STUDIES ON THE DEVELOPMENT OF FLAVOUR IN COCONUT AND OIL PALM KERNEL ON HEATING

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TO

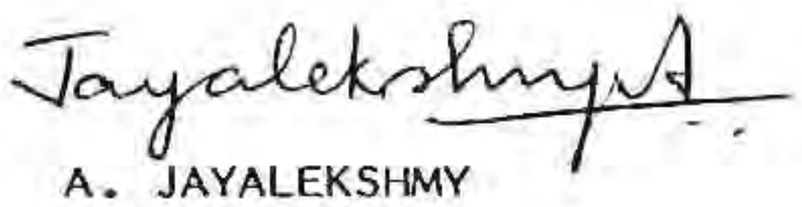
THE MEMORY

OF

MY FATHER

DECLARATION

I, A. Jayalekshmy do hereby declare that this thesis **"Studies on the development of flavour in coconut and oil palm kernel on heating"**, has not been submitted by me for the award of any Degree, Diploma, Title or Recognition before.



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This is to certify that the thesis bound herewith is an authentic record of the research work carried out by Mrs. A. Jayalekshmy, M.Sc. under my supervision in partial fulfilment of the requirements for the **Degree of Doctor of Philosophy** of the **University of Kerala** and further that no part thereof has been presented before for any other degree.

Dr. A.G. Mathew
Supervising Teacher

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C O N T E N T S

SYNOPSIS

The thesis entitled **"Studies on the development of flavour in Coconut and Oil palm kernel on heating"** is a scientific investigation of the development of roasted flavour in coconut and palm kernel during heating, which is due to the chemical changes taking place in the non-volatile constituents.

Coconut is an important vegetable oil source of India and also a major food item in the State of Kerala. Generally, roasting enhances the flavour of most of the food items. Coconut is heated for domestic and commercial oil extraction purposes with the final result of flavour enhancement and browning. Oil palm is an emerging oil crop of India, which can provide palm oil (from mesocarp) and palm kernel oil (from the endosperm). Oil palm cultivation is very much encouraged in Southern states like Kerala, Karnataka and Andhra Pradesh, due to favourable agro-climatic conditions. Foreseeing the potential of the kernel oil as a cooking medium, methods like mild heat treatment are attempted for improving its flavour.

Chapter 1 includes an exhaustive review of literature on thermally generated flavours, especially

pyrazines and on the chemistry of coconut and palm kernel. It is well known that heating of food leads to Maillard reaction between amino acids and sugars resulting in flavour enhancement. A number of volatile heterocyclic compounds like pyrazines (1,4-diazines) are formed which are considered to be responsible for the roasted aroma. Considerable research work has progressed on the chemistry of flavour changes in roasted cocoa, coffee, peanuts, meat products, potato products etc. However, no systematic investigation on the new flavours produced or the causative changes leading to it are studied in an important food item like coconut or in a promising oil seed like palm kernel. A survey of the literature on chemistry of coconut and palm kernel also indicates that studies are not adequate and a re-investigation with the help of modern techniques is felt necessary. With this background, and realising the importance of coconut and oil palm in India, especially in Southern states, the studies have been undertaken.

Chapter 2 includes the details of the materials used and the methodologies followed. The experiments were planned to study the changes in the volatile and non-volatile components of coconut and palm kernel on roasting.

The results and findings of the experiments with coconut and palm kernel are detailed in Chapters 3 and 4. A brief outline of the work carried out can be summarised as follows: Roasting conditions of coconut and palm kernel were optimised. A temperature of 160°C for 15 minutes was found to give coconut and the coconut oil, a desirable flavour and better shelf life for oil. Dry coconut gratings were taken as the control sample. Coconut heated to three different temperatures, namely 130, 145 and 160°C which represent progressive stages of Maillard reaction, were chosen for detailed study. The flavour was isolated by hydrodistillation and the flavour compounds extracted with solvents from the distillate selectively, after pH adjustment. Thus, basic, neutral and acid flavour compounds were separated. The flavour concentrates were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), and 75 compounds identified in total, including 18 pyrazines in roasted coconut. δ-Lactones, ketones, esters and alcohols were also present. Identification of this many pyrazines in coconut is reported for the first time. In the case of palm kernel, the temperature of roasting was chosen as 150°C (for 5 minutes) and flavour isolated as described for coconut. The flavour analysis was carried out by GC and GC-MS

and 29 compounds have been identified in control and 41 in roasted palm kernel. The latter included 8 pyrazines and 2 furan derivatives also. The other flavour compounds present were esters, ketones, δ-lactones etc. The systematic flavour analysis and identification of compounds in control and roasted palm kernel is also reported for the first time.

The changes occurring in the non-volatile components of the food matrix were studied, namely lipids, carbohydrates and proteins. The physicochemical characteristics of lipids extracted from dried (control) and roasted samples of coconut and palm kernel were studied. Fatty acid composition was analysed by gas chromatography (GC). Results indicated that there was not much change in lipids. The detailed analysis of soluble sugars of coconut by high performance liquid chromatography (HPLC) confirmed the presence of glucose, fructose, galactose and sucrose. It also showed reduction in fructose and glucose during roasting. The sugars arabinose, fructose, glucose, rhamnose and mannose were present in palm kernel and these sugars decreased on heating. The detailed analysis of sugars of palm kernel, by newer instrumental techniques, was not carried out earlier. Moreover, the changes in

sugars of coconut and palm kernel during heating are also reported for the first time.

The changes in the proteins were studied by detailed amino acid analysis of control and roasted samples of coconut and palm kernel, using an amino acid analyser. The amino acid profiles of heated samples did not differ much from the control samples. The changes in the free amino acids were also studied using the same instrument. The results indicated that in coconut the free amino acids lysine, tryptophan, glutamic acid, aspartic acid, alanine, valine and glycine were more affected during heating. Among the free amino acids of palm kernel valine, isoleucine, leucine, lysine, glutamic acid, aspartic acid and threonine showed considerable reduction on roasting. Since coconut is consumed as a food item also, the protein study was slightly more extended as follows: Solubility profile of the four samples of coconut (control and three heated samples) at different pH values (2 to 12) were studied and found to be affected during heating. The Osborne classification of coconut proteins was carried out and albumin and globulin fractions were found to be predominant and also more affected by heat. The sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of albumin and globulin fractions

of coconut samples was carried out. In heated samples, lower and higher molecular weight peptides were observed, indicating breakdown and cross-linking of existing peptide chains respectively.

Finally, based on the results obtained for the various compositional changes observed during heating of coconut and palm kernel, model systems were constituted, which very closely simulated the actual food matrix of coconut and palm kernel. The flavour was isolated and identified using GC-MS. The pyrazines identified in roasted coconut and palm kernel could be detected in the model systems also. Moreover, the lower aldehydes, alcohols and furans were also identified. Thus the interaction of free amino acids and simple sugars leading to formation of roasted flavour could be confirmed by the model system studies. These results are discussed in Chapter 5.

Thus, the study could provide rather a complete overview of the chemical changes taking place in the volatile and non-volatile constituents of coconut and palm kernel, during heating, in addition to providing new information on some of their important constituents.

CHAPTER 1

INTRODUCTION AND LITERATURE SURVEY

1.1 FLAVOUR

The sensation of flavour is one of the delights of eating. It is this subtle and complex sensation that helps us to distinguish good food from a bad one. To a great extent, it regulates the preferences of the consumer. According to Hall[1], flavour is defined as the sum of those characteristics of any material taken in the mouth, perceived principally by the senses of taste and smell, and also by the general pain, tactile and temperature receptors in the mouth as received and interpreted by the brain. Thus, flavour is constituted by taste, smell and texture.

Basically, there are four true tastes namely, sweet, sour, salty and bitter. The perception of taste is made possible by the numerous taste buds present in our mouth. Smell or odour is the sensation produced by the molecules of compounds, when they impinge on the olfactory epithelium,lining the nasal cavity. This normally happens during respiration or while swallowing food. The threshold values of odour compounds are much lower than taste threshold levels. Texture or

mouthfeel is related to the flow and deformation of the material in the mouth.

Of the above mentioned three factors, odour is the most important one which has got maximum influence on flavour. In this study, the role of odour is considered more important and dominant that the term 'flavour' is used synonymously with odour, unless otherwise stated.

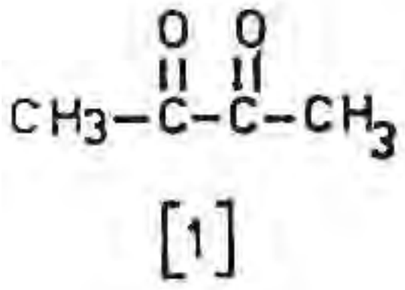
Food flavours can be broadly classified into two- natural and formed aromas. The natural flavour comprises those of fruits, vegetables, spices, nuts, raw meat, fish etc. They may be considered as metabolites produced in the tissues during intracellular biogenetic pathways. 'Formed flavours' include mainly those aroma compounds which are produced during processing, storage or deterioration. Examples are sugar-amine reactions, originally reported by Maillard[2] on heating such systems, ripening of cheese by microbial action, fermentation (eg.beer) and enzymic reactions (eg.in garlic, horse-radish, onion etc). Deterioration of food results in development of off-flavours such as those produced by microbial action or by autoxidation resulting in rancidity.

During processing, food may be roasted, toasted, baked, grilled or pressure cooked. In all these cases, the flavour profile can change due to (i) loss of volatiles, (ii) degradation of the existing flavour compounds and (iii) formation of new compounds as a result of interaction between the components of the food material. Processing operations generally affect the proteins, carbohydrates and lipids, proteins being affected most[3]. These can interact among themselves and produce a series of volatile and non-volatile compounds.

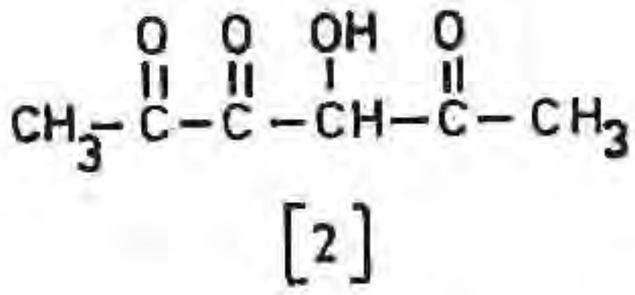
Processing conditions are generally selected in such a way that there is maximum enhancement of overall flavour and minimum loss of nutritive value. While subjecting to high temperatures such as grilling, roasting, smoking etc the possibility of formation of toxic compounds cannot be ignored. There is a global awareness about carcinogens like benzopyrenes that are identified in foods heated to very high temperatures[4]. The compounds formed during heat treatments can be scrutinised for the extent of toxicity also. Heat treatments of food is more important in the Indian context since Indian cookery has intricate steps which involve boiling, broiling, frying and also seasoning

with spices and condiments. In most of these heating procedures food is cooked at a high temperature (100°C and above) and there is considerable texture improvement along with the generation of a series of appetising aromas.

When food is heated, the carbohydrates and proteins undergo thermal degradations separately and also react together[5]. Thus, when sugars are heated in the absence of water or in concentrated solution form, browning occurs with the formation of characteristic caramel flavour. This change is referred to as **'caramelisation'**. The fused brown mass is known as **'caramel'** and is widely used in confectionery, soft drinks and alcoholic beverages. The typical caramel flavour is due to a number of sugar fragments and dehydration products[5]. Some of them are diacetyl **[1]**, acetic acid, formic acid, acetyl formoin **[2]** and 4-hydroxy-2,5-dimethyl-3(2H)-furanone **[3]** The last two compounds have typical caramel flavour. Other carbohydrates also undergo degradation at higher temperatures (150 to 200°C) giving volatile products like furans **[4]** and γ-lactones **[5]**. Cyclotene **[6]** has been identified during caramelisation of sucrose[5]. Amino acids undergo thermal changes



[1]



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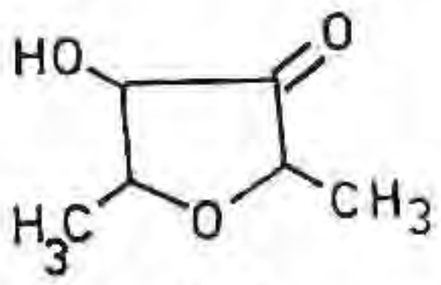

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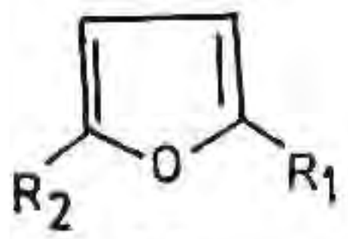
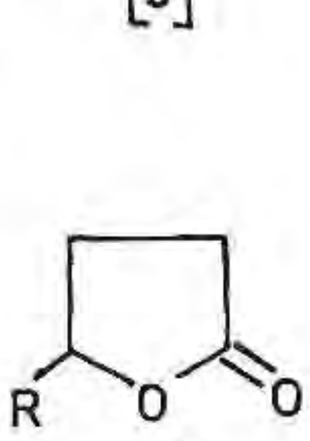
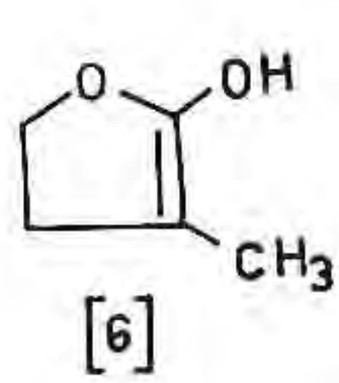


[4] R_1, R_2=H, alkyl



[5] R=H, alkyl



[6]

only at temperatures above 250°C, giving rise to amines[5]. However, sugars and amino acids interact at lower temperatures, mostly above 100°C, producing a number of volatile aroma compounds. The reaction is referred to as **Maillard reaction**[2]. The mechanistic aspects of this reaction will be dealt with in detail separately. Review[6] on the literature shows that a number of studies

have been conducted on the volatiles of roasted peanuts, filberts, almonds, cocoa, coffee, barley, potato chips, milk products etc. In all these cases, several nitrogen containing heterocyclic compounds have been identified, of which pyrazines are the most important[6,7]. They are directly or synergistically responsible for the roasted or cooked aromas[6,7].

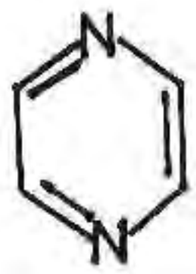
The role of lipids in the formation of flavours cannot be overlooked. Small amounts of methyl ketones, lactones and fatty acids are formed[5,8]. In addition to these, oxidation products of lipids can combine with amino acids of the food material and thus form Maillard reaction products[5].

The significance of Maillard reaction in food can be best understood by reviewing the research work pertaining to pyrazines which are the major flavour compounds produced.

1.1.1 Pyrazines

Structurally pyrazines are six membered hetero-cyclic compounds with two nitrogen atoms in 1,4-position. Thus, chemically they are 1,4 diazines [7]. The organic chemist had a long association with these compounds, but their occurrence in heated and processed foods and their significance as flavour imparting materials

were understood only towards the middle of the 1960s. Since then, they have been recognised as major contributors in the flavour of roasted and toasted foods.



[7]

Recently, chemists are focusing their research work on synthesising new pyrazines that can be better utilised in food processing industry[6]. This may help in imparting characteristic, roasted or nutty aromas without much heat treatment of the food. Apart from heated foods, pyrazines occur naturally In green peas[9], bell pepper[10], carrots[9], tomatoes[9], asparagus[9] etc which are not subjected to any heat treatment. But most of these naturally occurring pyrazines [7] are alkoxy derivatives, whereas in 'formed' flavours, alkyl and cycloalkyl pyrazines are found.

1.1.2 Food systems containing pyrazines

As mentioned earlier, pyrazines occur in a number of heated food products. Comprehensive reviews have been written by Maga and coworkers[6,11]. A few food systems which are relevant and more similar to the

present raw materials alone will be reviewed here. In most of the cases, the temperature of roasting was above 100°C. Table 1 gives a few pyrazines, which have been identified in some of the common food products.

Bakery products

A total of 12 pyrazines were isolated and identified by Von Sydow and Anjou[12] from rye crisp bread. They also postulated that compounds present in the pyrazine fraction were important in producing typical bread aroma. Sizer et al[13] have reported formation of major pyrazines in American type white bread crust as influenced by baking time. Using internal standards of major pyrazines, they showed that about 0.1 to 6 ppm of pyrazines were formed during a baking time of 10 to 30 minutes at 218°C. Based on threshold values available from literature, these authors could prove that pyrazines significantly contributed to the aroma of bread. The pyrazines identified were pyrazine (unsubstituted), 2-methyl, 2,3-dimethyl, 2,5-dimethyl, 2-ethyl-3-methyl, 2-ethyl-6-methyl, 2-ethyl, 2,3,5-trimethyl, n-propyl, 2-methyl-5-propyl, 3-methyl-2, 5-dimethyl, vinyl and 2-vinyl-6-methyl pyrazines.

Table 1 : Pyrazines identified in some common food products

Products	Pyrazine	Methyl pyrazine	2,3-Dimethyl pyrazine	2,5-Dimethyl pyrazine	2,6-Dimethyl pyrazine	Ethyl pyrazine	2-Ethyl-3-methyl pyrazine	2-Ethyl-5-methyl pyrazine	2-Ethyl-6-methyl pyrazine	Vinyl pyrazine	Acetyl pyrazine	Propyl pyrazine	Isopropyl pyrazine	2,3,5-Trimethyl pyrazine	Dimethyl, isobutyl pyrazine	Tetramethyl pyrazine	Alkoxy pyrazines
Bakery products	*	*	*	*		*	*		*	*		*		*			
Roasted barley	*	*	*	*			*	*						*			
Tea (black)		*	*	*	*	*		*	*					*		*	
Coffee	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*
Cocoa products		*	*	*	*	*		*	*				*	*	*	*	
Meat products		*	*	*	*	*		*						*		*	
Peanut products	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	
Popcorn	*	*	*	*				*			*			*			
Potato products		*	*	*		*	*	*	*	*			*	*	*		
Roasted Mungdal	*	*				*		*					*	*	*	*	*
Roasted brown mustard		*	*								*	*		*	*		
Tamarind	*	*		*	*	*											
Sesame seed (roasted)		*	*	*	*	*	*	*	*		*			*		*	
Rice (cooked)	*	*	*	*	*									*			

Roasted barley

This was first studied by Wang et al[14] who could identify as many as five pyrazines in barley, roasted at 180°C. They postulated that pyrazines play an important role in roasted barley flavour since their removal resulted in the loss of typical roasted aroma. In addition to this, they had also discussed the chemistry of formation of pyrazines. Later, more pyrazines were identified[15] by roasting at a higher temperature of 225°C. The pyrazine derivatives identified were dimethyl, trimethyl and ethyl methyl pyrazines.

Coffee and tea

Pyrazines were first reported in coffee in the mid-Sixties by Viani et al[16], followed by other researchers[17-21]. Later, Vitzthum and Werkhoff[22] reported a number of cyclopentapyrazines in coffee aroma. More than 45 pyrazines have been identified out of the 500 volatiles reported in coffee[23]. In recent times, coffee aroma has been extensively studied by Baltes and his group[24,25] using model systems also.

In the case of tea alone, 11 pyrazines were identified[26] among the 56 volatile nitrogenous compounds. Supercritical carbon dioxide was used for the extraction[26]. Pyrazines were also found in green tea, roasted at about 200°C for a short time[27].

Dietrich et al[28] had detected tetramethyl and 2,6-dimethyl pyrazines in cocoa products. This was followed by Rizzi[29] who identified 7 alkyl pyrazines in cocoa butter. Pyrazines which represented 0.05 percent by weight of original cocoa butter, were found to be important in cocoa butter aroma[29]. Later, 23 pyrazines were reported by two more groups of workers[30,31]. Pyrazines were also identified in the steam distillate of cocoa nibs by Van Praag and coworkers[32] and they postulated that these compounds imparted a 'nut like' odour to the basic fraction of steam distillate. Later, more number of pyrazines were added to the list[33]. The factors affecting formation of pyrazines in cocoa beans, such as variety of the beans, temperature and time of roasting were studied by Reineccius[34], whereas Maniere and Dirinck[35] studied the effect of conching time on volatiles. Vitzthum[36] identified 57 pyrazines with 34 new additions.

The total number of volatiles identified till recently in cocoa products is more than 400, of which nearly 40 are pyrazines[23]. It is believed that the fermentation step produces sufficient precursors like free sugars and amino acids which are supposed to intereact during conching process to give the unique flavour compounds.

Nuts and nut products

Pyrazines were identified by Mason et al[37] in roasted peanuts. Among the pyrazines identified, 2-ethyl-5-methyl pyrazine was found to possess the potent, nutty aroma of roasted peanuts[37]. 19 Alkyl pyrazines were identified in peanut oil[38,39] from roasted nuts and other studies included different varieties of the plant grown under different conditions[39]. The results could not be correlated well, except that a mineral deficiency of boron might result in higher pyrazine levels[39]. Walradt et al[40] extensively studied volatiles from roasted peanuts and identified 36 pyrazines among which bicyclic and oxygenated pyrazines as well as a few tetrahydroquinoxaline derivatives were also found. Vasundhara and Parihar[41] identified pyrazines in a heated system of peanut oil containing α-amino acids. They had used thin layer chromatography (TLC) for separation and identification. Roasting studies and pyrazine detection have been reported in other nuts also. Thus, Wang and Odell[42] identified 8 alkyl pyrazines in pecan nuts dry roasted at 170°C. In the case of filberts[43], the oil extracted from nuts roasted at 177°C for 18 minutes, was analysed and 9 pyrazines reported among the 32 compounds identified. Later, an exhaustive study was carried out by Kinlin et al[44] and 42 pyrazines

were identified among the 228 volatiles obtained by dry roasting of filberts with shells at 200°C for 5 minutes. Alkyl and cycloalkyl pyrazines had also been formed. However, quantitative determination was not attempted in these studies. In macadamia nuts[45] 7 pyrazines were reported whereas in roasted almonds[46] as many as 17 pyrazines could be identified. More derivatives were identified in almonds, later[47].

Miscellaneous

Other common food items in which pyrazines have been identified are potato chips[48-50], cereals[51], roasted cereal flours[52], mung dal[53], sesame seeds[54], tamarind[55], mustard seeds[56], soy products[57], egg products[58], meat[59-61] and shrimp products[62].

Pyrazines are also found in raw vegetables like carrot[9], tomato[9], asparagus[9], peas[9] etc. These are supposed to be formed through biogenic pathways. Riboflavin (Vitamin B_2) has a condensed pyrazine ring in its structure.

A number of patents[63-65] involving synthesis and use of different pyrazines in food systems have appeared which reiterate the importance of this field of study.

In addition to pyrazines, other heterocyclic compounds like furans, pyrroles, thiazoles, etc are also

formed in heated foods[66]. These, along with other compounds like aldehydes and other already existing compounds like carbonyls, lactones, esters and the like, can change the overall flavour and give a new version of the sensation.

1.1.3 Threshold values and flavour properties of pyrazines

Several researchers[67,68] have attempted to measure the threshold values of pyrazines. Guadagni et al[68] compared the threshold values of a few alkyl pyrazines in water and in oil and showed that thresholds in water were 1.5 to 60 times lower than threshold values in oil. The synergistic effect of pyrazines at subthreshold levels also offers an interesting area.

Nearly 35 to 40 pyrazines are recognised as GRAS (Generally Recognised As Safe) substances that have been assigned FEMA (Flavours and Extract Manufacturers Association) numbers[11]. Many researchers have attempted to explain the aroma properties associated with both naturally occurring and synthetic pyrazine derivatives. However, in some of the studies, concentration of the compound was not considered and hence resulted in different descriptive odours for the same compound.

The effect of the nature and position of substitution on the flavour was studied by Parliment and Epstein[69]. They showed that methoxy alkyl pyrazines give a strong bell pepper note whereas an alkyl derivative, not longer than a propyl group in the 3rd position of the pyrazine ring, engenders nutty, earthy or green aroma. According to Calabretta[70], the greater chance of hydrogen bond formation in methoxy pyrazine is related to their potent odour and this is also applicable to the observation that isopropyl and isobutyl pyrazines are stronger odourants than dialkylated or trialkylated pyrazines. It was also found that substitution generally enhances the flavour potential of pyrazine molecule. Recently, Shibamoto[71] reported odour thresholds of some pyrazines.

1.1.4 Toxicological studies of pyrazines and heated systems

Since pyrazine compounds are wide spread in our diet, a few references to their toxicological properties would be most appropriate. Two studies by Posternak et al[72,73] are notable in this respect. In their first study[72], they evaluated methyl methoxy, 2-methyl-3-ethyl, and 2,3-diethyl pyrazines. In the second study[73], they evaluated 2-methyl-3,5-methyl thio, 3-ethyl-2, 6-dimethyl, 2-methyl-3,5-furfuryl thio, and acetyl

pyrazines and pyrazinyl ethanethiol. Male and female rats were fed the above compounds at more than 100 times the equivalent of human daily intake, along with a basic diet for more than a month. No adverse effect on rat growth, food intake or organ weights could be noticed. No abnormality was noticed in chemical or pathological tests. According to Maga, "this was indeed fortunate since,if pyrazines had been found to be harmful, the utilisation of most of our food supply would be in jeopardy". However, experiments on mutagenicity of heated systems in general are limited and further investigation is recommended by most researchers and reviewers[74] to ensure the safety of foods undergoing prolonged thermal processing. The toxicological studies should be extended to the non-volatile,brown, polymeric components also. Many studies[75,76,77] are being conducted on mutagenicity of browned products.

1.1.5 Formation pathways of pyrazines

Koehler et al[78] had clearly demonstrated that the primary carbon source for pyrazine formation came mainly from sugars, as shown by using labelled sugars . Their proposed formation pathways will be discussed under chemistry of Maillard reactions. Koehler and Odell[79] also showed that fructose was very reactive.

Shibamoto and his coworkers have also extensively studied the formation pathways of pyrazines[80,81]. They used model systems containing D-glucose and ammonia under various conditions of reactant ratio, temperature and duration of heating[80]. The total pyrazine yield under optimum condition was 5.5 percent of the amount of sugar used. The role of the sugar entity in such model systems was investigated by Shibamoto and Bernhard[82] and it was found that mannose, fructose and galactose resulted in similar pyrazine distribution patterns but the utilisation of galactose resulted in lower pyrazine yields. In this respect pentoses were better than hexoses[82]. Between aldoses and ketoses unsubstituted pyrazine was more easily formed with aldoses[82].

1.2 CHEMISTRY OF MAILLARD REACTION

The term **'Maillard reaction'** is used to refer to a group of chemical reactions involving the amino and carbonyl functions present in food stuffs and leading to browning and enhancement of flavour. The reaction is named after the French chemist, Louis Maillard, who reported[2] for the first time in 1912, the formation of brown pigments called melanoidins, while heating a mixture of glucose and lysine in solution. The parameters affecting the Maillard reaction are temperature,

water content and pH[66]. Rate of reaction increases with temperature. Perfectly dry and very dilute systems do not favour the reaction whereas 20 to 30 percent moisture is optimum for browning. pH plays an important role and highly acidic conditions retard and alkaline pH generally favours the reaction. Presence of phosphates and citrates appear to increase the rate of browning due to their catalytic activity and buffering capacity[66]. The reaction is different from caramelisation occurring when pure sugars are heated[5]. However, most of the reactions observed during heating of sugar occur in the early stages of Maillard reaction also. For example, when simple sugars are in alkaline solution, there is a migration of the carbonyl function along the chain (Fig.1). The transformation is known as Lobry-de-Bruyn-Van-Ekensten transposition[83]. This explains the variety of carbonyl moieties produced during caramelisation[83]. Similar transformations take place in the early stages of the Maillard reactions also.

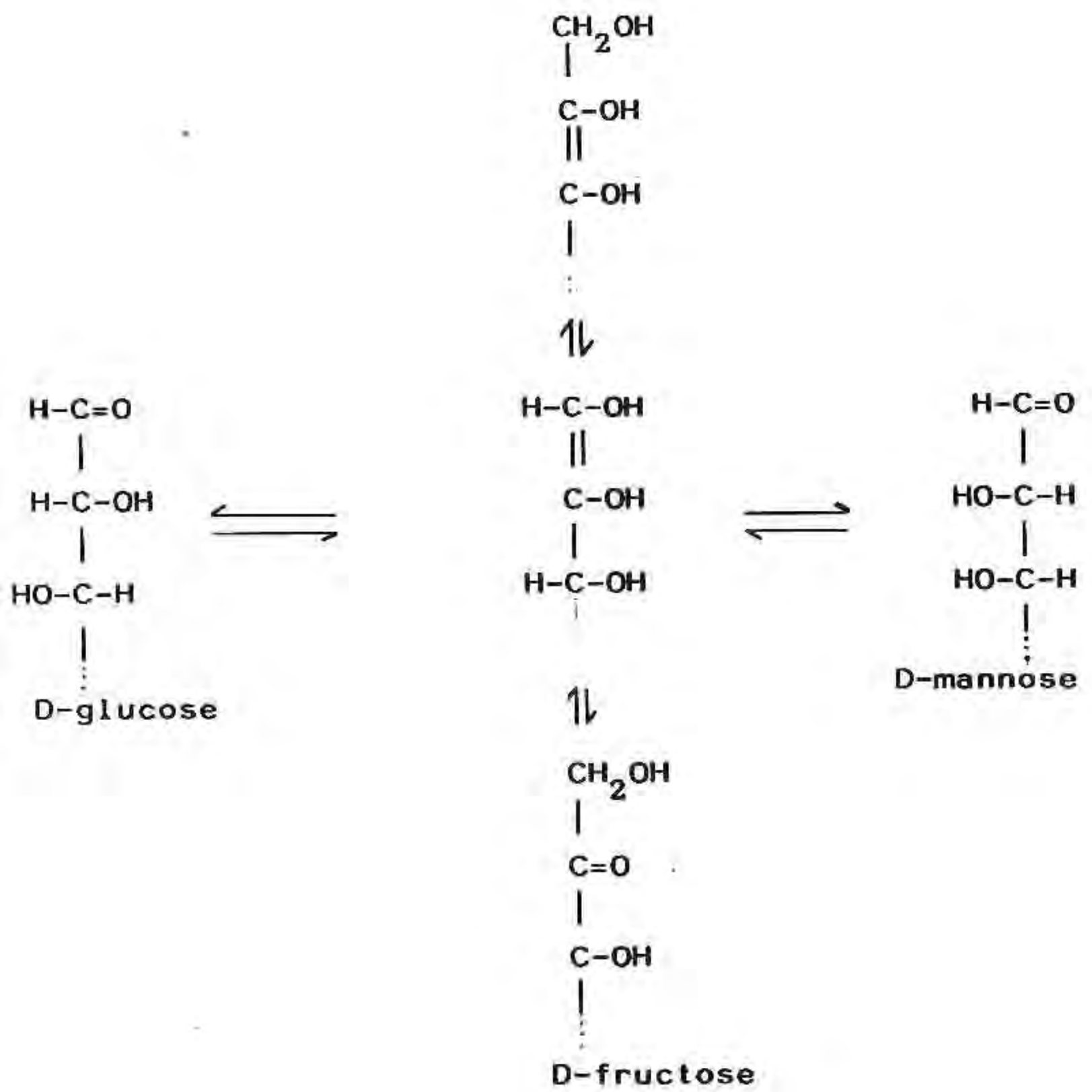


Fig.1 : Primary isomerisation steps common to thermal degradation of sugars and sugar-amine reactions

Essentially, the Maillard reaction comprises interaction of aldehydes, ketones or reducing sugars with amines, amino acids or free amino groups of peptides and proteins. In food materials, the reaction takes place between carbonyl groups of reducing sugars or

oxidised fat products and amino groups of amino acids of proteins[83]. Most primary amino groups are presented by the ε-amino groups of lysine and to a small extent by the α-amino groups of N-terminal amino acids. In addition to this, a small quantity of free amino acids are always present in food stuff[83].

Hodge[84] in 1953, put forward the first coherent scheme of the reaction. Further investigations were summarised by Ellis[85], Heyns and Paulsen[86], Reynolds[87] and Baltes[88]. According to Mauron[83], the Maillard reaction can be conveniently divided into three stages viz. early, advanced and final Maillard reactions. The first step involves chemically well defined reactions without causing any browning. The second step involves numerous reactions resulting in volatile or soluble substances and in the final step a series of reactions take place resulting in the formation of insoluble brown polymers.

1.2.1 Early Maillard reactions

Preliminary steps[66,84] in the Maillard reaction are represented by Figure 2. The first step is the condensation reaction between reducing sugars (eg.glucose) [8] and amino group [9] of amino acids or proteins.

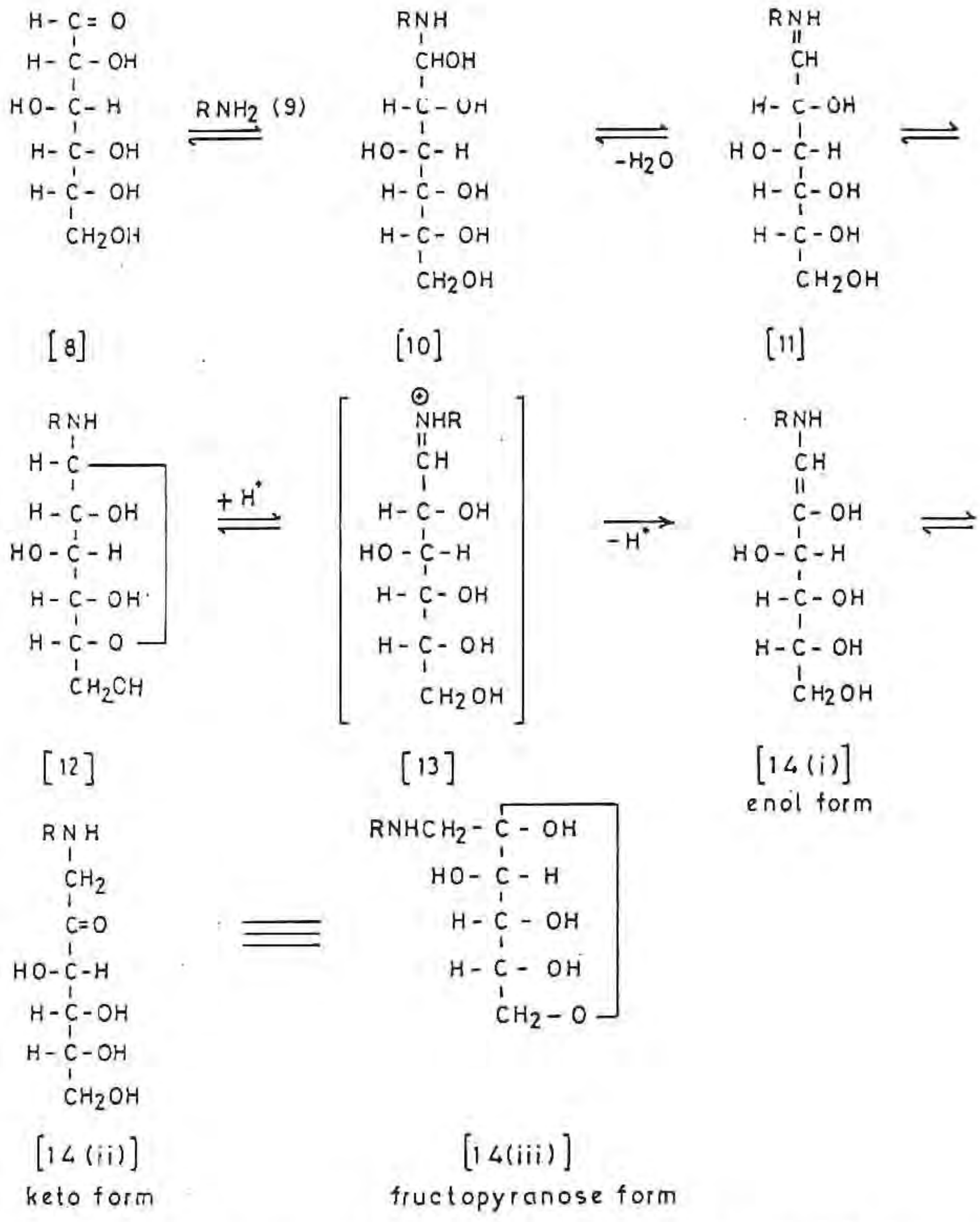


Fig. 2. Early steps in Maillard reaction showing Schiff base (11) formation and Amadori rearrangement (12 to 14)

The condensation product **[10]** rapidly loses a molecule of water and is converted into a Schiff base **[11]**. This undergoes cyclisation to the corresponding N-substituted glycosylamine **[12]**. The reaction is reversible upto this step since the glycosylamines can be hydrolysed back to the parent compounds in aqueous media or in the presence of strong acids. The glycosyl amine **[12]** is converted to N-substituted 1-amino-1-deoxy-2-ketose **[14]** through an intermediate product which is cation of Schiff base **[13]**. The transformation is known as **Amadori rearrangement** and it involves the transition from an aldose to a ketose sugar derivative. The rearrangement is catalysed by weak acids and in food, this is provided by the free amino acids. Moreover, glycosyl amines of amino acids, more readily undergo Amadori transformation than those derived from amines. Hence, in the usual pH range of food materials which is nearly neutral or weakly acidic and in the presence of free amino acids which occur in most of the foods, the Amadori rearrangement readily takes place.

Figure 3 represents the initial steps[89] of Maillard reaction in which a ketose is participating.The reaction between ketose (eg. fructose) **[15]** and an amino group **[9]** results in the formation of an addition compound

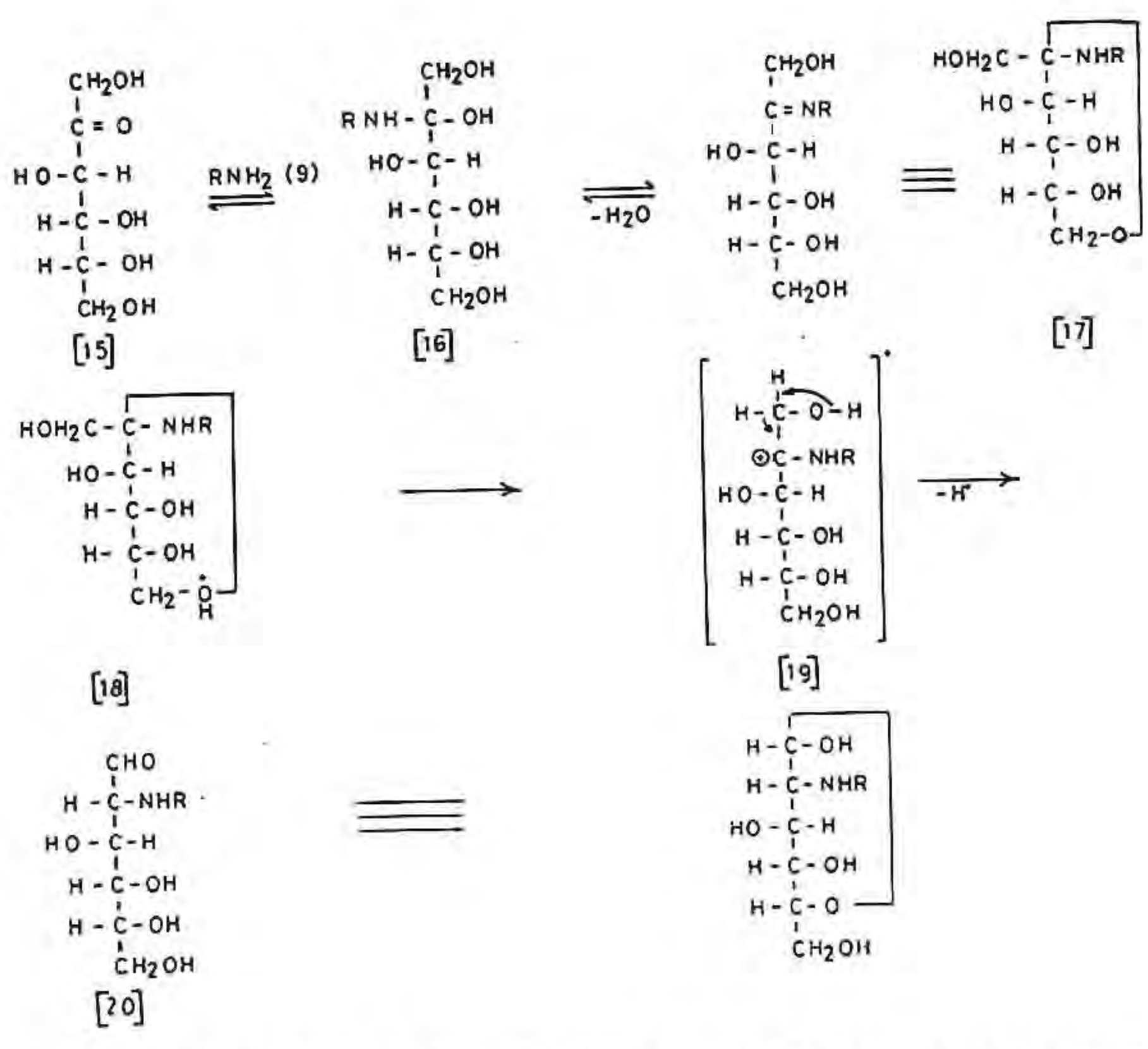


Fig. 3. Initial steps of Maillard reaction and Heyns rearrangement (18-20)

[16] and later in the formation of ketosylamines or ketosamines [17]. This undergoes the **Heyn's rearrangement** (Fig.3), which is the reverse of Amadori rearrangement . The ketosylamines (ketosamines) formed by the reaction between ketoses and amines are transformed into aldose derivatives in Heyn's rearrangement. The steps involved in this transformation (Fig.3) are very similar to those in Amadori rearrangement (Fig.2). The mechanism of the Amadori rearrangement (Fig.2) involves protonation of the nitrogen atom at carbon 1 (C_1),whereas in Heyn's rearrangement (Fig.3), it involves protonation of the oxygen atom at carbon 6 **[18]**, **[19]** which on rearrangement, eliminates one proton and gives the 2-amino-2-deoxy aldose **[20]**. (2-Amino-2-deoxy-glucose is shown to be formed since the starting sugar was fructose)[90].

These early Maillard reactions do not cause browning or give flavour to food systems although they can severely reduce nutritional value[91]. The Amadori compounds are however, important non-volatile flavour precursors[92].

1.2.2 Advanced Maillard reactions

According to Hodge[93,94], there are three main pathways for the advanced Maillard reaction - two starting

directly and the third indirectly from the Amadori product. The possible pathways are represented in Fig.4. During Amadori rearrangement, at some stage the intermediates are found to be highly prone to browning[94]. These have been identified to be the enol forms of amino deoxy ketoses. According to Hodge[94], the 1-amino-1-deoxy-2-ketose **[21]** enolises in positions 2 and 3 **[22]** irreversibly and eliminates the amine from C_1 to give an intermediate methyl dicarbonyl species **[23]**. This further breaks down into C-methyl aldehydes, ketoaldehydes, dicarbonyls and reductones**[24]**. Flavour compounds like acetaldehyde, pyruvaldehyde, diacetyl, acetic acid etc are formed.

The second pathway starts from 1,2 -enediol form **[25]** of the Amadori product. Elimination of the hydroxy group at C_3 is followed by deamination at C_1 and addition of one molecule of water gives 3-deoxyhexosone **[26]**[94,95]. Two molecules of water are removed in successive steps resulting in furaldehydes **[27]**. These compounds as well as the reductones **[24]** formed in the 2,3-enol pathway, can condense with amines to give brown polymeric compounds called **'melanoidins'**.

In both these pathways, the formation of various intermediates is not yet fully understood, though many

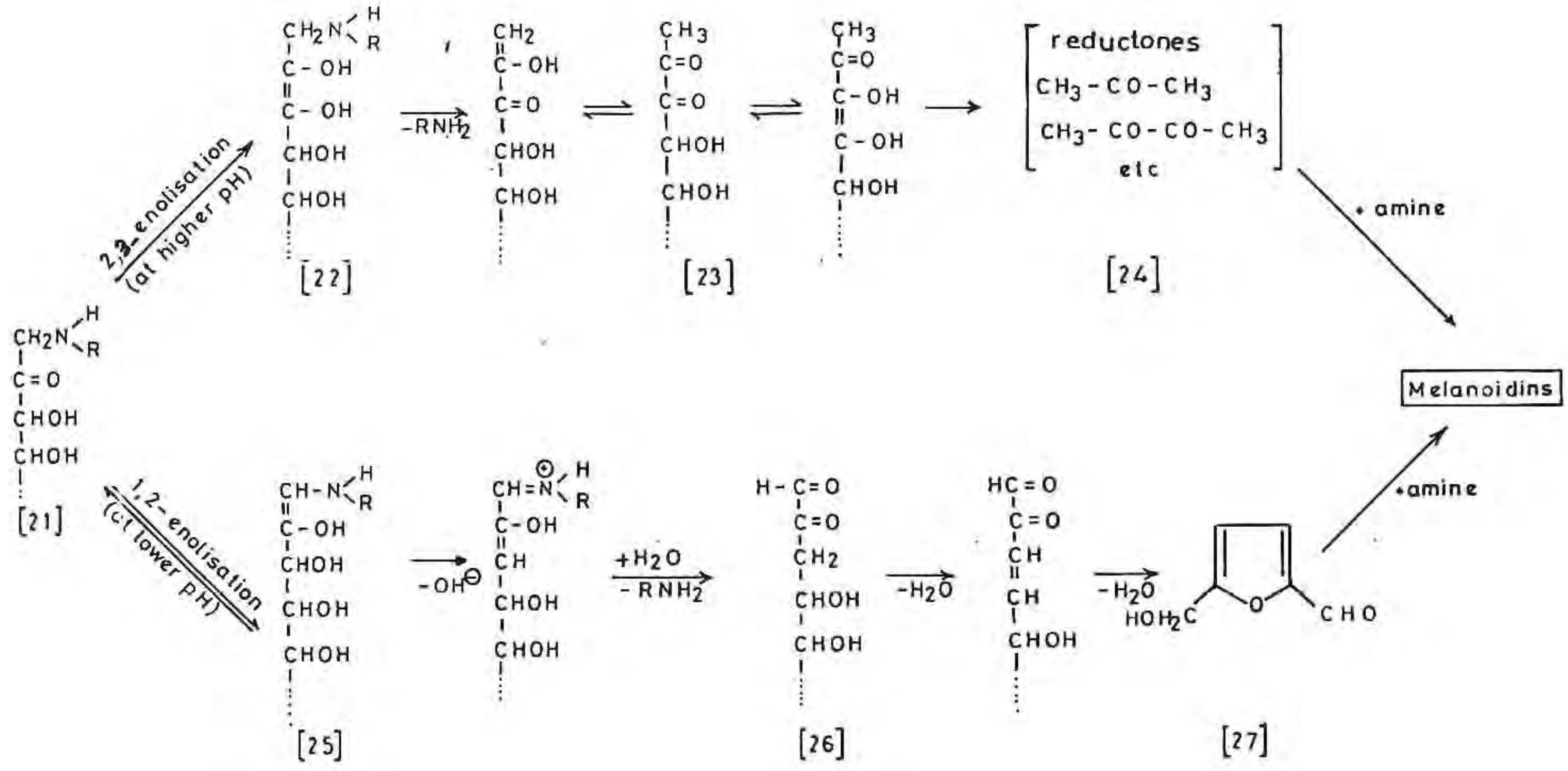


Fig. 4. Suggested pathways of advanced Maillard reactions.

eminent researchers have investigated in the past and are continuing their efforts[66,83]. Aldol condensations, amino-carbonyl polymerisation etc are believed to proceed and finally result in brown polymeric material. Heterocyclic, nitrogen containing compounds such as pyrazines, pyrroles and pyridines are also formed which are largely responsible for the roasted, nutty flavours of heated foods[95].

The third suggested pathway is the Strecker degradation[93,94,96] (Fig.5). In this, α-dicarbonyls [28] formed via the first two pathways, interact with amino acids producing the Schiff base **[29]**. This enolises and decarboxylates **[30]**. The α-dicarbonyls that are supposed to be formed are glyoxal, methyl glyoxal, diacetyl and 3-deoxy hexosones [26]. The decarboxylated -Schiff base **[30]** which is having one carbon less is easily hydrolysed into an amine and an aldehyde. The aldehyde known as **Strecker aldehyde** corresponds to the parent amino acid with one carbon atom less and are supposed to play a vital role in the overall flavour of heated foods[95,96]. However, they can continue to condense with other degradation products to form melanoidins. The amino ketones formed can self-condense

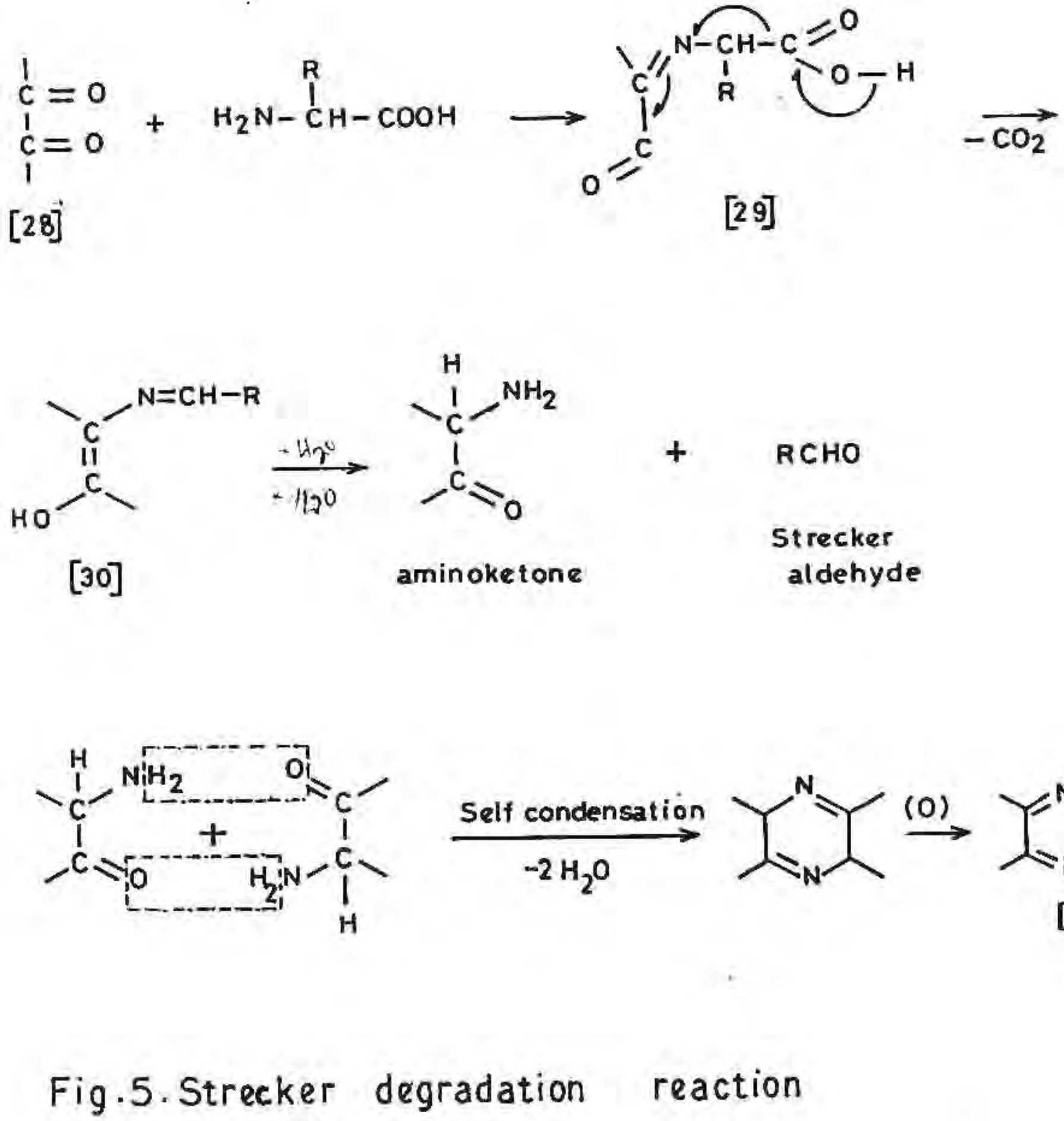


Fig.5. Strecker degradation reaction

to give pyrazines [7] which are important flavour compounds.

It is interesting to note that much of the carbon dioxide released during Maillard reaction is derived from the carboxylic groups of amino acids, in the Strecker degradation step[3,97,98].

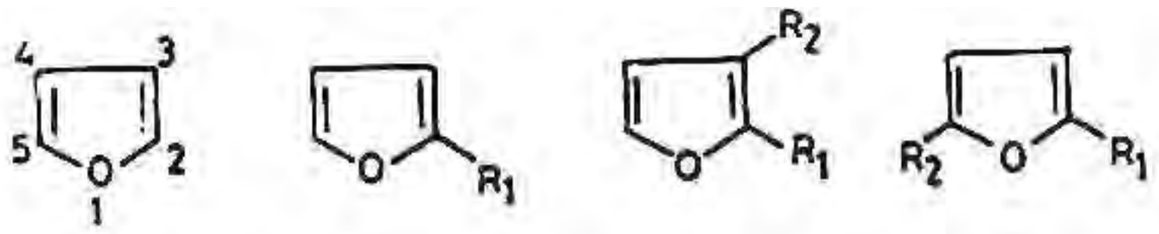
1.2.3 Final stage of Maillard reaction

The third and final phase of Maillard reaction also produces the non-volatile brown pigments called 'melanoidins'. The exact chemical nature of these brown coloured substances are not clearly known though many attempts have been made[99,100]. It is reported that they contain carbon, nitrogen and oxygen and are polymeric in nature[101]. Some are soluble in aqueous alcohol while others dissolve only sparingly. Moreover, the composition and hence molecular weight of the polymers varies with the nature of the reactants, pH, temperature and other conditions. The brown polymers show reductone reducing power, even after dialysis and antioxidant activity toward linoleic acid and in strongly browned foods and dark beers[102,103].

