RESULTS AND DISCUSSION

The various methods employed for the detection of adulteration of onion oil or garlic oil necessitated the application of accurate analytical methods to mixtures of such oil and a particular adulterant of well known composition. It is important to indicate that any foreign substance, when added in a variable quantities of authentic samples of the mentioned oils, could be looked upon as an adulterant. However the present investigation includes the various procedures which could be successfully used for the detection of adulterants usually employed by exporters and producers and it is common knowledge that these substances are characterized by their availability and their relatively cheap price besides the fact that they are structurally similar to one or more of the main constituents of onion and garlic oils .

The results obtained during the course of the present work necessitated the presentation of data under two main headings. The first one represents the results of onion oil from Allium Cepa L. (Bassel) and the second one represents the results of garlic oil from Allium sativum L. (Toam) although both present the Genus Allium which is placed in the family Liliaceae.

It should be pointed out at this stage that the analytical methods, which are indicated for each adulterant, are presented in a certain order which followed to a great extent the sequence of events aiming at the quantitative determination of minute quantities of the adulterant in question. In addition, more than one analytical method were employed to verify the results and to give those who are interested in such a problem the chance to choose between procedures on the basis of the availability of reagents.

IV-1. Part I: Onion volatile oil:

IV-1.1. Physical and chemical properties of onion volatile oil, aged onion volatile oil and adulterated samples with various adulterants

Onion volatile oil, aged onion volatile oil and adulterated onion volatile oil with various adulterants were subjected to the routine analytical determination employed in the field of essential oils, for identification and comparative purpose. These analysis include the specific gravity, refractive index, acid number and the solubility in ethanol and other organic solvents.

IV-1.1.1. Physical and chemical properties of fresh onion volatile oil:

Data tabulated in table (3) show the physico-chemical properties of onion volatile oil.

In general the obtained results are in agreement with those reported by Chiris (1937), Guenther (1952) and Nofal (1974).

The solubility results shown in table (4) indicated that the onion volatile oil was insoluble in diluted ethyl alcohol (75 and 85%), but soluble in 5.1 volume and more of 95% ethyl alcohol. Also these results indicated that onion volatile oil was completely soluble in

Table (3)

Physical and chemical properties of onion volatile oil and adulterated samples by different adulterants

Samples	Specific gravity	Refractive index	Acid number
Fresh onion volatile oil.	1.0965	1.5614	7.27
Fresh + 10% aged onion volatile oil	1.0977	1.5623	9.77
,, + 20% ,, ,, ,, ,,	1.0995	1.5629	10.57
,, + 30% ,, ,, ,, ,,	1.1015	1.5635	11.31
Fresh + 10% maize oil	1.0769	1.5517	6.88
,, + 20% ,, ,,	1.0557	1.5435	6.41
,, + 30% ,, ,,	1.0239	1.5342	5.29
Fresh + 10% ethyl alcohol	1.0697	1.5530	6.93
,, + 20% ,, ,,	1.0269	1.5396	6.33
,, + 30% ,, ,,	1.0142	1.4303	5.21
Fresh + 10% Paraffin oil	1.0770	1.5537	6.87
,, + 20% ,, ,,	1.0703	1.5461	5.74
,, + 30% ,, ,,	1.0544	1.5337	5.49
Aged onion volatile oil	1.1143	1.5703	12.33
Maize oil	0.9202	1.4755	0.27
Ethyl alcohol	0.7618	1.3645	0.13
Paraffin oil	0.9698	1.4864	0.28

Table (4)

The solubility in ethyl alcohol of onion volatile oil and adulterated samples by different adulterans

Sample	Solubility in ethyl alcohol			
	75%	85%	95 %	99 %
Fresh onion volatile oil	Turbid	Turbid	Sol. in 6.1 vol. and more	Sol. in 1 vol. and more
Fresh + 10% aged onion volatile oil	Turbid	Turbid	Sol. in 3.1 vol. and more	Sol. in 1 vol. and more
„ + 20% „ „ „	„	„	Slightly turbid	Slightly turbid
„ + 30% „ „ „	„	„	Turbid	Turbid
Fresh + 10% maize oil	Turbid	Turbid	Turbid	Sol. in 1 vol. and more
„ + 20% „	„	„	„	„
„ + 30% „	„	„	„	„
Fresh + 10% ethyl alcohol	Turbid	Turbid	Sol. in 4.6 vol. and more	Sol. in 1 vol. and more
„ + 20% „ „	„	„	Sol. in 3.6 vol. and more	Sol. in 1 vol. and more
„ + 30% „ „	„	„	Sol. in 3.6 vol. and more	Sol. in 1 vol. and more
Fresh + 10% Paraffin oil	Turbid	Turbid	Slightly turbid	Slightly turbid
„ + 20% „ „	„	„	„ „	„ „
„ + 30% „ „	„	„	„ „	„ „
Aged onion volatile oil	Turbid	Turbid	Turbid	Sol. in 3.6 vol. and more
Maize oil	„	„	„	Turbid
Ethyl alcohol	soluble	soluble	soluble	soluble
Paraffin oil	Turbid	Turbid	Turbid	Sol. in 1 vol. and more

carbon tetrachloride diethyl ether, n-hexane, and chloroform. These results are in partial agreement with the results obtained by Chiris (1937) and Nofal (1974).

IV-1.1.2. Physical and chemical properties of aged onion volatile oil and adulterated samples with aged onion volatile oil:

The aged onion volatile oil represent one of the most important adulterants. The addition of quantities of such oil could easily pass the simple routine analysis usually employed. The choice of aged onion volatile oils was based on the fact that it is available in cheap price.

The physico-chemical properties of aged onion volatile oil and onion volatile oil adulterated with various concentration of aged onion volatile oil are shown in table (3). Results showed that adulteration with 30% aged onion volatile oil increased the specific gravity from 1.0965 for the fresh sample to 1.1015.

The refractive indeces of fresh onion oil and aged onion volatile oil were 1.5614 and 1.5703 respectively. It is obvious to note that the addition of aged onion oil caused a gradual increase in the refractive index of the onion oil. Such increase was directly proportional to the concentration of aged onion oil added. The onion oil adulterated with 30% aged onion oil gave the higher refractive index (1.5635) in comparison with fresh onion oil sample (1.5614).

The acid number of fresh onion oil gradually increased with increasing the percentage of aged onion oil i.e. the acid number increased from 7.2656 for fresh onion oil to 9.7685, 10.5721 and 11.3136 for fresh onion oil adulterated with 10, 20 and 30% of aged onion oil, respectively. This result may be attributed to the high acidity of the latter oil, which resulted from the oxidation of aged onion oil during long term storage at ambient temperature. It could be concluded that the acid number could be used to detect the adulteration of onion volatile oil with aged onion volatile oil.

The solubility of onion volatile oil in ethyl alcohol decreased after adulteration with various concentrations of aged onion volatile oil (10, 20 and 30%), while the fresh onion volatile oil is soluble in 95% and 99% alcohol, the aged onion volatile oil soluble in 3.6 volume and more of 99% alcohol, and insoluble in 75%, 85% and 95% alcohol.

The solubility of onion volatile oil adulterated with 30% aged onion volatile oil decreased and was turbid in 99% ethyl alcohol. However, oily separation was observed from these solutions after about 1-3 minutes. Results in table (4) indicate that the solubility could be used to detect adulteration using aged onion volatile oil. Thus, the solubility of essential oils in ethyl alcohol could be considered as a good criterion for its quality, Guenther (1960).

On the other hand, fresh and aged onion volatile oil were completely soluble in diethyl ether, carbon tetra chloride, n-hexane, benzene and chloroform.

IV-1.1.3. Physical and chemical properties of adulterated onion volatile oil with maize oil:

Maize oil represents the most common edible oil usually used as an adulterant in the field of essential oil. Since it is relatively cheap and available in large quantities. But, the chemical nature of maize oil is quite different from that of onion volatile oil. Accordingly, addition of maize oil to onion volatile oil will clearly affect the various properties of the investigated essential oils, especially, the chemical properties.

In the present study, onion volatile oil was mixed with increment proportion of pure maize oil at concentration of 10, 20 and 30% by volume.

The changes in physico-chemical properties of onion volatile oil adulterated with 10, 20 and 30% maize oil are shown in table (3). The results revealed that adding increment proportions of maize oil caused a gradual decrease in the specific gravity values of onion volatile oil, since, the specific gravity of maiz oil was lower than that of pure onion volatile oil (0.9202). The specific gravity decreased from (1.0965) to (1.0239) for onion volatile oil admixed with 30 percent of maize oil.

However, obtained results reveal that specific gravity can not be used as a reliable parameter for the detection of maize oil, since such values fall easily within the range of specific gravity of onion volatile oil which ranged from 1.0118 to 1.0980 as mentioned by Chiris (1937), Gunther (1952) and Nofal (1974).

The refractive indeces of fresh onion volatile oil and maize oil were 1.5614 and 1.4755 respectively. Addition of onion oil with various concentrations of maize oil caused gradual decrease for refractive index of onion volatile oil. Such decrease was directly proportional to the admixed concentration of, maize oil. The onion volatile oil adulterated with 30% maize oil had the lowest refractive index (1.5342).

The acid number of adulterated onion volatile oil with maize oil at different concentrations showed obvious decreases when compared with the pure oil. The acid number decreased from 7.27 for onion volatile oil to 6.88, 6.41 and 5.29 for onion volatile oil adulterated with 10, 20 and 30% of maize oil respectively. This could be atributed to the low acidity of maize oil, accordingly, the addition of maize oil caused the decrements of the acidity of onion volatile oil.

The solubility could be considered a good parameter for determining the quality of essential oils, any change in the solubility behaviour of an essential oil usualy demonstrates the presence of an impurities in the oil. In the present investigation all adulterated samples were subjected to a series of solubility measurements using different concentrations of ethyl alcohol 75, 85, 95 and 99% .

It was noticed that pure onion volatile oil was completely soluble in alcohol at concentration more than 95%. While, samples mixed with maize oil in the concentration of 10, 20 and 30% were soluble in alcohol at 99% concentration while it was insoluble at a concentration of 95% alcohol or below that percent. In this case a turbid solution formed and an oily layer separated immediately at room temperature.

On the other hand, the onion volatile oil samples adulterated with maize oil at concentration of 10, 20 and 30% were completely soluble in diethyl ether, carbon tetra chloride, n-hexane, benzene and chloroform.

IV- 1.1.4. Physical and chemical properties of adulterated onion volatile oil with ethyl alcohol:

Ethyl alcohol is considered as one of the most alcohols usually used by exporters and producers of essential oil in moderate quantities as an adulterant. Since it is comparatively a cheap source and commercially available as diluent for essential oil.

Essential oils usually Contains alcohols demonstrated as hydroxyl groups, consequently the addition of ethyl alcohol would be a common practice especially it would not affect either the odour or the flavour of the resulting adulterated mixture.

In the present study, onion volatile oil was mixed with increment proportion of absolute alcohol in the concentration of 10, 20 and 30% by volume.

The changes in the physico-chemical proporties of onion volatile oil adulterated with 10, 20 and 30% ethanol are shown in table (3).

These results indicated that the addition of increment proportions of ethyl alcohol caused a gradual decrease in specific gravity values of onion volatile oil adulterated with ethyl alcohol, since the specific gravity of ethyl alcohol is lower than that of pure onion oil (0.7618). Consequently, the specific gravity decreased from (1.0965) for pure onion oil to 1.0697, 1.0269 and 1.0142 for onion oil admixed with 10, 20 and 30 percent of ethyl alcohol.

It is important to note that specific gravity can not be used as a reliable parameter for the detection of ethyl alcohol, since such values fall easily within the range of specific gravity of onion volatile oil from 1.0118 to 1.0980 which was mentioned by Chiris (1937), Guenther(1952) and Nofal (1974).

The refractive indeces of fresh onion oil and ethyl alcohol were 1.5614 and 1.3645, respectively. Adulterated onion volatile oil with increment proportion of ethyl alcohol caused gradual decrease in the refractive index of onion volatile oil. The onion oil adulterated with 30% ethyl alcohol had the lowest refractive index.

The acid number of adulterated onion volatile oil with ethyl alcohol at a concentration of 10, 20 and 30%, showed obvious decreases when compared with the elutriated oil. The acid number decreased from 7.27 for fresh onion volatile oil to 6.93, 6.33 and 5.21 for onion volatile oil adulterated with 10, 20 and 30% of ethyl alcohol respectively. This could be ascribed to the low acidity of ethyl alcohol (0.13), accordingly the addition of ethyl alcohol caused the decrements of the acidity of onion volatile oil.

The solubility of onion volatile oil in alcohol could be considered as a good method for determining the quality of essential oils as mentioned before, any change in the solubility behaviour of an essential oil demonstrates the presence of impurities in tested volatile oil. The solubility test was undertaken using different concentrations of ethyl alcohol viz;99, 95, 85 and 75%.

It was noticed that the pure onion volatile oil was completely soluble in alcohol at concentration of 99%, whereas, it was also soluble in 95% alcohol using 6.1 volume and more, while it was insoluble in alcohol at concentration less than 95%. On the other hand, samples mixed with ethyl alcohol at the concentration of 10, 20 and 30% were soluble in 99% alcohol, while in 95% alcohol it was soluble using 4.6 volume ,and more for volatile onion oil plus 10% ethyl alcohol, and 3.6 volume and more for onion volatile oil adulterated with 20 and 30% of ethyl alcohol. However, samples adulterated with 10, 20 and 30% ethyl alcohol were insoluble at a concentration ranged from 75 to 85% ethyl alcohol with the immediate separation of an oily layer at room temperature.

Meanwhile, the onion volatile oil samples adulterated with ethyl alcohol at the concentration of 10, 20 and 30% were completely soluble in diethyl ether, carbon tetrachloride, n-hexane, benzene and chloroform.

IV-1.1.5. Physical and chemical properties of adulterated onion volatile oil with paraffin oil:

Paraffin oil especially that of light paraffin oil is usually used as adulterant for essential oil. Since paraffin oil is colourless and does not impart any strange odour or flavour to the adulterated essential oil.

Results in table (3) represent changes in physico-chemical properties of onion volatile oil adulterated with paraffin oil, these results indicated that the increment concentrations of paraffin oil used as adulterant in onion volatile oil namely 10, 20 and 30% by volume caused a proportional decrements in the specific gravity, refractive index and acid number. Since, the specific gravity of paraffin oil (0.9698) is less than pure onions volatile oil (1.0965). It is quite clear that adding increment concentrations of light paraffin oil to elutriated pure onion oil decreased its specific gravity from 1.0965 to 1.0770 , 1.0703 and 1.0544 for adulterated onion volatile oil plus 10, 20 and 30% paraffin oil respectively.

On the other hand, the refractive index of pure onion volatile oil and light paraffin oil were 1.5614 and 1.4864 respectively. The admixture of light paraffin oil with pure onion volatile oil in increment proportion caused an obvious decrease, since the refractive index of the admixed onion volatile oil with paraffin oil were: 1.5537, 1.5461 and 1.5337 for onion volatile oil samples contained 10, 20 and 30% paraffin oil respectively.

The acid number of pure onion volatile oil was 7.27, whereas, that of pure paraffin oil was 0.28. Accordingly, the acid number of adulterated onion volatile oil proportionally decreased upon increasing the percentage of paraffin oil. Results obtained in table (3) had shown the same above mentioned trend, since the acid number was 6.87, 5.74 and 5.49 for adulterated onion volatile oil mixed with 10, 20 and 30% paraffin oil respectively.

Solubility of pure onion oil adulterated with light paraffin oil was determined using different concentration of ethanol viz., 75, 85, 95 and 99%. The results in table (4) indicate that pure onion oil was insoluble in alcohol at concentration ranged from 75 to 85% but started to turn soluble in 95% alcohol using 6.1 volume and more and 99% alcohol using 1 volume and more. Meanwhile, the pure parafffin oil is insoluble in all concentrations except that of 99% alcohol, since it turned soluble in 1 volume and more. The admixed samples of pure onion oil and light paraffin oil at different concentration were insoluble. However, the degree of solubility differed from turbid to slightly turbid as the concentration of ethyl alcohol increased from 75 to 99%. At proportionally low concentration 75 and 85% alcohol the adulterated oil together with ethyl alcohol were turbid and formed two layers, whereas, at higher concentration at 95 and 99% alcohol the mixture of the adulterated oil with the solvent was slightly turbid.

Generally, the results obtained for the physico chemical properties i.e. specific gravity, refractive index, acid number and solubility of adulterated onion volatile oil with different adulterants are in good agreement with results obtained by Osman et al. (1974) on geranium oil, El-Masry (1978) on geranium oil and peppermint oil, Oda (1982) on geranium oil and cumin oil and El-Masry (1985) on marjoram oil, petitgrain bigrade oil and fennel oil.

IV-1.2. UV spectra of onion volatile oil, aged onion volatile oil and adulterated samples with various adulterants:

IV-1.2.1. UV spectrum of fresh onion volatile oil:

The ultra-violet absorption spectrum of onion volatile oil showed a maximum absorption at 278 mu Fig (1). This might be attributed to the presence of the same chromophoric groups in the molecules of these oils especially unsaturated linkages (Bauman, 1963 and Rao, 1967) .

IV-1.2.2. UV spectra of aged onion volatile oil and adulterated samples with aged onion volatile oil:

The UV apsorption of aged onion volatile oil (Fig. 2) and onion volatile oil adulterated with aged onion volatile oil at different concentrations 10, 20 and 30% Fig. (3) reveal that the maximum absorption was at 278 for all examined samples, also minute changes were observed in the absorption intensities.

IV-1.2.3. UV spectra of adulterated onion volatile oil with maize oil:

The UV absorption of onion volatile oil adulterated with maize oil with the concentrations 10, 20 and 30% reveal that upon using the same concentration a proportional different absorption intensities were obtained for the different adulterated samples Fig. (4). The absorption rate decreased with increasing the concentration of maize oil percent. Since the absorption percentage were, 1.23, 1.16 and 1.02 for adulterated onion volatile oil with 10, 20 and 30% maize oil,respectively.

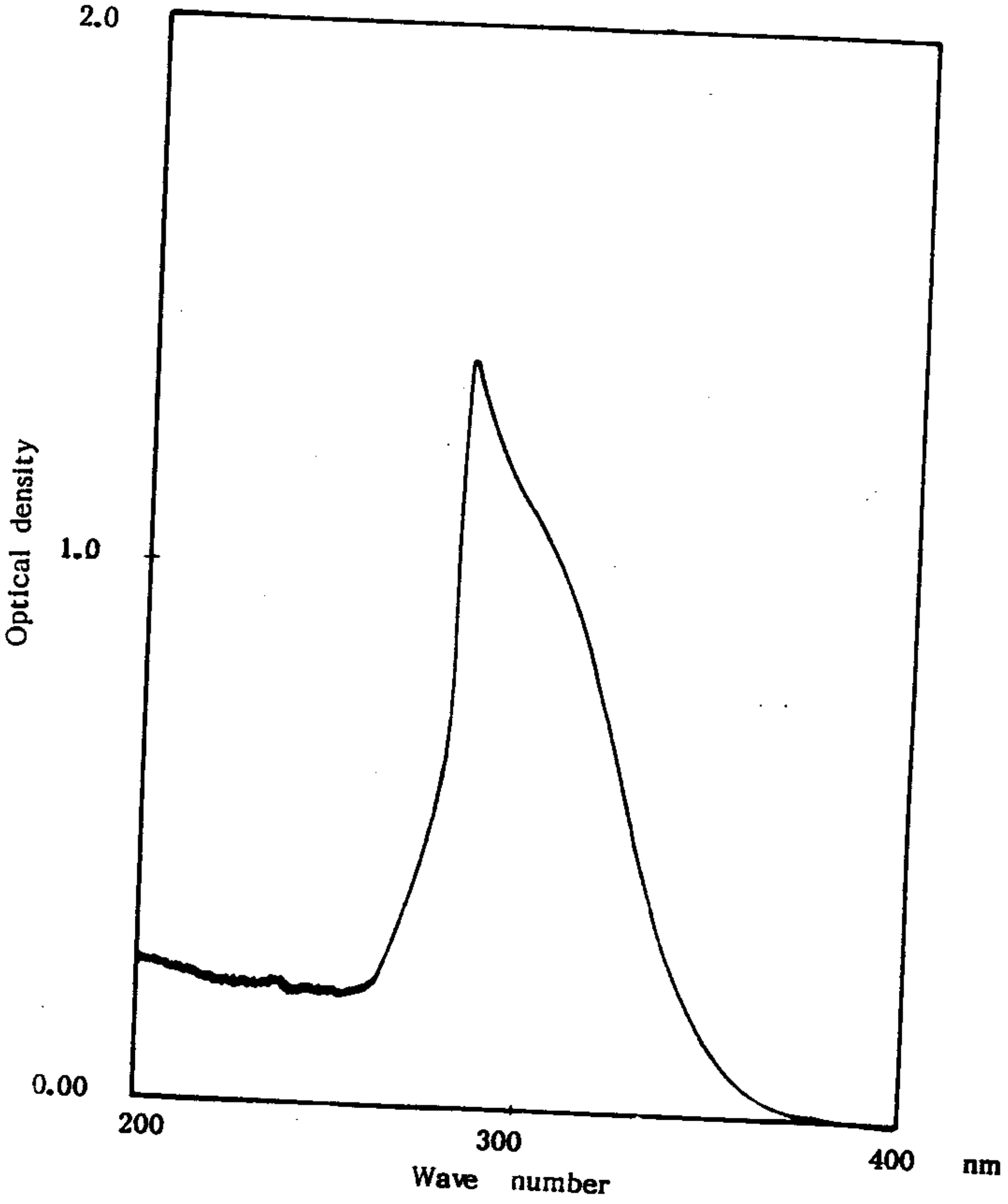


Fig. (1): U V absorption for fresh onion volatile oil.

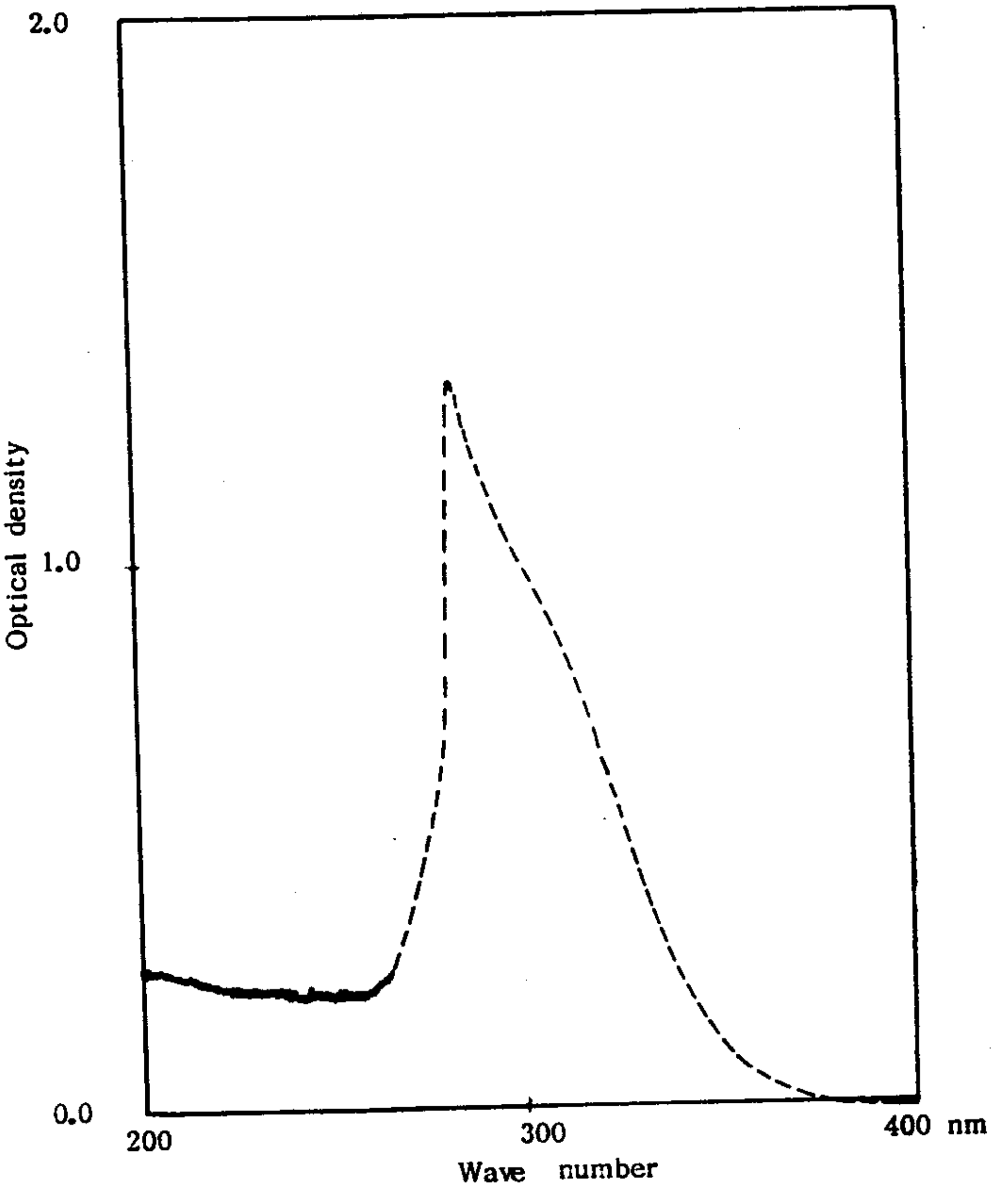


Fig. (2): U V absorption for aged onion volatile oil.

