RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

Studies on evaluation and utilization of by-products of some citrus fruits; Baladi orange, bitter orange, grapefruit and mandarin, processed commercially in Egypt are presented in this section.

An outline of different operations and products obtained from by-products of the investigated citrus fruits are presented in a skematic presentation shown in Fig 2.

Data obtained throughout the course of the present investigation are presented and discussed under five sub-headings, as follows:-

Part I : By-products obtained from different citrus fruits.
Part II : Characterization of citrus peel oils.
Part III: Characterization of citrus pectin pomace.
Part IV.: Characterization of citrus seed oils.
Part V : Characterization of citrus seed meal.

Part I : By-products obtained from different citrus fruits:

Estimation of edible and residual part from fruits of Baladi orange, bitter orange, grapefruit and mandarin were undertaken in the laboratory on many batchs of these fruit, as much as ten or even more, within the mid of their processing season in Egypt.

4.1. Amount of juice and components of residue obtained from different citrus fruits:

Estimation of amount of extracted juice and by-products of pressing the same weight of fresh citrus fruits indicated that they differed among themselves in amount of juice extracted, and also in amount of total residues (peel, pulp and seeds) as well as in amount of each component of residue (Table 8). Bitter orange had the highest amount of total residue and lowest amount of juice extracted (65 and 35% respectively). Total residue from grapefruit resembled that obtained from Baladi orange (59%). However, mandarin exhibited the lowest amount of wastes and also the highest amount of juice.

Peels constituted the major part of citrus residues. The highest amount of peels was found in bitter orange (41%) followed by Baladi orange and mandarin fruits (36%), grapefruit had the lowest amount of peels (31.5%).

The different citrus fruits varied in their content in flavedo layer of peels. The highest amount of flavedo was obtained from bitter orange (12%) followed by Baladi orange (10%) and then grapefruit (8%). The lowest amount was found in mandarin (7%). Differences in flavedo content of the different citrus fruits could be attributed to the observed differences in amount of peels obtained from each kind. For example, the lower amount of flavedo in grapefruit is due to its smaller amount of peel than that in Baladi or bitter orange. Mandarin was a special case, practically, it was difficult to remove flavedo from mandarin because of nature of the fruit and because of the method of removal of flavedo from fresh fruit. Such operation was made on the loose peels of mandarin but not on the whole fruits as was the case with the other citrus fruits investigated.

Residual part of citrus by-products which was left after removing the flavedo layer by grating as well as seeds constituted great proportion amounting 50% in grapefruit, 47% in Baladi orange, 46.2% in bitter orange and only 38.5% in mandarin. Except for mandarin, this part of residue was used for obtaining pectin pomace by the method of extraction applied in the present study. However, albedo of mandarin which represented 28.5% was used for obtaining pectin since amount of pulp was too small (10%) and also it was difficult to obtain pulp separately from seeds, especially under the condition of commercial processing of mandarin.

Albedo constituted the second great fraction in by-products of Baladi orange, bitter orange and mandarin where they did not differ greatly in its amount (26.5, 28.7 and 28.5%, respectively). The grapefruit had the lower albedo layer. In contrast to other citrus investigated, grapefruit had lower weight of albedo than pulp. Grapefruit, exhibited the highest amount of pulp or membrane (27%). Pulp in Baladi orange and bitter orange represented 20.5 and 17.5% of the residue, respectively. The smallest amount of pulp was found in mandarin (10%).

Seed constituted the least fraction in citrus. The highest amount of seeds were found in residues of bitter orange (6.8%) followed by mandarin (4.5%) and the Baladi orange (2%), while the lowest content of seeds were obtained from grapefruit. In the present investigation, this part of residue was used for obtaining citrus seed oil and citrus seed meals.

It should be mentioned origin, size, skin-thickness, growing conditions of different citrus fruits influence the amount of edible part as well as amount of residues. It was reported that large and/or thick-skinned citrus fruits contain high proportion of waste (Apfelsinen and Griepenburg, 1975).

Part II : Citrus peel oils:

The present study was conducted to compare yield, physicochemical properties and content in volatile components of peel oils obtained from four different citrus fruits, namely Baladi orange, bitter orange, mandarin, and grapefruit. Moreover, effects of the method of extracting these citrus peel oils on their properties and composition were investigated, where essential oils were recovered from fresh fruits by cold-pressing and water and steam distillation techniques.

4.2. Essential oil content in different citrus fruits:

Amount of essential oils in the fresh weight of fruits varied with the different citrus fruit (Table 9). The highest amount of essential oil was found in peels of bitter orange (0.68% of the fresh weight of fruits), Baladi orange come in the second order (0.58%). Grapefruit had lowest amount in essential oil, being 0.36 %.

In case of grapefruit, it should be noted that its oil glands are known to be located deeply in peels and,in addition, its peel is known to have a very thick layer of a sponge-like albedo which has a tendency to absorb the oil as soon as it is expressed from flavedo, and to retain it deppite the application of very high temperature (Guenther, 1960). It has been stated that yield of oil extracted from grapefruit is always low compared with orange (Guenther, 1960).

Table 9 - The yield of essential oils produced from various species of citrus fruits.*

Citrus species	Percent of flavedo in whole fresh fruit	Percent of essential oil in flavedo	Percent of essential oil in fresh weight of fruits
C. reticulata Blanco (Mandarin)	7	6.4	0.45
C. sinensis L. (Baladi orange)	10	5.8	0.58
C. aurantium L. (Bitter orange)	12	5.6	0.68
C. paradisi (Grapefruit)	8.0	4.5	0.36

* Average of six determinations.