1.2.4 Formation of volatile, heterocyclic compounds

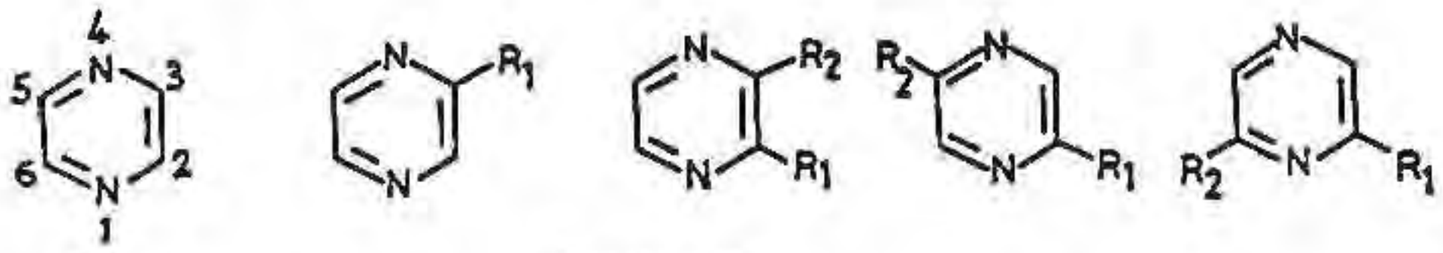
Nitrogen, sulphur and oxygen containing volatile heterocyclic compounds are fomred during the advanced

stages of Maillard reaction. They are important flavour compounds of heated food systems. Comprehensive reviews and chapters in books have been written on Maillard flavours by many researchers[6,11,66,104]. Figure 6 gives the structures of some of these compounds. The major class of compounds formed are furans **[4]**, pyridines **[31]**, pyrazines **[7]**, pyrroles **[32]**, oxazoles **[33]**, thiazoles **[34]** etc. The furans are suggested to be mainly formed by degradation of sugars. Maga[105] has reviewed the occurance and formation of furans in foods. The formation of pyrazines is already explained. Pyridines are also identified in heated food systems like coffee[18], barley[14], fried beef[106] etc. The mechanism of formation of pyridines in food systems is not well defined. Pyrroles are nitrogen containing five-membered heterocyclic compounds which are identified in coffee[107] and cocoa[108]. They are likely to be formed by the reaction of furfural or similar compounds with α-amino acids[109]. Oxazoles are oxygen and nitrogen containing heterocyclics that may be formed during Maillard reaction. Rizzi[110] has suggested the formation of oxazolines from valine **[35]** and diacetyl **[1]** (Fig.7). The oxazoline **[40]** formation is favoured when the electron distribution of the intermediate **[37]**, after the decarboxylation of the Schiff base **[36]**, enables a cyclisation**[39]**



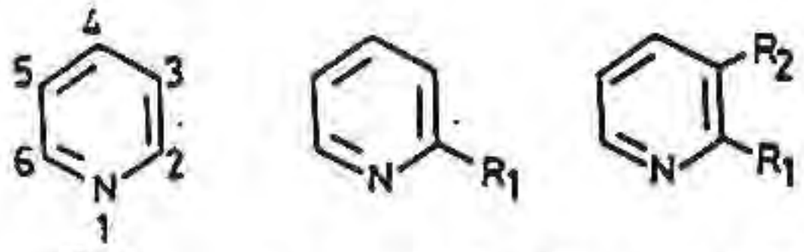
[4]

R_1, R_2 = H, CH_3, C_2H_5, C_3H_7 etc



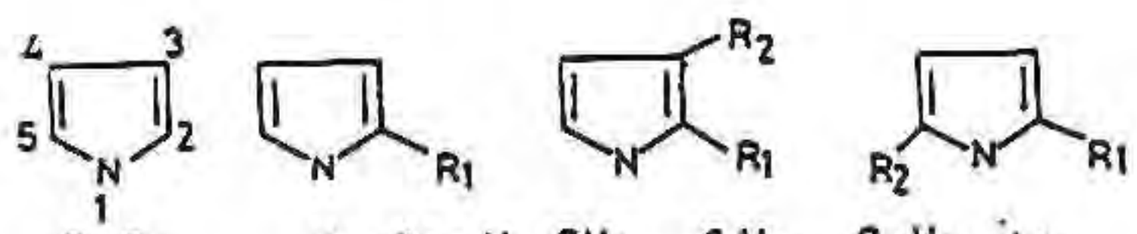
[7]

R_1, R_2 = H,
CH_3, C_2H_5, C_3H_7 etc.
$CH=CH_2$, $CH=CH-CH_2$ etc



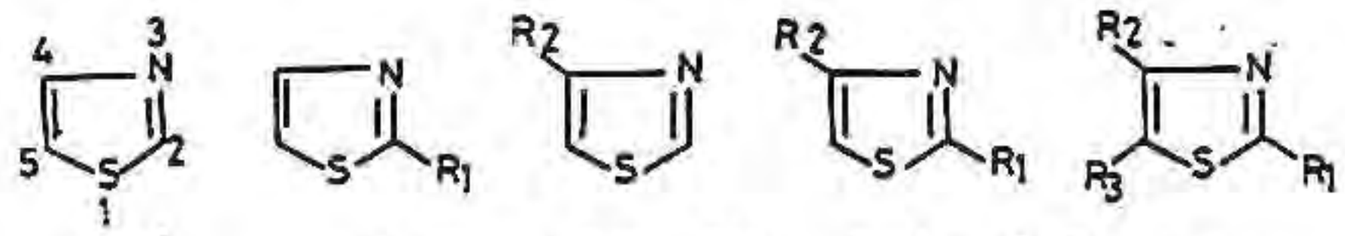
[31]

R_1, R_2 = H, CH_3, C_2H_5, C_3H_7 etc.



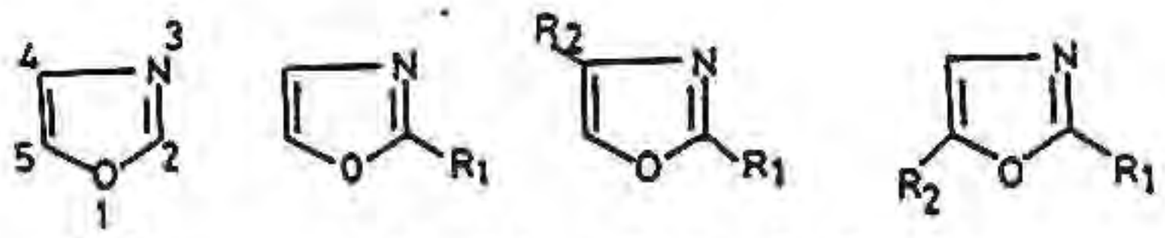
[32]

R_1, R_2 = H, CH_3, C_2H_5, C_3H_7 etc.



[33]

R_1, R_2, R_3 = H, CH_3, C_2H_5, C_3H_7 etc



[34]

R_1, R_2 = H, CH_3, C_2H_5, C_3H_7 etc.

Fig. 6. Structure of some of the heterocyclic compounds formed during Maillard reaction.

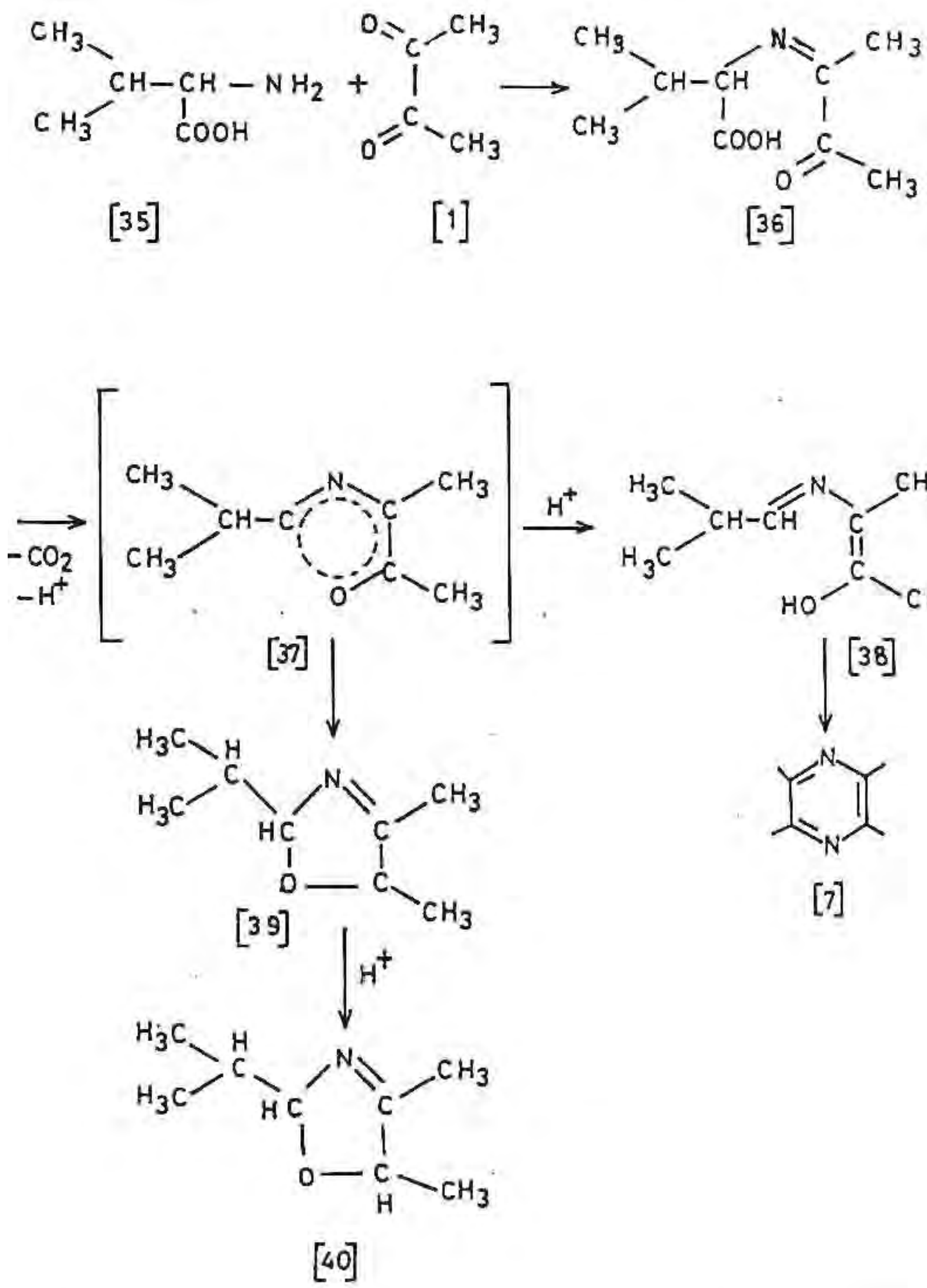


Fig.7. Formation of Oxazoline via Strecker degradation.

before the enolization [38] occurs. In the latter case, the pyrazine [7] is formed.

Thiazoles [34] are compounds which contain both nitorgen and sulphur in their ring structure (Fig.6) that they are also formed during Maillard reaction, and have been identified in roasted coffee[111], peanuts[112], potato products[113], cooked beef[114] etc. Their mechanism of formation during heating is not elucidated well. However, they have been reported to have 'nutty' or 'roasted' flavour[115].

1.2.5 Nutritional and toxicological studies of Maillard reaction systems

The most obvious negative consequence of Maillard reaction is the decrease in the nutritive value of proteins[86]. In general, this can be correlated with the decrease in digestibility and biological inactivation resulting from the blocking of certain essential amino acids. In most cases, this is attributed to the decrease in lysine availability or destruction of sulphur amino acids[83].

The toxicological studies carried out with heated glucose-amino acid mixtures showed that LD_{50} of the system was 50 percent lower than that of free amino acid[116]. Even then it showed only very low toxicity, being of

the order of 5 to 12 g/kg body weight by injection, intraperitonially. Much confusion was created by wrongly identifying nitrosamines as Maillard products by Dewik[117]. This was later shown to be only pyrazines by Dewik as well as by others[118] and were shown to be devoid of any toxicity[119]. In most of the feeding trials with browned food, the changes noticed in rats were principally due to malnutrition arising out of protein deficiency and not due to melanoidins. These polymers were not fully absorbed in the intestines and probably got deposited in kidneys which were slightly darkened[120]. In most cases, the unabsorbed matter was excreted through intestine or urinary track.

1.3 TECHNIQUES OF ANALYSIS OF FLAVOURS

The intriguing nature of flavour research can be understood from the fact that flavour compounds, especially of a heated food, represent chemicals of a wide variety of classes and a broad range of molecular weights, occurring at ppm levels. The degree of volatility and reactivity of these compounds also vary very much and they are distributed in a highly specific manner throughout the bulk of the food matrix consisting of carbohydrates, proteins, lipids and water. In general, flavour analysis includes isolation, concentration

and identification of the volatile flavour compounds. The flavour chemist has to be cautious in avoiding enzymatic or other chemical changes in the constituents. This may result in the formation of artifacts during isolation and concentration steps. Quite often, the major components may have only minor role in the flavour quality of the product and the reverse also may be true. Since the food matrix consists of lipids, proteins, carbohydrates and minerals, these may bind the volatile components to a large extent and careful selection of isolation techniques is very essential. Thus, extraction of flavour compounds is a challenging task.

1.3.1 Isolation methods

The sample is usually taken in a finely homogenised state for efficient isolation of the flavour compounds. Active enzymes if present, can be arrested by heat, methanol[121,122] or sodium fluoride[123] treatment.

A few commonly used isolation procedures will be discussed here.

Head space analysis

This is a simple and gentle method of isolation of flavour compounds from a food system, in which formation of artifacts by heat is rather eliminated.

Analysis of head space above a food product may give a closer approximation of flavour compounds that the nose perceives normally. Concentration of head space volatiles[124] is accomplished by using cryogenic, porous polymer, charcoal or on-column traps, while collecting. The method does not give all the volatiles but only the most volatile ones.

Distillation

Distillation methods take the advantage of volatility of flavour compounds and nonvolatility of most of the other food constituents under the same conditions. Steam distillation and codistillation with water were some of the earliest techniques adopted by flavour chemists for isolation, and these are still used even today. Since most of the volatile, flavour compounds are steam distillable, the condensate will contain the aroma compounds. The method is very effective in isolating volatiles from fats and fatty foods[124]. Moreover, the distillation can be repeated until the volatiles are more or less completely stripped off. The condensate is usually dilute and further extraction with a solvent becomes necessary. Occasionally, sodium chloride is used to saturate the distillate so as to improve separation and extraction by solvent[124]. The flavour compounds are extracted from the distillate

with organic solvents like pentane, isopentane, hexane, heptane, ether, dichloromethane, fluoro-hydrocarbons (eg. 'Freon' 113),chloroform etc. The solvent must be immiscible with water and must be a good flavour extractant. The solvent must be distillable at low temperature also. Distillation may be done at reduced pressure to get the flavour concentrate. Ether, though a good extractant for polar and non-polar compounds, suffers from its lack of purity, high volatility and affinity for water. Dichloromethane is more preferred by flavour chemists since it has practically no affinity for water and can be obtained in pure form.

The one disadvantage of steam distillation, as often pointed out, is the possibility of formation of artifacts during the entire procedure. To minimise this, steam distillation can be conducted under reduced pressure. In such cases, efficient cold traps are arranged in line to prevent escape of volatiles. Usually dry ice (-40°C) or liquid nitrogen (-180°C) traps are used.

Both distillation and extraction steps are combined in the simultaneous distillation extraction (SDE) unit which in principle is based on liquid-liquid extraction

of the flavour compounds. The apparatus was first developed by Likens and Nickerson[125] and later modified by Macleod and Cave[126] and Schultz[127]. The food under study, is mixed with water in a flask and heated whereas low boiling solvents like pentane, ether etc are distilled from another flask. The vapour coming from the aqueous slurry of the sample carries the flavour compounds also and these are extracted by the solvent vapours in an extraction head which is simultaneously connected to both the flasks. The extraction head is provided with efficient vertical condensers. The extractor is so designed that condensed liquids separate and flow back to the respective flasks. Thus, the flavour compounds can be effectively extracted into limited volume of solvent.

Selective extraction of flavour compounds

Flavour compounds from roasted foods are of complex chemical composition and usually contain organic acids, nitrogenous compounds like pyrazines and neutral compounds like carbonyls, alcohols, esters, lactones etc. These compounds can be selectively extracted with solvents from the steam distillate after adjusting its pH. Generally, hydrochloric acid or sodium hydroxide

is used to adjust the pH of the distillate in acidic or alkaline ranges, before extraction.

A few other methods like adsorption on polymers, charcoal etc, molecular distillation, liquid and super-critical carbon dioxide extraction are also employed for flavour isolation[124].

1.3.2 Methods of concentration of flavour compounds

Usually, simple evaporation under atmospheric or reduced pressure, over a fractional distillation column at low temperature is practised. Poor recoveries can result due to ineffective washing of the exposed glass surface of the concentration vessel, which invariably becomes coated with the flavour components during evaporation of the solvent. The Kuderna-Danish evaporator can be used for final concentration of volatiles also. The apparatus consists of a pear shaped glass vessel with provision to attach a sample tube at the bottom and a fractionating column at the top, all joints being made of ground glass (Fig.8). The taper towards the top of the vessel allows the walls to be washed continuously by the condensing solvent during evaporation. The detachable sample tube eliminates the need for transferring residue into another container for

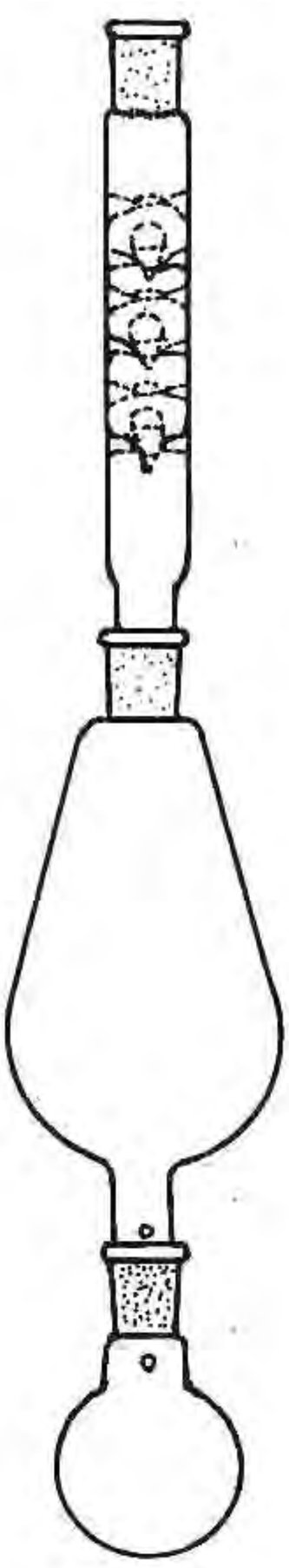


Fig.8. Kuderna-Danish evaporator

storage. If graduated tubes are used, concentration can be stopped at desired stage. Heat is applied by immersing it in hot water bath during concentration. Freeze concentration and zone melting are two techniques used to concentrate organic compounds in solution. These low temperature procedures do not incur artifact formation. In freeze concentration[128], pure water is frozen out of an aqueous extract leaving the organic compounds in the unfrozen liquid portion. Careful freezing is required to minimise loss of volatiles in the form of occluded droplets in ice mass, entrapment in air pockets and surface evaporation during stirring. In zone melting, instead of water, a solvent extract is taken. The frozen mass is heated at narrow zones, and the pure liquid flows and refreezes in colder regions, leaving the solutes (here flavour compounds) in the heated region. By repeated heating and cooling under equilibrium conditions, concentrated samples have been prepared. Raspberry[129] flavour isolate in benzene was concentrated by this technique. However, practical difficulties restrict wider applications.

Adsorbents like charcoal and porous polymers (eg. Porapak) are also used for separating the volatiles from aqueous slurries which have been mentioned earlier.

The volatiles are recovered from the adsorbents by Soxhlet extraction[124].

Due to the complexity of flavour concentrate, a preliminary fractionation of the compounds upto some form of groups (eg. basic, acid or neutral compounds) is practised[6,124,130] by many flavour chemists. The procedure has been described briefly under isolation methods. The method helps in reducing the ambiguity of analytical data in a later stage. Prior to analysis, fractionation by acid-base separation and also column separation on silicic acid are useful. A number of workers have adopted these procedures before instrumental analysis as in the case of potato chips[131], lamb meat[132], roasted sesame[133] and roasted cocoa[32].

1.3.3 Identification techniques used in flavour research

Since flvour compounds occur at ppm levels, sophisticated instruments like gas chromatograph (GC), gas chromatograph coupled to mass spectrometer (GC-MS), ultraviolet (UV) absorption spectrometer and infra red (IR) spectrophotometer are most suited to this purpose.

For preliminary identification and classification, simpler techniques like thin layer, column and very rarely, paper chromatography are used[124]. However,

for ultimate identification, GC and GC-MS are the choice of the flavour chemist.

Gas chromatography is the single, most widely used technique in flavour studies since separation and identification procedures are combined in one step. When it is coupled with a reasonably sensitive detector like flame ionisation detector (FID), compounds occurring at ppm levels can be detected. Identity of the peaks is made possible by retention time data (RT). For better comparison, relative retention time based on co-injection of n-paraffins is done. Kovats[134] had developed the Kovats Index System of relative retention time. Esters as standards were used by Van den Dool and Kratz[135]. Analysis of the sample in two different columns - one polar and the other non-polar - and comparison of retention time in each against standards is also useful.

Mass spectrometry has become the second most commonly used instrumental technique in flavour research. It can be readily coupled to a gas chromatograph and has an appreciable sensitivity of 10 to 100 picogram levels. It can also provide structural information about the compounds identified. The technique finds wider use

after the introduction of computer-aided spectra matching systems. A survey of literature reveals that no major flavour work is complete without GC and GC-MS analysis in these days.

Before the advent of coupled GC-MS, IR spectroscopy was the second most commonly used technique in flavour analysis. Compounds separated by GC were trapped and subjected to IR analysis. The spectrum provides valuable information about the type of functional groups present in the unknown molecule. One of the problems in employing this technique to flavour anaysis, is the difficulty in getting sufficient pure sample (1 to 10 μg). When IR is coupled to a GC, suitable modifications of the detector is necessary for fast scanning of the eluting GC peaks. Some modifications have been effected by Brown et al[136] and Crooks and coworkers[137] for flavour work. Fourier transform infra red spectroscopy (FT-IR) permits direct coupling of GC and IR and with computerised recording and matching facilities, the technique can be made very effective in identification of complex mixtures[138].

Application of high pressure liquid chromatography (HPLC) to flavour analysis is a recent development and promises a useful method of fractionation of flavour

concentrates. A preliminary separation based on molecular sieves or adsorption on silica gel can be carried out. The individual fractions can be further separated on a normal or reversed phase HPLC column which will considerably simplify the GC profile. Teitelbaum[139] has used HPLC for separation of volatiles from cocoa butter. The use of reverse phase HPLC has been reported by Parliment[140] in recent past. The advent of LC-MS, which is HPLC coupled to MS, can largely help flavour analysis[141].

Nuclear magnetic resonance (NMR) spectra has found only limited applications in flavour research since it requires samples at mg levels. Collection of this much quantity of the pure, individual component is a tedious task.

1.4 STUDIES ON CHANGES IN FOOD COMPOSITION

In the case of formed flavours, the extent of the Maillard reaction can be studied by following the changes in volatile and non-volatile components. The methods to study the former have been dealt with in detail. Among the non-volatile constituents, the maximum changes occur in proteins, followed by carbohydrates and to some extent in lipids.

1.4.1 Studies on changes in proteins

The changes occurring in proteins can be followed by studying the solubility at various pH values and in different solvents, amino acid composition of total and free amino acids, using amino acid analyser. Heat-denaturation of proteins is usually studied by separation and purification of proteins by gel permeation chromatography and electrophoresis. In recent times, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is often employed for such studies[142].

1.4.2 Nutritional study of heated proteins

The nutritional value or quality of a particular protein depends on two factors viz. (1) its content of the essential amino acids and (2) its digestibility. Proteins differ considerably in the relative proportion of amino acids they contain. Some proteins have a complete set of essential amino acids, in the required proportion, while others may be deficient in one or more essential amino acids. The nutritional quality of proteins can be determined in two ways. In the first method, the protein is completely hydrolysed and its amino acid composition analysed and compared with that of egg or milk protein as a standard. This 'chemical score' of a protein indicates the potential of the protein.

A more accurate measure is the 'biological value' of the protein which is inversely proportional to the amount of a particular protein source that must be consumed to keep an adult human subject or experimental animal in nitrogen balance (a condition in which the intake of protein nitrogen exactly balances the loss of nitrogen in the urine and faeces). If the protein provides all the essential amino acids in the proper proportions and all are released in free form and absorbed, it will have a biological value of 100, much higher than a protein that is complete in amino acid content, but is incompletely digested. A completely digestible protein, with inadequate amounts of essential amino acids or without some of them, will also show a lesser biological value. Also, if one of the essential amino acids is totally deficient, its biological value will be zero. All essential amino acids must be available simultaneously for body protein synthesis.

1.4.3 Nutritional consequences of heat processing

Most of the food processing treatments involve at least some heat processing steps also. Depending on the controlling factors like time, temperature, moisture content, presence or absence of reducing substances etc , heat treatment has either a beneficial

or detrimental effect. The beneficial effects include inactivation of inhibitors, antinutritional components and so on. The detrimental effects include reduction in palatability and nutritive value. This may be due to oxidation of amino acids or change in the linkages between amino acids, with the result that these amino acids are released slowly during digestion. Occasionally new amino acid bonds are formed which are not hydrolysed by digestive enzymes[143]. Delayed release of amino acids during digestion may disrupt protein synthesis in body tissues by preventing the availability of all essential amino acids simultaneously. Those that can not be utilised are biologically oxidised[143,144]. The presence of reducing sugars can initiate Maillard reaction in food, by which amino sugars, polymerised products, new carbon-nitrogen linkages are formed which substantially reduce the nutritive value. Nutritional evaluation of the heated proteins can be carried out by finding out chemical score as well as biological value.

Although some damage or destruction of proteins invariably results from normal heat treatments, such losses are considered as relatively insignificant, when a diet consisting of more than one particular type of food is included in the same meal.

1.4.4 Changes in carbohydrates and lipids

The changes occurring in carbohydrates are largely confined to the reducing sugars like glucose and fructose[74]. Sucrose does not directly involve in Maillard reaction unless it is hydrolysed at a very high temperature[144]. Degradation of polysaccharides (eg. starch, cellulose) is likely to occur under heated conditions[145]. Standard methods for estimation of total and reducing sugars are anthrone[146] and phenol sulphuric acid[147] methods for the former, and Nelson-Somogyi[148], Schaffer-Somogyi[149] methods etc for the latter. Starch and dextrins can be estimated by the method of Clegg[150] following modified anthrone method. The estimation of individual sugars can be made possible by HPLC[151] using amino bonded silica columns or by GC[152] after silylation of the sugars.

The lipids undergo only very small changes under the conditions of roasting[5]. However, their oxidation products[153] like carbonyls can react with free amino groups to form Schiff bases and thus take part in advanced Maillard reactions also. Studies on lipids of roasted groundnut[154] and pistachio kernels[155] are reported.

Precursor studies in food products are very much limited and only few reports[156,157] are seen. But model system studies[158,159] using amino acids and simple sugars are more common. The flavour compounds are also analysed in such cases.

The appropriate selection of isolation procedures, coupled with sophisticated instrumental analysis and supporting data on changes in the non-volatiles of the food matrix together, can give a comprehensive idea about the formed flavours and their formation pathways.

1.5 RELEVANCE OF THE FLAVOUR STUDIES ON HEATED COCONUT AND PALM KERNEL

The study of formed flavours becomes all the more important in the Indian context because Indian cookery has various steps like boiling, broiling, frying and grilling, which are different from Western cooking. There is also seasoning with spices and condiments. In most of these heating procedures, food is cooked at a higher temperature and there is considerable texture improvement along with generation of a series of new appetising aromas. Coconut which is widely used as a culinery item in India, especially in the State of Kerala, is subjected to roasting as part of

the making of certain curries. Heating results in the development of roasted nutty aroma which is inherited by the oil pressed from it. This is practised by many oil mills in our country. (Beside this, heating of coconut milk at higher temperatures yields coconut oil with pleasant nutty aroma. This oil is traditionally used for bathing babies). The chemistry behind these flavour changes are not studied and reported in the literature.

Oil palm is emerging as a valuable oil source in India. Palm oil is widely used for cooking purposes, whereas the palm kernel oil which is very similar to coconut oil, is practically not utilised in our country. Flavour constituents of the palm kernel oil as well as its enhancement on heating are worthy of a detailed investigation.

The present study is undertaken with a view to analyse the flavour compounds of these tree nuts when heated, and the changes occurring in the non-volatile matrix as a result of the heat treatment. The latter part can explain the formation of various aroma compounds generated during thermal processes. A detailed literature survery on coconut and palm kernel has been

carried out. Their regional importance and relevant aspects of published literature will be given in subsequent pages.

1.6 COCONUT

Coconut *(Cocos nucifera,Linn.)* isone of the most important vegetable oil sources in the world. It belongs to the natural order 'palmae' , which comes under monocotyledons. It is grown in more than 80 countries of the tropics, with an annual world production of 33,700 million nuts per year[160]. The country of origin is still under dispute. It is assumed that .coconut existed in India nearly 3000 years ago[161]. The tree thrives well in hot, tropical, rainfed areas with well drained soil. The exact number of distinct varieties of coconut has not been determined due to the wide distribution and lack of genetic purity owing to cross pollination. In India, the tall form of some varieties are gorwn and one such variety is the West Coast Tall. This is abundant in Kerala State and has good nut characteristics[161]. Hybrid varieties of West Cost Tall and Orange Dwarf palms are reported to give good yields[161].

1.6.1 Economic importance of the crop

India is the third largest coconut producing country in the world, after Philippines and Indonesia[160,161]. Of the 6,800 million nuts produced annually in India, the State of Kerala alone accounts for 4,080 million nuts[160]. The name Kerala itself means 'land of kera' (coconut) in vernacular. The other states which cultivate coconut are Karnataka, Tamil Nadu, Andhra Pradesh, Orissa and West Bengal.

Coconut can be considered as an important oil seed crop since it is one of the richest vegetable oil sources in the world. Copra, the dried kernel of the coconut, has an oil yield of 65 percent[162] on milling which is the highest among oil sources. Total world exports of copra and coconut are estimated at about 1.77 million tonnes of oil equivalent[162]. This approximates nearly 10 to 11 percent of world's total export trade in oils and fats. Coconut oil is a popular cooking medium and is widely used as hair oil and massage oil in many parts of India. It has wide ranging industrial applications including soap making. The current production of coconut oil is 200,000 tonnes which is 8 to 10 percent of the vegetable oils produced in India[162].

In India, as well as in other producing countries, coconut is very much relished and consumed as a food item also. Of the total nuts produced, nearly 50 to 60 percent goes for culinary, religious and cultural purposes[160]. About 8 percent is consumed as ball copra and 3.5 percent as tender nuts[160]. The remaining are converted to copra and milled[160].

Coconut is cultivated as a plantation crop of small holdings in Kerala State. Its economic importance can be understood from the fact that coconut constitutes about one-third of the total agricultural income and one-sixth of the annual income of the state[162]. The production of copra and coconut oil in Kerala are estimated to be 294,000 tonnes and 192,000 tonnes respectively, which is 90 percent of the total Indian production[162]. Moreover, nearly 10 million people are directly or indirectly dependent on its cultivation for their livelihood[162]. Coconut also provides raw materials for a number of processing industries like copra, coconut oil, oil cake, desiccated coconut, toddy, coir and coir products. The trunk of the tree provides wood for constructing roof of houses, while the leaves are used for thatching them. Fronds, spadices, husk,

shell etc are also utilised as fuel materials in rural areas. Unfortunately, the production of coconuts have come down in Kerala in recent years due to root wilt disease and unexpected droughts.

Because of the internal demand and consequent high price, coconut does not figure as an export item of India. As a measure of protection to growers, import is not encouraged also.

Many researchers have investigated the major chemical constituents of coconut like fat, protein, sugars etc.

1.6.2 Proximate compostion

The kernel or endosperm of the ripe coconut is an important article of food in the coconut growing countries. It is used for culinery purposes in various forms. In the wet stage, the kernel has a moisture content of about 40 percent. Fat is the major constituent in coconut ($\approx$ 40 percent). Table 2 represents the proximate composition[161] of mature coconut of the West Coast Tall variety.

1.6.3 Extraction and physicochemical characteristics of coconut oil

The major constituent of coconut is the oil. It is extracted after drying the split coconut cups

Table 2: Proximate composition (%) of coconut[161]

Moisture	44 to 45
Fat	37 to 38
Protein	3.6 to 4.6
Carbohydrates	9.7 to 10.0
Crude fibre	2.3 to 3.1
Mineral matter	1.0 to 1.3

usually in the sun, to a moisture level of 5 to 7 percent. The dried kernel, 'copra' is pressed in rotary or expeller mills. The kernel has an oil content of 68 to 70 percent on dry weight basis, and the yield of oil from copra in India is estimated to be nearly 62.5 percent[161]. The residual oil cake is sold as a cattle feed. Further solvent extraction of the cake yields almost defatted meal. The oil is refined, bleached and deodourised and used for making cosmetics and toiletries.

Table 3 gives the physicochemical characteristics of coconut oil[161,163].

Coconut oil is a colourless to pale, brownish-yellow oil. It is a fluid in the warm tropical climate, but solidifies in cold and temperate climate. The solid coconut oil melts in the range 23 to 26°C and is classified among the 'solid seed fats' containing glycerides of mainly lauric and myristic acids. Its high saponification value and low iodine value indicate abundance of short chain, saturated fatty acids in the glyceride structure. The Reichert-Meissl value (R.M. value) reported for coconut oil is 6 to 8, and Polenske value (P. value) is 15 to 18. These values are a measure of the volatile, water soluble and volatile,

Table 3: Physicochemical characteristics of coconut oil[161,163]

	range
Relative density, 40°/25°C	0.908 to 0.913
Refractive index, $n^{D}_{40°}$	1.448 to 1.450
Melting range, °C	23 to 26
Titer, °C	20 to 24
Acid value, mg KOH/g oil	1 to 10
Saponification value (SV)	251 to 264
Iodine value (IV)	7 to 10
Reichert-Meissl value (RM value)	6 to 8
Polenske value (P value)	12 to 18
Unsaponifiable matter, %	0.15 to 0.6

water insoluble fatty acids respectively. Fatty acid composition[164] of coconut oil is given in Table 4. The constituent fatty acids are C_6 to C_{18}, with $C_{18:1}$ and $C_{18:2}$ also. The latter are present 6 and 2 percent respectively. The predominent saturated fatty acid is lauric ($C_{12:0}$,47 percent) followed by myristic ($C_{14:0}$,17 percent) and $C_{8:0}$,$C_{10:0}$and $C_{16:0}$occurring approximately in 5 to 8 percent. The glyceride composition of coconut oil has been reported by Bezard[165]. Table 5 gives the mole percent of major glycerides.

1.6.4 Development of rancidity in coconut oil

Deterioration of fats and oils, mostly during storage, is indicated by the development of undesirable flavours and this is often referred to as 'rancidity'. Such oils usually show poor quality like increased acidity, lower smoke point and so on. Oils become rancid due to oxidation, lipolysis etc. During oxidation, unsaturated fatty acids present in the glycerides are attacked by oxygen, by a free radical mechanism, in presence of light, heat etc and catalysed by metals like copper and iron. Peroxides and hydroperoxides are the primary products formed and are flavourless. These undergo further reactions to give alcohols, aldehydes, ketones, acids etc. The low molecular weight

Table 4: Fatty acid composition (%) of coconut oil[164]

Saturated acids	range
Caproic ($C_{6:0}$)	0 to 0.8
Caprylic ($C_{8:0}$)	5.4 to 9.5
Capric ($C_{10:0}$)	4.5 to 9.7
Lauric ($C_{12:0}$)	44.1 to 51.3
Myristic ($C_{14:0}$)	13.1 to 18.5
Palmitic ($C_{16:0}$)	7.5 to 10.5
Stearic ($C_{18:0}$)	1.0 to 3.7
Arachidic ($C_{20:0}$)	0 to 1.5
Unsaturated acids	
Hexadecenoic ($C_{16:1}$)	0 to 1.3
Oleic ($C_{18:1}$)	5.0 to 8.2
Linoleic ($C_{18:2}$)	1.0 to 2.6

Table 5: Major glycerides of coconut oil[165]

Glycerides	Mole (%) in oil
Trilaurin (12, 12, 12)	10.6
Dilauromyristin (12, 12, 14)	10.8
Caprylodilaurin (8, 12, 12)	11.9
Capridilaurin (10, 12, 12)	5.7
Dilauropalmitin (12, 12, 16)	3.8
Laurodimyristin (12, 14, 14)	3.1

aldehydes, and acids are largely responsible for the off-flavours of stale or rancid food[166]. In lipolysis, the ester linkages of fats and oils are hydrolysed, mostly by fat splitting enzymes, in the presence of moisture. This can also lead to off-flavour development and the rancidity is referred to as lipolytic rancidity or hydrolytic rancidity. The necessary enzyme may be derived from parent plant tissue or from adventitious microorganisms such as moulds. The liberated free fatty acids, especially the short chain ones (C_4 to C_{12}), contribute to the rancid odours[167].

Coconut oil has a great tendency to become rancid. Since the oil is of a saturated nature, possibility of oxidative rancidity is very limited in it. On the other hand, it is reported to be highly susceptible to a type of rancidity known as 'ketonic rancidity' or 'perfume rancidity'. This is mostly noticed in lauric oils only. As a first step, hydrolysis of the glyceride takes place. In clean dry copra, very little fat-splitting occurs and lipolysis of coconut oil takes place, mostly in infected copra, due to enzymes produced by moulds[167]. Though coconut oil is not a good medium for the growth of microorganisms, when the oil is derived from moist and infected copra, the

oil may contain moisture and sufficient amounts of nutrients dissolved in it for the growth of micro-organisms[167]. These attack the oil directly. Free fatty acids produced from coconut oil are mostly short chain fatty acids and may directly contribute to off-odours. Moreover, these are highly susceptible to β-oxidation by dry moulds[168] and finally decarboxylate to give methyl ketones which have very penetrating odours[167]. While this type of chemical action may occur side by side with fat-splitting causing acidity, the two processes need not proceed necessarily at the same rate. Thus an oil may be perceptibly rancid without showing high acidity. Methyl heptyl ketone and methyl nonyl ketone are reported in crude samples of coconut oil[167] and are supposed to be formed from caproic and lauric acids.

There are only a few practical methods to measure rancidity in coconut oil like organoleptic test and the Taüfel and Thaler Colour Test[169]. During storage, moisture, free fatty acid (FFA) and peroxide value increase progressively but the increase in peroxide value is meagre[170]. Prevention and estimation of rancidity in coconut oil are not yet fully achieved and the efforts continue still further.

1.6.5 Proteins of coconut

The main protein present in coconut is a globulin[171,172]. The amino acid composition of coconut proteins has been reported[173,174] by many workers. They are moderately good sources of all essential amino acids. It has been reported that methionine content of the protein decreased on storage[175]. Mitchell and Villegas[176] reported a biological value of 58 for coconut cake proteins, whereas others had reported higher biological values[177-179]. Later Mitchell[180] reported a digestibility coefficient of 86 percent and biological value of 71 for the protein. However, drastic heat treatment involved in the expeller extraction, reduced the biological value to 58[180,181]. Krishnamoorthy and coworkers[182] studied nutritive value of coconut proteins both by nitrogen balance and growth methods. They reported that coconut cake protein had a digestibility coefficient of 74, biological value of 68 and protein efficiency ratio of 2.1.

1.6.6 Flavour of coconut

The people of coconut growing areas relish coconut and coconut oil for their unique flavour characteristics. The flavour constituents of coconut oil contain C_7, C_9, C_{11}, C_{13} and C_{15} straight chain methyl ketones

and C_6, C_8, C_{10}, C_{12} and C_{14} δ-lactones[183]. The volatile constituents of coconut meat consists of C_6, C_7, C_8 and C_9 alcohols and C_8 to C_{11} δ-lactones[184].

1.7 OIL PALM KERNEL

Oil palm (*Elaeis guineensis,Jacq.*) is an important plantation crop of the world and is an emerging oil source in India. It is indigenous to Africa and is extensively cultivated in Malayasia and Indonesia. The palm was introduced in India about 20 years ago. The chief attraction of this crop is that it has the highest oil yield per unit area among oil crops[185]. It gives 5 to 7 tonnes of oil per hectare as reported from Malayasia[185]. In India, the agroclimatic conditions limit its cultivation to the Western Ghats and Andaman and Nicobar Islands. In the southern part of Kerala State, in Quilon District, at Anchal, nearly 4,000 hectares have been utilised for cultivation[186] and the plantation under 'Oil Palm India Limited' is in harvesting stage. Besides, the Central Plantation Crop Research Institute (CPCRI) of Indian Council of Agricultural Research (ICAR) is working on the genetic improvement for evolving suitable hybrids for the region and supply seedlings to meet future demand. They also sustain a plantation at Palode, (Experimental Station) in Trivandrum District.

Future programme includes cultivation in large tracts in Karnataka and other Southern states also.

Several varieties of the oil palm differing in the size and colour of the fruit, amount of pericarp and thickness of the seed and shell have been recognised[187]. Based mainly on shell thickness, four economic varieties have been identified. They are (i) macro carya (shell 50 percent of fruit weight) (ii) dura variety (shell 30 percent (iii) tenera variety (shell 10 percent) and (iv) pisifera variety (shell absent, fruit only)[187].

The palms at present cultivated in India are a hybrid of pisifera and dura varieties. The resultant fruits have more mesocarp and less seed percentages.

Figure 9 is the photograph showing oil palm fruit bunch. Figure 10 shows oil palm fruit (I), longitudinal cross section of the fruit (II), shell containing palm kernel (III), its cross section (IV) and the palm kernel endosperm (V). The oil palm fruit is a drupe, the outer pulp of which provides the palm oil of commerce; within the pulp i.e. mesocarp, lies hard shelled nut, enclosing the oblong or round shaped palm kernels. The kernel has brown testa over it. The fresh palm kernel has a moisture content of 20 percent[191].On dry weight basis[187],

Fig.9 : Oil palm fruit bunch

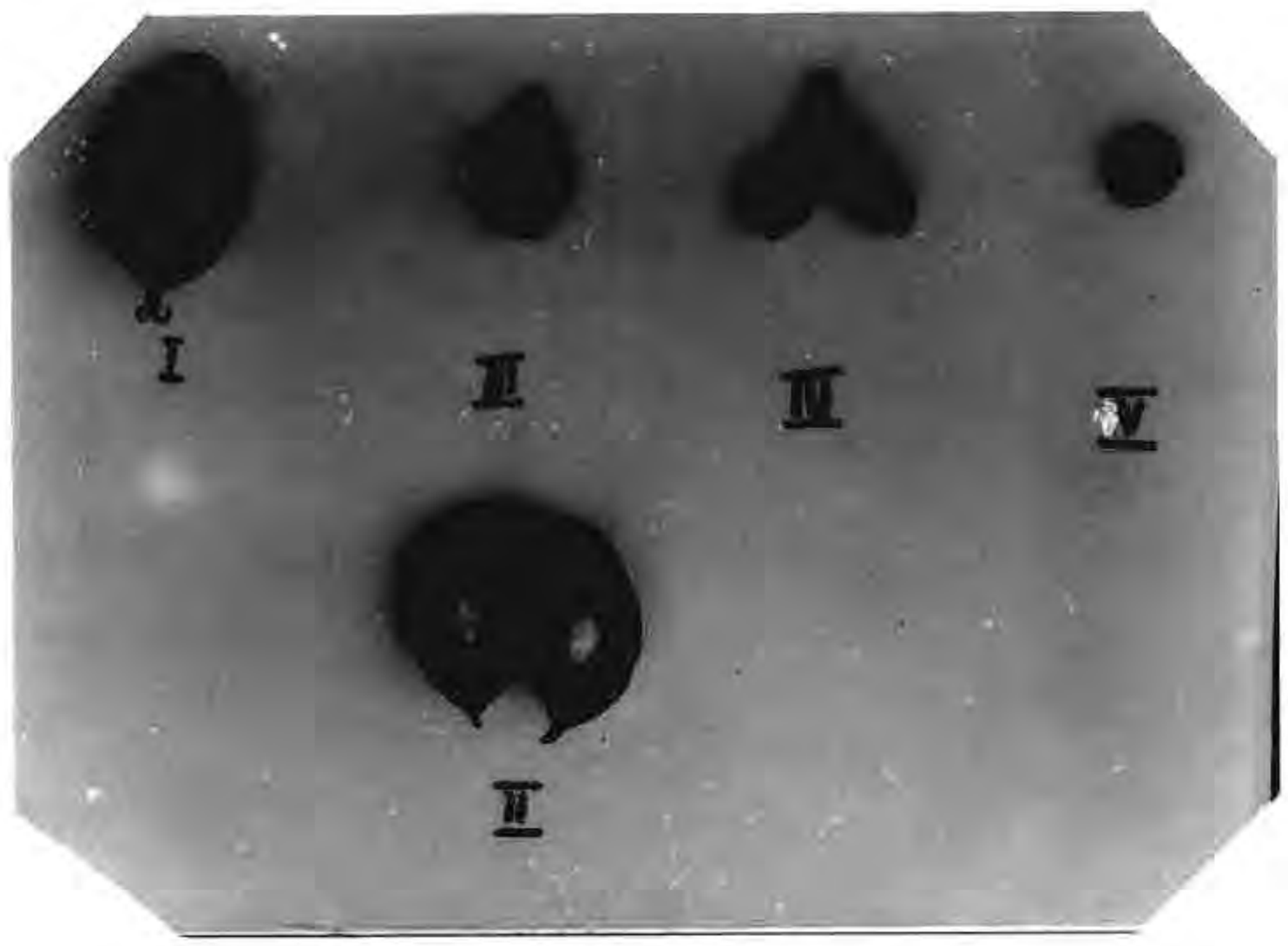


Fig.10: Different parts of Oil palm fruit - (I) A single fruit (II) Dissected fruit showing inner parts (III&IV) Shell containing kernel (V) Kernel

it has an oil content of approximately 50 percent, protein 8 to 9 percent, carbohydrates of 37 to 39 percent and ash 1 to 2 percent (Table 6).

1.7.1 Mesocarp oil (palm oil)

The oil palm fruit when mature, is deep orange red in colour. Crude palm oil, extracted from mesocarp, is also deep orange red in colour and is mostly used for edible purposes after refining, bleaching and deodourisation (RBD oil)[186]. The oil is a semi-solid at a temperature of 20°C and melts over a range of temperatures from 25 to 50°C[186]. It is composed mainly of fatty acids which are present as glycerides. Though palm oil is considered as a saturated fat, the fatty acid profile shows it has a high percentage of unsaturated fatty acids also[186,187]. The main component acids are palmitic ($C_{16:0}$, 42 percent), oleic ($C_{18:1}$, 37 percent) and linoleic ($C_{18:2}$, 11 percent); myristic ($C_{14:0}$) and stearic ($C_{18:0}$) acids occur below 5 percent[186]. The oil has a saponification value (SV) in the range 195 to 205[186] and an iodine value (IV) in the range 45 to 56[186]. Raw palm oil owes its deep red colour to carotenoids (700ppm), 90 percent of which are α- and β-carotenes[186]. Raw palm oil is reported to be one of the richest natural sources of β-carotenes[186]. It also contains tocopherols

Table 6:Proximate composition (%) of dried palm kernels[187]

Moisture	6 to 8
Oil	47 to 52
Protein	7 to 9
Extractable non-nitrogen	23 to 24
Cellulose	5
Ash	2

(800 ppm)[186] which have vitamin E activity. The tocopherols protect the oil, to some extent, against oxidation and rancidity. The sterol content of the oil is about 300 ppm[188].

In modern processing raw or red palm oil is subjected to refining and fractionation to suit the requirements of international market. The fact that one gram of palm oil is equivalent to about 100 I.U. of Vitamin A and about 300 I.U. of Vitamin E, make the oil nutritionally important when consumed in raw form[188]. Crude palm oil has formed part of the Nigerian dietary for a long time[189]. The 'palmolein' available in Indian market is the liquid portion of the refined palm oil imported from Malayasia. Recently studies on the extraction and evaluation of raw palm oil for edible use are being carried out in some research institutions including Regional Research Laboratory, Trivandrum,in India[186].

1.7.2 Palm kernel oil

Palm kernel oil has a composition very similar to coconut oil and is rich in lauric acid ($C_{12:0}$). The kernel oil has not become a popular cooking medium, probably due to the bland nature of the oil and also

due to the poor storage stability[187]. It is mainly used in soap industry in India, though it is widely used for making margarine, shortening etc in other countries. Table 7 gives, the physicochemical characteristics of palm kernel oil[163,190].

Palm kernel oi is white, yellowish to light brown in colour and is rather bland. Crude oil has a characteristic tenacious taste and smell. The melting point range is 24°C to 30°C. It has a high saponification value (SV) like coconut oil and low iodine value (IV) of 13 to 23 which is more than coconut oil. Reichert-Meissl value (RM value) of 4 to 7 and Polenske value (P value) of 8 to 12 have been reported for the oil[163,190]. Like coconut oil, its predominent fatty acid is lauric ($C_{12:0}$) followed by myristic ($C_{14:0}$)and palmitic ($C_{16:0}$) Caproic ($C_{6:0}$) caprylic ($C_{8:0}$)and capric ($C_{10:0}$)are present in small amounts. Among unsaturated fatty acids $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ also occur. Table 8 represents the fatty acid composition of palm kernel oil[163,190]. Mixed glycerides of palm kernel oil have been reported by Bezard and coworkers[191]. Table 9 gives some of the major glycerides present in palm kernel oil.

Table 7: Physicochemical characteristics of palm kernel oil[163,190]

Relative density, 40°C/25°C	0.899 to 0.914
Refractive index, $n^{D}_{40°C}$	1.448 to 1.452
Melting range, °C	24 to 30
Titer, °C	20 to 28
Saponification value (SV)	230 to 254
Iodine value (IV)	13 to 23
Reichert-Meissl value (RM value)	4 to 7
Polenske value (P value)	8 to 12
Unsaponifiable matter, %	0.02 to 0.10

Table 8: Fatty acid composition (%) of palm kernel oil[163,190]

Saturated acids	range
Caproic ($C_{6:0}$)	0.5
Caprylic ($C_{8:0}$)	2.4 to 6.2
Capric ($C_{10:0}$)	2.6 to 7.0
Lauric ($C_{12:0}$)	41.0 to 55.0
Myristic ($C_{14:0}$)	14.0 to 20.0
Palmitic ($C_{16:0}$)	6.5 to 11.0
Stearic ($C_{18:0}$)	1.3 to 3.5
Arachidic ($C_{20:0}$)	≈1.0
Unsaturated acids	
Hexadecenoic ($C_{16:1}$)	0.4 to 0.6
Oleic ($C_{18:1}$)	10 to 23
Linoleic ($C_{18:2}$)	0.7 to 5.4

Table 9: Major glycerides of palm kernel oil[191]

Glyceride	Mole (%)
Trilaurin (12, 12, 12)	19.8
Dilauromyristin (12, 12, 14)	14.1
Caprylodilaurin (8, 12, 12)	6.4
Caprylolauromyristin (8, 12, 14)	3.6
Capridilaurin (10, 12, 12)	4.7
Dilouropalmilin (12, 12, 16)	3.5
Laurodimyristin (12, 14, 14)	3.6

1.7.3 Proteins, carbohydrates and flavour compounds of palm kernel

Plam kernel cake is used as feed for livestock. Pressed cake[190] contains moisture 10.0, ash 3.9, crude protein 14.0, fat 9.0, crude fibre, 6.8, and N-free extract 57.2 percent.Solvent extracted cake[190] contains moisture 12.0, ash 3.2, crude protein 14.8, fat 2.1, crude fibre 7.3 and N-free extractives 60.9 percent. It is almost flavourless and suitable for admixture with other ruminant feeds[187].

The amino acid composition of palm kernel meal has been reported[192-194] and compares somewhat well with ground nut meal, eventhough the protein content is 2.5 times less.

The carbohydrates are not much studied. TLC analysis of an aqueous extract has been carried out by Crombie[195], according to method of Stahl and Kaltenbach[196]. According to him[195], no glucose was detected and only sucrose and mannose were present. Starch could not be identified by iodine method[195].

A fairly high ash content of 2.0 percent is reported for the kernels[197]. Detailed analysis of mineral constituents is not reported elsewhere.

The flavour of palm kernel has not been studied at all in recent times. Very old references[198-200] suggest methylnonyl ketone to be present in the oil.

With this background of work in previous years, the study is chosen after realising the importance of coconut and future potential of palm kernel in this country and the topic has much scientific relevance also. A study of the flavour constituents of coconut and palm kernel and their changes during heating are interesting and hitherto not reported. Moreover, the changes taking place in non-volatile food matrix are important in understanding the non-enzymic browning reactions of coconut and palm kernel. The study will be interesting from nutritional point of view also.

CHAPTER 2

EXPERIMENTAL

2.1 MATERIALS

Chemicals

All chemicals used in the study were either Analar grade of BDH, India or GR grade of E. Merck, India.

Solvents

All solvents used in the study were either Analar grade of BDH or GR grade of E. Merck. The solvents used for flavour analyses and other chromatographic analyses were double-distilled before use. The solvents used in high performance liquid chromatographic (HPLC) methods were of HPLC grade, procured from Spectrochem Pvt. Ltd., Bombay.

Standards

Most of the authentic reference compounds like pyrazines, pyridines, thiazoles, oxazoles, lactones, esters, ketones, aldehydes etc were gift samples from Colorado University, USA. Other standards were procured from Sigma Chemical Co., USA.

Enzymes

Amyloglucosidase enzyme was purchased from NOVO, Denmark (activity 12000 IU per ml).

Glucose oxidase-peroxidase dye kit was procured from Sigma Chemical Co., USA.

Raw Materials

Coconut: Mature coconuts (11 months old) harvested from coconut trees of West Coast Tall variety, available in the laboratory campus, were used in the present investigation.

Palm kernel: Mature palm kernels were taken from the fresh, mature, oil palm fruits (≈ 180 days) of tenera variety, collected from the plantations of Central Plantation Crops Research Institute (CPCRI) Experimental Station, Palode, Kerala. These palm kernels were used in the present investigation.

Following , general, laboratory appliances were used in the study.

All heat treatment studies were carried out in a 'Memert' (model TV10) air oven with thermostatic control supplied by J.T. Jagatiani, National House Appliances, Bombay.

A 'Sumeet' mixer/homogeniser (Power Control & Appliances Co., Bombay) was used for disintegrating, blending and homogenising purposes.

All pH measurements were made in an 'Elico' (model LI-10T) pH meter with an accuracy of ±0.05. The pH meter was calibrated with standard pH solutions (4.0, 7.0 and 9.2).

All centrifuging operations were performed in a 'Remi' (model K24) refrigerated centrifuge.

For all colorimetric estimation, a Bausch and Lomb Spectronic-20 spectrophotometer was used.

2.2 FLAVOUR DEVELOPMENT BY HEAT TREATMENT

2.2.1 Sample preparation

Coconut samples for the study were prepared as follows. Mature coconuts of West Coast Tall variety were harvested from the trees available in the campus. For the various experiments in this study, nuts of different trees and bunches were pooled together for sample preparation. Dehusked nuts were split and grated in a hand grater. The wet gratings of 40 to 45 percent moisture were dried in a cross flow drier (SHAMCO Co. Bombay) at 60°C to a final moisture content of 3 to 5 percent and kept in air tight glass bottles with screw caps. The dried gratings passed through 4 mm sieve. Palm kernel samples were prepared as follows:

Fresh palm fruits of tenera variety were collected from the plantations at Palode, near Trivandrum. The mesocarp and shell were removed and the inner kernels cleaned. The fresh whole kernels of 20 to 22 percent moisture were dried in a cross flow drier at 60°C and disintegrated in a mixer-grinder and dried in a cross flow drier to a final moisture level of 5 percent.

samples were kept in air tight glass bottles with screw cap. The grating size was almost same as in the case of coconut.

2.2.2 Heat treatment

Coconut

Coconut gratings of 250g lots were heated for 30 minutes in an air oven, maintained at constant temperatures of 100 to 200°C at 5° interval. The samples were immediately used for further studies. Dry coconut gratings (moisture 3 percent) were taken as control sample.

In commercial practice copra pieces of 1 cm cube size are cut and sometimes are heated and are then extracted in rotaries and expellers to get coconut oil with enhanced, roasted flavour. To simulate these conditions, same type of copra pieces (moisture content 5 percent) were heated in an air oven for 30 to 40 minutes. The copra pieces were then disintegrated and kept in air-tight glass bottles with screw cap, before oil extraction. Dried copra pieces (without roasting) were taken as control samples.

Palm kernel

200g lots of palm kernel gratings were heated in an aluminium pan with constant stirring to a final

temperature of 150°C and were held at this temperature for 5 minutes. Dry palm kernel gratings(moisture 5 percent) were chosen as control sample for these studies.

2.2.3 Extraction and storage study of oil samples

Both coconut and palm kernel oil samples were extracted in a hydraulic press (Universal Testing Machine, UTM German make) at a pressure of 10,000 psi. 200g lots of the dry gratings were pressed. The oil samples were allowed to settle for two days in the dark, in stoppered conical flasks with minimum head space above the sample. The oil was then filtered through Whatman 1 filter paper.