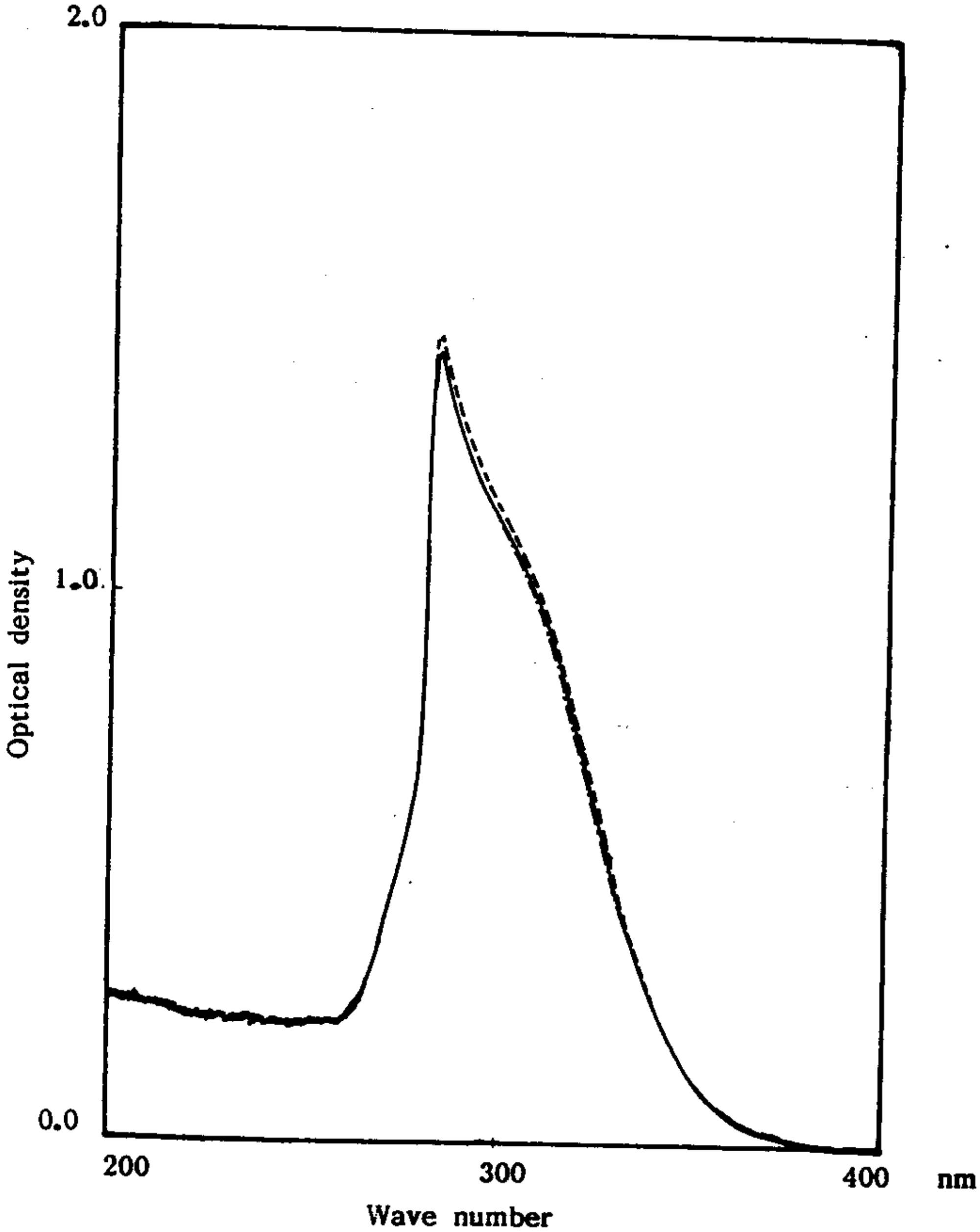


Fig. (3): U V absorption for onion volatile oil adulterated with aged onion volatile oil.

(———) : Onion volatile oil + 10% Aged onion volatile oil.

(- - - -) : ,, ,, ,, + 20% ,, ,, ,, ,,

(-·-·-·) : ,, ,, ,, + 30% ,, ,, ,, ,,

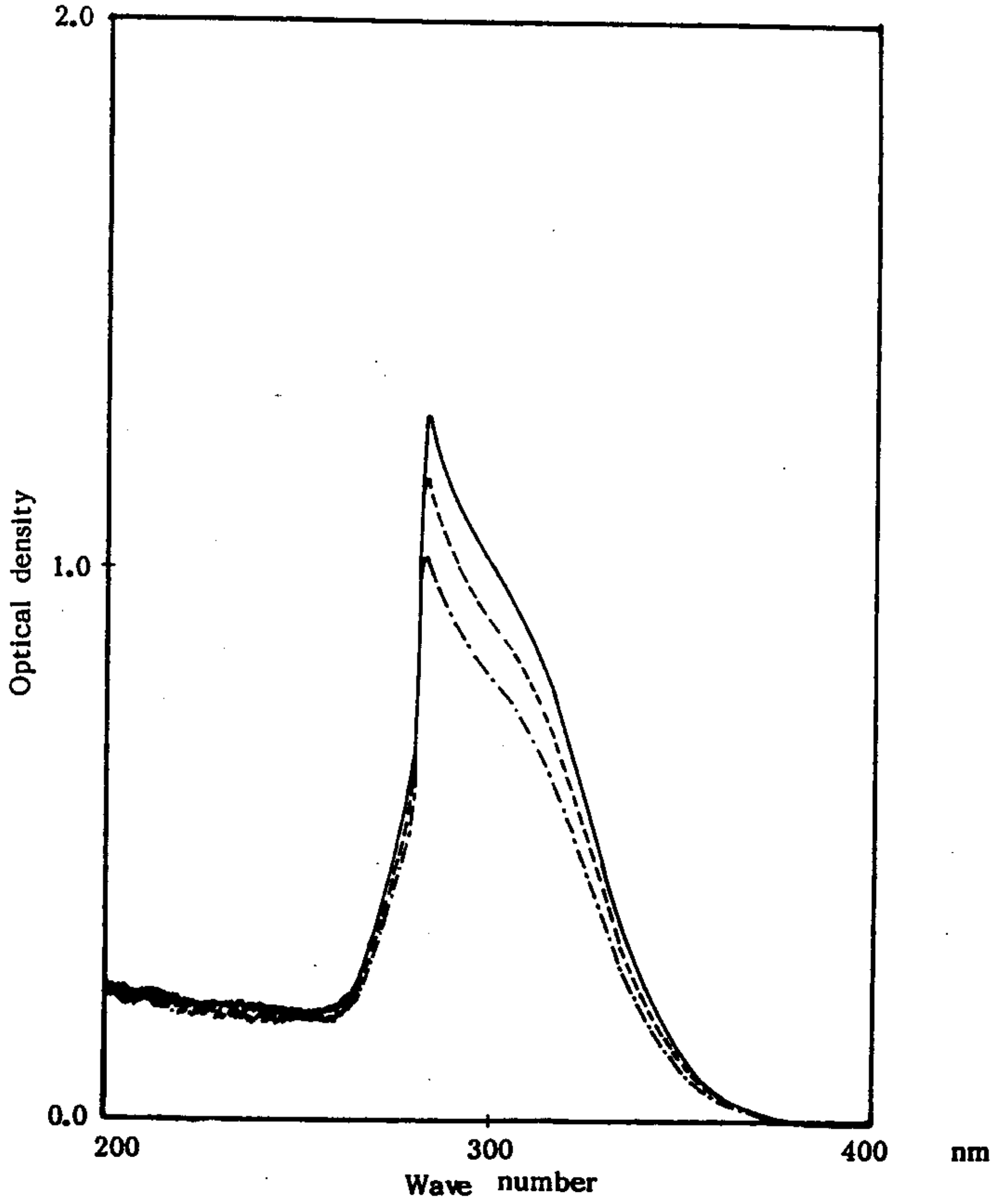


Fig. (4): U V absorption for onion volatile oil adulterated with maize oil.

(———) : Onion volatile oil + 10% Maize oil.

(- - - -) : ,, ,, ,, + 20% ,, ,,

(—.—.) : ,, ,, ,, + 30% ,, ,,

According to the obtained results, this test could be used as a qualitative test to indicate the adulteration practice with maize..oil.

IV-1.2.4. UV spectrum of adulterated onion volatile oil with ethyl alcohol:

The UV absorption of onion volatile oil samples adulterated with different concentration of ethyl alcohol viz; 10, 20 and 30% indicate that upon using the same concentration different absorption intensities were obtained for the different adulterated sample Fig. (5). The absorption intensities decreased obviously with increasing the concentration of ethyl alcohol percent. However, the intensity of decreasing was not proportionally related to the amount of ethyl alcohol added.

IV-1.2.5. UV spectrum of adulterated onion volatile oil with paraffin oil:

The UV absorption of onion volatile oil adulterated with paraffin oil in increment concentration namely 10, 20 and 30% is shown in Fig. (6). It could be observed that upon using the same concentration proportional different absorption intensities were obtained for the different adulterated samples. The absorption intensity decreased with increasing the concentration of the paraffin oil. Since, the absorption intensities were, 1.39, 1.16 and 1.11 for adulterated onion volatile oil with paraffin in the concentration 10, 20 and 30% respectively. These results may be attributed to that the added paraffin oil plays as a diluent.

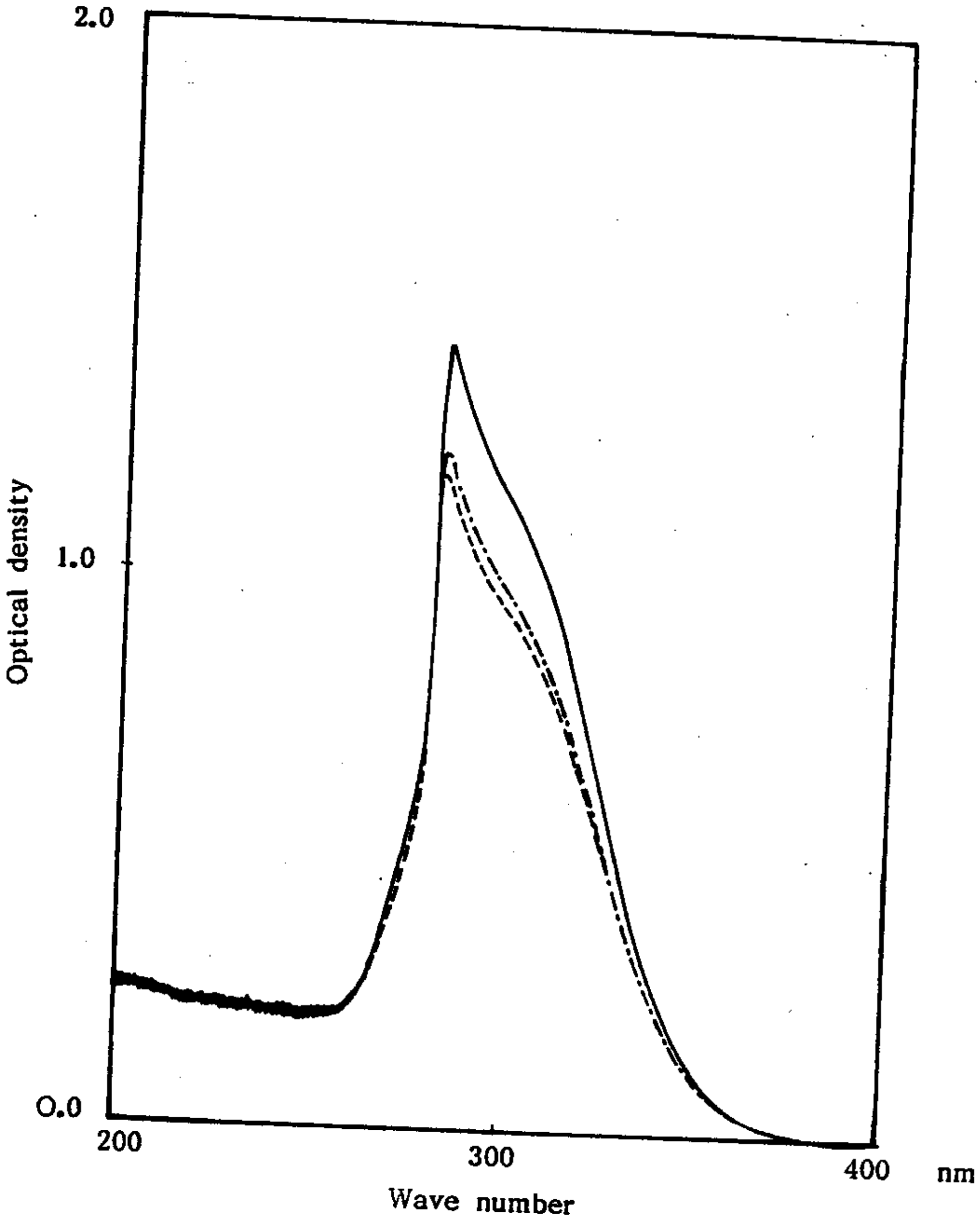


Fig. (5): U V absorption for onion volatile oil adulterated with ethyl alcohol.

(——) : Onion volatile oil + 10% Ethyl alcohol .

(-.-.-.) : ,, ,, ,, + 20% ,, ,,

(- - -) : ,, ,, ,, + 30% ,, ,,

Accordingly, it seems that the UV absorption rates could be used as a qualitative test to indicate the adulteration of onion volatile oil.

IV-1.3. I.R spectra of onion volatile oil, aged onion volatile oil and adulterated samples with various adulterats:

IV-1.3.1. I R. spectrum of fresh onion volatile oil:

I R spectrum of fresh onion volatile oil is shown in Fig. (7) and tabulated in table (5).

The absorption band at 665 Cm^{-1} indicates the probable presence of (C-S), as mentioned by Finar (1973). The absorption bands at 730 and 755 Cm^{-1} refer to alkenes $(CH_2)_n$-rock and $(CH_2)_n$-rock (Smaller n) respectively as it was reported by Goergy (1978). While, the absorption band at 935-952 Cm^{-1} may be assigned to $\rangle C = C \langle$ trans doubole bond (Gore 1972). The absorption bands at 1035 and 1048 Cm^{-1} are due to sulphoxide group $(-\overset{\overset{O}{\|}}{S}-)$ as mentioned by Bhati (1968), while those at 1085 Cm^{-1} and 1110 Cm^{-1} is due to the presence of sulphinic acid (-SO-OH) and thioketone (Bellamy 1962). Furthermore, the absorption band at 1150-1170 Cm^{-1} is attributed to sulphone group $(\rangle SO_2)$ as stated by Bellamy (1962), Nakanishi (1964) and Bhati (1968).

The absorption band at 1285 Cm^{-1} refers to the presence of propyl sulphide, while the absorption band at 1375 Cm^{-1} resulting from (CH_3-bending). Also, the absorption band 1411-1460 Cm^{-1} indicates the presence of active methylene group (Goergy 1978). The absorption band at 1700 Cm^{-1} refers to carbonyl group as reported by Rao (1963) and Bhati (1968). The absorption band at 2250 Cm^{-1} may be assigned to alkyne disubstituted (Pecsok and Shields 1968).

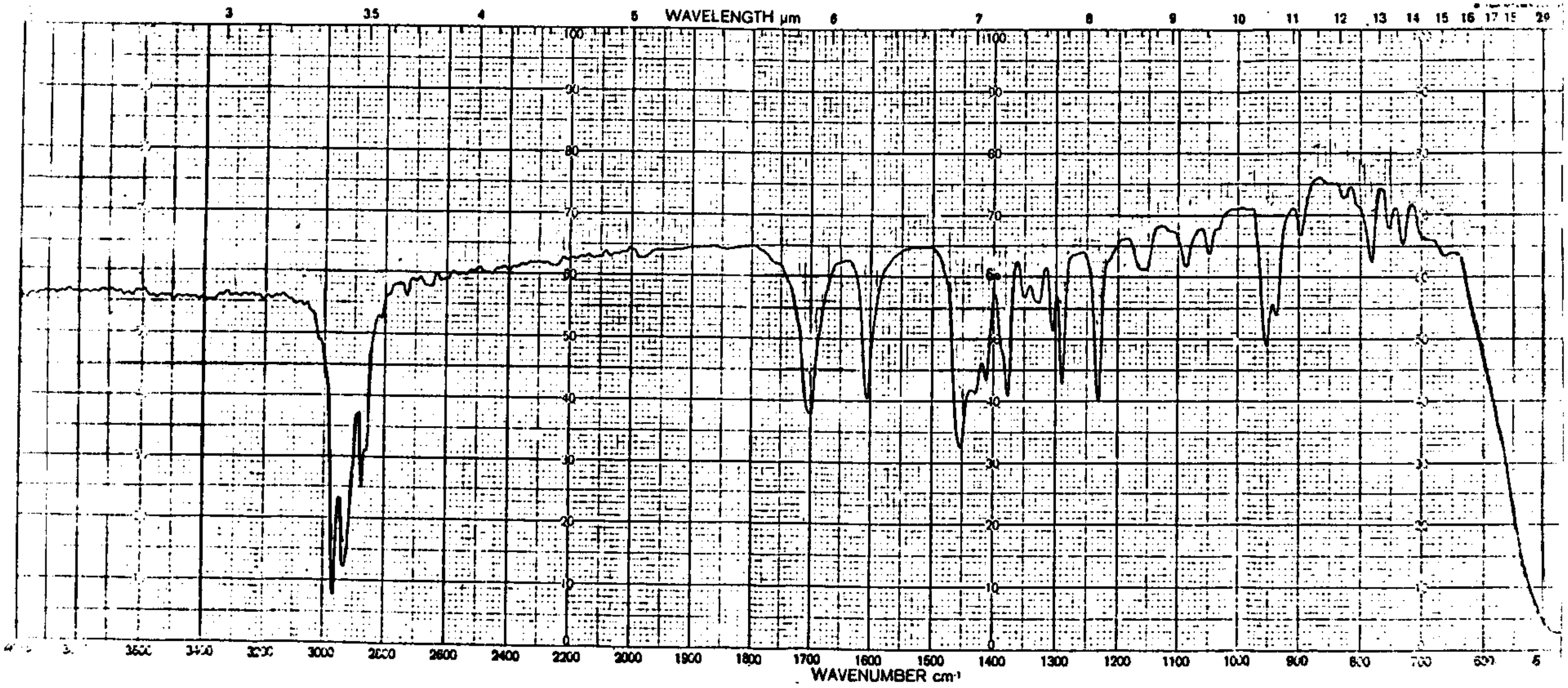


Fig. (7): IR spectrum of fresh onion volatile oil.

Table (5)

I R spectrum of fresh onion volatile oil

Band at wave number Cm^{-1}		Bond or group
665	w	C-S
730	w	$(CH_2)_n$- rock
755	w	$(CH_2)_n$- rock (smaller n)
935-952	b.s	$>C = C<$ trans double bond
1035		Sulphoxide group
1048	w	" "
1085	w	Sulphinic acid-SO-OH
1150-1170	b.w	Sulphone group $>SO_2$
1230	s	C-OH stretching
1285	s	Propyl sulphids
1305	m	Sulphone group $>SO_2$
1325-1355	b.w	Sulphone group $>SO_2$
1375	s	CH_3- (bending)
1410-1480	b.s	Active methelene group
1605	s	Amines N-H
1700	s	Carbonyl group
1765	sho	C=O stretching
2250	v.w	C=C alkyne disubstituted
2600-2660	b.w	(-SH) group
2730	w	C=O stretching
2818	sho	C=O stretching
2850-2990	b.s	CH_3-CH_2 - Alkane

Where: s = strong
w = weak
sho = shoulder
b.w = broad weak

m = medium
v.w = very weak
b.s = broad strong
b.m = broad medium

Whereas, the absorption bands at 2600 and 2660 Cm^{-1} refer to (-SH) group Finar 1973). The absorption band at 2730-2890 Cm^{-1} may be due to bonded oH (Pecsock and Shields 1968). While, the absorption bands at 2920-2980 Cm^{-1} may be due to methyl and methelene group (Goergy 1978).

From the above mentioned results, the infrared spectrcscopy can be used as a good qualitative analysis method for the volatile oils in onion. Since, the whole spectrum from 3000 Cm^{-1} to 650 Cm^{-1} could be considered as fingerprint for the different constituents of this essential oil.

IV-1.3.2. I R spectra of aged onion volatile oil and adulterated samples with aged onion volatile oil:

IR spectra of aged onion volatile oil and onion volatile oil adulterated with different percentages of aged onion volatile oil are illustrated in Figs (8 and 9) and tabulated in table (6).

The obtained results showed that no new bands were detected in the I R spectra of onion volatile oil adulterated with different concentration of aged onion volatile oil. The adulteration caused only an increase in the absorption bands intensities.

IV-1.3.3. I R spectrum of adulterated onion volatile oil with maize oil

IR spectra of onion volatile oil adulterated with different percentage of maize oil and the spectrum of pure maize oil are illustrated in Figs. (10 and 11) and tabulated in table (7).

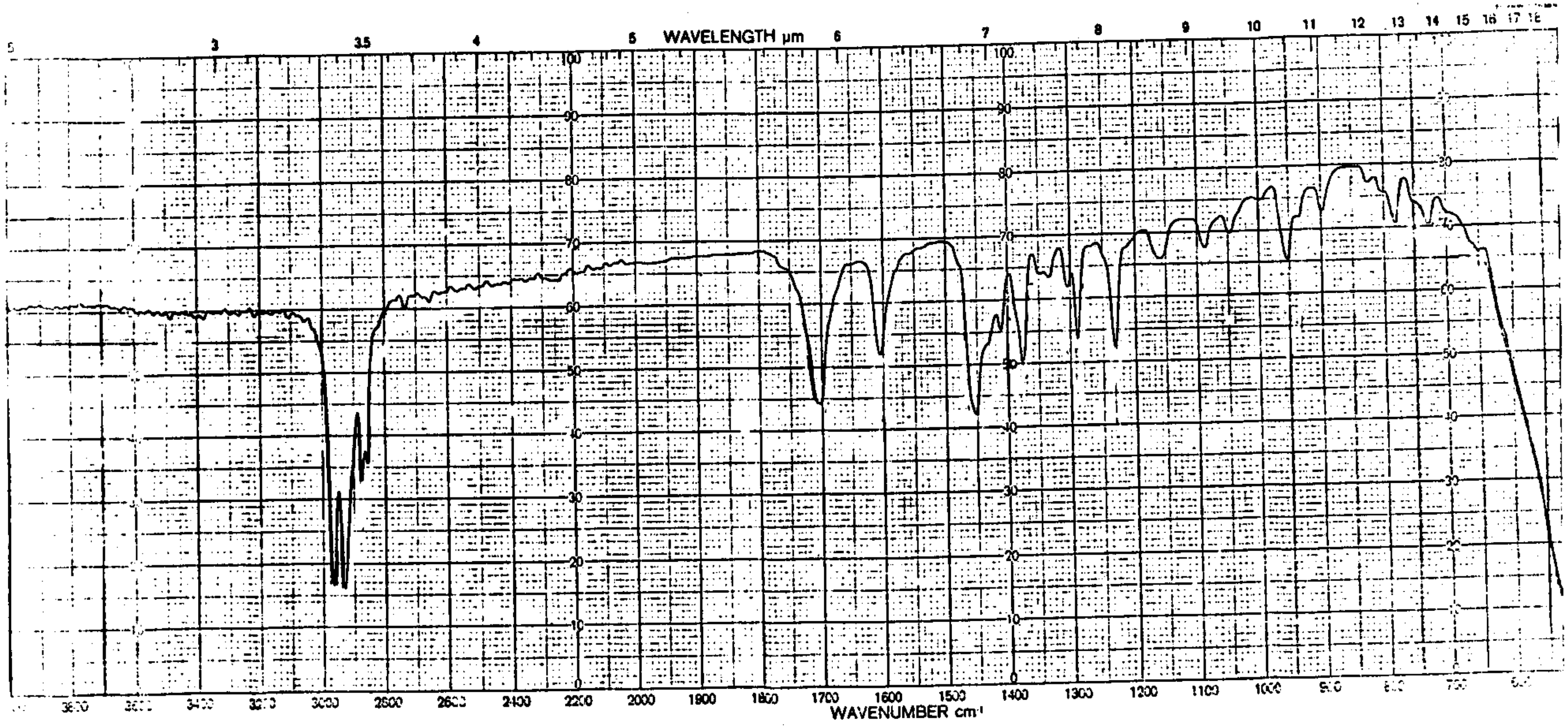


Fig. (8)) IR spectrum of aged onion volatile oil.

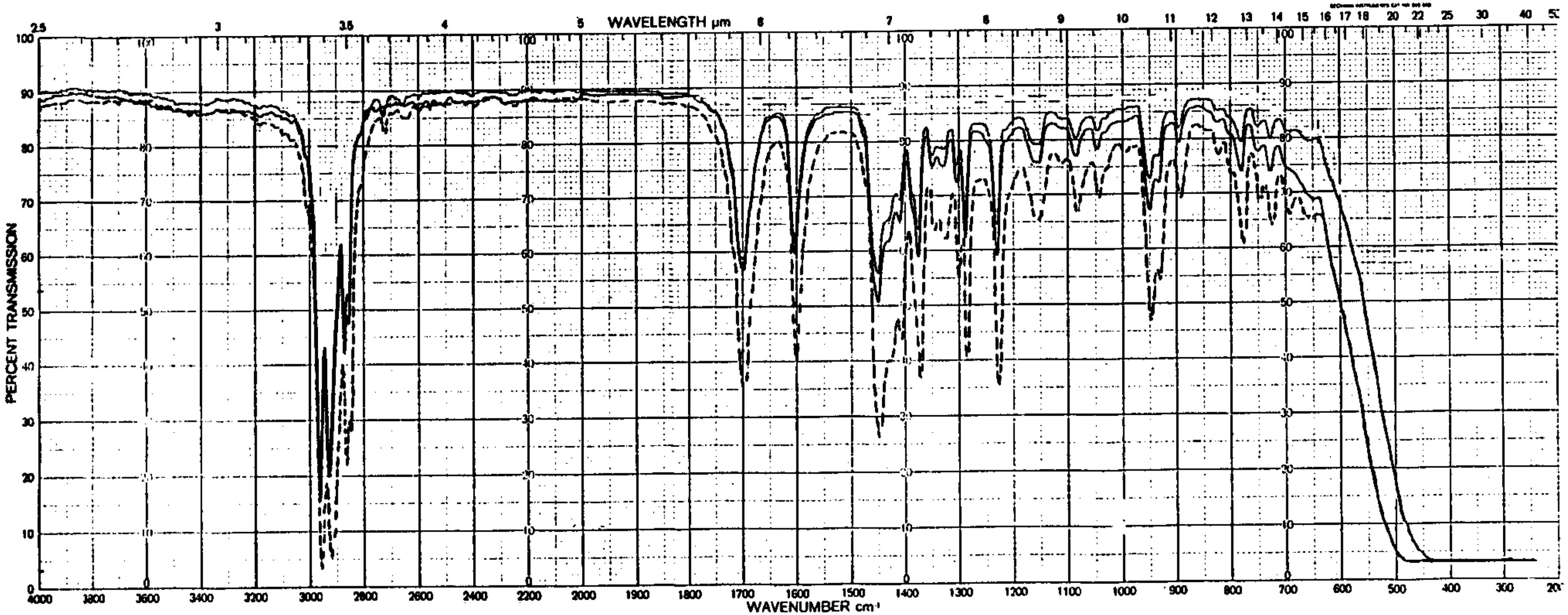


Fig. (9): I.R. spectra of onion volatile oil adulterated with aged onion volatile oil.

(·········): Onion volatile oil + 10% Aged onion volatile oil.