Although mandarin had a lower amount of essential oil in fresh weight of fruit, it showed the highest amount in these oils in flavedo layer obtained from fruits by grating compared with the other citrus fruits. Similarly, bitter orange contained lower percent of essential oil in its flavedo layer than Baladi orange. Moreover, amount of essential oil was not found correlated with the amount of flavedo layer of peels.

Results of the present study on amount of essential oil are consistent with those reported by Ghoniem (1980) for Egyptian cultivars of bitter orange, Baladi orange and mandarin. Moreover the found values for citrus peel oil content is higher than that reported by Hafez (1964) and lower than that reported by Salib et al. (1978), Ashour and El-Kebeer (1980), and Ashour and El-Saadany (1981). The cultivated Egyptian citrus varieties seem to contain higher amount of essential oils compared to other varieties grown in different parts of the world (Manelski, 1932; Patel, 1937). If the average content in citrus peel oils is considered to be about 0.5% one would except that one ton of citrus fruits processed in any citrus processing plant would produce 5 kg of essential depending on variety of citrus and other conditions.

4.3. Physical and chemical properties of different citrus peel oils:

The determination of physical and chemical properties of a given essential oil is of great importance in characterization of that oil, in determining effect of its extraction or processing methods, and in detection of adulteration with other oil. Determination of or physical and chemical properties of citrus peel oil obtained by water and steam distillation and by cold-pressing procedures included specific gravity, refractive index, optical rotation, solubility in alcohol, odour, colour, acid number, ester content and aldehyde content (Tables 10 and 11).

4.3.1. Physical properties:

4.3.1.1. Odor and color:

Odor and color characteristics of the investigated citrus peel oils were determined by five trained panelists from the Aromatic Plants Dept. of the Agriculture Research Institute, Cairo, Egypt. Baladi orange oil obtained by water and steam distillation was colourless while that obtained by cold-pressing was reddish yellow. Mandarin oil was slight yellow upon extraction by distillation while that obtained by pressing was reddish yellow. Similarly grapefruit oil obtained by cold-pressing was more yellow than that obtained by hydro-distillation. It could be noticed that method of extraction seemed to influence the color of citrus peel oil investigated.

Generally, odor of cold-pressed citrus oils was more pleasant than those oils obtained by hydrodistillation. However, odor of each kind of the various citrus peel oils tested was actually a characteristic of the fruit from which it was extracted.

4.3.1.2. Specific gravity (sp. gr.):

Examination of tables 10 and 11 reveals that peel oils extracted by both methods from all the citrus fruits, had values for sp. gr. lower than 0.9. This finding could be taken as an indication of the presence of higher percentage of terpene hydrocarbons in these essential oils. It is well known, that, oxygenated compounds have the highest sp. gr. value over all the other constituents found in an essential oil (Guenther, 1961). Moreover, variation in ratio of terpene hydrocarbon to oxygenated constituents certainly influences the value sp. gr. of the essential oil. If the sum of values of acids, esters and aldehydes could be taken as an arbitrary estimate of oxygenated compounds, ane would expect a positive correlation between amount of oxygenated compounds; and therefore, a- negative correlation between terpene hydrocarbons, and the value for specific gravity. The relative higher sp. gr. value for mandarin and grapefruit peel oils than Baladi and bitter orange oil could be attributed to the higher amount of oxygenated compounds in mandarin and grapefruit over present in Baladi and bitter orange peel oils.

4.3.1.3. Refractive index:

The tested citrus peel oils exhibited, in general, high refractive indexes. Volatile oils are characterized by high refractive index (Guenther, 1961). However, there was no great differences among peel oils from the different citrus types. Values obtained ranged from 1.4729 to 1.4740 for those extracted by water and steam distillation and from 1.4738 to 1.4762 for cold-pressed peel oils. It is evident that refractive indexes of oils extracted by cold-pressing is slightly higher than those obtained by the hydrodistillation method. Such finding is similar to those mentioned by Guenther (1961) and Ashour and El-Saadany (1981) for steam distilled and cold-pressed oils. The ranges obtained coincided with those stated by Hafez (1964), Salib et al. (1978), Ghoneim (1980) and Ali (1983) for different citrus peel oil extracted by different methods. It should be noted that Warigar (1954) reported that refractive index of citrus oil increases with storage.

4.3.1.4. Optical rotation:

Among the different citrus oils, mandarin peel oil prepared by both extraction methods had lower optical rotation (+70°) than the others. However, oils of Baladi orange, bitter orange and grapefruit peel oil obtained by water and steam distillation had the same value of optical rotation. On the other hand, all cold-pressed citrus oils exhibited slightly lower values than all those obtained with hydrodistillation method. Similarly, Ashour and El-Saadany (1981) found that cold-pressed had lower optical rotation than steam-distilled peel oil of Baladi orange.

The higher specific rotation in orange peel oil could be related to the higher amount of D-limonene in these oils. Pure d-limonene was reported to have specific rotation value of +116 (Guenther, 1961). The optical rotation of an essential oil is a function of its optically active constituents since oil consists of a mixture of organic compounds which differ in their assymmetry which could be influenced by method of extraction. Therefore, the observed difference in optical rotation between citrus oils extracted by the two methods could partly be explained by the difference in their content of d-limonene and other constituents.

The obtained data for optical rotation of citrus peel oils are in agreement with those stated by Guenther (1961), Hafez (1964), Shabana (1969), and Ashour and El-Kebeer (1980). However, they are different from those mentioned by Ali (1983) who reported much lower values for cold-pressed orange peel oil (+45).