Coconut and palm kernel oil samples were stored for one year at room temperature(30±0.5°C), in stoppered test tubes of 20x1 cm dimension, out of contact with light. Following characteristics were studied before and after storage. Sensory evaluation of oil samples was carried out by the method of scoring, according to Jacobsen[201] by a panel of eight, untrained judges, who were familiarised with the qualities to be evaluated. Free fatty acid content (FFA%) and peroxide value (PV) were determined according to A.O.C.S. method[202]. Colour was measured in a Lovibond Tintometer using 1 inch cell and absorbance measured at 400 nm wavelength.

A sample of a commercial coconut oil with roasted flavour was also procured for comparison and sensory evaluation and storage studies were carried out with this sample also.

Effect of moisture content on the development of rancidity in coconut oil

The effect of moisture on the development of rancidity in coconut oil was studied by adding 0.05, 0.1, 0.5 and 1.0 percent levels of water to 5g of oil, kept in petridishes of 10 cm diameter and 1 cm depth. Coconut oil from dry coconut and that obtained from heated coconut (160°C/15 minutes) were subjected to these experiments.

2.2.4 Proximate composition analysis

Samples of dry coconut (control) and coconut heated at 160°C for 15 minutes were chosen for proximate composition analysis. In the case of palm kernel, dry palm kernel (control) and the sample heated at 150°C for 5 minutes were subjected to proximate analysis.

Moisture: This was determined by air oven method as outlined in AOAC[203].

Fat: Fat was extracted from 10g of samples using solvent hexane in a Soxhlet apparatus according to AOAC procedure[203]. The final traces of solvent were

removed by gently bubbling dry nitrogen gas through the extract until constant weights were reached.

Protein: Protein was estimated by Kjeldahl method[203]. A conversion factor of 6.25 was used to calculate the protein from nitrogen content.

Crude fibre: This was determined by acid and alkali digestion, as given in AOAC[203].

Mineral analysis: Ash content was determined according to AOAC[203]. The acid insoluble ash content was also estimated[203]. Mineral composition was studied as follows.

Potassium and sodium were estimated by flame photometry using an 'Elico' (model EI 220A) Flame photometer. Copper was determined by atomic absorption spectrometry using a 'Pye Unicam' (SP 2900) Absorption spectrometer. Iron was estimated by thiocyanate method[204]. Calcium and magnesium were estimated[205] by complexometric titration using ethylene diamine tetra acetic acid (EDTA) sodium salt. Chlorine was determined according to AOAC[203], by argentometry. Sulphur was estimated[203] gravimetrically after precipitating as barium sulphate. Phosphorus was estimated by a micro-colorimetric method[206], in which phosphomolybdate was converted to molybdenum blue and absorbance measured at 820 nm.

2.3 ISOLATION OF FLAVOUR COMPOUNDS FROM COCONUT AND PALM KERNEL SAMPLES

2.3.1 Steam distillation and extraction

Coconut oil (200g), pressed out from control and heated coconut (160°C/15 minutes) samples were subjected to steam distillation. The simple set up consisted of a steam generator (water in an RB-flask was heated up) connected to a long Vigreux column (60 cm x 2.5 cm i.d.) which was connected through a multiple adapter with parallel necks, to the condenser system of a distillation assembly and an oil reservoir. Oil was added from the top, to the Vigreux column at the rate of 3 to 4 drops per minute. The distillation rate was adjusted to be 5 to 6 drops per minute. About 2 litres of the steam distillate were collected.

The flavour compounds were extracted with dichloromethane (DCM) solvent. The solvent extract was first concentrated over a water bath, at low temperature (≈ 40°C) and finally concentrated in a Kuderna Danish evaporator (Fig.8 Chapter 1) to a final volume of 0.5 ml. The weight of flavour concentrate was found out. The samples were stored in sealed tubes at -10°C.

2.3.2. Hydrodistillation and selective extraction

Following samples were analysed: Dried coconut gratings (moisture ≈ 3%) taken as control sample and

those heated at 130, 145 and 160°C for 15 minutes, were subjected to hydrodistillation, and detailed flavour analysis. In the case of palm kernel, dried gratings (moisture 5 percent), taken as control and those heated at 150°C for 5 minutes were subjected to hydrodistillation and detailed flavour analyses.

200g lots of samples were codistilled with 3.5 litres of water (2.0+1.5 litres) at atmospheric pressure. Ice-cooled water was circulated through condenser. The receiver flask was cooled in an ice bath and was further connected to cold trap containing dichloromethane solvent at -10°C. About 2 litres of the distillate were collected for each batch. The pH of the distillates were found out.

Selective extraction of the flavour compounds was carried out after pH adjustment of the distillate. The scheme of separation is outlined in Figure 11. The solvent extracts were dried over anhydrous sodium sulphate. The dried extracts were concentrated over a low temperature bath (≈ 40°C) and final concentration was done in a Kuderna Danish evaporator to 0.5 ml. The experiment was repeated 5 times (total 1 kg sample) and the final concentrates pooled together for subsequent analysis. The samples were stored in a sealed tube,

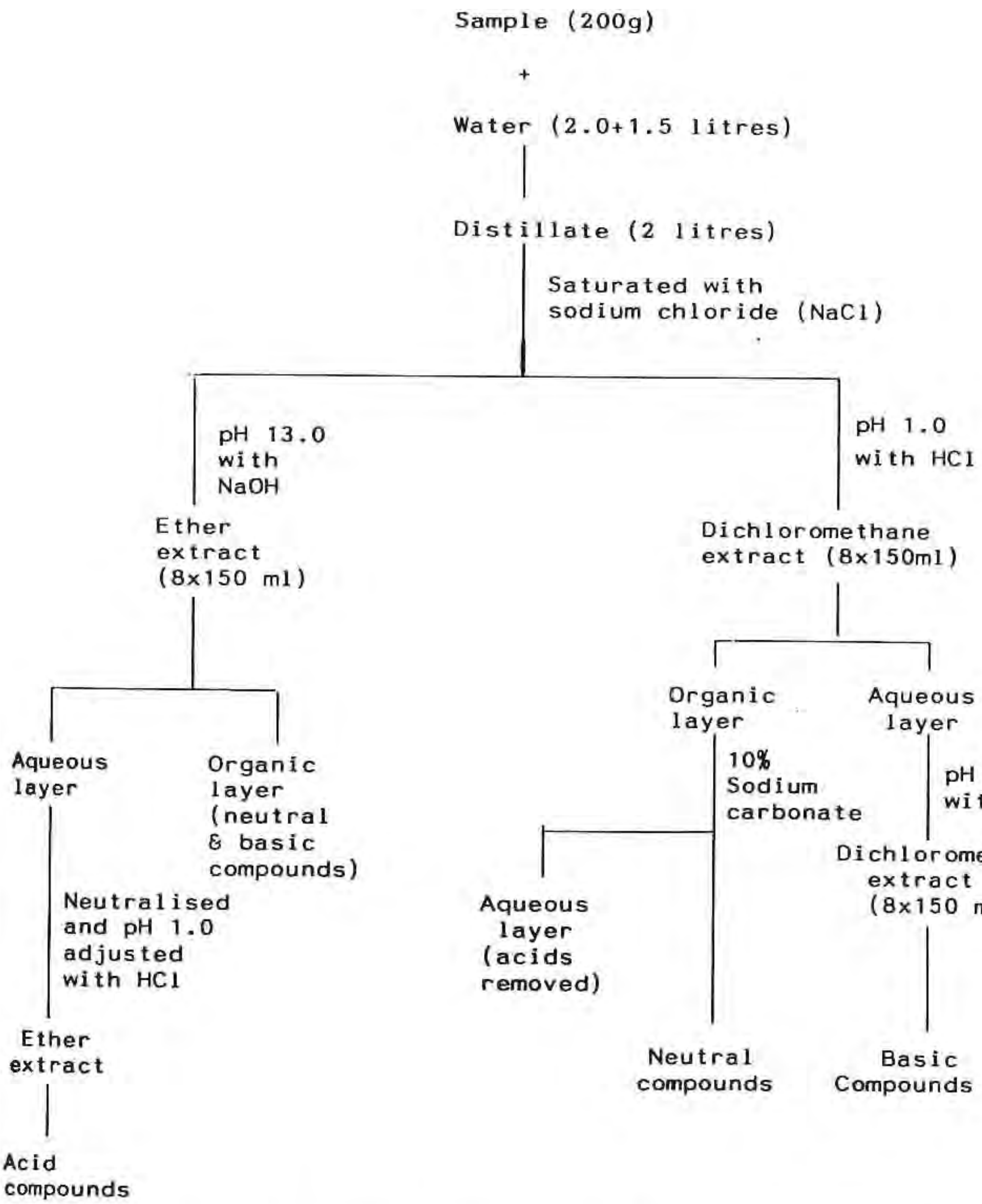


Fig.11 : Selective extraction of volatile, flavour compounds

below -10°C. The acid fraction was methylated by refluxing with methanol-sulphuric acid (50:1) reagent. The methylated samples were washed free of sulphuric acid and extracted with hexane and stored as mentioned earlier.

2.4 IDENTIFICATION OF FLAVOUR COMPOUNDS OF COCONUT AND PALM KERNEL (CONTROL AND HEATED)

2.4.1 Thin layer chromatographic (TLC) analysis

Silica gel (G) of E. Merck Co. was used for preparing 0.25 mm thick plates. The plates were activated at 110°C for 1 hour before the experiment.

The total flavour extract from coconut oil was subjected to TLC analysis using the optimised system of petroleum ether-ethyl acetate (90:10) and later the spots were detected under UV light and by Iodine absorption. The plate was also sprayed with dinitrophenyl hydrazine reagent[207].

Basic and neutral flavour fractions were subjected to TLC analysis. Mixture of petroleum ether (60-80°) and ethyl acetate (90:10, v/v) was the optimised solvent system used. After development, the plates were exposed to UV radiations and later to iodine vapours. In the case of basic fraction, after development, the plate was sprayed with Dragendorff's reagent prepared according to Stahl[208]. Preparative TLC was carried out with basic

fraction of the isolated flavour using silica gel (G) plates (0.6 mm thick) and the solvent system, petroleum ether-ethyl acetate (90:10). The separated bands were eluted with spectroscopic grade dichloromethane solvent and UV spectra were recorded in a Hitachi 200 UV-Vis spectrometer, and compared with the spectra[6] of pyrazines.

TLC analysis of the acid fraction (before methylation) was carried out with silica gel G plates using the optimised solvent system, ethyl acetate:ammonia (95:5).

2.4.2 Gas chromatographic (GC) analysis of flavour compounds

A Hewlett Packard 5840A model gas chromatograph, with a built in electronic integrator was used for all the GC analyses. Both thermal conductivity detector (TCD) and flame ionisation detector (FID) were available with the instrument and these were utilised in this study. The chart speed was 1 cm/min and the attenuation was 2 † 8 Individual peaks were quantitated from the relative area percentage of the separated peaks. The GC analysis was repeated three times and mean value of area percentage was taken.

2.4.2.1 GC retention time indices (I_E)

The I_E values were calculated following the method of Van den Dool and Kratz[135]. A series of standard methyl esters of normal carboxylic acids were injected and the retention time noted for each standard peak. The GC conditions were maintained same as that of the GC analysis of sample. The I_E value of each methyl ester standard was arbitrarily given the value of the carbon number of the acid of the ester; thus the I_E value of ethyl hexanoate was taken as 6.0. A graph was plotted with carbon number (same as I_E value) of standards on the X-axis and their retention time on the Y-axis. From this, I_E values of sample peaks were calculated by linear interpolation of their retention times. The I_E values of separated peaks of experimental samples, as well as those of authentic standards injected for comparison and identification, were calculated and compared.

2.4.2.2 GC analysis of total flavour extract isolated by steam distillation of coconut oil

The GC analysis was carried out under the optimum conditions given below:

Column : S.S. column (1.83m x 3mm i.d.) of OV-17 (3 percent) on Chromosorb WHP (80 to 100 mesh).

Carrier gas : Nitrogen at a flow rate of 20 ml/min.

Detector : FID at 300°C

Injection temperature : 250°C

GC programme : Isothermal at 100°C for 5 minutes and then temperature programmed to 225°C at the rate of 5°/min with a final hold up of 10 minutes at 225°C.

2.4.2.3 GC analysis of basic fraction of flavour compounds

The basic fractions of flavour, isolated from coconut and palm kernel samples were analysed in a GC under the following, optimum conditions. The samples included flavour fractions from control and heated samples of coconut and palm kernel.

Column : S.S. column (1.83m x 3mm i.d.) of OV-17 (3 percent) on Chromosorb WHP (80 to 100 mesh).

Carrier gas : Nitrogen at a flow rate of 20 ml/min.

Detector : FID at 300°C

Injection port : 250°C

GC programme : Isothermal at 100°C for 5 minutes and then temperature programmed to 200°C at the rate of 5°/min with a final hold up time of 15 minutes.

Identification : Comparison of I_E values with those of authentic compounds, like pyrazines, pyrroles, pyridines, thiazoles etc.

2.4.2.4. GC analysis of neutral fraction of flavour compounds

Neutral fractions of the flavour concentrates isolated from control and roasted samples of coconut and palm kernel were analysed in a GC under the optimum conditions given below:

Column : S.S. column (1.83m x 3mm i.d.) of OV-17 (3 percent) on Chromosorb WHP (80 to 100 mesh).

Carrier gas : Nitrogen at a flow rate of 20 ml/min.

Detector : FID at 300°C

Injection port : 250°C

GC programme : Isothermal at 150°C for 1 minute and then temperature programmed to 225°C at the rate of 5°/min with a final hold up time of 5 minutes.

Identification : Comparison of I_E of authentic compounds namely lactones, alcohols, aldehydes, ketones, esters etc.

2.4.2.5 GC analysis of the acid fraction of flavour compounds

The methylated acid fractions were analysed under the optimum conditions given below:

Column	:	S.S. Column (1.83m x 3 mm i.d.) of Diethylene Glycol Succinate (DEGS), 10 percent, on Chromosorb WHP (80 to 100 mesh).
Carrier gas	:	Nitrogen at a flow rate of 20 ml/min.
Detector	:	FID at 300°C
Injection port	:	200°C
GC conditions	:	Isothermal at 100°C for 1 minute and temperature programmed to 190°C at the rate of 5°/min and kept at 190°C for 15 minutes.
Identification	:	Comparison of I_E values of sample peaks with those of methylated fatty acid standards.

2.4.2.6 Aromagrams-GC profile with odour descriptions of the flavour compounds of coconut and palm kernel

GC analyses with odour descriptions (aromagrams) of the flavour concentrates, especially basic and neutral fractions, were carried out. Aromagrams of control and heated samples of coconut and palm kernel were prepared. For this purpose, a thermal conductivity

detector (TCD) was used. The column and GC conditions were maintained same as that for neutral and basic fraction analyses carried out with FID. The carrier gas was hydrogen at a flow rate of 20 ml/min. A small teflon tube (3 mm i.d.) was connected to the exit port of the GC and the free end of the tube was attached to a small funnel (2 cm i.d.). As and when each compound was being eluted i.e. when the peak was being traced, the gas coming out of the exit port was sniffed and the flavour note written down descripively. The experiment was repeated thrice.

For the acid fraction, since methylated samples were analysed, aromagrams were not prepared.

2.4.3 Capillary GC-MS analyses

The three flavour fractions of coconut and palm kernel (control and roasted) were analysed by GC-MS. All GC-MS analyses were carried out in a coupled GC-MS instrument, namely Hewlett-Packard 5995B model, quadrupole mass spectrometer, provided with a Data Base System. The MS conditions for the various samples, analysed were maintained the same, as given below:

Ionisation voltage	70eV
Source temperature	150°C
Transfer line temperature	280°C
Analyser	180°C
Electron multiplier	1800V

The instrument was initially tuned and calibrated with perfluoro tributyl amine (PFTBA).

All GC-MS analyses were carried out in capillary columns made of fused, flexible silica. The details of GC conditions in GC-MS analyses are given below:

Carrier gas : Helium

Capillary injection mode: Split mode (50:1)

Injection port of GC : 250°C

For basic fraction

Column : Carbowax 20M (50m x 0.2mm i.d.)

GC programme : Isothermal at 90°C for 15 minutes and then temperature programmed to 200°C at the rate of 15°/min with a final hold up of 15 minutes.

For neutral fraction

Column : Cross-linked Methyl Silicone (12m x 0.2mm i.d.)

GC programme : Isothermal at 90°C for 3 minutes and temperature programmed to 250°C at the rate of 5°/min with a final hold up of 20 minutes.

For acid fraction (analysed as methyl esters)

Column : Cross-linked Methyl Silicone (12m x 0.2mm i.d.)

GC programme : Isothermal at 90°C for 3 minutes and then temperature programmed to 200°C at the rate of 3°/minute, with a hold up of 15 minutes at final temperature.

Identification

Identification of the recorded peaks in the GC-MS analyses was carried out by comparing the spectra with the NBS library of Flavour and Fragrances in the Data Base System provided with the instrument. Available authentic standards were also analysed by GC-MS and their spectra recorded and matched with those of sample peaks. Spectra of a few compounds which were not available as authentic standards and which were not covered by the NBS library, were compared with published mass spectral data[6,209,210].

Quantitation

All GC-MS analyses were carried out in duplicate and the quantitation of individual compounds was done based on relative ion intensity.

2.5 ANALYSIS OF LIPIDS OF COCONUT AND PALM KERNEL SAMPLES (Control and heated)

2.5.1 Extraction

Neutral lipids were extracted with solvent hexane in a Soxhlet extractor for 16 hours[203]. 100g each of

coconut and palm kernel samples were extracted with 1.5 litres of solvent as described above. The solvent was evaporated off over a water bath (50°C) under reduced pressure and traces of solvent removed by passing a stream of dry nitrogen gas through the extract. For quantitative estimation, 10g samples were extracted in triplicate and mean value found out.

Total lipids were extracted according to Folch procedure[211]. Chloroform-methanol in the ratio 2:1 was used as the solvent mixture . 100g each of the samples were extracted with 1 litre of the solvent mixture in three steps of 500 ml, 250 ml and 250 ml. The extraction was done by blending the material with 500 ml of solvent in a mixer-blender for 2 minutes and subsequently extracted twice with 250 ml each of the solvent mixture in the same manner. The combined extracts were pooled and washed with 10 percent sodium chloride solution twice. The organic layer was evaporated under reduced pressure and at a lower temperature (≈ 50°C). Traces of solvent was evaporated off by passing a stream of nitrogen gas through it. For quantitation, 10g samples were extracted in triplicate, and mean value found out.

2.5.2 Determination of Physicochemical characteristics

All determinations were carried out in triplicate and mean value taken. Colour was determined in Lovibond

Tintometer using 1" cell according to AOCS method[202]. Specific gravity (at 60°C), refractive index (at 40°C), acid value (AV), peroxide value (PV), iodine value (IV), saponification value (SV), Reichert-Meissl value (RM value), Polenske value (P value) and unsaponifiable matter were estimated for neutral and total lipids of coconut and palm kernel samples according to A.O.C.S. methods[202].

2.5.3 Fatty acid composition

Neutral and total lipids of control and heated samples of coconut and palm kernel were analysed for their fatty acid composition. The fatty acid methyl esters (FAME) were prepared by trans esterification of the lipid with sodium methoxide. Sample (50 mg) was treated with 1 ml of 0.05N sodium methoxide in a sealed tube and heated in water bath at 90°C for 15 minutes. The tubes were later cut open and subjected to GC analysis immediately. A Hewlett Packard 5840A gas chromatograph was used. The GC condition for FAME analyses was same as that described under 2.4.2.5.

2.6 ANALYSIS OF CARBOHYDRATES OF COCONUT AND PALM KERNEL SAMPLES (Control and heated)

Samples of coconut and palm kernel were defatted with petroleum ether (40 to 60°C) by Soxhlet extraction. The residue was powdered to pass through 200 micron

sieve. The defatted dried meal was used for subsequent analyses of carbohydrates and proteins. All estimations were carried out in triplicate.

2.6.1 Extraction and estimation of soluble sugars

1g of defatted meal was refluxed with 70 ml of aqueous methanol (80 percent, v/v) for 5 hours over a temperature controlled water bath[212]. The methanol was evaporated off under reduced pressure and at lower temperature (50 to 60°C). The concentrate was calrified with lead acetate and excess lead removed by potassium oxalate. The clarified solution was made upto 10 ml. Total soluble sugars were estimated by phenol-sulphuric acid method[147], and reducing sugars by the Nelson-Somogyi micro colorimetric method[148].

HPLC analysis of sugars: 5 ml of the clarified solution was further concentrated under vacuum to 1 ml, deionised by passing through columns (20 cm x 1.5 cm i.d.) of ion exchange resins (Amberlite IR-120, cation and Amberlite, IR-45, anion) and eluants and washings concentrated to 0.5 ml. This was filtered through 0.5 μm millipore filter before HPLC analysis.

A Waters Associate Liquid Chromatograph was used for analysis. It was equipped with a 6000A model solvent delivery systems, a differential refractometer

(R401 model), a 46K Universal injector and a variable speed omniscribe recorder was used. Conditions of analyses were:

Column	: µ-Bondapak Carbohydrate column (30cm x 0.39 cm i.d.) made of stainless steel.
Mobile phase	: Acetonitrile-water (85:15, v/v) degassed in ultrasonic bath before use.
Flow rate	: 2 ml/min.
Detector	: Refractive index (RI)
Quantitation	: By triangulation of peaks

The calibration was done with a mixture of standards viz. ribose, rhamnose, arabinose, fructose, glucose, mannose, galactose, sucrose, maltose and raffinose each at 1.percent level. Different combinations of the standard sugars were also tried to check resolution of individual sugars.

Glucose was separately estimated by glucose oxidase peroxidase-dianisidine method according to Holm et al[213].

2.6.2 Estimation of Starch

Perchloric acid method: The residue after extraction of soluble sugars, was digested with perchloric acid

(60 percent). The starch was estimated by modified anthrone method according to Clegg[150].

Amyloglucosidase enzyme method: In the case of palm kernel, the residue after extraction of soluble sugars, was hydrolysed with amyloglucosidase enzyme according to standard methods[214]. The hydrolyzed sugars were estimated by colorimetric method, using dinitro-salicylic acid (DNS)[215].

2.7 ANALYSIS OF PROTEINS OF COCONUT AND PALM KERNEL (Control and heated)

All estimations were carried out in triplicate. Defatted samples of coconut and palm kernel (preparation given under 2.6 carbohydrate analysis) were used for the various analytical procedures.

2.7.1 Protein and non-protein nitrogen contents

Total Protein: This was estimated by semi micro Kjeldahl method, according to AOAC[203]. A factor of 6.25 was used for conversion.

Non-protein nitrogen: 1g of defatted sample (2g, in the case of palm kernel) was extracted with 50 ml of 10 percent solution of trichloroacetic acid (TCA), over a magnetic stirrer for 2 hours and left aside for 16 hours. It was centrifuged at 10,000 rpm for 10 minutes and supernatant layer filtered through Whatman 1 filter

paper. The non-protein nitrogen content was estimated by semi-micro Kjeldahl method[203].

2.7.2 Protein solubility studies

The studies were limited to control and heated samples of coconut only.

Solutions with pH values of 3 to 11 were prepared by adding 0.1N hydrochloric acid (HCl) or 0.1N sodium hydroxide (NaOH)solution to distilled water and adjusting the pH to required value. For preparing solutions having pH of 0, 1 and 2 and 14, 13 and 12, 1N, 0.1N and 0.01N HCl and 1N, 0.1N and 0.01N NaOH were accurately prepared[216].

0.5g of sample was stirred with 25 ml of the extractant at about 50 rpm speed over a magnetic stirrer at 30±0.2°C for one hour. The extract was centrifuged at 10,000 rpm in a centrifuge for 10 minutes and supernatant liquid decanted. The residue was re-extracted with another 25 ml of the extracting solution for one hour and centrifuged as before. The required pH was maintained during stirring. The combined extracts were filtered through Whatman 1 filter paper.

The soluble protein was precipitated from the solution with 50 ml of 20 percent solution of TCA so that final concentration of TCA was 10 percent. A contact

time of 16 hours was allowed, and the contents centrifuged at 6,000 rpm for 10 minutes. The residue (protein) was dissolved in 0.1N NaOH and made upto 25 ml. After four-fold dilution, the protein was estimated by Lowry's method[217]. Bovine serum albumin (BSA) was taken as the standard protein for calibration. The absorbance was meausred at 660 nm.

$$\text{Solubility (\%) of protein at a given pH} = \frac{\text{Dissolved protein}}{\text{Total protein}} \times 100$$

A graph was drawn connecting solubility of protein and the corresponding pH values.

2.7.3 Classification of coconut proteins

The classification of proteins based on their solubility in water (albumins), salt solutions (globulins), aqueous alcohol (prolamines) and dilute alkali or acid (glutelins) is well known. The sequential extraction of proteins with these solvents, forms the basis of this experiment. Osborne classification of proteins as modified by Senvaire[218] was carried out in the case of control and three heated samples of coconut.

Defatted, finely powdered sample (1g each) was stirred with the solvent as outlined in Figure 12.

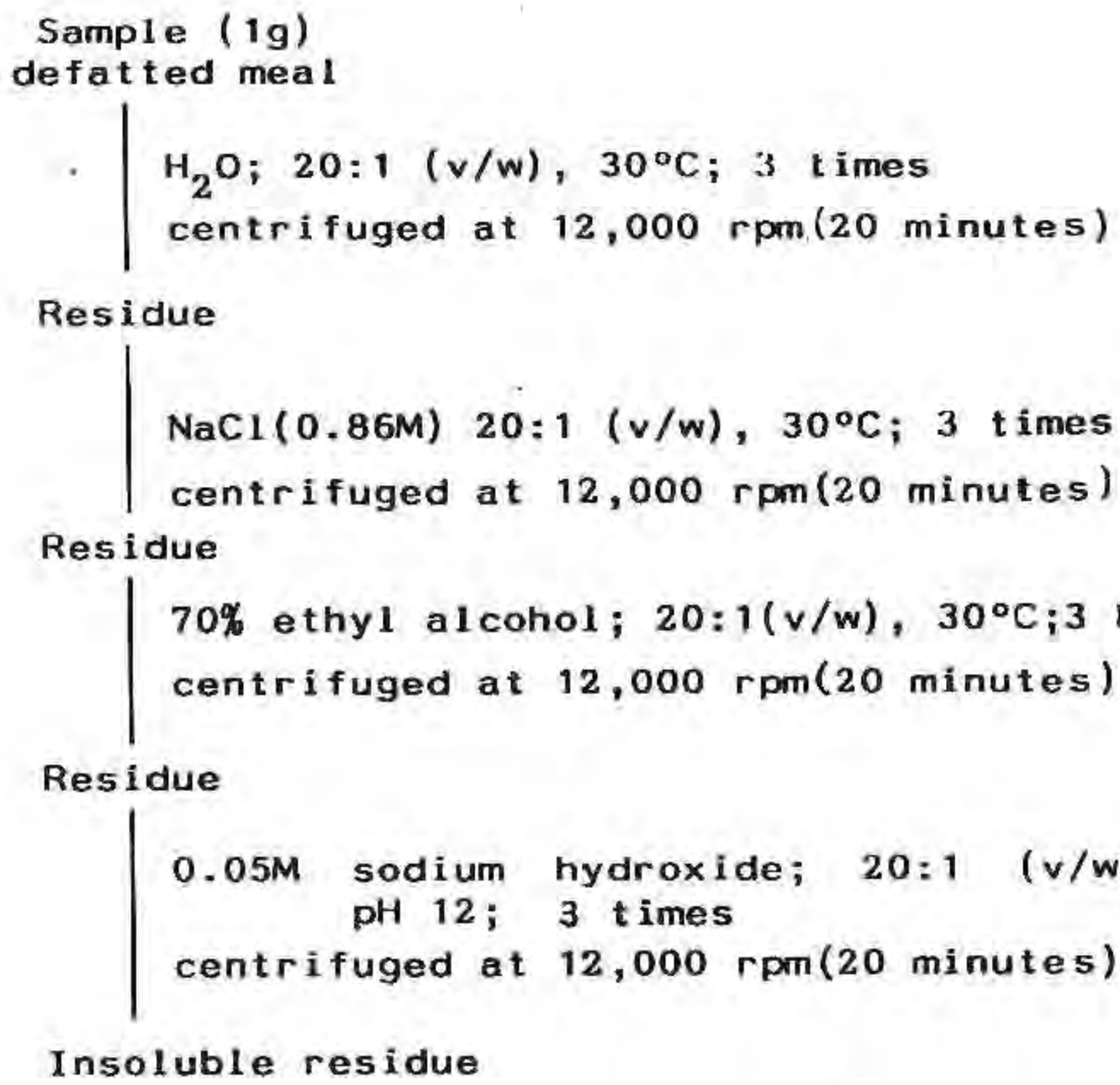


Fig. 12 : Scheme of fractionation of proteins

Centrifuging was done in a Remi centrifuge with thermostatic control. The albumin, globulin, prolamine and glutelin fractions were made upto 100 ml with respective solvents. Aliquots (40 ml) were pipetted out and the protein precipitated with 40 ml of 20 percent TCA (final concentration, 10 percent). The prolamine fraction (100 ml) was completely used for protein precipitation after concentrating to 40 ml. A contact time of 16 hours was given. The precipitated protein was centrifuged at 6,000 rpm for 10 minutes at 30±0.2°C

and redissolved in 0.1N sodium hydroxide solution and made upto 25 ml. The protein content in each fraction was estimated by Lowry's method[217], taking aliquots of 0.1 to 0.2 ml. The residue after extraction was washed free of alkali and vacuum dried and weighed. The protein content in the residue was determined by Kjeldahl method[203].

2.7.4 Electrophoresis of coconut proteins

The sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of coconut proteins was carried out. The albumin and globulin fractions of coconut were extracted as described before (2.7.3). 40 ml each of the extracts were pipetted out and the proteins precipitated[219] with ammonium sulphate at 50, 75 and 100 percent saturation. In each case, the precipitated protein was centrifuged at 10,000 rpm for 10 minutes, combined and deionised by dialysis against distilled water. The purified protein fractions were concentrated under vacuum and dissolved in phosphate buffer (pH 7.0) containing mercapto ethanol and bromophenol blue as indicator. Preparation of the reagents and standard protein solutions was carried out according to Sigma Technical Buletin No.MWS-877 for SDS-PAG electrophoresis of proteins, which is based on the work of Laemmli[220].

The molecular weight markers used were:

Albumin, bovine plasma	66,000
Albumin, egg (Ovalbumin)	45,000
Pepsin, porcine (Stomach mucose)	34,700
Trypsinogen (bovine, pancreas PFSF treated)	24,000
β-Lactoglobulin, bovine milk (sub-unit)	18,400
Lysozyme, egg white	14,300

10 percent polyacrylamide gel in phosphate buffer (pH 7.2) with 0.1 percent sodium dodecyl sulphate (SDS) was used. Vertical tube gel electrophoresis was performed in a 'Broviga' Disc Electrophoresis apparatus (model 1295) for 5 hours under a constant current of 8 mA per tube. The gels were fixed in methanol:acetic acid:water (400:70:530,v/v/v), stained with Coomassie Brilliant Blue and destained with methanol:acetic acid:water (50:75:875, v/v/v). The relative mobility (Rf) values of various bands were calculated and compared with those of standard proteins.

2.7.5 Amino acid analysis

Total (protein) and free amino acid analyses of coconut and palm kernel samples were carried out.

Total (protein) amino acids: Hydrolysis was carried out according to Moore and Steine[221]. 50 mg each of defatted samples were hydrolysed with 6N hydrochloric acid (HCl) under vacuum in a sealed tube at 110°C for 20 hours. After hydrolysis, the contents were filtered through Whatman 1 filter paper and the HCl was removed under vacuum and the contents dissolved in 5 ml of citrate buffer (pH 2.2)[219]. The amino acid analysis was carried out in a Technicon NC-2P automatic amino acid analyser using single column technique and three buffer system for elution as suggested by Spackman et al[222]. Conditions of analysis were:

Column	: Single column (23cm x 0.5cm i.d.) packed with Resin C_3-Technicon Cation Exchanger.
Conditions	: Temperature of column 60°C; elution rate 30 ml/hour.
Detection and estimation	: The reaction between ninhydrin and amino acid at 95°C developed a colour which was measured at 410 nm using a flow through flow cell.
Sample volume	: 20 to 30 µl.

A single channel continuous recorder was used. Norleucine was used as the internal standard, and calibration was done using 20 µl of a mixture of standard amino acids each at 0.0025 mole/litre concentration (i.e. 0.05 µmole/20 µl). Each amino acid was estimated as follows:

$$\mu\text{mole of amino acid} = \frac{\text{area of norleucine (NL)}}{\text{area of amino acid in the standard chromatogram}} \times \frac{\text{area of amino acid in the unknown chromatogram}}{\text{area of norleucine in the unknown}} \times 0.05 \text{ (concentration in } \mu\text{mole)}$$

Since tryptophan was destroyed during acid hydrolysis, it was separately estimated according to Sastri et al[223], after alkaline hydrolysis. The colorimetric estimation was based on the formation of a pink coloured complex of tryptophan (λ_{max} 500 nm), when treated with sucrose and thioglycollic acid under acid conditions.

Free amino acids: The free amino acids were extracted as given under 2.7.1 for non-protein nitrogen. The TCA extract was concentrated under vacuum and final traces of TCA removed[224] by washing with diethyl ether. The contents were then made upto 5 ml with citrate buffer (pH 2.2)[219] and the amino acid analysis was carried out as described earlier.

2.8 MODEL SYSTEM STUDIES OF COCONUT AND PALM KERNEL

Model systems simulating fresh coconut and palm kernel were constituted. Details of the composition of these systems will be given in Chapter 5.

Coconut: Calculated quantities of water, coconut oil, and sugars (ribose, rhamnose fructose, glucose, galactose and sucrose) were mixed. Calculated quantities of amino acids-aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenyl alanine, tryptophan, histidine, lysine and arginine - were dissolved in phosphate buffer (pH 7.0)[219]. Calculated quantity of sand was used in the place of protein and required amount of starch was added. Whatman 1 filter paper (weighed amount) in small bits was used to provide the 'body' or 'matrix' of the system. The whole mixture was intimately mixed and soaked for 2 hours and ground in a mixer-homogeniser and heated in an oven at 160°C for 15 minutes. The heated system was subjected to hydrodistillation, followed by flavour analysis by GC-MS as described earlier (2.3 and 2.4).

Palm kernel: Calculated quantities of water and palm kernel oil were taken. Weighed amounts of rhamnose, arabinose, fructose, glucose, mannose and sucrose were

added to water. Sand was added in equivalent amounts to protein content. The amino acids dissolved in pH 7.0 buffer, in required amounts, were aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, histidine, lysine and arginine. Whatman 1 filter paper (weighed quantity) was added in smaller bits, to provide body or matrix to the system. The whole system was intimately mixed and soaked for 2 hours. It was heated at 150°C for 5 minutes in the oven and flavour isolated and analysed as described earlier (2.3 and 2.4).

CHAPTER 3

DEVELOPMENT OF ROASTED FLAVOUR IN COCONUT

Coconut and coconut oil are very much relished by people of India , especially in the State of Kerala, for their unique flavour characteristics. Fresh coconut is widely used in food preparations, in this region and for certain curries like 'theeyal', roasted coconut is used for increased flavour. Since coconut is very rich in oil, it is not oil-roasted like peanuts or cashewnuts. Simple heating without oil itself produces roasted flavour. Heating of copra is practised by some of the oil mill owners, for getting coconut oil with an enhanced roasted flavour. Coconut oil with a pleasant nutty aroma is made in the households by heating the water extract of fresh coconut called coconut milk. At about 150 to 160°C, the oil separates out while the browned solids settle. The oil is traditionally used for bathing babies and is believed to be good for the skin. Thus, coconut is heated for domestic and commercial purposes with the final result of browning and development of roasted flavour.

Thermal generation of aromas is a topic of scientific interest even today. During non-enzymic browning, the amino acids and sugars interact, producing, a number of heterocyclic compounds like pyrazines. These are considered to be responsible for roasted aromas. Considerable research work has progressed on the chemistry

of flavour development in roasted coffee, cocoa, peanuts, potato products, meat products etc. A similar investigation in coconut appears to be essential since development of roasted flavour is most interesting to the flavour chemist while the changes in the lipids, carbohydrates and proteins are nutritionally important from a food chemist's angle.

The flavour constituents of coconut and coconut oil have been reported in the past, mainly, by two researchers. Allen[183] in 1965, analysed crude and fresh samples of coconut oil using GC with the help of a thermal conductivity detector. He identified quantitatively C_7, C_9, C_{11}, C_{13} and C_{15} methyl ketones and C_6, C_8, C_{10}, C_{12} and C_{14} δ-lactones. Of these, C_{11} and C_{13} methyl ketones were present in large quantities - 150 and 300 ppm respectively - in crude coconut oil, whereas in fresh oil, they were present below 1 ppm level and other methyl ketones were not detected at all. Probably methyl ketones are formed by microbial action on fatty acids in crude oils. The δ-lactones were present in 50 to 100 ppm levels.

The second important study was made by Lin and Wilkens[184] in 1970, in which, the volatile flavour compounds of coconut meat were analysed by GC-MS. No quantitation was attempted, but odour descriptions of eluting

peaks were noted. Only three δ-lactones (C_8, C_{10} and C_{11}) and two methyl ketones (C_9 and C_{11}) could be identified. However, the study was characterised by the identification of a few primary and secondary alcohols (C_7 to C_9) which were not reported earlier. Probably, these alcohols contribute to the fresh flavour of wet coconut. Both these flavour studies were carried out in GC, packed columns and identification and quantitation of separated peaks were not complete. This implies that a reexamination of the flavour constitutents of coconut with the help of modern instruments like capillary GC-MS is very essential.

In the present study, the flavour compounds of heated coconut as compared to its unheated control and the causative changes occurring in the food matrix, leading to development of roasted flavour, are investigated. The results are discussed in this chapter.

RESULTS AND DISCUSSION

Studies on the development of roasted flavour in coconut were preceded by standardisation of the heating procedure and optimisation of the same, based on sensory and chemical analysis. Storage study of the oil extracted from heated coconut was also carried out.

3.1 STANDARDISATION AND OPTIMISATION OF THE HEAT TREATMENT

Dry coconut gratings were heated in an oven at different temperatures from 100 to 200°C at 5° interval, for 30 minutes. The flavour of oil pressed from these gratings were compared organoleptically. Pleasant, rcasted flavour was produced above 125°C and the intensity of colour and flavour increased with temperature. Beyond 160°C, the flavour became undesirable due to charring of coconut and consequent burnt smell in the oil. When the gratings were heated in a pan over Bunsen flame, pleasant, roasted flavour, with adequate intensity was produced in about 6 minutes' time. In commercial oil extraction, copra is usually cut into cubical pieces of 1 cm dimension. Hence, experiments with copra pieces were carried out at the above temperatures. Comparable flavours were produced when the time of heating was 35 minutes. When heated over a Bunsen flame, copra pieces gave desirable flavour in 8 to 10 minutes time.

3.1.1. Storage study of oil extracted from heated coconut

The oil was pressed in a hydraulic press and the samples were used for storage studies. Table 10 represents the results of the sensory evaluation of coconut oil samples during storage. It could be seen that coconut oil samples with roasted flavour were rated higher than

Table 10 : Sensory evaluation of coconut oil extracted from heated coconut gratings

Samples	Colour (Maximum score 10 marks)		Aroma (Maximum score 10 marks)	
	Initial	After storage over one year	Initial	After storage over one year
Control (without heating)	8.0	6.0	6.8	7.1
105°C	8.0	5.7	7.3	6.6
125°C	7.5	5.7	9.2	8.0
130°C	7.6	5.5	9.4	7.1
135°C	7.0	5.6	9.7	7.9
140°C	6.5	5.4	9.4	7.7
145°C	4.5	4.0	8.7	7.2
150°C	3.4	4.0	7.7	8.3
160°C	3.7	2.2	6.3	8.8
Fried over flame for 6 min.	6.0	5.0	8.0	7.0
Commercial sample	5.0	5.0	9.2	8.6

the control oil sample, from unheated dried coconut. Samples heated at 130 to 145°C were given best scores. Sensory evaluation of samples after one year storage indicated that most of the heat treated samples were better than the control. They also showed more satisfactory keeping quality than the control sample during storage. The free fatty acid content of all heated samples were lower than that of unheated sample (Table 11). A commercial sample of coconut oil with roasted flavour (developed by heat treatment) was found to be comparable with the experimental samples heated at 140 to 145°C in flavour. In keeping quality, the commercial sample was comparable with the experimental sample heated at 160°C. Samples heated above 125°C and the commercial sample showed small but positive peroxide values. However, organoleptic evaluation showed that small differences in peroxide values had little significance on aroma. The control sample though rancid, had a lower peroxide value while oil from coconut heated at 160°C had no detectable rancidity but showed measureable peroxide value (Tables 10 and 11).

The colour values were measured in a Lovibond Tintometer using 1" cell. Both before and after storage, the samples heated upto 140°C showed a lighter colour (Table 12). Above 140°C the colour became more intense

Table 11 : Changes in free fatty acid content and peroxide value of oil samples of heated coconut* during storage

Samples	Free fatty acid (%)				Peroxide value as milliequivalents of peroxide oxygen per kg fat	
	Initial	4 months	8 months	One year	Initial	After one year of storage
Control	0.08	0.26	0.51	0.45	0	0.55
105°C	0.10	0.21	0.29	0.37	0	0.54
125°C	0.08	0.23	0.30	0.36	0	0.43
130°C	0.08	0.10	0.25	0.27	0	1.03
135°C	0.08	0.14	0.14	0.21	0	1.16
140°C	0.08	0.11	0.13	0.16	0	0.91
145°C	0.08	0.09	0.14	0.23	0	1.12
150°C	0.10	0.18	0.20	0.24	0	1.14
160°C	0.09	0.12	0.17	0.21	0	1.16
Fried over flame for 6 min.	0.08	0.12	0.18	0.27	0	1.57
Commercial sample	0.13	0.16	0.17	0.19	0	1.16

* gratings

Table 12 : Changes in colour and transmittance of coconut oil extracted from heated coconut*, during storage

Sample	Colour in Lovibond units (Y+5R) using 1" cell		Transmittance (%) at 400 nm	
	Initial	After storage over one year	Initial	After storage over one year
Control	0.2Y	0.6Y	Taken as reference sample	
105°C	0.5Y+0.5R	0.3Y	96.5	100.0
125°C	0.7Y+0.5R	0.3Y	93.0	100.0
130°C	0.7Y+0.5R	0.3Y	92.5	100.0
135°C	0.8Y+0.5R	0.5Y	89.0	98.5
140°C	0.9Y+0.5R	0.4Y	89.0	100.0
145°C	1.1Y+1.5R	1.6Y	81.0	92.5
150°C	3.2Y+2.5R	2.4Y+1.0R	58.0	80.0
160°C	4.0Y+6.0R	3.0Y+2.0R	21.0	46.5
Fried over flame for 6 min.	1.7Y+1.5R	2.0Y+0.5R	79.0	90.0
Commercial reference sample	1.0Y+1.0R	1.9Y+0.3R	83.5	83.5

* gratings.

(Table 12) and undesirable (see Table 10). The sample heated at 160°C was highly coloured but keeping quality was better (Table 11). In the early stages of storage, the samples heated at 140 to 145°C were preferred by many judges. Therefore, short time heating of copra pieces and gratings at 160°C for 5, 10, 15 etc upto 30 minutes were carried out, to arrive at the optimum time of heating. Sensory evaluation of the samples indicated that heat treatment at 160°C for 15 minutes for gratings and 20 to 25 minutes for copra pieces, provided the required intensity of flavour and adequate colour. These were comparable with samples that were earlier obtained by heating coconut at 135 to 145°C in colour and flavour. The storage study indicated that samples have not deteriorated (Tables 13 and 14). Hence, heating of coconut gratings at 160°C for 15 minutes was chosen as the optimum condition. Gratings simulated domestic use

In the experiments to study the effect of moisture on development of rancidity, different levels of water -0.05, 0.10, 0.50 and 1.0 percent were added to oil samples extracted from control and heated coconut (160°C for 15 minutes). The effect was insignificant and almost same in both the cases.

Table 13 : Free fatty acid contents of coconut oil from copra pieces heated at 160°C for different duration of time

Samples	Initial			After one year		
	Sensory score		FFA%	Sensory score		FFA%
	Colour	Aroma		Colour	Aroma	
Unheated	9.0	5.0	0.087	9.0	3.0	0.467
160°C 5 min.	7.5	6.0	0.082	7.5	5.0	0.231
10 min.	7.0	7.0	0.080	7.0	6.0	0.229
15 min.	6.8	7.5	0.078	6.5	7.0	0.219
20 min.	6.5	8.0	0.077	6.5	7.8	0.216
25 min.	6.0	8.5	0.077	6.5	8.0	0.216
30 min.	5.5	7.8	0.076	6.0	6.5	0.217

Table 14 : Free fatty acid (FFA) content of coconut oil from coconut gratings heated at 160°C for different duration of time

Sample	Initial			After one year		
	Sensory score (Max. 10)		FFA%	Sensory score (Max. 10)		FFA%
	Colour	Aroma		Colour	Aroma	
Control	9.0	5.0	0.087	9.0	3.0	0.467
160°C 5 min	7.5	7.0	0.082	7.5	5.0	0.227
10 min	6.5	7.5	0.080	7.0	7.0	0.229
15 min	6.0	8.5	0.081	6.5	8.0	0.214
20 min	5.0	8.0	0.078	5.5	7.5	0.212

3.1.2 Discussion

On the whole, the heat treatment studies indicated that browning and flavour development began only above 100°C. However, precursors of browning reaction would have already formed during initial stages of heating itself. Visual browning is accelerated only above 100°C. The storage study showed that free fatty acid contents of oil samples of heated coconut were less than the control during storage. In general, it is known that heating results in cleavage of glycerides, to a small extent, producing minute amounts of free fatty acids[225], but these will be below measurable quantities in ordinary estimations.Coconut oil from heated samples showed small but measurable peroxide values. Kashani and Valadan[155] had also observed higher peroxide values for oils from heated samples in the case of roasted pistachios. Probably, heating results in production of chemically reducing species like reductones. This is indirectly supported by results of sensory evaluation also, since coconut oil with no measurable peroxide value is adjudged highly rancid while the heated samples with peroxide values were rated better. The development of rancidity in heated samples is found to be much slower in heated samples organoleptically also (Table 10). Since coconut

oil is of a saturated nature, development of rancidity in coconut oil has been mainly attributed to β-oxidation of lower fatty acids by lipase which are released by moulds in the presence of moisture[167]. The increased shelf stability of coconut oil from heated samples can be explained by the general observation that some of the products of Maillard reaction exert antioxidant properties[226] and the antioxidant activity has been reported to be comparable with butylated hydroxy anisole (BHT)[227].

3.2 FLAVOUR ISOLATION AND IDENTIFICATION

From the roasting experiments, it could be inferred that browning and associated changes were initiated at 125 to 130°C and were more advanced at 140 to 145°C. There was further intensification of reaction at 160°C. There were significant differences in colour and flavour at each interval. Hence the three temperatures namely 130, 145 and 160°C, with a gap of 15 degrees, were selected to study the changes in flavour profile as well as other compositional changes. Duration of heating was fixed as 15 minutes, since the optimum heating time was 15 minutes at 160°C. Hence for comparative study, the same duration of heating was followed in other two cases also. Dry coconut gratings (moisture 3.0 percent) without any heat treatment were taken as control sample.

The proximate composition of the control and the maximum heated sample viz. 160°C for 15 minutes were compared. The results are given in Table 15. It can be seen that the overall composition did not change significantly. Detailed mineral analysis of the above two samples of coconut was also done and showed that heating did not affect mineral composition (Table 16).

3.2.1 Steam distillation, extraction and preliminary flavour analyses of coconut oil obtained from control and heated samples of coconut

Isolation of flavour compounds by steam distillation of coconut oil from unheated coconut gratings (control) and heated samples (160°C, 15 min) and subsequent extraction with solvent ether gave a total flavour extract, amounting to approximately 300 ppm. Thin layer chromatographic (TLC) analysis of these extracts were carried out with the solvent system petroleum ether-ethyl acetate (90:10). After development, the plates were exposed to iodine vapours and UV light in a UV chamber (Chromocab). 4 spots could be detected on exposure to iodine (Fig.13). Of these, two spots showed fluorescence under UV light. Two spots gave bright orange red coloration with dinitrophenyl hydrazine reagent (DNPH). On subsequent spraying with alkaline potassium ferricyanide, these spots turned greenish yellow in colour indicative of aldehydes. Results

Table 15 : Proximate composition of control and heated samples of coconut gratings (g/100g on dry weight basis) *

	Unheated (control)	Heated (160°C/15 min)
Moisture	3.00	2.40
Fat	70.50	71.70
Protein	8.30	8.03
Carbohydrates (by difference)	16.93	16.57
Crude fibre	4.65	5.05
Ash (total)	1.27	1.30
Acid insoluble ash	0.04	0.04

* Mean of three determinations

Table 16 : Mineral composition of coconut gratings (mg/100g on dry weight basis)

	Control	Heated (160°C/15 min)
Potassium	560.00	548.00
Sodium	50.00	51.00
Calcium	22.10	21.98
Magnesium	67.20	69.03
Iron	4.00	4.05
Copper	1.50	1.37
Phosphorus	121.40	122.00
Sulphur	43.10	43.70
Chlorine	320.00	317.40

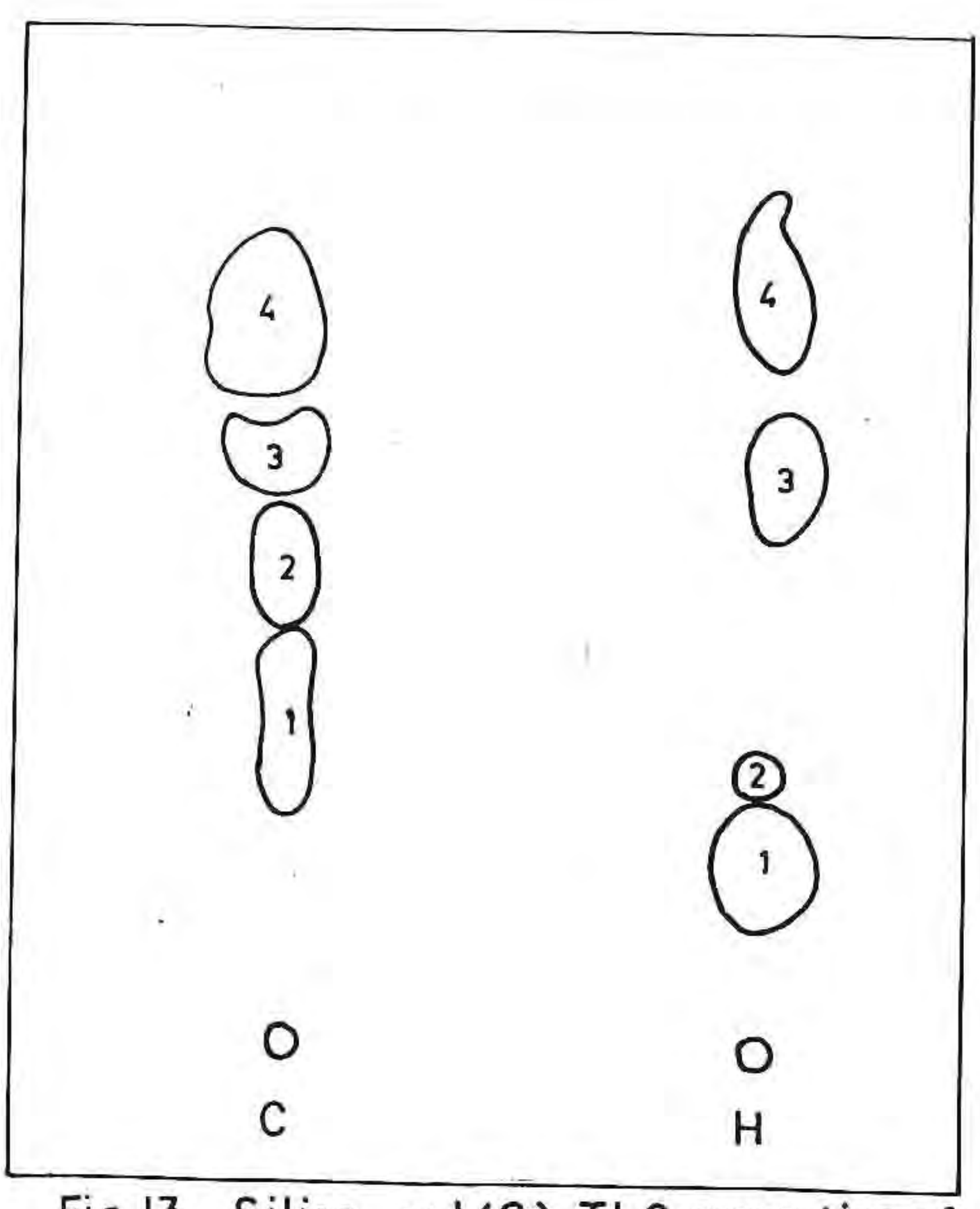


Fig.13. Silica gel(G) TLC separation of total flavour extracts isolated from coconut oil. Control sample-C; Heated (160°C/15 min) sample-H. Solvent system: Petroleum ether-ethyl acetate (90:10)

of TLC analysis are included in Table 17. In the case of oil with roasted flavour, spraying with Dragendorff reagent, after TLC separation with the solvents petroleum ether-ethyl acetate (90:10), one of the spots with R_f value 0.17 developed pinkish red colour. The colour development is characteristic of nitrogenous bases. In general, TLC analysis indicated that aldehydes, carbonyls, lactones and some nitrogenous compounds were present.

GC analysis of the flavour extract

GC analysis in packed OV-17 column of the total flavour extract from coconut oil (control) registered 15 peaks and that of roasted coconut 23 peaks. For heated sample, the retention values of GC peaks agreed well with the compounds methyl pyrazine, ethyl methyl pyrazines, dimethyl pyrazines, 2-undecanone, δ-octa lactone, δ-deca lactone and δ-dodeca lactone. For the control sample of coconut oil, only 15 peaks were separated under the same conditions, of which octanol, 2-undecanone, δ-octa lactone, δ-deca lactone, δ-dodeca lactone, ethyl octanoate, ethyl decanoate etc were positively present. The major difficulty in identification of experimental peaks was the overlapping of retention time (RT) values of different authentic

Table 17 : TLC analysis of total flavour extracts isolated by steam distillation of coconut oil from control and heated (160°C/15 min) samples of coconut
Solvent system: Petroleum ether:ethyl acetate (90:10)

Characteristics observed	Coconut oil (control)				Coconut oil from heated coconut (160°C/15 min)			
	1	2	3	4	1	2	3	4
Rf value	0.22	0.43	0.54	0.65	0.17	0.25	0.53	0.69
Exposure to iodine vapours	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Exposure to UV light	slight fluo-rescence	slight fluo-rescence	no fluo-rescence	slight fluo-rescence	slight fluo-rescence	no fluo-rescence	no fluo-rescence	slight fluo-rescence
Spraying with DNPH reagent	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve
Spraying with Dragendorff's reagent	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Probable identity			carbonyls (aldehydes)	carbonyls (aldehydes)	carbonyls, nitro-genous compounds		carbonyls (aldehydes)	

compounds at a particular RT value. On the whole, the GC profile was complicated due to this overlapping and hence confirmative analysis was difficult.

To overcome this difficulty, flavour extraction was carried out to separate flavour compounds selectively into different chemical categories. Gratings were hydrodistilled to isolate a more 'complete flavour' representative of coconut.

3.3 HYDRODISTILLATION, SELECTIVE EXTRACTION AND TLC ANALYSES OF FLAVOUR FRACTIONS

Hydrodistillation, followed by selective extraction after pH adjustment was the second method adopted for flavour isolation. The basic, neutral and acid flavour fractions of control sample of coconut and samples heated at 130, 145 and 160°C for 15 minutes were separated and estimated quantitatively. Table 18 represents quantitative distribution of the flavour fractions of coconut. From the table, it can be seen that hydrodistillation followed by selective extraction of flavour compounds was more effective compared to steam distillation of oil and subsequent solvent extraction.

