85 at 30.2 ^{0}C

Brix value 65.4

Reducing sugars 12.7 %

Total sugars 65.2 %

Acidity 0.18 %

4.3.1.2 Volatile components and chromatographic data identified in market sample

Table 4.3 Volatile flavor components of treacle sample

Peak No	Compound	RT (min)	Area %
1	Benzene, 1, 3 – dimethyl	3.73	0.21
2	Ethanone	5.03	0.35
3	Benzaldehyde	6.52	0.14
4	2 – Furancarboxaldehyde	6.68	0.20
5	2, 2' – Bifuran	9.15	0.18
6	Benzeneacetaldehyde	9.35	0.98
7	Benzene, 1, 2 – dimethyl	17.62	0.11
8	Safrole	17.89	1.62
9	α-Cubebene	19.81	0.32
10	Eugenol	20.31	5.96
11	α.-Copaene	20.63	0.46
12	β- Cubebene	21.11	0.36
13	Nonadecane	21.41	0.13
14	Methyl Eugenol	21.75	0.52
15	β-Caryophyllene	22.02	2.67
16	α-Humulene	23.09	0.90
17	Naphthalene	23.82	0.29
18	Germacrene-D	23.95	0.51
19	β-Selinene	24.11	0.23
20	α- Selinene	24.39	0.23
21	Pentadecane	24.50	0.34
22	Methyl Isoeugenol	24.66	1.44

23	α-amorphene	24.97	0.43
24	Delta.cadinene	25.24	2.00
25	α-Calacorene	25.87	0.11
26	Caryophyllene oxide	27.02	1.42
27	β-Selinene	27.50	1.74
28	12-Oxabicyclo	27.79	1.26
29	Unknown(may be Junipene)	28.35	0.14
30	Delta.cadinene	28.77	0.13
31	Unknown (may be Aromadendrene)	29.12	0.56
32	Unknown(may be Bycyclo oct-2-ene)	29.32	0.64
33	Unknown(may be 1,2-Benzenedicarboxylic acid)	29.58	1.74
34	Unknown(may be α-farnesene)	29.95	0.68
35	Zerumbone	31.26	4.02
36	Unknown(may be 2,4-dimethylfuran)	32.56	8.13
37	Octadecane	32.85	0.13
38	Ethyl 5-methoxy-1,2-dimethylind	33.58	0.20
39	Unknown(may be carr's ketol)	34.01	2.06
40	Ferrocenyldimethylborane	34.34	0.99
41	1,2-Benzenedicarboxylic acid	34.70	5.34
42	Nonadecane	35.38	0.17
43	Hexadecanoic acid	36.07	0.50
44	Tetradecanioc acid	37.27	3.13
45	Eicosane	37.79	0.51
46	Unknown	39.17	1.14
47	Unknown	39.52	0.44
48	9-Octadecanoic acid	40.24	2.96
49	Docosane	42.05	4.34
50	Heneicosane	43.46	14.14
51	Octadecane	44.64	22.81

The volatile flavor components of the selected kithul treacle sample are listed in the table 4.3. According the chromatogram 51 compounds are detected and 43 compounds were tentatively identified, when their mass spectra matched those in the

Mass Spectrometer library but for others there was no perfectly matching reference compounds were available for confirmation of retention indices and mass spectra.

According to the results most abundant compound in that sample is octadecane. About 17 compounds gives major contribution to the volatile flavor and other compounds are minor volatile constituent that give contribution to the kithul flavor.

According to the sample major volatiles contribution to the flavor comes from octadecane, heneicosane, docosane, zerumbone, tetra decanoic acid, α-octadecanoic acid, β-caryophyline, β-selinene, safrole, caryophyline cxide and 12- cxabicyclo.Others give minor contribution to the volatile flavor.