4.3.1.5. Solubility in alcohol:

Since solubility in alcohol is dependent on temperature (Guenther, 1961) the determination was made at a constant temperature of 20°C. Citrus oils extracted by water and steam distillation were found soluble in 4.5 to 5 volumes and more up to 10 volumes of 90% ethanol. Moreover, they were soluble in 0.5 to 1 volumes and more, up to 10 volumes, of 95% ethanol (Table 10). In contrast, cold-pressed citrus oil were found insoluble in 90% ethanol. Grapefruit peel oil was also insoluble in 95% ethanol; but Baladi orange and mandarin oils were only soluble with hazy turbidity The present observations could be explained on the basis of the high content of terpene hydrocarbons in these citrus oils, since oils rich in oxygenated constituents are known to be readily soluble in dilute alcohol than those rich in terpene hydrocarbons (Guenther, 1961).

Data on solubility are in general agreement with other studies on some Egyptian citrus peel oil especially those of Hafez (1964) and Shabana (1969), but differed from those of Ashour and El-Saadany (1981) who reported comparable solubilities in 70%, 80% and 90% alcohol for both cold-pressed and steam distilled Baladi orange peel oils.

4.3.2. Chemical properties:

4.3.2.1. Acid number:

Although acid number does not help in characterization of essential oil, it serves as an estimate of the small amounts of free acids present and reflects the deteriorative effects of extraction method, processing and storage conditions. Acidity in all the investigated citrus peel oil was low and ranged from 0.15 to 0.42 for oils extracted with water and steam distillation and from 0.8 to 1.3 for those obtained by cold-pressing. Similar acid numbers were reported for steam-distilled citrus oils, which were also lower than the corresponding cold-pressed oils (Ghoneim, 1980). Among the water and steam distilled oils tested, mandarin had the highest while bitter orange showed the lowest acid number. Baladi orange and grapefruit had comparable acid number. In contrast, cold-pressed Baladi orange possesed the highest, while mandarin had the lowest acid number. It seems that acidity of freshly prepared Egyptian citrus peel oils is always low since many investigators reported such observation (Salib et al., 1978; Ghoneim, 1980; El-Samahy et al., 1982; and Ali, 1983). Higher acid number was found for solvent extracted citrus peel oils (Háfez, 1964 ; Shabana, 1969).

4.3.3.2. Ester content:

Examination of table 10 and 11 reveals that percentage of esters, calculated as linalyl acetate ranged from 1.73 to 4.08 in oils extracted by water and steam-distillation and from 4.3 to 8.5 in cold-pressed citrus peel oils under investigation. It is evident that ester content is higher in cold-pressed oils than those extracted by hydrodistillation method. Consistent conclusions were obtained from studies of Ghoneim (1980) and Ashour and El-Saadany (1981) who compared cold-pressed and steam-distilled peel oils of different Egyptian citrus varieties.

Upon recovery by both methods of extraction, peel oils of mandarin showed the highest while that obtained from Baladi orange had the lowest ester content. Moreover, grapefruit and bitter orange peel oils had also higher ester content than Baladi orange despite of the method of extraction applied.

Values obtained for ester content in the present study either are higher than or in general agreement to the ranges found in the literature for different citrus peel oils extracted by different method (Kesterson & McDuff 1948; Guenther, 1961; Hafez, 1964; Ashour and El-Kebeer, 1980; Ghoneim, 1980 ; El-Samahy et al., 1982).

4.3.2.3. Aldehyde content:

Among the many constituents present in citrus peel oils, aldehydes content is considered to be an indication of flavouring potential of every individual oil. As shown in Table (10) Baladi orange peel oil extracted by water and steam distillation had the highest aldehyde (as citral) content compared to the other tested oils which showed a relatively comparable aldehyde content (as decyl aldehyde) having a range between 0.90-0.95%. Cold-pressed mandarin oil (Table 11) had the highest aldehyde content (1.58%) which was slightly higher than that found in mandarin oil extracted by hydrodistillation. The other two cold-pressed oils had similar aldehyde content (1.5%) which was greatly higher than that of the corresponding oil obtained by hydrodistillation method. Ghoneim (1980) reported also that Baladi orange oil had higher aldehyde content than those from other citrus peels. Ashour and El-Saadany (1981) also found that cold-pressed contained more citral than the steam-distilled orange peel oil. The obtained values for aldehyde content for each of the tested citrus oils are within the ranges reported by many investigators (Manelski, 1932; Braverman, 1933; Hafez, 1964; El-Samahy *et al.*, 1982).

The different investigated citrus peel oil showed also variation in upon calculation of the aldehyde: ester ratio, where Baladi orange oil had the highest ratio compared to the other citrus oils, regardless of the method of its extraction.

Baladi orange oil obtained by water and steam distillation method had a ratio of 0.83. However, the other cold-pressed Baladi orange oil had only about half the ratio present in that obtained by hydrodistillation (only 0.35). Bitter orange and grapefruit oil showed similar trend with aldehyde: ester ratio which ranged 0.43-0.48 in oils obtained by hydrodistillation, but was reduced to an average 0.18 in oils obtained by cold-pressing. Mandarin peel oil obtained by hydrodistillation exhibited the lowest ratio among the different citrus oils investigated, being 0.23 which also was even lower in mandarin obtained by cold-pressing (0.18).