3.3.1 TLC analyses

TLC analyses were carried out with basic and neutral fractions of control and heated samples of coconut (160°C/15 min) using the solvent system, Petroleum ether (60-80°)-ethyl acetate (90:10). The plates were later

Table 18 : Quantitative distribution of the flavour fractions of control and heated coconut isolated by hydrodistillation and selective extraction *

	Control	130°C	145°C	160°C
Basic	5	75	125	150
Neutral	300	280	300	310
Acid	25	40	60	80
Total	330	395	485	540

* Mean of three determinations

Calculated as follows:

$$\text{Quantity (mg/kg)} = \frac{\text{Weight of flavour extract (mg)} \times (100 - \text{solvent peak area \% in GC})}{\text{Wt. of dry sample (kg)}}$$

exposed to iodine vapours. The basic fraction of flavour of the control sample did not show any distinct spot whereas the neutral flavour fraction of control and heated samples were very similar. Exposure to iodine vapours revealed 5 spots for basic fraction of heated sample and 4 spots for neutral fraction in control and heated samples. Under UV light, 3 spots showed fluorescence in basic fraction and one spot in the neutral fraction (Fig.14). The basic fraction of heated sample was sprayed with Dragendorff's reagent and all the spots developed pinkish orange colour, which is characteristic of nitrogenous bases. In the case of neutral fraction, only one spot showed fluorescence under UV and 3 spots developed colour with DNPH reagent showing presence of carbonyl compounds. These results are given in Tables 19 and 20. Preparative TLC was also performed with the basic fraction and the spots eluted with dichloro methane solvent. UV spectra of the various spots were taken and compared with spectral data of a few pyrazines. These results are included in Table 19.

TLC analysis of the acid fractions using ethyl acetate and ammonia (2.5 percent) in the ratio 95:5, gave 3 spots (Fig.15). The fastest moving spot (Rf 0.687) had comparable Rf value with octanoic acid. Further

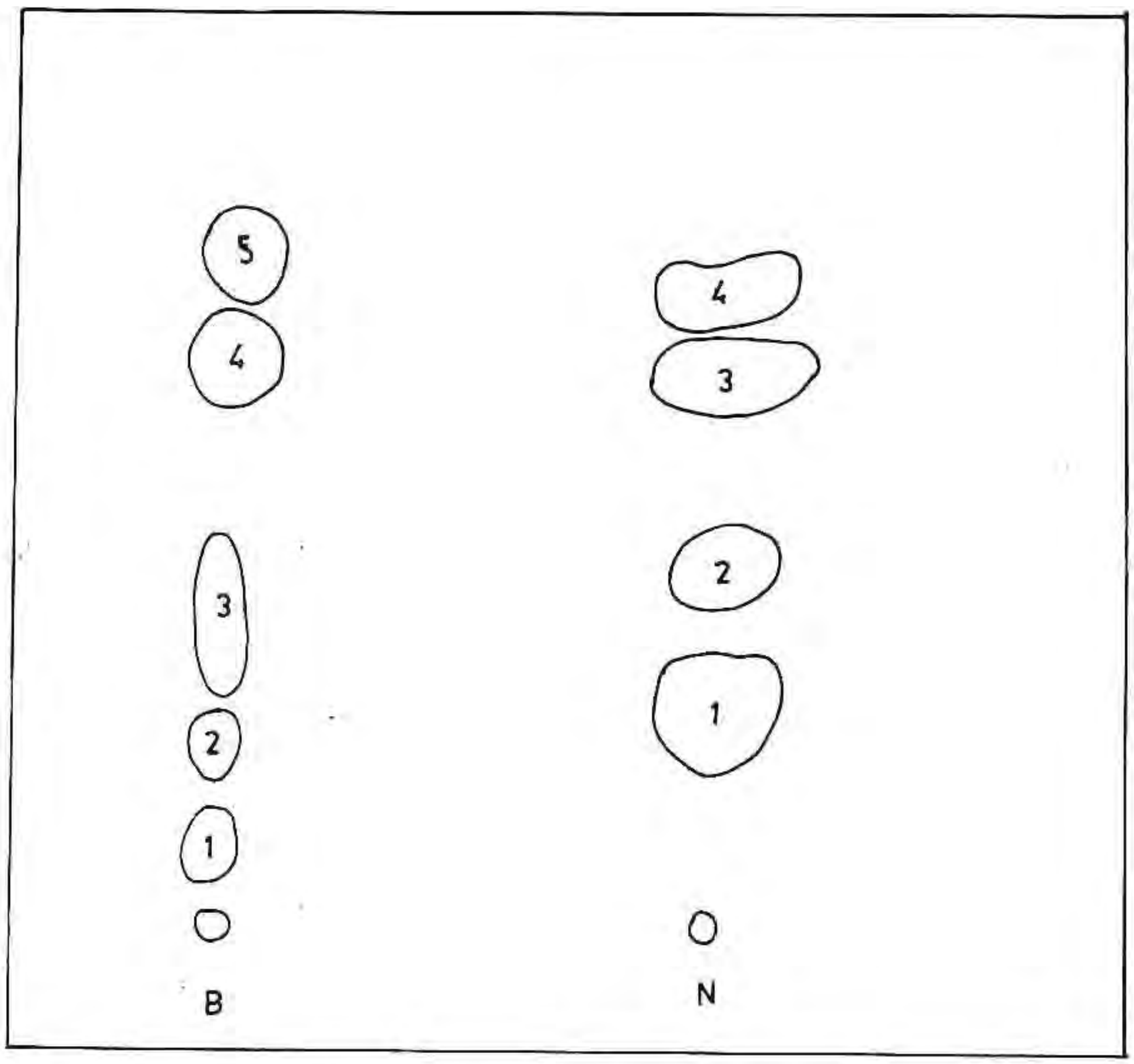


Fig.14. Silica gel (G) TLC separation of basic (B) and neutral (N) fractions of flavour isolated from coconut heated at 160°C for 15 minutes.

Solvent system: Petroleum ether–ethyl acetate. (90:10)

Table 19 : TLC analysis of the basic fraction of flavour, isolated from control and heated samples of coconut by hydrodistillation and selective extraction. Solvent system : Petroleum ether-ethyl acetate (90:10)

Characteristics studied	Spots				
	1	2	3	4	5
R_f value	0.07	0.11	0.16	0.55	0.67
Exposure to UV light	Blue fluoro-scence	Blue fluoro-scence	Blue fluoro-scence	No fluoro-scence	No fluoro-scence
Exposure to iodine vapours	+ve	+ve	+ve	+ve	+ve
Colour reaction with Dragendorff's reagent	+ve	+ve	+ve	+ve	+ve
UV absorption (nm)	300,271	300,269, 227	300,274, 227	282,274, 228	272,266, 264,228
Aroma characteristics	Nutty with hydro-carbon-like smell	Nutty	Roasted, nutty	Earthy, raw	Earthy, nutty
Probable identity	Trisub-stituted pyrazines	Ethyl pyra-zines, ethyl, methyl pyra-zines	Pro-penyl pyra-zine	Vinyl pyra-zine, dimethyl pyra-zine	Pyra-zine, methyl pyra-zine

Table 20 : TLC analysis of neutral fraction of flavour isolated from coconut heated at 160°C for 15 minutes. Solvent system: Petroleum ether-ethyl acetate (90:10)

Characteristics studied	Spots			
	1	2	3	4
R_f value	0.25	0.42	0.63	0.70
Exposure to iodine vapours	+ve	+ve	+ve	+ve
Exposure to UV radiations	light blue floure-scence	nil	nil	nil
Spraying with DNPH	No colour	+ve	+ve	+ve (intense)
DNPH + alkaline ferri-cyanide			bluish tinge	bluish tinge
Aroma characteri-stics	pleasant, nut-like	pleasant, fruity	nut-like	coconut oil-like
Probable identity	lactones	carbonyls	carbonyls, lactones	carbonyls lactones

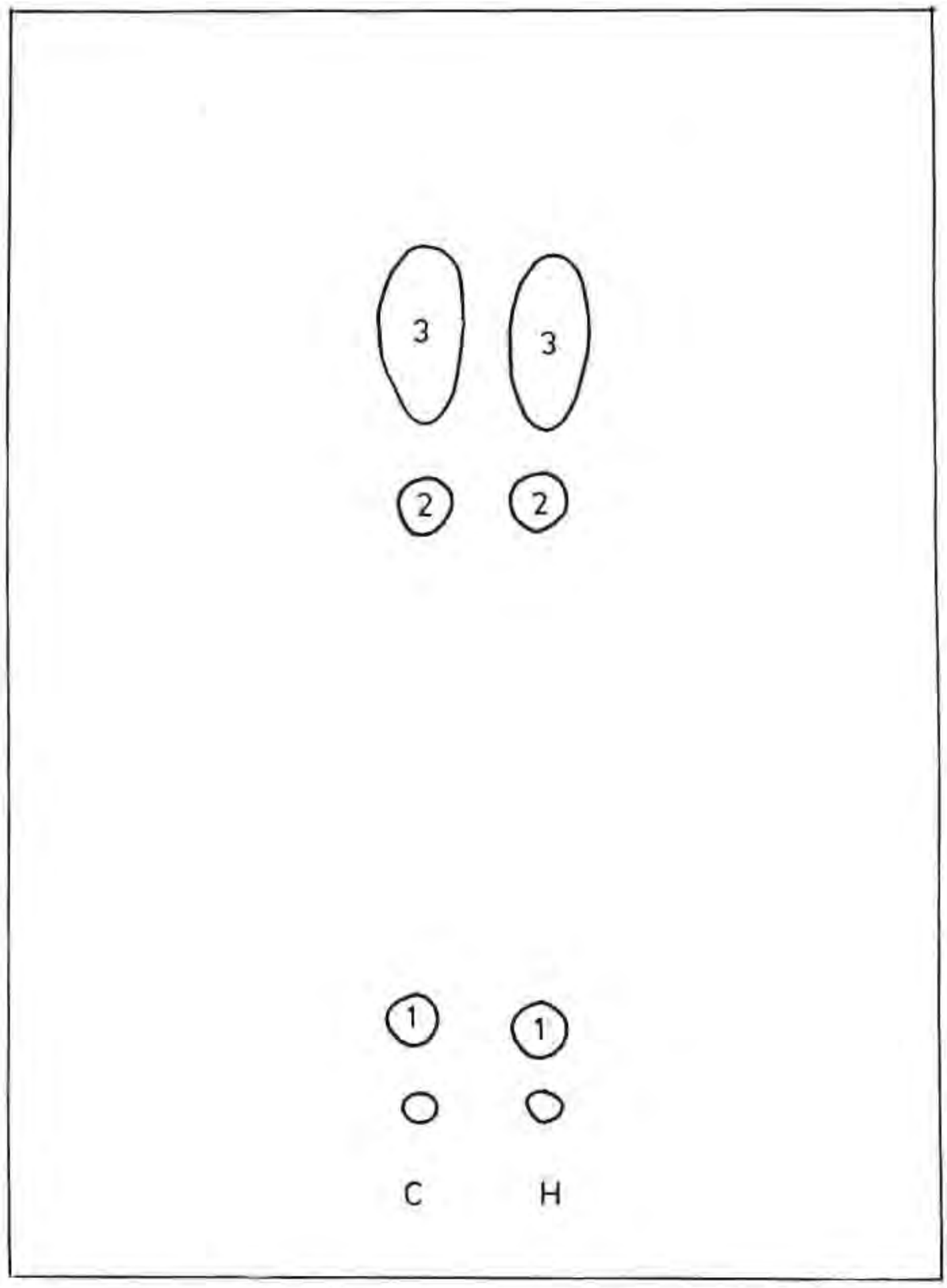


Fig.15. Silica gel (G) TLC separation of acid fraction of flavour isolated from control(C)and heated (H, 160°C/15 min) samples of coconut. Solvent system: Petroleum ether- ethyl acetate (90:10)

trials and even two dimensional chromatography of individual spots did not give complete separation. Subsequent analyses were carried out in a gas chromatograph using packed and capillary columns.

3.3.2 Discussion

From these analyses it can be inferred that the basic fraction of flavour of roasted coconut consists of nitrogen containing compounds, whereas similar fraction from dry coconut do not show these spots. UV spectra of the compounds separated as spots agree well with spectra of some of the pyrazine derivatives. The aroma characteristics of these separated spots are also noteworthy, since most of them possess nutty, roasted, earthy odour characteristics. Thin layer chromatographic analyses, along with the spectral data, could give only limited information about the nature of compounds present.

3.4 GC AND GC-MS ANALYSES OF THE FLAVOUR FRACTIONS ISOLATED FROM CONTROL AND HEATED SAMPLES OF COCONUT

The GC analyses of the different flavour fractions of control and heated samples of coconut were carried out first in packed columns of OV-17 (basic and neutral fractions) and DEGS (methylated acids) and peaks compared with authentic compounds. The GC picture of the flavour fractions obtained by selective extraction of the aqueous

distillate was very much simplified compared to total flavour extract isolated by steam distillation of oil. For basic fraction analysis, authentic compounds like pyrazines, pyridines, thiazoles, oxazoles etc were injected to compare the RT values. These compounds were primarily considered, based on information from literature, on similar roasted foods. For the analysis of neutral fraction, a series of standard γ- and δ-lactones, aliphatic and aromatic alcohols, aldehydes, ketones, esters, hydrocarbons etc were injected under same GC conditions, to identify the separated peaks. The acid fraction was methylated before GC analysis which was carried out in DEGS column. The peaks were compared with a series of methyl esters of odd and even numbered fatty acids. Methylated butter fat was also used to compare position of GC peaks of lower fatty acids, hydroxy fatty acids and some of the unsaturated fatty acids. In these experiments, the retention index (I_E) was calculated for all the peaks, registered in each of the experimental samples and also for the peaks corresponding to authentics. In general, the gas chromatographic analyses gave definite ideas about most of the separated peaks. Preliminary GC-MS analysis of basic fraction (160°C/15 min) was carried out in a Jeol D-300 coupled instrument using OV-1(3 percent) column (1.83 m x 3 mm i.d.), before capillary GC-MS analysis.

The flavour analyses were also carried out in a coupled GC-MS instrument using capillary columns. Capillary Carbowax 20M column was used for analysis of basic fraction. For neutral and acid fraction (after methylation) capillary columns of cross-linked Methyl Silicone were used. The capillary GC analysis gave very good separation of compounds. A few peaks which had not resolved in packed columns were separated in capillary GC analysis. Identification was done by comparison of RT values with those of standards and by mass spectral matching.

3.4.1 Identification of flavour compounds in the basic fraction

The basic fraction concentrate of control sample of coconut did not have any characteristic roasted smell but only an indistinguishable heavy odour. On the other hand, the basic fraction from heated coconut samples possessed an earthy, raw, mildy roasted aroma, characteristic of pyrazine mixtures (by comparison with authentic compounds).

GC analysis of basic fraction of flavour concentrates of control and heated samples of coconut were first carried out in OV-17(1.83m x 3mm i.d) column. In the heated samples, 25 to 30 peaks were separated. The analysis could give valuable information about the presence of pyrazine, methyl pyrazine, vinyl pyrazine,

dimethyl pyrazines, methyl ethyl pyrazines, methyl propyl pyrazines etc. In contrast, the basic, flavour fraction from dry coconut (control) showed only 3 peaks including solvent peak and no pyrazines could be detected in the sample. For further confirmation of the identified compounds, capillary GC-MS analysis was carried out using Carbowax 20 M column. Figure 16 represents the capillary GC separation of basic fraction compounds of roasted coconut (160°C/15 minutes). Since capillary analysis gave better separation of compounds, identification and quantitation were invariably based on these analyses too. The results of GC and GC-MS analyses are represented in Table 21. It could be seen that no pyrazines were detected in control sample. 2-Butyl benzothiazole was the most predominent compound. Benzothiazole has been reported to be present in fresh coconut meat by Lin and Wilkens[184]. In the present study pyrazines were found to be present in all heated samples. Thus, 20 pyrazines were identified in roasted coconut. 20 peaks were separated in the sample heated at 130°C, out of which 19 could be identified positively. At 145°C, 30 peaks were recorded of which 22 could be identified. At 160°C, 35 peaks were separated by capillary analysis and 26 compounds could be identified

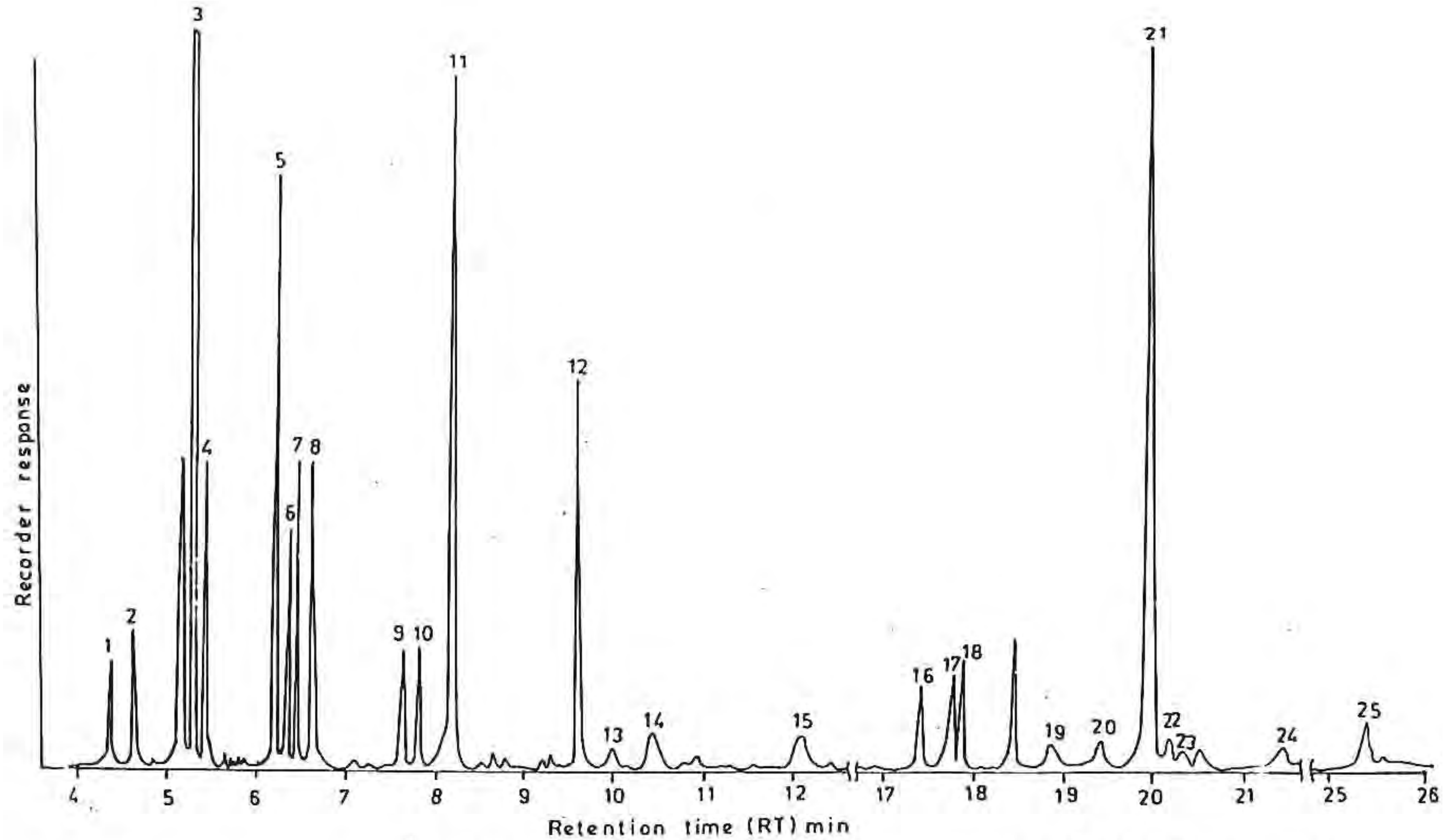


Fig.16. Gas chromatogram of the basic fraction of heated coconut (160°C/15min) Column: capillary Carbowax 20 M (50 m x 0.2 mm)

Table 21 : Compounds identified by GC and GC-MS analyses of basic fraction of flavour, isolated from control and heated samples of coconut

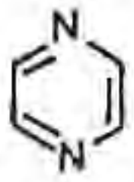
Peak No.	RT (min) in capillary Carbowax column	I_E value in OV-17 column	Compounds	MS fragment ions (in order of abundance)	Control		130°C		145°C		160°C	
					Rel. conc. %	Actual conc. in ppm	Rel. conc. %	Actual conc. in ppm	Rel. conc. %	Actual conc. in ppm	Rel. conc. %	Actual conc. in ppm
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	4.26	4.70	Pyridine	79,52,51,50,39	-	-	-	-	-	-	1.40	2.10
2	4.34	4.70	Pyrazine	80,53,81,52	-	-	-	-	1.60	2.00	2.00	3.00
3	5.18	5.40	Methyl pyrazine	94,67,54,43,53	-	trace	6.40	4.81	7.30	9.10	18.80	28.20
4	5.42	5.86	Vinyl pyrazine	106,52,53,79,80,105	-	-	0.40	0.30	3.20	4.00	4.60	6.20
5	6.14	5.90	2,5-Dimethyl pyrazine	42,108,82,45,79,80	-	-	7.81	5.80	6.60	8.30	8.90	13.40
6	6.26	5.94	2,6-Dimethyl pyrazine	108,42,47,41,109,81	-	-	2.60	2.00	1	1.90	2.90	4.40
7	6.40	6.20	Ethyl pyrazine	107,108,80,53,52	-	-	3.10	2.30	3	2.40	3.80	5.70
8	6.62	5.60	2,3-Dimethyl pyrazine	108,67,109,43,42	-	-	5.20	4.01	4.60	5.80	4.10	6.10
9	7.68	7.60	2-Ethyl-6-methyl pyrazine	121,122,44,94,56	-	-	3.40	2.62	3.00	3.80	2.00	3.00
10	7.82	6.40	2-Ethyl-5-methyl pyrazine	121,122,39,56,94	-	-	3.01	2.30	2.80	3.50	2.20	3.30
11	8.24	6.40	2-Ethyl-3-methyl pyrazine	121,122,67,94,81	-	-	14.60	11.04	15.70	19.60	12.70	19.10
12	9.76	8.30	2,6-Diethyl pyrazine	135,136,108,53,56,39	-	-	11.90	9.00	9.30	11.60	6.40	9.60
13	10.00	7.80	5-Methyl-4-butenyl pyrrole	81,80,121,79,41,53, 136	-	-	-	-	0.60	0.80	0.80	1.20
14	10.32	8.10	2,3,-Dimethyl 5-ethyl pyrazine	135,136,54,42,53, 39,108	-	-	2.80	2.10	2.30	2.90	2.40	3.60
15	12.02	8.70	3,5,-Dimethyl-2n. propyl pyrazine	149,122,150,135,43, 42,122	-	-	3.40	2.60	3.00	3.80	1.50	2.30
16	17.42	9.08	2-(trans-1-propenyl)-pyrazine	119,120,39,67,41, 51	-	-	1.40	1.10	0.71	0.90	1.00	1.50
17	17.66	6.15	5-Methyl furfural	110,109,53,39,81	-	-	1.00	0.70	2.00	2.50	1.00	1.40
18	17.92	9.40	6,7-Dihydro-5H-cyclopenta pyrazine	119,120,39,41,66,65	-	-	-	-	-	-	1.40	2.10
19	18.80	9.50	2,5-Dimethyl 3-vinyl pyrazine	133,134,42,54,91,66, 108	-	-	-	-	0.80	1.00	1.00	1.50

Table 21 contd.....

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
20	19.46	Not identified	2-Methyl-6,7-dihydro-5H-cyclopenta pyrazine	134,133,66,39,40,65	-	-	-	-	-	-	1.30	1.90
21	19.88	7.20	2-Ethyl-3-isopropenyl pyrazine	147,148,133,122,94,67	-	-	7.80	5.80	12.30	15.40	18.30	27.00
22	20.38	11.30	β-Terpeneol acetate	68,93,43	-	-	1.10	0.80	0.50	0.63	0.30	0.50
23	20.40	5.00	Tetradecane	43,57,71,85,41	-	-	0.30	0.20	0.40	0.50	0.50	0.70
24	21.54	24.80	Tetradecanoic acid	57,85,43,41,55,71,83,105	-	-	-	-	-	-	0.50	0.80
25	25.42	23.68	2-Butyl benzothiazole	149,41,56,57,150,223	93.00	4.50	3.00	5.00	3.10	3.80	3.00	4.50

and confirmed as present. Structures of a few flavour compounds identified in the basic fraction are given in Figure 17.

Pyrazine , methyl pyrazine, isomers of dimethyl pyrazine and ethyl methyl pyrazine, and cyclopenta pyrazine were found to be present. The relative concentration and individual concentration of each compound was calculated from area percentages of peaks in GC-MS studies. From the results (Table 21) it could be seen that methyl pyrazine **[41]**, 2,5-dimethyl pyrazine **[43]**, 2-ethyl-3-methyl pyrazine **[46]** and 2-ethyl-3-isopropenyl pyrazine **[50]** were present in higher amounts in the roasted samples. At 160°C, methyl pyrazine and 2-ethyl-3-isopropenyl pyrazine were formed in large quantities (29 and 27 ppm respectively). Most of the pyrazines increased in concentration during heating which indicated increased formation of pyrazines with temperature. This was reflected in the quantitative distribution of flavour fractions of control and heated samples also, wherein basic fraction steadily increased with temperature (Table 18) in the latter. The 2,3- and 2,6-isomers of dimethyl pyrazine **[42]**, **[44]** and ethyl pyrazine **[45]** were present to the extent of 4 to 5 ppm level, whereas 2,5-dimethyl pyrazine was present abundantly. 2-ethyl-3-methyl pyrazine**[46]**



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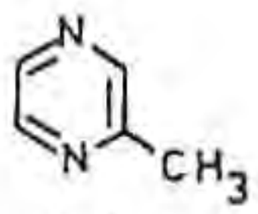

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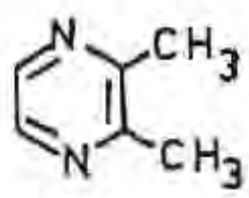

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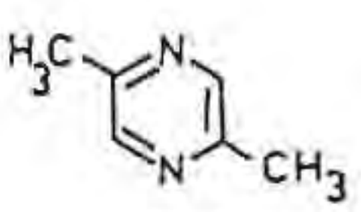

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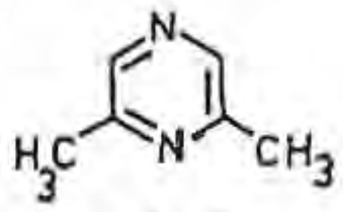

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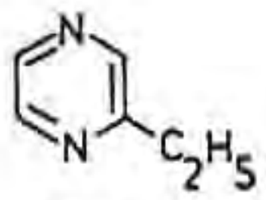

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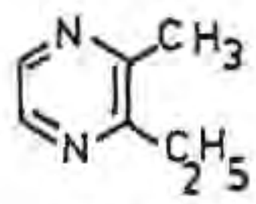

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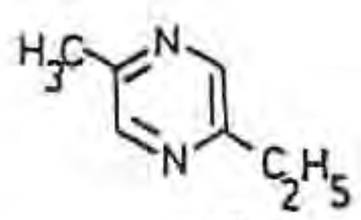

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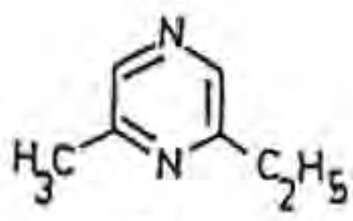

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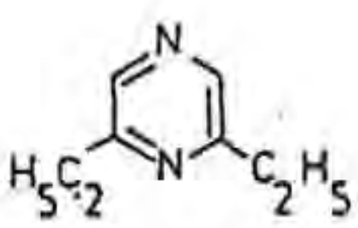

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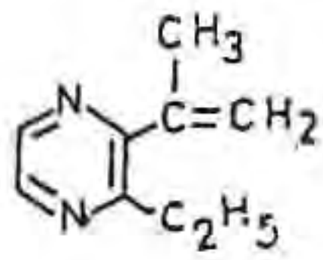

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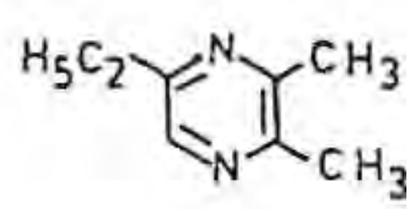

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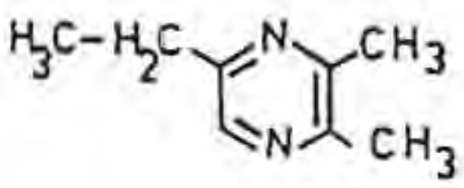

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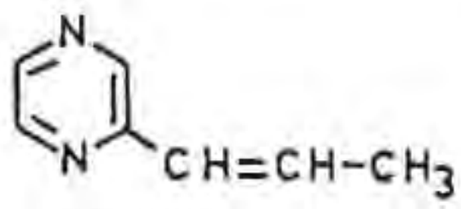

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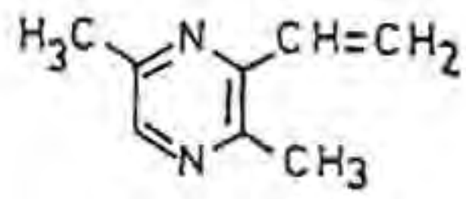

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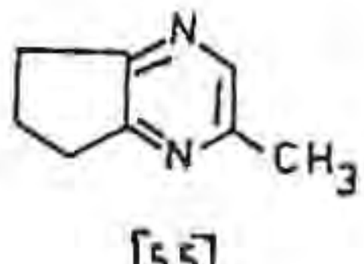

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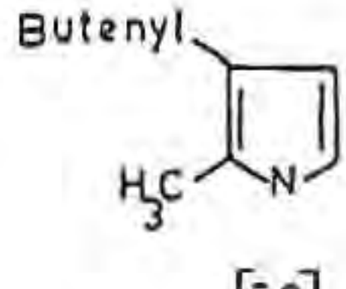


[56]

Fig 17. Structure of a few flavour compounds – pyrazines – identified in the basic flavour fraction of heated coconut.

was formed in higher amounts (19 ppm). 2-Ethyl-5-methyl [47] and 2-ethyl-6-methyl [48] pyrazines were present in lesser amounts. 2,6-Diethyl pyrazine [49] was also present to the extent of 10 to 13 ppm. 2,3-Dimethyl-5-ethyl pyrazine [51]. 3,5-dimethyl-2n-propyl pyrazine [52] and 2(trans-1-propenyl) pyrazine [53] were found to occur in small amounts (2 to 3 ppm). Vinyl pyrazine and its dimethyl derivative [54] were found to be formed in larger proportions at higher temperatures. Interestingly two bicyclic pyrazines were also identified in coconut heated at 160°C. The 6,7-dihydro 5H-cyclopenta pyrazine and its 2-methyl derivative [55] are reported in roasted almonds[47], coffee[224], cocoa butter[228] etc. The only pyrrole derivative identified in this study was 5-methyl-4-butenyl pyrrole [56] which was present in small quantities (1 ppm). β-Terpenyl acetate, reported for the first time was found in very small amounts only (0.5 ppm).

In addition to these compounds, other compounds like 5-methyl furfural, tetradecane, pentadecane, tetradecanoic acid and 2-butyl benzothiazole were also found in roasted samples. The last one, namely 2-butyl benzothiqzole was found to occur in unroasted sample too.

3.4.2 Aromagram of basic fraction

The odour evaluation of the eluting GC peaks separated in the packed OV-17 column gave interesting results. Since 160°C was chosen as the optimum temperature, odour description of this sample alone was attempted. Moreover, most of the peaks observed in this sample were present in the corresponding fractions of other two temperatures also. Figure 18 gives the gas chromatogram of the basic fraction for which odour descriptions were made. Aroma properties of the numbered peaks and their identity based on GC analysis are included in Table 22.

3.4.3 Discussion of the results of basic fraction analysis

From Table 18, it can be understood that during heating, basic compounds are formed more and more with temperature, upto 160°C. It has been reported that pyrazine concentration increased with temperature upto 150 to 160°C in the case of potato chips and diminished thereafter due to volatile loss or by degradation[50]. Development of roasted flavour in coconut and oil was first reported by Jayalekshmy et al[229] in 1980. The identification of flavour compounds of basic fraction in coconut, heated at 160°C was presented at a seminar in 1984 by the author and her colleagues[230] and was communicated to

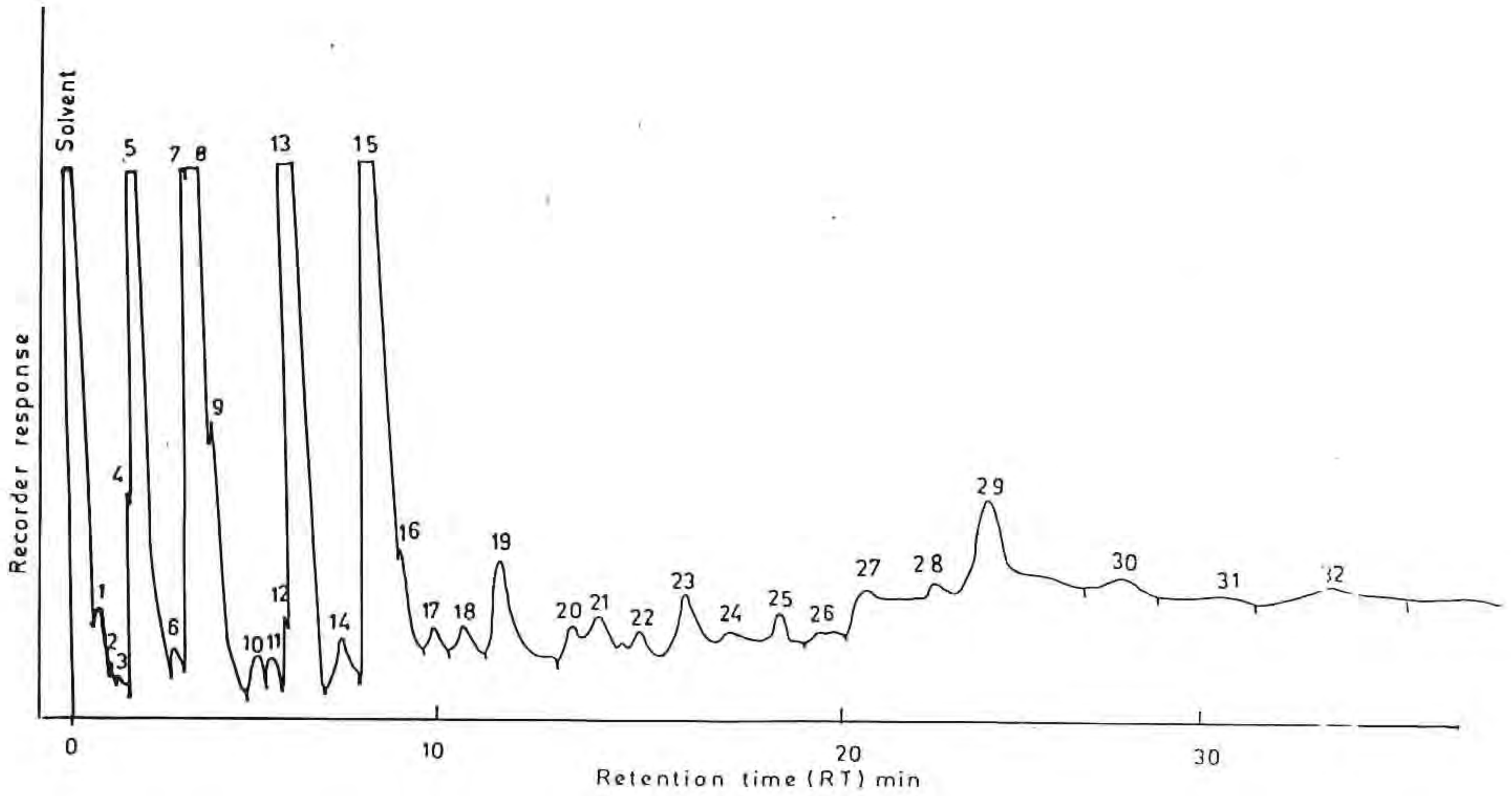


Fig.18. Gas chromatogram of basic fraction of flavour isolated from heated coconut (160°C/15 min). Column: OV-17 (1.83 m x 3 mm id). See Table 22 also.

Table 22 : Odour descriptions of the separated GC peaks (aromagram) of basic fraction of coconut

Peak No..	Odour description	Compound
1	pungent, greeny, roasted	pyrazine
2		unknown
3	hydrocarbon-like	tetradecane
4	hydrocarbon-like	pentadecane
5	raw, roasted	methyl pyrazine
6	raw, green	unknown
7	good roasted	vinyl pyrazine
8	fine, roasted	2,5-dimethyl pyrazine
9	roasted, nutty	2,6-dimethyl pyrazine
10	pleasant, roasted	2,3-dimethyl pyrazine
11	green roasted	2-ethyl pyrazine
12	cooked aroma	5-methyl furfural
13	green, musty, roasted	2-methyl-3-ethyl and 2-methyl-5-ethyl pyrazine
14	green, nutty, roasted	2-methyl-6-ethyl pyrazine
15	musty, roasted	2-ethyl-3-isopropenylpyrazine
16	fragrant but slightly musty	5-methyl-4-butenyl pyrrole
17	green beany	2,3-dimethyl-5-ethyl pyrazine

Peak No.	Odour description	Compound
18	mildy roasted	3,5-dimethyl-2-propenyl pyrazine
19	green, bell pepper-like	2,6-diethyl pyrazine
20	pungent, penetrating, mildly roasted	2(trans)-1-propenyl pyrazine
21	penetrating, musty	unknown
22	slightly fermented	2,5-dimethyl-3-vinyl pyrazine
23	mildly roasted	6,7-dihydro-5H-cyclopenta pyrazine
24	Nothing characteristic	unknown
25	Nothing characteristic	unknown
26	pleasant aromatic, mango-like	β-terpeneol acetate
27	harsh, oily, burnt smell	tetradecanoic acid
28	unpleasant	unknown
29	harsh, medicinal	2-butyl benzothiazole
30	pungent, irritating (stored oil-like)	unknown
31	harsh and pungent	unknown
32	harsh pungent smell continues	unknown

the journal, Lebensmittel & Wissenchauft (Food Science + Technology) in 1984 itself. The paper was published in 1985 December issue[231]. However, it appears that simultaneous work on identification of flavour compounds was being carried out by a few Japanese researchers independently at our reported optimum temperature of 160°C and the results were published in Agricultural and Biological Chemistry in September 1984.[232] They had reported only 6 pyrazines in roasted coconut meat and more pyrazines in defatted, roasted coconut meal. The research paper of the author was in press during this period and this is proof enough to conclude that the work was carried out independently.

In the case of roasted coconut (temperature 160°C) basic fraction contributes more than 30 percent of the total flavour extract. Identification of pyrazines, furans,pyrrole etc is reported first time in coconut.The major pyrazines, namely, methyl pyrazine, 2-ethyl-3-isopropenyl pyrazine, 2-ethyl-3-methyl pyrazine, 2,5-dimethyl pyrazine and 2,6-diethyl pyrazine occur above 10 ppm concentration. However, this does not mean that other compounds are not important in the total flavour of roasted coconut. Quite often, synergistic effects are reported to exist between compounds that occur even at sub-threshold levels and as a result they impart

noticeable olfactory impact[6]. From the aromagram, it could be seen that typical roasted aroma was elicited, mostly, by methyl pyrazine, vinyl pyrazine, 2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine and 2,6-dimethyl pyrazine. Eventhough, it is difficult to pinpoint or identify a particular compound to be associated with the roasted flavour of coconut, it is confirmatory that roasted flavour is largely contributed by pyrazines.

Temperature of heating is very important as far as pyrazine formation is concerned. In the case of potato chips, the formation of pyrazines was estimated at different temperatures from 120 to 180°C for 5 minutes and also at different time intervals, at 150°C. The pyrazine concentration was found to increase with temperature[50]. A similar study was carried out in the case of roasted cocoa beans also[34]. In the present study too, the author could find a gradual increase in pyrazine concentration in coconut, from 130 to 160°C.

The single, alkyl pyrazines identified in coconut are present in roasted coffee[17,21,233], cocoa[36] almonds[46], beef products[60,234], filbert[44] etc. Roasted coconut is also found to contain two cyclopentyl derivatives of pyrazine, namely, 6,7-dihydro 5H-cyclopenta pyrazine

and its methyl derivative. Such bicyclic pyrazines are reported in roasted filberts[44] almonds[46] etc also.

Possible formation pathways of various pyrazine derivatives

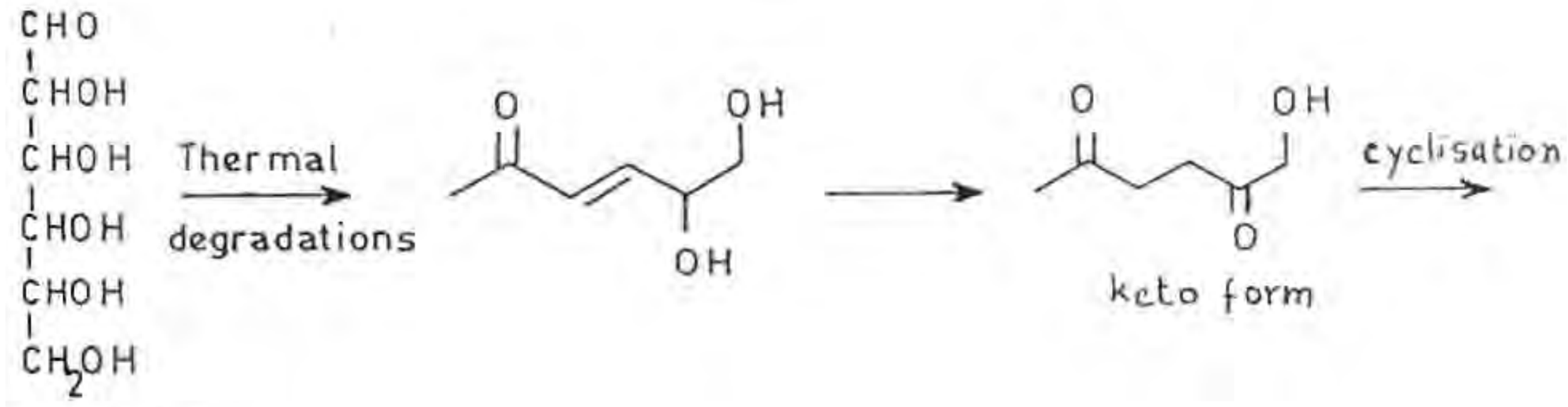
The proposed mechanism of formation of single alkyl pyrazines is discussed in Chapter 1. Aldehydes like pyruvaldehyde can condense with amino acids and the resulting product can undergo Strecker degradation (Fig. 7 in Chapter 1) producing amino reductones which, through other steps like self condensation and oxidation, finally result in compounds like dimethyl pyrazines.

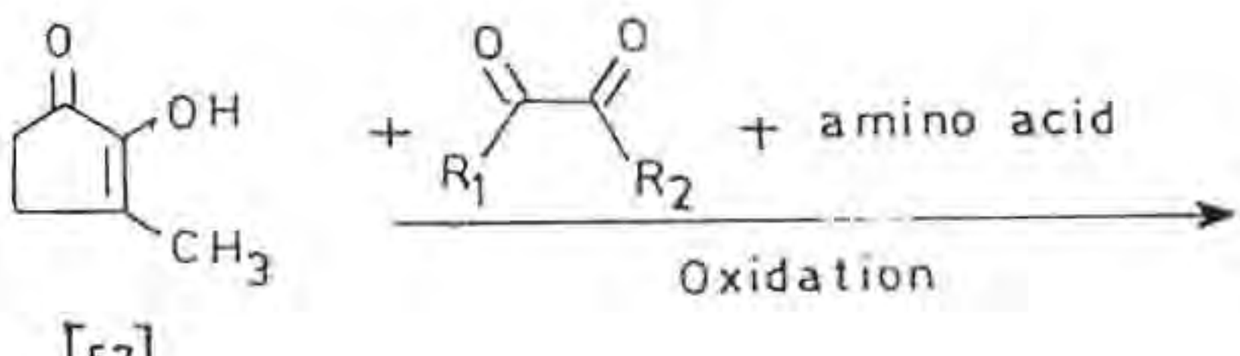
Manley et al[133] proposed the mechanism of formation of cyclopenta pyrazines from sugar dehydration products like cyclopentanones[57],diketones, furyl substituted diketones etc. Condensation of these compounds with amino acids can finally lead to the formation of cyclopenta pyrazine **[58]** as can be seen from Figure 19.

The formation of pyrazines as related to the changes in amino acids and sugars of coconut will be discussed along with model system studies in Chapter 5.

3.4.4 Identification of flavour compounds in the neutral fraction

The neutral fraction of the flavour concentrate of coconut had the characteristic coconut-like, sweet,





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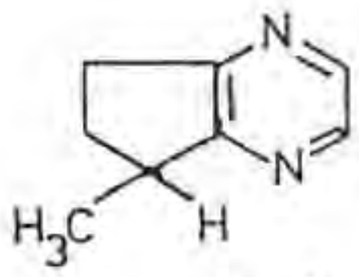


[58] $R_1, R_2 = H, CH_3$

Fig.19. Formation of cyclopentapyrazine.

oily, fruity aroma. Corresponding fractions from roasted coconut samples showed a pronounced nutty, oily, sweet flavour note reminiscent of coconut, but mixed with a tinge of cooked smell also.

The neutral flavour fractions of both control and heated coconut samples were found to occur almost to the same extent of about 300 ppm. However, relative concentration of the neutral fraction in coconut was 90 percent, whereas that in heated (160°C) samples was only 60 percent (see Table 18).

Table 23 represents the compounds identified by GC and GC-MS analyses of the neutral fractions. The GC analysis was carried out in packed OV-17 column as described in Chapter 2. It could be seen that the packed column also gave very good separation of the individual compounds and the GC profile was not very different from capillary analysis using cross-linked Methyl Silicone column. This was true of the relative area percentages also. Figure 20 represents the gas chromatographic separation on neutral fraction of flavour compounds, when analysed in a packed OV-17 column. The GC profile was dominated by C_8, C_{10}, C_{12} and C_{14}, δ-lactones in control and heated samples. Except δ-octalactone, other lactones were found to

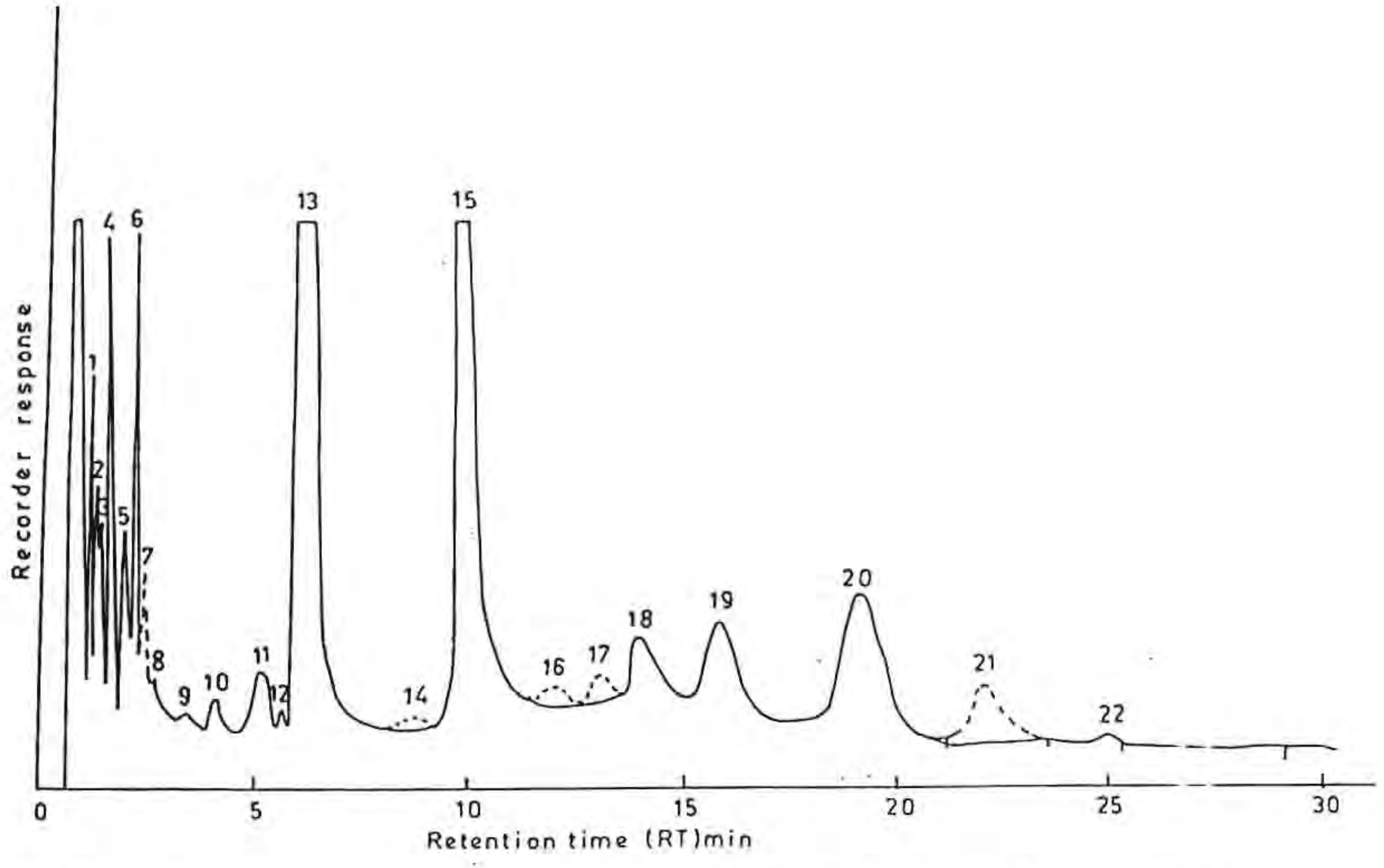


Fig.20. Gas chromatogram of neutral fraction of flavour isolated from heated coconut (160°C/15min) Column OV-17 (1·83 m x 3mm). See Table 24 also. Dotted lines indicate peaks which appeared in control and other heated samples.

Table 23: Compounds identified by GC and GC-MS analyses of neutral fraction of flavour, isolated from control and heated samples of coconut

Peak No.	RT(min) in capillary column of cross-linked Methyl Silicone	I_E value in OV-17 column	Compounds	MS fragment ions (in order of abundance)	Control		130°C		145°C		160°C	
					Relative conc. %	Actual conc. in ppm	Relative conc. %	Actual conc. in ppm	Relative conc. %	Actual conc. in ppm	Relative conc. %	Actual conc. in ppm
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	2.30	5.60	2-Pentanol	56,41,43,55,83,84	1.70	5.00	1.20	3.00	0.40	1.20	3.10	9.00
2	2.60	5.81	2-Hexanol	45,69,87,56,57,44	3.00	9.00	1.10	3.00	1.10	3.01	0.90	3.00
3	3.10	6.50	2-Heptanol	56,70,43,55,42,41, 83,98	1.25	4.00	3.52	10.50	1.60	4.80	1.50	4.50
4	3.80	6.72	Octanol	56,55,43,70,84,83 41,112	9.00	27.00	1.42	4.20	2.00	6.00	5.40	16.00
5	4.06	7.20	2-Furyl methyl ketone*	95,110,39,43,67,111	-	-	2.05	6.00	1.00	3.00	2.00	8.00
6	4.32	7.40	Octanal	43,44,57,56,69,84, 41,100,110	2.60	7.00	1.41	4.20	2.02	6.00	5.40	16.00
7	4.78	7.80	Phenyl ethyl alcohol	91,90,122,65,78, 104	0.90	2.70	-	-	0.40	1.20	-	-
8	5.03	8.41	Phenyl acetal-dehyde	119,120,91,77,42 64	-	-	1.30	3.90	0.60	1.80	0.10	0.30
10	5.10	9.05	Nonanal	57,44,56,43,55,70, 82,98,124	-	-	0.50	1.50	0.80	2.40	1.00	3.00
12	6.18	9.18	2-Tridecanone	58,43,40,71,59,88 105	0.13	0.40	0.20	0.60	0.30	1.50	tr.	tr.
13	5.30	11.20	δ-Octalactone	99,71,42,43,70,55, 114	27.20	81.00	48.00	130.00	40.00	120.00	32.00	96.00
14	5.10	11.70	Ethyl octanoate	88,101,57,41,43,55 114,152	-	1.00	-	-	-	0.30	-	-
15	10.32	12.10	δ-Decalactone	99,42,41,71,70,55, 114,152	28.00	84.00	29.60	80.00	25.60	77.00	18.40	75.00

Table 23 contd...

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
16	8.74	13.85	Ethyl decanoate	88,43,41,101,105, 70,57	0.30	1.00	0.30	1.00	-	-	-	-
17	14.72	14.70	Ethyl-5-hydroxy-decanoate	88,99,71,55,145,43	0.30	1.00	-	-	-	-	-	-
18	15.39	14.90	δ-Dodecalactone	99,41,42,43,55,57, 70,114	9.00	27.00	3.40	10.20	3.00	9.00	2.60	7.80
19	13.54	16.91	Ethyl dodecanoate	88,43,41,101,105,70, 57	2.00	6.00	-	-	2.80	8.40	3.50	11.00
20	16.40	16.80	δ-tetra-decalactone	99,42,41,55,70,114	14.40	42.00	6.60	19.00	9.50	29.00	6.10	26.00
21	17.63	17.20	Ethyl tetra-decanoate	88,101,70,157,143, 213,227,256	1.30	4.00	0.20	0.60	0.80	2.40	-	-
22	18.80	20.40	2-Butyl benzothiazole	149,41,56,57,150	0.60	2.00	0.30	1.00	0.30	1.00	0.30	1.00

* Traces - 0.1 to 0.5 ppm

decrease during heating. On the whole, δ-lactones, which represented nearly 80 percent of the neutral flavour fraction, were reduced to 60 percent at the final temperature of heating.

Another group of compounds detected in heated as well as control samples, were the aliphatic alcohols. n-Octanol was present in coconut (control) in considerable quantities (27 ppm) and was partially lost during heating (16 ppm). 2-Pentanol, 2-hexanol and 2-heptanol were present in 5 to 10 ppm levels. While concentration of 2-pentanol increased during heating, 2-hexanol was found to decrease in heated samples. 2-Heptanol increased with temperature and finally decreased to original concentration. The increase in concentration can be attributed to formation of these compounds during heating by mild degradation of the glycerides[235]. Phenyl ethyl alcohol which had been reported by other researchers like Lin and Wilkens[184] in coconut meat, was found to decrease in heating and became undetectable (<0.1ppm). Octanal and nonanal were two aliphatic aldehydes identified in coconut, the former increasing with temperature. Phenyl acetaldehyde was found to occur only in heated samples, though in small quantities. 2-Tridecanone

was the only methyl ketone identified in control sample, whereas both 2-furyl methyl ketone and 2-tridecanone were present in heated ones. Another important group of flavour compounds identified in coconut were a series of ethyl esters of even numbered fatty acids. Thus, ethyl octanoate, decanoate, dodecanoate and tetradecanoate were identified in coconut. Small quantities of ethyl-5-hydroxydecanoate was also detected in control sample. Except ethyl dodecanoate, all other esters were reduced to undetectable levels in the final stages of healing. Ethyl dodecanoate was not detected at 130°C but thereafter increased with temperature. 2-Butyl benzothiazole which was identified in basic fraction of roasted coconut was detected in the neutral fractions of all the four samples. Benzothiazole had been reported earlier in coconut meat by Lin and Wilkens[184].