4.3.2 Gas Chromatography-Mass Spectrometer analysis for prepared sample

(Sample 4)

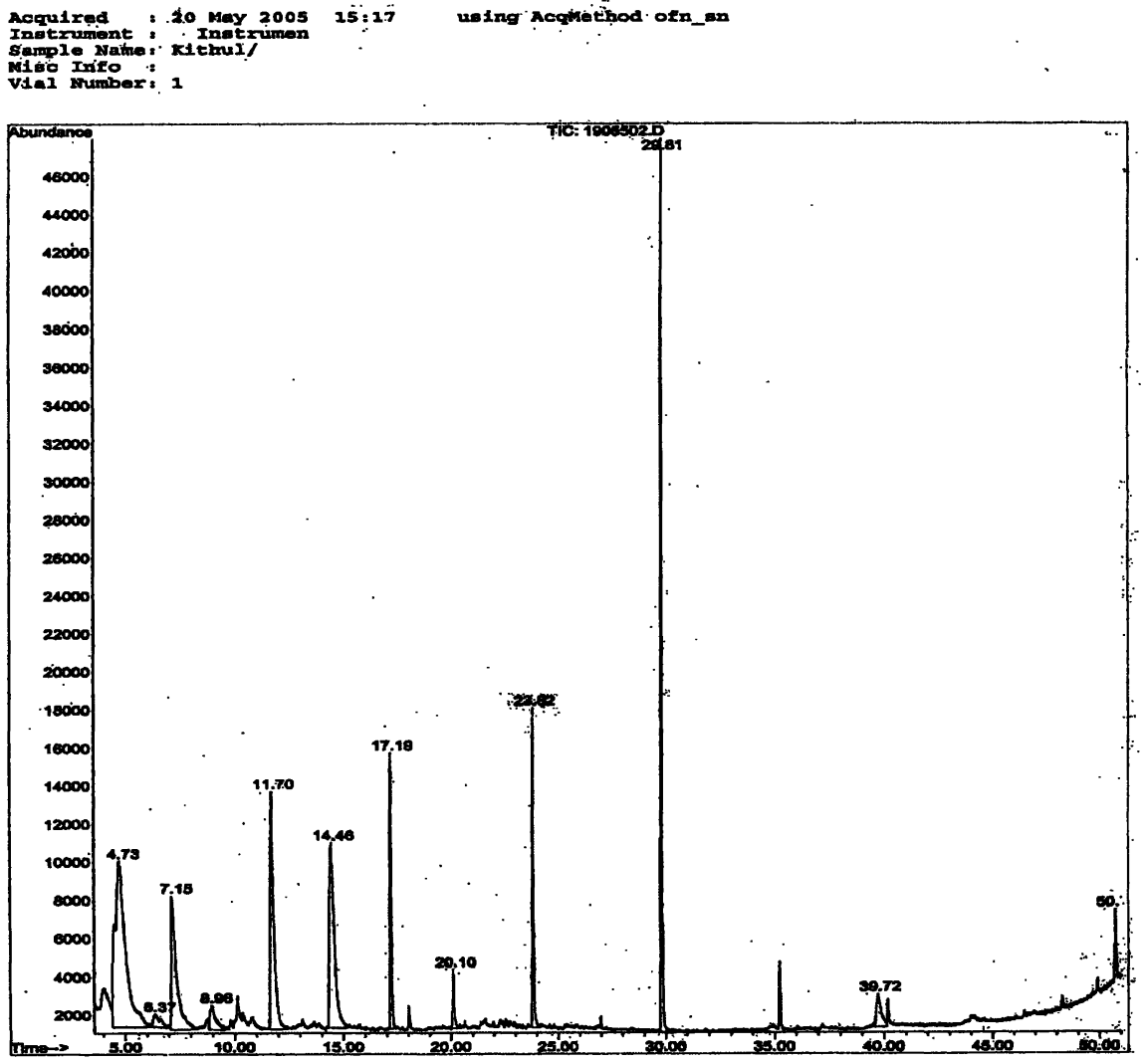

Figure 4.2 Chromatogram of the prepared sample

4.3.2.1 Chemical parameters of selected market sample

pH value 5.08 at 27.5 ^{0}C

Brix value 67.2

Reducing sugars 7.26 %

Total sugars 67.07%

Acidity 0.17 %

4.3.2.2 Volatile components and chromatographic data identified in prepared sample

Table 4.4 Volatile flavor components of treacle sample

Peak No	Compound	RT (min)	Area %
1	2-Furancarboxaldehyde	4.73	25.62
2	Unknown(may be 4-Cyclopentane)	6.37	1.4
3	Pyrazine	7.15	10.09
4	2-Furancarboxaldehyde	8.96	2.01
5	Benzeneacetadehyde	11.7	12.07
6	Benzeneethanol	14.46	13.48
7	Ethyl caprylate	17.19	5.33
8	Benzene	20.10	1.48
9	Decanoic Acid / Ethyl Caprate	23.82	5.85
10	Ethyl Laurate	29.81	19.07
11	Palmatic acid	39.72	2.32
12	Unknown (may be Phthalic acid)	50.74	1.29