(———): ,, ,, ,, + 20% ,, ,, ,, ,,

(– – – –): ,,, ,,, ,, + 30% ,, ,, ,, ,,

Table (6)

I R. spectra of aged onion volatile oil and adulterated onion volatile oil with aged onion volatile oil

Aged onion Volatile oil		Onion oil + 10% aged oil		Onion oil + 20% aged oil		Onion oil + 30% aged oil		Bond or group
665	sho	665	sho	665	sho	665	sho	C-S
690	sho	690	sho	690	sho	690	w	Alkenes - disubstituted - cis
730	w	728	m	726	m	725	m	$(CH_2)_n$ - rock
755	sho	750	w	750	w	745	w	$(CH_2)_n$ - rock (smaller n)
935- 952	b.s	935- 950	b.m	935- 950	b.m	930- 945	b.s	>C=C< trans double bond
995	w	995	sho	995	sho	995	sho	
-		1030	sho	1030	sho	1030	sho	Sulphoxide group
1048	w	1048	w	1048	w	1045	m	Sulphoxide group
1085	m	1085	w	1085	w	1080	m	Sulphinic acid
1150-1170	b.m	1150-1170	b.m	1150-1170	b.m	1145-1160	b.m	Sulphone group $>SO_2$
1230	s	1230	s	1230	s	1230	s	C-0H stretching
1265	sho	-		1260	sho	1260	sho	=C-0-C=antisym. stretching
1289	s	1289	s	1289	s	1289	s	Propyl sulphide
1305	w	1305	w	1305	w	1300	m	Sulphone group $>SO_2$
1330-1355	b.w	1325-1352	b.w	1325-1352	b.w	1320-1350	b.w	Sulphone group ,,
1375	s	1375	s	1375	s	1375	s	SH_3- bending
1410-1480	b.s	1408-1465	b.m	1408-1465	b.w	1408-1460	b.s	Active methelene group
1605	s	1605	s	1605	s	1600	s	Amines N-H
1705	s	1700	s	1700	s	1700	s	Carbonyl group
2240	v.w	2240	w	2240	w	2240	w	C ≡ C Alkyne disubstituted
2660	w	2660	w	2 640	w	2650	w	(- SH) group
2730	w	2730	w	2730	w	2730	w	C=O stretching
2850-2980	b.s	2850-2930	b.s	2850-2980	b.s	2840-2980	b.s	CH_3-CH_2- Alkane

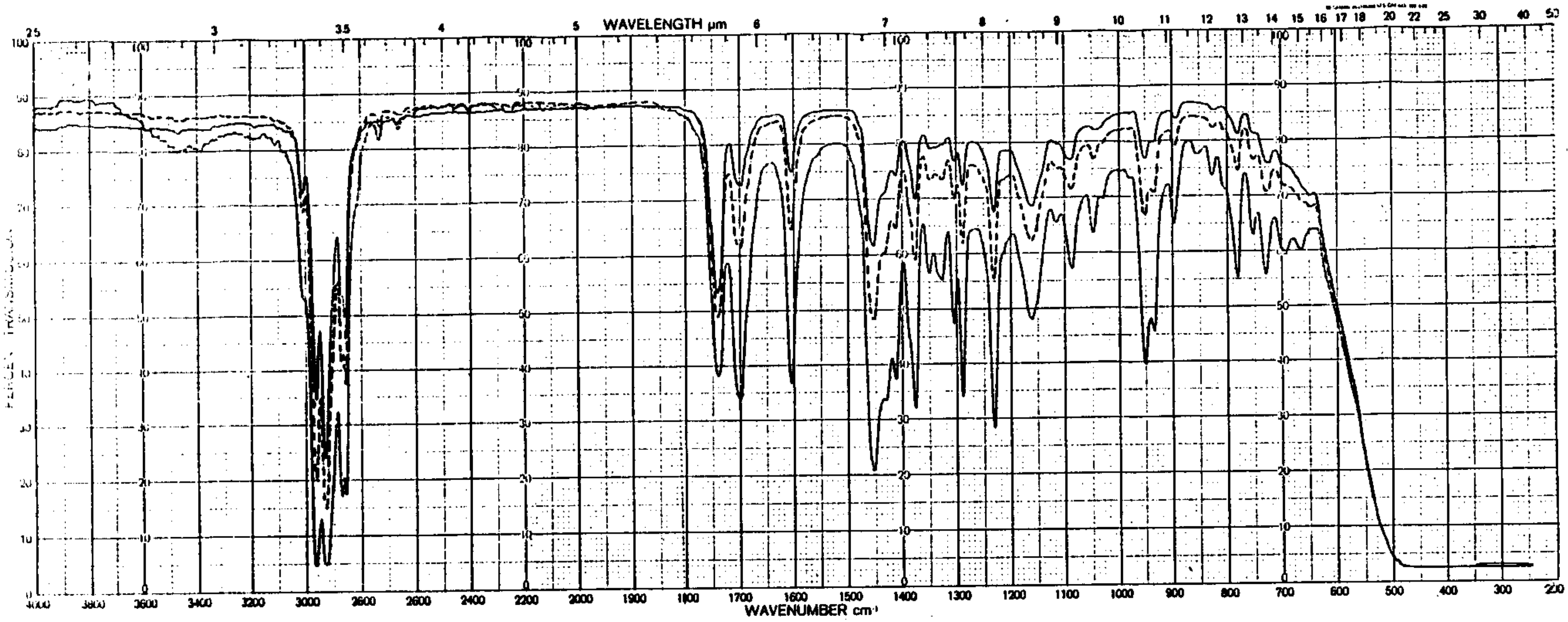


Fig. (10): I R spectra of onion volatile oil adulterated with maize oil.

(........) : Onion volatile oil + 10% Maize oil.

(– – –) : ,, ,, ,, + 20% ,, ,,

(——) : ,, ,, ,, + 30% ,, ,,

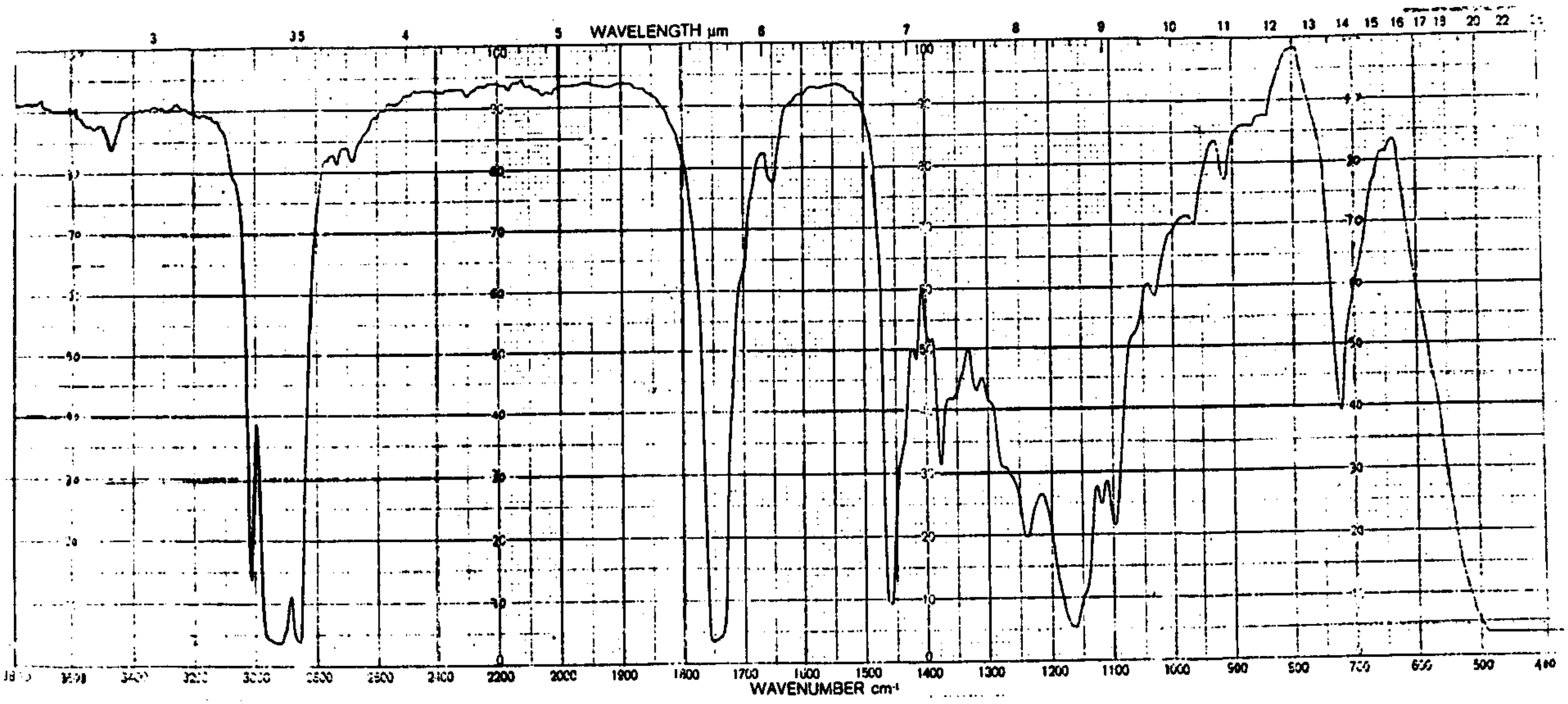


Fig. (11) : IR spectrum of maize oil.

Table (7)

I R spectra of adulterated onion volatile oil with maize oil and pure maize oil.

Pure maize oil		Onion oil + 10% maize oil		Onion oil + 20% maize oil		Onion oil + 30% maize oil		Bond or group
-		665	w	665	w	665	sho	C-S
722	m	730	w	725	w	725	w	$(CH_2)_n$ rock
-		755	w	755	w	755	w	$(CH_2)_n$ rock (smaller n)
915	n	910- 980	s	910- 980	s	910- 980	s	$>C=C<$ trans double bond
965	w							$>C=C<$,, ,, ,,
1030	w	1030	sho	1030	sho	1030	sho	Sulphoxide group
-		1048	m	1046	w	1040	w	Sulphoxide group
-		1085	m	1085	m	1085	w	Sulphinic acid -SO-OH
1096	s	-		-		-		C-O stretching, aliphatic ethers
1115	m	1115	w	1115	sho	1115	sho	C-O in primary alcohol
1165	s	1160	s	1160	s	1160	s	Sulphone group or tertiary alcohol
1238	m	1230	s	1230	s	1230	s	C-OH stretching
-		1288	s	1288	s	1288	m	Propyl sulphide
-		1305	m	1305	w	1305	w	Sulphone group $>SO_2$
1318	w	-		-		-		
-		1325	m	1325	w	1325	w	Sulphone group $>SO_2$
-		1350	w	1350	w	1350	w	Sulphone group $>SO_2$
1375	s	1375	s	1375	s	1375	s	CH_3 - (bending)
1410-1470	b.s	1410-1470	b.s	1410-1470	b.s	1410-1470	b.s	Active methelene group
n -		1605	s	1605	s	1605	m	Amines N-H
1650	m	-		-		-		Alkenes disubstituted, cis
-		1700	s	1700	s	1700	s	Carbonyl group
1740	s	1740	s	1740	s	1740	s	C=O stretching
-		2660	w	2660	w	2660	w	(-SH) group
-		2730	w	2730	w	2730	w	C=O stretching
2300-3000	b.s	2840-3000	b.s	2840-3000	b.s	2840-3000	b.s	CH_3-CH_2- Alkane
3010	s	3010	sho	3010	w	3010	w	Methyl and methelene group
3465	w	-		-		-		O-H Alcohol

It is obvious that new band appeared in the three obtained spectra as compared with the spectrum of onion volatile oil. The new band appeared at 1740 Cm^{-1} refering to C=O stretching of aliphatic esters. Moreover the adulteration of onion volatile oil led to broading in absorption bands at 910-980 Cm^{-1} and 2840-3000 Cm^{-1}. The first band indicates to trans double bond, while the second band refers to -CH- stretches, as showed by Gore (1972).

As it is shown from the obtained data, that I R spectra of onion volatile oil adulterated with maize oil differ from the spectrum of pure onion volatile oil, therefore it can detect the adulteration of onion volatile oil with another edible oil.

IV-1.3.4. I R spectrum of adulterated onion volatile oil with ethyl alcohol:

I.R. spectra of onion volatile oil adulterated with 10, 20 and 30% ethyl alcohol and pure ethyl alcohol are shown in Figs. (12 and 13) and table (8).

The obtained results showed that no new bands appeared in the spectra, as a result of adulteration onion volatile oil with ethyl alcohol. The absorption bands increased in intensity by adding ethanol to onion volatile oil.

IV-1.3.5. I R spectrum of adulterated onion volatile oil with paraffin oil:

I.R spectra of onion volatile oil adulterated with 10, 20 and 30%

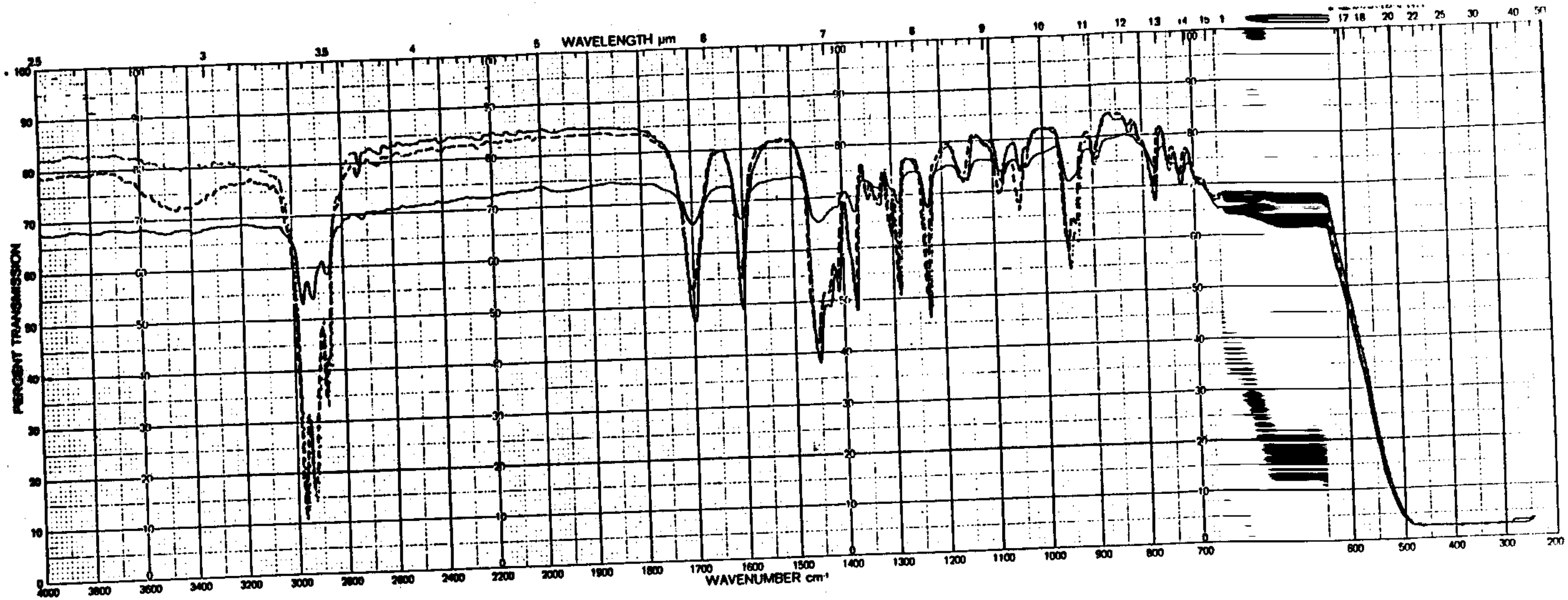


Fig. (12): I R. spectra of onion volatile oil adulterated with ethyl alcohol

(.........) : Onion volatile oil + 10% Ethyl alcohol .

(– – – –) : ,, ,, ,, + 20% ,, ,,

(———) : ,, ,, ,, + 30% ,, ,,

Table (8)

I R spectra of adulterated onion volatile oil with ethyl alcohol and pure ethyl alcohol.

Pure ethyl alcohol		Onion oil + 10% ethyl alco.		Onion oil + 20% ethyl alco.		Onion oil + 30% ethyl alco.		Bond or group
-		665	sho	665	sho	665	sho	C-S
-		725	w	730	w	725	w	$(CH_2)_n$ - rock
-		750	w	752	w	740	sho	$(CH_2)_n$ - rock (smaller n)
-		920-970	bs	920-970	bs	920-970	bm	$>C=C<$ trans double bond
-		1040	w	1050	w	1040	w	Sulphoxide group $-\overset{\overset{O}{\|}}{S}-$
-		1082	m	1085	m	1080	w	Sulphinic acid
1098	w	-		-		-		C-0-H bend primary alcohol
-		1115	w	1115	sho	-		Thioketone
-		1155	m	1155	m	1155	m	Sulphone group $>SO_2$
-		1228	s	1230	s	1230	m	C-OH stretching
-		1285	s	1290	s	1288	m	Propyl sulphide
-		1305	m	1308	w	1300	sho	Sulphone group
-		1325	w	1325	w	1325	v.w	Sulphone group
-		1348	w	1348	w	-		Sulphone group
-		1374	s	1376	s	1375	w	CH_3- bending
-		1400-1470	b.s	1400-1470	b.s	1400-1470	b.s	Active methelene group
-		1605	s	1605	s	1605	m	Amines N-H
-		1700	s	1700	s	1700	m	Carbonyl group
-		2250	v.w	2250	v.w	-	v.w	C≡C alkyne disubstituted
-		2650	v.w	2650	v.w	2650	v.w	(- SH) group
-		2730	w	2725	w	2710	w	C=0 stretching
-		2840-2990	b.s	2840-2990	b.s	2820-3000	b.m	-CH- stretches
2980		"		"		"		CH_3 - stretching.
3400	b.s	3460	v.w	3460	b.m	-		H-bonded OH stretch

paraffin oil and the spectrum of paraffin oil are illustrated in Figs. (14 and 15) and showed in table (9).

Four new bands appeared in the obtained spectra as a result of adulteration of onion oil with paraffin oil. The new absorption bands are at 705, 1125, 1270 and 1730 Cm^{-1}. The four absorption bands refer to alkenes, C-0 in primary alcohols, C-0-C antisymmetrical stretching and C=O stretching in aliphatic esters, respectively as mentioned by Silverstein and Bassler (1967).

Adding paraffin oil with different concentration to onion volatile oil caused also broading . two absorption bands namely at 740 and 2840-3000 Cm^{-1}. The previous absorption bands refer to $(CH_2)_n$ and -CH stretches respectively, as announced by Gore (1972).

As it is shown from obtained data, I R spectrum of onion volatile oil adulterated with paraffin oil differ from the spectrum of pure onion oil. Therefore, the adulteration of onion oil with paraffin oil can be dected. by using this method.

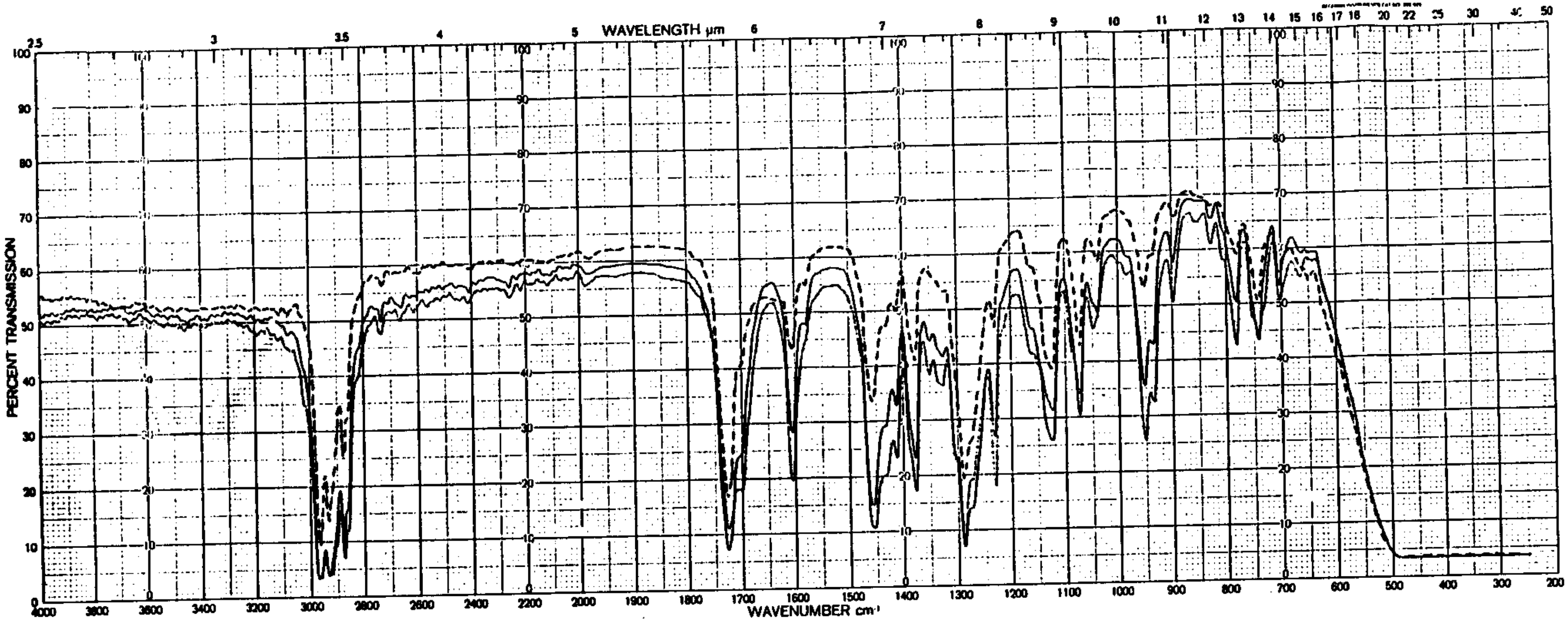


Fig. (14): I R spectra of onion volatile oil adulterated with paraffin oil.

(........) : Onion volatile oil + 10% Paraffin oil.

(———) : ,, ,, ,, + 20% ,, ,,

(-----) : ,, ,, ,, + 30% ,, ,,

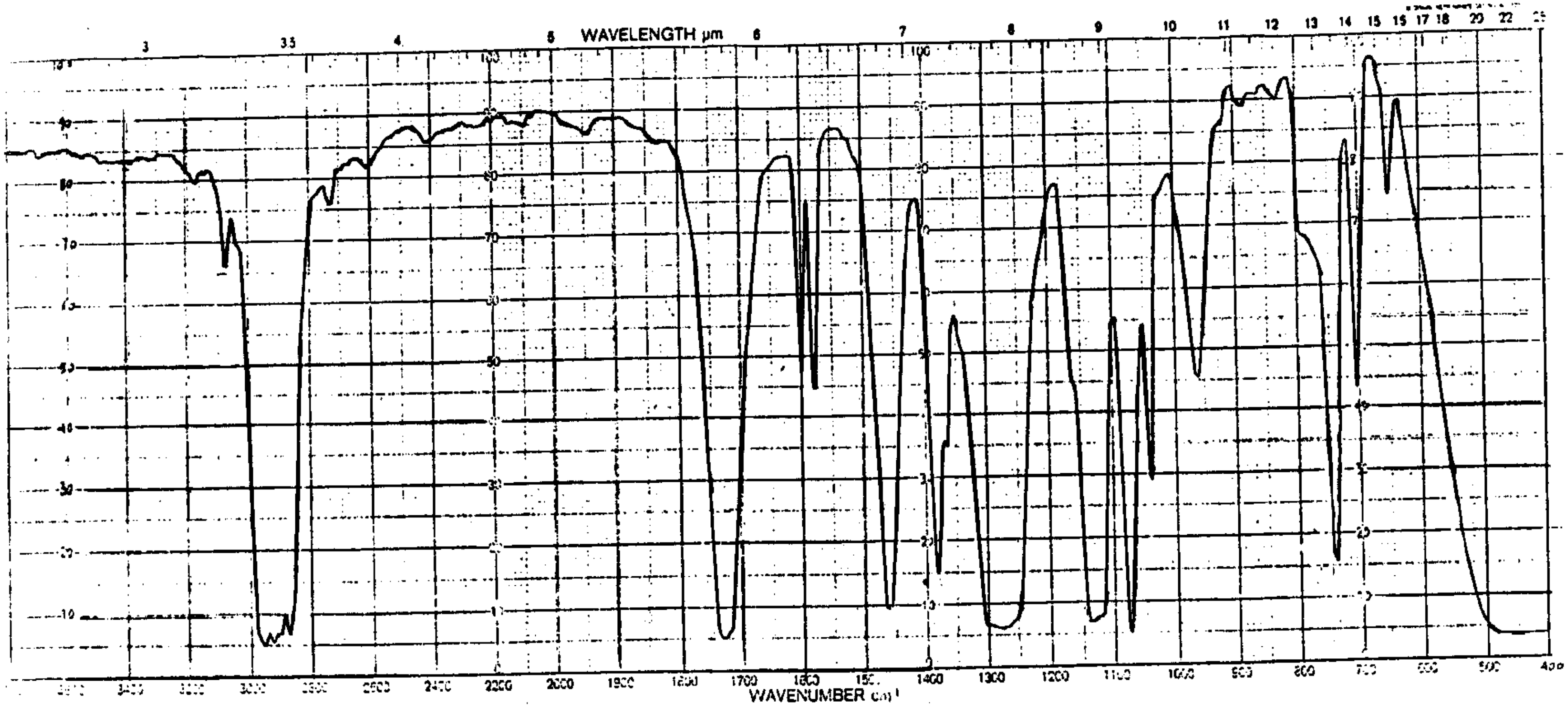


Fig. (15): IR spectrum of paraffin oil.