3.4.5 Aromagram of neutral fraction

From preliminary GC analysis it could be seen that a few peaks were missing in roasted samples compared to the GC profile of coconut. Hence the odour assessment of eluting GC peaks was carried out first with neutral fraction of control sample and later with that of roasted (160°C) sample. A thermal conductivity detector was used for this exerpiment and

sensory analysis was carried out, three times, for each sample before finalising description of each eluting peak. Figure 20 represents the gas chromatogram of the neutral fraction of control sample, for which odour evaluation of peaks was carried out. The peaks in dotted lines represent the compounds eluted additionally in the roasted sample. The odour descriptions of the various peaks and their identity are detailed in Table 24. The flavour profile indicated that peaks corresponding to δ-octa, δ-deca and δ-tetradeca lactones exhibited characteristic coconut-like, oily aroma, δ-octalactone and δ-decalactone being most predominantly contributing to this. The ethyl ester, particularly, ethyl decanoate and ethyl-5-hydroxy decanoate also elicited a fruity, oily, coconut-like,pleasant flavour note. The peaks which had oily, nutty, aroma were found to be mostly the ethyl esters. The lower alcohols were characterised by a fresh, green, grassy flavour note, whereas n-octanol imparted a strong, citrus-like aroma. Peaks corresponding to octanal and nonanal had a mildly pungent flavour note also. The peak No.5 corresponding to RT 2.5 minutes had a cooked smell. Later, this peak was identified as 2-furyl methyl

Table 24 : Odour descriptions of the separated GC peaks (aromagram) of neutral fraction of coconut

Peak No.	Odour description	Compound
1	grassy green	2-pentanol
2	green, grass-like	2-hexanol
3	pleasant, over ripe fruit-like	3-heptanol
4	citrus-like	octanol
5	cooked, mildly roasted	2-furyl methyl ketone
6	pungent, slightly fragrant	octanal
7	fragrant, rose-like	phenyl ethyl alcohol
8	aromatic, fragrant	phenyl acetaldehyde
9	pleasant, flower-like	unknown
10	slightly pungent	nonanal
11	oily, pleasant	unknown
12	oily, old nut-like slightly harsh	2-tridecanone
13	typical coconut-like	δ-octalactone
14	pleasant oily	ethyl octanoate
15	Coconut-like	δ-decalactone
16	oily, nutty, fruity	ethyl decanoate
17	pleasant, fruity and nutty	ethyl hydroxy decanoate
18	heavy, nutty, oily	δ-dodecalactone

Table 24 contd....

Peak No.	Odour descriptions	Compounds
19	oily, nutty	ethyl dodecanoate
20	coconut-like, oily	δ-tetradecalactone
21	pleasant, nutty, oily	ethyl tetradecanoate
22	pleasant, medicinal, slightly pungent	2-butyl benzo-thiazole

kelone. 2-Tridecanone produced a harsh, stored oil-like, sensory effect. The peak corresponding to 2-butyl benzothiazole had a sharp pungent, medicinal flavour. On the whole, in the aromagram, δ-decalactone, δ-octalactone and δ-dodecalactone had typical, coconut-like flavour. The lower alcohols also indicated a significant contribution. The ethyl esters played an auxiliary role. The loss of these esters as well as change in concentration of the lower alcohols, together with the formation of pyrazines must be the reason for the flavour changes on roasting of coconut.

3.4.6 Discussion of the results of neutral fraction analysis

The neutral fraction constituted nearly 90 percent of the total flavour extract of coconut. As a result of heating upto 160°C this was reduced to 60 percent. At the same time, there was no drastic change in the total amount of neutral compounds during heating except that, the additional formation of pyrazines made their relative abundance less. This is probably, responsible for the changes in the overall flavour of heated coconut. Possibly, the presence of δ-lactones, alcohols, esters and other compounds, though present at different relative concentrations, are largely responsible for making the roasted flavour, characteristic of coconut.

This explains why a roasted peanut is different from a roasted coconut, or roasted cocoa especially when some of the pyrazine derivatives are present, in all of them. The possible answer is that the native flavour compounds, originally present, are also important and contribute to the total falvour.

From the chemical composition of neutral fraction, it can be seen that δ-lactones are predominant. Next to the δ-lactones, compounds like 2-pentanol, 2-hexanol, 2-heptanol, n-octanol, octanal, and nonanal are present in considerable quantities. Lin and Wilkens[184] had reported C_7, C_8, C_9 and C_{11} secondary alcohols (the position of -OH group was not mentioned) in fresh coconut meat. However, Allen[183] could not identify these volatile alcohols in his study on flavour constituents of coconut oil. They reported C_7, C_9, C_{11}, C_{13} and C_{15} methyl ketones, in addition to the δ-lactones. Recently, Saittagaroon et al[232] have reported on basic and neutral compounds of roasted coconut. They have not reported these lower alcohols in roasted or unroasted samples. In the present work, all these compounds, which eluted closely after the solvent, could be positively identified. The ethyl esters and δ-lactones have been reported by Lin and Wilkens[184]. An interesting work had been carried out by Pai et al[236] on the

volatile composition of coconut oil subjected to heating in air and vacuum with and without moisture. From this study, they could conclude that small amounts (1 ppm) of lower ketones, esters, fatty acids, γ- and δ-lactones and hydrocarbons were formed during heating by the degradation of oil. In the present work, furyl methyl ketone and phenyl acetaldehyde are also detected, which can be considered as products of Maillard reaction and Strecker degradation. Phenyl acetaldehyde has been considered to be a contributing factor in roasted cocoa flavour. The possible mechanism of formation of phenyl acetaldehyde **[60]** from phenyl alanine **[59]** has been suggested by Manley et al[133]. This is shown in Figure 21.

3.4.7 Identification of flavour compounds in the acid fraction

Quantitatively, the acid compounds in the flavour extract of coconut, increased with temperature of roasting, as can be seen from Table 18, i.e. from 25 ppm, it increased to 80 ppm at 160°C. However, the relative concentration of these volatile compounds in the total flavour was not altered much (8 to 10 percent). Since the acid fraction isolate was methylated before GC analysis, odour descriptions of the individual compounds as and when they eluted from the GC exit port was not

Fig.21. Formation of phenyl acetaldehyde from phenyl alanine through Strecker degradation.

carried out in this case. Before methylation, the acid fraction concentrates from control and heated samples were noticed to have the typical odours of fatty acids and predominantly that of octanoic acid (with reference to the available authentic sample), which smells rancid, acidic and disgusting.

GC analysis of methylated acid compounds was first carried out in a packed DEGS column using FID. The separation was reasonably good. Figure 22 represents the GC separation of methylated acid fraction of flavour, isolated from coconut roasted at 145°C. The sample corresponding to this temperature had more number of peaks, most of which were present in other samples too. Position of any additional peak identified in other samples is marked in dotted lines. Since the first few peaks eluted with solvent during GC-MS analysis, the calculation of the concentration of individual compounds was based on area percentages printed out in GC analyses.

Table 25 represents the results of the GC and GC-MS analyses of the acid fractions of flavour, isolated from control and heated coconut. Results indicated the presence of lower acids like acetic, propionic, butyric and pentanoic and most of the straight chain

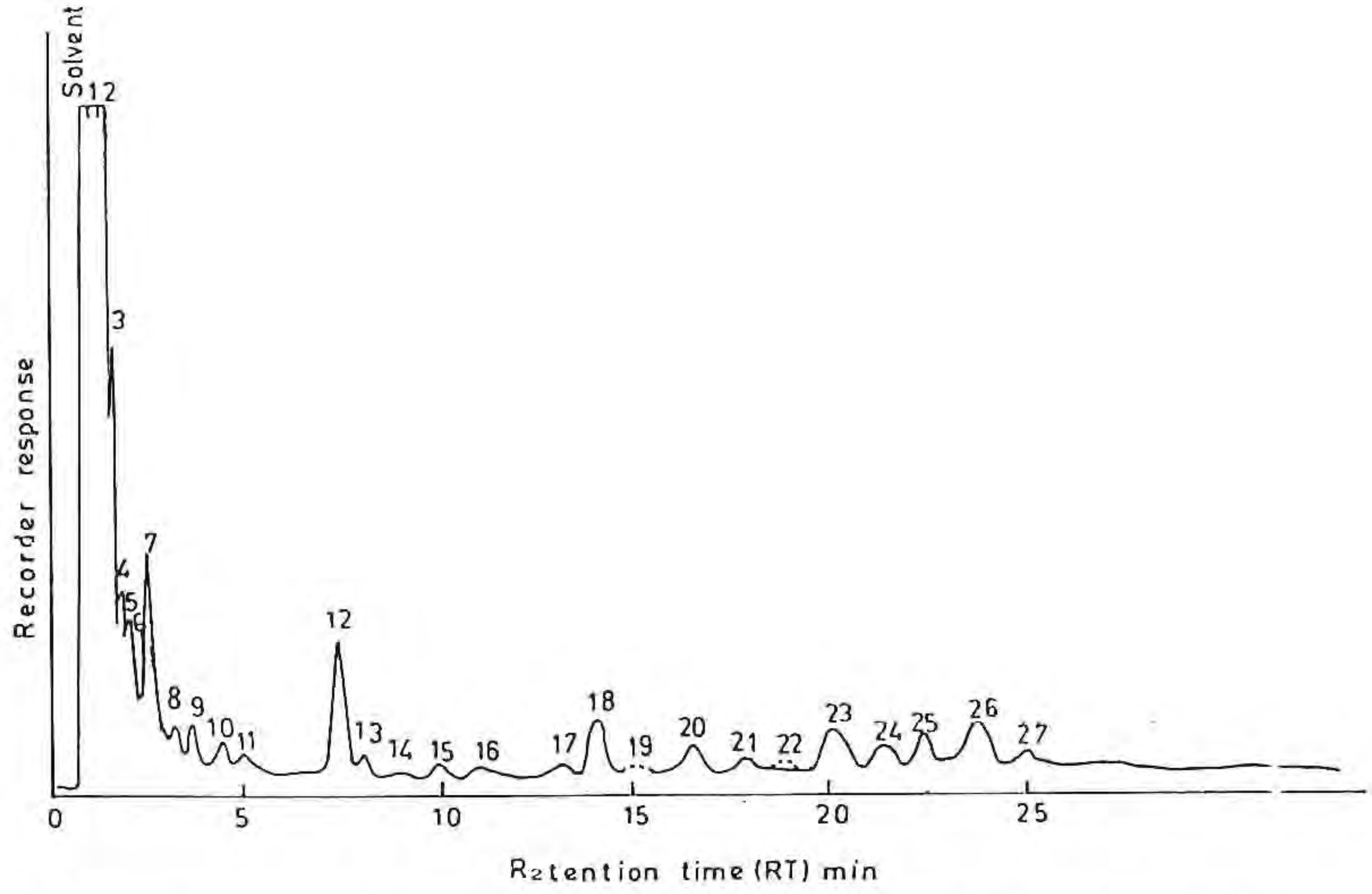


Fig.22. Gas chromatogram of methylated acid fraction of flavour isolated from heated coconut (145°C/15 min). Column: DEGS (1.83 m x 3 mm id). Dotted lines indicate peaks which appeared in other samples.

Table 25 : Compounds identified by GC and GC-MS analyses of methylated acid fraction of flavour, isolated from control and heated samples of coconut

Peak No.	RT(min) in capillary column of cross-linked Methyl Silicone	I_E value in DEGS column	Compounds	MS fragment ions (in order of abundance)	Control		130°C		145°C		160°C	
					Relative conc. %	Actual conc. in ppm	Relative conc. %	Actual conc. in ppm	Relative conc. %	Actual conc. in ppm	Relative conc. %	Actual conc. in ppm
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	-	3.80	Acetic & Propionic acids	Eluted with solvent in GC-MS analysis	46.00	11.50	65.70	26.00	28.00	17.00	3.40	2.70
2	-	4.60	Butyric	"	0.70	0.20	21.00	8.40	17.70	10.60	-	-
3	-	5.81	Pentanoic	"	6.70	1.70	1.10	0.40	1.00	0.60	0.14	0.10
4	0.98	6.80	Hexanoic	74,84,51,47,41,88, 49	3.50	0.90	-	-	0.10	0.06	-	-
5	1.23	7.40	Heptanoic	74,87,43,36,113, 55,41	0.10	0.02	0.10	0.04	0.50	0.30	0.50	0.40
6	2.01	8.30	Hydroxy octanoic	74,99,130,101,140,174	Tr.	Tr.	0.10	0.04	-	-	-	-
7	3.18	8.70	Octanoic	74,87,55,41,69,158	0.05	0.01	0.80	0.30	5.10	3.10	1.60	1.30
8	4.34	9.10	Nonanoic	74,87,43,41,55,172	-	-	-	-	0.60	0.40	0.80	0.60
9	5.60	10.02	Hydroxy decanoic	74,99,127,156,202	-	-	-	-	1.70	1.00	1.70	1.40
10	7.50	10.70	Decanoic	74,87,143,43,55,186	0.06	0.02	0.20	0.10	0.60	0.40	4.10	3.30
11	8.10	11.30	Decenoic	74,87,41,184	-	-	-	-	0.20	0.10	1.60	1.30
12	12.50	12.00	Hydroxy dodecanoic	99,74,41,106,230	Tr.	Tr.	-	-	5.70	3.40	11.60	9.30
13	12.94	12.80	Dodecanoic	74,87,41,55,43,214	0.50	0.13	0.60	0.20	0.20	0.10	9.80	7.80
14	13.40	13.41	Dodecenoic	74,87,41,212	-	-	-	-	0.10	0.06	3.50	2.80
15	17.26	14.10	Tetradecanoic	74,87,41,43,55,242	Tr.	Tr.	-	-	1.50	0.90	7.70	6.20

Table 25 contd....

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
16	17.18	14.60	Tetradecenoic	74,87,41,240	0.20	0.05	0.20	0.10	0.80	0.50	9.50	7.80
17	18.62	15.63	Pentadecanoic	74,87,43,113,41,55, 256	-	-	-	-	1.30	0.80	-	-
18	19.34	15.90	Pentadecenoic	74,87,41,254	Tr.	Tr.	Tr.	Tr.	3.20	2.00	11.10	8.90
19	20.58	16.41	Hydroxy hexadecanoic	99,74,130,144,41, 286	0.30	0.08	-	-	-	-	-	-
20	24.16	17.40	Hexadecanoic	74,87,43,75,41,227, 270	0.20	0.05	0.80	0.30	2.00	1.20	8.0	6.40
21	20.72	16.52	Hexadecenoic	74,87,255,268,43, 41	0.90	0.23	0.20	0.10	2.60	1.60	-	-
22	24.70	17.90	Octadecanoic	74,87,43,55,41,143, 255,298	-	-	0.20	0.10	-	-	-	-
23	24.10	18.48	Octadecanoic ($C_{18:1}$, oleic)	55,74,69,67,83,43, 81,222	0.31	0.08	0.50	0.20	4.20	2.50	5.80	4.60
24	25.92	19.40	9,12-Octadeca-dienoic ($C_{18:2}$, linoleic)	74,87,255,43	4.20	1.10	0.80	0.30	2.90	1.70	4.10	3.30
25	26.30	20.20	Eicosanoic (C_{20})	74,87,43,326	5.80	1.50	2.20	0.90	5.10	3.10	3.20	2.60
26	27.10	21.10	9,12,15-Octadeca-trienoic ($C_{18:3}$, lindenic)	74,87,43,255	6.70	1.70	2.00	0.80	6.50	4.00	3.10	2.50

fatty acids from hexanoic ($C_{6:0}$) to eicosanoic ($C_{20:0}$) 5-Hydroxy fatty acids of octanoic ($C_{8:0}$), decanoic ($C_{10:0}$) dodecanoic ($C_{12:0}$) and hexadecanoic ($C_{16:0}$) were also found to be present. Unsaturated fatty acids like 5-decenoic ($C_{10:1}$), 9-dodecenoic ($C_{12:1}$), 7-tetradecenoic ($C_{14:1}$) and 10-hexadecenoic ($C_{16:1}$) were also present. The unsaturated fatty acids of C_{18} present were oleic ($C_{18:1}$), and linoleic ($C_{18:2}$). In total, 21 different acids were identified to be present in control sample, the major ones being acetic, propionic and butyric. These acids were found to increase in concentration with temperature of heating upto 145°C, after which the content decreased, possibly due to evaporational loss. Among saturated acids, octanoic ($C_{8:0}$), nonanoic ($C_{9:0}$) decanoic ($C_{10:0}$) dodecanoic ($C_{12:0}$) tetradecanoic ($C_{14:0}$) hexadecanoic ($C_{16:0}$), octadecanoic ($C_{18:0}$) and eicosanoic ($C_{20:0}$) marked an overall increase in concentration during heating. Among unsaturated acids 5-decenoic ($C_{10:1}$), 9-dodecenoic ($C_{12:1}$), 7-tetradecenoic ($C_{14:1}$), 9-pentadecenoic ($C_{15:1}$), and 10-hexadecenoic ($C_{16:1}$) increased upto 145°C, and 9-octadecenoic ($C_{18:1}$, oleic acid), 9,12-octadec-di-enoic ($C_{18:2}$, linoleic) showed an increase in concentration at higher temperatures. The 5-hydroxy acids increased only slightly during

heating, the most noticeable change being in the case of 5-hydroxydodecanoic (C_{12-OH}) acid. No phenolic compounds were identified in any of the acid fractions.

3.4.8 Discussion of the results of acid fraction analysis

The presence of fatty acids, except dodecanoic acid (lauric acid) has not been reported in the previous research work of Lin and Wilkens[184] on the volatile aroma compounds of coconut meat. Allen[183] also could not identify any of these acids in the volatile compounds of coconut oil. Presence of a few fatty acids was, however reported by Pai et al[236], in a system of heated coconut oil. The reported work on aroma constituents of roasted coconut by Saittagaroon et al[232] also did not report the details of acid fraction compounds. In this context, the acidic flavour compounds identified in the present work are reported for the first time in the volatiles of coconut. The formation of fatty acids during heating[237] has been reported in the case of roasted peanut[112,238], meat[239], cocoa[32], heated milk fat[240], sesame[138] etc. Recently Jayalekshmy and Narayanan[241] identified and reported a few fatty acids in roasted cashewnut also. In most of these foods, acids like acetic, propionic, isobutyric, butyric etc have been reported to be formed

during roasting, due to thermal hydrolysis of the triglyceride. In coconut also, some of these lower acids are found to occur in control and heated samples.

The role of fatty acids in the overall flavour of a product is under estimated quite often. Studies have been conducted with fatty acids on their odour thresholds in aqueous phase and lipid phase[242]. The results showed that short chain fatty acids which are more polar, and hence more soluble in aqueous phase had a higher threshold in aqueous medium compared to lipid medium. In other words, short chain fatty acids will be more readily detected (by nose) in a lipid than in an aqueous medium, because the threshold concentration is lower in the lipid medium. Conversely, long chain fatty acids which are less polar, and hence more hydrophobic, will be less soluble in aqueous phase than in lipid phase. Thus, they will be more easily sensed in an aqueous medium than in a lipid environment. For example, in coconut oil, short chain fatty acids will be directly contributing to head space volatiles, whereas long chain fatty acids will exert an olfactory effect, more in coconut milk.

In short, the flavour studies of coconut indicate that the flavour profile of coconut is dominated by

δ-lactones which contribute to the characteristic, oily, nut-like aroma. Esters and ketones are also present, which impart fresh, fruity, and estery shades of odours to the overall flavour. Heating results in the formation of heterocyclic compounds, especially pyrazines which are formed in larger proportions. Pyrazines are considered to be mainly responsible for the roasted flavour. Besides pyrazines, pyridines pyrroles and furans are also found to be present. A number of fatty acids have been identified in heated and unheated samples of coconut which are likely to contribute to the overall flavour of the product.

A classification of the type of flavour compounds identified in coconut is given below.

	Control	Heated(160°C/15min)
Pyrazines	-	17
Pyridines	-	1
Pyrroles	-	1
Furans	-	1
Benzothiazole	-	1
Hydrocarbons	-	1
Alcohols	5	4
Aldehydes	1	3
Ketones	2	3

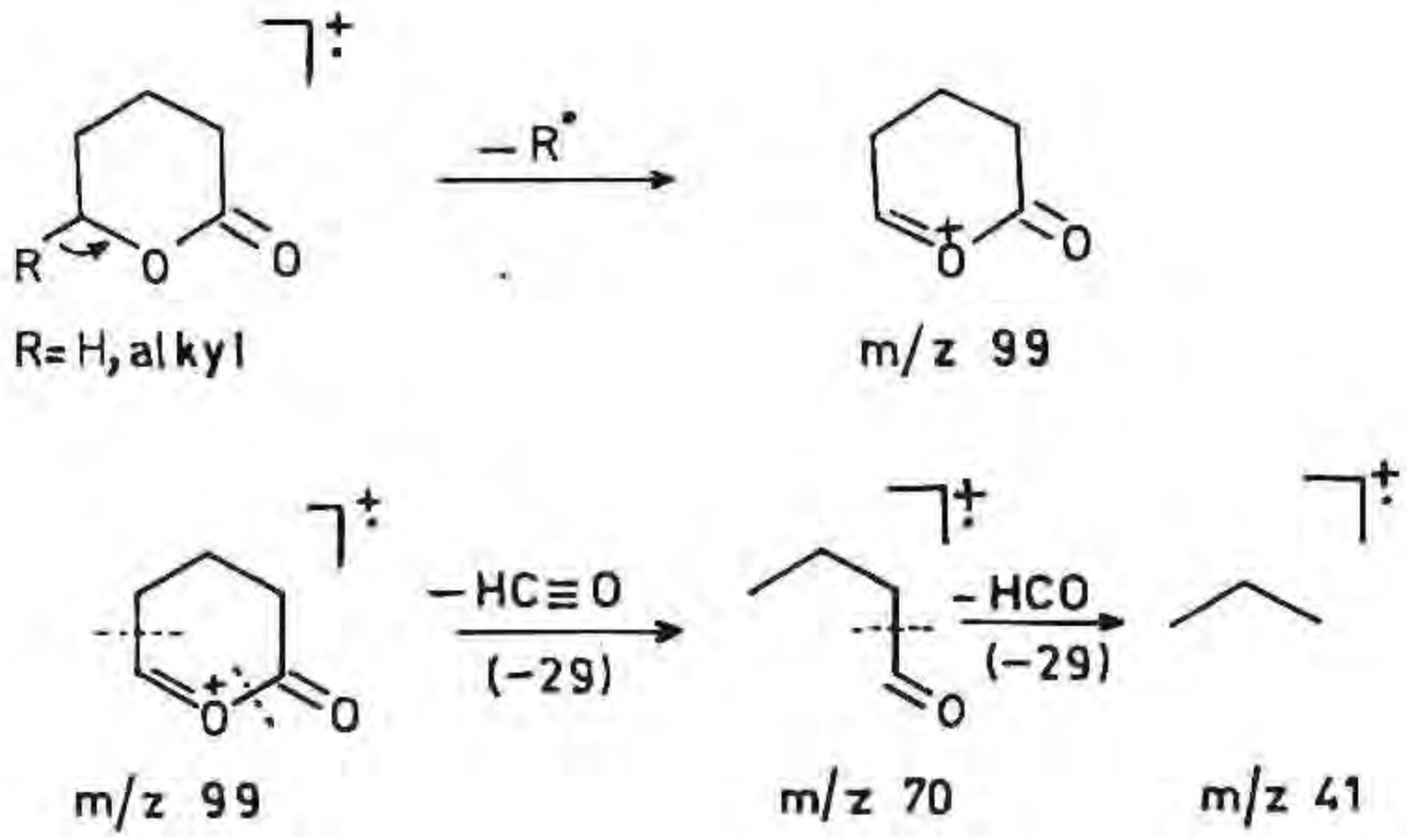
δ-lactones	4	4
Esters	5	2
Acids	21	20

3.5 MASS SPECTRAL FRAGMENTATION MODES OF IMPORTANT FLAVOUR COMPOUNDS IDENTIFIED IN THE STUDY

The scheme of mass spectral fragmentation of some of the important class of flavour compounds of coconut will be discussed here in brief.

δ-Lactones

The δ-lactones show characteristic base peak at m/z 99. Scheme of fragmentation of δ-lactones is shown below[243]:



Cleavage adjacent to the oxygen atom is the characterislic fragmentation mode of δ-lactones. In addition to this most abundant peak, others corresponding to alkyl side chain are also seen and give valuable clues as to the identity of parent molecule. The peaks at m/z 42, 70 and 71 are also typical of δ-lactone spectra which are formed by the breaking of cyclic structures.

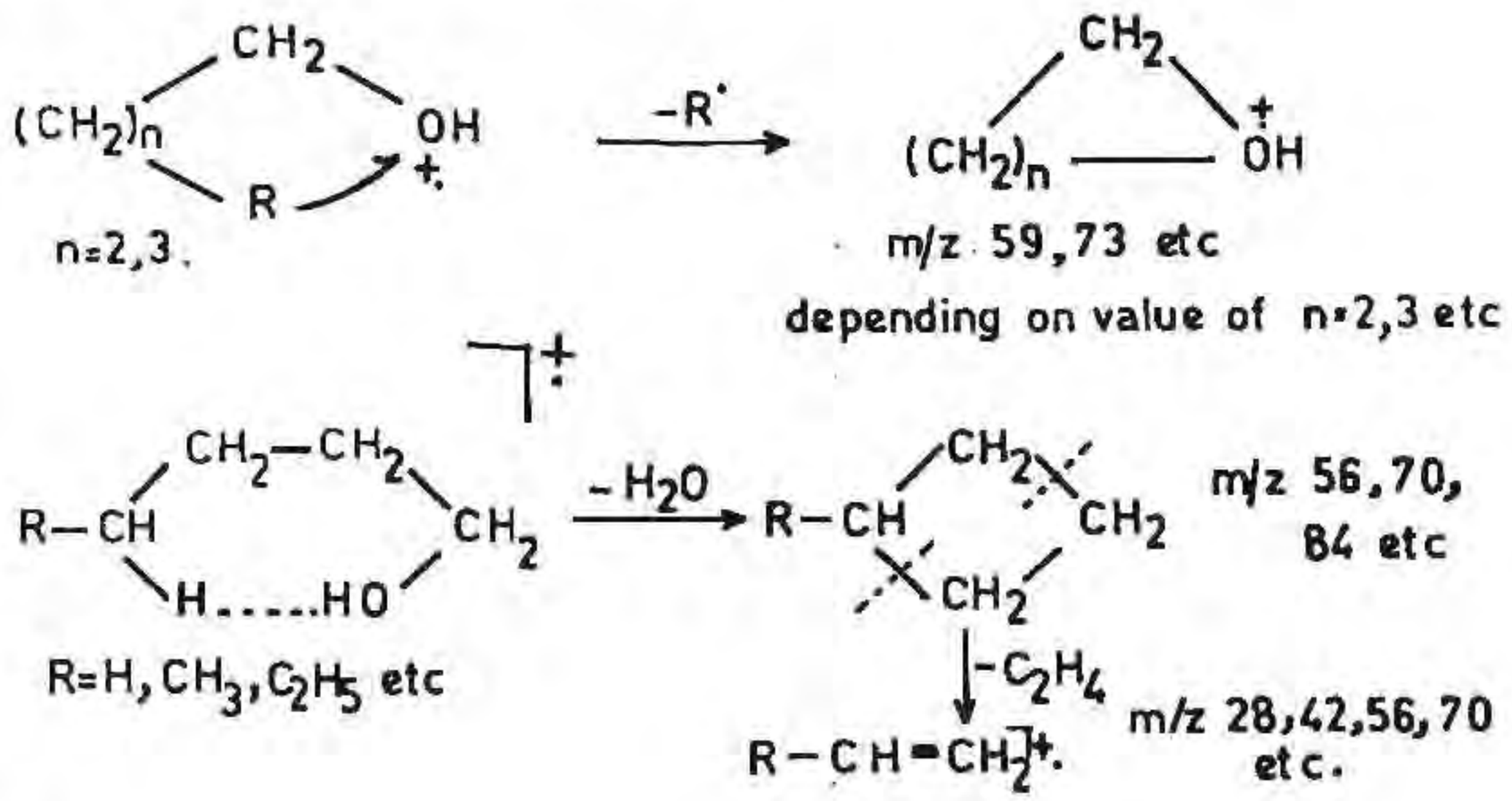
Alcohols

Molecular ions of primary and secondary alcohols are formed in relatively small amounts only. The mosl preferred fragmenlation mode is α-cleavage in which the C-C bond adjacent to hydroxyl group is affected[244]. The fragment with m/z value 31 due to the oxonium ion $CH_2{=}\overset{+}{O}H$ is characteristic of primary alcohols. They also undergo β-, γ- and δ-cleavages to give fragments with m/z 45, 59, 73 etc. Secondary alcohols give R-CH=$\overset{+}{O}$H fragments with m/z values 45, 59, 73 etc depending on the nature of the alkyl group. Formation of cyclic oxonium ion is possible during such fragmentation.

$$R-CH_2-CH_2-\overset{+}{O}H \longrightarrow R-CH_2^{\cdot} + CH_2=\overset{+}{O}H \quad (m/z\ 31)$$

$$R-\underset{\overset{+}{O}H}{CH}-CH_3 \xrightarrow{-CH_3^{\cdot}} R-CH=\overset{+}{O}H \quad \text{Where } R = H, CH_3, C_2H_5 \text{ etc}$$

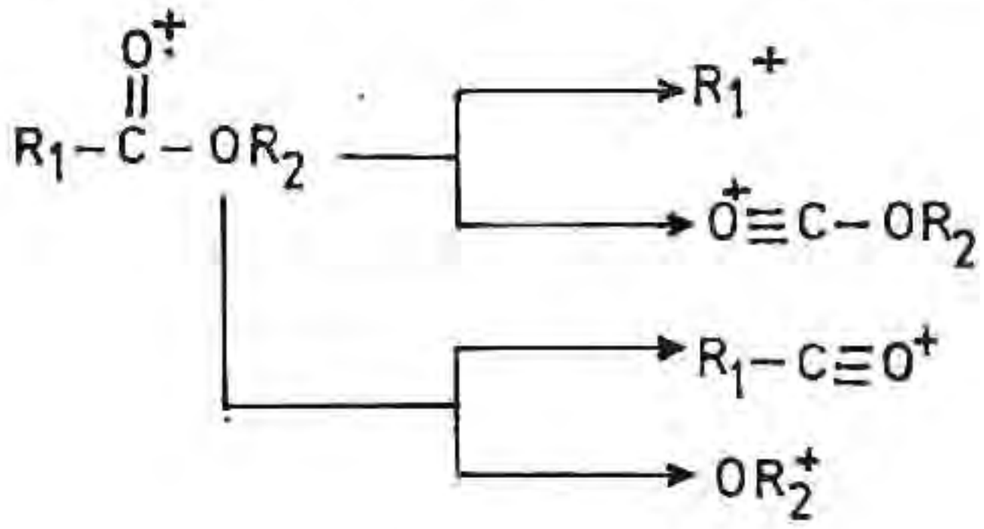
In addition to this, parent ion can lose two to three hydrogen atoms producing (M-H), (M-2H) etc ions. Water molecules are also eliminated (M-18), which is sometimes accompanied by elimination of an ethylene molecule also [(M-H_2O-C_2H_4, i.e. (M-18)-(28)] in alcohols, containing more than four carbon atoms. Elimination of water molecule takes place through a six membered cyclic transition state.



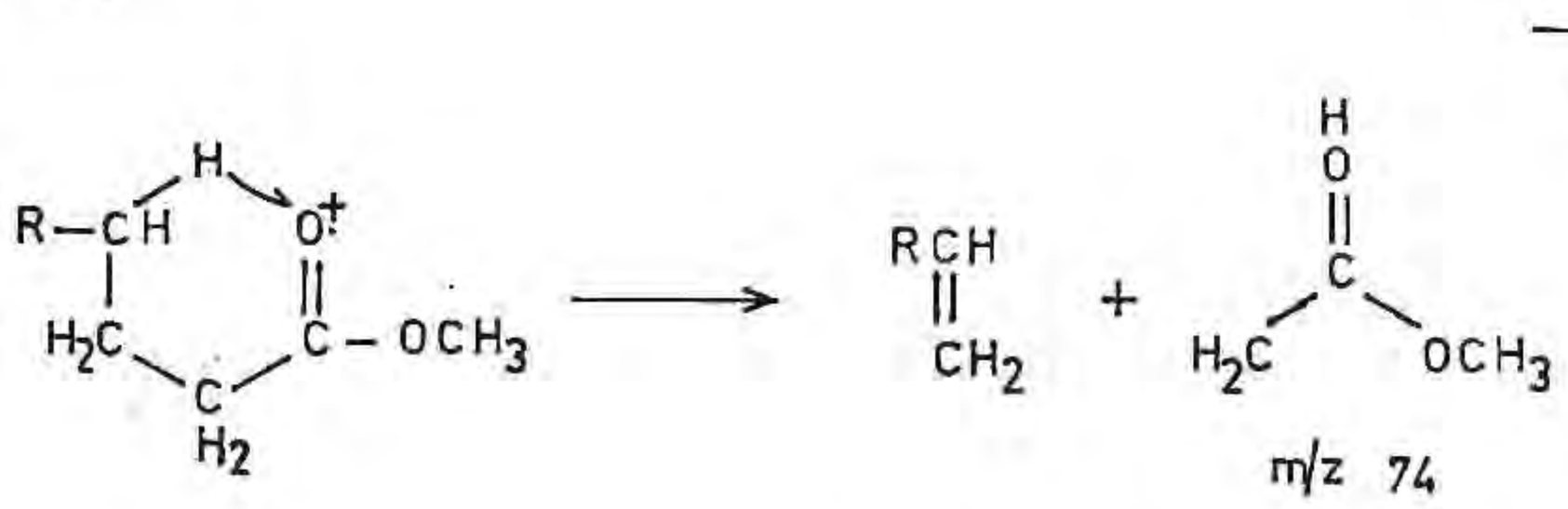
Esters

The characteristic fragmentation modes of esters are α- and β-cleavages. α-Cleavage usually gives four different ions as shown here. Of these, alkyl ion and acyl ion are more important. Methyl esters of long chain fatty acids fragment by McLafferty rearrangement with the production of base peak at m/z 74[245]. The

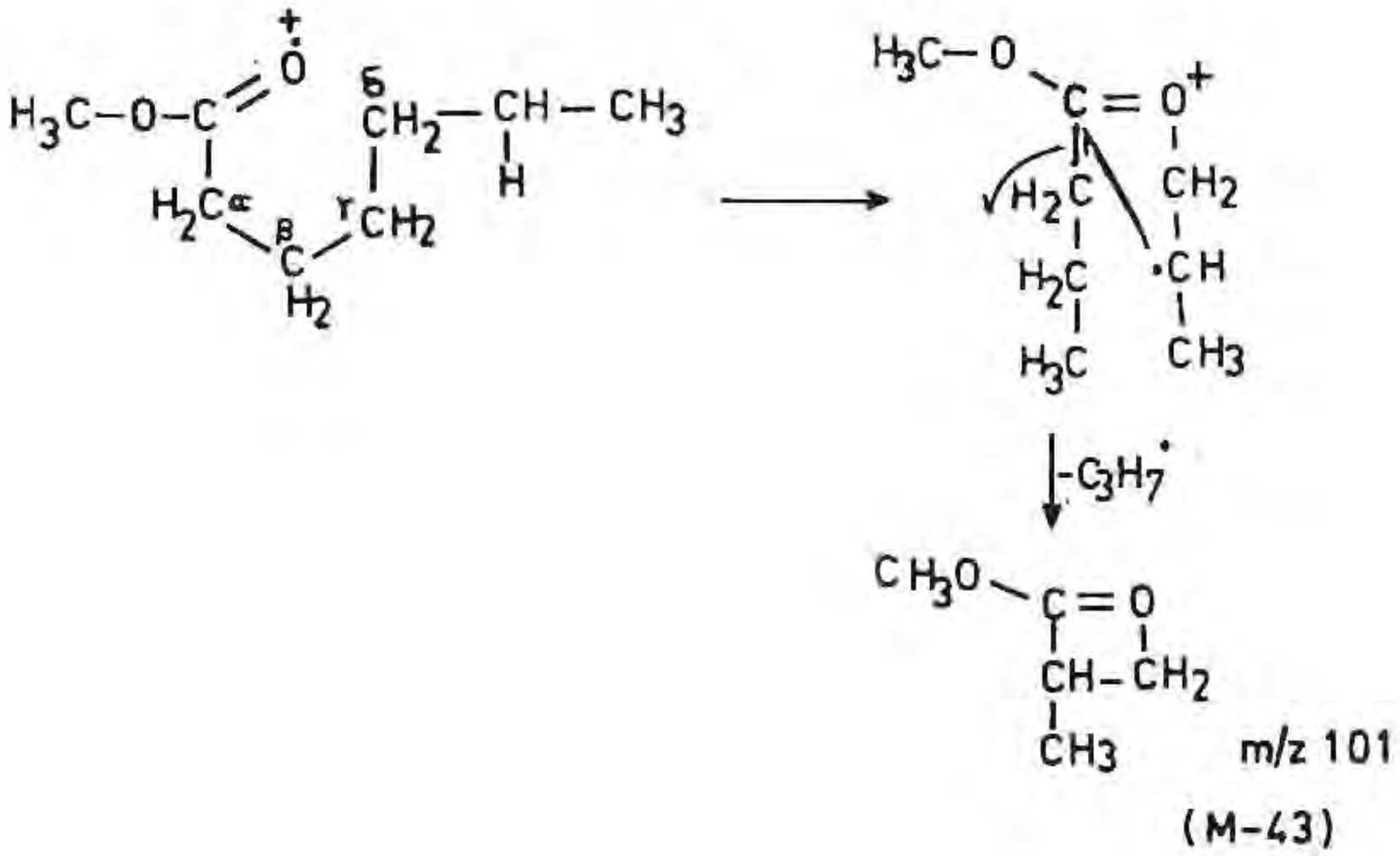
scheme can be outlined as follows:



Fragmentation of long chain methyl esters can be depicted as:



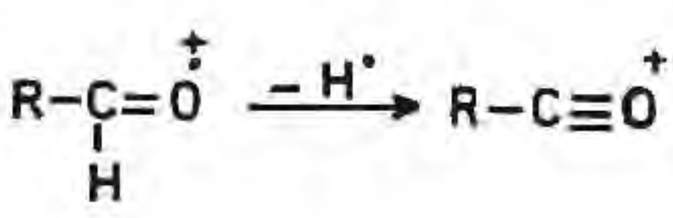
In the case of ethyl esters, the base peak is at m/z 88. The $(M-C_3H_7)$ ion is also significant. This is illustrated below:



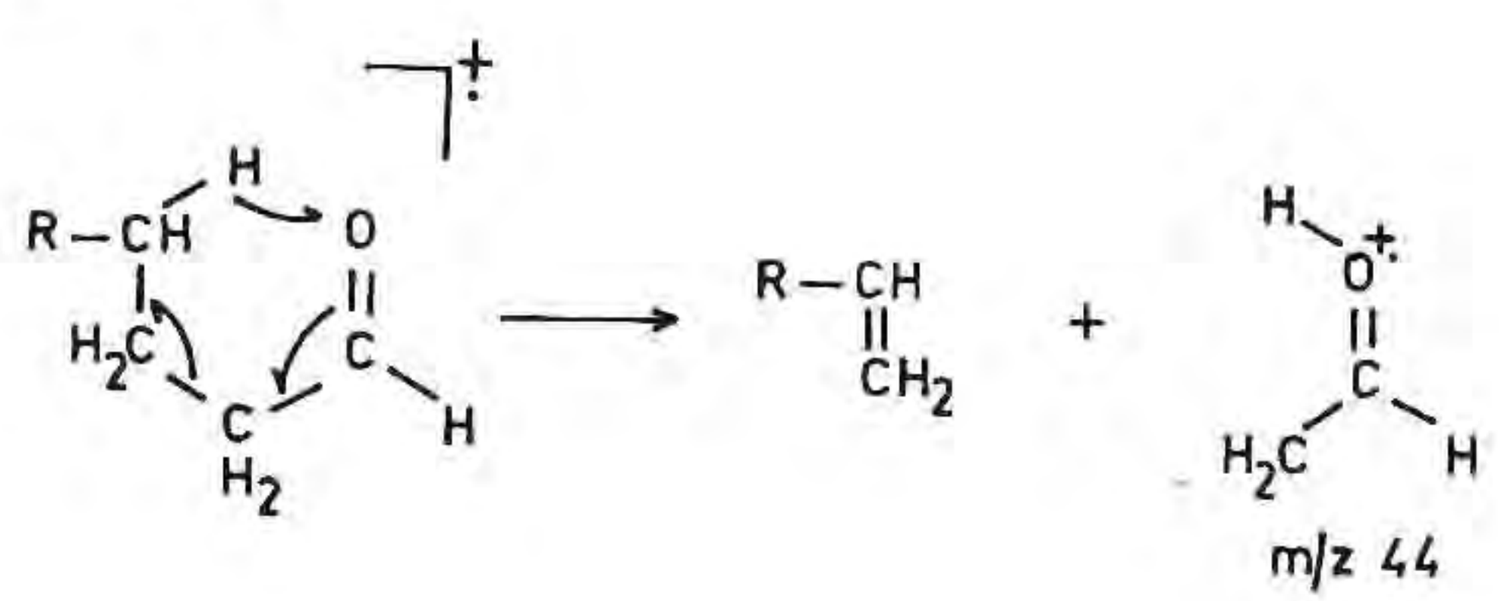
Aldehydes

Intensity of molecular ion peak decreases as the alkyl chain length increases. Both α- and β-cleavages with γ-hydrogen transfer are noticed. Higher aldehydes undergo McLafferty rearrangement. Some of these fragmentation modes are given below:

Lower aldehyde (α-cleavage important)

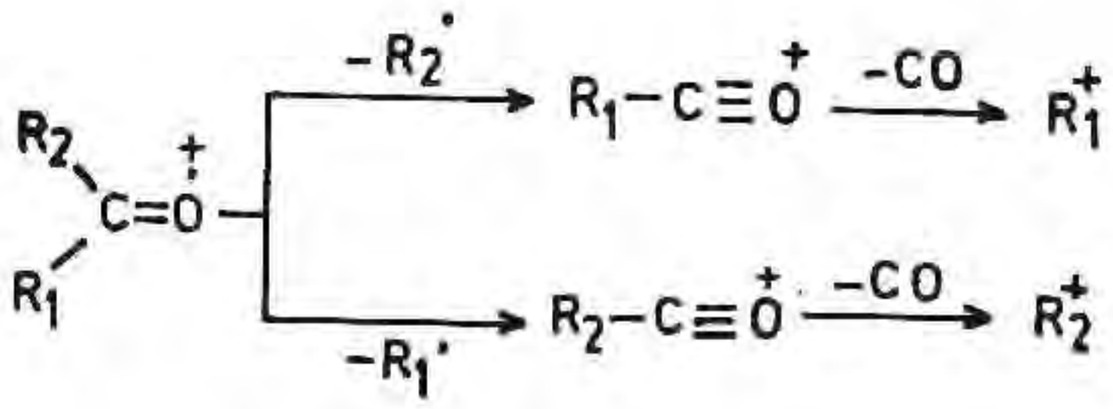


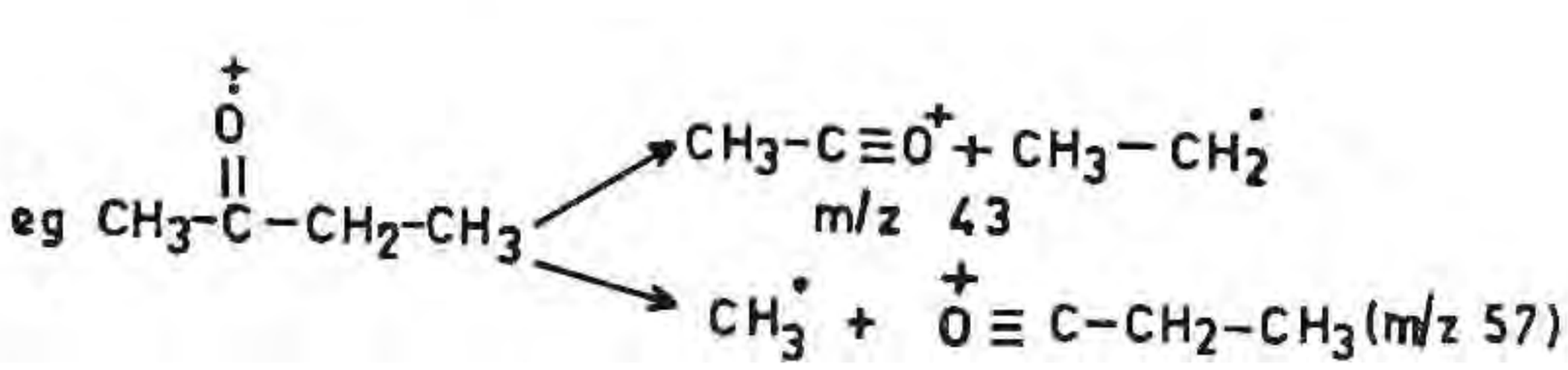
Higher aldehydes



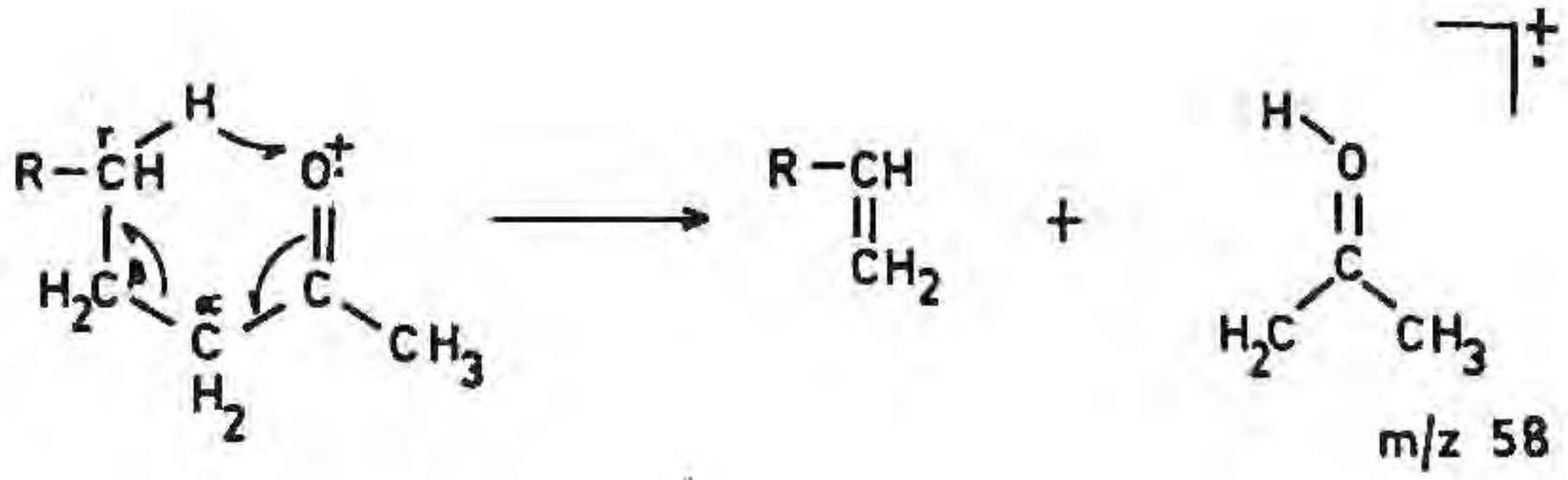
Ketones

Molecular ion peak is prominent. α- and β-cleavages are predominant. Higher ketones show fragments characteristic of McLafferty rearrangement. Following scheme represents the possible fragmentation modes.



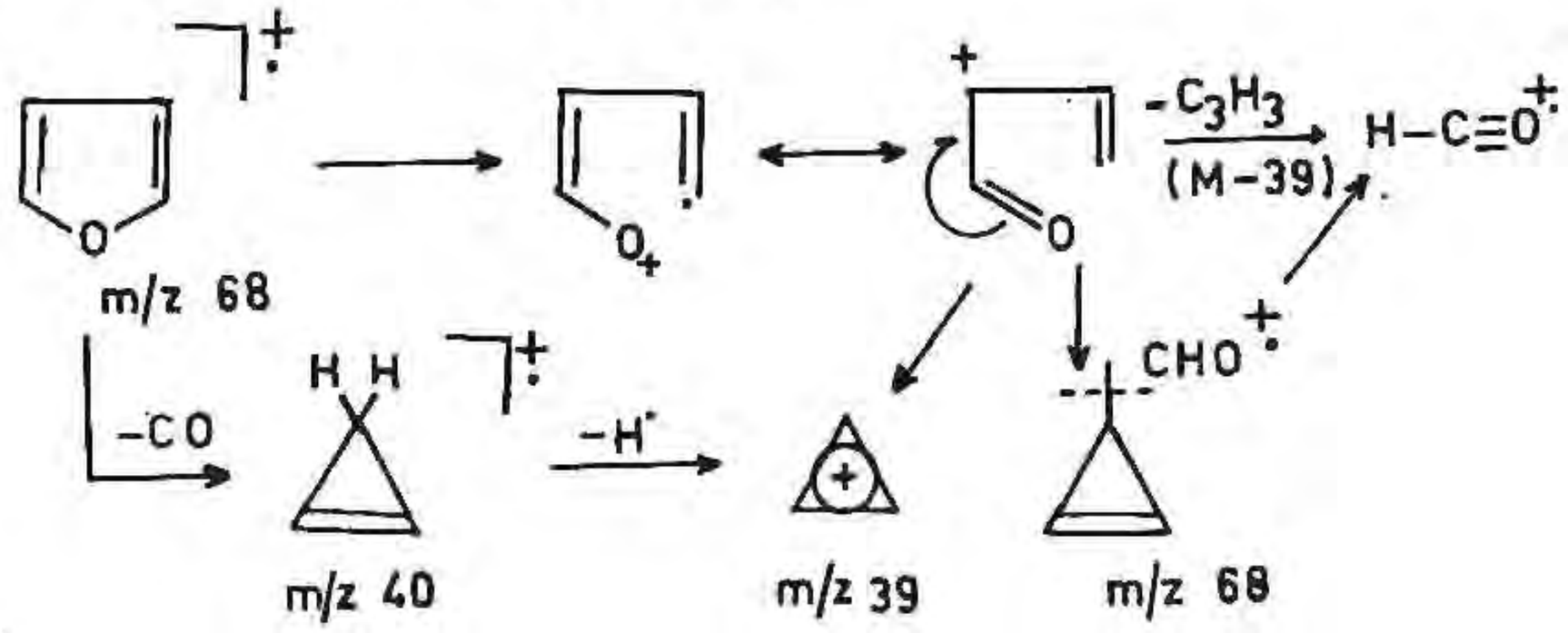


Higher ketones

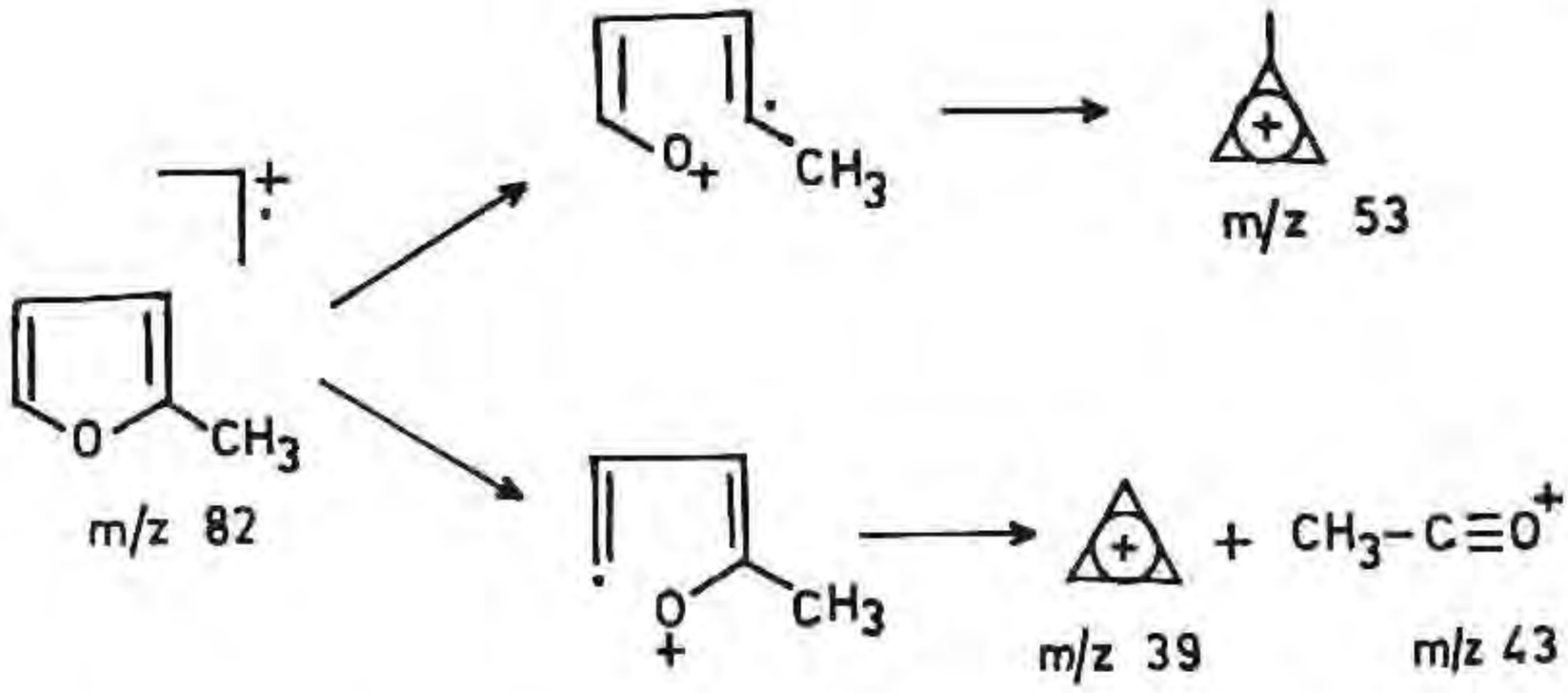


Furans

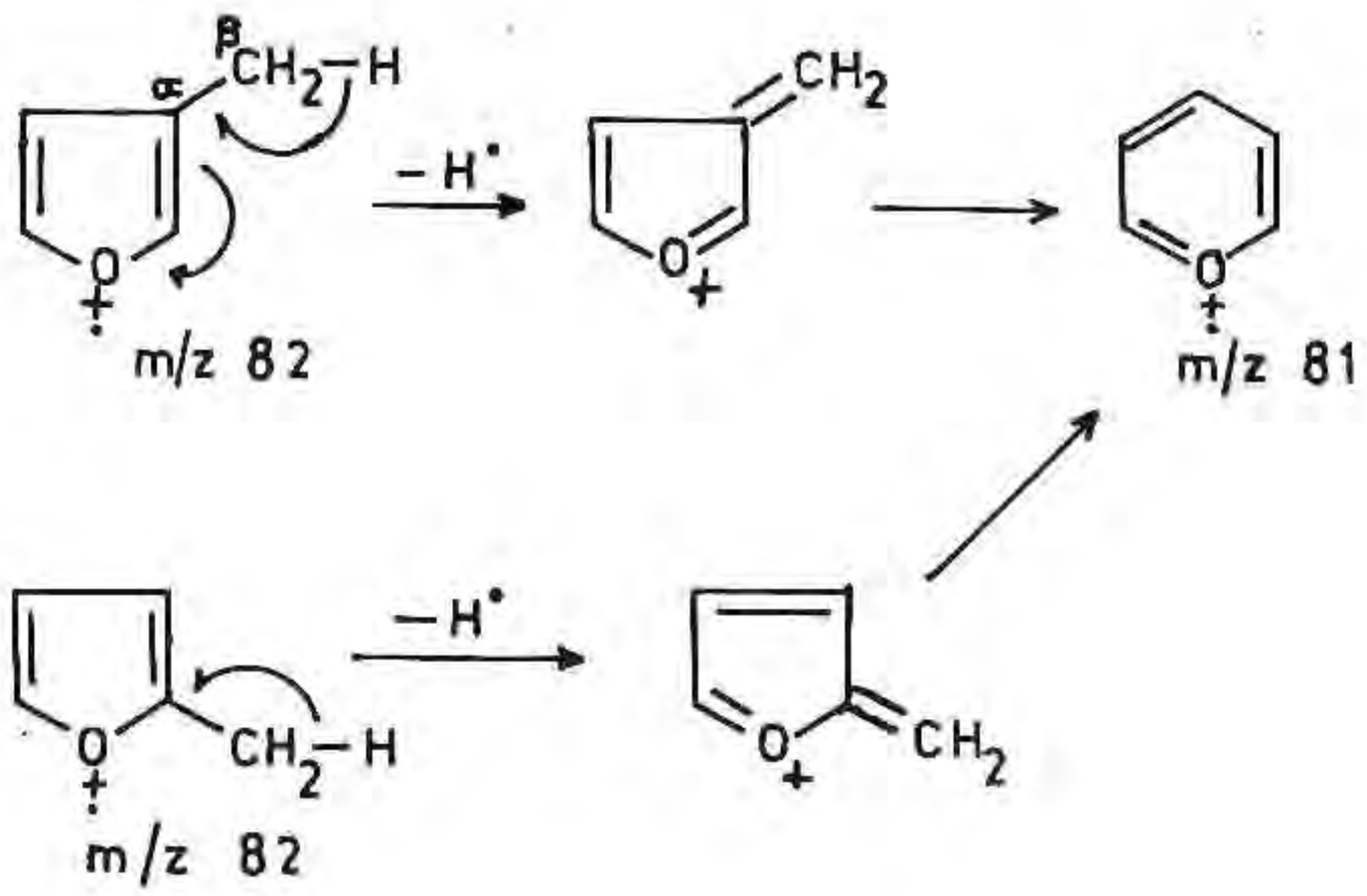
Furans being aromatic in nature give intense molecular ion peak. Usual fragmentation mode is the cleavage of C-O bond, as depicted here.