Table 4.4 gives the results obtained for the analysis of the volatile flavor compounds of the pure kithul treacle. From the results, it can be seen that the essence contained 12 main compounds of which 6 (comprising 65.9% of the sample) have been positively identified, with a further 2 (3.8%) partially characterized. Only 2 compounds remain unidentified. It can be observed that the major volatile component is 2-furancarboxaldehyde. Middleditch (1989) noted that this compound was formed during an analysis of products of the Maillard reaction, and termed it an artifact. These are formed when glucose solutions are heated in the presence of oxygen at 100^0 C for five

days. Ethyl caprylate and ethyl caprate are other major compounds and according to Middleditch (1989) that is encountered during the analysis of maple syrup.

4.3.3 Gas Chromatography-Mass Spectrometer analysis for blank

```
Acquired   : 13 May 2005  11:45      using AcqMethod ofn_sn
Instrument :   Instrumen
Sample Name: Kthul/Blank
Misc Info  :
Vial Number: 1
```

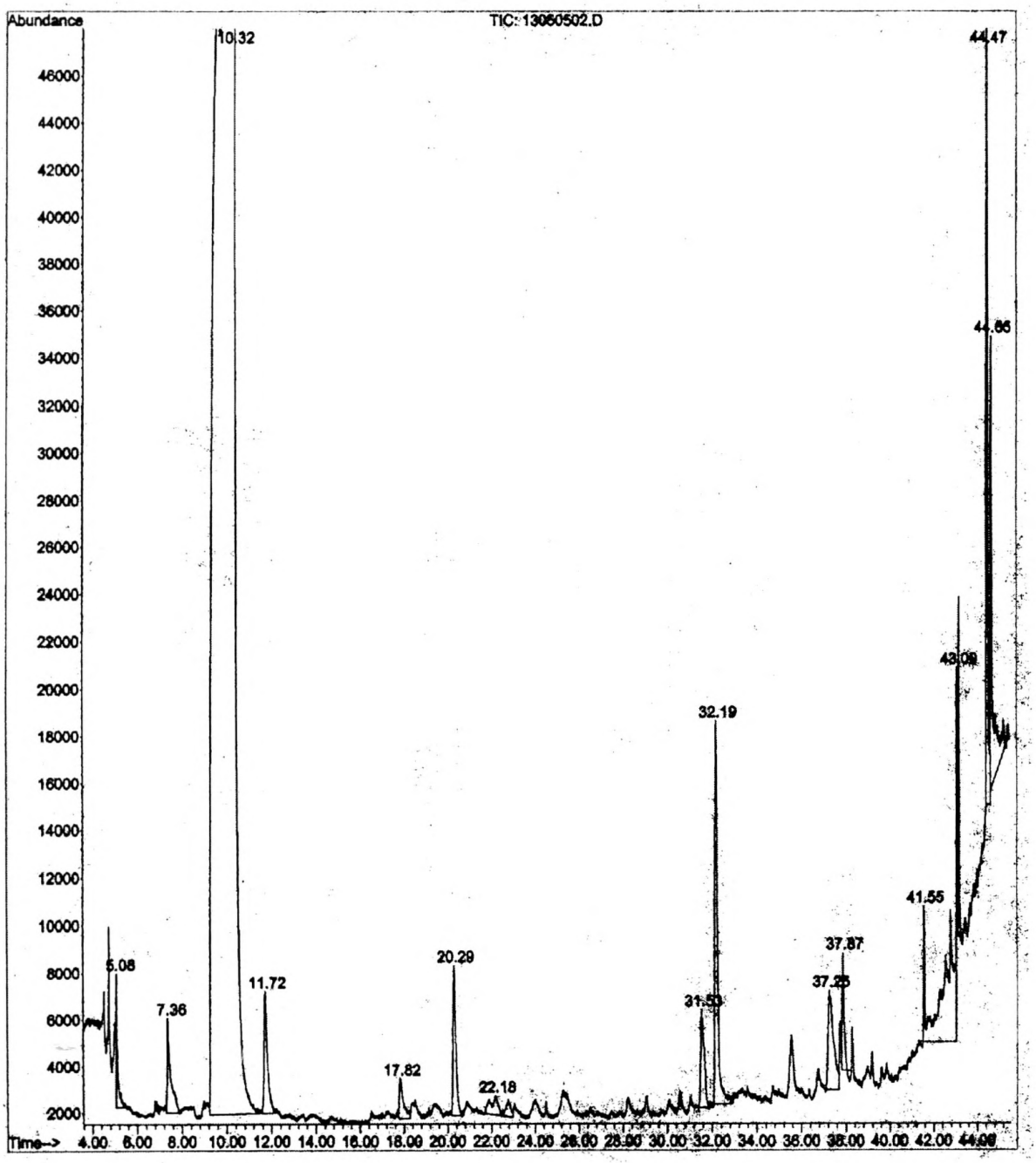

Figure 4.3 Chromatogram of blank

4.3.3.1 Volatile components and chromatographic data identified in blank

Table 4.5 Volatile compounds present in the blank distillation

Peak No	Compound	RT (min)	Area %