Table (9)

I R spectra of adulterated onion volatile oil with paraffin oil and pure paraffin oil

Pure paraffin oil		Onion oil + 10% paraffin oil		Onion oil + 20% paraffin oil		Onion oil + 30% paraffin oil		Bond or group
650	m	650	sho	650	sho	650	w	Olefinic C-H bending vibrations
-		665	w	665	sho	-		C-S
705	s	705	w	705	w	705	w	Alkenes (distributed)
740	s	740	s	740	s	740	s	$(CH_2)_n$- rock
920	sho							
965	s	920-970	b.s	920- 970	b.s	920- 970	b.s	$>C = C<$ trans double band
1040	s	1048	s	1040	m	1040	w	C-O stretching in primary alcohol
1075	s	1075	s	1075	s	1075	s	C-O stretching in primary alcohol
1120-1140	b.s	1125	s	1125	s	1125	s	C-O in primary alcohol
-		1165	sho	1165	sho	1165	sho	Sulphone group $>SO_2$
-		1232	s	1232	s	1232	m	C-OH stretching
1240-1310	b.s	1275	sho	1275	sho	1275	sho	=C-0-C antisymmetrical stretching
		1288	b.s	1288	b .s	1288	b.s	Primary alcohol
		1305	sho	1305	sho	1305	sho	Sulphone group $>SO_2$
		1325	w	1325	w	1325	sho	,, ,, ,,
		1350	w	1350	w	1350	sho	,, ,, ,,
1375	sho	1378	s	1378	s	1378	m	CH_3 - bending
1380		-		-		-		CH_3 - bending
1460	s	1410-1470	b.s	1410-1470	b.s	1410-1470	b.s	OH bending in carboxylic acids
1580	s	1580	sho	1580	w	1580	w	C=O stretching (carboxylate anion stretching)
1600	s	-		-		-		
-		1605	s	1605	s	1605	m	Amines N-H
1730	s	1730	s	1730	s	1730	s	C=O stretching in aliphatic ester
2410	w	2410	v.w	2410	v.w	2410	v.w	Disubstituted acetelene
-		2660	v.w	2660	v.w	-		(-SH) group
2720	m	2730	w	2730	w	2730	w	C=O stretching
2840-3035	b.s	2840-3040	b.s	2840-3060	b.s	2840-3060	b.s	CH_3-CH_2- Alkane
3080	m	3080	w	3080	w	3080	w	C -H stretching

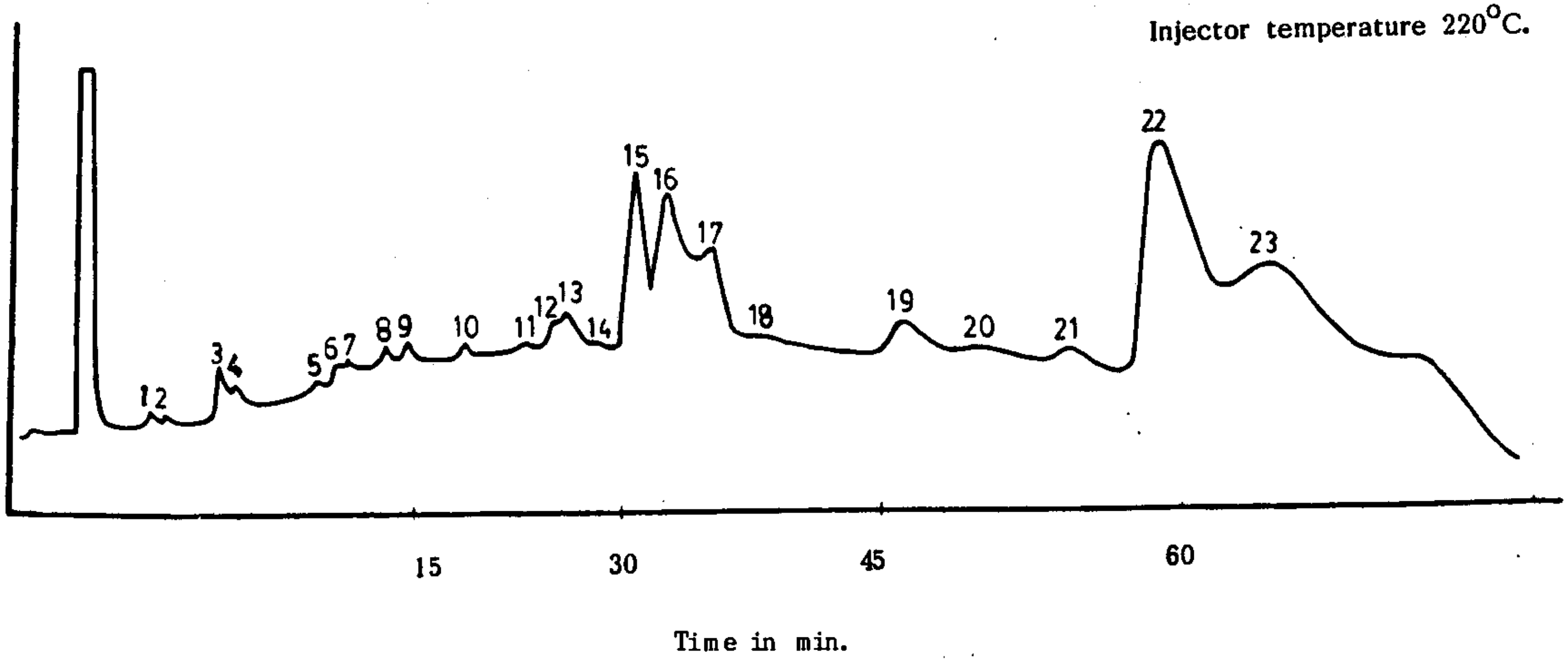
IV-1.4. G.L.C. of onion volatile oil, aged onion volatile oil and adulterated samples with aged onion volatile oil and ethyl alcohol:

Gas liquid chromatography was accomplished for the separation of the components of onion volatile oil which are responsible for the aroma and flavour of essential oils. The onion volatile oil, aged onion volatile oil and adulterated samples with aged onion volatile oil and ethyl alcohol were analyzed using gas liquid chromatography under the conditions previously mentioned in the methods section:

IV-1.4.1. G.L.C. of fresh onion volatile oil:

The gas chromatogram of onion volatile oil is shown in Fig.(16). The identified compounds of this oil are listed in table (10). Results in this table indicate that the total peaks of the fresh onion volatile oil were 23. Sixteen volatile compounds had been identified, while seven compounds were still unknown with concentration ranged from 0.14 to 33.91%, these unknown peaks would require further studies by other technique of analysis to be identified.

The identified flavour components of the onion volatile oil could be classified into four categories according to (Boelens _et al._ 1971) namely oxygenated compounds, disulphide, trisulphide and thiol compounds. The oxygenated compounds namely; acetaldehyde, propionaldehyde, nonanal, decanal, octanal, undecanal, propyl alcohol, hexyl alcohol, decanol and methyl taurine. The total percentage of these oxygen compounds were 35.21%.



Speed chart 1 Cm/min.
Nitrogen flow rate 30 ml/min.
Hydrogen flow rate 30 ml/min.
Air flow rate 300 ml/min.
Column temp. 90~190°C. with
programing rate 4°C/min.
Detector temperature 300°C.
Injector temperature 220°C.

Fig. (16): G.L.C. of fresh onion volatile oil.

Table (10)

G.L.C. of fresh onion volatile oil

Peak No.	Percentage	Name of component
1	0.46	Acetaldehyde
2	0.30	Propionaldehyde
3	1.46	Propyl alcohol
4	0.50	Octanal
5	0.34	Hexyl alcohol
6	0.60	Nonanal
7	0.23	1-propanethiol
8	0.82	Decanal
9	0.56	Disulphide propane
10	0.56	Decanol
11	0.49	Propane trisulphide
12	0.60	Propyl disulphide propane
13	2.58	Unknown
14	0.14	,, ,,
15	12.95	Methyl propyl trisulphide
16	7.53	Dipropyl trisulphide
17	9.16	Undecanal
18	0.38	Unknown
19	3.10	,, ,,
20	0.34	,, ,,
21	1.87	,, ,,
22	33.91	,, ,,
23	21.01	Methyl taurine

These results are in partial agreement with those obtained by Bandyopadkyoya et al. (1970), Brodnitz and Pollock (1970), Boelens et al. 1971), Nofal (1974) and Mazza et al. (1980) who identified, propyl alcohol, propionaldehyde, acetaldehyde, 2-methyl-2-pentanal and acetic acid as oxygenated compounds of the onion volatile oil. Also, the same results indicated that methyl taurine undecanal and propyl alcohol are the main oxygenated compounds in the onion volatile oil, their percentages are 21.01, 9.16 and 1.46% , respectively. On the other hand propionaldehyde and hexyl alcohol represented the lowest amount of oxygenated compounds, their percentages are 0.30 and 0.34%, respectively.

The identified disulphide compounds in the onion volatile oil were, disulphide propane and propyl disulphid propane, their percentages were 0.56 and 0.60%, respectively. The total percentages of these compounds are 1.16%. Whereas the identified trisulphides were; propane trisulphide, methyl propyl trisulphide and dipropyl trisulphide their amounts are 0.49, 12.95 and 7.53% ,respectively and hence, the total percentage of these compounds is 20.97.

The obtained results are in good agreement with these obtained by Carson and Wong (1961 B), Saghir et al.(1964), Carson (1967), Chua et al. (1968), Bernhard (1969), Brodnitz et al. (1969), Boelens et al (1971), and Mazza et al. (1980) , as reported in the literature.

IV-1.4.2. G.L.C. of aged onion volatile oil and adulterated samples with aged onion volatile oil:

Gas chromatography was used to separate the volatile components of adulterated onion volatile oil using aged onion volatile oil with ratios 10 and 20% by volume.

The gas chromatograms of aged onion volatile oil and adulterated samples of onion volatile oil with aged onion volatile oil are illustrated in Figs (17, 18 and 19), whereas, the identified compounds of these oils are listed in table (11). Results indicate that the total peaks of fresh onion volatile oil, aged onion volatile oil and two adulterated samples with (10 and 20%) aged onion volatile oil were 23 compound. Sixteen volatile compounds were identified whereas seven compounds were considered as unknowns with the concentration ranged from 0.14 to 33.91, 0.29 to 47.12, 0.20 to 36.21 and 0.21 to 42.69% for fresh onion volatile oil, aged onion volatile oil and adulterated onion volatile oil with ratios 10 and 20% aged onion volatile oil, respectively. The unknow percentages were higher in aged onion volatile oil than in the fresh ones.

The identified volatile compounds can be classified to four categories namely; oxygenated compounds, disulphide, trisulphide and thiol compound.

The total percentages of the oxygenated compounds in these volatile oils were 35.21%, 24.8%, 34.98% and 29.17% for fresh onion volatile oil, aged onion volatile oil and adulterated onion volatile oil with 10 and 20% of aged oil, respectively.

Results indicated that some of the oxygenated volatile compounds decreased upon storage such as methyl taurine, undecanal, decanal,

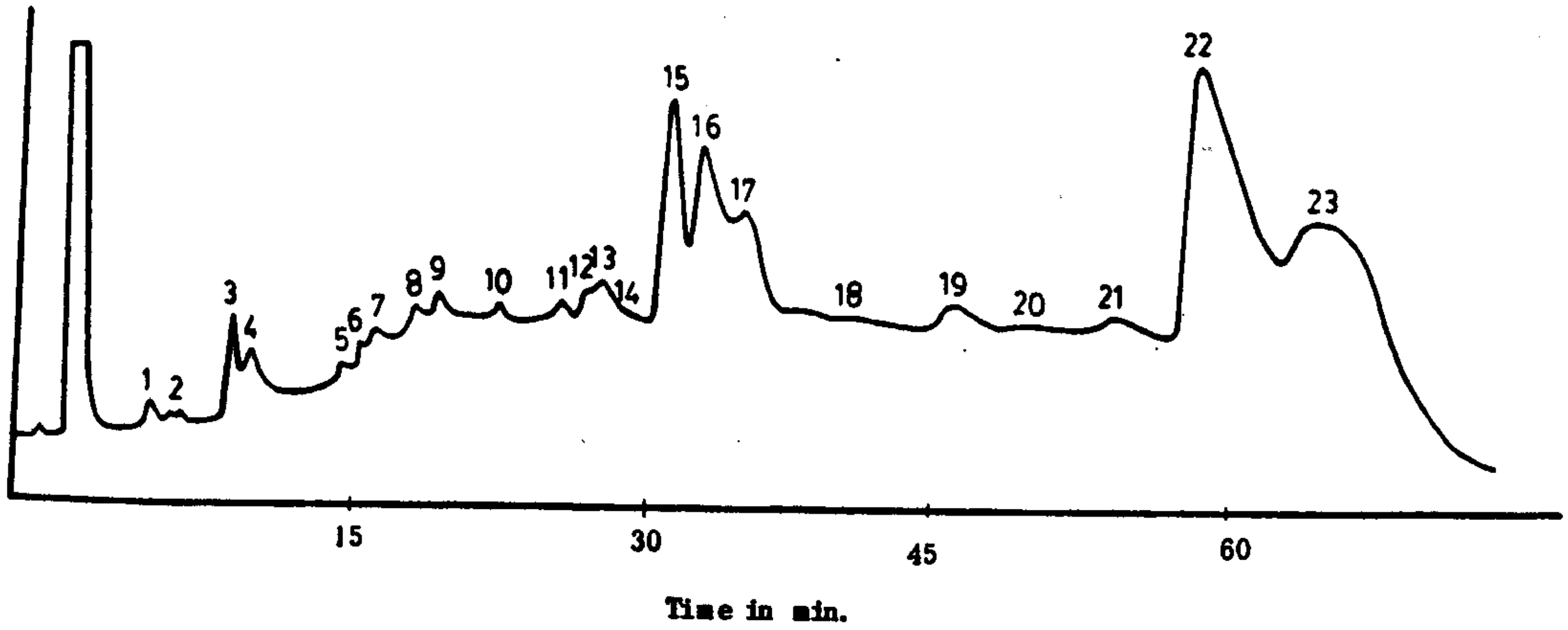


Fig. (18): G.L.C. of fresh onion volatile oil adulterated with 10% aged onion volatile oil.

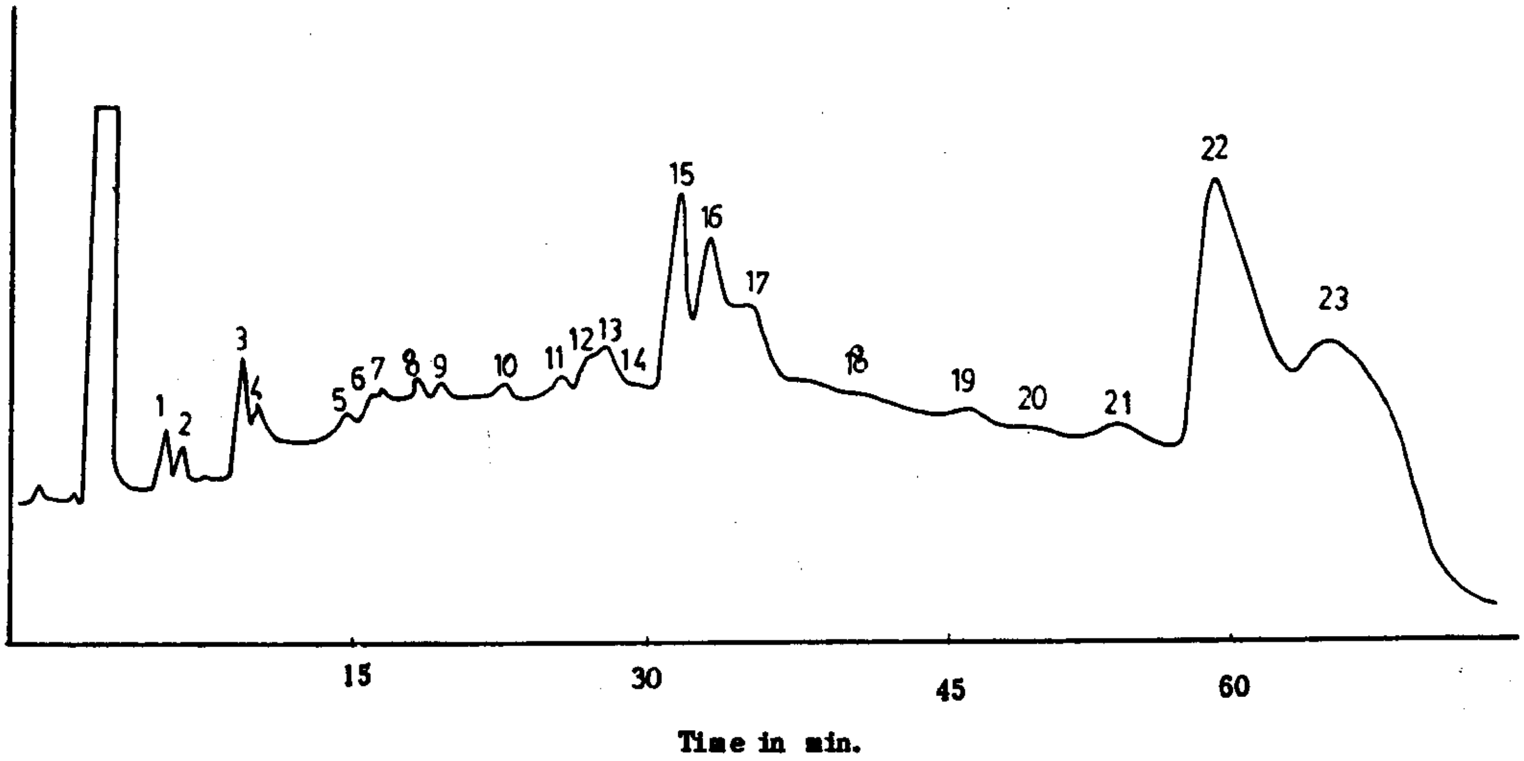


Fig. (19) : G.L.C. of fresh onion volatile oil adulterated with 20% aged onion volatile oil.

Table (11)

G.L.C. of pure onion volatile oil and adulterated samples with aged onion volatile oil

Peak No.	Fresh onion oil	Aged onion oil	Fresh o. + 10% aged oil	Fresh o. + 20% aged oil	Name of compound
1	0.46	trace	0,44	0.40	Acetaldehyde
2	0.30	trace	0.30	0.41	Propionaldehyde
3	1.46	2.89	2.70	2.82	Propyl alcohol
4	0.50	1.15	0.80	1.13	Octanal
5	0.34	0.69	0.38	0.50	Hexyl alcohol
6	0.60	0.81	0.68	0.72	Nonanal
7	0.23	0.41	0.59	0.33	1-Propanethiol
8	0.82	0.37	0.80	0.55	Decanal
9	0.56	0.31	0.52	0.45	Disulphide propane
10	0.56	0.35	0.55	0.52	Decanol
11	0.49	0.35	0.47	0.45	Propane trisulphide
12	0.60	1.01	0.71	0.94	Propyl disulphide propane
13	2.58	0.96	1.55	1.46	Unknown
14	0.14	0.29	0.20	0.21	,, ,,
15	12.95	13.93	13.12	13.17	Methyl propyl trisulphide
16	7.53	3.40	6.30	5.90	Dipropyl trisulphide
17	9.16	3.64	8.63	4.90	Undecanal
18	0.38	0.34	0.37	0.35	Unknown
19	3.10	0.53	2.70	1.58	,, ,,
20	0.34	4.52	0.72	1.14	,, ,,
21	1.87	1.77	1.50	1.73	,, ,,
22	33.91	47.12	36.21	42.69	,, ,,
23	21.01	14.90	19.70	17.22	Methyl taurine.

decanol, acetaldehyde and propionaldehyde. While other oxygenated compounds increased such as propyl alcohol, nonanal, octanal and hexyl alcohol. The adulterated samples obviously contained intermediate amounts of oxygenated compounds between fresh and aged onion volatile oils. These results are in partial agreement with those obtained by Bandyopadhyaya et al. (1970), Brodnitz and Pollock (1970), Boelens et al. (1971), Nofal (1974) and Mazza et al. (1980).

The total percentage of the identified disulphide compounds in the onion volatile oil was 1.16%. Whereas, in aged onion volatile oil was 1.32%. The same data obviously indicate that in most cases the adulterated samples contained an intermediate percentages of the above-mentioned compounds according to percentage of aged oil added to the adulterated samples.

On the other hand, identified trisulphide compounds percentage in genuine onion volatile oil was 20.97%, whereas, in aged onion volatile oil their amount was 17.68%, these trisulphide had a decreasing pattern in aged onion volatile oil in comparison with fresh onion volatile oil. However, methyl propyl trisulphide slightly increased.

The obtained results are in line with those obtained by Carson and Wong (1961 B) and Bandyopadhyaya et al. (1970).

Conclusively, the gas chromatography could be used to indicate the adulteration of fresh onion volatile oil with aged onion volatile oil.

IV-1.4.3. G.L.C. of adulterated onion volatile oil with ethyl alcohol:

Gas liquid chromatographic analysis was applied in the present study on the adulterated samples of onion volatile oil with 10, 20 and 30% ethanol.

Results illustrated in figures (20, 21 and 22) indicate the analysis of onion volatile oil adulterated with 10, 20 and 30% ethanol. The peaks obtained showed that ethyl alcohol could be identified in the adulterated sample since, its peak located directly after the solvent peak.

Results tabulated in table (12) show that there was a noticeable decrease in the concentration of chemical constituents of the studied oils by increasing the concentration of added ethanol. Also, the results show that there was an obvious decrease in the percentages of the oxygenated compounds since, the total percentages of these compounds in fresh onion volatile oil was 35.17. This value decreased and reached 19.88 - 16.05% by adulterating fresh onion volatile oil with 10 - 30% ethanol.

Other compounds such as disulphides had a total percentage of 1.16 for fresh onion volatile oil. This amount decreased to 1.01 - 0.83% when onion volatile oil was adulterated with 10 - 30% ethanol.

On the other hand, the total percentage of trisulphide compounds was 20.97 for fresh onion volatile oil and decreased to 14.10 - 9.01% by adulterating onion volatile oil with 10 - 30% ethanol.

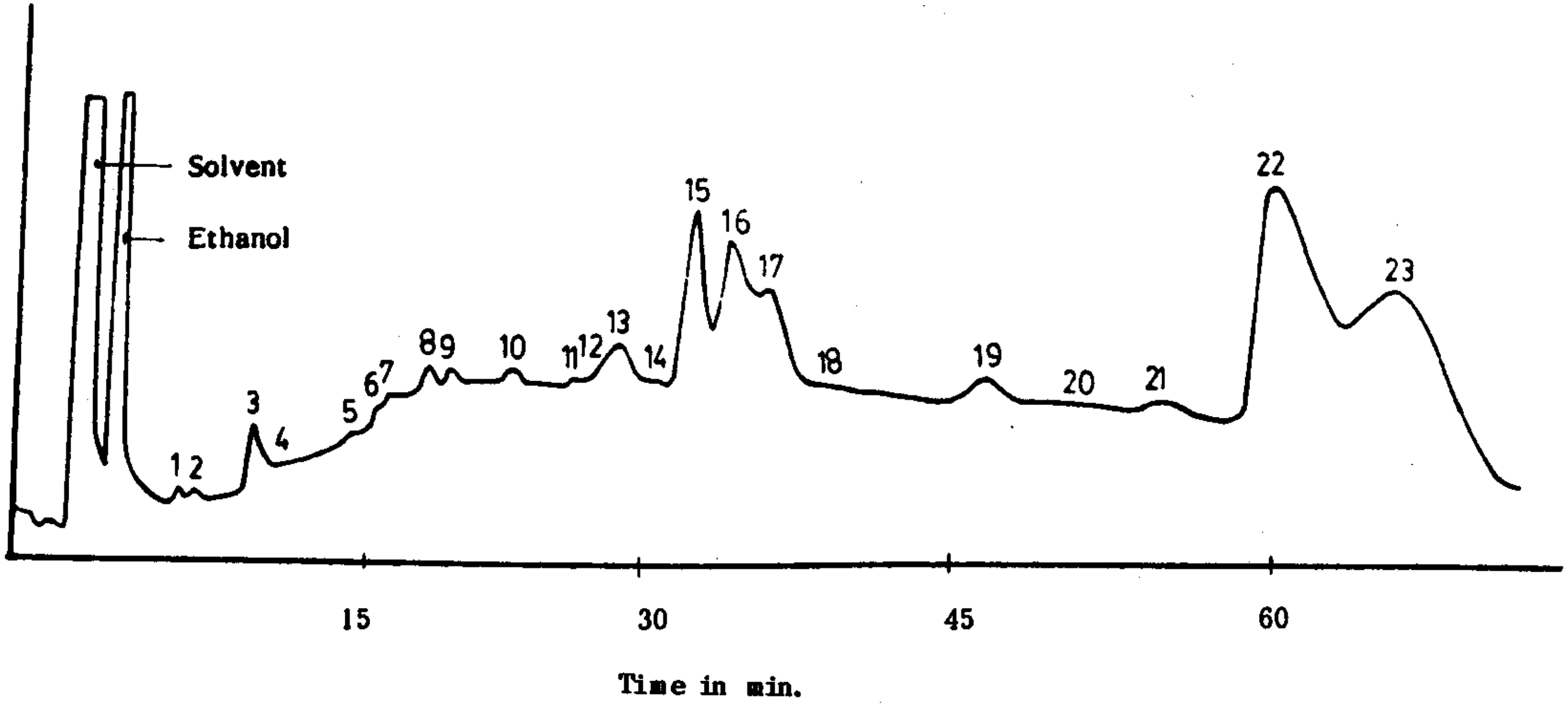


Fig. (20): G.L.C. of onion volatile oil adulterated with 10% ethyl alcohol.

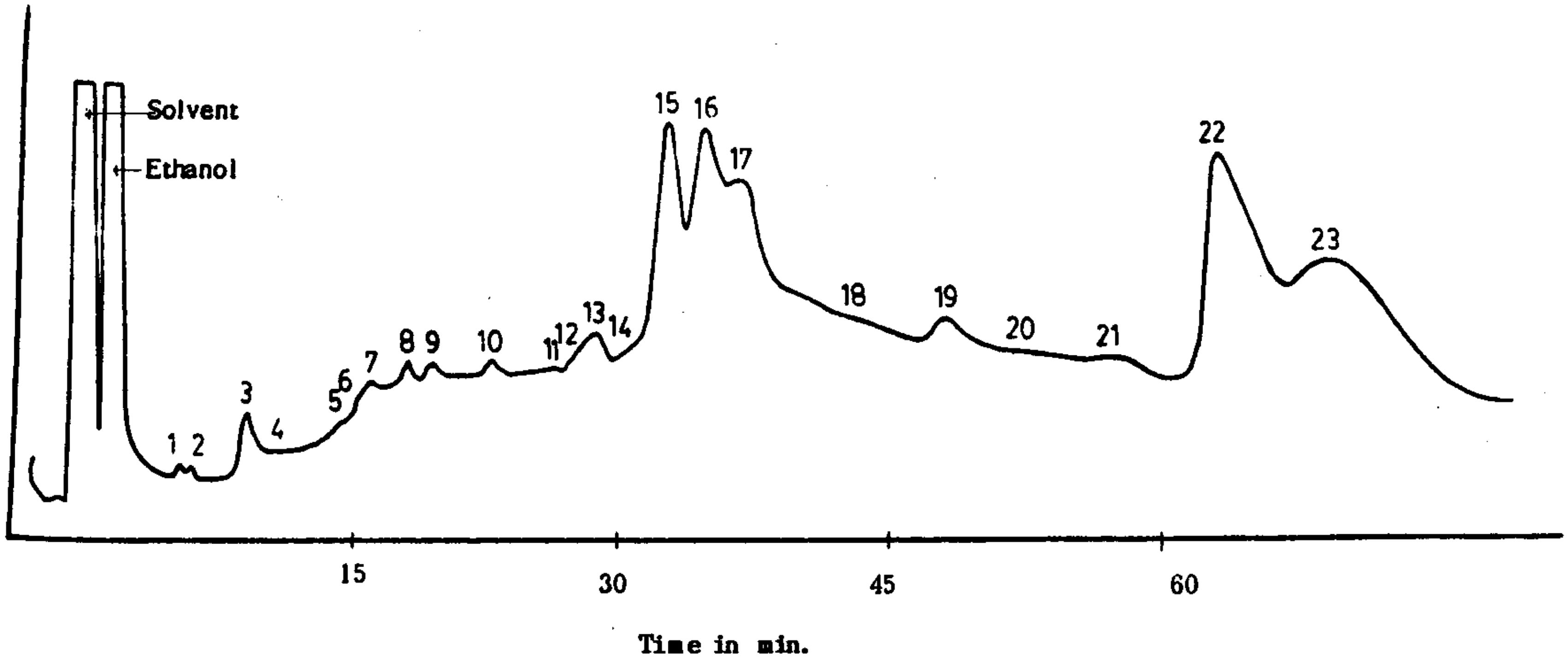


Fig. (21): G.L.C. of onion volatile oil adulterated with 20% ethyl alcohol.