The found aldehyde/ester ratios are consistent with those reported by El-Samahy et al. (1982) who mentioned that cold-pressed Baladi orange and bitter orange had ratio of 0.8 and 0.56, respectively.

It should be mentioned, that results of the present investigation indicate that citrus peel oils, in general, have much esters as aldehydes. This result is similar to that observed by Braddock and Kesterson (1976), especially in grapefruit oil. Moreover, the present results indicate also that Baladi orange had lowest content in esters, and the highest content in aldehydes despite of method of its extraction among the citrus peel oils investigated.

4.4. Gas-liquid chromatographic analysis of citrus peel oil:

The application of gas chromatography for characterization and purity determination for several aroma substances or volatile constituents in many essential oils extracted from various plants has been demonstrated. In fact, the use of chromatography and supplementary analytical technique has greatly accelerated interest in the identification of specific flavour producing compounds in these essential oil. Aknowledge of the nature of constituents responsible for odour and flavour of citrus peel oils is quite necessary to provide a base for comparing the different citrus oils, detect any adulteration with oil of inferior quality and eventually to provide a foundation for understanding the chemistry of these compounds.

For the best of our knowledge, scarce information are present in literature concerning properties and volatile components of different citrus peel oils obtained by water and steam distillation. Therefore, comparison of the present data on oils obtained by such method to others will be only limited to those oils obtained by steam distillation method. Moreover, a comparison of the present results with literature reports is a difficult task as various methods or procedures for GLC analysis of citrus peel oils or present in literature. However, the major concern of each of these methods is to detect or identify maximum volatile constituents or to improve their resolution from the essential oil.

The GLC analysis of each oil under investigation was followed according to the enrichment method in which known compounds were added individually to each sample chromatographed and then compared in each instance to the oil sample alone. Gas liquid chromatograms of the volatile constituents separated from each essential oil investigated are presented in separate figures (Figures 3, 4, 5, 6, 7, 8 and 9).

4.4.1. Volatile constituents separated from different citrus peel oils obtained by water and steam distillation:

Gas-liquid chromatographic chromatograms indicated that different numbers of peaks (between 7 and 10 peaks) were detected in the different citrus peel oils investigated under the used conditions of GLC analysis in the present study. About 10 peaks were separated from mandarin and grapefruit oils, 8 peaks from bitter orange and only 7 peaks from Baladi orange. However, only 9 compounds were identified in these oils as α-pinene, B-pinene,

