SECTION A

ANALYSIS OF LEMONGRASS OIL
CHAPTER IV

INTRODUCTION
4. Analysis of Lemongrass (Cymbopogon flexuosus) Oil

4.1 Introduction

The quality of lemongrass oil is determined by its citral content. Various methods were reported in literature for the estimation of citral in lemongrass oil\(^1\),\(^2\),\(^3\) and also for the separation of citral from lemongrass oil\(^4\),\(^5\),\(^6\),\(^7\). The common methods for the estimation and separation of citral are discussed below:

4.1.1 Bisulphite method\(^1\),\(^4\)

The bisulphite method is based on adduct formation process as shown below:

\[
\text{H}_\text{C} = \text{C} = \text{OH} \quad \xrightarrow{\text{NaHSO}_3} \quad \text{H}_\text{C} = \text{C} = \text{OH} \quad \text{SO}_3\text{Na}
\]

Upon shaking a measured quantity of the oil with a hot aqueous solution of sodium bisulfite, an adduct is formed, which dissolves on heating the solution. The noncitral portion of the oil separates as an oily layer which can be measured conveniently in the neck of a Cassia flask and thereby determine the citral content of the oil. In the case of citral (1) due to the presence of two ethylenic linkages, and an
aldehyde group, the adduct formation is complex. A normal addition compound (2) is formed when one molecule of sodium bisulfite combines with the carbonyl group of citral, from which citral can be regenerated by treatment with alkalis such as sodium carbonate. A labile disulfonate (3) is formed in which the sulfonate radical is apparently attached to carbonyl group and also to the ethylenic linkage conjugated to the carbonyl group. The disulfonate adduct formed is water soluble and citral can be regenerated from it by treatment with sodium or potassium hydroxide. But there will be a loss of 10 to 15% in the recovery. With sodium bisulfite for several hours in acid medium citral give a disulfonate(4) which is stable and from which citral cannot be regenerated. Since it combines with tolenyl hydrazine, it contains a free aldehyde group. The sulfonate radical evidently shifts on altering the acidity of the solution. Thus the labile disulfonate gets converted to the stable derivative by treatment with acids.

According to Dodge (5), it is possible to obtain besides the normal addition product, a water soluble monosulfate where the sulfonate radical is attached to the double bond conjugated to the carbonyl group. The carbonyl compound can be regenerated from this product. However in the case of citral, if the sulfonate radical is attached to position 7(5), the compound is stable and
from it citral cannot be regenerated. A mixture of these three types may also be formed simultaneously and theoretically it is possible to get seven sulfonates. Thus any mono or poly sulfonate which has a sulfonic radical in position 7 of the citral molecule is a stable compound from which citral cannot be regenerated, thus having sulfonate groups attached to positions 1, 3 or 7. Citral can be regenerated if the sulfonate groupings are at 1, 3 or 1 and 3, but cannot be regenerated if the sulfonate groupings are at 1, 7 and 1, 7 and 3 or 7 and 3 and 7 and 1.
Commonly used method of estimation of citral especially in trade and commerce is the bisulfite adducting method. But this method has many disadvantages as an estimation method for citral in lemongrass oil. Since lemongrass oil contains aldehydes other than citral like citronellal, n-decyl aldehyde etc. and also methyl ketones like methyl heptenone and all aldehydes and methyl ketones will get adducted with sodium bisulfite, the value obtained will be much higher than the actual citral content.

As a method of separation also this method has many defects. All the aldehydes and methyl ketones present in lemongrass oil will get adducted and on regeneration all of these components will be regenerated thereby reducing the purity of citral obtained. In this process, the oil is shaken with saturated sodium bisulfite solution - and the resulting crystalline solid is separated and purified by washing with alcohol or ether. Citral is regenerated by decomposition of the adduct with sodium carbonate, sodium hydroxide or hydrochloric acid. Even though the normal adduct can be easily decomposed, quantitative regeneration of citral is difficult. Usually a loss of 10-15% is observed. The loss is reported to be due to the formation of a cyclic bisulfite compound in presence of alkali from which recovery of citral is found to be difficult. Acid
initiated decomposition of the adduct usually leads to cyclisation and polymerisation of the product which is undesirable. Because of these reasons the yield as well as the purity of the citral separated will be poor.

4.1.2 Neutral sulfite method

This is also an adduct formation reaction as shown below:

\[
\text{CHO} \quad \xrightarrow{\text{Na}_2\text{SO}_3, \text{H}_2\text{O}} \quad \text{OH} \quad \text{H} \quad \text{SO}_3\text{Na} \quad \text{NaOH}
\]

In this method, sodium hydroxide liberated has to be neutralised periodically with acid to permit the reaction to go to completion. Neither must the solution be permitted to turn acidic, as this would result in the formation of the stable dihydrosulfonic compound, from which citral cannot be regenerated. For this purpose, Tiemann suggested the following modification.