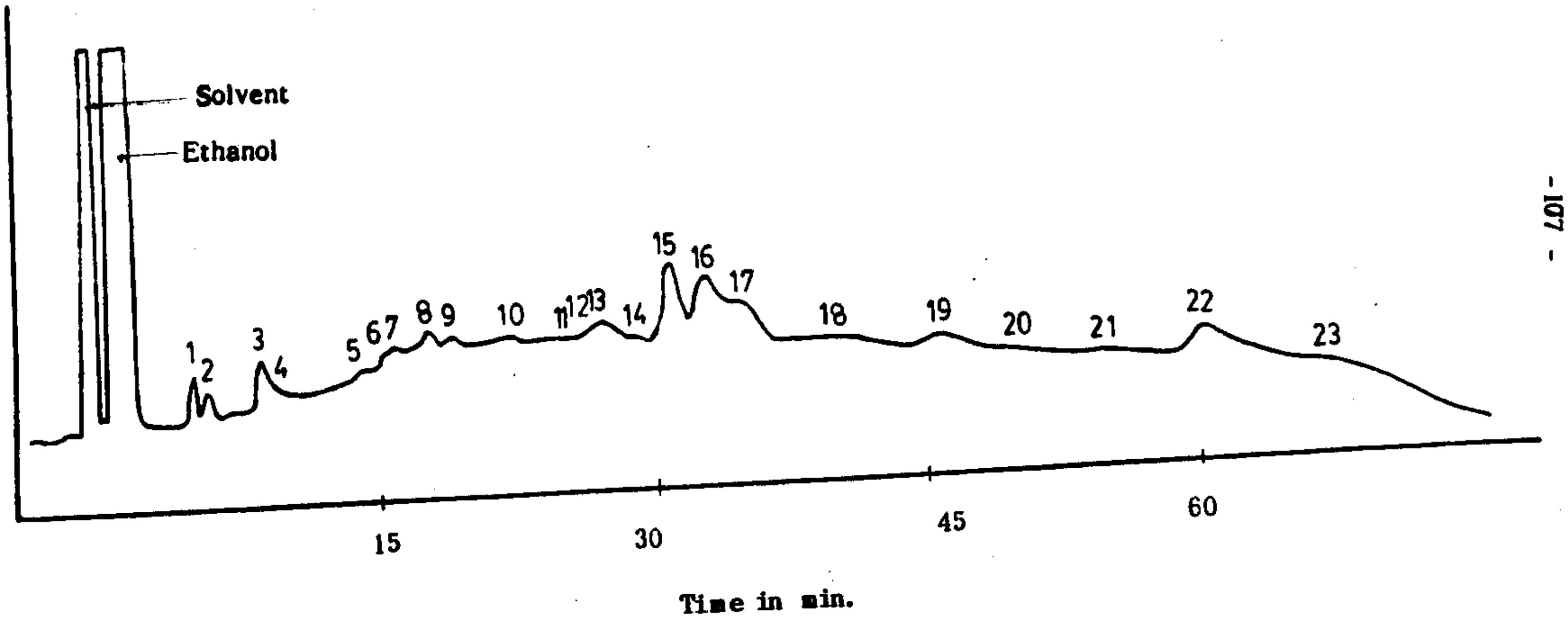


Fig. (22): G.L.C. of onion volatile oil adulterated with 30% ethyl alcohol.

Table (12)

G.L.C. of pure onion volatile oil and adulterated samples with ethanol

Peak No.	Fresh onion oil	Fresh onion oil + ethanol			Name of component
		10 %	20%	30%	
1	0.46	0.36	0.34	1.14	Acetaldehyde
2	0.30	0.29	0.27	0.62	propionaldehyde
3	1.46	1.80	1.69	2.17	Propyl alcohol
4	0.50	0.58	trace	trace	Octanal
5	0.34	0.22	0.14	0.22	Hexyl alcohol
6	0.60	0.22	0.20	0.21	Nonanal
7	0.23	0.29	0.41	0.21	1-propane thiol.
8	0.82	0.72	0.75	0.72	Decanal
9	0.56	0.43	0.68	0.52	Disulphide propane.
10	0.56	0.58	0.61	0.41	Decanol
11	0.49	0.36	0.34	0.31	Propane trisulphide
12	0.60	0.58	0.68	0.31	Propyl disulphide propane
13	2.98	2.01	2.17	0.62	Unknown
14	0.14	trace	trace	trace	"
15	12.95	8.42	8.54	4.97	Methyl propyl trisulphide
16	7.53	5.32	6.17	3.73	Dipropyl trisulphide
17	9.16	2.95	3.05	2.07	Undecanal
18	0.38	0.14	0.20	0.41	Unknown
19	3.10	2.88	3.66	3.11	" "
20	0.34	0.14	0.54	0.31	" "
21	1.87	1.87	1.83	1.55	" "
22	33.91	33.67	30.58	10.14	" "
23	21.01	12.16	8.07	8.49	Methyl taurine
	-	13.88	29.08	56.94	Ethanol

IV-1.5. Determination of glycerol content in onion volatile oil adulterated with maize oil:

Results shown in table (13) represent the glycerol content of adulterated onion volatile oil with maize oil.

It is quite clear that glycerol was not detected in the pure sample of onion volatile oil, since it has no glycerides. Whereas, glycerol increased proportionally with the increase of added maize oil as adulterant. Since, the glycerol measurements increased to reach 0.9657, 1.9447 and 2.8523 g/100 gm by adding maize oil in different percentages 10, 20 and 30%, respectively.

Accordingly, the glycerol determination by periodic acid could be considered as a reliable method for the quantitative determination of maize oil used as adulterant in the field of essential oils .

Table (13)

Glycerol content of onion volatile oil adulterated with maize oil.

Samples	Glycerol content in gm/100 gm of sample		Recovery
	Theoretically	practically	percent
Pure onion volatile oil	0.0	0.0	—
Pure maize oil	9.7592	9.7592	—
Onion volatile oil + 10% maize oil	0.9759	0.9657	98.95 %
Onion volatile oil + 20% maize oil	1.9518	1.9447	99.64 %
Onion volatile oil + 30% maize oil	2.9277	2.8523	97.42 %

IV-1.6. Determination of ethyl alcohol in adulterated onion volatile oil:

IV-1.6.1. Qualitative determination of ethanol (Iodoform test):

Results obtained concerning the iodoform test reveal that no crystals could be observed for the pure onion volatile oil, onion volatile oil adulterated with aged onion volatile oil, maize oil and paraffin oil. Whereas, samples adulterated with 10, 20 and 30% ethyl alcohol form a flat yellow crystals.

Thus, iodoform test can be considered as an excellent and rapid qualitative method to detect adulteration of essential oil with ethyl alcohol, especially when applied to the water layer resulted after washing the adulterated samples with water using saturated sodium chloride solutions.

IV-1.6.2. Quantitative determination of ethanol:

Results in table (14) represent the ethanol percent in adulterated onion volatile oil with ethanol at different percentages namely; 10, 20 and 30% which was determined by two different methods i.e. volumetrically and colorimetrically.

The data obtained reveal that the volumetric method was less sensitive in comparison with the colorimetric method, its recovery on average for the volumetric method was 97.87%, whereas it was 99.31 for the colorimetric method. Ethyl alcohol percent increased proportionally upon increasing its percent in the adulterated samples,

Table (14)

Ethanol content in onion volatile oil adulterated with ethyl alcohol

Samples	Weight of added ethanol	Ethanol content gm/100 gm sample			
	gm/100 gm sample	volumetrically	recovery	colorimetrically	recovery
Pure onion volatile oil	0.0	0.0	—	0.0	—
Onion volatile oil +10% ethanol	7.618	7.442	97.69 %	7.613	99.93 %
Onion volatile oil + 20% ethanol	15.236	15.152	99.45 %	15.217	99.88 %
Onion volatile oil + 30% ethanol	22.850	22.048	96.49 %	22.423	98.13 %

since, the ethanol percentages were; 7.442 and 7.613 for onion volatile oil plus 10% ethanol, 15.152 and 15.217 for onion volatile oil plus 20% ethanol and 22.048 and 22.423 for onion volatile oil plus 30% ethanol determined volumetrically and colorimetrically respectively. On the other hand, pure onion volatile oil samples did not contain any ethyl alcohol.

IV-1.7. Determination of paraffin oil content in onion volatile oil adulterated with paraffin oil:

Determination of paraffin oil in pure onion volatile oil and adulterated samples with paraffin oil in the concentration 10, 20 and 30% were carried out using the TLC technique described by Dhingar and Sharma (1978).

As early mentioned the following solvent systems were tried to obtain a good separation of the adulterated samples of onion volatile oil with paraffin oil.

1- Benzene (79-80.5°C) : ether (34-36°C) : Petroleum ether (60:80°C) (6 : 1 : 1, v/v).

2- Benzene (79 - 80.5°C) : ethyl acetate (40 - 60°C) : ether (34 - 36°C). (9 : 1 : 1, v/v).

3- Petroleum ether (60 - 80°C) : ether (34 - 36°C) : garlic acetic acid (90 : 10 : 1, v/v).

This last solvent system proved to be the most suitable, since, it gave a good separation without any overlapping . It is important to notice that all separated compounds in onion volatile oil appeared when the iodine vapour used to visualize the spots, whereas, the paraffin oil spots only appeared when the reagent 2,7-dichloro flouroscien 0.2% in 95% ethanol was used to visualize the spots in onion volatile oil adulterated with paraffin oil. For determination paraffin oil quantitatively the spots were measured colorimetrically at 535 nm.

Results obtained concerning the determination of paraffin oil used as an adulterant in different increment 10, 20 and 30% by volume are represented in Fig. (23) and table (15). These results indicate that the recovery of paraffin oil content in adulterated samples of onion volatile oil was 80.96% on average, which emphesized the possibility of using such method to detect the presence of paraffin oil in the essential oil. However, such method could be considered a tedious and time consuming procedure to be used as a routine method to detect this type of adulteration. It is easily to use solubility tests for paraffin detection and this mentioned method for confirmation.

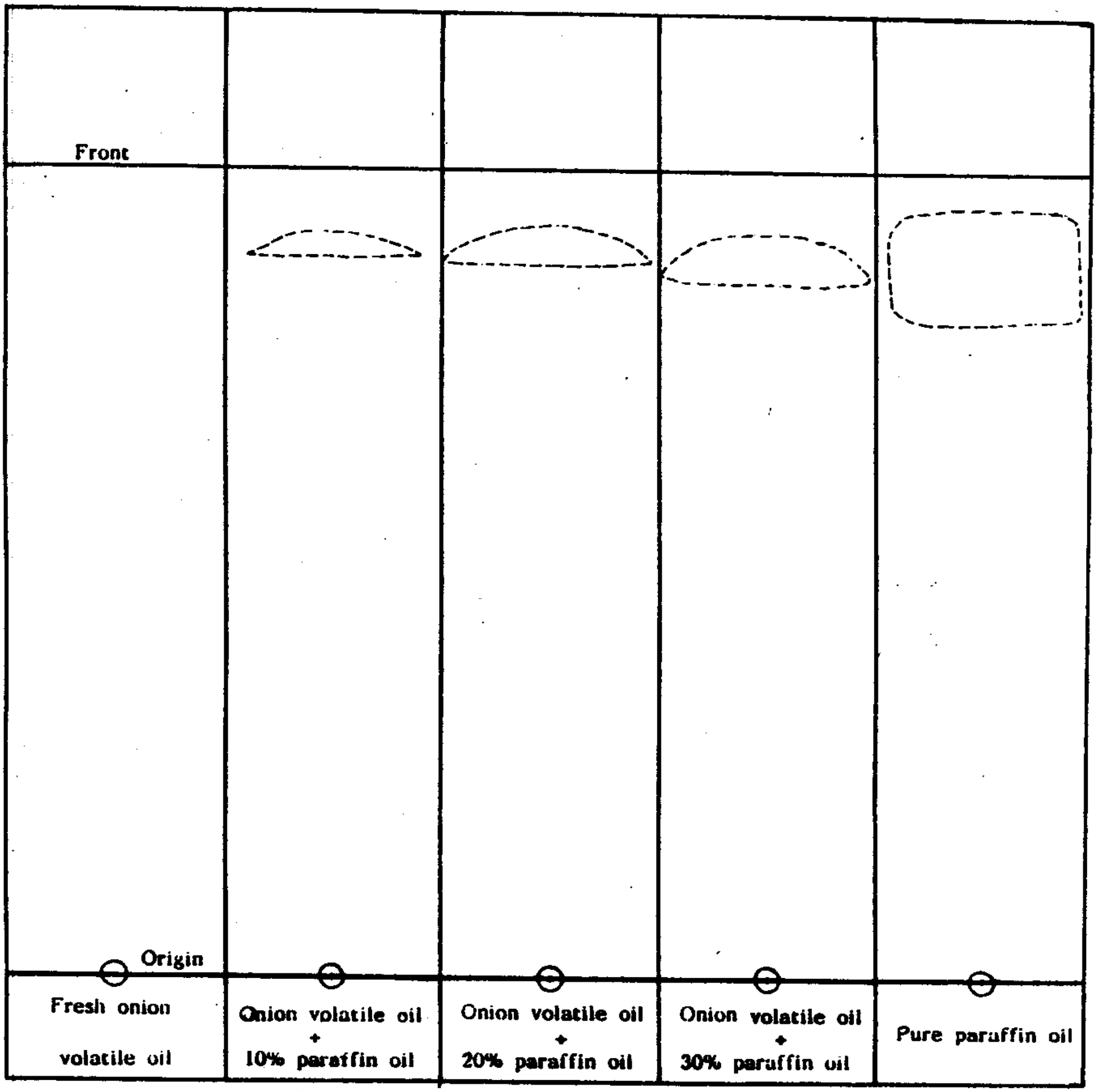


Fig. (23): T.L.C. of onion volatile oil adulterated with paraffin oil.

Table (15)

Paraffin oil content in adulterated onion volatile oils with paraffin oil

Samples	Weight of added paraffin oil gm/100 ml	Paraffin oil content gm/100 ml	Recovery
Pure onion volatile oil	0.0	0.0	—
Onion volatile oil + 10% paraffin oil	9.698	7.90	81.46 %
Onion volatile oil + 20% paraffin oil	19.396	15.36	79.19 %
Onion volatile oil + 30% paraffin oil	29.094	23.92	82.22 %

IV-2. Part II. Garlic volatile oil:

The aim of the present investigation is to detect and determine the adulteration of garlic volatile oil by using different adulterants commonly used in such practices. These adulterants were; aged garlic volatile oil, maize oil, ethyl alcohol and light paraffin oil.

IV-2.1. Physical and chemical properties of garlic volatile oil, aged garlic volatile oil and adulterated samples with various adulterants:

Garlic volatile oil, aged garlic volatile oil and adulterated samples with various adulterants were subjected to the routine analytical determination which are considered common procedures for the identification and comparative purpose. These analysis include the specific gravity, refractive index, specific optical rotation, acid number and solubility in both ethanol and other some organic solvents.

IV-2.1.1. Physical and chemical properties of fresh garlic volatile oil:

The results in table (16) show the physico-chemical properties of garlic volatile oil.

These results are similar to that obtained by Feneralli (1923), Guenther (1952) and Atallah (1980).

The solubility results in table (17) indicate that garlic volatile oil was soluble in 5.6 volume and more of ethanol 85%, and was insoluble in the concentration less than 85% ethanol. Also, the

Table (16)

Physical and chemical properties of fresh and aged garlic volatile oil and adulterated samples with various adulterants.

Samples	Specific gravity	Refractive index	Optical rotation	Acid number
Fresh garlic volatile oil	1.0770	1.5665	0.0°	0.88
,, + 10% aged garlic volatile oil	1.0671	1.5665	0.0°	0.89
,, + 20% ,, ,, ,, ,,	1.0642	1.5656	0.0°	1.03
,, + 30% ,, ,, ,, ,,	1.0577	1.5630	0.0°	1.05
Fresh + 10% maize oil	1.0509	1.5565	0.0°	0.84
,, + 20% ,, ,,	1.0389	1.5478	0.0°	0.80
,, + 30% ,, ,,	1.0226	1.5383	0.0°	0.77
Fresh + 10% Ethyl alcohol	1.0391	1.5549	0.0°	0.82
,, + 20% ,, ,,	1.0226	1.5502	0.0°	0.78
,, + 30% ,, ,,	0.9884	1.5427	0.0°	0.69
Fresh + 10% Paraffin oil	1.0587	1.5585	0.0°	0.83
,, + 20% ,, ,,	1.0479	1.5495	0.0°	0.73
,, + 30% ,, ,,	1.0375	1.5412	0.0°	0.70
Aged garlic volatile oil	1.0350	1.5522	0.0°	1.0833
Maize oil	0.9202	1.4755	0.0°	0.27
Ethyl alcohol	0.7618	1.3645	0.0°	0.13
Paraffin oil	0.9698	1.4864	0.0°	0.28

Table (17)

Solubility in ethanol of fresh and aged garlic volatile oil and adulterated samples with various adulterants.

Samples	Solubility in ethyl alcohol			
	75 %	85 %	95 %	99 %
Fresh garlic volatile oil	Turbid	ol. in 5.6 vol. and more	Sol. in 1 vol. and more	Sol. in 1 vol. and more
Fresh + 10% aged garlic volatile oil	,,	Sol. in 5.6 vol. and more	,, ,,	,, ,,
Fresh + 20% ,, ,, ,, ,	,,	Sol. in 6.6 vol. and more	,, ,, ,, ,,	,, ,, ,, ,,
Fresh + 30% ,, ,, ,, ,,	,	Sol. in 7.1 vol. and more	,, ,,	,, ,,
Fresh + 10% maize oil	Turbid	Turbid	Sol. in 1 vol. and more	Sol. in 1 vol. and more
,, + 20% ,, ,,	,,	,,	Slightly turbid	Sol. In 1 vol and more
,, + 30% ,, ,,	,,	,,	Turbid	Sol. in 1 vol. and more
Fresh + 10% ethyl alcohol	Turbid	Sol. in 5.6 vol. and more	Sol. in 1 vol. and more	Sol. in 1 vol. and more
,, + 20% ,, ,,	,,	,, ,,	,, ,,	,, ,,
,, + 30% ,, ,,	,,	,, ,,	,, ,,	,, ,,
Fresh + 10% Paraffin oil	Turbid	Sol. in 6.1 vol. and more	Sol. in 1 vol and more	Sol. in 1 vol. and more
,, + 20% ,, ,,	,,	Sol. in 6.6 vol and more	,, ,,	,, ,,
,, + 30% ,, ,,	,,	Sol. in 6.3 vol. and more	,, ,,	,, ,,
Aged garlic volatile oil	Turbid	Sol. in 6.0 vol. and more	Sol. in 1 vol. and more	Sol. in 1 vol and more
Maize oil	,,	Turbid	Turbid	Turbid
Ethyl alcohol	soluble	soluble	soluble	soluble
Paraffin oil	Turbid	Turbid	Turbid	Soluble

solubility results indicate that garlic volatile oil is completely soluble in carbon tetrachloride , diethyl ether, n-hexane, benzene and chloroform.

IV-2.1.2. Physical and chemical properties of aged garlic volatile oil and adulterated samples with aged garlic volatile oil:

The aged garlic volatile oil represents one of the most important adulterants. The addition of quantities of such oil could easily pass the simple routine analysis usually employed. The choice of aged garlic volatile oil was based on the fact that it is available in cheap price.

The changes in physico-chemical properties of garlic volatile oil adulterated with different concentrations of aged garlic volatile oil are shown in table (16). Results obtained reveal that adding increment amount of aged garlic volatile oil to fresh garlic volatile oil decreased the specific gravity from 1.0770 to 1.0671, 10642 and 1.0577 for garlic volatile oil plus aged garlic volatile oil at the concentrations of 10, 20 and 30% by volume,respectively.

The refractive indeces of both fresh garlic volatile oil and aged garlic volatile oil were 1.5665 and 1.5522,respectively. From these results it could be observed that the addition of aged garlic volatile oil caused a slight gradual decrease in the refractive index of the garlic volatile oils. Such decrease was directly proportional to the concentration of the added aged garlic volatile oil. The garlic volatile oil adulterated with 30% aged garlic volatile oil gave lower refractive index (1.5630) when compared with fresh garlic volatile oil sample (1.5665).

The obtained results pertaining adulteration with aged garlic volatile oil could not be taken as a mean for the detection of adulteration since, results fall within the range of specific gravity and refractive index for fresh garlic volatile oil.

On the other hand, the optical rotation of both fresh and aged garlic volatile oil were (0.0). It is logical that, fresh garlic volatile oil adulterated with aged garlic volatile oil at the concentrations of 10, 20 and 30% had the same above mentioned value.

The acid number of fresh garlic volatile oil gradually increased with the addition of increment percentages of aged garlic volatile oil. This may be attributed to the high acidity of the aged garlic volatile oil due to its oxidation during storage for long time at ambient temperature. The acid number increased from 0.88 for fresh garlic volatile oil to 0.89, 1.03 and 1.05 for fresh garlic volatile oil adulterated with 10, 20 and 30% of aged garlic oil respectively.

It could be concluded that the acid number could be used to detect the adulteration of garlic volatile oil with aged garlic volatile oil.

The solubility of garlic volatile oil in ethyl alcohol decreased after adulteration with various concentrations of aged garlic volatile

The obtained results reveal that adding increment amounts of maize oil decreased the specific gravity of garlic volatile oil adulterated with maize oil, since, the specific gravity of maize oil (0.9202) was lower than that of pure garlic volatile oil (1.0770). The specific gravity decreased from 1.0770 for pure garlic volatile oil to 1.0226 for garlic volatile oil admixed with 30 percent of maize oil.

The results obtained indicate that specific gravity could not be used as a reliable parameter for the detection of maize oil at this concentration since, the specific gravity of adulterated garlic volatile oil using this concentration of maize oil was in agreement with that reported by Guenther (1952) and Atallah (1980). On the other hand, increasing the percent of added maize oil to 20 and 30% the specific gravity of the mixture became less than the specified range i.e. 1.0389 and 1.0226, respectively.

Whereas, the refractive indeces of fresh garlic volatile oil and maize oil were 1.5665 and 1.4755, respectively. Admixed garlic oil with various concentrations of maize oil caused a gradual decrease in the refractive index of garlic volatile oil, which was in proportion to the added percent of maize oil. The garlic oil adulterated with 30% maize oil had the lowest refractive index (1.5383).

The optical rotation of the adulterated garlic volatile oil was not influenced by the adulteration practices, since the compounds of maize oil have not any asymmetrical carbon atoms.

Results of the acid number of adulterated garlic volatile oil with maize oil at different concentrations show obvious decreases in comparison with the elutriated oil. Since, the acid number decreased from 0.88 for fresh garlic oil to 0.84, 0.80 and 0.77 for garlic volatile oil adulterated with 10, 20 and 30% maize oil, respectively. These results could be attributed to the addition of maize oil which had low acidity to pure garlic volatile oil.

The results of solubility cited in table (17) show that the pure garlic volatile oil was soluble in 5.6 volume and more of 85% ethanol, whereas pure maize oil was turbid even with high concentration i.e. 99% alcohol. But after mixing the garlic volatile oil with 10% maize oil the mixture became soluble in 1 volume and more of 95% alcohol while turbid in 85%. Garlic volatile oil plus 20% maize oil turned soluble in 1 vol. and more of 99% alcohol, but became slightly turbid using 95% alcohol. Whereas, mixing the pure garlic volatile oil with 30% maize oil was soluble only in 1 vol. and more of 99% alcohol. Conclusively, it is clear that the solubility gradually decreased with the increasing quantities of maize oil as adulterant.

On the other hand, all samples adulterated with different concentration of maize oil were completely soluble in diethyl ether, carbon tetrachloride , n-hexane, benzene and chloroform.

IV-2.1.4. The physical and chemical properties of adulterated garlic voltile oil with ethyl alcohol:

Ethyl alcohol is considered as one of the most alcohols usually used in the adulteration of essential oil.

In the present study, garlic volatile oil was mixed with increment proportion of absolute ethyl alcohol in the concentration of 10, 20 and 30% by volume.

The changes in physico-chemical properties of garlic volatile oil adulterated with 10, 20 and 30% ethanol are shown in table (16). These results indicate that adding increment proportions of ethyl alcohol caused a gradual decrease in the specific gravity values of garlic volatile oil adulterated with ethyl alcohol, since, the specific gravity of ethyl alcohol (0.7618) was lower than that of garlic volatile oil (1.0770). Consequently, the specific gravity decreased from 1.0770 for pure garlic volatile oil to 1.0391, 1.0226 and 0.9884 for garlic volatile oil admixed with 10, 20 and 30% ethyl alcohol.

It is important to note that specific gravity can be easily used as a reliable parameter for the detection of adulteration with ethyl alcohol, since the specific gravity decrements were less than the normal range of specific gravity for garlic volatile oil (1.046 to 1.2741) mentioned by Guenther (1952) and Atallah (1980).

The refractive indeces of fresh garlic volatile oil and ethyl alcohol were 1.5665 and 1.3645 , respectively. Adulterated garlic volatile oil with increment proportion of ethyl alcohol caused a gradual decrease in values of refractive index of the samples of garlic volatile oil adulterated with ethyl alcohol, since, it became

1.5549, 1.5502 and 1.5427 for garlic volatile oil plus 10, 20 and 30% ethyl alcohol. These results are in agreement with the results obtained by El-Masry (1978 and 1985).

On the other hand, the optical rotation of the adulterated garlic volatile oil was not influenced by the adulteration practices. Since, these compounds are optically inactive.

Meanwhile, the acid number of adulterated garlic volatile oil with ethyl alcohol at the concentration of 10, 20 and 30% showed a gradual decrease when compared with the elutriated oil. Since the acid number decreased from 0.88 for pure garlic volatile oil to 0.82, 0.78 and 0.69 for garlic volatile oil adulterated with 10, 20 and 30% of ethyl alcohol, respectively. This could be ascribed to the low acidity of ethyl alcohol (0.13), accordingly the addition of ethyl alcohol caused the decrement of acidity of garlic volatile oil.

The solubility test was undertaken using different concentrations of ethyl alcohol Viz; 99, 95, 85 and 75%. The solubility of garlic volatile oil adulterated with ethyl alcohol did not change, since the solubility behaviour of garlic volatile oil and adulterated with ethyl alcohol at concentration 10, 20 and 30% were the same. Where samples of both pure garlic volatile oil and garlic volatile oil adulterated with the different concentration of ethyl alcohol were soluble in 1 volume and more of 95% and 99% alcohol, but soluble only in 5.6 volume and more of 85% alcohol.

On the other hand, garlic volatile oil samples adulterated with ethyl alcohol at different concentrations were completely soluble in diethyl ether, carbon tetrachloride , n-hexane, benzene and chloroform.