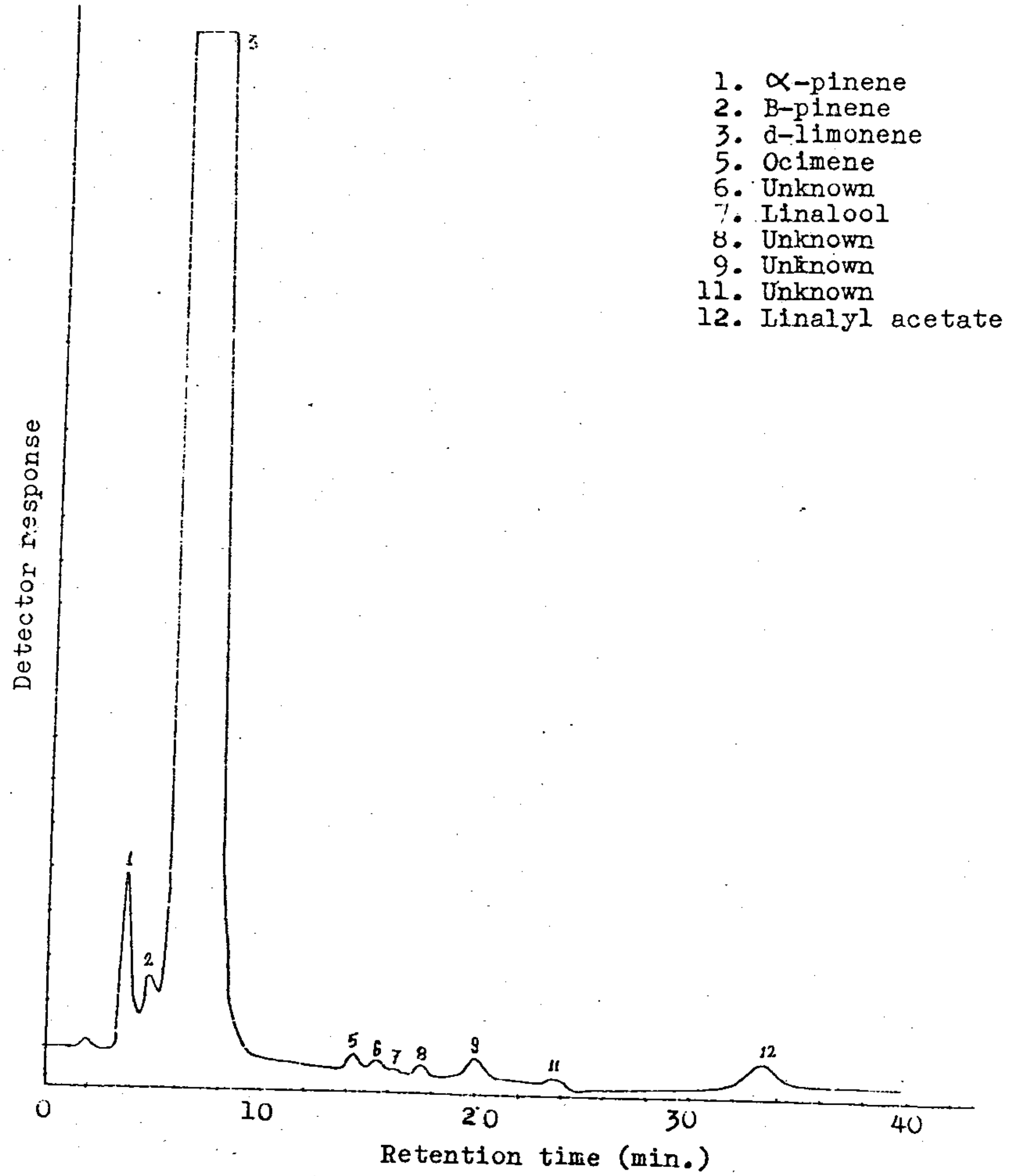

Fig. 3 - GLC separation of mandarin peel oil obtained by water and steam distillation.

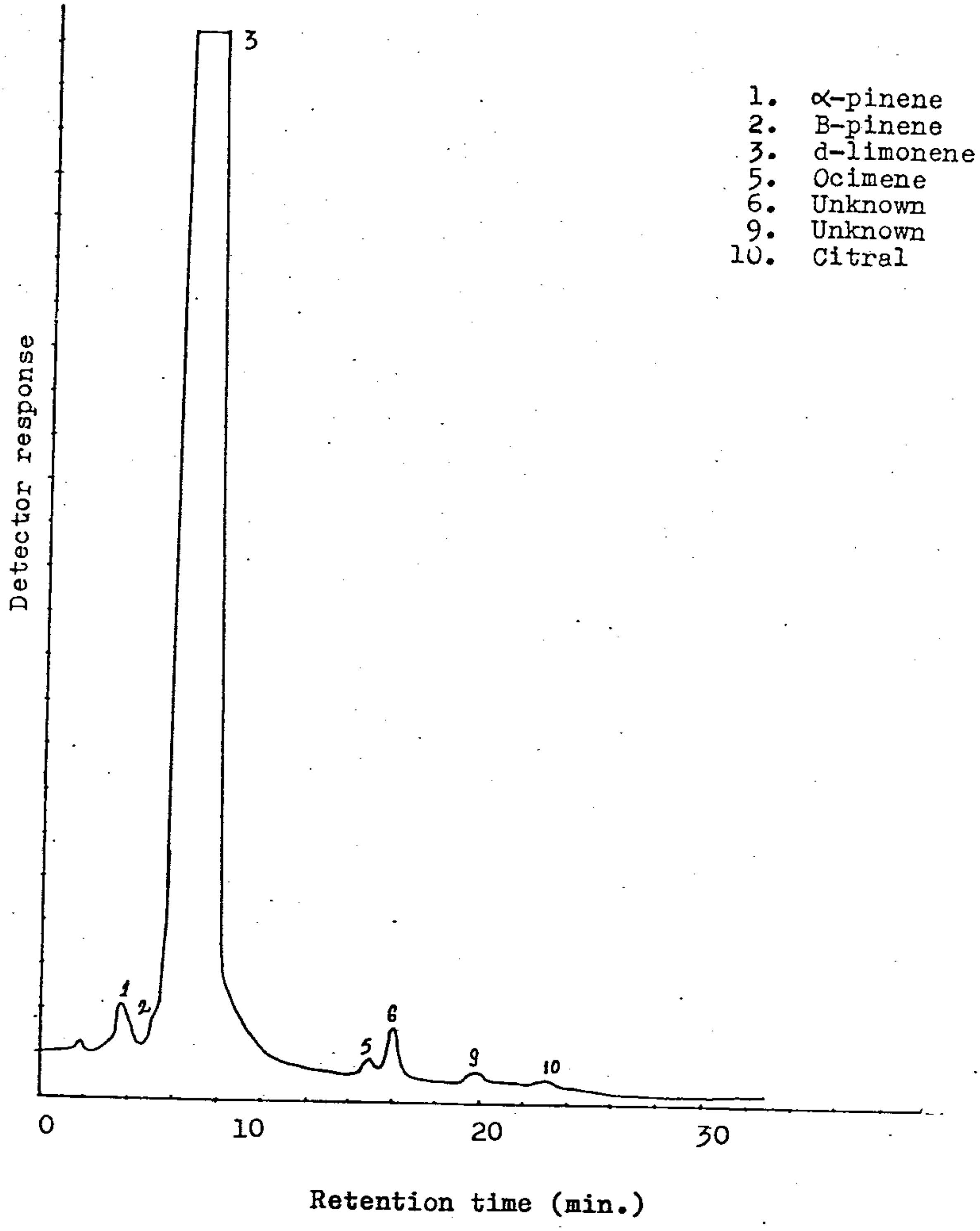

Fig. 4 - GLC separation of Baladi orange peel oil obtained by water and steam distillation.