Shake a solution of 350 gm of sodium sulfite in 1 litre of water with 100 gm. of pure citral, after adding a few drops of phenolphthalein. Reduce the strongly alkaline reaction to be produced, from time to time by gradually adding standardised sulfuric acid of about 20% strength. Keep the solution always slightly alkaline.
This method also has all the disadvantages of the bisulfite method as a method of estimation and also of separation. However it offers certain advantages over bisulfite method. By using the indicator (phenolphthalein) the exact end point of the reaction can be determined\textsuperscript{2}.

Erman and Coworkers\textsuperscript{10} found that when citral was treated with Na\textsubscript{2}SO\textsubscript{3} in aqueous base at pH 11.2, (6) was obtained in 77% yield, which was cyclised to give 81% (7). At pH 3 - 8.8, 87% (3) was obtained. Additional products obtained were (2), (4) and (8).

\begin{align*}
\text{(6)} & \quad \text{SO}_3\text{Na} \quad \text{S} \quad \text{Na} \quad \text{CHO} \\
\text{(7)} & \quad \text{SO}_3\text{Na} \quad \text{S} \quad \text{O} \quad \text{H} \\
\text{(8)} & \quad \text{SO}_3\text{Na} \quad \text{S} \quad \text{O} \quad \text{H} \\
\end{align*}

4.1.3 Hydroxylamine Method\textsuperscript{11,12}

This method is also used for the estimation of citral in lemongrass oil. This method makes use of both hydroxylamine and hydroxylamine hydrochloride and is based on the equation,
After the reaction of this with the carbonyl group the mixture is titrated with standard alkali.

The hydroxyl amine method also has some defects. All the carbonyl groups present in lemongrass oil will react with hydroxyl amine and the value obtained will be much higher. However this method offers some advantages over the adduct formation process.

1. Relatively small amounts of the oil are required for an estimation.

2. The reaction of hydroxylamine with aldehyde is rapid, thereby shortening the time required for the estimation.

3. This method proves exceptionally applicable to oils which contain large amounts of aldehydes.

4. The solution used for the standard procedure are stable and can be kept for longer periods.
The titration can also be done potentiometrically. For that known amount of titrant are added to the solution to be titrated. After each addition, the pH is determined and end point of titration found out. The end point is the increase in volume with highest pH difference.

4.1.4 Colorimetric Method

The citral content of lemongrass oil has also been estimated by the coloring agent - that of Ehrlich Miller. This coloring agent has been found to give better results and development of colour takes place rapidly and remain quite stable for a long time. The colouring agent has been prepared according to Ehrlich Miller and consist of the solutions.

1. 5% p-dimethylaminobenzaldehyde solution in acetic acid.
2. 10% phosphoric acid solution in acetic acid.

One ml each of the above solutions are added to different amounts of citral in acetic acid, whereby a marked colour change from blue to pink can be observed. The percentage absorbance and extinction of the coloured citral is then measured using colourimeter and calibration graphs are plotted. The amount of citral in solutions can be compared with that of known strength and thus the percentage of citral can be determined.
Here also we need solutions of citral with known strength.

Since all these methods discussed have defects as a method of estimation of citral, it was thought to develop a new method of estimation of citral in lemongrass oil, having no defects. With this aim in view a new method for the estimation of the correct percentage of citral in lemongrass oil by physical separation of citral quantitatively in pure form, was developed by column chromatographic technique.

Preliminary experiments were done using neutral alumina (grade I) as the adsorbant and hexane as the eluent. Eventhough it was possible to separate all the hydrocarbons using this system, it was found that on prolonge column chromatography, the column developed heat and the chromatography could not be done satisfactorily. The development of heat can be attributed to the polymerisation of the highly reactive \( \alpha \)-unsaturated aldehydes namely geranial and neral on the alumina column. Hence further chromatographic studies were undertaken using the milder adsorbant silicagel. Preliminary studies gave promising results. After repeated column chromatographic studies it was found possible to isolate citral quantitatively in pure form (99+% – purity checked by GLC) and thus determining the correct percentage of citral in lemongrass oil. This
happens to be a new method of estimation of citral.

Different varieties of lemongrass oil obtained from different regions of India (RRL - Jammu, RRL - Bhubaneswar, RRL - Jorhat, Odakkali, Bhuttan and Cochin) were analysed by the newly developed column chromatographic technique and the correct percentage of citral present in these oils were determined.