IV-2.1.5. The physical and chemical properties of adulterated garlic volatile oil with paraffin oil:

Paraffin oil commonly used as adulterant for essential oils as it was mentioned before. In the present study garlic volatile oil was mixed with paraffin oil at 10, 20 and 30% by volume. Trials to determine the paraffin oil were made.

Results in table (16) represent changes in physico-chemical properties of garlic volatile oil adulterated with paraffin oil.

These results indicate that the increment of light paraffin oil used as adulterant in garlic volatile oil at 10, 20 and 30% by volume caused a proportional decrements in the specific gravity, since the specific gravity of light paraffin oil (0.9698) is lower than the specific gravity of pure garlic volatile oil (1.0770). It is quite clear that the addition of light paraffin oil at concentrations 10, 20 and 30% to pure garlic volatile oil caused a drop in its specific gravity from 1.0770 to 1.0587, 1.0479 and 1.0375, respectively.

On the other hand, the refractive index of pure garlic volatile oil and light paraffin oil were 1.5665 and 1.4864, respectively. The admixtion of light paraffin oil with pure garlic volatile oil in increment proportion caused an abvious decrease, since, the refractive indeces of the admixed garlic volatile oil with paraffin oil were; 1.5585, 1.5495 and 1.5412 for 10, 20 and 30% paraffin oil, respectively.

The optical rotation results revealed that no optical rotation for both pure garlic volatile oil and paraffin oil. Consequently, the addition of different concentration of paraffin oil did not cause any change for its optical activity. Accordingly, the optical rotation could not be considered as a parameter to detect the adulteration of garlic volatile oil with light paraffin oil.

The acid number of pure garlic volatile oil was 0.88 whereas, that of paraffin oil was 0.28. Accordingly, the expected acid number of adulterated garlic volatile oil is irreversally proportional to the percentage of added paraffin oil. Results obtained in table (16) showed the same above mentioned trend, since, the acid number were, 0.83, 0.73 and 0.70 for adulterated pure garlic volatile oil with 10, 20 and 30% paraffin oil, respectively.

The solubility of pure garlic volatile oil adulterated with light paraffin oil was determined using different concentration of ethanol at 75, 85, 95 and 99% and the results are shown in table (17). These results indicate that pure garlic volatile oil insoluble in 75% alcohol, whereas, it started to be soluble in 85% alcohol using 5.6 volume and more and in 95% alcohol using 1 volume and more. Meanwhile the paraffin oil is insoluble in all used concentration except that of 99% alcohol, since, it turned soluble in 1 volume and more. The admixed samples of pure garlic volatile oil and light paraffin oil started to be soluble in 6.1 volume and more, 6.6 volume and more and 6.8 volume and more of 85% ethyl alcohol, for garlic volatile oil plus 10, 20 and 30% paraffin oil, respectively. These results clearly indicate that addition of paraffin oil to garlic volatile oil decreased the degree of solubility. Meanwhile, all adulterated samples were soluble in 95% and 99% alcohol in 1 volume and more.

On the other hand, all samples completely soluble in diethyl ether, n-hexane, benzene, carbon tetra chloride and chloroform.

These results obtained concerning the physico-chemical properties of garlic volatile oil adulterated with various adulterants are in agreement with the results obtained by Osman et al. (1974) on geranium oil, El-Masry (1978) on geranium oil and peppermint oil, Oda (1982) on geranium oil and Cumin oil and El-Masry (1985) on marjoram oil, petitgrain bigrade oil and Fennel oil.

IV-2.2. U V Spectra of garlic volatile oil, aged garlic volatile oil and adulterated samples with various adulterants:

IV-2.2.1. U V spectrum of fresh garlic volatile oil:

The ultra - violet absorption spectrum of fresh garlic volatile oil from fresh garlic cloves showed a maximum absorption at 278 nm (Fig. 24). This could be due to the presence of same chromophoric groups in the volatile oil molecules especially, unsaturated bonds in these compounds (Bauman, 1963 and Rao, 1967).

IV-2.2.2. U V spectra of aged garlic volatile oil and adulterated samples with aged garlic volatile oil:

The results obtained concerning the U V absorption of aged garlic volatile oil and adulterated samples with aged garlic oil in increasing amounts namely 10, 20 and 30% Figs. (25 and 26) indicate that upon using the same concentration a proportional different absorption rates were observed.

The absorption intensity decreased with increasing the concentration of the added aged garlic oil, since the absorption intensities were 1.83, 1.78 and 1.73 for adulterated garlic volatile oil with aged garlic oil in the concentration of 10, 20 and 30% respectively.

IV-2.2.3. U V spectrum of adulterated agrlic volatile oil with maize oil:

The results obtained concerning with the U V absorption of different samples of garlic oil adulterated with different concentrations

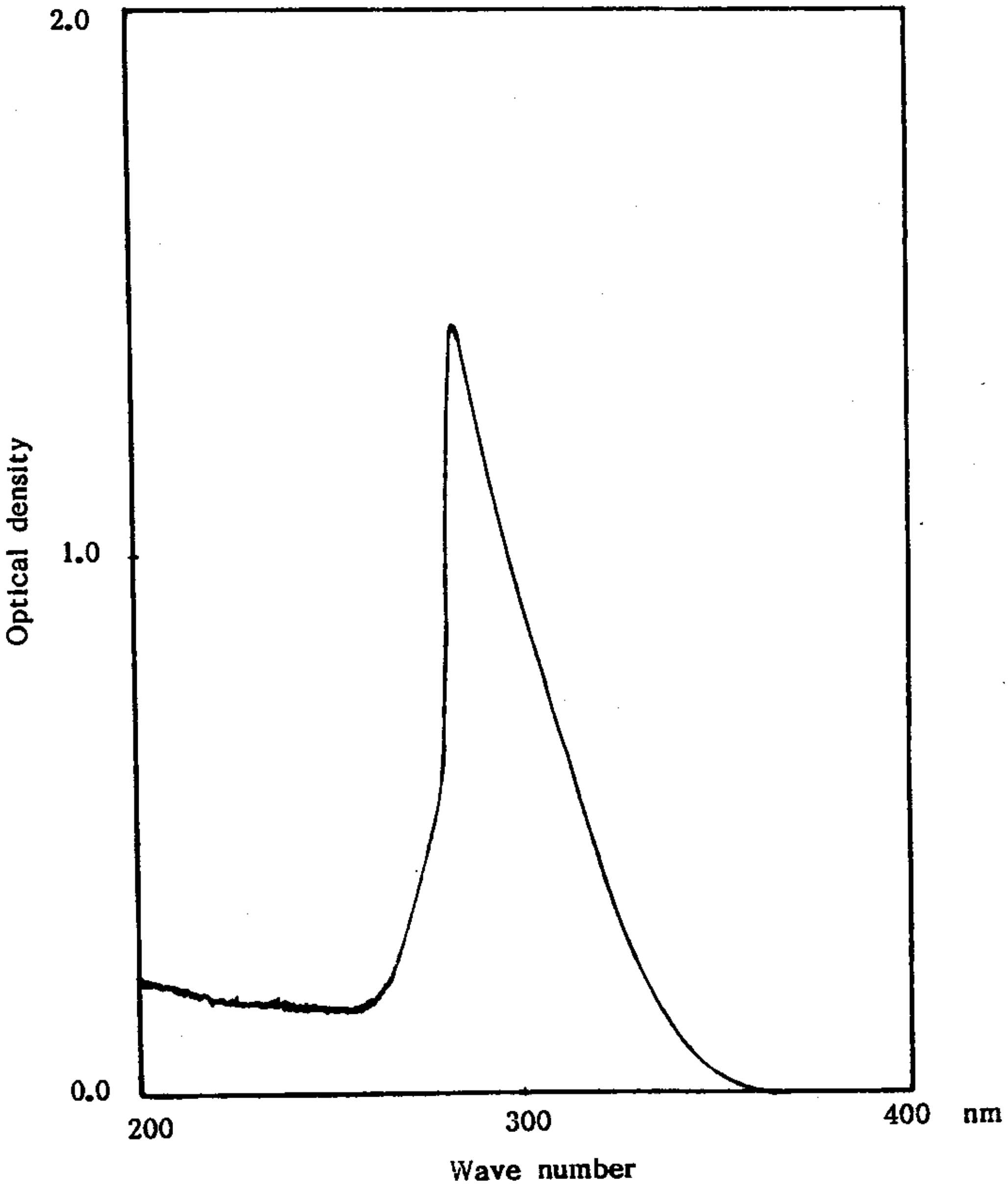


Fig. (25): U V absorption for aged garlic volatile oil of maize oil in increment i.e. 10, 20 and 30% , reveal that different proportional absorption intensities were obtained for the different adulterated samples as shown in Fig. (27). The absorption intensity at 278 nm, which is considered the maxium absorption, decreased with increasing the concentration of the maize oil percent. The absorption intensities were 1.62, 1.50 and 1.34 for adulterated garlic volatile oil with maize oil at 10, 20 and 30% , respectively.

Accordingly, this test could be used as a rapid qualitative test to indicate the adulteration practice of garlic volatile oil with maize oil.

IV-2.2.4. UV spectrum of adulterated garlic volatile oil with ethyl Alcohol:

UV spectrum of garlic volatile oil samples adulterated with different concentrations of ethyl alcohol in viz, 10, 20 and 30% are shown in Fig. (28). It could be observed that upon using the same concentration different absorption intensities were obtained for the different adulterated samples, but the maximum absorption was the same in all samples at 278 nm. The absorption rate decreased with increasing the concentration of ethyl alcohol. However, the rate of decreasing was not proportionaly related to the amount of ethyl alcohol added.

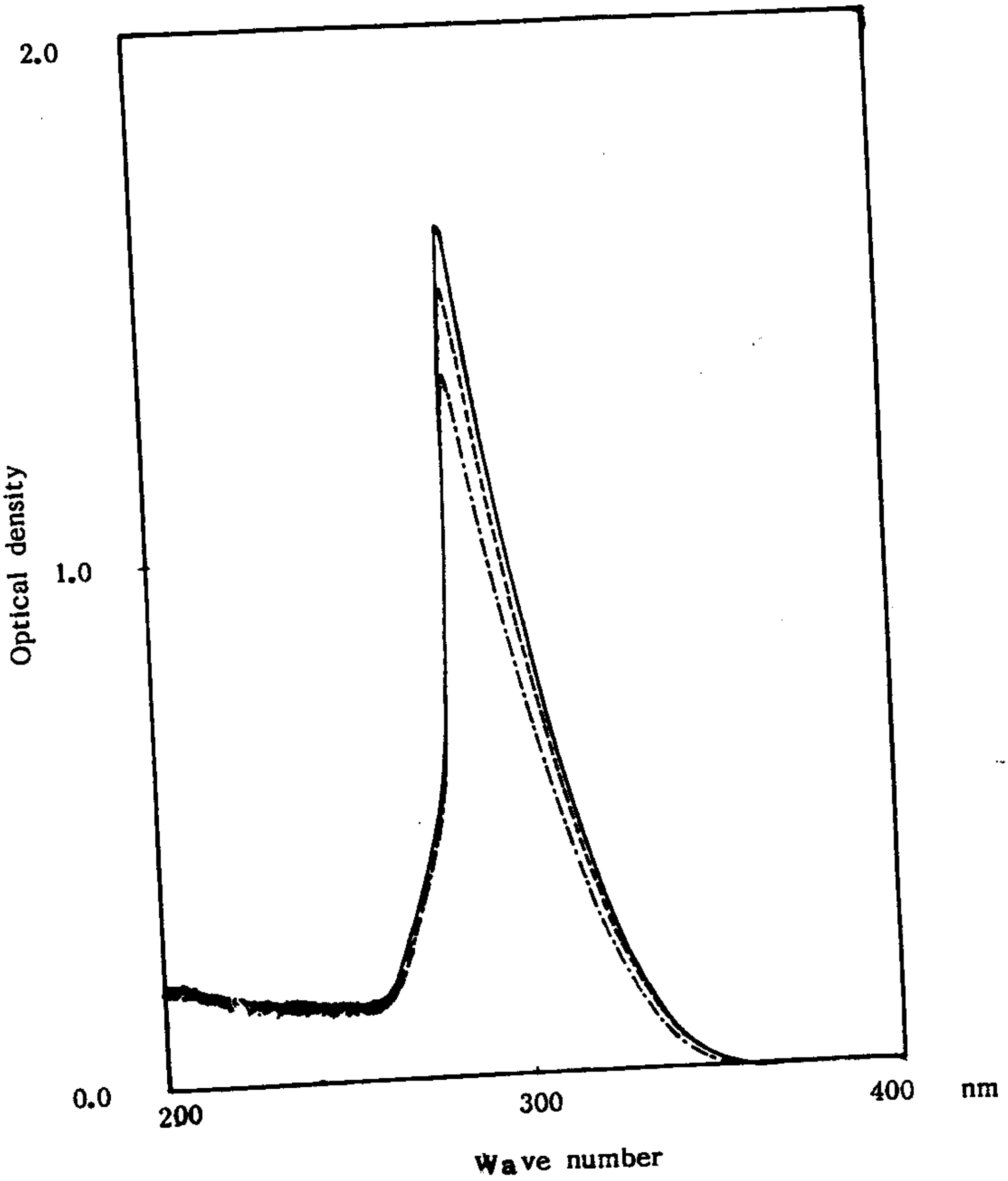


Fig. (27): U V absorption for garlic volatile oil adulterated with maize oil.

(———) : Garlic volatile oil + 10% maize oil.
(- - - - -) : ,, ,, ,, + 20% ,, ,,
(—.—.—.—) : ,, ,, ,, + 30% ,, ,,

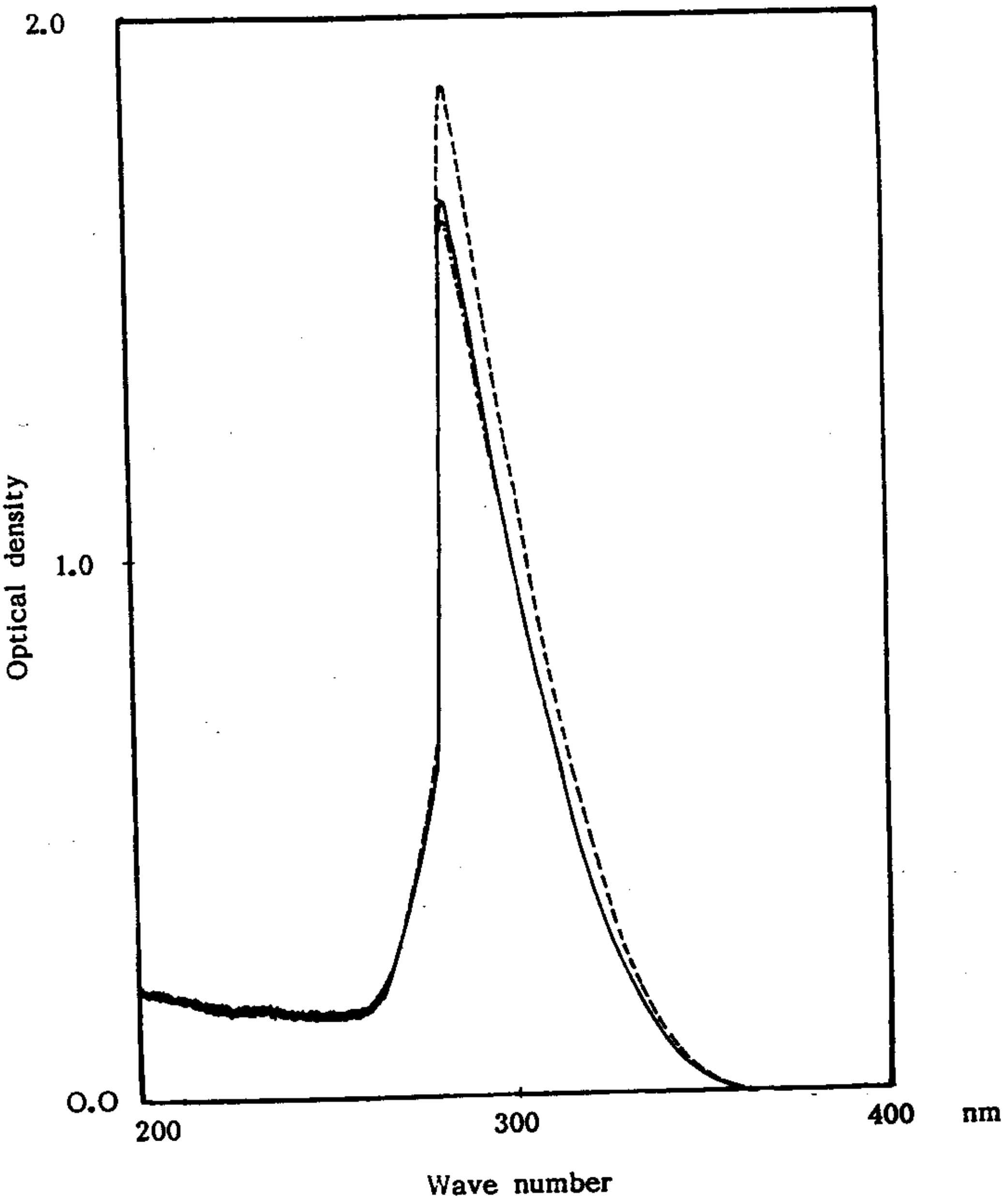


Fig. (28): U V. absorption for garlic volatile oil adulterated with ethyl alcohol.

(-----) : Garlic volatile oil + 10% Ethyl alcohol.
(———) : ,, ,, ,, + 20% ,, ,,
(—.—.—.) : ,, ,, ,, + 30% ,, ,,

IV-2.2.5. U V specterum of adulterated garlic volatile oil with paraffin oil:

Results obtained concerning the U.V. absorption of garlic volatile oil samples adulterated with paraffin oil in increment concentration at 10, 20 and 30%, reveal that upon using the same concentration a proportional different absorption rates were obtained according to the percentages of paraffin oil in the different adulterated samples Fig. (29). The absorption rate decreased with increasing the concentrations of the added paraffin oil. Since, the absorption rates were 1.82, 1.69 and 1.52 for adulterated garlic volatile oil with paraffin oil at 10, 20 and 30%, respectively.

Accordingly, this test could be considered as a reliable qualitative test to indicate the adulteration of garlic volatile oil with aged garlic volatile oil, maize oil, ethyl alcohol and paraffin oil.

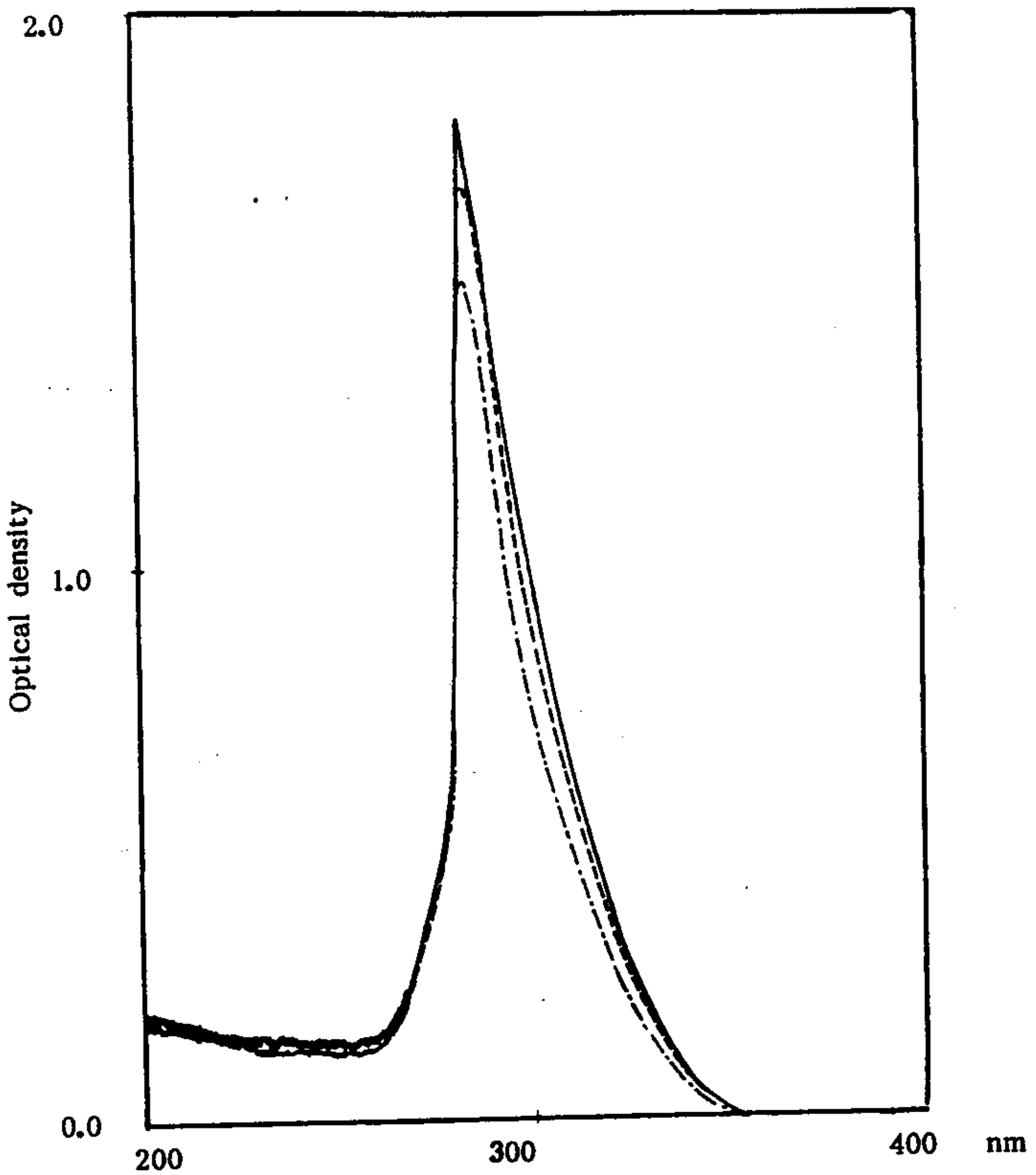


Fig. (29): U V absorption for garlic volatile oil adulterated with paraffin oil.

(———) : Garlic volatile oil + 10% Paraffin oil.
(- - - - -) : ,, ,, ,, + 20% ,, ,,
(—·—·—) : ,, ,, ,, + 30% ,, ,,

IV-2.3. I R spectra of garlic volatile oil, aged garlic volatile oil and adulterated samples with various adulterants :

IV-2.3.1. I R spectrum of fresh garlic volatile oil:

I R spectrum of fresh garlic volatile oil shown in table (18) and Fig. (30), which represents a series of absorption bands which take place: at 650 Cm^{-1} indicates the probable presence of (C-S) accodring to Finar (1973), at 720 Cm^{-1} refers to $-(CH_2)_n-$ in which $n \geqslant 4$ as it was reported by Goergy (1978), at 825 Cm^{-1} assigns to epoxide compound, as it was mentioned by Sen Gupta (1946), at 920 and 950 Cm^{-1} indicate the presence of $>C=C<$ trans double bond Gore (1972), at 987 Cm^{-1} refers to propenyl sulphide as it was mentioned by Nofal (1974) at 1075 Cm^{-1} indicates the presence of C-0 stretching characteristic of primary alcohol as it was mentioned by Finar (1973), at 1218 Cm^{-1} may be assigned to C-C stretch, as it was mentioned by Gore (1972), at 1258 Cm^{-1} indicates the probable presence of =C-O-C= antisymstretching (Gore 1972), and at 1290 Cm^{-1} refers to propyl sulphide (Goergy 1978). Whereas, at 1305 and 1320 Cm^{-1} indicate the presence of the sulphone group ($>SO_2$) as mentioned by Bellamy (1962), Nakanishi (1964) and Bhati (1968). While at 1395 and 1420 Cm^{-1} refer to the presence of $(-CH=CH_2)$as mentioned by Nakanishi (1964) whereas, at 1555 and 1609 Cm^{-1} indicates the probable presence of (C=0 stretching) in esters, carboxylate onion stretching as suggested by Pecsok and Shields (1968). Also, the absorption band at wave number 1630 Cm^{-1} refers to Alkenes C=C nonconjugated (Silverstein and Bassler 1967). Furthermore, the absorption

Table (18)

I R spectra of fresh garlic volatile oil.

Band at wave number cm^{-1}		Bond or group
650	w	C-S
720	s	$-(CH_2)_n-$ n $\geqslant$ 4
752	m	$-(CH_2)_n$ - rock (smaller n)
825	sho	Epoxide compound
857	m	C-C stretch
920	s	$>C=C<$ trans double bond
950	w	$>C=C<$ trans double bond
987	s	Propenyl sulphide
1075	m	C-0 of primary alcohol
1197	sho	C-O stretch
1218	s	C-C stretch
1258	w	=C-O-C= antisym. stretching.
1290	m	Propyl sulphide
1305	m	Sulphone group $>SO_2$
1395	s	$(-CH=CH_2)$
1420	s	$(-CH=CH_2)$
1555	w	C=0 stretching (esters)
1609	sho	C=0 carboxylate anion stretching.
1630	s	Alkenes C=C nonconjugated.
1700	w	C=0 carbonyl group (ester)
1842	m	C=0 stretching
1968	w	
2915	s	Alkane CH_3-CH_2-
2950	s	Methyl and methelene group.
3010	s	C-H stretching alkene mono substituted
3080	s	C-H stretching alkene mono substituted

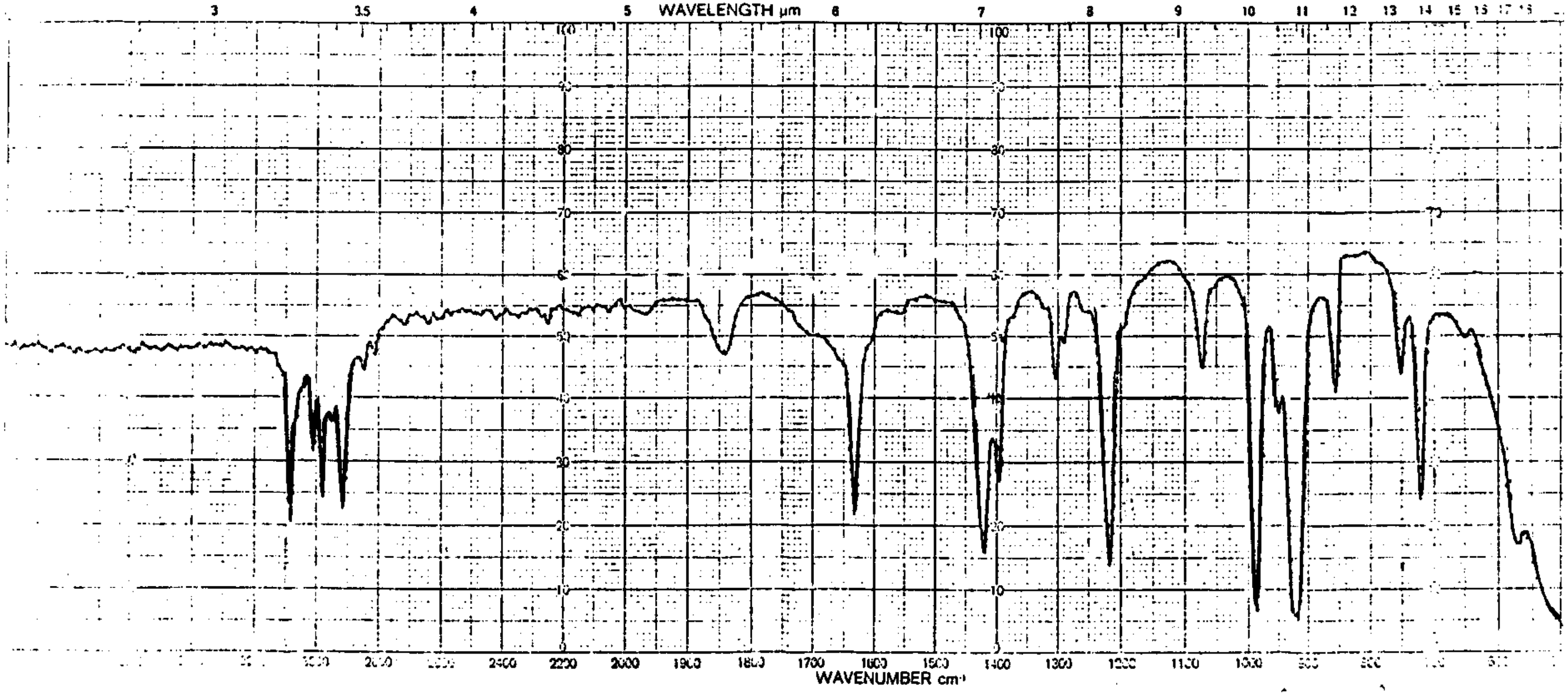


Fig. (30) : IR spectrum of fresh garlic volatile oil.

band at 1700 Cm^{-1} refers to carbonyl groups (ester) as reported by Bellamy (1962), Rao (1963) and Bhati (1968). Also, the absorption band at 1842 Cm^{-1} refers to (C=0 stretching) anhydrides as mentioned by Pecsok and Shields(1968), while at 2718 Cm^{-1} indicates the presence of (C-H stretching) as mentioned by Gore (1972). Also, at 2850 and 2915 Cm^{-1} refers to presence alkane (CH_3-CH_2-) (Gore, 1972). Whereas, at 2950 Cm^{-1} refers to methyl and methelene group (Goergy 1978), while at 3040 and 3080 Cm^{-1} refer to C-H stretching (alkene) mono substituted as mentioned by Pecsok and Shields(1968).