1	Unknown	4.74	1.08
2	Unknown (may be Propionic acid)	5.08	0.97
3	Unknown	7.36	20
4	Benzyl alcohol	9.5	39.43
5	Unknown (may be Arsenous acid)	11.72	3.96
6	Eugenol	20.29	4.29
7	Unknown	31.53	3.64
8	1,2 Benzedicorboxylic acid	32.19	10.78
9	Hexadecanoic acid	37.25	2.39
10	8,9,10,11 – Tetrahydro – 7 methylbenzene	37.87	2.88
11	Unknown	38.28	0.79
12	Octadecane	41.55	1.65
13	Unknown	43.09	2.82
14	Heneicosane	43.17	3.23
15	Pentadecane	44.47	14.7
16	Hexanedioic acid	49.65	5.4

According to the Chromatogram obtained from the blank sample ten compounds were identified and 6 compounds were not identified. In both market samples and blank Eugenol can be identified. But it is a major compound in cinnamon bark oil and this equipment is mostly used for cinnamon oil analysis. Hence it is suspected eugenol that can be a contaminant from the distillation apparatus. For the determination that, whether the contaminant from distillation unit or Gas Chromatography-Mass Spectrometer equipment or from the solvent, Gas Chromatography-Mass Spectrometer was carried out for only n-pentane.

4.3.3.2 Chromatogram of n-pentane

```
Acquired   : 20 May 2005  14:12     using AcqMethod ofn_sn
Instrument :   Instrumen
Sample Name: Kithul/solvent
Misc Info  :
Vial Number: 1
```