For the separation of citral from lemongrass oil both chemical and physical methods are used at present. The chemical methods used were discussed earlier in the bisulfite and neutral sulfite adducting methods. On an industrial scale it is commonly separated from lemongrass oil till now by vacuum fractionation of the oil. In vacuum fractionation an enrichment of citral happens and citral of only 95% purity is generally obtained. Moreover removal of components like geraniol(2) nerol (10) etc., which have boiling points differing only by few °C from that of citral is found to be difficult even when high efficiency fractionating columns are used. Being a mixture of α-β unsaturated aldehydes, citral is heat labile and excessive heat treatment is likely to lead to rearrangements, polymerisation and eventual destruction of the material. Hence in this method there is invariably a loss in the yield.
Hence in both the vacuum fractionation and in the adducting methods, used at present for the isolation of citral from the oil, the purity as well as the yield of citral is unsatisfactory. Since estimation of the correct percentage of citral in lemongrass oil by the physical separation of pure citral in quantitative yields was achieved, column chromatographic method for the separation of citral from lemongrass oil was tried. With a view to check the possibility of commercialising the isolation of pure citral in quantitative yields from lemongrass oil, slight modifications were made on the estimation method using column chromatography. Changes were made on the column parameters like the ratio of adsorbant to silicagel, eluents and rate of elution. It was found possible to isolate pure citral, (99+% purity checked by GLC) in near quantitative yields by this method. This process being a physical process not involving the use of any chemicals, the possibility of rearrangements of citral during separation is minimal. This process also
excludes excess of heat treatment which is undesirable in the case of thermally labile molecules like citral. The adsorbant used can be regenerated and reused after proper cleaning and activation and also the eluents used can be recovered and recycled. Because of these advantages this method is far superior to other existing physical (distillation) and chemical methods, available, for the separation of citral of high purity and that too in near quantitative yields.

Total analysis of lemongrass oil was also tried using column chromatographic method. In a single column it was possible to separate lemongrass oil into four fractions – namely mixture of hydrocarbons, gernayl acetate together with carbonyl components other than citral, citral and mixture of alcohols. The third fraction obtained was pure citral. The other three fractions were repeatedly chromatographed so as to separate individual components.

The first fraction was found to be a mixture of 3 hydrocarbons namely myrcene, limonene and dipentene. It was found difficult to separate these three components. On a long column using silicagel as adsorbant and hexane as eluent, myrcene was separated from this mixture.

8% of the lemongrass oil (commercial sample from Cochin) was found to be a mixture of 4 components namely n-decyl aldehyde, citronellal, geranyl acetate and
methyl heptehone. From this mixture pure geranyl acetate and pure methyl heptenone were separated.

12.33% of the lemongrass oil analysed was found to be alcohols - linalool, geraniol, nerol, citronellol and methyl heptenol. By column chromatography linalool was obtained in pure form. Using column chromatography the non-citral portion of lemongrass oil and also the ionone tops were analysed. They are used as cheap perfumes in soaps. On chromatographic analysis, it was found that 50-60% of these fractions are valuable alcohols. If these alcohols can be separated these fractions will command more attractive price. With this target in mind we separated the alcohols present in the non-citral portion as well as that in the ionone tops.

Citral the main component of lemongrass oil is known to be a mixture of geranial (11) and neral (12) in the ratio of 5:3. Complete separation of these two isomers has not yet been reported even though isolation of one isomer by chemical method is claimed. It is reported that geranial can be obtained free from neral, during regeneration from the bisulfite adduct by
taking advantage of the fact that crystalline sodium bisulfite adduct of gernial is sparingly soluble while the corresponding adduct of neral is readily soluble in water. By this method the yield of gernial obtained will be less since its adduct is sparingly soluble in water and also neral cannot be regenerated. It is also reported that neral can be isolated from citral by shaking for a short time with alkaline cyanoacetic acid solution. Gernial is said to react with this acid much more readily than neral. This separation is also not likely to be complete. Separation of these two isomers were also reported through their semicarbazones. Semicarbazones of gernial and neral differ in melting points (semicarbazone of gernial M.P. 164°C and semicarbazone of neral M.P. 171°C).

By repeated recrystallisation it was possible to separate the two semicarbazones. Partial separation of citral isomers by vacuum spinning band distillation in small quantities was also reported recently. By this method, of the six fractions collected, the sixth fraction contains above 95% gernial.

With a view to separate the cis-trans isomers in pure form and without loss of either of the isomers, column chromatographic studies were undertaken. Preliminary results indicated the possibility of separation of the two isomers. The initial experiments showed a reversal of the ratio of the two isomers in GLC. Finally
separation of citral into pure neral, mixture of neral and geranial and pure geranial was achieved\textsuperscript{21}. Complete separation of these two isomers in quantitative yields can be considered as the ultimate achievement in the field of lemongrass oil technology. This work is of great relevance commercially since neral is highly priced and also nerol obtained by the reduction of neral is an expensive perfumery chemical. Source for pure neral and nerol in India are rare.

Column chromatographic separation of the cis-trans isomers were also checked in the alcohol level. But it was found to be more difficult than in the case of aldehydes. For this citral was reduced using aluminium isopropoxide in anhydrous isopropanol. The product obtained on analysis showed the presence of 20% hydrocarbons and the rest being alcohols (geraniol/nerol). Hydrocarbons were formed as the side product of Meerwein Pondroff Verley reduction. Finally the product was separated into hydrocarbons, pure nerol, mixture of nerol and geraniol and pure geranial.