From the results shown in table (18) the I R spectroscopy can be used as a qualitative analysis of the garlic volatile oil. The whole spectrum from 3080 Cm^{-1} to 650 Cm^{-1} could be empirically used as a **fingerprint** of the molecules mainly found in garlic volatile oil.

IV-2.3.2. I R spectra of aged garlic volatile oil and adulterated samples with aged garlic oil:

The obtained data in figs (31 and 32) and table (19) showed that no new bands were detected in I R spectra as a result of adding aged garlic oil to fresh garlic oil.

There are insignificant changes in intensities of absorption bands when the aged garlic oil was added to fresh garlic oil.

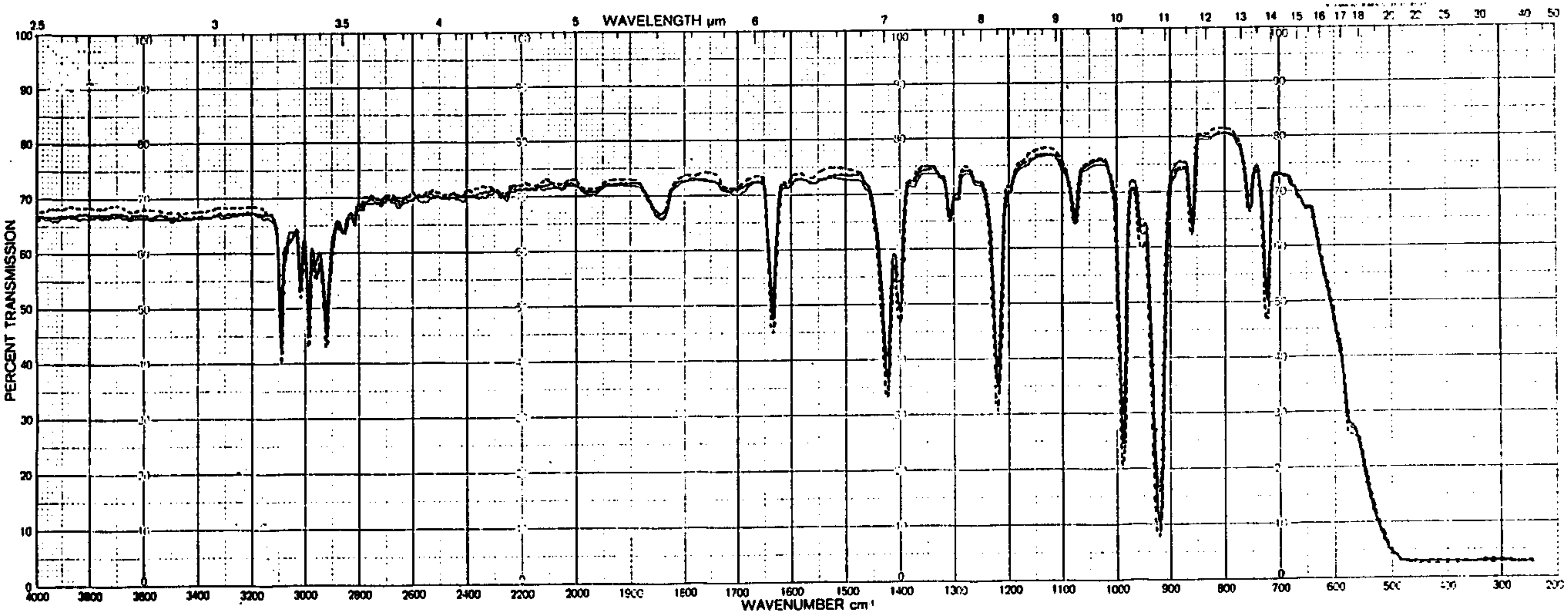


Fig. (32): I.R spectra of garlic volatile oil adulterated with aged garlic volatile oil.

(..........) : Garlic volatile oil + 10% aged garlic volatile oil;

(— — — —) : ,, ,, ,, + 20% ,, ,, ,, ,,

(————) : ,, ,, ,, + 30% ,, ,, ,, ,,

Table (19)

I.R. spectra of aged garlic volatile oil and adulterated garlic volatile oil with aged garlic volatile oil.

Aged garlic volatile oil		Garlic oil + 10% aged oil		Garlic oil + 20% aged oil		Garlic oil + 30% aged oil		Bond or group
722	s	725	s	725	s	725	s	$-(CH_2)_n-$ n $\geqslant$ 4
755	m	755	w	755	w	755	w	$-(CH_2)_n-$ rock (smaller n)
830	sho	830	sho	830	sho	830	sho	Epoxide compound
860	s	860	s	860	s	860	s	C-C stretch
920	s	920	s	920	s	920	s	$>C=C<$ trans double bond
955	sho	955	w	955	w	955	w	$>C=C<$ trans double bond
990	s	989	s	989	s	989	s	Propenyl sulphide
1075	m	1075	w	1075	w	1075	w	C-0 of primary alcohol
1200	sho	1200	sho	1200	sho	1200	sho	C-0 stretch
1220	s	1220	s	1220	s	1220	s	C-C stretch
1258	sho	1255	sho	1255	sho	1255	sho	=C-0-C = antisym stretching
1295	w	1290	sho	1290	sho	1290	sho	Propyl sulphide
1305	sho	1308	s	1308	s	1308	s	Sulphone group $>SO_2$
1400	s	1400	m	1400	m	1400	m	$(-CH=CH_2)$
1424	s	1425	s	1425	s	1425	s	(-CH = CH 2)
1562	w	1560	w	1560	w	1560	w	C=0 stretching in esters
1605	w	1608	sho	1608	sho	1608	sho	C=0 carboxylate anion stretching
1635	s	1635	s	1635	s	1635	s	Alkenes C=C nonconjugated
1700	w	1700	w	1700	w	1700	w	Carbonyl group (esters)
1840	w	1840	m	1840	m	1840	m	C=0 stretching
1968	w	1970	w	1970	w	1970	w	
2918	s	2920	s	2920	s	2920	s	CH_3-CH_2- Alkane
2980	s	2990	s	2990	s	2990	s	Methyl and methelene group
3020	s	3018	m	3018	m	3018	m	C-H stretching alkene monosubstituted
3090	s	3090	s	3090	s	3090	s	C-H stretching alkene monosubstituted

IV-2.3.3. I R spectra of adulterated garlic volatile oil with maize oil

The adulteration of garlic volatile oil with different concentrations of maize oil can be detected from the spectra obtained after adding maize oil to garlic oil, (fig. 33 and table 20).

In comparison of I R spectrum of garlic oil, and the I.R. spectra of garlic and maize oils mixtures, it can be deduced that four new absorption bands appeared in the last spectrum. The new absorption bands appeared are at 1090 cm^{-1} (C-O in primary alcohol), 1460 cm^{-1} (OH bending in carboxylic acids), 1740 Cm^{-1} (C=O stretching in aliphatic esters and 3455 Cm^{-1} (bonded OH). By the way, these four absorption bands are distinguished for maize oil but do not exist in I R spectrum of garlic oil. Therefore, it can be consideres as an indicator to detect the adulteration of garlic volatile oil with maize oil. The increase in intensity absorption bands is corresponding to the percentage of maize oil added to garlic oil.

IV-2.3.4. I R spectra of adulterated garlic volatile oil with ethyl alcohol:

As it is shown from fig. (34) and table (21), no new bands appeared as a result of adding ethanol to garlic volatile oil. The intensities of absorption bands increased by increasing the amounts of ethanol added to garlic volatile oil.

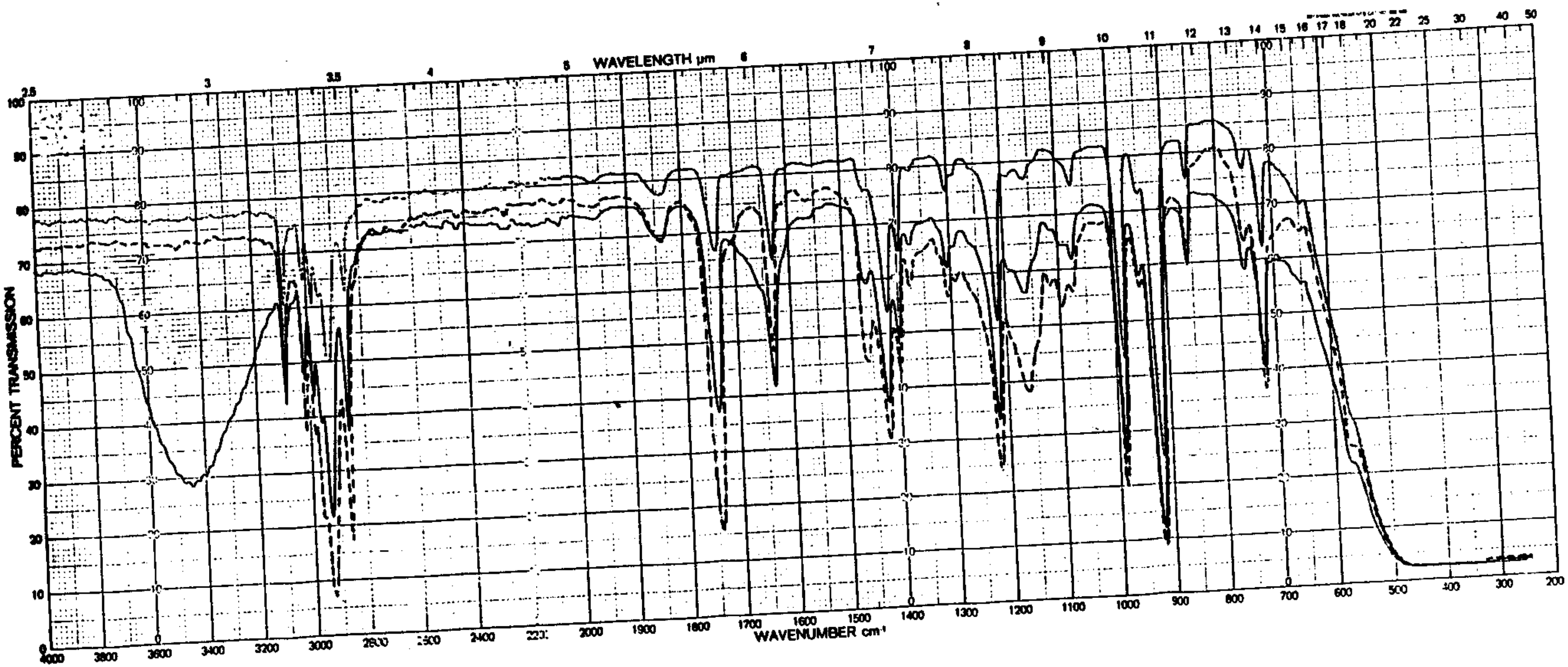


Fig. (33): I R spectra of garlic volatile oil adulterated with maize oil.

(........) : Garlic volatile oil + 10% maize oil.
(———) : ,, ,, ,, + 20% ,, ,,
(– – – –) : ,, ,, ,, + 30% ,, ,,

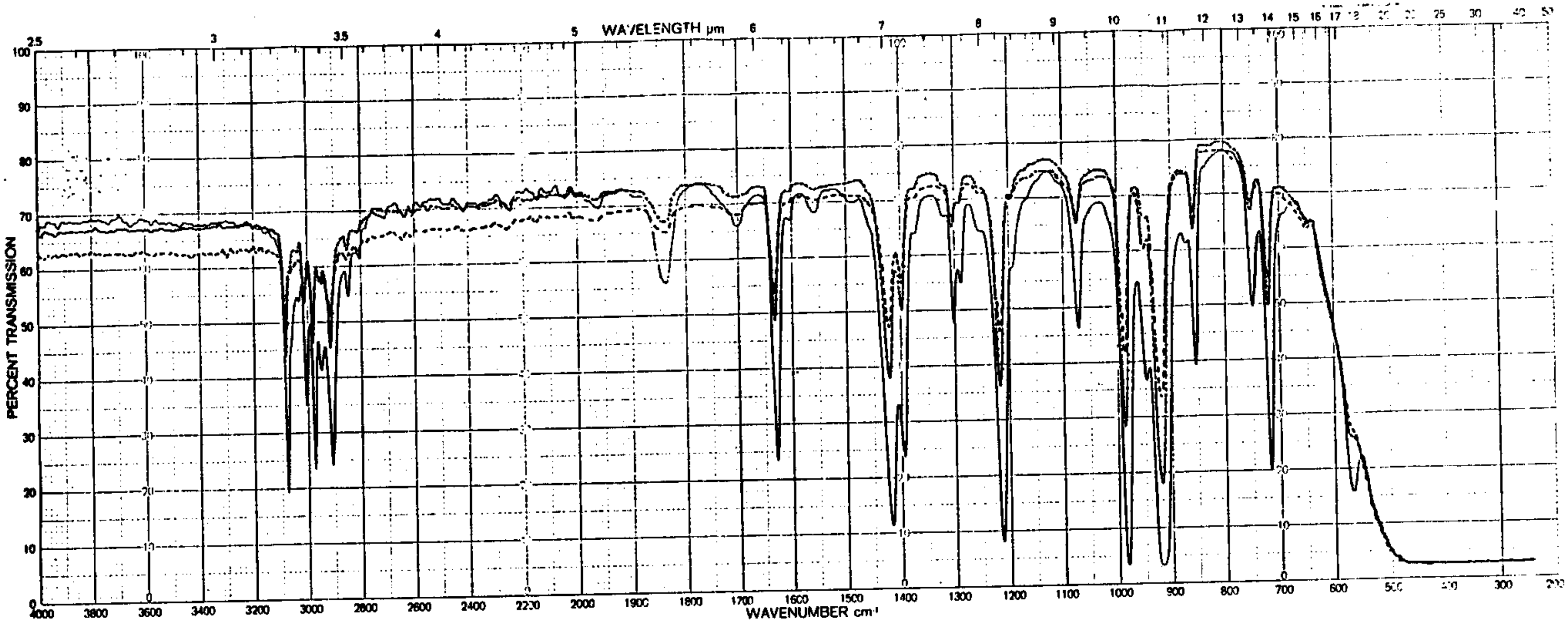


Fig. (34): I R spectra of garlic volatile oil adulterated with ethyl alcohol.

(———) : Garlic volatile oil + 10% Ethyl alcohol.
(........) : ,, ,, ,, + 20% ,, ,,
(— — —) : ,, ,, ,, + 30% ,, ,,

IV-2.3.5. I R spectra of adulterated garlic volatile oil with paraffin oil

New bands appeared in IR spectra of garlic and paraffin oils mixtures, in comparison with I R spectrum of garlic oil, fig (35) and table (22). The new bands appeared at 700, 1120, 1460 and 1725 Cm^{-1} which refer to alkens C-O in primary alcohols, OH bending in carboxylic acids and C=O stretching in aliphatic esters,respectively.

The previous absorption bands are distinguished to paraffin oil, and therefore it can be used I R technique to detect the adulteration of garlic volatile oil with paraffin oil.

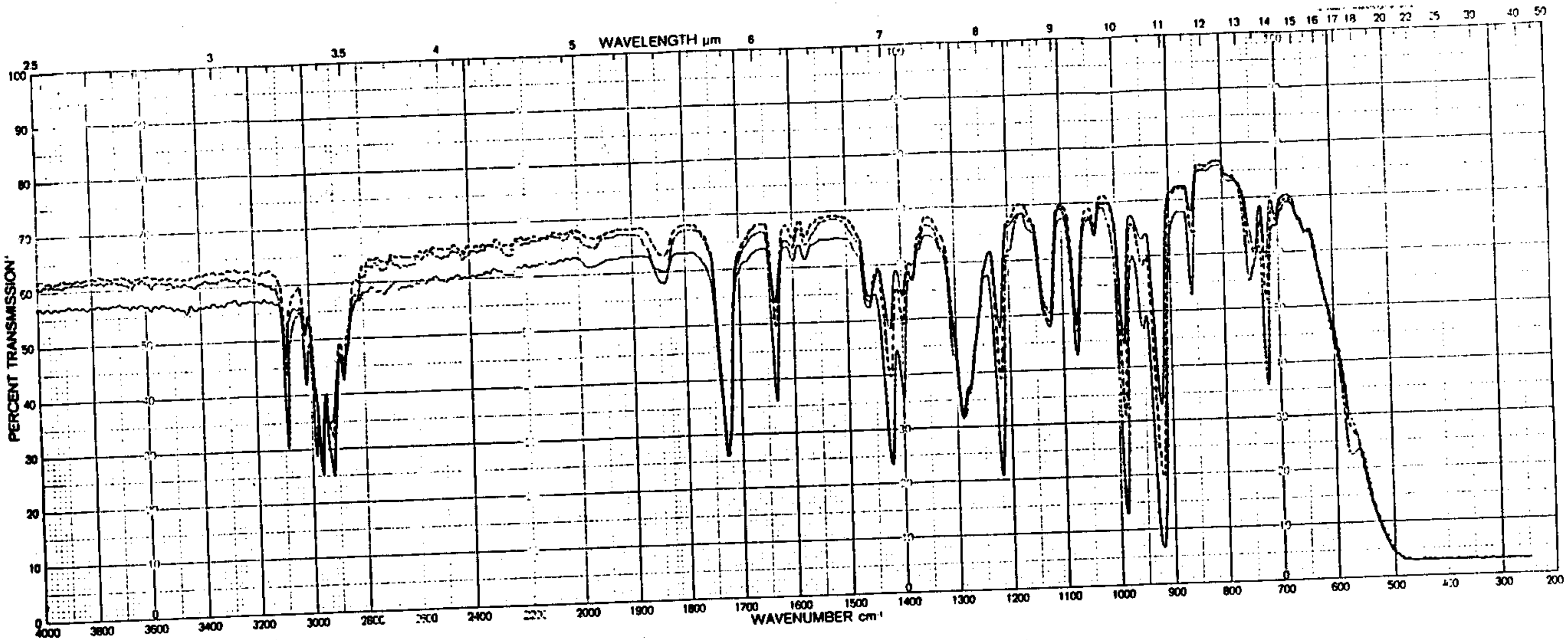


Fig. (35): I.R. spectra of garlic volatile oil adulterated with paraffin oil.

(........) : Garlic volatile oil + 10% Paraffin oil.
(- - - -) : ,, ,, ,, + 20% ,, ,,
(———) : ,, ,, ,, + 30% ,, ,,

IV-2.4. G.L.C. of garlic volatile oil, aged garlic volatile oil and adulterated samples with aged garlic volatile oil:

IV- 2.4.1. G.L.C. of fresh garlic volatile oil:

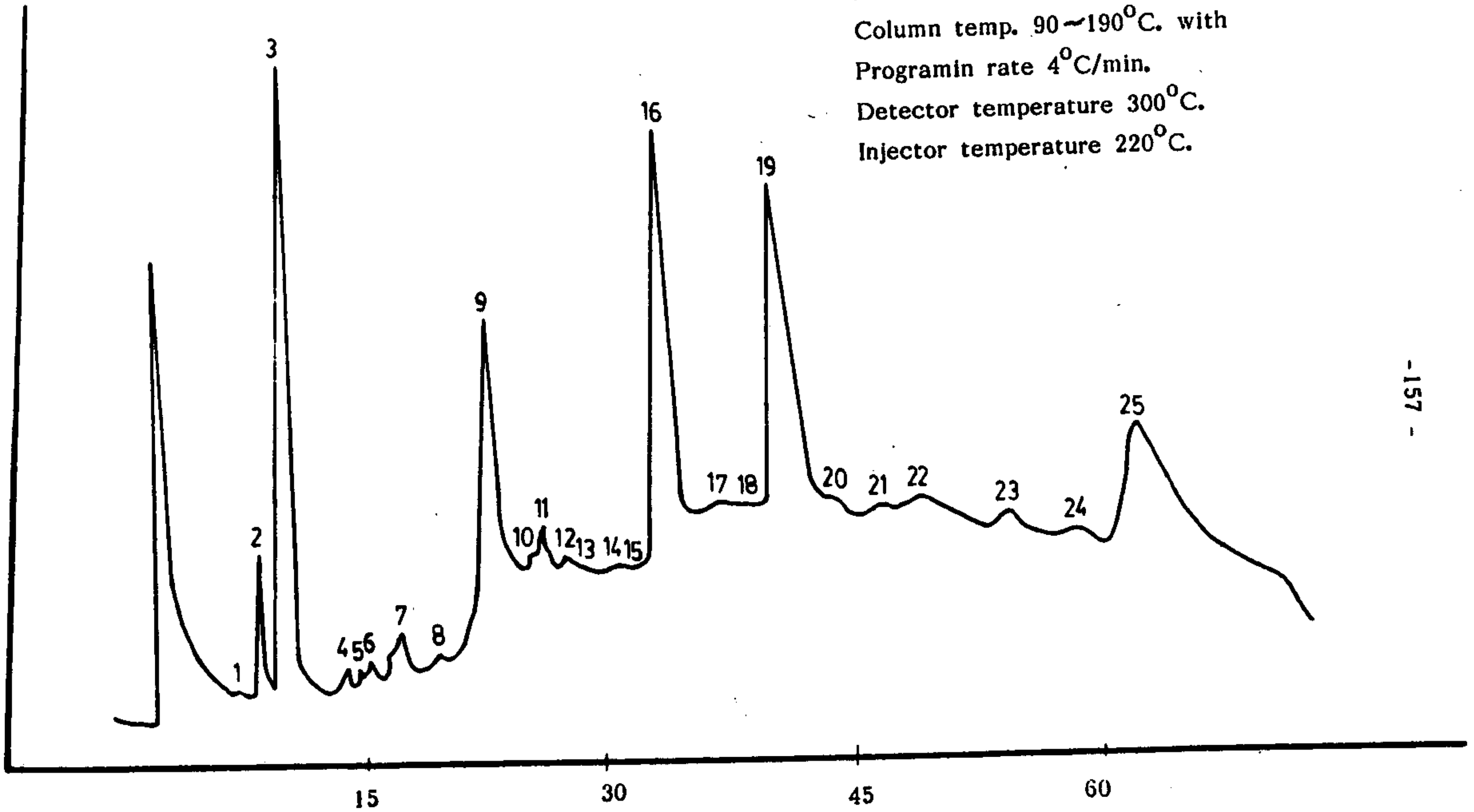
Gas liquid chromatography was accomplished for the separation of the components of garlic volatile oil which are responsible for the aroma and flavour of essential oils.

The garlic volatile oil sample was analyzed using gas liquid chromatography under the conditions previously mentioned in the methods section. The gas chromatogram of garlic volatile oil is shown in Fig (36). The identified compounds of this oil are listed in table (23). Results in this table indicated that the total peaks numbers of the fresh garlic volatile oil were 25. Thirteen volatile compounds were identified, while, twelve compounds were still unknown with concentrations ranged from 0.15 to 15.07%.

The identified flavour components of garlic volatile oil could be classified into five categories namely; monosulphides, disulphides, trisulphides, thiophene compounds and thiosulphonate compounds.

The monosulphide compound namely; diallyl sulphide represented the fourth peak of the chromatogram of the garlic volatile oil and its percentage was 0.37%.

The identified disulphide compounds in fresh garlic volatile oil were; methyl allyl disulphide and diallyl disulphide, their



Speed chart 1 Cm/min.
Nitrogen flow rate 30 ml/min.
Hydrogen flow rate 30 ml/min.
Air flow rate 300 ml/min.
Column temp. 90~190°C. with
Programin rate 4°C/min.
Detector temperature 300°C.
Injector temperature 220°C.

Fig. (36): G.L.C. of fresh garlic volatile oil.

Table (23)

G.L.C. of fresh garlic volatile oil

Peak No.	Percentage	Name of component
1	0.13	Methyl propyl trisulphide
2	3.03	Dipropyl trisulphide
3	18.61	2-propenyl-2-propane thiosulphonate
4	0.37	Diallyl sulphide
5	0.13	Methyl allyl disulphide
6	0.34	Diallyl disulphide
7	0.15	Unknown
8	1.18	” ”
9	0.34	”
10	12.18	Diallyl trisulphide
11	0.13	Unknown
12	1.06	”
13	0.30	Thiophene
14	0.17	Unknown
15	0.07	Methyl thiophene
16	19.10	Dimethyl thiophene
17	0.20	Trimethyl thiophene
18	0.13	Unknown
19	15.07	”
20	0.30	”
21	0.17	Allyl thiophene
22	1.38	Unknown
23	1.01	”
24	0.40	”
25	24.05	Diallyl thiophene

percentages were 0.13 and 0.03%, respectively. The total percentages of these compounds was 0.16%.