For alkyl substituted furans, ring opening takes place in two ways, resulting in ring contraction[245].



In another mode, β-cleavage, followed by ring expansion takes place.

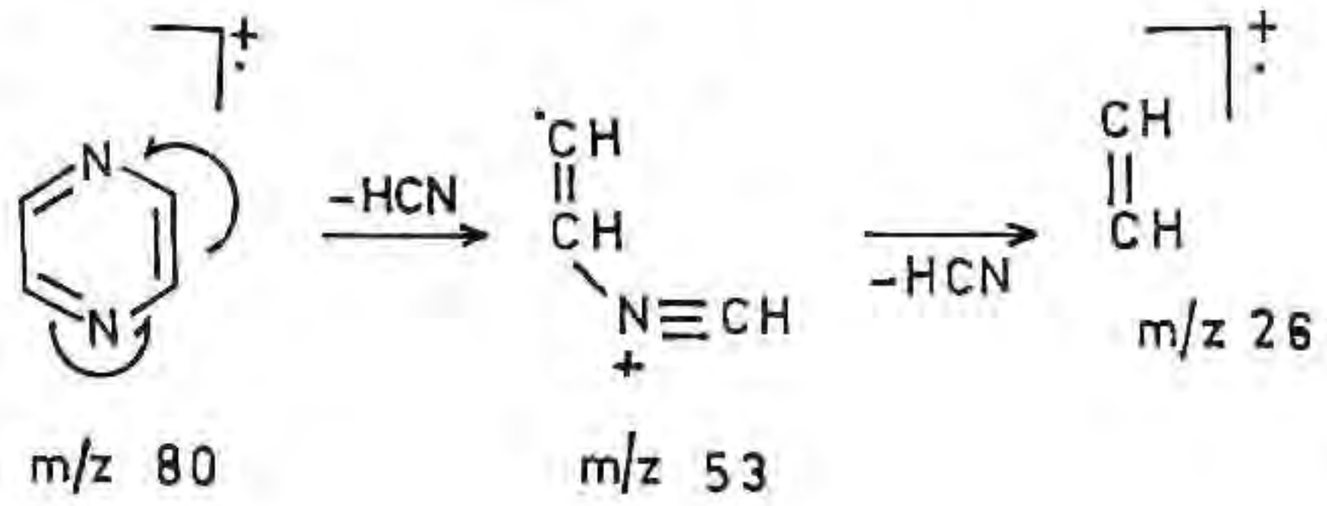


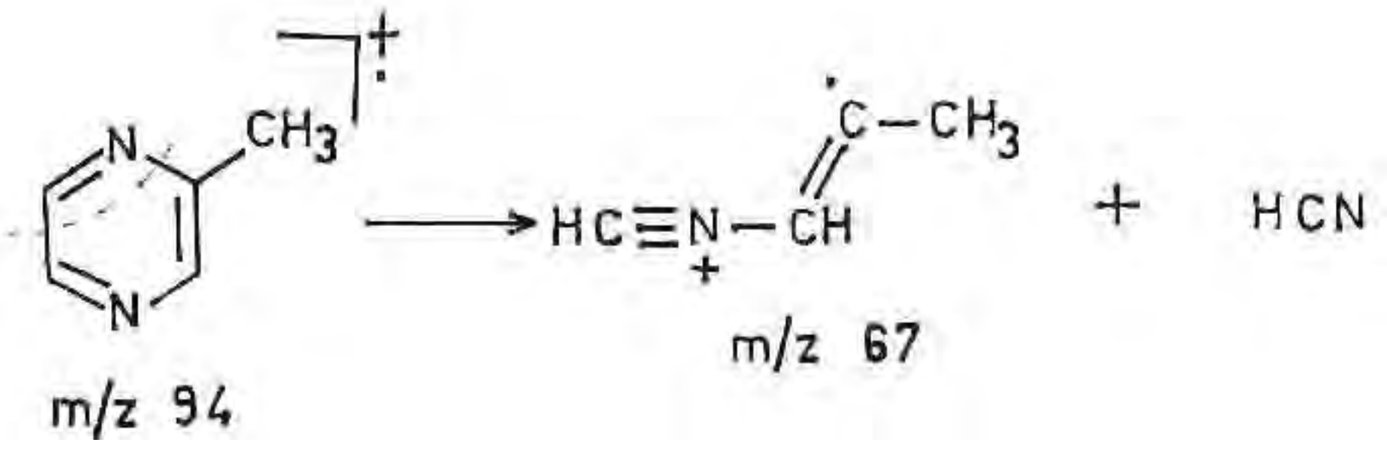
So fragments will be with m/z values 81, 82, 53, 29, 39, 43.

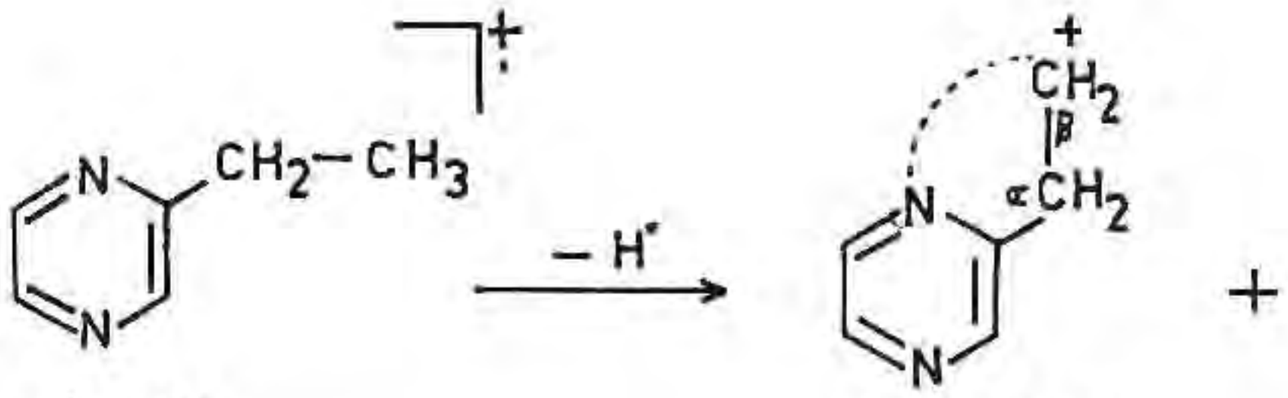
Pyrazines

Fragmentation modes of the different pyrazines are discussed only by a few authors in the literature[6,24,246]. Since the pyrazine nucleus itself is

relatively stable and resistant to fragmentation, the mass spectra of substituted pyrazines are greatly infuenced by the nature of the substituents. The first member, pyrazine itself has a mass spectrum consisting of three principal ions m/z 80 (100 percent), 53 (45 percent), 26 (40 percent). In monosubstituted pyrazines like methyl pyrazine, a large peak appears, corresponding to the loss of HCN from the parent ion. In the case of ethyl pyrazine, a large (M-1) peak appears. Scheme of formation of these ions is outlined below:

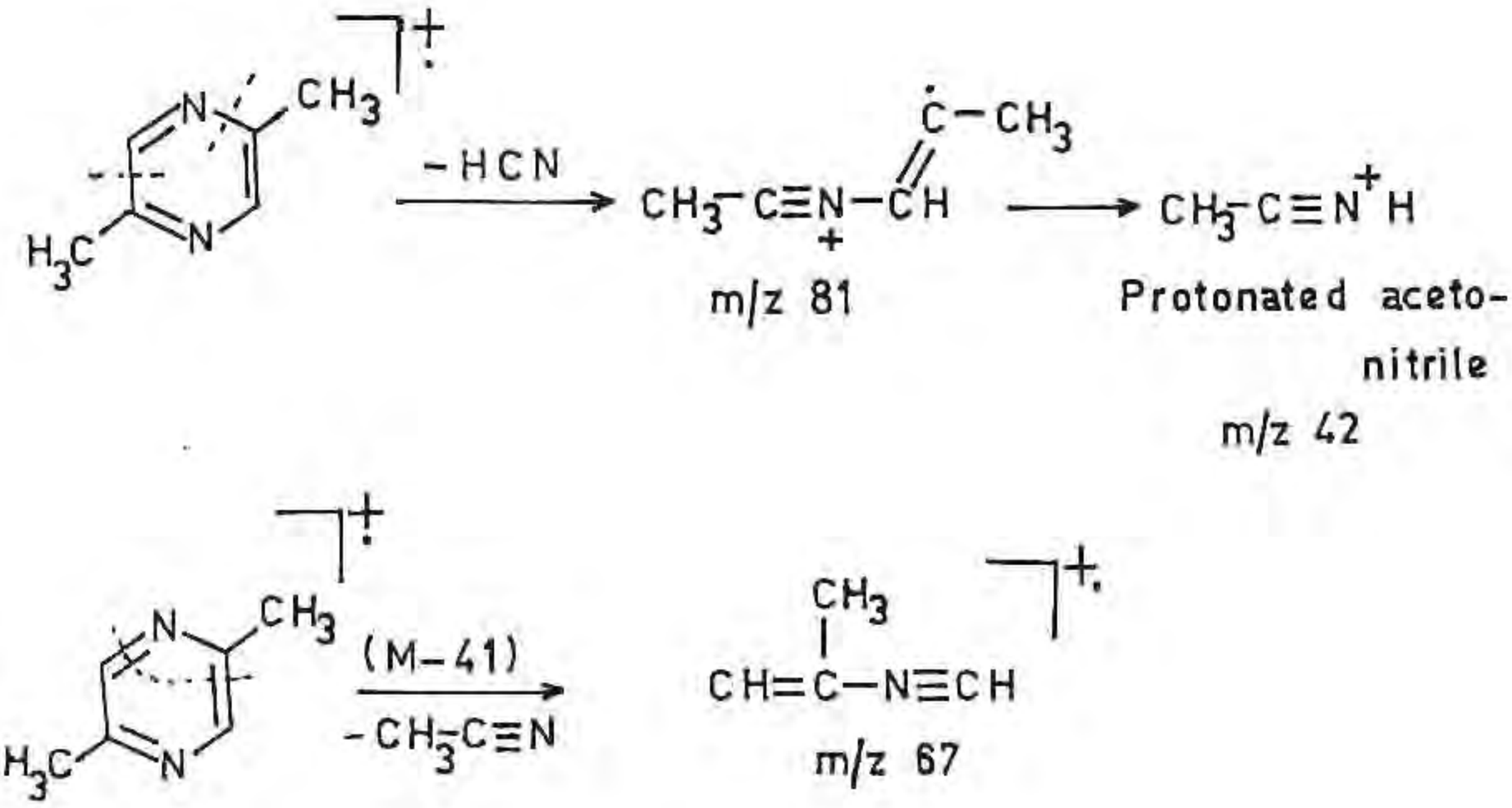






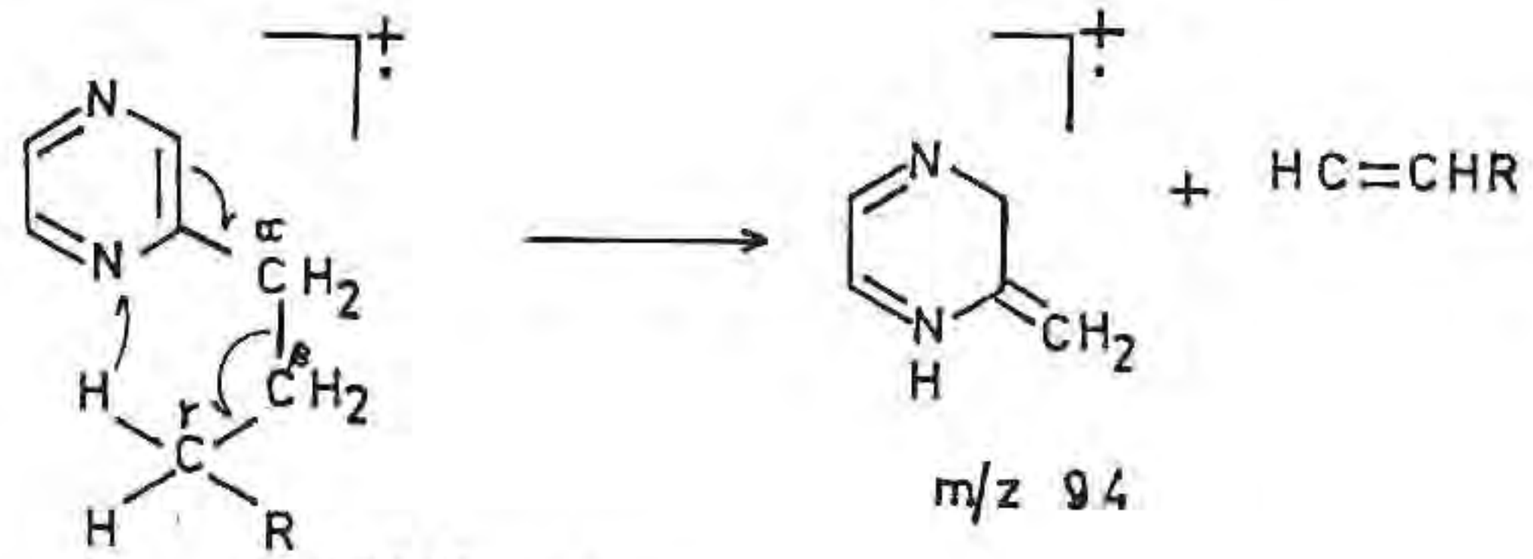
m/z 108 $(M-1)^+$ m/z 107

In the case of dimethyl pyrazines, elimination of a molecule of methyl cyanide (acetonitrile) corresponds to (M-41) fragment. Thus, in 2,5-dimethyl pyrazine we get m/z 67 as a prominent peak, in addition to molecular ion base peak.



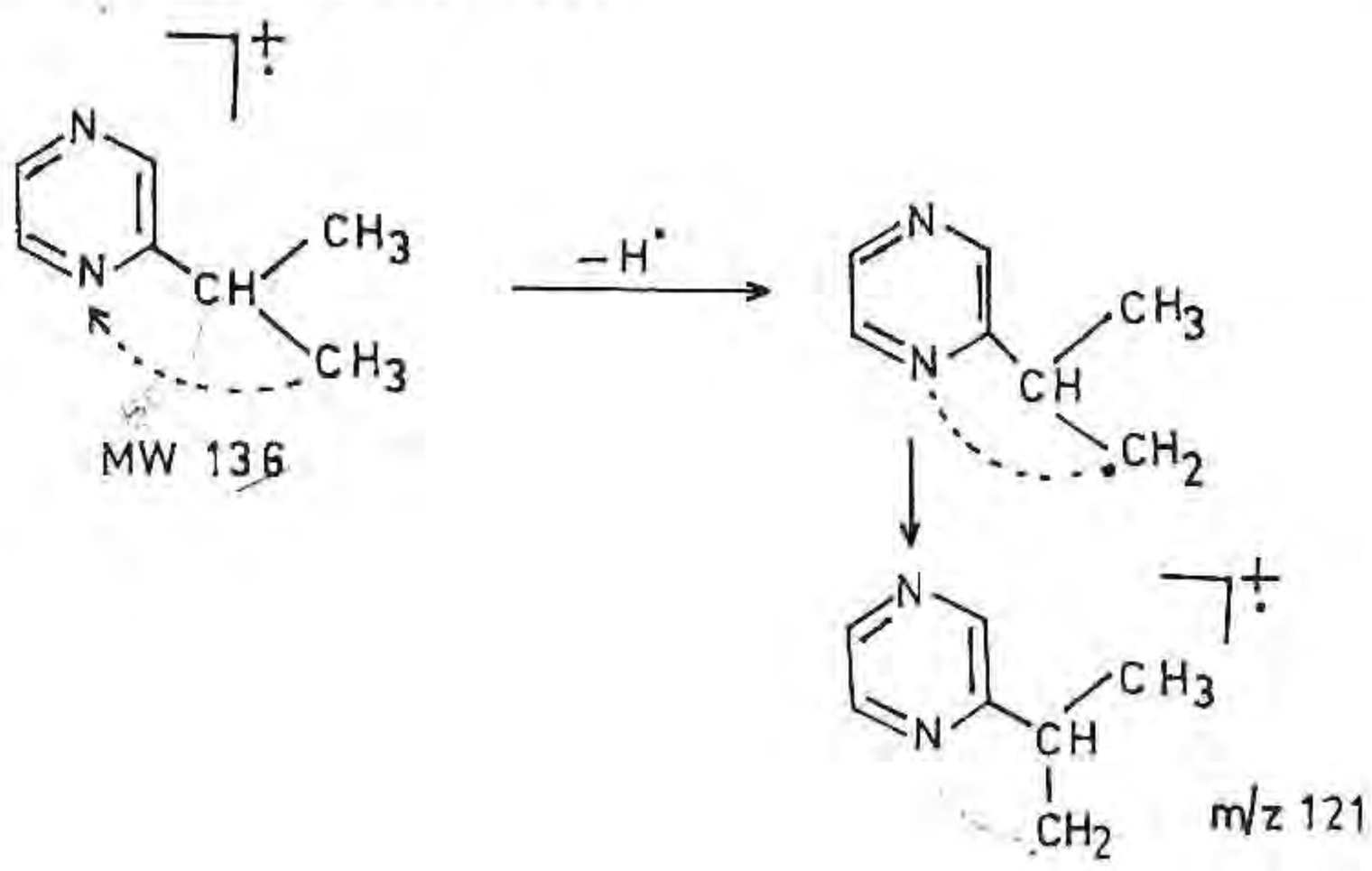
Pyrazines, substituted with an alkyl side chain with a length of three or more carbons undergo a McLafferty type rearrangement clearing the bond between the C_1

and C_2 carbons. In a normal propyl pyrazine, this results in a large (M-28) peak. γ-Hydrogen is transferred to the pyrazine nitrogen via a 6-membered transition state.

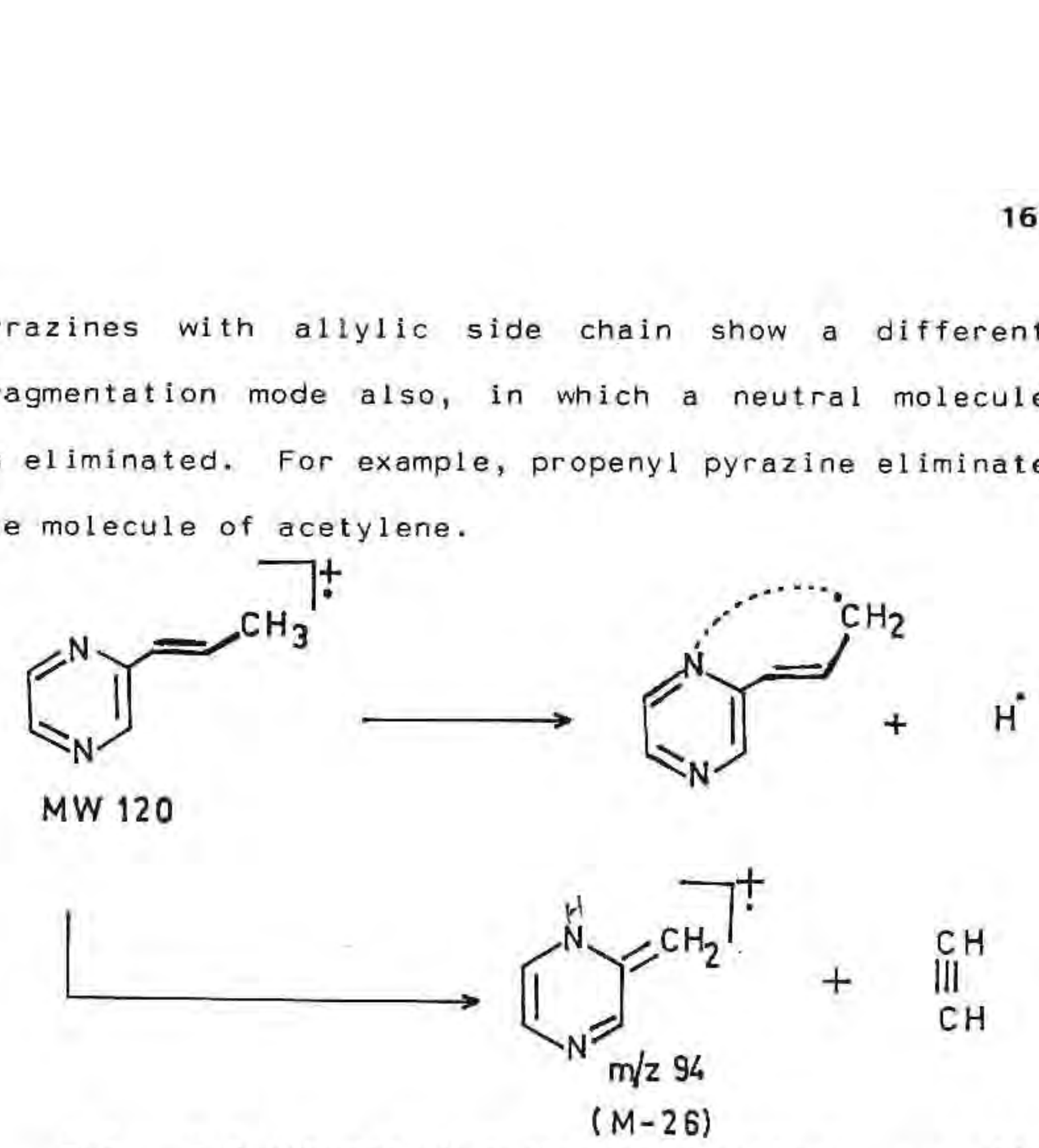


MW - Molecular Weight.

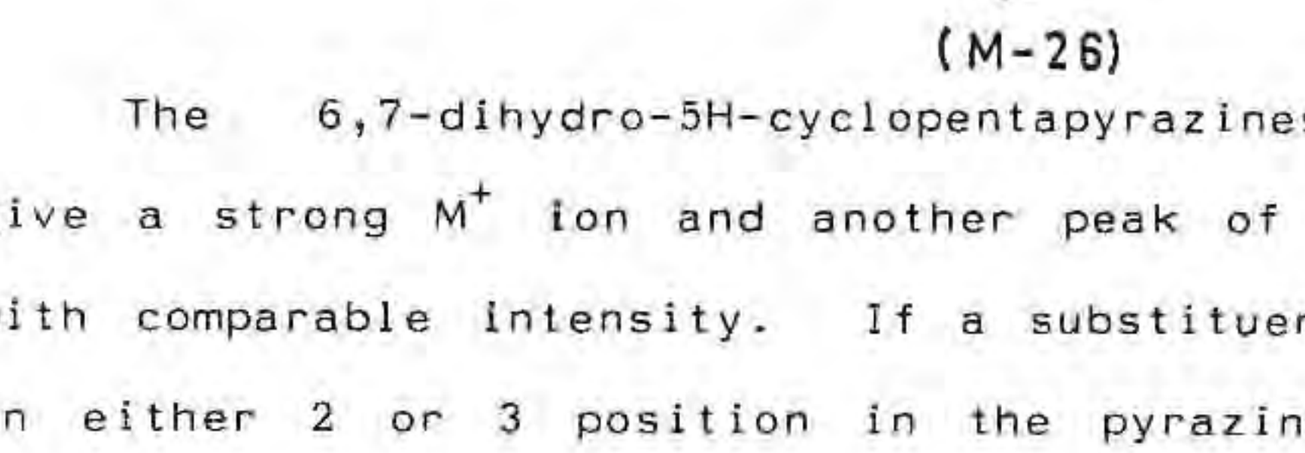
If the side chain has secondary or tertiary carbon atom, containing substituents, usually a γ-hydrogen is eliminated. Thus, isopropyl pyrazine shows a prominent peak at m/z 121.



Pyrazines with allylic side chain show a different fragmentation mode also, in which a neutral molecule is eliminated. For example, propenyl pyrazine eliminates one molecule of acetylene.

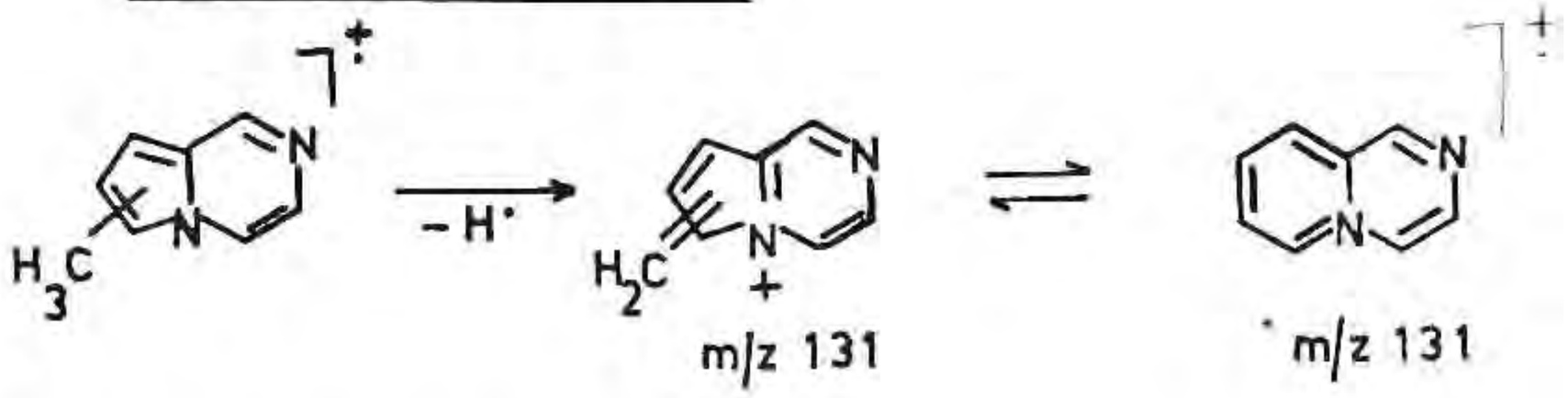


The 6,7-dihydro-5H-cyclopentapyrazines usually give a strong M^+ ion and another peak of $(M-1)^+$ ion with comparable intensity. If a substituent is there in either 2 or 3 position in the pyrazine ring, it will not be lost easily. On the other hand, if it is in cyclopentane ring, (M-15) ion forms a prominent peak.

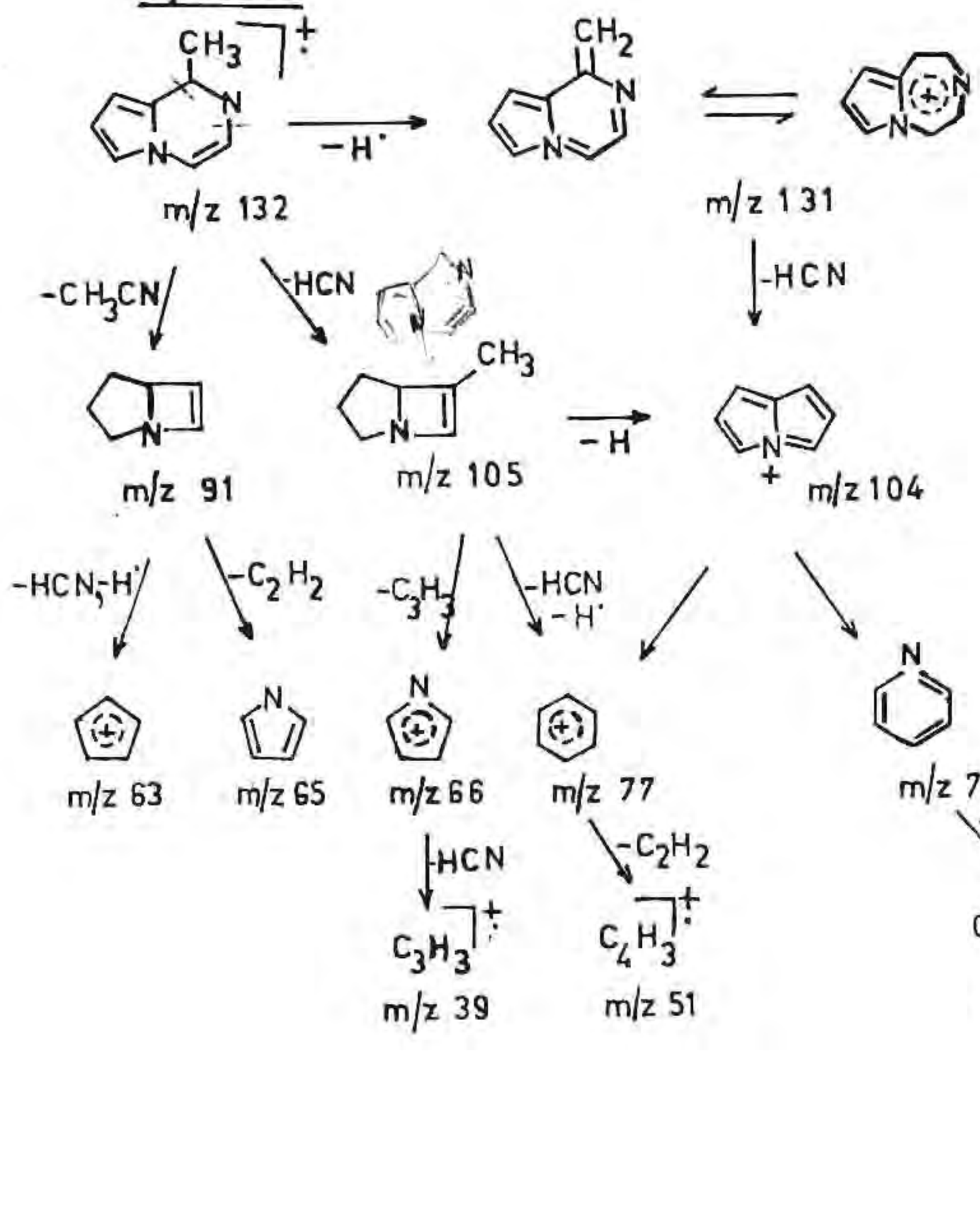


Unsubstituted as well as alkyl derivatives of

6(7,8) Methyl pyrrolo(1,2-a) pyrazine



1 Methyl pyrrolo(1,2-a) Pyrazine



pyrrolo(1,2-a) pyrazine give very similar spectra[24]. The principal ions being M^+, $(M-1)^+$ and $(M-28)^+$. Modes of fragmentation have been discussed in a recent paper by Baltes and Bochmann[24].

On the whole, the mass spectra of pyrazines are useful in the determination of the type of groups attached to the nucleus, but not necessarily their position in some derivatives. The sequence of elution of the original standards in a capillary column is also helpful. Moreover, spectra of unknown peaks should be comparable with spectra of authentics under the conditions of experiment.

CHANGES IN THE NON-VOLATILE CONSTITUENTS OF COCONUT ON HEATING

The flavour enhancement of coconut during heating appears to be due to non-enzymic browning reaction between amino acids and reducing sugars. To understand these changes, the investigation was directed towards the changes occurring in the food constituents like lipids, carbohydrates and proteins, as a result of heating.

3.6 CHANGES IN THE LIPIDS OF COCONUT ON HEATING

Coconut is a rich source of vegetable oil and the dried kernel contains 68 to 70 percent of it. When the kernel is heated it is seen that flavour of both

coconut and coconut oil are greatly enhanced. Since lipids form the major constituents of coconut, estimation of their physicochemical characteristics, and fatty acid composition are studied in heated and unheated (control) samples of coconut.

3.6.1 Extraction and physicochemical characteristics of lipids

Table 26 represents the neutral and total lipid contents of coconut heated at different temperatures and of the control sample without heating. The neutral lipids in all the samples were found to be about 71 percent and the total lipids were estimated to be about 75 percent. The difference between the two values represents the polar lipids (2.5 to 3.6 percent). Thus, it was found that the neutral lipids were the major components in the total lipids of coconut. As a result of heating, there was no significant change in neutral lipids, whereas some decrease was noted in the case of total lipids viz. in polar lipids.

In order to study the changes occurring in lipids during heating, the physicochemical characteristics were also found out. The results, represented by Table 26 indicated that the changes were not significantly different. The free fatty acid contents of control and heated samples of both neutral and total lipids

Table 26 : Changes in the content and physicochemical characteristics of lipids of coconut during heating*

	Mode of extraction							
	Petroleum ether (Neutral lipids)				$CHCl_3$/MeOH extract (total lipids)			
	Control	130°C	145°C	160°C	Control	130°C	145°C	160°C
Lipid content (total solvent extractives)	71.40	70.80	71.00	70.80	74.90	74.10	74.50	73.30
Polar lipids					3.50	3.30	2.50	2.50
Free fatty acid content (FFA %)	0.10	0.10	0.12	0.11	0.12	0.13	0.14	0.13
Peroxide value	0.00	0.00	0.00	0.00	0.30	0.30	0.40	0.50
Sp.Gr. (relative density) at 60°C	0.9183	0.9183	0.9183	0.9183	0.9184	0.9183	0.9183	0.9182
Refractive Indiex, n_{40}^{D}	1.4553	1.4553	1.4552	1.4547	1.4553	1.4552	1.4552	1.4547
Colour in Lovibond units (Y+5R) using 1" cell	0.7Y	0.7Y+0.5R	1.6Y+1.5R	2.6Y+5.0R	2.3Y+1.0R	2.5Y+1.0R	2.0Y+2.5R	3.3Y+5.0R
Iodine value (IV)	9.00	8.60	8.66	8.57	8.19	7.66	7.58	7.50
Saponification value (SV)	262.2	262.1	260.0	260.3	262.7	262.0	261.0	261.0
Reichert-Meissl value (RM value)	8.52	8.51	8.29	8.35	9.20	9.30	9.50	9.50
Polenske value (P value)	14.2	14.0	14.6	14.5	14.0	14.2	14.4	14.2
Unsaponifiable matter (%)	0.147	0.209	0.164	0.154	0.152	0.220	0.178	0.169

* Mean of three determinations

were almost same only. The same trend was noticed in specific gravity and refractive index values also. However, the peroxide value was nil in neutral lipids, whereas positive values were obtained for total lipids. The colour values of total lipids was higher than that of neutral lipids and increased with temperature of heating. The iodine value of neutral lipids of coconut (control sample) was slightly more than that of total lipid and the value decreased in heated samples. The decrease was in total lipids. The saponification values of lipids of control and heated coconut samples were found to be in the range 260 to 262; the value slightly decreased in heated samples. The Reichert-Meissl value (RM value) of control sample agreed with reported value for coconut oil. The values were found to be slightly higher for total lipids indicating that polar lipids contained more of steam volatile, water soluble (short chain) fatty acids. The change in RM value on heating was very little only. A value of 14 to 15 was obtained for Polenske value (P value) which is a measure of steam volatile, water insoluble fatty acids. As a result of heating, not much change could be noticed in these values. The unsaponifiable matter ($\approx$ 0.15 percent) content was more or less same in neutral and

total lipids and in both cases, the value increased to a small extent and then decreased.

3.6.2 Discussion

The total, neutral and polar lipids (difference of the first two) contents of control samples of coconut agreed with the values reported earlier by Krishnamoorthy and Chandrasekhara[247], who had reported 74±0.9 percent and 71±1.2 percent for total and neutral lipids respectively. As a result of heating, a slight decrease in polar lipids was noticed which indicated either destruction or degradation of polar lipids during heating and a consequent reduction in solubility. It has been reported that as a result of heating, polar lipids in general and phospholipids in particular, were affected during roasting of pistachios[155] also. Polar lipid content has been reported to decrease during roasting of soyabeans too[248] and our observation is valid in this respect. The effect of heating on lipids, has been investigated in meat[249] and cocoa butter[250] and has been shown that they are largely responsible for the development of characteristic flavour notes which distinguish one type from the other.

The minor changes in lipids due to heating are not seen to reflect in the values of physicochemical

characteristics. The more noticeable changes are in colour values which show a deepening tendency, with heating. The colour of total lipids is much higher compared to neutral lipids. This can be due to solubility of the brown colour formed additionally during heating. The exact chemical composition of these brown pigments are not clearly understood but they have been reported[101] to contain carbon, nitrogen and oxygen, the chemical nature being complex and polymeric. They have been reported to be non dialysable too. The antioxidant property of non-enzymic browning reaction products towards oxidative rancidity, is reported in literature[226,227]. It is possible that these have phenolic type of (-OH) groups which make them more soluble in polar solvents.

The iodine value of total lipids is lower than that of neutral lipids which is different from the observation of Krishnamoorthy and Chandrasekhara[247]. Upon heating, the iodine values decreased slightly, both in neutral and total lipids, which is an observation consistent with other reports also[155]. These changes can be attributed to degradation of unsaturated fatty acids by heat or by cross-linking through the unsaturated sites, reducing their availability. The saponification value is not affected noticeably which indicates

more or less intact nature of the glyceride structure. The Reichert-Meissl values and Polenske values do not exhibit major changes. The possible reason is that the minute changes taking place at the glyceride level are far below the sensitivity of the methods generally used for these estimations. The unsaponifiable matter content of the samples show slight increase in value at 130°C and then decrease at higher temperatures. Browning prdoucts which are formed at 130°C, are likely to be extracted with solvent and may remain along with other unsaponifiables. On further heating, either these products are changed to high molecular weight polymers which become less soluble or partly destroyed by heat and thus the content may be reduced.

The storage stability of coconut oil extracted from heated gratings was mentioned earlier in this thesis. The lipids extracted from all the heated samples contained more of unsaponifiable matter compared to control sample. This is probably due to the extracted 'melanoidins' also. Though coconut oil is a saturated oil and the browning products exhibit antioxidant effect towards oxidative rancidity[227], the possibility of Maillard reaction products improving stability of coconut oil can not be ignored.

3.6.3 Fatty acid composition of lipids of control and heated samples of coconut

Results of the GC analysis of the methylated fatty acids of neutral and total lipids of control and heated coconut samples are detailed in Table 27. Analysis showed presence of even numbered fatty acids from C_6 (caproic) to C_{20} (arachidic) and also unsaturated fatty acids like $C_{18:1}$ (oleic), $C_{18:2}$ (linoleic) and very small amounts of $C_{18:3}$ (linolenic). Lauric acid ($C_{12:0}$) was the most predominant fatty acid (≈ 48 percent) followed by myristic ($C_{14:0}$, 18 to 19 percent). Stearic ($C_{16:0}$, 7 to 8 percent), and caprylic ($C_{8:0}$, 8 percent). Coconut lipids were predominantly saturated in nature (≈ 93.0 percent). Between neutral lipids and total lipids, the latter contained lower percentage of unsaturated fatty acids. As a result of heating, unsaturation decreased and this was reflected in the fatty acid profile also. The decrease in iodine value, discussed earlier, also support the results of GC analysis. Heating slightly affected the most abundant acid, namely lauric acid ($C_{12:0}$). $C_{6:0}$, $C_{8:0}$ and $C_{10:0}$ acids showed only minor variation on heating and their relative concentration in neutral and total lipids were also comparable. Only very minor variations were noticed in $C_{14:0}$, $C_{16:0}$ and $C_{20:0}$ acids. The unsaturated fatty acids showed

Table 27 : Fatty acid composition (%) of lipids of coconut heated to different temperatures

Fatty acid	Mode of extraction							
	Petroleum ether				Chloroform/methanol (2:1)			
	Control	130°C	145°C	160°C	Control	130°C	145°C	160°C
6:0	0.43	0.36	0.40	0.43	0.27	0.38	0.19	0.25
8:0	8.08	8.07	8.10	9.02	8.08	8.52	6.95	7.54
10:0	5.73	5.73	5.65	5.24	5.67	5.92	5.20	5.37
12:0	47.55	46.11	46.76	46.88	48.10	46.85	45.60	45.10
14:0	19.02	17.59	19.24	17.39	18.74	18.88	18.21	16.60
16:0	7.03	7.89	8.31	6.55	7.58	7.47	7.90	6.14
18:0	3.41	4.20	3.95	3.90	3.11	3.30	4.12	4.10
18:1	4.56	4.66	4.21	4.20	4.51	3.68	3.48	3.22
18:2	2.75	2.32	2.71	2.78	2.28	2.41	2.47	2.54
18:3	-	0.12	-	0.04	0.19	0.47	0.09	-
20:3	0.54	0.22	0.75	0.32	0.61	0.33	0.15	-
Total unsaturated fatty acids	7.31	7.10	7.67	7.02	6.98	6.56	6.04	5.76

a definite decrease as a result of heating, and the change was more for oleic acid ($C_{18:1}$) in the total lipid extract.

3.6.4 Discussion

From the fatty acid profiles of neutral and total lipids of control and heated coconut, it can be seen that unsaturated fatty acid content is less in total lipids. The observation is different from that of Krishnamoorthy and Chandrasekhara[247], who had reported higher iodine values for total lipids. As a result of heating, there is not much change in the overall fatty acid profile of neutral and total lipids except at 160°C. In this sample, a decrease is noticed in the unsaturated fatty acid content of total lipids, which again indicates changes in polar lipids. Similar observations have been reported in baked potato products[251] and roasted pistachio kernels[155]. The unsaturated fatty acids which are lost, are supposed either to form flavour compounds like alcohols, aldehydes, ketones etc or to cross-link, giving polymeric compounds[153]. The unsaturated fatty acids of polar lipids must be more affected in this way, since the change in unsaturated fatty acids was more in the total lipid extract.

3.7 CHANGES IN THE CARBOHYDRATES OF COCONUT ON HEATING

The sweet taste of coconut and its delicate falvour are largely responsible for its ready acceptability as a food item. When coconut is heated, the aroma characteristics are greatly enhanced but the sweet taste of coconut changes possibly due to changes in the sugars present. Since, carbohydrates are a major class of food constituents, the changes they undergo during heating of coconut are important from flavour as well as nutritive points of view.

3.7.1 Changes in the sugars and starch of coconut on heating

Table 28 represents the changes in carbohydrates of coconut during heating. Total sugars, reducing sugars, starch (and dextrins) of dry coconut gratings were found to be 8.32, 0.82 and 3.36 percent respectively. Total available carbohydrates were also estimated to be 11.68 percent for the same sample. As a result of heating, total soluble sugars and hence the total available carbohydrates decreased noticeably (9 and 7 percent respectively). The reducing sugars decreased and then slightly increased to become almost steady at 145 and 160°C. High performance liquid chromatographic (HPLC) analysis showed that sucrose was the major sugar in coconut, followed by glucose

Table 28 : Changes in the carbohydrates of coconut during heating (values as % on dry weight basis)

	Control	130°C	145°C	160°C
Total available carbohydrates	11.68	10.46	10.96	10.83
Total soluble sugars	8.32	7.68	7.70	7.57
Reducing sugars	0.82	0.68	0.70	0.70
Ribose	trace	trace	trace	trace
Rhamnose	trace	trace	trace	-
Fructose	0.20	0.17	0.18	0.18
Glucose	0.32	0.25	0.26	0.24
Galactose	0.28	0.26	0.26	0.26
Sucrose	7.50	7.00	6.90	6.85
Starch and dextrins	3.36	3.28	3.26	3.26

* Mean of three determinations

trace - 10 to 30 mg/100g

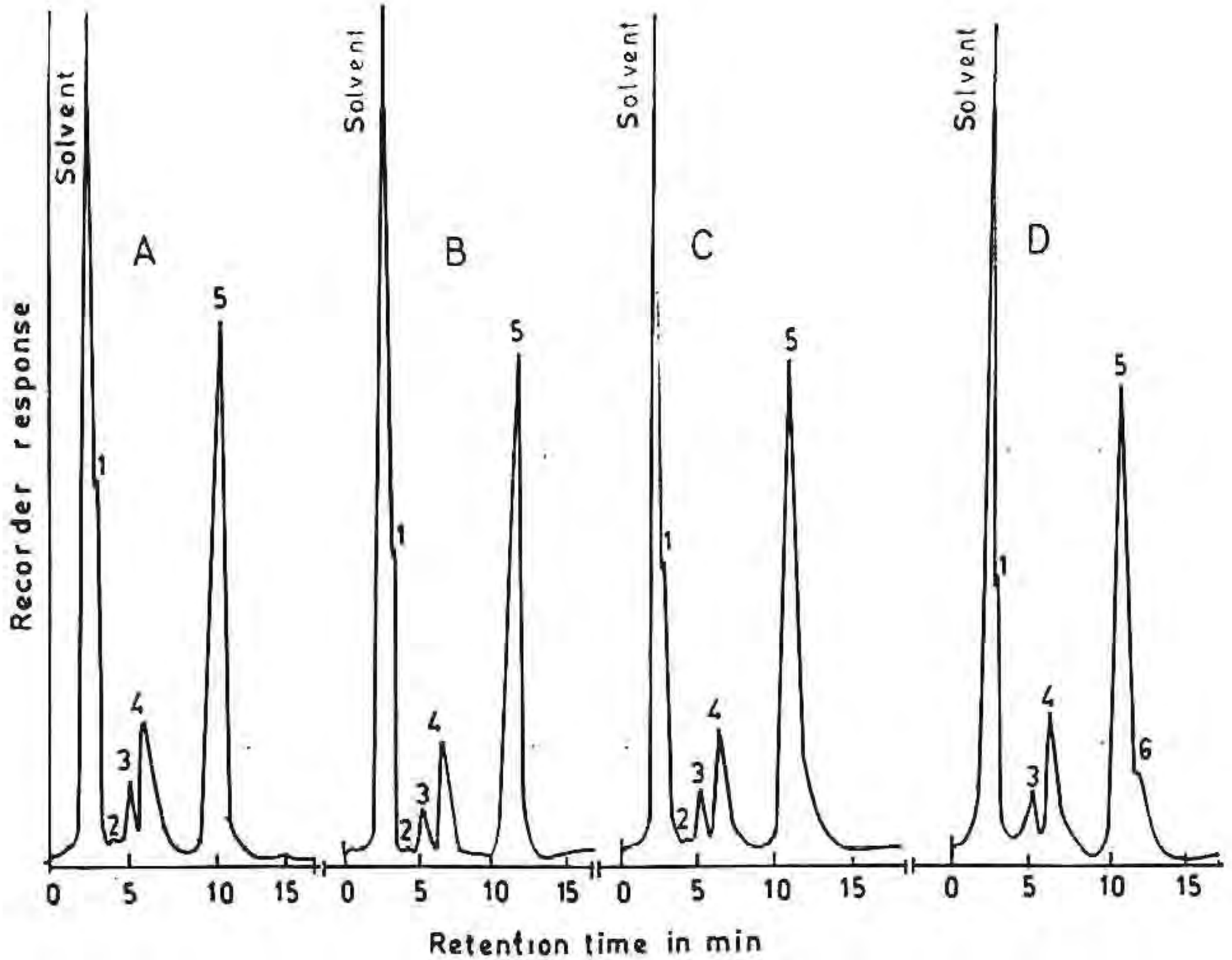


Fig.23. HPLC Separation of sugars of heated coconut samples.
A.Control B.130°C C.145°C D.160°C.
1.Ribose 2. L-Rhamnose 3. Fructose 4. Glucose/Galactose 5. Sucrose
6. Maltose.

and fructose. Trace amounts (less than 10 to 20 mg/100g) of ribose and L-rhamnose could also be detected. Earlier workers like Caray[252] and Bhown[253] had reported galactose also in coconut. However, in the HPLC column used, and under the conditions followed in this study, glucose and galactose could not be separated and so eluted as a single peak. Hence, glucose was estimated separately by glucose oxidase-peroxidase enzyme method, according to Holm et al[213] and from this, glucose and galactose contents were separately estimated. The presence of galactose was further confirmed by paper chromatography as suggested by Bhown[253]. The analysis of samples, heated at different temperatures showed that the total sugars decreased by 7.7 percent in the first stage of heating. The reducing sugar value was brought down from 0.82 to 0.68 (about 17 percent decrease). The detailed analysis of sugars by HPLC (Fig.23, Table 28), showed that traces of ribose were present in heated samples, whereas rhamnose could not be detected in final stages. Fructose and glucose were affected much more than galactose. While the sucrose content decreased slightly during heating, glucose and fructose decreased and then the values remained almost steady. The starch content showed only slight decrease from 3.36 to 3.26 percent.)

3.7.2 Discussion

As a result of heating, the reducing sugars are affected noticeably (17 percent decrease in content) which indicates that during roasting of coconut, reducing sugars are utilised in some chemical reactions. From literature, we understand that Maillard type of reactions take place during roasting of nuts, kernels etc in which reducing sugars and amino acids play a major role. Sugars have been reported to be affected during oil roasting of peanuts, by Oupadissakoon and Young[156]. The decrease in sucrose content and simultaneous increase in glucose and fructose may be attributed to hydrolysis of sucrose at higher temperatures, giving glucose and fructose, as suggested by Evans and Butts[254] and Anantharaman and Carpenter[255]. At the same time, glucose and fructose are being consumed, for browning, in a parallel reaction also. The sucrose may be undergoing hydrolysis and possibility of caramelisation reaction also exists. Rohan and Stewart[256] had studied the changes in sugars of roasted cocoa and they reported almost complete destruction of reducing sugars. Sucrose was found to participate in the browning reaction, after being hydrolysed in the hot acid medium of cocoa beans. In pistachio kernels also, during roasting, reducing sugars have been found to

be affected much, by Kashani and Valadon[257]. According to them, starch is also affected during the process. In the present study on coconut, it is found that starch content is slightly affected at temperatures of 145 and 160°C. Starch is likely to break down into smaller units, especially maltose units, under the impact of heat[145]. In our study the appearance of maltose peak, eluting with the receding sucrose peak, in the corresponding HPLC profiles, lends support to this.

3.8 CHANGES IN THE PROTEINS OF COCONUT ON HEATING

The nutritive value of any food material is correlated with the quality of protein it has. Proteins also play important role in retaining the texture, flavour etc of the food. Since they are heat sensitive compounds, roasting can affect their solubility and nutritive value. Moreover, the participation of amino acids, which are the constituents of proteins in Maillard reaction, are discussed quite often in literature. They play a vital role in producing volatile heterocyclic compounds. In this context, the study of the changes in proteins and amino acids of coconut during roasting, are very relevant and can possibly explain the enhancement of flavour observed on heating.

3.8.1 Changes in the protein and non-protein nitrogen contents of coconut on heating

Table 29 represents the changes in total nitrogen, non-protein nitrogen and crude protein contents of coconut, heated at 130, 145 and 160°C with reference to heated coconut (control). It can be seen that total nitrogen and hence, the crude protein present are not very much affected during heating. The non-protein nitrogen (NPN) content of coconut was found to be 0.189 percent on dry weight basis, which is nearly 13 percent of the total nitrogen. On heating, the NPN decreased to 0.156 at 130°C, marking a sharp fall of 17 percent and was further reduced to 0.152 and 0.149 during subsequent heating. The overall reduction in NPN content was a significant 21 percent.

3.8.2 Discussion

The values reported in Table 29 for total nitrogen and crude protein content are comparable with reported values[161,164]. From the preliminary experiments, it can be seen that on roasting, total nitrogen and crude protein contents do not change much. This is understandable because the volatile nitrogenous compounds, which would have possibly escaped the nitrogen estimation are present in 100 to 120 ppm levels only. These changes will not be reflected in the semimicro kjeldahl

Table 29 : Total nitrogen, non-protein nitrogen, crude protein and actual protein contents of control and heated samples of coconut (g/100g on dry weight basis)

	Control	130°C	145°C	160°C
Total nitrogen	1.37	1.35	1.33	1.33
Non-protein nitrogen (NPN content)	0.189	0.156	0.152	0.149
% NPN in total nitrogen	13.70	11.40	11.10	10.90
Crude protein (by Kjeldahl method)	8.54	8.43	8.37	8.37
Actual protein (from amino acid analysis)	7.42	7.31	7.28	7.25
Actual protein (calculated) [(Kjeldahl N-NPN) x 6.25)]	7.38	7.36	7.36	7.38

estimation results. The non-protein nitrogen (NPN) content of coconut is reported first time and is moderately high (0.189 percent) and is comparable with NPN contents of pistachio kernels[257], beef[258], pork[258], cashewnuts[241] etc. Nitrogenous species like free amino acids, lower peptides and nucleotides are generally present in the non-protein nitrogen extract. A reduction in NPN content on roasting of coconut, indicates that NPN compounds are taking part in non-enzymic browning reactions, during heating. Participation of NPN species in such reactions has been reported in roasted cocoa[34,157], peanuts[259], cooked beans[260], potato products[261] etc accompanied by roasted or cooked flavour development. In roasted cocoa, the NPN-precursor, contributing maximum to the flavour development, was identified to be a smaller peptide. Under the conditions of heating, followed in the present study, it can be assumed that coconut provides a suitable environment for Maillard reaction to take place, since it is rich in reducing sugars and NPN species.

3.8.3 Changes in the protein solubility of coconut during heating

The protein solubility of heated and unheated coconut were determined at pH values ranging from 0 to 14. Usually, the solubility studies are limited to

the pH range of 2 to 12 since the proteins soluble in this pH range are more available to the human systems. In the present study, solubility in the entire pH range (0 to 14) was determined mainly for academic interest. Figure 24 gives the graphic representation of this. In the control sample, the solubility was maximum at pH 12 and 13 and also at pH 1.0. Maximum solubility was noticed at pH 3.0. As heating progressed, the solubility came down significantly. At 130°C, the solubility was reduced at all pH values except at pH 14. The reduction in solubility was more or less 50 percent, in the pH range 3 to 11. At 145°C the solubility was found to be lowered, at all pH values, still further. Thus, the maximum solubility at pH 12.0, was reduced by 40 percent. Except at a few pH values, highly acidic or alkaline, the solubilities at all other pH values. were found to be less than 10 percent. At 160°C, the change was even more drastic, and at pH values in the range 3 to 10, the solubility was below 1 to 2 percent. Table 30 gives the solubility of various samples at different pH values. On the whole, the protein solubility was very much affected as a result of roasting.