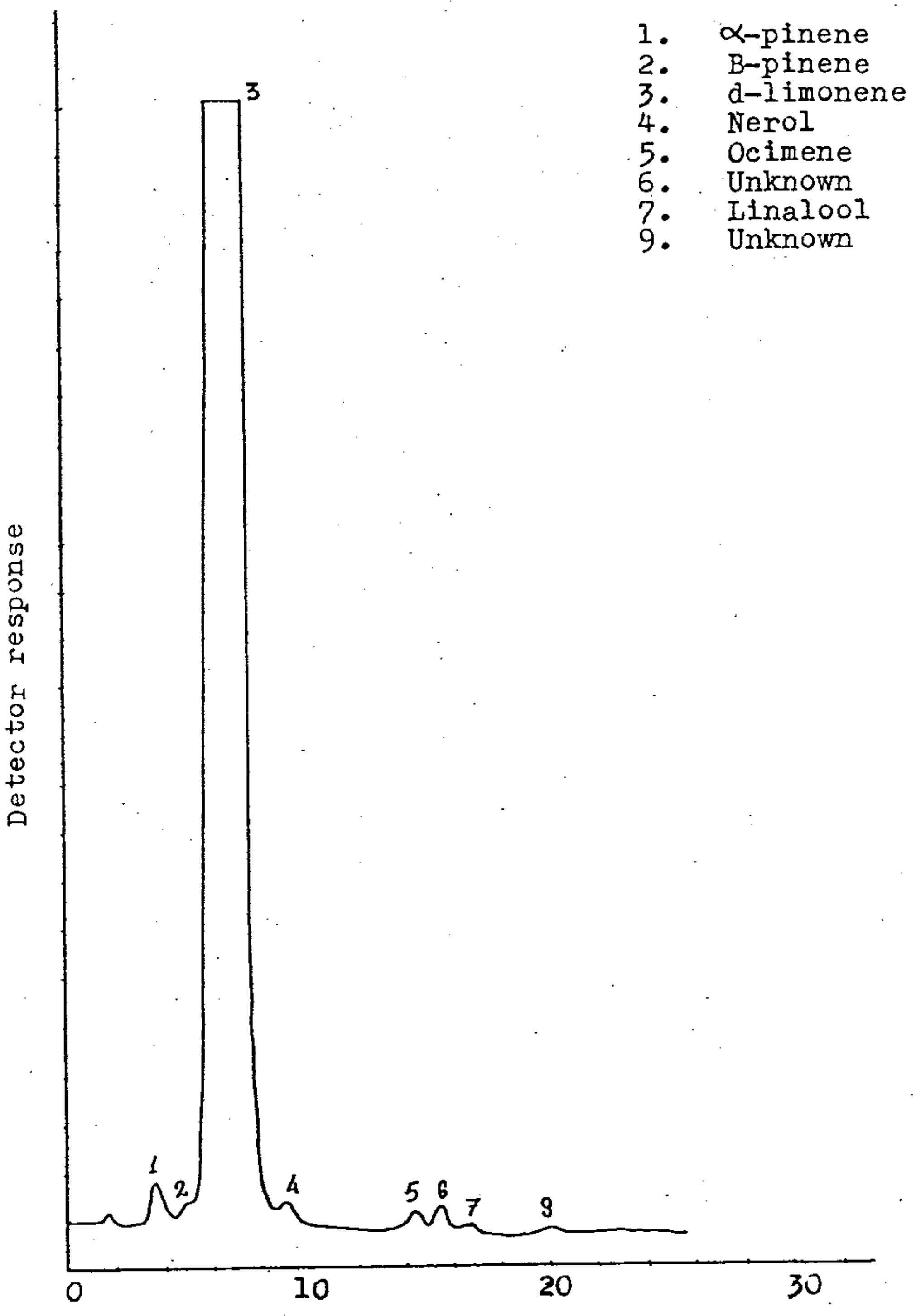

Fig. 5 - GLC separation of bitter orange peel oil obtained by water and steam distillation.