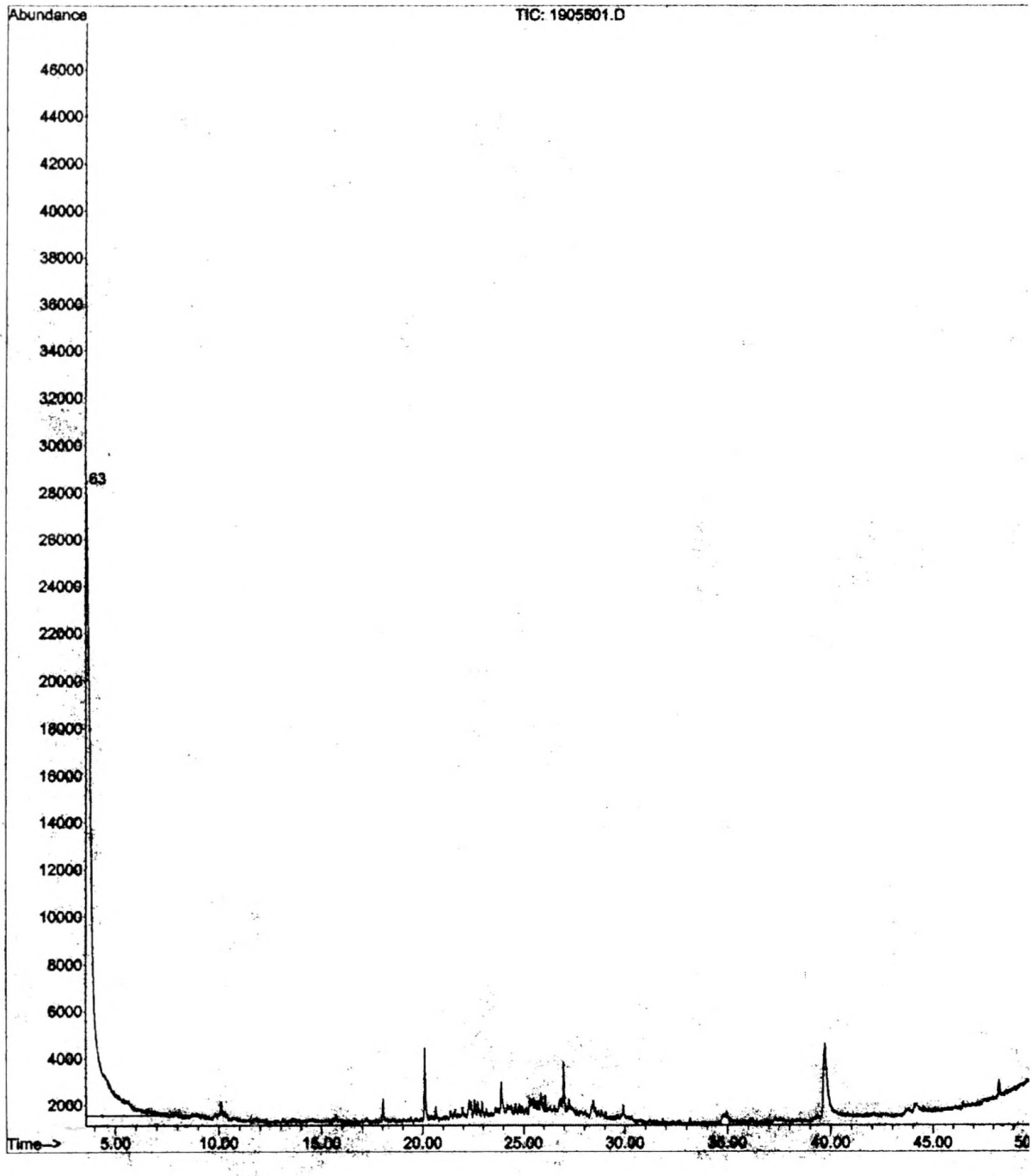

Figure 4.4 Chromatogram for n-pentane

According to the Chromatogram for n-pentane, eugenol could not be observed. Therefore eugenol, it was suspected that it must have come from distillation unit and it is not a volatile present in kithul treacle. To avoid the error obtained due to contamination thorough cleaning process was used for distillation apparatus before the analysis of second sample. Benzene can be identified in all chromatograms and it was found that is a major contaminant in n-pentane.

According to the chromatograms of treacle samples and blank, hexadecanoic acid and pentadecane can be identified. The area percentages of above compounds are high in the blank chromatogram. Therefore it was confirmed that, those are contaminants from the distillation. Both heneicosane and octadecane are obtained in both chromatograms of treacle samples and blank. But the area percentages are high in chromatogram of treacle sample. Due to that those are volatiles that are present in kithul treacle. In chromatogram of prepared sample there is no contaminant due to thorough cleaning process.

It can be observed that benzene acetaldehyde and 2-furancarboxaldehyde common in chromatograms of both treacle samples. These may be common volatiles for kithul treacle. Other volatiles varied and that can be due to the different chemical conditions of two samples.