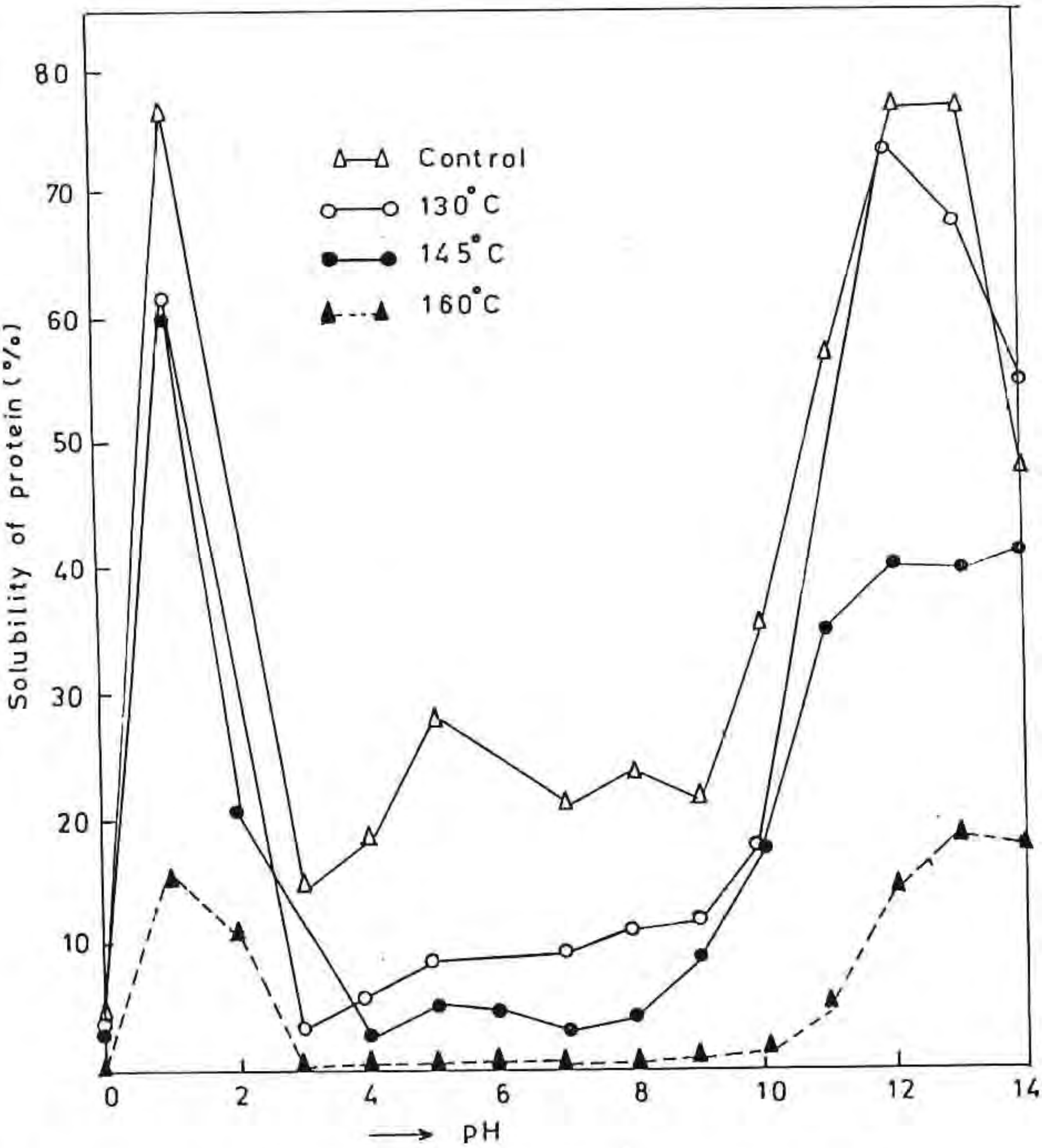


Fig.24. Solubility profiles of heated coconut at various pH values.

Table 30 : Changes in solubility profile of coconut proteins as a result of heating, at different pH values

pH	% Solubility of samples studied			
	Control	130°C	145°C	160°C
0	4.8	3.3	2.9	0.2
1	76.7	61.5	60.0	15.7
2	47.4	20.7	20.7	11.1
3	14.1	3.7	4.1	0.0
4	18.5	5.5	2.9	0.2
5	28.9	8.5	5.2	0.4
6	24.4	4.4	4.8	0.4
7	20.7	9.3	3.3	0.5
8	23.7	11.1	4.1	0.5
9	21.5	11.8	8.9	0.9
10	35.5	18.5	17.8	1.5
11	57.4	37.0	35.5	5.0
12	77.4	74.1	40.7	14.8
13	77.0	68.1	40.0	19.3
14	48.1	55.5	41.5	18.5

3.8.4 Discussion

Protein solubility, also expressed as nitrogen solubility index (NSI), is often considered as a measure of protein denaturation. Samson[262] has determined the NSI values of coconut meat heated in the range 60 to 120°C and coconut meal heated to 150°C. However, for unexplained reasons, as Samson[262] himself has described it, the solubility of heated coconut meat (with full fat) was found to be lower than that of coconut meal. This is unusual to happen because heat denaturation of coconut meal which is a defatted powder, will always be more severe than coconut meat as such. From the present study, it can be generalised that solubility of protein in the range 3 to 10, is drastically reduced. The proteins soluble in this range are more accessible to human digestive systems. The heating regime followed in this study is similar to the practice adopted in (coconut) oil milling industry for the extraction of coconut oil with enhanced flavour. Even in ordinary oil extraction procedures followed in oil mills, heat is generated in the second stage of expeller extraction (temperature 130°C) and solubility of coconut cake is found to be less than that of original coconut[182]. The reduction in solubility is a clear indication of protein denaturation in any case.

3.8.5 Osborne classification of proteins of heated coconut

The preliminary study on protein solubility of coconut necessitated further study of how the different types of proteins were affected during heating. Results of the Osborne classification study of the proteins is given in Table 31. In coconut, globulins which are salt soluble proteins, were found to be the most predominant protein followed by albumins, which are water soluble. Prolamines (aqueous alcohol soluble) and glutelins (dilute alkali soluble) were less than 1 percent. The residual protein constituted about 19 percent. Upon heating, albumin was drastically reduced by 90 percent even in the initial stages of heating. The globulins were also affected but to lesser extent (50 percent reduction in solubility at 130°C) compared to albumin. However, severe changes in solubility were noticed at higher temperatures. Prolamines were reduced to one-third of their initial content. Interestingly, the glutelins showed an increase in relative concentration. Similar observation has been reported in roasted soyabeans also[248]. In the heated samples, the residual protein content was much higher and the value reached 86 percent at 160°C.

Table 31 : Osborne classification of proteins of coconut heated to different temperatures*

Protein fractions	Samples studied			
	Control	130°C	145°C	160°C
Albumin	18.63	1.62	0.85	0.42
Globulin	60.43	27.70	14.17	3.36
Prolamine	0.27	0.24	0.18	0.08
Glutelin	0.83	2.18	13.63	9.95
Residual protein	19.00	68.00	70.63	86.00

* Mean of three determinations

3.8.6 Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of coconut proteins

Since albumins and globulins were found to be the major protein fractions of coconut, electrophoretic study of these two major proteins were carried out using SDS-PAGE. Figure 25 is the photograph of the electropherograms obtained for albumins and globulins of coconut samples. The standard proteins with known molecular weight (molecular weight markers) were run in the same experiment. These are indicated along with their molecular weight as against the respective bands. Table 32 gives details of the separated protein bands of coconut samples. The results of the electrophoretic study can be explained, in brief, as follows.

The albumin fraction of coconut showed distinct bands - 3 prominent and 3 week ones. The highest molecular weights observed were 52,000 which gave a prominent band. The next one was 39,000 which was rather low in concentration. The other units were with molecular weights 35,000, 29,000, 22,000 and 14,000. Of these 35,000 and 22,000 were intense. In the case of heated samples, namely, that heated at 130°C, the albumin fraction of proteins showed faint protein bands at molecular weights 71,000 and 25,000. Two prominent bands at 15,000 and 11,000 were also noted. At 145°C,

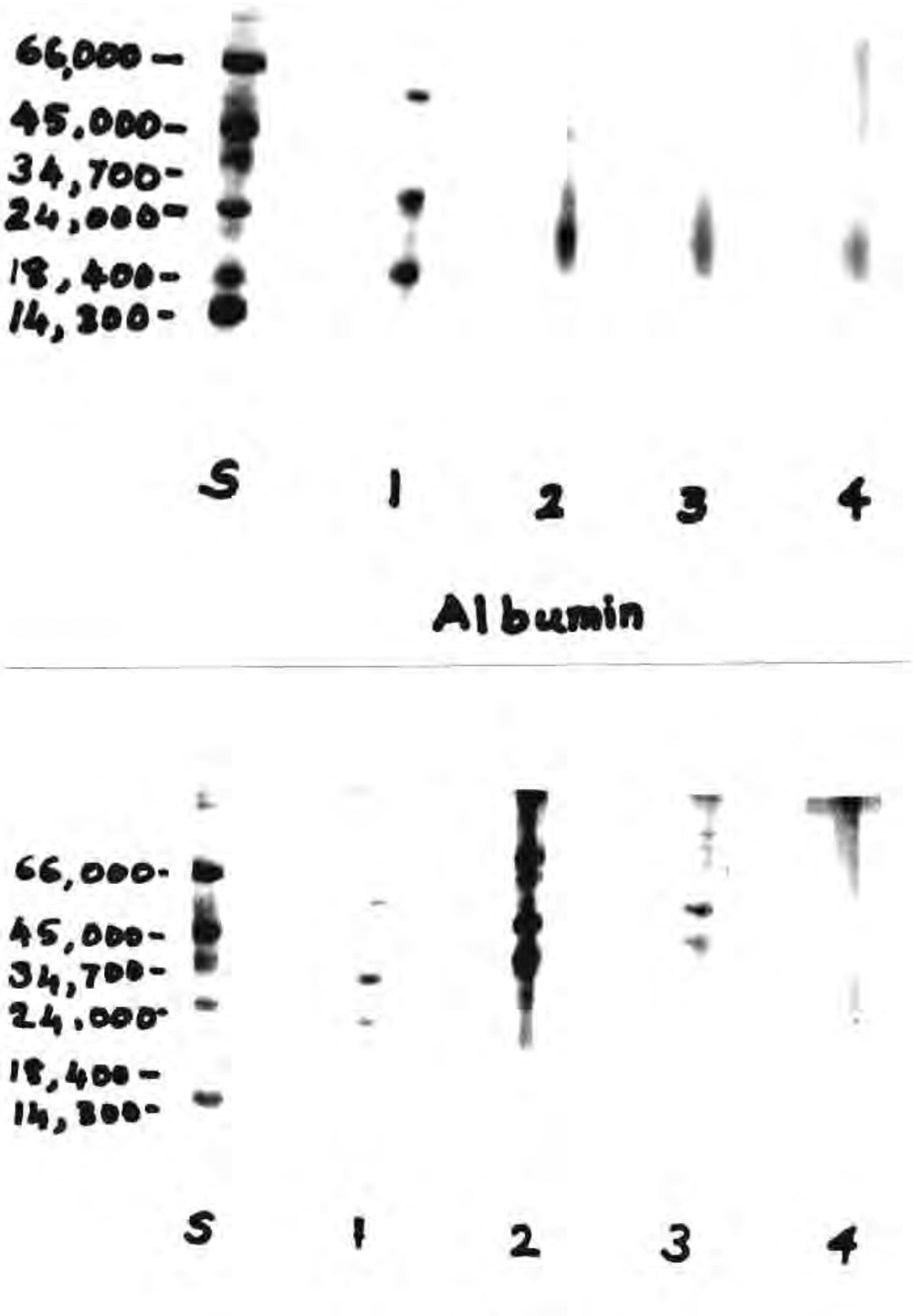


Fig.25 : SDS-PAG Electrophoretic separation of albumins and globulins of coconut heated to different temperatures. S. standard proteins (1) control sample (2) 130°C (3) 145°C (4) 160°C

Table 32: Characterisation of albumin and globulin fractions of proteins of control and heated samples of coconut by SDS-PAG electrophoresis

Standard proteins	Albumins				Globulins			
	Control	130°C	145°C	160°C	Control	130°C	145°C	160°C
Albumin (bovine) (R_f 0.210) MW.66,000	52,000 (R_f 0.310) (intense)	71,000 (R_f 0.190) (faint)	74,000 (R_f 0.170) (faint)			100,000 (R_f 0.033 to 0.055) (two bands, faint)	62,000 (R_f 0.233) (two bands, faint)	
Albumin (egg) (R_f 0.360) MW.45,000	39,000 (R_f 0.400)		64,000 (R_f 0.225) (faint)		45,000 (R_f 0.360) (intense)	56,000 (R_f 0.270)		
Pepsin (R_f 0.440) MW.34,700)	35,000 (R_f 0.450) (intense)				27,000 (R_f 0.550) (intense)	48,000 (R_f 0.331)	54,000 (R_f 0.290)	
Trypsinogen (R_f 0.570) MW.24,000	29,000 (R_f 0.520)				23,000 (R_f 0.610) (intense)	31,500 (R_f 0.497) (prominent)	35,000 (R_f 0.452) (prominent)	
β-Lactoglobulin (R_f 0.700) MW.18,400	22,000 (R_f 0.630) (intense)	25,000 (R_f 0.570) (faint)		25,000 (R_f 0.590)	17,500 (R_f 0.710)	22,500 (R_f 0.625) (prominent)	22,000 (R_f 0.635) (prominent)	
Lysozyme (R_f 0.790) MW.14,300	14,000 (R_f 0.81)	15,000 (R_f 0.780) (intense)	11,500 (R_f 0.885) (intense)	15,000 to 11,000 (R_f 0.780 to 0.900)		15,000 (R_f 0.768)	15,000 (R_f 0.780)	15,000 to 11,000 (R_f 0.780 to 0.900) (faint)
		11,000 (R_f 0.900) (intense)				11,000 (R_f 0.898)	11,500 (R_f 0.0885)	

MW - Molecular Weight

faint bands were seen at 74,000 and 64,000, and a prominent band at 11,500. At 160°C, the number of bands were still reduced and only two bands, namely one at 25,000 and a diffused band in the molecular weight range 11,000 to 15,000, were noted.

Globulins, which form the major proteins of coconut separated into 4 different bands of medium molecular weight, under the conditions of the electrophoretic study. The bands were having molecular weights 45,000, 27,000, 23,000 and 17,500. Most of the bands were visibly intense as can be seen from Figure 25. The details of the electrophoretic study are included in Table 32. In the heated samples, the globulin fraction showed more number of bands with high, medium and low molecular weight proteins. At 130°C, 7 bands were visible and the maximum molecular weight of the separated bands was also higher i.e. 100,000. Among the other bands which separated, those with molecular weights 56,000, 31,500 and 22,500 were more prominent than the bands at 48,000, 15,900 and 11,000. At 145°C, the protein consisted of a pair of faint bancs at 62,000, a single band at 54,000 and 2 prominent bands at 35,000 and 22,000. Two low molecular weight bands at 15,000 and 11,500 were also noticed. The electrophoretic pattern

of proteins (globulins) of coconut heated at 160°C showed only a diffused band with approximate molecular weight of 15,000 to 11,000. The results indicated protein denaturation on heating.

3.8.7 Discussion

Heating affects both albumins and globulins which can be understood from the observation that their solubility is drastically reduced by more than 90 percent. From the study, globulins have been found to be most abundant protein type in coconut followed by albumins. The same observation has been reported by other researchers[172,182]. The prolamines are present only in very small quantities even in unheated coconut. The glutelins show an increase in relative concentration, during heating. Similar trend has been reported by Ishii et al[248] in the case of roasted soyabeans; the reason is unexplained. The significant increase in residual protein is an indication of the extent of heat denaturation at different temperatures. The reduction in solubility of various proteins can result due to extensive cross-linking and changes in tertiary structure[142].

The electrophoretic pattern of albumins and globulins of coconut shows that individual peptides are not of very high molecular weight. However, on heating

peptide bands having higher and lower molecular weights, compared to unheated sample, are formed. There are more protein bands in the albumin fraction of control sample. In the heated samples, albumins show higher molecular weight bands at 71,000 and 64,000, and in lower molecuar weight range, three bands at 25,000, 15,000 and 11,000. At 160°C there are only 2 bands, one at 25,000 and a faint diffused band in the range 11,000 to 15,000. In the case of globulin only 3 bands are there in the control sample, whereas bands with higher and lower molecular weights have appeared at 130°C, with molecular weights as high as 100,000. At 145°C, the band corresponding to 100,000 had disappeared and at 160°C, only a faint diffused band in the molecular weight range of 11,000 to 15,000 is seen. One explanation for the disappearance of most of the peptide bands is reduction in solubility of most of these due to cross-linking and formation of very high molecular weight species. The decrease in solubility is further supported by the lower values obtained for albumins and globulins in heated samples of coconut (Table 31).

It is reported that the dye binding capacity of proteins is very much lowered by heat treatment[263].

This factor may interfere with the quantitative estimation of separated bands also and for the same reason estimation is not done. Formation of lower and higher molecular weight peptides has been reported in heated meat proteins which are subjected to SDS-PAG electrophoresis[142]. Extensive cross-linking (resulting in high molecular weight peptides) and break down of existing peptides into smaller units (resulting in lower molecular weight peptides) has been suggested by Hoffmann[142]. Similar observation has been made in the case of peanut proteins also[264]. The SDS-PAGE study of albumin and globulin proteins of coconut and their comparison with similar fractions from heated coconut are reported for the first time. Heating has definitely affected proteins and reduced its solubility, cross-linking between peptide units, has resulted in large molecular weight proteins and under the influence of heat, breakdown of already existing peptides has resulted in smaller peptide units also.

3.8.8 Changes in the amino acids of coconut on heating

Total (protein) amino acid analysis of heated and unheated samples of coconut

Effect of heating on the amiro acid composition of coconut proteins was studied. The results are represented in Table 33. As many as 18 amino acids

Table 33 : Changes in the protein and free amino acids of coconut during heating*

Amino acids	Quantity in mg/100 g dry sample								% reduction of free amino acids in final stages of heating
	Protein amino acids				Free amino acids				
	Control	130°C	145°C	160°C	Control	130°C	145°C	160°C	
Aspartic acid (+ asparagine, if present)	771	769	762	766	30.97	21.76	19.52	9.42	70
Threonine	255	250	251	256	7.20	4.77	4.06	2.55	65
Serine	343	339	347	340	8.57	7.84	3.58	5.30	40
Glutamic acid (+ glutamine, if present)	1460	1453	1452	1456	38.05	20.48	20.70	11.30	70
Glycine	288	282	286	279	5.47	1.96 u.i.p.	2.49	1.71 u.i.p.	70
Alanine	307	296	299	303	26.67	14.79	13.20	7.91	70
Valine	398	392	396	397	6.79	4.22	3.62	2.42	65
Cysteine (as half-cystine)	90	88	89	86	-	1.43	2.86	1.71	
Methionine	142	138	134	137	1.74	0.73	1.05	0.60	65
Isoleucine	256	255	252	257	3.84	2.49	3.33	1.28	67
Leucine	510	506	504	504	0.32	1.34 u.i.p.	1.41 u.i.p.	0.56	
Tyrosine+	190	185	189	186	trace	trace	1.55	1.92	
Phenylalanine	379	376	369	374	0.30	-	-	23.50	
Tryptophan	126	121	127	124	74.00	53.70	69.10	5.50	93
Histidine	181	187	188	177	4.06	1.30	1.97	4.58	
Lysine	300	269	230	207	2.86	0.96	1.87	-	100
Ammonia	-	-	-	-	0.16	0.60	0.71	-	
Arginine	1049	1042	1037	1030	15.69	9.28	10.60	5.39	66
Total amino acids	7416	7311	7279	7248	236.43	156.39	168.50	90.59	62

* Mean of two determinations; u.i.p. unidentified peaks; + Trace : 10 to 20 µg/100g

were found to be present. Glutamic acid (including glutamine, if present) was the most abundant amino acid followed by arginine and aspartic acid (including asparagine, if present). The effect of heating was not very much reflected in the protein amino acid profile except in lysine and arginine, of which, the former registered a noticeable decrease.

Free amino acid analysis of heated and unheated (control) samples of coconut

The results of the free amino acid analysis of control and heated samples of coconut are included in Table 33, for comparison with protein amino acid profiles. The free amino acid analysis confirmed the decrease noticed in NPN content. As amino acids, the reduction was 60 percent in the final stage of heating. Most of the amino acids present in total protein hydrolysate could be identified in this case also. Tryptophan was found to be the most abundant amino acid followed by glutamic acid, aspartic acid, alanine and arginine. As a result of heating, lysine was destroyed fully and tryptophan largely, followed by aspartic acid, alanine, glycine and glutamic acid. Threonine, valine, arginine, methionine, isoleucine, proline and serine were also affected during roasting(60 percent reduction).

Free ammonia was also detected and its content was found to vary during heating. Interestingly, cystine, tyrosine and phenyl alanine marked a positive change and the change was very significant in the case of phenyl alanine. This may be due to the release of these amino acids in free form by break down of peptides during heating. A few unknown peaks were also noticed in the amino acid chromatograms.

3.8.9 Discussion of amino acid analyses

Amino acid composition of proteins of coconut was comparable with reported values[182,265], except for slightly lower values for proline, aspartic acid, glycine, and tryptophan. The presence of large quantities of glutamic acid in the free as well as in bound form, can probably explain the wholesome taste perceived while taking coconut preparations. Moreover, when coconut is added to any food preparation, the overall flavour is greatly enhanced. During roasting, it is seen that the protein amino acids are not affected much except lysine and arginine which appear to have been affected, even in the bound form. The observation is, however, supported by the results of Evans and Butts[254] who had found that lysine and arginine interacted with sugars even in the peptide (bound)

form. Kashani and Valadon[257] had studied the changes in proteins of pistachio kernels (nuts) during roasting. They could not find any major change in protein amino acid composition as a result of heating. Thus, it is likely that it is preferably the free amino acids which readily intereact with reducing sugars.

Free amino acid composition of coconut and the changes in it, during heating are reported for the first time. Baptist[266] had analysed the aqueous extract of coconut endosperm and coconut water, for free amino acids, at varying stages of maturity, using paper chromatography, rather qualitatively. γ-Amino butyric acid was reported to be present in large proporation. However, collection of quantitative data or further systematic analysis of free amino acids, was not carried out thereafter. In the present study, tryptophan was found to be most abundant followed by glutamic acid, aspartic acid, alanine and arginine. The presence of γ-amino butyric acid could not be confirmed or estimated.

The participation of free lysine in non-enzymic browning reaction can be understood since, even in the bound state, the ε-amino group of lysine is found

to be reactive. In the free state, the activity of lysine will be very much enhanced due to availability of two free amino groups. Lysine, arginine and methionine have been reported to be significantly involved in the reactions during roasting of pistachio nuts also[257]. During roasting of peanuts, amino acids like aspartic acid, glutamic acid, alanine, isoleucine and phenyl alanine, in free form, were found to be affected more[156,259]. In the case of cocoa beans also, the free amino acids threonine, glutamic acid, phenyl alanine, isoleucine, histidine and phenyl alanine were found to be destroyed more during heating[157]. Maga and Sizer[261] have reported methionine, glycine, lysine, phenyl alanine, tyrosine, serine and isoleucine were more heat damaged in extruded potato products. In short, the reactivity of most of the affected amino acids noticed in this study is supported by results reported in other systems also. Moreover, participation of free ammonia also in Maillard reaction in food systems is quite possible, since model reaction studies have been carried out with ammonia as nitrogen source and the volatile products with roasted aroma have been analysed[81]. The occasional increase noticed in the case of a few free amino acids can be explained as

due to the release of these amino acids in free form by break down of peptides during heating[142]. A few unknown peaks were noticed in the free amino acid profiles of heated samples. This possibly represents the Amadori products and smaller peptides.

CHAPTER 4

DEVELOPMENT OF ROASTED FLAVOUR IN PALM KERNEL

As already indicated in Chapter 1, the versatility of the oilpalm fruit lies in providingtwo types of oil- palm oil from mesocarp and palm kernel oil from endo- sperm. India has been facing vegetable oil shortage for quite sometime and vegetable oil is imported from other countries to meet the growing requirements of the society. The Oil Seed Mission programmes recently introduced in the country, are the result of a con- scious effort to improve the vegetable oil situation in India. In this context, the importance of oil palm cultivation is understandable since it gives the highest oil yield per hectare. Fortunately, agroclimatic con- ditions, prevailing in some parts of India are favourable for its cultivation. Under Indian conditions, the yield of palm oil is about 4 to 5 tonnes/hectare/year[186]. Usually, kernel oil will be approximately one-fourth of the oil yield obtainable from mesocarp.

The composition of palm kernel oil is very similar to coconut oil. Poor handling and storage of palm kernels may lead to lipolysis and high acidity[187]. Technical aspects of palm kernel oil extraction and processing have been given much attention. The incidence of browning of palm kernels leading to coloured oil is reported[192] in literature. This can happen during

processing of oil palm fruits under pressure for a long time or during storage of palm kernels. Fresh, unsterilised kernels have a moisture content of 22 percent and rapid drying is necessary for getting quality kernels and oil. Even when dried kernels are stored under humid conditions or in big heaps, browning of kernels is noticed due to spoilage and biological heating. The discoloration is more in the case of broken kernels. Studies on the browning of kernels during storage have been carried out earlier and it is inferred that moisture is very important in browning reaction[194]. In addition to this, mannose is supposed to be particularly effective in browning[192]. The α,β-unsaturated aldehydes produced from unsaturated fatty acids also lead to browning reaction[192]. Other than these references on non-enzymic browning of palm kernels, changes in the flavour profile of palm kernel during heating are not studied earlier. Literature reveals only a few very old references on flavour compouds of palm kernel oil[198-200]. Hence the present study on development of roasted flavour in kernel and in the oil, on heating, is new.

The studies consisted of development of roasted flavour in palm kernel by heating and isolation (by

hydrodistillation) and identification (by GC and GC-MS) of flavour compounds of heated and unheated palm kernel. The chemical changes taking place in lipids, carbohydrates and proteins of palm kernel during heating were studied too.

RESULTS AND DISCUSSION

4.1 Development of roasted flavour by heating palm kernel and sensory evaluation and storage study of the oil

As in the case of coconut, different time-temperature combinations were tried in palm kernel also and guided by organoleptic appraisal, a temperature of 150°C for 5 minutes was selected. Since, in the case of palm kernel gratings, the problem of oozing out of oil was more, the roasting studies could not be continued in the temperature controlled oven and hence the roasting was done in a pan, with constant stirring over a Bunsen flame.

Palm kernels, freshly removed from mature oil palm fruits of freshly harvested oil palm bunches, had a moisture content of 20 to 22 percent. The kernels were dried and disintegrated and samples were heated at 150°C for 5 minutes. The proximate compositions of control (dried) and heated samples were found out. The results are included in Table 34. The analysis showed that moisture content of heated samples was

Table 34 : Proximate composition of control and heated samples of palm kernel (as % on dry weight basis)

	Control	Heated
Moisture	5.00	3.00
Fat	50.20	50.80
Protein	8.79	8.53
Carbohydrate (by difference)	34.16	34.88
Crude fibre	6.75	6.25
Ash (total)	1.85	1.79
Acid insoluble ash	0.78	0.80

* Mean of three determinations

3 percent as against 5 percent of the control sample. The fat content of 50 to 51 percent was within the range of reported values[187,190] and there was not much difference in fat content between control and heated samples. The protein content also did not differ much. The carbohydrate content (by difference) was found to be higher (34 percent) than the reported[187] value of 28 percent. The crude fibre content was 6.7 percent and was not much affected by heating. The ash content was found to be 1.85 percent and the acid insoluble ash was higher. The mineral composition of palm kernel was also determined and the results are given in Table 35. The chloride content was very high. Potassium was found to be present in large quantities. Heating did not affect the mineral composition.

The oil samples which were extracted using a hydraulic press were subjected to sensory evaluation according to Jacobsen[201] and storage study, as in the case of coconut oil. The ratings were given compared to imaginary sample of coconut oil with an arbitrary score of 10. This was chosen because in this region, coconut oil is the most preferred oil for cooking purposes.

Results of the sensory evaluation are given in Table 36. Results indicated that, though both samples

Table 35 : Mineral composition of palm kernel (mg/100g on dry weight basis)

	Control	Heated
Potassium	300..00	298.00
Sodium	0.02	0.02
Calcium	14.41	13.70
Magnesium	50.20	51.02
Iron	3.10	3.25
Copper	1.10	1.30
Sulphur	54.00	55.00
Phosphorus	92.90	93.40
Chlorine	548.00	547.60

Table 36 : Changes in colour, free fatty acid content (FFA%), peroxide value and sensory evaluation scores during storage of palm kernel oil from control and heated samples

	Control		Heated	
	Initial	After storage	Initial	After Storage
Colour (in Lovibond using 1" cell)	0.3Y	0.2Y +0.5R	2.3Y +1.5R	2.1Y +1.0R
Free fatty acid content (FFA%)	0.10	0.50	0.10	0.25
Peroxide value (milli eqvts/kg)	0.00	0.28	0.00	1.03
Sensory scores*				
Colour	6.0	5.5	4.5	5.0
Aroma	5.0	4.0	7.0	6.5

* Maximum 10

did not score comparably with coconut oil, the palm kernel oil from roasted samples were more preferred by judges, compared to control sample of palm kernel oil.

The initial free fatty acid (FFA) content of heated samples were much lower compared to unheated ones. The colour was much deeper in roasted sample and peroxide values for both samples were below measurable limits. During storage, the free fatty acid content did not increase much compared to control. There was only a marginal increase in peroxide value. The sensory scores were slightly better for heated sample. The colour values had decreased during storage, but still colour values were rated inferior compared to unheated, control. The overall flavour scores were better for the heated ones.

4.2 DISCUSSION

The proximate composition of palm kernel is comparable with the reported values[187] except for a higher value of 34 percent for carbohydrate content (by difference). The maximum reported value is only 28 and the difference is likely to be due to variation in geographical location, agroclimatic conditions, nutritional status and varietal difference. The fibrous

nature of the palm kernel can also be correlated with this higher content of carbohydrates which include non-available polysaccharides also. The value is almost double that of coconut (see Table 15) which is comparatively soft textured. The mineral composition and the acid insoluble ash content of palm kernel are not reported earlier. On the whole, the proximate composition does not reflect any major change on heating. This is probably due to the fact that changes are too small to be reflected in proximate analysis.

As a result of heating, flavour of palm kernel oil is enhanced much as indicated by sensory analysis. The storage stability of oil is also found to be better. This may be attributable to antioxidant effects of Maillard products[226]. As in the case of coconut, here also, peroxide value of oil from heated kernels is found to be more than that of control sample. Probably heating produces compounds which also answered peroxide tests[155].

4.3. Isolation and identification of flavour compounds of control and heated palm kernels

Based on the results of the experiments on flavour isolation of coconut, the hydrodistillation technique alone was adopted in palm kernel. The distillate

containing volatile flavour compounds was selectively extracted after pH adjustment as described in Chapter 2, and the basic, neutral and acid fractions of flavour compounds were separated. The pH of the aqueous distillate of palm kernel was found to be 7.3 and that of heated kernel was 5.4, which showed a shift towards acidic region. The basic fraction concentrate of unheated sample had no characteristic smell except a 'heavy' odour, whereas a similar concentrate from heated sample possessed an 'earthy' odour, reminiscent of certain pyrazines, combined with a subdued, cooked, nutty smell. The neutral fraction from control and heated samples were almost similar in sensory characteristics, namely a nutty, oily, hydrocarbon-like, heavy aroma, except that the heated sample had a sweet odour note also. The acidic fractions possessed an unpleasant flavour, characteristic of fatty acids like octanoic acid. The quantitative distribution of the basic, neutral and acid fractions of flavour of control and heated samples of palm kernel are given in Table 37.

As in the case of coconut, the flavour fractions were first analysed in a packed column in GC and later in the capillary column in a GC-MS instrument. The acid fractions were methylated and analysed. The

Table 37 : Quantitative distribution of the different flavour fractions of control and heated palm kernel

Flavour fractions	Concentration of flavour compounds in mg/kg of dry sample	
	Control	Heated
Basic	5	100
Neutral	150	170
Acid	50	75
Total (by addition)	205	345

authentic compounds used for identifying experimental peaks were pyrazines, pyridines, furans, thiophenes, thiazoles, oxazoles etc for basic fraction and aliphatic and aromatic alcohols, aldehydes, ketones, esters, γ- and δ-lactones etc for neutral fraction. For the methylated acid fraction, methyl esters of acetic and propionic acids and odd and even fatty acids (C_4 to C_{20}) including unsaturated acids, were injected. Methylated butterfat was also used to compare GC positions of lower fatty acids and other hydroxy fatty acids as well as some of the unsaturated fatty acids. In all these cases, the retention index (I_E) was calculated for all the peaks registered in each of the experimental samples and also for the peaks corresponding to authentic compounds. More than 80 percent of the separated peaks could be identified in each fraction of both the samples.

4.3.1 Identification of flavour compounds in the basic fraction

There was a considerable increase in the basic fraction content as a result of roasting (Table 37). Figure 26 represents the capillary GC separation of the basic fraction of roasted samples. Compounds identified are listed in Table 38 along with GC and GC-MS data. It could be seen that unheated palm kernel did not contain any pyrazines or even any of the basic

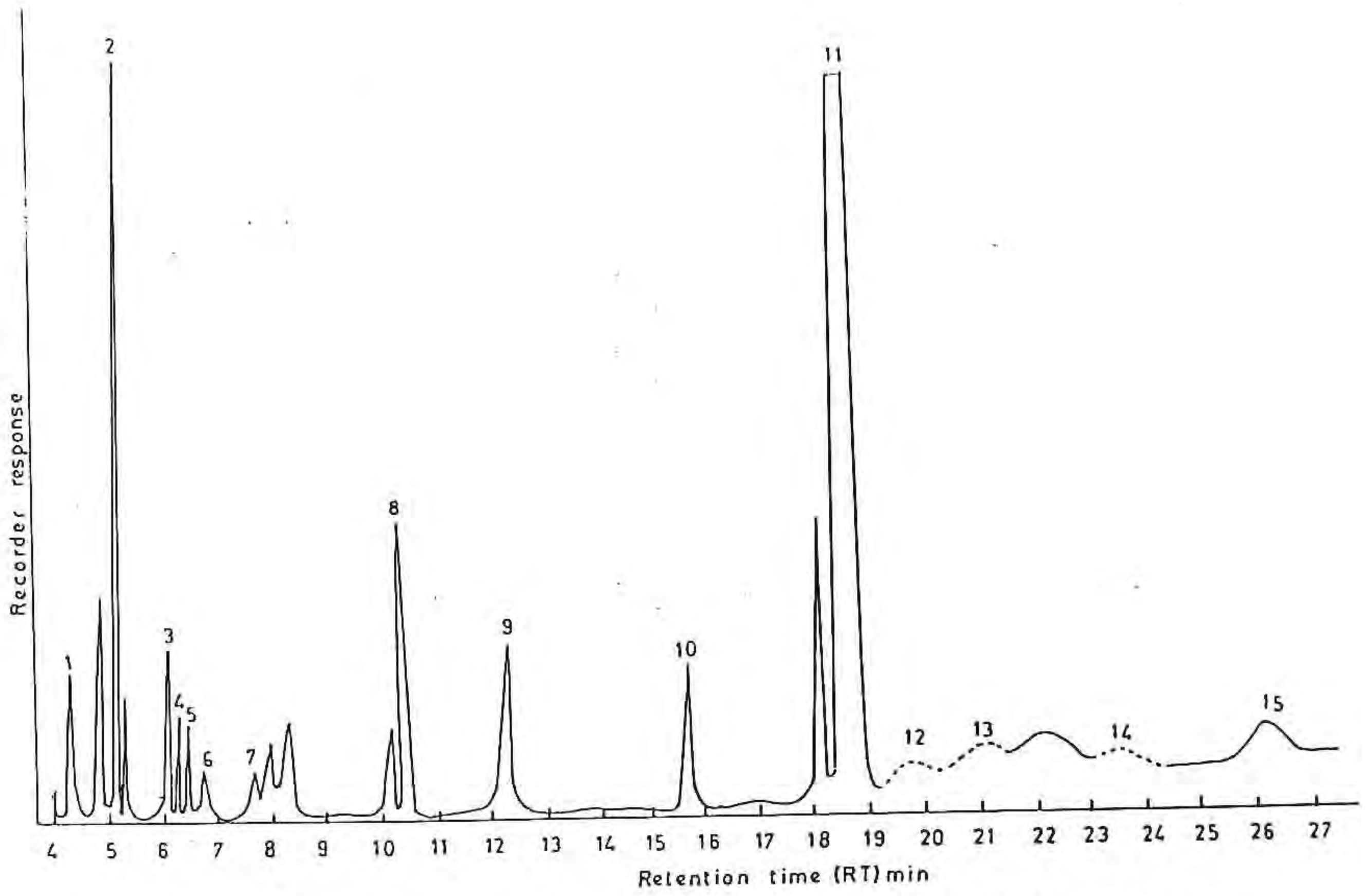


Fig.26. Gas chromatogram of the basic fraction of flavour isolated from heated palm kernel. Column: capillary Carbowax 20M (50m x 0·2mm i.d) Dotted lines indicate peaks which appeared in control sample

Table 38 : Compounds identified by GC and GC-MS analyses of the basic fraction of flavour isolated from control and heated samples of palm kernel

Peak No.	RT (min) in capillary Carbowax column	I_E value in OV-17 column	Compounds	MS fragment ions (in order of abundance)	Samples analysed			
					Control		Heated	
					Relative conc. %	Actual conc. mg/kg (ppm)	Relative conc. %	Actual conc. mg/kg (ppm)
1	4.56	4.70	Pyrazine	80,53,52,51,44	-	-	3.00	3.00
2	5.24	5.40	Methyl pyrazine	94,67,54,43,53	-	-	15.89	15.89
3	6.20	5.90	2,5-dimethyl pyrazine	42,108,82,45,79,52,80	-	-	3.45	3.45
4	6.32	5.90	2,6-dimethyl pyrazine	108,42,47,41,109,81	-	-	2.96	2.96
5	6.40	6.20	Ethyl pyrazine	107,108,80,53,52	-	-	1.88	1.88
6	6.68	5.60	2,3-dimethyl pyrazine	108,67,109,43,42	-	-	1.88	1.88
7	7.70	5.30	2-Ethyl-6-methyl pyrazine	121,122,44,94,56	-	-	0.67	0.67
8	8.24	6.85	Trimethyl pyrazine	42,122,121,44	-	-	1.14	1.14
9	10.22	7.60	Furfural	96,95,67,42,41,50,51	-	-	6.70	6.70
10	12.06	8.50	2-Ethyl hexanal	57,72,41,43,55,56,42	8.30	0.42	3.67	3.67
11	15.98	5.50	5-Methyl furfural	110,109,53,51,43,81	-	-	3.23	3.23
12	18.66	5.70	Dimethyl furan + Furfuryl alcohol	96,95,43,57,98,97,81 43,53,69,70	-	-	33.67	33.67
13	20.56	5.50	Dodecane	57,43,71,41,85	11.96	0.60	-	-
14	21.98	-	Tetradecane	43,57,71,85,41	12.30	0.62	-	-
15	23.16	-	2-Propyl oxetane	Tentative	11.70	0.58	-	-
16	26.30	13.60	2-Butyl benzothiazole	149,41,56,57,150,223	-	-	1.98	1.98

compounds. Presence of 2-ethyl hexanal, dodecane, tetradecane and 2-propyl oxetane were confirmed in this fraction. On the other hand, the basic fraction of heated palm kernel was characterised by the presence of pyrazines and furans. Among the 8 pyrazines identified, methyl pyrazine was the most abundant one (approximately 16 percent of the basic fraction). Pyrazine, 2,3-dimethyl pyrazine, 2-ethyl-6-methyl pyrazine, 2,3,5-trimethyl pyrazine and ethyl pyrazine were present in lesser amounts only (upto 3 percent). Furans constituted a greater proportion of the basic compounds. Eventhough they are less basic in nature compared to pyrazines, pyridines etc, furans have been found to be extracted along with basic compounds. Dimethyl furan and furfuryl alcohol together contributed nearly one-third of the basic fraction concentrate. Furfural and 5-methyl furfural were also present, though in lesser amounts. 2-Ethyl hexanal, which was identified in control sample was present in heated sample also. 2-Butyl benzothiazole was identified in the basic fraction of heated palm kernel only. As in the case of coconut, the sensory characteristics of eluting GC peaks were noted by sniffing at the GC exit port in an experiment, using a TC detector. Figure 27 represents GC profile of basic fraction of roasted palm kernel for which odour descriptions have been given in Table 39. From analysis, it was found that methyl pyrazine,

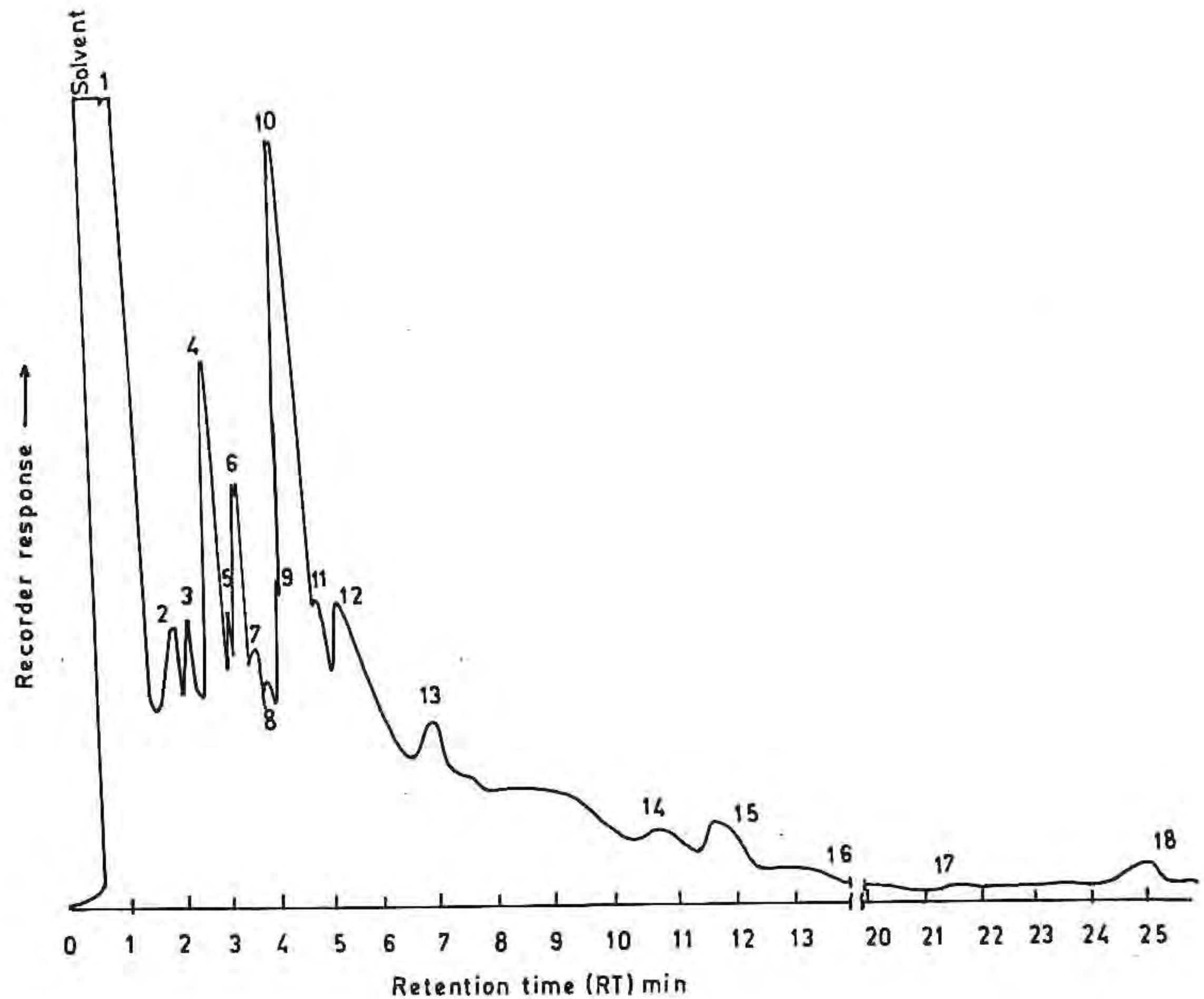


Fig.27. Gas chromatogram of basic fraction of flavour isolated from heated palm kernel. Column: OV-17(1.83 m x 3 mm id). See Table 39 also.

Table 39 : Odour description of the separated GC peaks of basic fraction of heated palm kernel

Peak No.	Odour description	Compound
1	musty, roasted	unknown
2	green, roasted	pyrazine
3	hydrocarbon-like	unknown
4	raw, roasted	methyl pyrazine
5	fine, roasted	2,3-dimethyl pyrazine
6	good, roasted aroma	2,5- and 2,6-dimethyl pyrazines
7	green, earthy	ethyl pyrazine
8	green, roasted	2-ethyl-6-methyl pyrazine
9	pungent, cooked	5-methyl furfural
10	burnt, caramelised	dimethyl furan + furfuryl alcohol
11	mild roasted	trimethyl pyrazine
12	pungent, stinging	furfural
13	pungent	unknown
14	unpleasant	unknown
15	raw, green	2-ethyl hexanal
16	nothing characteristic	unknown
17	hydrocarbon-like	unknown
18	slightly harsh, mild roasted aroma	2-butyl benzothiazole

2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine and 2,6-dimethyl pyrazine were found to have roasted flavour notes. The peaks corresponding to the furan derivatives gave mainly caramel like, fruity or burnt sensory effects.

4.3.2 Identification of flavour compounds in the neutral fraction

Table 40 represents the compounds identified by GC and GC-MS in the neutral flavour fraction of unheated (control) and heated palm kernel. The neutral flavour compounds consisted of 6-lactones, ethyl esters and methyl ketones. Most of the neutral flavour compounds identified in control sample were present in the neutral fraction of heated samples also. The relative concentration of each compound was slightly different in the two samples. GC separation of the compounds in OV-17 packed column was reasonably good and did not differ much from the GC-MS capillary separation with respect to relative concentration. Capillary separation is represented by Figure 28 and the GC profile of the aromagram by Figure 29. Table 41 represents odour descriptions of the peaks separated by GC analysis (also refer Figure 29).

The most predominant volatile compound was ethyl benzoate occurring in the range of 80 to 100 ppm in unheated and heated samples. The sensory characteristics

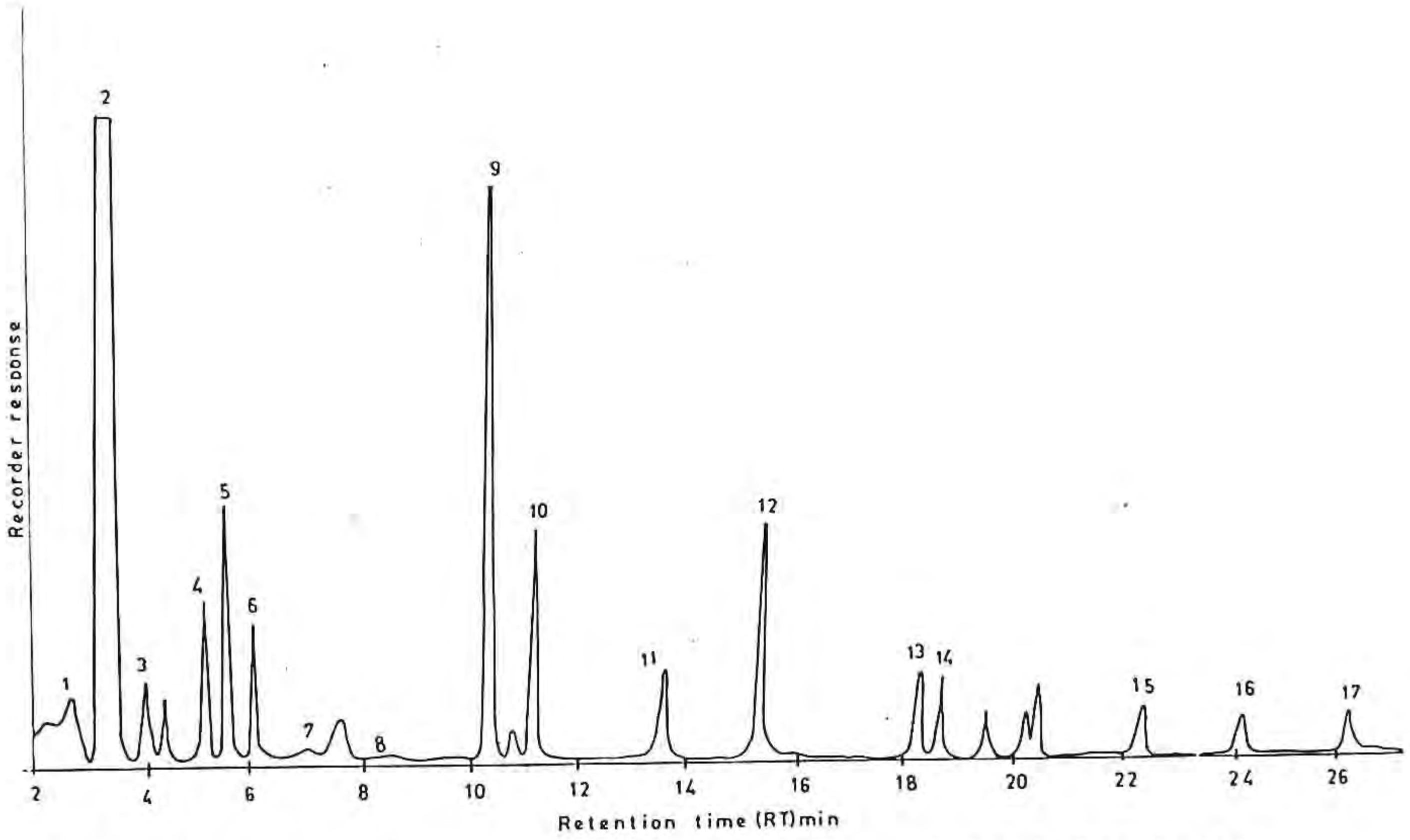


Fig.28. Gas chromatogram of the neutral fraction of flavour isolated from heated palm kernel. Column: capillary, cross-linked MethylSilicone (12 m x 0.2 mm id)

Table 40 : Compounds identified by GC and GC-MS analyses of the neutral fraction of flavour isolated from control and heated samples of palm kernel

Peak No.	RT (min) in capillary column of cross-linked Methyl Silicone	I_E value in OV-17 column	Compound	MS fragment ions (in order of abundance)	Samples analysed			
					Control		Heated	
					Relative conc. %	Actual conc. mg/kg(ppm)	Relative conc. %	Actual conc. mg/kg(ppm)
1	2.44	5.45	Tert-butyl benzene	119,91,41,77,57,51	1.50	2.25	0.60	1.02
2	3.62	8.75	Ethyl benzoate	105,122,77,51,150,151	65.00	97.50	48.00	81.60
3	4.18	8.10	Ethyl octanoate	88,57,43,101,127	3.20	4.80	1.00	1.70
4	5.04	Not identified	3,3,4,8-tetramethyl 1-tetralone	146,118,147,117,93	-	-	1.98	3.37
5	5.30	11.20	δ-Octalactone	99,71,42,43,70,55,114	3.20	4.80	3.17	5.39
6	6.16	6.32	2-Undecanone	58,43,40,71,59	1.35	2.02	1.01	1.72
7	7.14	Not identified	2-Hexylamino tetralin	Tentative	-	-	trace	trace
8	8.74	13.05	Ethyl decanoate	88,43,41,101,105,70,57	0.78	1.17	-	-
9	10.40	13.12	δ-Decalactone	99,42,41,71,70,55,114,152	7.35	11.02	8.0	13.60
10	11.10	15.95	2-Tridecanone	58,43,41,59,71,85,198	3.71	5.56	5.0	8.50
11	13.54	13.90	Ethyl dodecanoate	88,43,41,101,105,70,57	-	-	1.01	1.72
12	15.30	13.90	δ-Dodecalactone	99,41,42,43,55,57,70,71,114	1.00	1.50	4.54	7.72
13	18.42	Not identified	3,3-dimethyl oxirane	Tentative	0.80	1.20	0.85	1.45
14	18.80	16.15	2-Butyl benzothiazole	149,41,56,57,150,223	-	-	1.23	2.09
15	22.36	6.00	Tetradecane	57,71,85,99,43,55	2.57	3.86	0.76	1.29
16	24.20	Not identified	Tetradecanoic acid	57,85,43,41,55,71,83,105	0.92	1.38	0.40	0.68
17	25.70	Not identified	1,1,2-trichloro-1,2,2-trifluoro-ethane	Tentative	1.00	1.50	0.8	1.36

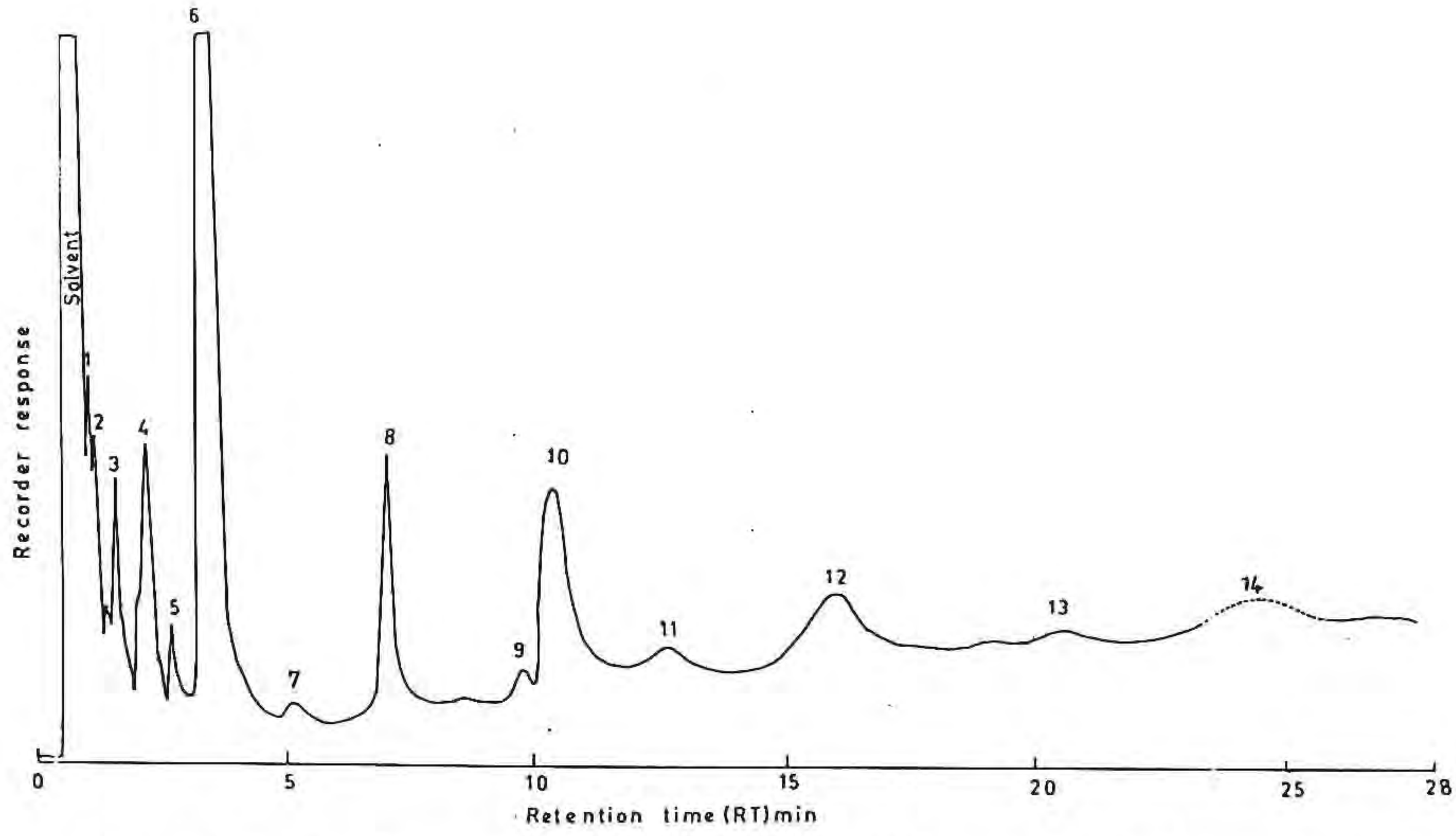


Fig.29. Gas chromatogram of the neutral fraction of flavour isolated from palm kernel (Control). Column: OV-17 (1.83 m x 3 mm id). See Table 41 also.