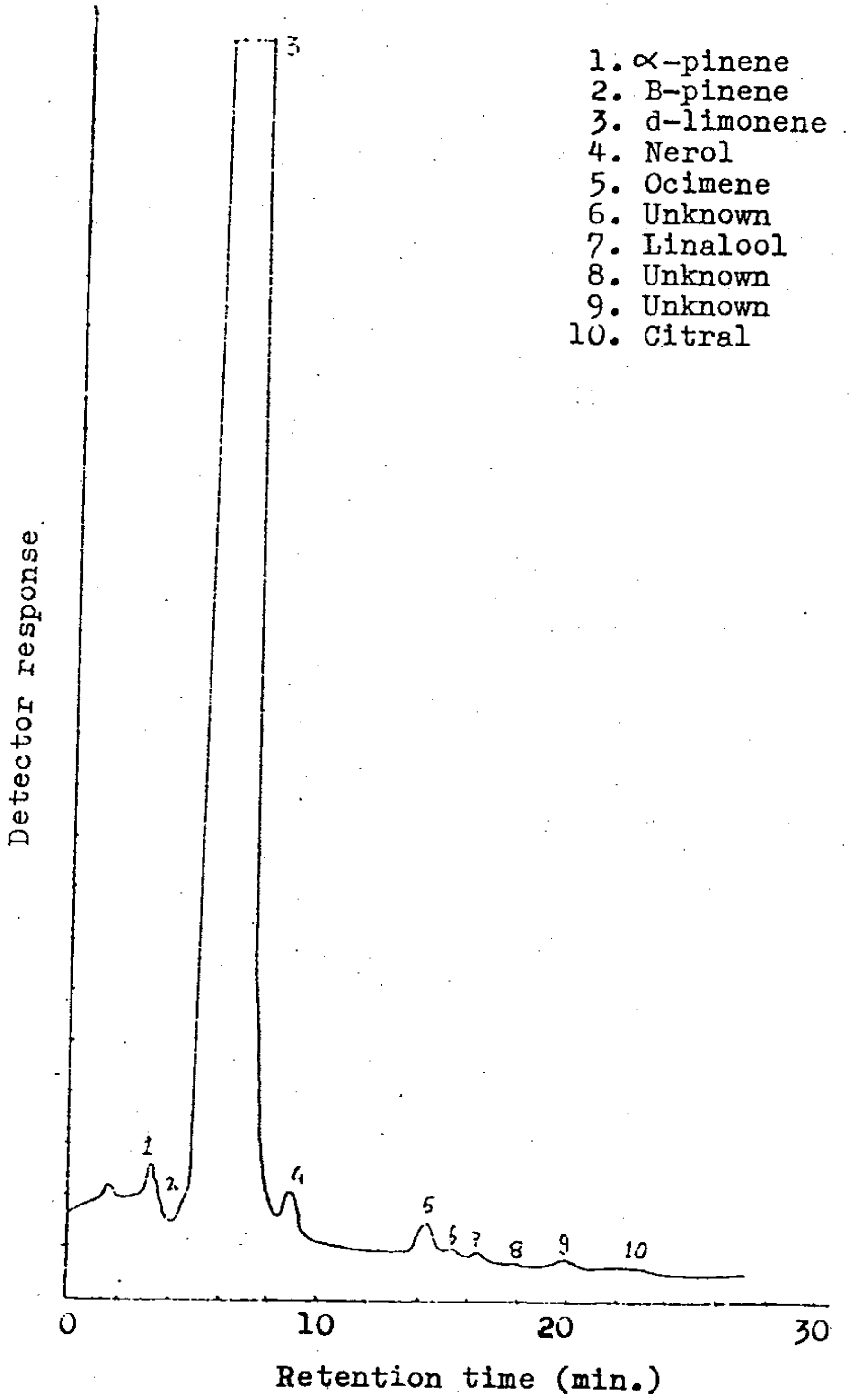

Fig. 6 - GLC separation of grapefruit peel oil obtained by water and steam distillation.

d-limonene, nerol, ocimene, linalool, citral and linalyl acetate. Moreover, it was experimentally impossible to distinguish between d-limonene and myrecene using the chromatographic separation method of the present study. Myrcene was found to have a close relative retention time to that of d-limonene, under the conditions of the present study. Therefore, myrecene is believed to be included in the area of d-limonene peaks.

Furthermore, two unknown compounds were present in peel oils of Baladi orange and bitter orange, 3 unknowns in grapefruit and 4 unknowns in mandarin oils. Relative percentage of various constituents separated by GLC from oils obtained by hydrodistillation as presented in table (12), where compounds are arranged according to their retention time.

4.4.1.1. Terp ydrocarbons:

The identified terpene hydrocarbons in chromatograms of different citrus peel oils obtained by water and steam distillation were α -pinene, B-penene, d-limonene (probably myrecene) and ocimene. The principle terpene hydrocarbon in all the investigated oils was d-limonene which showed a range of 95.49-97.43%. Bitter orange had the high d-limonene content (97.43) followed by Baladi orange (96.70). On the other hand, α-pinene was more concentrated in mandarin oil (1.77%) followed by grapefruit (1.18%) while Baladi and bitter orange oil showed camparable contents (0.87 and 0.77%, respectively). B-pinene was lower than α-pinene, while ocimene was the smallest terpene hydrocarbon fraction detected in mandarin and Baladi orange oils. In contrast, ocimene was higher than B-pinene in bitter orange and grapefruit oils. Of all the oils tested, mandarin had the highest B-pinene content (0.50%), while grapefruit had the highest ocimene content (0.59%).

In spite of the observed difference in concentration of different terpene hydrocarbons fractions in different citrus oils, the amount of total terpene hydrocarbons in all the tested oils was closely comparable (Table 13). The higher content in total amount of α -pinene, B-pinene and ocimene, especially in mandarin and grapefruit (2.44% and 2.07%, respectively) compansated the differences in

Table 13 - Relative percentages of main groups present in citrus peel oils obtained by water and steam distillation as calculated from gas-liquid chromatogram.

Groups	Mandarin peel oil	Baladi orange peel oil	Bitter orange peel oil	Grape-fruit peel oil
Terpene hydrocarbons	98.34	98.33	98.69	97.56
Oxygenated compounds	0.78	0.11	0.67	2.11
Unidentified compounds	0.89	1.57	0.64	0.34
Total	100.0	100.0	100.0	100.0

d-limonene content observed among the tested citrus oils. It is evident from table 13 that terpene hydrocarbons are the major component of hydrodistilled oils at the citrus fruits tested where it showed a proportion ranged from 97.56 to 98.69% with an average of 98.23% of the total constituent.

Steam-distilled oils of different Egyptian citrus cultivars were reported to contain the same terpene hydrocarbons detected in the present study (Osman et al., 1970; Karawya et al., 1971; Ghoneim, 1980; Ashour and El-Kebeer 1980; Ashour and El-Saadany, 1981). Moreover, d-limonene was reported to be the major constituent and at certain proportion comparable to that found by the present investigation, especially in bitter orange oil (Ashour and El-Kebeer, 1980) and in Baladi orange (Ashour and El-Saadany, 1981). In contrast Karawya et al. (1971) and Ghoneim (1980) found much lower d-limonene content in steam distilled citrus peel oils.

Numerous terpene hydrocarbons, other than those detected in the present study, were identified in steam distilled oils of Egyptian citrus fruits. Sabinene, camphene p-cymene and terpene were found in sweet orange, but only sabinene, p-cymene, B-phellandrene were found in bitter orange steam distilled oils (Karawya et al., 1971). P-cymene and B-phellandrene were detected in Baladi orange oil (Ashour and

El-Saadany, 1981). However, Ashour and El-Kebeer (1980) stated that camphene, terpinene, p-cymene were present in steam distilled oils of the setting and immature fruits of bitter orange, but not in that of ripened fruits.