Chapter 05

Conclusions

5.1 Conclusions

There is sufficient evidence to conclude that the qualities of kithul treacle available in the market are varying according to the Sri Lanka specification with respect to chemical parameters. Although 50% of samples were adulterated according to the results, it is difficult to conclude whether the samples are adulterated or not. Because the amount of reducing sugars varied with acidity.

There was a clear relationship with chemical and sensory data, but it is difficult to conclude weather people can identify natural kithul flavor by taste.

Preliminary study of the detection of kithul volatile flavor components using Gas Chromatography-Mass Spectrophotometer, it was observed that benzene acetaldehyde and 2-furancarboxaldehyde are common in both Gas Chromatography-Mass Spectroscopy chromatograms. Due to that, they are common volatiles in kithul treacle that contribute to volatile flavor. Other volatiles also give major contribution to volatile flavor. But it can be concluded that those varied with parameters such as preparation process, pH, and brix etc....

5.2 Recommendations for further work

In order to find the volatile flavor components that contribute to the volatile flavor profile, further studies are needed with various types of kithul samples from different parts of the island and with various method of preparation etc...

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Appendix 01

PREFERENCE TEST FOR TREACLES

Name: **Date :**

Time :

Instructions:

- You have been given three types of treacle samples, in random order.
- Please assess the characters of the samples given below in the given order using the following scale.
- At the beginning & in between samples wash your mouth with water.
- Don't compare the samples.

Character	Code No.		
Color			
Consistency			
Taste & Flavor			
Overall Acceptability			

9 – Like extremely
8 – Like very much
7 – Like moderately
6 – Like slightly
5 – Neither like nor dislike
4 – Dislike slightly
3 – Dislike moderately
2 – Dislike very much
1 – Dislike extremely

Comments:

.....
Signature

THANK YOU.

Appendix 02

Ranks of each parameter in preference test

Sample No Parameter	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
color	7, 8, 9, 7, 7, 7, 8, 8, 7, 7, 5, 8, 8, 8, 6, 8, 7, 7, 8, 6	8, 9, 7, 7, 5, 8, 9, 7, 7, 8, 7, 8, 7, 7, 7, 8, 8, 8, 7, 6	7, 4, 9, 8, 7, 7, 8, 7, 8, 6, 5, 7, 9, 7, 7, 8, 8, 7, 7, 6	7, 6, 8, 7, 7, 7, 9, 7, 7, 8,5, 8, 9, 7, 7, 8, 8, 7, 6, 6	7, 6, 9, 7, 7, 8, 9, 8, 8, 8, 5, 8, 9, 7, 7, 8, 7, 7, 8, 6	7, 6, 7, 7, 7, 7, 9, 7, 7, 7, 5, 8, 9, 6, 4, 8, 7, 7, 7, 6
Consistency	5, 5, 3, 6, 9, 6, 6, 8, 2, 8, 4, 8, 6, 8, 8, 6, 7, 3, 4, 8	7, 7, 9, 7, 6, 7, 8, 6, 8, 2, 5, 8, 8, 7, 4, 8, 7, 3, 7, 7	7, 6, 8, 7, 6, 7, 8, 5, 8, 7, 3, 7, 8, 6, 6, 8, 8, 3, 8, 7	8, 7, 8, 8, 8, 7, 8, 6, 8, 8, 5, 8, 8, 8, 6, 8 7, 6, 7, 7	8, 6, 9, 7, 8, 8, 8, 7, 8, 7, 4, 8, 8, 7, 6, 8, 8, 6, 6, 6	4, 4, 7, 8, 5, 7, 7, 4, 6, 6, 3, 7, 8, 6, 2, 8, 4, 6, 5, 5
Taste & Flavor	6, 4, 2, 4, 6, 6, 6, 3, 2, 7, 4, 4, 5, 8, 4, 5, 4, 3, 3, 6	7, 8, 8, 7, 7, 8, 9, 6, 7, 6, 6, 8, 8, 6, 3, 8, 8, 3, 9, 9	7, 8, 9, 7, 6, 7, 8, 7, 8, 8, 5, 8, 8, 6, 8, 8, 8, 3, 8, 8	6, 7, 8, 7, 6, 7, 9, 6, 8, 7, 4, 8, 8, 8, 8, 8, 6, 5, 8, 8	8, 7, 9, 6, 4,8, 6, 8, 7, 5, 5, 7, 6, 7, 7, 8, 8, 5, 7, 7	2, 2, 6, 8, 4, 6, 5, 6, 6, 5, 3, 7, 5, 6, 3,8, 5, 4, 6, 6
Overall acceptability	4, 4, 3, 4, 8, 6, 6, 6, 2, 8, 4, 4, 6, 8, 6, 6, 4, 3, 4, 6	7, 8, 8, 7, 6, 8, 9, 7, 7, 7, 6, 8, 8, 6, 4, 8, 7, 3, 8, 8	7, 8, 9, 7, 7, 7, 8, 7, 8, 7, 4, 7, 8, 6, 8, 8, 8, 3, 8, 8	6, 6, 8, 8, 7, 7, 9, 7, 8, 7, 5, 8, 8, 8, 4, 8, 6, 6, 7, 6	8, 7, 9, 7, 6, 8, 7, 8, 7, 6, 4, 7, 7, 7, 6, 8, 8, 6, 6, 7	3, 2, 6, 8, 6, 6, 6, 7, 6, 3, 3, 7, 6, 6, 3, 8, 5, 5, 5, 5