Table 41 : Odour descriptions of the separated GC peaks of neutral fraction of heated palm kernel

Peak No.	Odour description	Compound
1	hydrocarbon-like (aromatic)	tert-butyl benzene
2	hydrocarbon-like	tetradecane
3	rancid oil-like	2-undecanone
4	pleasant, oily	ethyl octanoate
5	heavy smell	unknown
6	pleasant, fruity, ester-like	ethyl benzoate
7	mild, nut-like	unknown
8	pleasant, coconut-like	δ-octalactone
9	oily, nut-like	ethyl decanoate
10	nut-like, pleasant	δ-decalactone
11	oily, nutty	δ-dodecalactone, ethyl dodecanoate
12	oily, rancid, nut-like	2-tridecanone
13	heavy, hydrocarbon-like	unknown
14	medicinal, slightly harsh	2-butyl benzothiazole

of the corresponding peak was pleasant, aromatic and fruity. Octa, deca and dodeca δ-lactones were present in 5 to 15 ppm range only. Of the ethyl esters identified, ethyl octanoate was present in control and heated samples. Ethyl decanoate was found only in unheated sample indicating its loss during roasting whereas ethyl dodecanoate was identified only in heated samples. 2-Undecanone and 2-tridecanone were present in both samples. The occurrence of tert-butyl benzene, though in smaller amounts could be confirmed by mass spectral data. 2-Butyl benzothiazole which was identified in basic fraction of heated palm kernel was present in neutral fraction also. Compounds whose mass spectral data did not match above a correlation cut off of 0.93 were marked as tentatively identified.

4.3.3 Identification of flavour compounds in the acid fraction

Acid fraction constituted 20 to 25 percent in unheated (control) and heated samples. In the case of palm kernel, acidic compounds were high (20 percent) even in control samples. 12 fatty acids with carbon numbers from 6 to 18 were present in palm kernel and hexadecanoic ($C_{16:0}$) and cis 9-octadecenoic (oleic)were predominant. 5-Hydroxy tetradecanoic acid [C_{14}(OH)] and cis 6-hexadecenoic ($C_{16:1}$) were found in palm kernel.

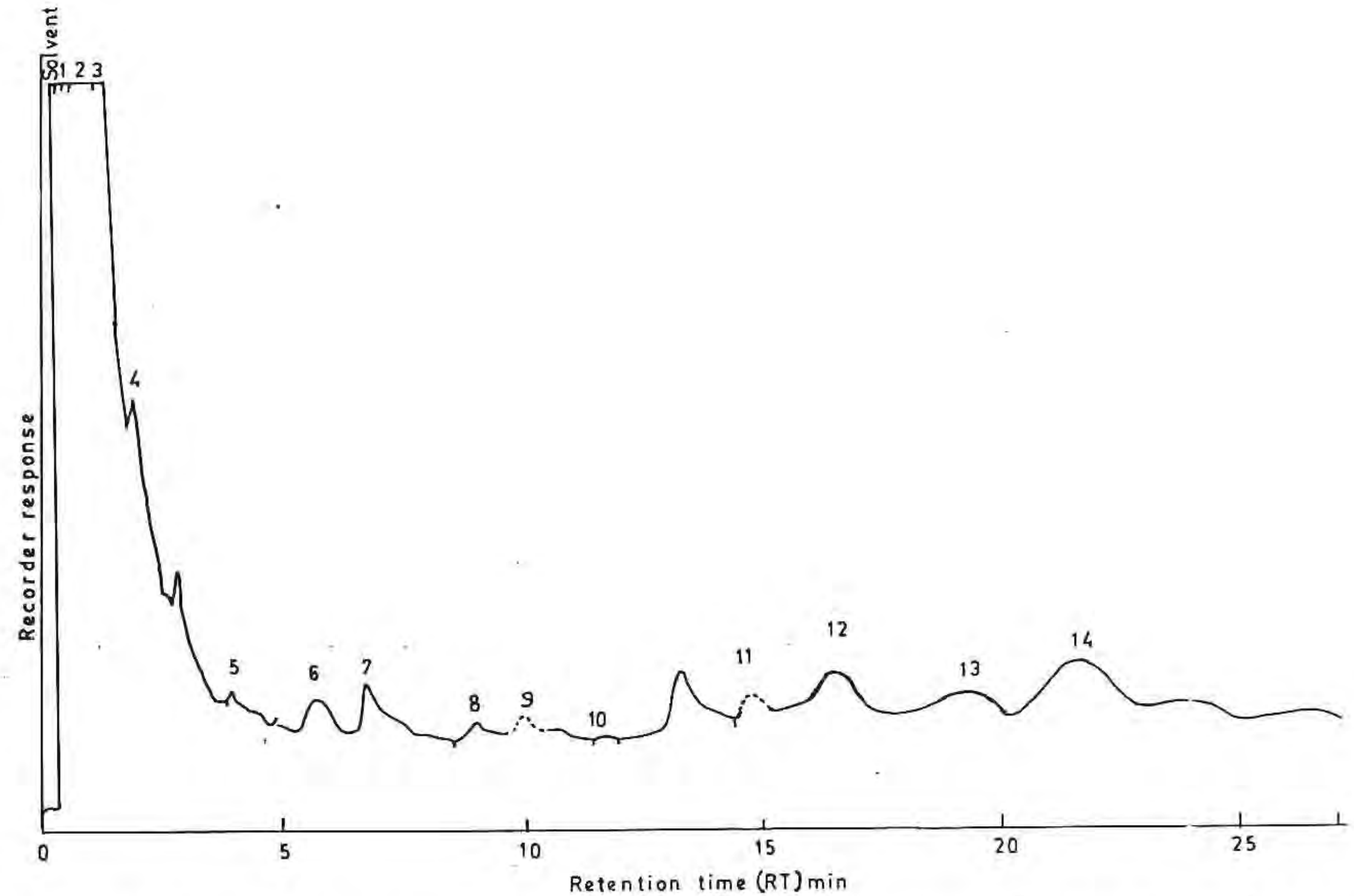


Fig. 30. Gas chromatogram of the methylated acid fraction of flavour isolated from heated palm kernel. Column: DEGS (1.83m x 3mm id) · Dotted lines indicate peaks which appeared in control sample.

Table 42 : Compounds identified by GC and GC-MS analyses of the methylated acid fraction of flavour isolated from control and heated samples of palm kernel

Peak No.	RT(min) in capillary column of crosslinked Methyl Silicone	I_E value in DEGS column	Acid compounds (analysed as methyl esters)	MS fragment ions (in order of abundance)	Samples analysed			
					Control		Heated	
					Relative conc. %	Actual conc. in mg/kg(ppm)	Relative conc. %	Actual conc. in mg/kg(ppm)
1	Not identified	3.50	Acetic and propionic	Eluted with solvent in MS analysis	-	-	8.25	6.10
2	-do-	3.80	Butyric	-do-	-	-	27.10	20.32
3	0.98	6.05	Hexanoic	74,84,51,47,41,86,49	8.49	4.24	13.52	10.14
4	3.18	8.10	Octanoic	74,87,55,41,59,158	trace	trace	0.36	0.27
5	7.50	10.00	Decanoic	74,87,143,43,55,186	trace	trace	0.22	0.16
6	12.50	11.80	5-Hydroxy dodecanoic	Tentative	5.38	2.69	1.17	0.88
7	12.94	11.98	Dodecanoic	74,87,41,43,55,243	10.03	5.01	1.53	1.15
8	17.08	13.50	5-Hydroxy tetradecanoic	Tentative	3.85	1.92	0.52	0.39
9	17.18	14.00	Tetradecenoic	Tentative	2.72	1.36	-	-
10	17.26	14.59	Tetradecanoic	74,87,41,43,55,243	3.31	1.65	0.95	0.71
11	20.72	16.05	Hexadecenoic	74,87,255,298,43,41	7.56	3.78	-	-
12	21.16	17.15	Hexadecanoic	74,87,43 ,75,41,227,270	19.16	9.83	3.33	2.50
13	24.70	18.20	Octadecanoic	74,87,43,55,41,143,255,298	7.44	3.72	7.44	3.72
14	24.10	18.50	Octadecenoic (oleic)	55,74,69,67,83,43,41,87,222	23.30	11.15	23.30	11.15

In the heated sample, lower acids like acetic, propionic and butyric were also present and butyric acid was most abundant too. Table 42 represents the results of the GC and GC-MS analyses and Figure 30 represents the GC separation of fatty acid methyl esters of heated palm kernel.

4.3.4 Discussion of the results of flavour studies

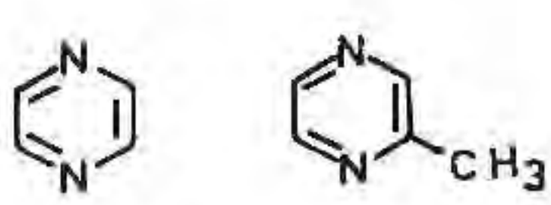
Flavour compounds of palm kernel are systematically analysed and reported for the first time. Very old reference by Jamieson[199] indicated 2-undecanone and 2-tridecanone to be present as volatile aroma compounds of palm kernel oil. Presence of δ-lactones is indicated in the work of Vander Ven and Koene[267] in connection with optical activity of naturally occurring δ-lactones of vegetable oils. Apart from these, literature is rather blank regarding the flavour constituents of palm kernel and its oil. In the present study, 29 compounds have been identified in palm kernel which include 3 esters, 3 δ-lactones, 2 methyl ketones, 4 hydrocarbons and 12 fatty acids.

There is a considerable increase in the amount of basic fraction due to heating (Table 37). It can be seen that basic fraction of control sample contains only neutral type of compounds. In the unroasted stage,

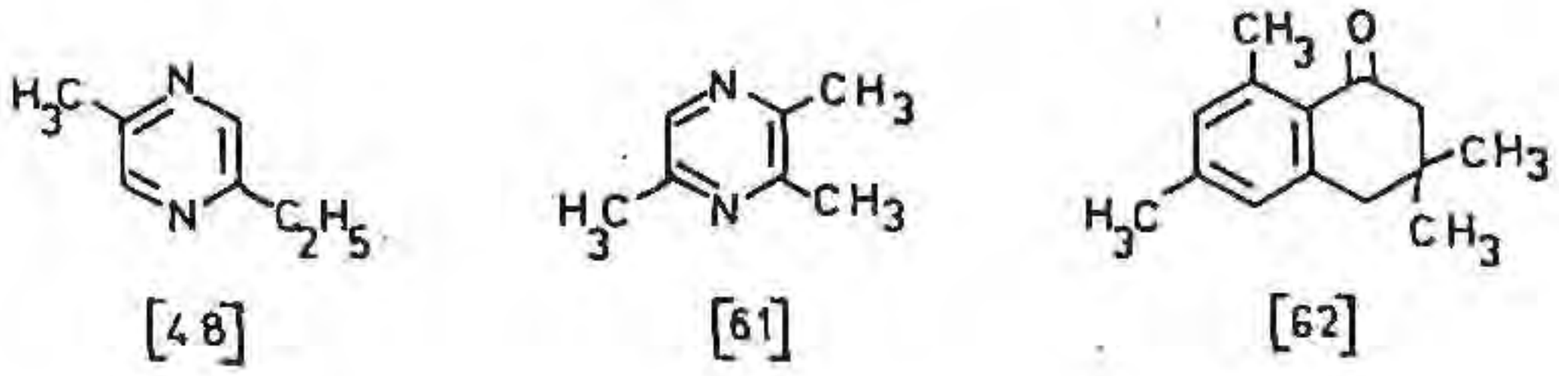
neutral compounds dominate the flavour profile. Of the 14 compounds identified, the ethyl esters together contribute more than 70 percent by weight. Ethyl benzoate is present in significant quantities (65 percent relative concentration), ethyl octanoate and decanoate exist in smaller amounts only. These esters can together explain the pleasant smell of neutral fraction concentrate. The latter two esters and the δ-lactones have been identified in coconut also by other researchers[183 184] as well as in the present study. The identification of tert-butyl benzene in palm kernel can not be unusual since methyl substituted xylenes are reported in the volatiles of crude palm oil[268]. The trichloro-trifluoro derivative could not be confirmed due to unavailability of authentic compound and also due to insufficient mass spectral data and hence was marked as 'tentative'.

The acid fraction of palm kernel contains a number of fatty acids. Presence of 5-hydroxy fatty acids could not be confirmed from mass spectral data and have been marked as 'tentative' in Table 42. The fatty acids can be considered as formed during the biosynthesis of triglycerides. Also, δ-lactones identified in the neutral fraction are formed from hydroxy fatty acids

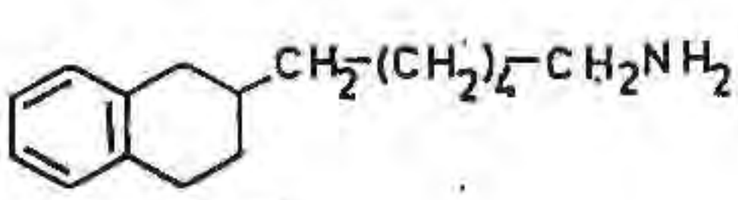
during biosynthesis of fats. This may involve catalytic δ-oxidation of saturated fatty acids or δ-hydroxylation of unsaturated fatty acids to produce the δ-lactones. The ethyl esters identified in the neutral fraction are probably the esterified fatty acids produced during biosynthesis. In heated palm kernel, 41 compounds (volatile) have been identified. The basic fraction is characterised by the presence of a number of pyrazines that are responsible for the roasted aroma of heated palm kernel. Among the eight pyrazines identified, methyl pyrazine is the most abundant one. Other pyrazines may also contribute to overall roasted flavour through synergistic effect reported for flavour compounds[6]. Pyrazine[7],methyl pyrazine **[41]**, 2,3-dimethyl pyrazine **[42]**, 2-ethyl-6-methyl pyrazine **[48]**, trimethyl pyrazine **[61]** and ethyl pyrazine **[45]** are known to occur in roasted foods[6] and contribute to roasted aroma. In the case of coconut also, most of these pyrazines could be identified. The formation of pyrazines can be attributed to the series of changes taking place by interaction of reducing sugars and free amino acids during heating and is confirmed by studies conducted in a number of model systems[78,79]. Another interesting observation is that the derivatives of furan are more abundant in



[7] [41] [42] [43] [44]



[48] [61] [62]



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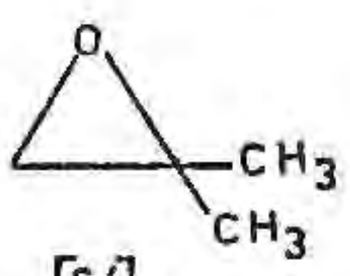


[64]

Fig.31. Structure of a few compounds identified in the volatiles of heated palm kernel.

roasted palm kernel volatiles than in roasted coconut. Furans contribute nearly 40 percent of the basic fraction concentrate. The formation of larger quantities of furans in palm kernel indicates more of carbohydrate degradation and possibly, preferential 1,2-enolisation pathway in Maillard reaction[95]. Furans are important flavour compounds in heated foods and are identified in roasted coffee, cocoa, peanut, filberts etc[105]. The presence of 2-butyl benzothiazole in heated palm kernel indicated involvement of sulphur containing amino acids during the Maillard reaction.

Neutral fraction of heated palm kernel contains most of the ethyl esters, δ-lactones, ketones and aliphatic and aromatic hydrocarbons and other miscellaneous compounds identified in control sample of palm kernel. 2-Ethyl hexanal, which was identified in basic fraction of unheated palm kernel, has been found in the neutral fraction of heated palm kernel volatiles. Interestingly tetramethyl tetralone **[62]**, 2-hexylaminotetralin **[63]** and 3,3-dimethyl oxirane **[64]** have been found in the roasted palm kernel. The possibility of formation of the first two compounds could not be visualised.

The acid (flavour) fraction of heated samples included most of the fatty acids which appeared in

the control sample. In addition, acetic, propionic and butyric acids were also found. Such acids have been reported in roasted cocoa[32], peanuts[112] cashew-nuts[241] etc. In coconut also, acids have been identified in the present work. The formation of lower acids is supposed to be due to sugar degradation[269], lipid decomposition[270] and according to a few researchers, by the deamination of free amino acids[271] during heating. Structure of the various compounds identified ir palm kernel are given in Figure 31. The mass spectral fragmentation of most of the identified compounds have been already discussed in Chapter 3.

In short, the results of the flavour studies of palm kernel can be generalised as follows:

Class of compounds	Control	Heated
Pyrazines	-	8
Furans	-	4
Benzothiazole	-	1
Oxetane	1	-
Oxirane	1	1
Chloro compound	1	1
Hydrocarbons	3	3
Alcohols	-	1
Aldehydes	1	1
Ketones	2	3
Esters	3	3
Amines	-	1
Acids	12	13

4.4 CHANGES IN THE LIPIDS OF PALM KERNEL ON HEATING

As in the case of coconut, changes in the lipids of palm kernel during heating were studied. Palm kernel is rich in lauric fat and the changes taking place in the lipid constituent on heating was not hitherto studied. Physicochemical characteristics and fatty acid composition of lipids extracted from control and heated samples of palm kernel were carried out.

4.4.1 Extraction and analysis of total and neutral lipids of control and heated samples of palm kernel

Table 43 gives the content and physicochemical characteristics of lipids extracted from control and heated palm kernels. The total lipid content was slightly higher than neutral lipid content by 2 to 2.5 percent which represented the polar lipids. As a result of heating the neutral or total lipid content was not altered much. There was slight reduction in polar lipids of roasted sample (from 2.5 to 2.1 percent). Peroxide value was measurable in total lipid extracts and also in both extracts of heated samples. Also, total lipid extract was more coloured than petroleum ether extract. Specific gravity, refractive index, and free fatty acid content (FFA %) did not show any decrease. Iodine values of total lipids was slightly

Table 43 : Changes in the content and physicochemical characteristics of lipids of palm kernel on heating

	Mode of extraction			
	Petroleum ether		Chloroform/methanol	
	Control	Heated	Control	Heated
Lipid content (%) in dry palm kernel	50.20	50.80	52.70	52.90
Polar lipids (%)	-	-	2.5	2.1
Free fatty acid content (FFA%)	0.80	0.82	0.81	0.82
Peroxide value (milli eqvts/kg)	0.00	0.30	0.10	0.50
Sp. gravity (60°C)	0.9140	0.9147	0.9142	0.9145
Refractive index n_{40}^{D}	1.4483	1.4482	1.4482	1.4481
Colour in Lovibond units (Y+5R)	0.5Y+ 0.5R	2.0Y+ 2.5R	1.1Y+ 1.5R	2.5Y+ 3.5R
Iodine value (IV)	17.50	17.06	18.02	17.57
Saponification value (SV)	251.0	252.0	252.0	252.7
Reichert-Meissl value (RM value)	6.32	6.50	6.70	6.48
Polenske value (P value)	10.25	10.05	10.50	10.37
Unsaponifiable matter (%)	0.197	0.209	0.203	0.210

higher and a slight decrease was noticed on heating, generally. Saponification value, Reichert Meissl value (RM value), and Polenske value (P value) did not show any major change. The unsaponifiable matter content was slightly more in total lipid extracts.

4.4.2 Discussion

Palm kernel lipids contain only a small amount of polar lipids (2.5 percent) which is slightly reduced on heating. No detailed analysis of lipids of heated palm kernel or palm kernel cake is available in literature. The polar lipids are reported to be affected during roasting, in pistachios[155], soybeans[248], baked potatoes[251] etc also. There is no significant change in physicochemical characteristics of palm kernel during roasting. The colour value increases because of browning during heating. The noticeable increase in unsaponifiable matter must be due to the brown coloured, Maillard polymers which are also being extracted into solvent along with lipids. The antioxidant activity of brown polymers in general are under study by many researchers[272,273] and in the present work also we find that in the roasted sample, the FFA content of oil does not increase much during storage. The increase in peroxide value in roasted sample, can be explained as in the

case of coconut, as due to formation of some reducing species during roasting, which are extracted into lipid layer. Other researchers have noticed this type of change in a few other systems[155,248].

4.4.3 Fatty acid composition of lipids of control and heated samples of palm kernel

Table 44 gives the composition of fatty acids of palm kernel samples. As in the case of coconut, palm kernel also belongs to lauric fat group and $C_{12:0}$ acid (lauric acid) is the most predominant one (49 percent), followed by $C_{14:0}$ acid (myristic $C_{14:0}$, 16 percent). The fatty acid composition of palm kernel oil was very similar to coconut oil, the main difference was the higher percentage of $C_{18:1}$ in the former. Also, $C_{10:1}$ acids were present in palm kernel though in traces. Iodine value of palm kernel lipids (Table 43) was almost double the value for coconut. Composition of total lipid was not very different from neutral lipid extract and on heating there was no significant change in fatty acid profile as a result of roasting.

4.4.4 Discussion

The fatty acid composition of palm kernel lipids compared well with reported values[163,190]. The percentage of unsaturated fatty acids is slightly more in palm kernel oil compared to coconut oil. The fatty

Table 44 : Fatty acid composition (%) of lipids of control and heated palm kernel

Fatty acids	Mode of extraction			
	Petroleum ether extract		Chloroform/methanol extract (2:1)	
	Control	Heated	Control	Heated
6:0	0.14	0.15	0.14	0.14
8:0	3.95	3.84	3.90	3.79
10:0	3.60	3.52	3.58	3.53
10:1	0.15	0.10	0.12	0.09
12:0	49.61	48.62	50.01	49.38
14:0	16.51	16.69	16.38	16.28
16:0	7.61	7.69	7.4	7.12
16:1	0.15	0.10	0.20	0.15
18:0	1.20	1.00	1.20	1.10
18:1	16.02	16.51	16.80	16.73
18:2	1.21	1.19	1.13	1.08
18:3	trace	0.08	trace	0.10
20:0	0.20	0.20	0.10	0.20
Total unsaturated fatty acids	17.38	17.88	18.13	18.06

Trace : less than 0.05%

acid profiles of control and heated palm kernel did not show any major change. It is understandable that no severe degradative changes take place in the temperature range used for roasting kernels and the changes, if there are any, are far too minute to reflect in the fatty acid profiles.

4.5 CHANGES IN THE CARBOHYDRATES OF PALM KERNEL ON HEATING

Reports on carbohydrate composition of palm kernel are very few in literature. The development of roasted flavour in palm kernel during heating and the identification of furans in large quantities in flavour volatiles will prompt one to study the sugar profile of this oil rich seed. The detailed sugar analysis and the changes thereof on roasting, are reported for the first time.

4.5.1 Extraction of soluble sugars and starch

Table 45 represents the changes in the sugars of palm kernel during roasting. Total soluble sugars, reducing sugars and non-reducing sugars of palm kernel were found to be 3.16, 0.50 and 2.66 percent respectively. Very small quantities of starch only could be found in palm kernel (0.6 percent). The reducing sugar content was only 1.6 percent of the total sugars present. It was found that total extractable sugars reduced

Table 45 : Changes in the carbohydrates of palm kernel during heating (as g/100g on dry weight basis)

	Control	Heated
Total soluble sugars	3.16	2.40
Reducing sugars	0.50	0.32
Non-reducing sugars	2.66	2.08
Ribose	0.040	0.102
L-Rhamnose	0.050	trace
Arabinose	0.020	0.024
Fructose	0.280	0.146
Glucose	0.050	0.030
Mannose	0.080	0.044
Sucrose	2.610	2.110
Starch + dextrins	0.63	0.60

* Mean of three determinations

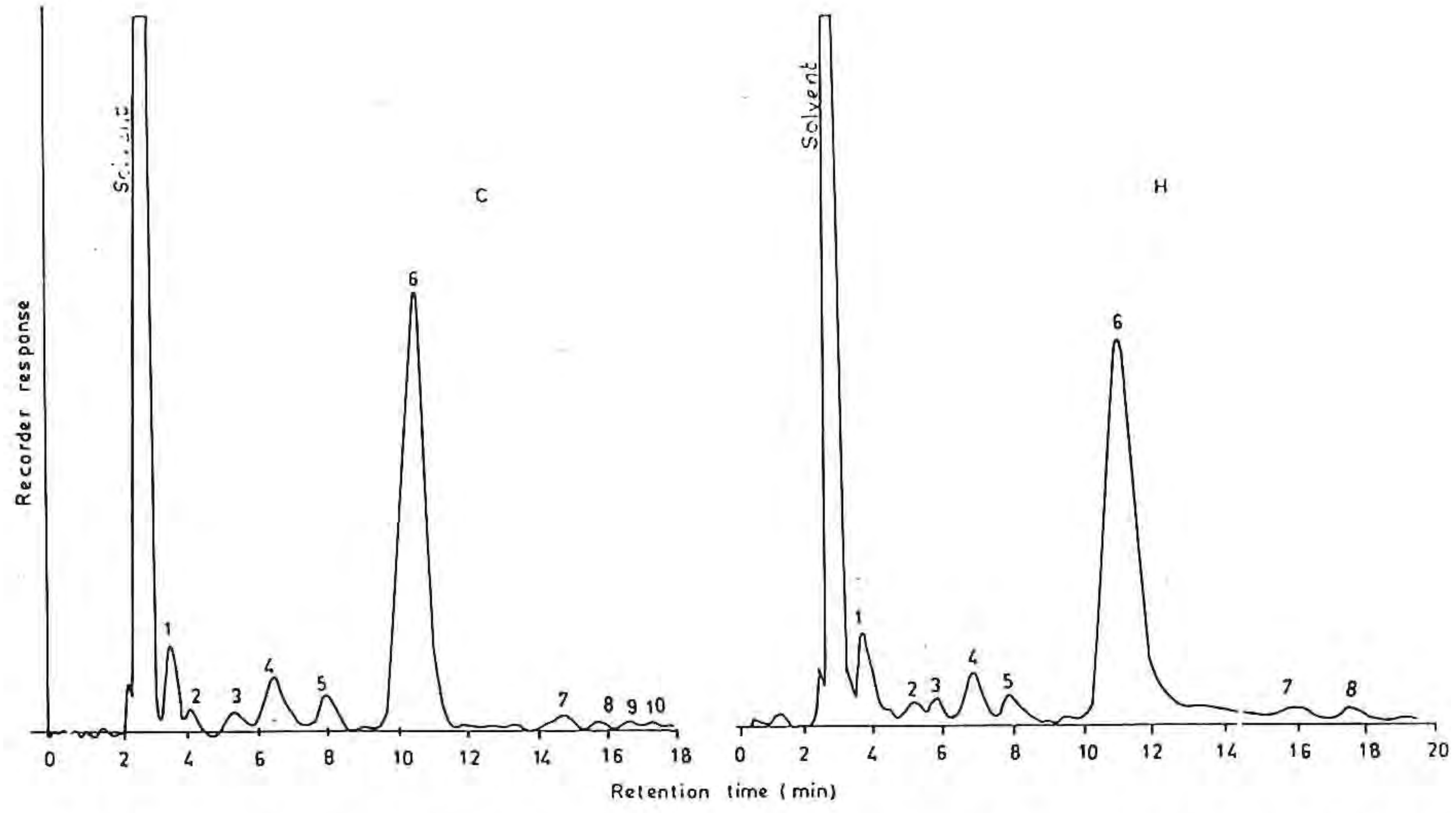


Fig.32. HPLC Separation of soluble sugars of control (C) and heated (H) samples of palm kernel

1. Ribose, 2. L-Rhamnose, 3. Arabinose, 4. Fructose, 5 Glucose/mannose, 6. Sucrose, 7. Unknown, 8. Uknown, 9. Unknown, 10. Unknown.

to 2.4 (21 percent loss) and reducing sugars became 0.32 (36 percent loss); correspondingly non-reducing sugars were 2.09 percent. The starch content was very low, only 0.6 percent and there was not much change in starch content as a result of roasting.

4.5.2 HPLC analysis of soluble sugars

Figure 32 represents the separation of soluble sugars of palm kernel by HPLC. The detailed analysis of the methanolic extract containing soluble sugars, showed that sucrose was the most abundant sugar (85 percent) followed by fructose and mannose.L-Rhamnose. ribose, glucose and arabinose were also found to be present, in lesser amounts. Presence of sucrose and mannose were reported by earlier workers also[195,274]. Glucose and starch were not convincingly reported by Crombie[195]. Moreover, the methods adopted were mostly TLC, followed by colorimetric detection. Under the conditions of HPLC analysis followed in this study, mannose and glucose eluted together so that glucose was separately estimated by glucose-oxidase peroxidase
213
method . The presence of glucose and mannose was also tested by paper chromatographic analysis[275]. Table 45 represents the sugar composition of palm kernel.

As a result of heating L-rhamnose was almost completely utilised, whereas glucose, mannose and fructose

were reduced by 40 to 50 percent. Sucrose content changed from 2.6 to 2.11 (20 percent reduction). Ribose content increased 2.5 times, whereas arabinose content was more or less steady after heating.

4.5.3 Discussion

Though coconut endosperm and oil palm kernel are comparable in many ways like fatty acid composition of their lipids, protein content and amino acid composition, the sugar content and sugar profile of palm kernel are slightly different. The total extractable sugars is only 3 percent against 11 percent in coconut. Though sucrose is the major sugar in both the oil seed endosperms, its content is less in palm kernel. Mannose and arabinose could not be detected in coconut whereas, L-rhamnose is present in measurable quantities in palm kernel. Also starch content is much less in palm kernel. The polysaccharide composition of palm kernel is not subjected to detailed investigation. It is this low sucrose content and high fibrous content which limits palm kernel's use as an edible item for human consumption. Earlier studies conducted, indicated its usefulness as feed stock for pigs[276].

The changes in carbohydrate profile on heating, indicate that L-rhamnose, mannose, fructose and glucose

are consumed for browning reaction. The activity of these reducing sugars, especially L-rhamnose, is well proved in model system studies[32,82]. The effectiveness of mannose in browning of palm kernels has been reported by McWeeny[274]. The decrease in sucrose content is also not unexpected since caramelisation[74], hydrolysis[254,255] etc are suggested under heated conditions. The participation of simple sugars can be inferred, from the studies on coconut discussed in this thesis. Consumption of sugars is reported in roasted cocoa[256], peanuts[156], pistachio[257] etc also.

4.6 CHANGES IN THE PROTEINS OF PALM KERNEL ON HEATING

The changes occurring in the protein and non-protein nitrogen contents of palm kernel during heating are not subjected to systematic study so far. The present work is a first step towards this investigation. Changes in protein and free amino acid composition of roasted and unroasted (control) palm kernels have been studied by amino acid analysis.

4.6.1 Changes in the protein and non-protein nitrogen (NPN) contents

The total nitrogen content and the crude protein content of palm kernel were found to be 1.406 and 8.79 percent respectively (Table 46). The NPN content

Table 46 : Changes in total nitrogen, crude protein and non-protein nitrogen (NPN) contents of control and heated palm kernel(as % on dry weight basis)

	Control	Heated
Total nitrogen (by Kjeldahl method)	1.406	1.365
Crude protein (Nx6.25)	8.79	8.53
Non-protein nitrogen (NPN)	0.092	0.077
% NPN in total nitrogen	6.54	5.64
Total protein amino acids (experimental)	8.30	8.21
Actual protein content [(total N-NPN) x 6.25)]	8.21	8.05

was found to be 0.092 which represented only 6.5 percent of the total nitrogen content; this is only half of NPN content in coconut. From Table 46 it can be seen that total nitrogen and crude protein decreased only slightly, whereas the NPN content reduced from 0.092 to 0.077 marking a 16 percent loss during heating. Eventhough, this loss is less than the 21 percent loss in NPN content of coconut, it is clear from the preliminary analysis that NPN species is taking part in thermal reactions.

4.6.2 Changes in protein amino acids of palm kernel during heating

Results of the protein amino acid analysis of control and heated samples of palm kernel are presented in Table 47. The analysis showed presence of 18 amino acids. Glutamic acid was found to be the most abundant acid, as reported by others also[277]. This was followed by arginine anc aspartic acid. On the whole, the total amino acid profile did not show any major changes as a result of heating. However, slight reduction was noticed in the case of lysine, arginine, aspartic acid, methionine, cystine, histidine and tyrosine. Lysine and arginine are reported to interact with sugars even in bound form[254]. The sulphur amino acids cystine and methionine are known to be heat labile[278]. The

Table 47 : Changes in the total and free amino acids of palm kernel on heating*

Amino acid	Total amino acids (mg/100g dry sample)		Free amino acids (mg/100g dry sample)		% reduction in free amino acids after heating
	Control	Heated	Control	Heated	
Aspartic acid	824.5	812.0	9.15	3.66	60
Threonine	260.0	258.0	2.16	0.85	60
Serine	400.0	394.0	2.51	1.46	42
Glutamic acid	1520.0	1516.0	7.10	2.93	60
Proline	326.0	318.0	3.15	2.59	20
Glycine	439.0	440.0	1.05	0.88	16
Alanine	406.0	422.0	2.11	1.05	50
Valine	497.0	500.0	2.26	-	100
Cysteine (as half-cystine)	162.0	153.5	-	-	-
Methionine	198.0	188.2	-	-	-
Isoleucine	364.0	298.0	1.50	-	100
Leucine	551.0	548.0	1.72	-	100
Tyrosine	237.5	229.0	-	-	
Phenyl alanine	308.0	301.5	u.i.p	u.i.p	
Tryptophan	100.0	102.1	-	-	
Histidine	165.0	159.0	2.41	1.55	
Lysine	293.5	270.0	1.92	0.68	
Ammonia	-	-	-	-	
Arginine	1305.0	1293.0	9.30	7.60	20
Total amino acids	8296.5	8212.3	46.34	23.25	50

u.i.p. - unidentified peak * Mean of two determinations

amino acid alanine marked a slight increase. An increase in alanine content is reported in heated caseine-glucose system whereby aspartic acid is supposed to be decarboxylated to alanine during heating[279].

4.6.3 Changes in the free amino acids of palm kernel during heating

Results of the free amino acid analysis of palm kernel samples are presented in Table 47, along with total amino acid changes, for comparison. Most of the amino acids present in total protein hydrolysate were present in NPN fraction also; 13 amino acids were identified. Tryptophan, phenyl alanine, tyrosine (all with aromatic side chain), isoleucine and methionine were not found to be present. Arginine, aspartic acid and glutamic acid were found to be most abudant in the free form.

As a result of heating, the free amino acid content reduced to half its original value. The change in NPN content on heating was thus confirmed by free amino acid analysis. It was found that, valine, isoleucine, and leucine were completely lost during heating whereas lysine, aspartic acid, threonine and glutamic acid marked 60 percent reduction. Alanine was lost by 50 percent, whereby serine and histidine were reduced by 35 to 40 percent. Arginine was only slightly affected

(about 20 percent). In general, the amino acids with alkyl side chain were more affected during heating.

4.6.4 Discussion

Crude protein content and total (protein) amino acid composition do not change much, whereas NPN content and free amino acid profile change as a result of heating. The observation is not unusual. In the case of pistachios also similar observation has been made[256]. In the present work itself on coconut, the same trend is noted. It has been observed that free amino acids interact with sugars producing Maillard flavours and earlier studies on roasted cocoa[157], peanut[259], baked potato[261], cooked beans[260] etc support this. The pyrazine content in palm kernel is found to be only 30 ppm as against 100 ppm in coconut. The lower NPN content may be correlated with the lesser pyrazine content in palm kernel.

As in the case of coconut, glutamic acid, arginine and aspartic acid dominate the total amino acid profile. Among the free amino acids, aspartic acid, arginine and glutamic acid are in greater amounts. As a result of heating, the free amino acids are found to decrease (Table 47), which shows individual participation by free amino acids. No free ammonia was detected in

palm kernel as can be seen from Table 47. A few unknown peaks that were observed in the chromatogram of heated sample could be due to the Amadori products or smaller peptides. The quantitative free amino acid analysis and its changes during roasting of palm kernel are reported for the first time.. An effort is made to correlate these changes and the changes in sugars with flavour formation, with the support of results of model system studies and is discussed in Chapter 5.

4.7 COMPARATIVE STUDY OF COCONUT AND PALM KERNEL

The flavour profile of coconut is dominated by δ-lactones, whereas in palm kernel, methyl benzoate is the most abundant volatile compound. The δ-lactones and ethyl esters though present in palm kernel, are in much lesser quantities compared to coconut. This difference in the volatile, flavour composition is likely to be responsible for the difference in aroma characteristics. Quantitatively the basic fraction of roasted coconut is 1½ times that of palm kernel. While 17 pyrazines are identified in roasted coconut, only 8 pyrazines are identified in roasted palm kernel. Furans are noticeably present in palm kernel, and fatty acids which are supposed to be formed from lipids on heating are more or less similar in both coconut and

palm kernel. The analysis of non-volatile components also reveal the differences in composition of the two lauric fat rich oilseeds. The neutral lipids are the major class in both. Polar lipids account for 2.5 to 3.5 percent only. The higher value for iodine value in the case of palm kernel oil is supported by fatty acid composition also. Oleic acid is present to the extent of 16 percent as against 4 percent in coconut. The problem of rancidity in palm kernel oil is reported to be much more than in coconut oil, possibly due to more of unsaturated fatty acids also.

Qualitative and quantitative differences are there in sugar profile also. Coconut contains simple sugars like glucose, fructose, galactose and sucrose. Traces of rhamnose is also found. Starch content of coconut is ony 3 percent. In palm kernel, ribose, rhamnose, arabinose, fructose, glucose, mannose and sucrose are there. The starch content of palm kernel is very low. The polysaccharides of palm kernel are not well characterised in literature and in the present study also it was not undertaken. The sucrose content is much higher in coconut and explains the sweetness of the nut.

The (protein) amino acid profile of coconut and palm kernel show that most of the amino acids present in coconut are present in palm kernel also. In both cases, the protein amino acids are not much affected by heat. There is change in the non-protein nitrogen (NPN) content and also in free amino acid composition. Arginine, aspartic acid and glutamic acid were found to be abundant in palm kernel, whereas tryptophan, glutamic acid, aspartic acid, alanine and arginine were more in coconut. The changes in amino acids on heating is also different. This is discussed in Chapter 5.

CHAPTER 5

MODEL SYSTEM STUDIES AND SUGAR-AMINO ACID INTERACTIONS IN COCONUT AND PALM KERNEL

The chemical reactions occurring in the food matrix, on heating, leading to flavour enhancement, have been studied extensively. The food industry has used this knowledge to control processes and hence to develop better tasting food products. One of the most important uses of this information is in the development of flavourings. Flavour industry increasingly offers roast aromas which can be added to convenient food products to fortify their sensorial qualities. Roast aroma essences of caramel, meat preparations, bread, peanut, biscuit, coffee, cocoa and malt products are already marketed abroad. In India, these flavours have not been produced or introduced in the market.

'Processed flavours', as the manufacturers describe it, mainly consist of flavours which have been created by thermal processing of foods, ingredients or by the use of blends of synthetic chemicals and extractives which have been known to be formed during heating. The evaluation of artificial roast aromas requires the exact knowledge of their composition.

A number of flavour studies have been reported recently, in which model systems consisting of important amino acids, sugars and rarely the lipids are reacted. Conditions of the reaction like nature of the reactants,

their concentration, water content, pH, temperature, presence of solvents etc are important. The nature of the volatiles produced depends much on blanching the 1,2- and 2,3-enolisation of Amadori compounds and aldolisation and dealdolisation reactions. The nature of sugars - oligomers and monosaccharides - and the amino acids are also important.

In the present study, based on the results of flavour analysis as well as carbohydrate and protein analyses, model systems were constituted simulating coconut and palm kernel and then heated. Results of these experiments and the sugar-amino acid interactions in coconut and palm kernel are discussed in this chapter.

RESULTS AND DISCUSSION

The volatile products formed during heating of the model systems were analysed by GC and GC-MS. About 75 percent of the recorded peaks were identified.

5.1 MODEL SYSTEM STUDIES OF COCONUT

Table 48 represents the composition of the model system used. The pyrazines identified in the roasted coconut sample could be identified in model system also. In addition to the pyrazines and 5-methyl furfural,

Table 48 : Composition of the model system simulating coconut

	g/100g
Water	40.0
Coconut oil	42.0
Sand (equivalent to protein content)	4.8
Sucrose	4.2
Glucose	0.6
Fructose	0.3
Starch	1.8
Cellulose (Filter paper)	6.0

Free amino acids were taken based on the free amino acid analysis of coconut (Table 33)

a few more furans also could be identified. Thus, 2-methyl, 2-ethyl, 2-methyl-3-ethyl, 2,3-dimethyl and 2-pentyl furans were also identified. Table 49 gives the list of compounds identified additionally in the model system. In the neutral fraction, γ-lactones, benzaldehyde, 2,4-undecadienal furan derivatives etc were also noticed, in addition to the δ-lactones, alcohols, ethyl esters and hydroxy esters which were identified in control and heated samples. More number of hydrocarbons were formed in the model system. Methyl ketones like 2-nonanone and 2-undecanone were also noted in the model system. The acid fraction showed straight chain odd and even numbered fatty acids and this included additionally two acids which were not identified in heated coconut.

5.2 MODEL SYSTEM STUDIES OF PALM KERNEL

The composition of the model system used for the study is represented in Table 50. The compounds identified in roasted palm kernel could be identified in model reaction system also. All the pyrazines were identified and the furans were formed in higher proportions. 2-Methyl furan, 2-ethyl furan, 2,3-dimethyl furan and 2-pentyl furan were identified only in the model system. Table 51 gives the list of compounds additionally identified. These compounds included a few hydrocarbons,

Table 49 : Compounds identified additionally in the volatiles of model system similar to coconut

2-Methyl furan

2,3-Dimethyl furan

2-Ethyl furan

2-Methyl-3-ethyl furan

2-Pentyl furan

Benzaldehyde

2,4-Undecadienal

2-Nonanone

2-Undecanone

Ethyl hexanoate

Ethyl nonanoate

Ethyl undecanoate

δ-Hexalactone

γ-Nonalactone

γ-Undecalactone

Decane

Undecane

Tridecane

Pentadecane

Hexadecane

Undecanoic acid

Tridecanoic acid

Table 50 : Composition of model system simulating palm kernel

	g/100g
Water	20.0
Palm kernel oil	40.0
Sand (equivalent to protein content)	7.0
Sucrose	2.0
Other sugars*	0.5
Starch	0.6
Cellulose (Filter paper)	5.4

Free amino acids were taken based on the free amino acid analysis of palm kernel (Table 47)

* Other sugars (mg/100g)

Ribose	32
L-Rhamnose	42
Arabinose	16
Fructose	220
Glucose	52
Mannose	60

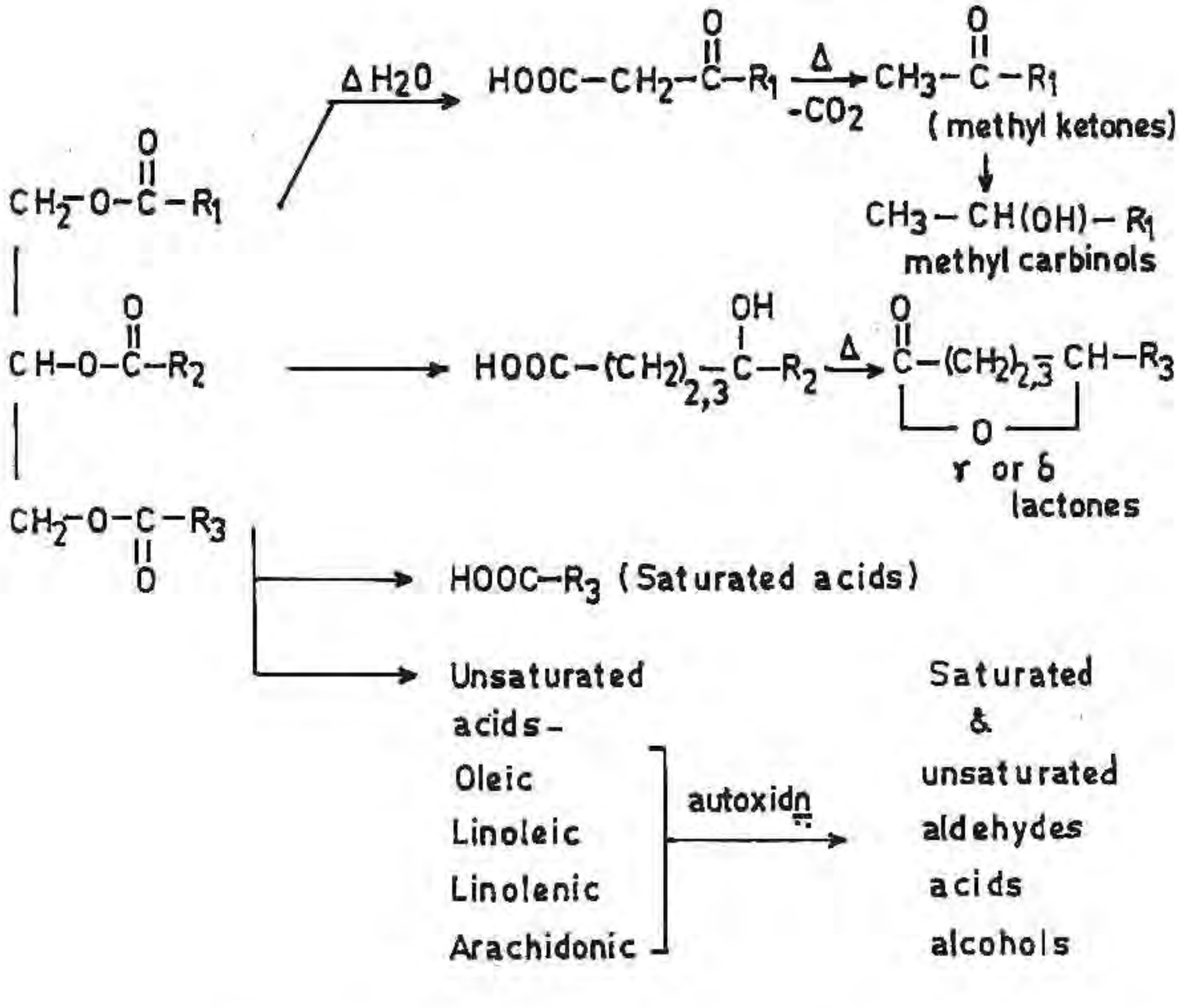
Table 51 : Compounds identified additionally in the model system of palm kernel

2-Methyl furan
2-Ethyl furan
2-Pentyl furan
Nonane
Decane
Undecane
Tridecane
Pentadecane
Hexadecane
Ethyl undecanoate
Ethyl hexanoate
Benzaldehyde
2-Nonanone
2,4-Undecadienal
Pentanoic acid
Heptanoic acid
Nonanoic acid
Undecanoic acid
Pentadecanoic acid

ethyl esters and a few odd carbon numbered fatty acids also.

5.3 DISCUSSION OF MODEL SYSTEM STUDIES

More numbers of hydrocarbons, furans, ketones and esters have been found to be produced in the model system of coconut. Two γ-lactones, namely, γ-nonalactone and γ-undecalactone are noticed. The formation of more number of furans can be attributed to carbohydrate degradation; whatever be the intimate mixing effected in a model system, the difference in homogenity of the food matrix can lead to localised heating by which a few types of compounds are formed more. The hydrocarbons, methyl ketones, esters and γ-lactones are mostly lipid-derived[237] and in the model system constituted here, the lipids are certainly more exposed to the heat than they would have been in the endosperm cells. Formation of lipid derived volatiles can be outlined as shown here.[8]



No additional pyrazines could be detected in the model system. The difference in the relative concentration of pyrazines, if there be any, is not taken into account in this study, since it was planned mainly as a qualitative study.

In the case of palm kernel, the same pyrazines, which were identified in heated kernel were detected in model system studies also. However, more number of furans were detected. More number of hydrocarbons have been formed from fat degradation. The ethyl esters and

methyl ketones which are additionally formed are volatiles that are mostly lipid derived. Here also, the minor differences in the volatile composition of model system can be attributed to the slight differences in the homogenity of the food matrix. The oil is more exposed to heat treatment in the constituted matrix than it would have been in the compartmentalised state inside the cells of the endosperm.

In coconut and palm kernel oils, the polar lipids are relatively low and the neutral lipids are the major class. So the pressed oils were used in the model systems. As far as pyrazine formation is concerned, it appeared that the polar lipids do not exert a major impact, since the same number of pyrazines could be detected in both natural and constituted systems. The free amino acids and sugars are responsible for pyrazine formation since even without the proteins, the pyrazines have been formed.

5.4 SUGAR-AMINO ACID INTERACTIONS IN COCONUT ON HEATING

The formation of pyrazines from amino compounds and carbonyl compounds is more or less established. In a food system, these are readily provided by the free amino acids and reducing sugars present in them. From the present study we find that non-protein nitrogen (NPN) content and the free amino acid concentration are affected

by heat and at the same time, increase in pyrazine content is also noticed. A few examples of relevant model system studies carried out to correlate reaction between amino acids and sugars with flavour changes as well as intensity of browning on heating, are worth discussing here.

Koehler et al[78] and Koehler and Odell[79] used different amino acids as nitrogen source and sugars as carbon source for their studies and reported the formation of pyrazines during heating. Different combinations of amino acids and sugars were heated under different conditions. The results indicated that quantitatively a temperature range of 100 to 150°C increased pyrazine concentration and a basic pH favoured its formation. The temperature range agrees with the observation in this study also. Among the amino acids tried, asparagine, aspartic acid and glutamic acid gave better yield of pyrazines. Among sugars, pentoses were found to be more reactive than hexoses and fructose reacted more readily than glucose. In all these experiments, methyl pyrazine and 2,5-dimethyl pyrazine were predominantly formed irrespective of the amino acid and sugar used in the various combinations of sugars and amino acids tried, possibly due to differential fragmentation of the carbon skeleton in individual sugars. Recently, Arnoldi et al[250] have

reported a model system study simulating roasting of cocoa beans. In this, fructose was heated with major amino acids present in cocoa and the results indicated that methyl and 2,5-dimethyl pyrazines were formed in all cases, in higher amounts. Leucine was found to give as many as 10 different pyrazines, whereas lysine and aspartic acid gave only two different pyrazines. The amount of pyrazines formed in fructose-lysine system was also less. A few other model system studies are also reported in literature in which intensity of browning is correlated with activity of amino acids and sugars. Ashoor and Zent[280] had heated different amino acids and sugars and the degree of browning compared. It was seen that intensity of browning varied as ribose> fructose > glucose; and lysine > glycine > tryptophan > tyrosine. The formation of pyrazines in coconut and in palm kernel and the changes in the sugars and free amino acids of these nuts can be correlated in the light of the above studies.

Coconut contains glucose and fructose which are known to be active in browning as well as in pyrazine formation and in the present study they are found to be utilised during heating. Sucrose and starch are also found to be affected in roasted coconut. Formation of a few furans

in heated coconut can be attributed to carbohydrate degradation. Among free amino acids, lysine, tryptophan, glutamic acid, aspartic acid, alanine, valine and glycine are more affected. Of these, lysine, glycine and tryptophan are likely to contribute more towards actual browning process whereas aspartic acid, glutamic acid, alanine and valine may preferentially participate in pyrazine formation.

5.5 SUGAR-AMINO ACID INTERACTIONS IN PALM KERNEL ON HEATING

In the case of palm kernel, the important sugars present are fructose, glucose, mannose and L-rhamnose. Of these, L-rhamnose was reduced to nearly traces ($\leq$10mg/100g level) and fructose, glucose and mannose were all affected. The principal role of mannose in browning of palm kernels during heating has been proposed by McWeeny in one of his private communication as cited in a report[274]. Shibamoto and Bernhard[82] had carried out a few model system studies using hexoses and ammonium hydroxide. They found that total pyrazine yields from mannose and fructose were essentially same, whereas galactose produced less. Mannose produced most and fructose the least amount of unsubstituted pyrazine. Also these researchers[82] had reacted L-rhamnose with ammonia and isolated nearly 95 compounds which included unique pyrazines also. The changes in sugars of palm kernel can also be correlated with browning and formation of pyrazines based on these reported studies.

Among the free amino acids of palm kernel valine, isoleucine, leucine, lysine, glutamic acid, aspartic acid and threonine are affected more during roasting and these may contribute directly to pyrazine formation. Lysine-mannose system is assumed to contribute largely towards browning of palm kernel, by McWeeny[274]. This has been discussed in Chapter 4. The formation of furans in larger quantities in palm kernel must be due to preferential 1,2-enolisation in the intermediate stages of Maillard reaction (Fig.4). Also, compared to coconut, carbohydrate participation/degradation is likely to be more in palm kernel due to lower NPN content and lesser availability of reducing sugar.

Thus, eventhough changes in a particular amino acid or sugar cannot be identified with the formation of a specific pyrazine, the overall changes in carbohydrate and amino acid profiles of coconut and palm kernel on heating are explanatory of the flavour enhancement observed during roasting.

5.6 GENERAL OBSERVATIONS

A few observations made during the study are worth investigating further. The susceptibility of saturated oils like, coconut oil and palm kernel oil for ketonic rancidity is very high and is a major problem during

storage. Many mechanisms have been proposed to explain the development of rancidity in these saturated oils. However, retardation or prevention of rancidity is still in experimental stage. The area provides scope for further research work, especially when it has been observed that oil extracted from heated kernels has a better shelf life compared to unheated control. The antioxidant property of Maillard products in preventing oxidative rancidity is reported in the literature[272,273], quite often and found to be comparable with BHA, BHT etc[273]. The antioxidant property of Maillard reaction products, especially the non-volatile part, deserves a deeper study.

In coconut, the nutritional aspects of heated coconut, still remain to be investigated. Biochemical studies of coconut cake have been reported in the past[178]. Samson[262] had studied the lysine availability by chemical methods, in coconut heated to 120°C. Other studies conducted by him were on defatted coconut meal heated to different temperatures upto 160°C. These experimental conditions are different from the sample preparation followed in the present study because in a defatted meal, the changes due to heat are more severe. Even the studies of coconut cake cannot be compared with coconut because during oil extraction heat is generated by abrasion and

shearing of the material. Effect of heat treatment on coconut meal are much more drastic than in the case of cake or full fat coconut. To a large extent, the fat protects sensitive components like proteins from heat denaturation.

Recently, Baltes and Bochmann[24] had reported a number of coffee volatiles in their model system studies using serine and threonine with sucrose. According to them the head space volatiles are extremely important in preserving the fine aroma of fresh coffee. Simulated meat flavourings are already reported which are either 'reaction products' formed by heating relevant amino acid-sugar precursors or 'natural meat-based flavourings' in which meat slurries, extracts, dialysates etc are also involved. In addition to these, meat aroma chemicals are finely combined to get the overall flavour effect. In this context, other aspects of work that can be thought of are the development of flavour of roasted coconut which is more of a food item than an oil seed, and palm kernel oil with enhanced flavour characteristics. Since model system studies tried on the present work, could produce flavours very similar to roasted coconut and palm kernel, the possibility of developing their enhanced flavours by reacting most active ingradients rather than heating the entire food material is indicated. This approach

may avoid unnecessary heat denaturation of proteins and reduction in nutritive value. The possibility of encapsulating these flavours as molecular complexes, offers a field of immediate academic interest and in the long run, of some practical applications.

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LIST OF PUBLICATIONS

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