Whereas, the identified trisulphides were; methyl propyl trisulphide, dipropyl trisulphide and diallyl trisulphide, their amounts were 0.13, 3.03 and 12.18%, respectively, and hence, the total percentages of them was 15.34%.

On the other hand, the identified thiophene compounds namely; thiophene, methyl thiophene, dimethyl thiophene, trimethyl thiophene, allyl thiophene and diallyl thiophene and their total percentage was 43.89%

The amount of identified thiosulphonate compound, 2-propenyl-2-propane thiosulphonate was 18.61%.

IV-2.4.2. G.L.C. of aged garlic volatile oil and adulterated samples with aged garlic volatile oil:

G.L.C. analysis was used to separate the volatile components of adulterated garlic volatile oil using aged garlic volatile oil with ratios 10 and 20% by volume.

The gas chromatogram of fresh garlic volatile oil was illustrated in Fig. (36). Whereas, the identified compounds of these oils are listed in table (24) and illustrated in Figs (37, 38 and 39).

Table (24)

G.L.C. for fresh garlic volatile oil and adulterated garlic volatile oil with aged garlic volatile oil

Peak No	Fresh garlic volatile oil	Aged garlic volatile oil	Fresh garlic oil + 10% aged garlic oil	Fresh garlic oil + 20% aged garlic oil	Name of component
1	0.13	0.08	0.10	0.08	Methyl propyl trisulphide
2	3.03	0.08	0.08	0.10	Dipropyl trisulphide
3	18.61	15.75	18.27	17.99	2-propenyl-2-propane thiosulphonate
4	0.37	0.55	0.45	0.55	Diallyl sulphide
5	0.13	0.37	0.31	0.35	Methyl allyl disulphide
6	0.34	0.03	0.16	0.10	Diallyl disulphide
7	0.15	0.03	0.15	0.02	Unknown
8	1.18	0.31	0.42	0.40	"
9	0.34	0.24	0.30	0.29	"
10	12.18	6.43	9.87	7.36	Diallyl trisulphide
11	0.13	0.08	0.12	0.10	Unknown
12	1.06	0.73	1.02	1.00	"
13	0.30	0.31	0.30	0.30	Thiophene
14	0.17	0.02	0.12	0.08	Unknown
15	0.07	0.21	0.15	0.15	Methyl thiophene
16	19.10	58.10	30.61	36.60	Dimethyl thiophene
17	0.20	0.18	0.16	0.19	Trimethyl thiophene
18	0.13	0.18	0.18	0.18	Unknown
19	15.07	4.41	11.54	9.33	"
20	0.30	0.02	0.16	0.15	"
21	0.17	0.42	0.30	0.40	Allyl thiophene
22	1.38	0.31	0.63	0.30	Unknown
23	1.01	0.02	0.20	0.18	"
24	0.40	0.71	0.40	0.61	"
25	24.05	10.45	24.00	23.19	Diallyl thiophene

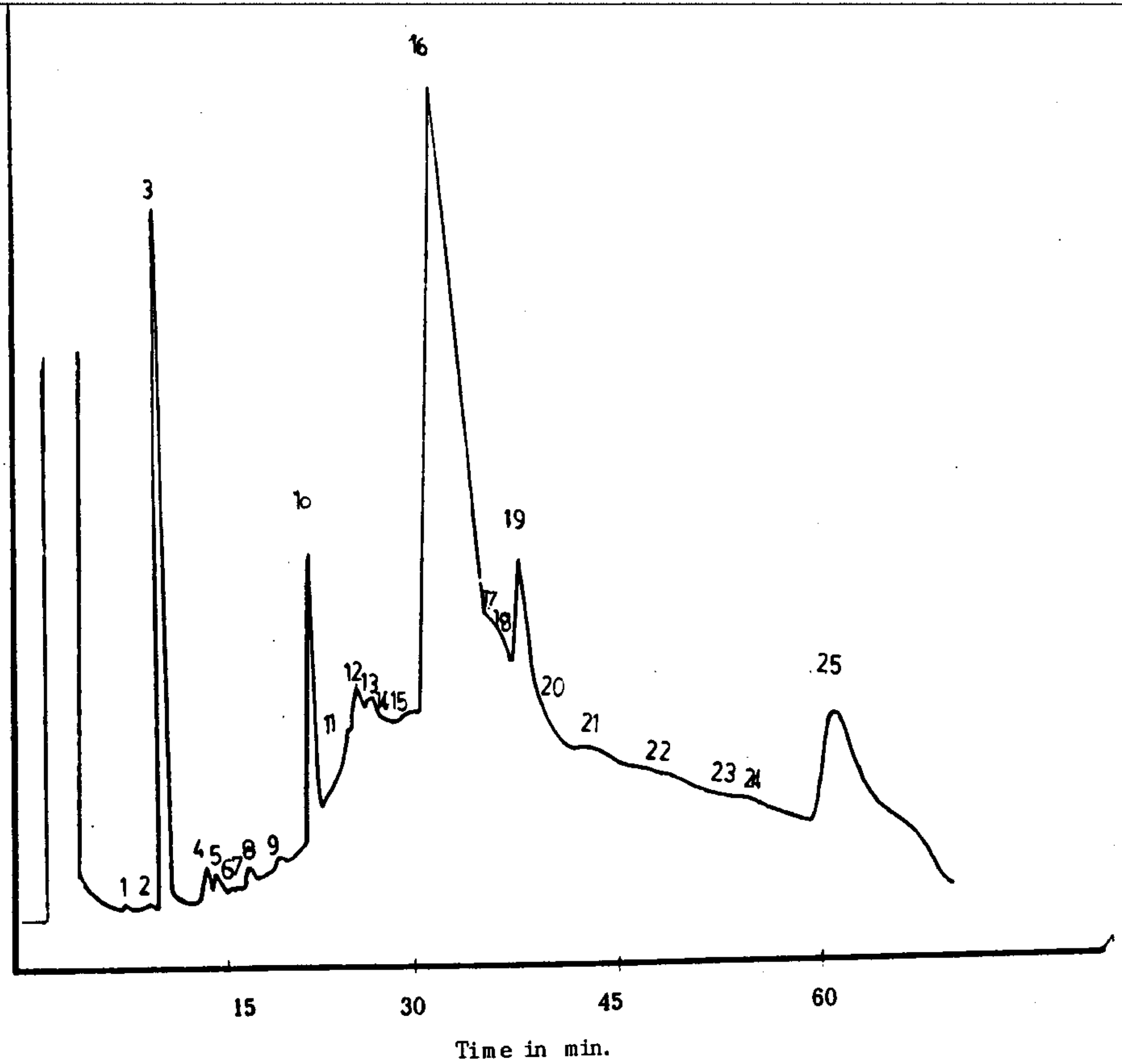


Fig. (37): G.L.C. of aged garlic volatile oil.

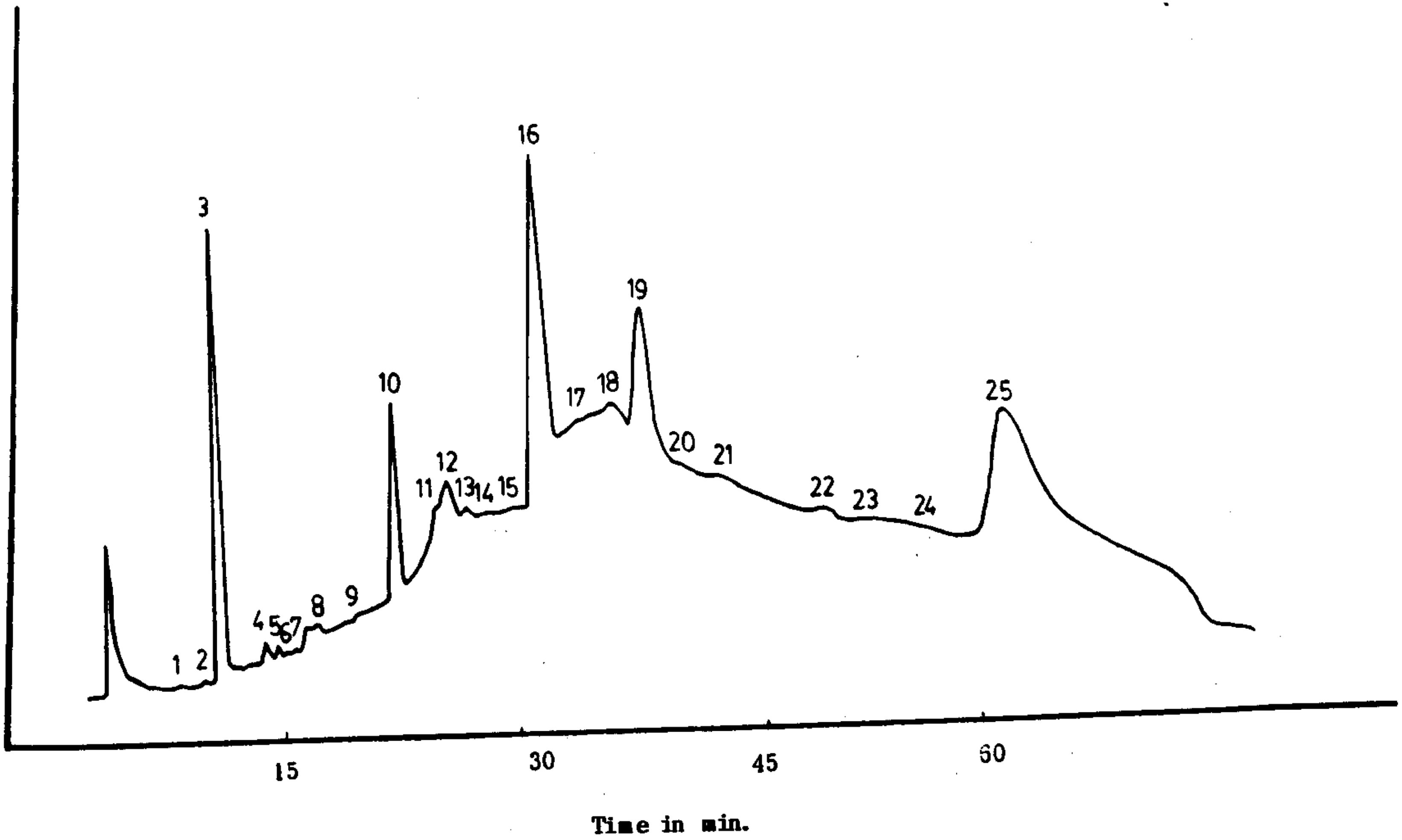


Fig. (38): G.L.C. of garlic volatile oil adulterated with 10% aged garlic volatile oil.

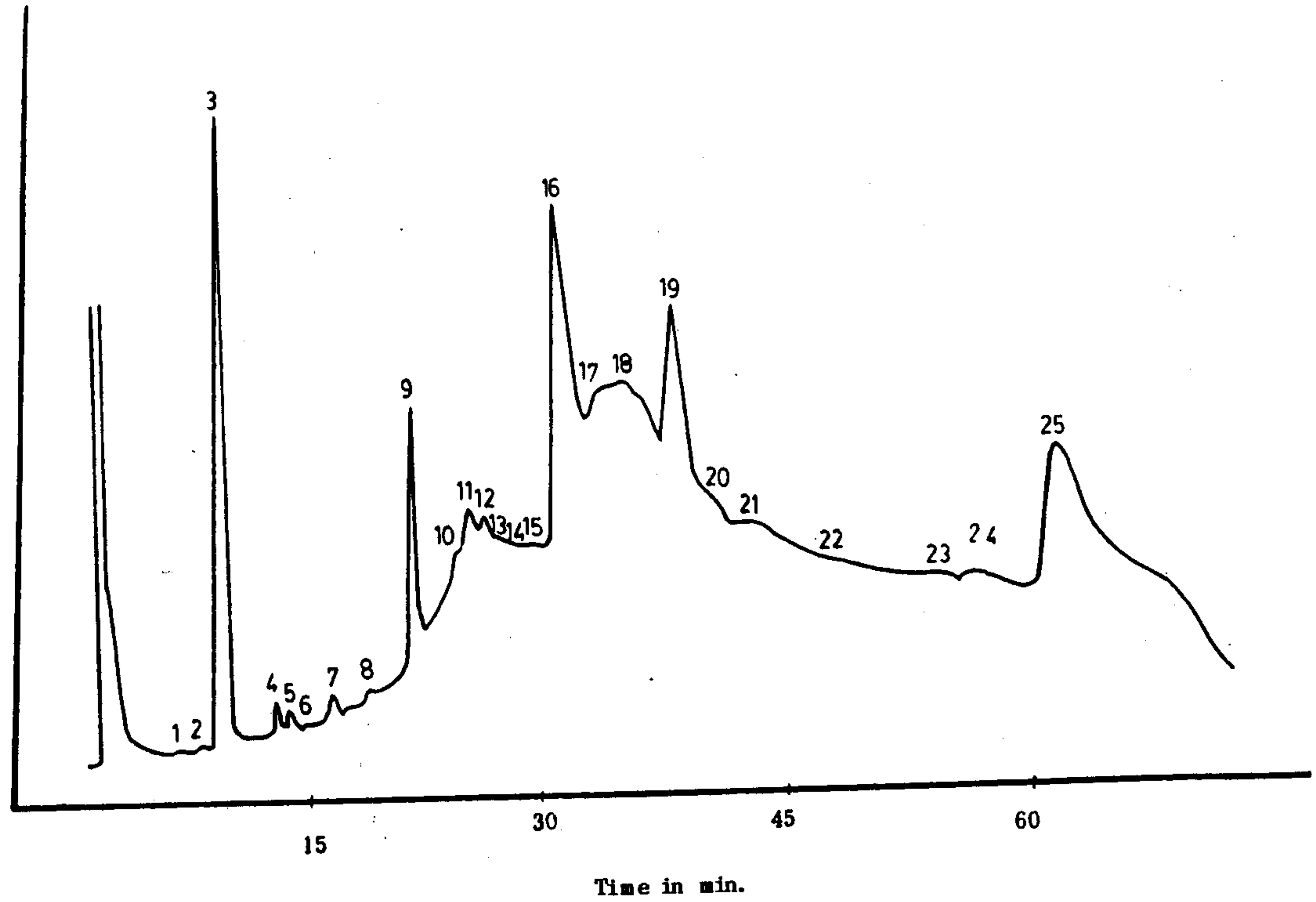


Fig. (39): G.L.C. of garlic volatile oil adulterated with 20% aged garlic volatile oil.

Results indicate that the total peaks for each sample of the fresh garlic volatile oil, aged garlic volatile oil and adulterated samples with 10 and 20% aged garlic volatile oil were 25, from which 13 peaks were identified, while the others were unknown with concentrations ranged from 0.02 to 4.41, 0.12 to 11.54 and 0.02 to 9.33% for aged garlic volatile oil and garlic volatile oil adulterated with 10 and 20% aged garlic volatile oil, respectively.

The identified monosulphide compound was diallyl sulphide and its percentage was 0.37, 0.55, 0.45 and 0.55 for fresh garlic volatile oil, aged garlic volatile oil and adulterated garlic volatile oil with 10 and 20%, respectively. These percentages indicated that monosulphide compound increased upon storage. The adulterated samples contained intermediate amounts of monosulphide more than that of fresh and less than that of aged garlic volatile oil.

The identified disulphide compounds in all samples of garlic volatile oil were 0.47, 0.40, 0.47 and 0.45% for fresh garlic volatile oil, aged garlic volatile oil and adulterated garlic volatile oil with 10 and 20% aged garlic volatile oil, respectively. These results reveal that the identified disulphide compounds slightly decreased as affected by storage. Whereas, the adulterated samples contained intermediate amounts of disulphide compounds between fresh and aged garlic volatile oil.

The identified trisulphide compounds were 15.34, 6.59, 10.05 and 7.54% for fresh garlic volatile oil, aged garlic volatile oil and adulterated garlic volatile oil with 10 and 20% aged garlic volatile oil, respectively. These percentages indicated that the trisulphide compounds decreased in the garlic volatile oil influenced by storage. Whereas, the adulterated samples of garlic volatile oil obviously contained intermediate amounts of trisulphide compounds.

The decreasing of the trisulphide compounds was clearly accompanished with an increasing in both di and monosulphide compounds. This may be attributed to the cleavage of sulphur bond during storage.

On the other hand, the total percentages of identified thiophene compounds were; 43.89, 69.67, 55.52 and 60.83% for fresh garlic volatile oil, aged garlic volatile oil and adulterated garlic volatile oil with 10 and 20% aged garlic volatile oil, respectively. The percentages of thiophene compounds indicated that the thiophene compounds obviously increased upon storage. The adulterated samples contained intermediate amount of thiophene compounds between fresh and aged garlic volatile oil.

The percentages of identified thiosulphonate compound 2-propenyl-2-propane thiosulphonate were; 18.61, 15.75, 18.27 and 17.99 for fresh garlic volatile oil, aged garlic volatile oil and adulterated garlic volatile oil with 10 and 20% aged garlic volatile oil. From these percentages it could be observed that the thiosulphonate compounds decreased as influenced by storage of garlic volatile oil. The amounts of thiosulphonate compound in the adulterated sample decreased and it is due to the addition of aged garlic volatile oil to fresh garlic volatile oil.

IV-2.5. Determination of glycerol content in garlic volatile oil adulterated with maize oil:

Results presented in table (25) represent the glycerol determination by periodic acid oxidation in the adulterated garlic volatile oil with maize oil. As it was expected glycerol could not be detected in the pure sample of garlic volatile oil. Whereas, glycerol increased proportionally with the increasing of maize oil used as adulterant. The glycerol measurements increased to reach 0.9583, 1.9501 and 2.8601 gm/100 gm of adulterated garlic volatile oil with maize oil at 10, 20 and 30%, respectively.

Accordingly, the glycerol determination by periodic acid could be considered as a reliable quantitative test for the detection of maize oil used as adulterant for garlic volatile oil. These results are in line with results obtained by El-Masry (1978 and 1985), who detected the adulteration of geranium oil, peppermint oil, marjoram oil, petitgrain bigrade oil and fennel oil with cotton seed oil by determining the glycerol content.

Table (25)

Glycerol content of garlic volatile oil adulterated with mize oil.

Samples	Glycerol content theoretical gm/100 gm	Glycerol content gm/IOO gm of sample	Recovery
Pure garlic volatile oil	0.0	0.0	--
Pure maize oil	9.7592	9.7592	100 %
Garlic volatile oil + 10% maize oil	0.9759	0.9583	98.20 %
Garlic volatile oil + 20% maize oil	1.9518	1.9501	99.91 %
Garlic volatile oil + 30% maize oil	2.9277	2.8601	97.69 %

IV-2.6. Determination of ethyl alcohol in adulterated garlic volatile oil:

IV-2.6.1. Qualitative determination of ethanol (Iodoform test):

The iodoform test is possitive with any compound containing the group (CH_3-CO-), Subsequently the iodofom test is expected to be possitive with ethanol and forming flat yellow crystals of iodoform.

Results obtained concerning the iodoform test revealed that no crystals were formed for the pure garlic volatile oil, garlic volatile oil adulterated with aged garlic volatile oil, garlic volatile oil adulterated with maize oil and garlic volatile oil samples adulterated with paraffin oil. Whereas, flat yellow crystals immediately observed at room temperature in samples of garlic volatile oil adulterated with 10, 20 and 30% ethyl alcohol. Accordingly, the iodoform test can be considered a qualitative method to detect adulteration of essential oil with ethyl alcohol, especially when applied to the water layer resulted after washing the adulterated samples with water and saturated sodium chloride solution.

IV-2.6.2. Quantitative determination of ethanol:

Results in table (26) represent the ethanol content in the adulterated garlic volatile oil samples with ethyl alcohol at different concentrations 10, 20 and 30% by two different methods. The data obtained revealed that the volumetric method was less sensetive when compared with the colorimetric method, since the recovery

Table (26)

Ethanol content in garlic volatile oil adulterated with ethyl alcohol.

Samples	Weight of added ethanol gm	Ethyl content gm/100 gm. sample			
		Volumetrically	Recovery	Colorimetrically	Recovery
Pure garlic volatile oil	0.0	0.0	--	0.0	--
Garlic volatile oil + 10% ethyl alcohol	7.618	7.389	96.99 %	7.5046	98.51 %
Garlic volatile oil + 20% ethyl alcohol	15.236	15.095	99.07 %	15.1422	99.38 %
Garlic volatile oil + 30% ethyl alcohol	22.850	22.017	96.35 %	22.686	99.28 %

percent on average for the volumetric method was 97.47%, whereas, it was 99.06% for the colorimetric method. Ethyl alcohol content increased proportionally upon increasing this percent in the adulterated samples. Where, the ethanol percentages were 7.389 and 7.5046 for garlic volatile oil plus 10% ethanol, 15.095 and 15.1422 for garlic volatile oil plus 20% ethanol and 22.017 and 22.686 for garlic volatile oil plus 30% ethanol determined by volumetric and colorimetric methods, respectively.

Conclusively both methods could be considered as reliable methods for determining ethanol in adulterated garlic volatile oil samples.

IV-2.7. Determination of paraffin oil content in garlic volatile oil adulterated with paraffin oil:

The quantitative determination of paraffin oil in garlic volatile oil and adulterated samples with paraffin oil were carried out using the TLC technique mentioned by Dhingar and Sharma (1978).

The following solvent systems were tried to obtain a good separation of the adulterated samples of garlic volatile oil with paraffin oil.

(1) Benzene (79-80.5°C) : ether (34-36°C): petroleum ether (60-30°C) (6:1:1, v/v).

(2) Benzene (79-80.5°C): ethyl acetate (40-60°C): ether (34-36°C) (9:1:1, v/v).

(3) Petroleum ether (60-80°C): ether (34-36°C): glacial acetic acid (90:10:1, v/v).

The last solvent system proved to be the most suitable, since, it gave a good separation without any over lapping.

It is important to notice that all separated compounds in garlic volatile oil appeared when the iodine vapor used to visualize the spots, whereas, the paraffin oil spots only appeared when the reagent 2,7-dichloroflouroscien 0.2% in 95% ethanol was used to visualize the spots in garlic volatile oil adulterated with paraffin oil. For the determination of paraffin oil quantitatively the spots were measured colorimetrically at 535 nm.

Results obtained concerning the determination of paraffin oil used as an adulterant in garlic volatile oil at the concentration 10, 20 and 30% are shown in table (27) and Fig. (40).

Results indicate that the paraffin oil content increased proportionally with increasing the percent of paraffin oil in garlic volatile oil at 10, 20 and 30%.

On the other hand, the recovery percent of paraffin oil mixed with garlic volatile oil was 81.06 on average which emphasize the possibility of using such method to detect the presence of paraffin oil in garlic volatile oil.

Table (27)

Paraffin oil content in garlic volatile oil adulterated with paraffin oil.

Samples	Weight of added paraffin oil gm.	Paraffin oil content gm/100 gm sample	Recovery
Pure garlic volatile oil	0.0	0.0	–
Garlic volatile oil + 10% paraffin oil	9.698	7.71	79.50 %
Garlic volatile oil + 20% paraffin oil	19.396	15.67	80.79 %
Garlic volatile oil + 30% paraffin oil	29.094	24.12	82.90 %

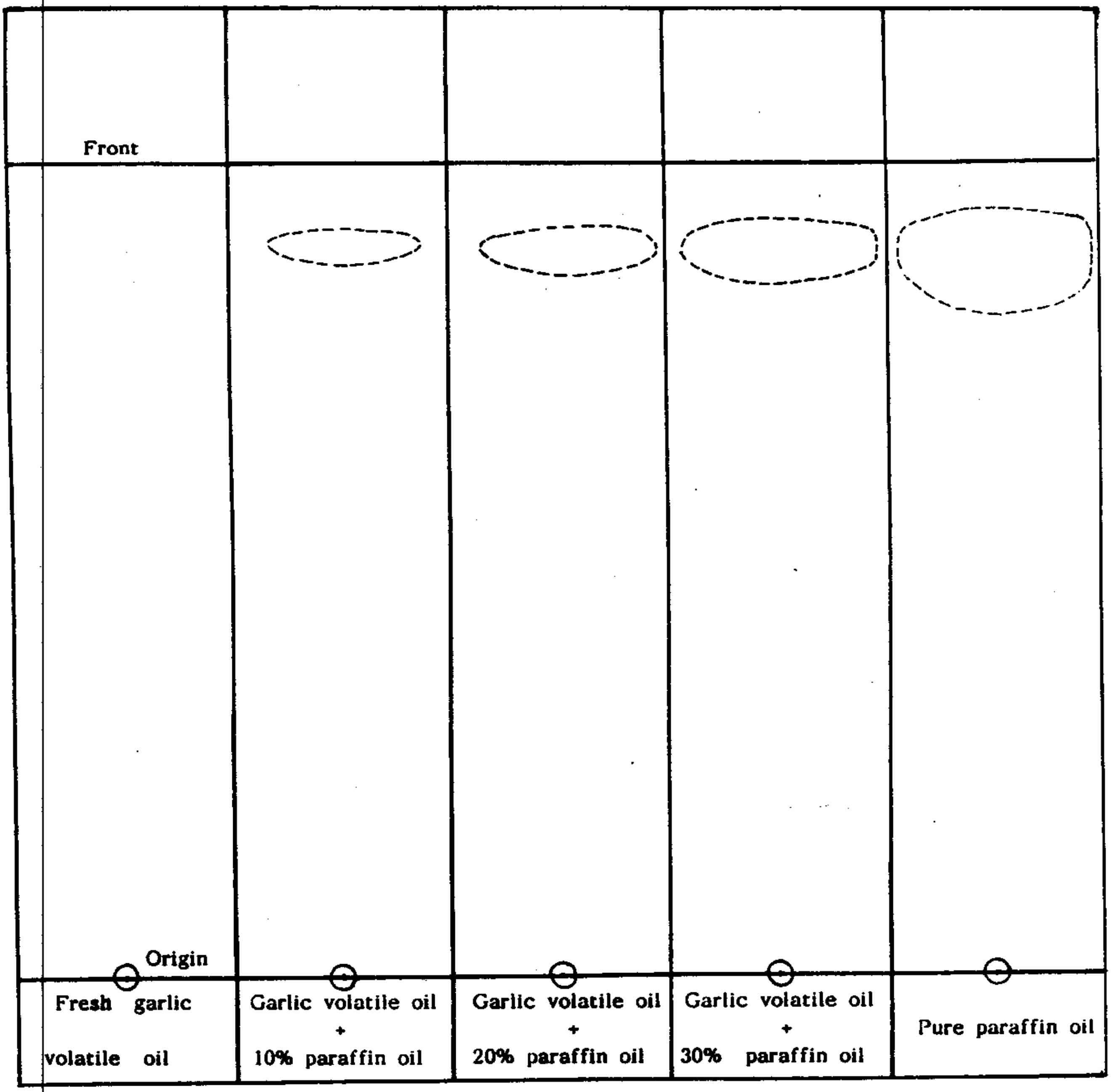


Fig. (40): T.L.C. of garlic volatile oil adulterated with paraffin oil.