Appendix 03

The SAS System
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
SAMPLE	6	1 2 3 4 5 6

Number of observations in data set = 120

Dependent Variable: COLOUR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3.17500000	0.63500000	0.57	0.7254
Error	114	127.75000000	1.12061404		
Corrected Total	119	130.92500000			

R-Square	C.V.	Root MSE	COLOUR Mean
0.024251	14.65177	1.05859059	7.22500000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	5	3.17500000	0.63500000	0.57	0.7254

T tests (LSD) for variable: COLOUR

Alpha= 0.05 df= 114 MSE= 1.120614
Critical Value of T= 1.98
Least Significant Difference= 0.6631
Means with the same letter are not significantly different.

T Grouping	Mean	N	SAMPLE
A	7.4000	20	5
A			
A	7.4000	20	2
A			
A	7.3000	20	1
A			
A	7.2000	20	4
A			
A	7.1000	20	3
A			
A	6.9500	20	6

Appendix 04

The SAS System
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
SAMPLE	6	1 2 3 4 5 6

Number of observations in data set = 120

Dependent Variable: Consistency

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	43.70000000	8.74000000	3.30	0.0081
Error	114	302.30000000	2.65175439		
Corrected Total	119	346.00000000			

R-Square	C.V.	Root MSE	Consistency Mean
0.126301	25.05263	1.62842083	6.50000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	5	43.70000000	8.74000000	3.30	0.0081

T tests (LSD) for variable: Consistency

Alpha= 0.05 df= 114 MSE= 2.651754
Critical Value of T= 1.98
Least Significant Difference= 1.0201

Means with the same letter are not significantly different.

T Grouping			Mean	N	SAMPLE
	A		7.3000	20	4
	A				
	A		7.1500	20	5
	A				
B	A		6.6500	20	3
B	A				
B	A	C	6.3000	20	2
B		C			
B		C	6.0000	20	1
		C			
		C	5.6000	20	6

Appendix 06

The SAS System
Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
SAMPLE	6	1 2 3 4 5 6

Number of observations in data set = 120

Dependent Variable: Overall acceptability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	94.11041667	18.82208333	9.12	0.0001
Error	114	235.28750000	2.06392544		
Corrected Total	119	329.39791667			

R-Square	C.V.	Root MSE	Overall acceptability Mean
0.285704	22.28784	1.43663685	6.44583333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SAMPLE	5	94.11041667	18.82208333	9.12	0.0001

T tests (LSD) for variable: Overall acceptability

Alpha= 0.05 df= 114 MSE= 2.063925
Critical Value of T= 1.98
Least Significant Difference= 0.9

Means with the same letter are not significantly different.

T Grouping	Mean	N	SAMPLE
A	7.1500	20	3
A			
A	7.1500	20	4
A			
A	7.0250	20	2
A			
A	6.9500	20	5
B	5.3000	20	6
B			
B	5.1000	20	1

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