4.4.2. Oxygenated compounds in oils obtained by water and steam distillation:

As shown in Table (12) the oxygenated compounds gave numerous peaks upon GLC analysis which included alcohols, esters and aldehydes. However, the different tested citrus oils deferred in kind and concentration of each of the oxygenated compounds detected.

The identified alcohols were nerol and linalool which were detected only in bitter orange and grapefruit oils. Nerol was found in higher amount than linalool. Moreover, nerol was higher in grapefruit (1.33%) than bitter orange (0.59%). On the other hand, linalool content was almost similar (0.08%) in both oils. Mandarin oil had traces of linalool.

Linalyl acetate was the only ester identified in citrus oils obtained by water and steam distillation, however, it was found in mandarin oil only and represented about 0.78% of the constituents.

Citral was the main aldehyde identified in the water and steam distilled oils, however, it was present in Baladi orange and grapefruit oils at a proportion of 0.11% and 0.7%, respectively. Citral was not detected in bitter orange or mandarin peel oils.

Upon calculation of the total amount of oxygenated compounds as identified by GLC, water and steam distillation citrus oils (Table 13), contained a variable amount which ranged between 0.11 to2.11%. Grapefruit oil had the highest total oxygenated compounds due to its higher neroland citral content, followed by mandarin due to linalyl acetate content. Baladi orange had the lowest total content being only 0.11%due to the presence of citral.

Comparing the obtained data with those in literature concerning steam-distilled oils of different citrus grown in Egypt revealed conflicting findings for each of the oxygenated compounds and for each type of citrus oils.

With respect to alcohols, nerol and linolool detected in the present study was also detected in bitter orange by Karawya et al. (1971) and Ghoneim (1980). In agreement with our findings, Ghoneim(1980) as well as, Ashour and El-Saadany (1981) did not identify nerol in steam-distilled oils of Baladi orange. Moreover, Ghoneim (1980) found only traces of nerol in mandarin oil. However, in contrast to our results, Karawya et al. 1971, working on steam-distilled oils, detected terpineol, geraniol and farnesol in bitter orange oils, while farnesol and geraniol were found in sweet orange, in addition to nerol and linalool. Ashour and El-Saadany (1981) identified, in addition to linolool, citronellol and terpineol in steam distilled Baladi orange oil. Ghoneim (1980) found linalool, farnesol in mandarin and bitter orange oils, while linalool and terpineol were present in Baladi orange oil. Ashour and El-Kebeer (1980) detected nerol and terpineol in steam distilled oils of ripened fruits bitter orange, however, linalool was present only in oil of immature fruits. Furthermore, borneol and citronellol were detectable in solvent extracted oils of Baladi orange and grapefruit, while mandarin oil contained citronellol and ergenol Salib et al. (1978).

With respect to esters, linalyl acetate was also detected in steam distilled oils of mandarin grown in Egypt by Ghoneim (1980) but at much lower proportion than that found in the present study for the water and steam distilled oil. In agreement to our results, Ashour and El-Saadany (1981) did not identify linalyl acetate or any other ester in steam distilled orange oil. In contrast to our data, linalyl was detected in steam distilled oils of sweet and bitter orange (Karawya et al. 1971, Ghoneim, 1980) and in ripened grapefruit oil at higher amount than that of the immature fruit (Ashour and El-Kebeer, 1980).

Steam distilled peel oils of different citrus fruits grown in Egypt were reported to contain other ester compounds which were not detected or identified in oils extracted by water and steam distillation in the present investigation. Methyl anthanilate, geranyl acetate were present in sweet and bitter orange by Karawya et al. (1971). Methyl anthranilate was detected in bitter orange and mandarin oils, but not in orange oil as found by Ghoneim (1980). Neralyl acetate was present in bitter orange oils. However, geranyl acetate was not detected (Ashour and El-Kebeer, 1980).

In agreement with our results concerning aldehydes, neither citral nor other aldehyde was detected by Ashour and El-Kebeer (1980) in steam distilled bitter orange oils. Ghoneim (1980) reported a low amount of citral in orange oil but higher amount in mandarin and bitter orange. Karawya _et_ _al_. (1970) found detected citral in sweet and bitter orange oils but at higher proportions (about 1%) than that found in orange oil in the present study.

Many Egyptian investigators indicated more aldehyde components than that found in the present study. Nonyl acetate and decyl acetate were present, in addition to citral, in steam-distilled oils of Baladi and bitter orange as mentioned by Ghoneim (1980). Citronellal was identified in steam-distilled oil of sweet and bitter orange by Karawya _et_ _al_. (1971) and of Baladi orange by Ashour and El-Saadany (1981).

4.4.1.3. Unidentified compounds:

The four unidentified peaks in chromatograms of oils of the different citrus oils constituted very small proportion of the total components. Two compounds were not identified in all citrus oils investigated, but Baladi orange peel oils contained an additional two unknown The analyzed oils of Baladi orange, mandarin, bitter orange and grapefruit comprised, 1.57, 0.89, 0.64 and 0.34%, respectively of these unidentified compounds.

4.4.2. Volatile constituents separated from different citrus oils obtained by cold-pressing technique:

Chromatograms of different citrus peel oils showed slight variation among themselves in number of peaks detected which ranged between 8 to 9 peaks under the condition of GLC analysis used in the present study(Fig.7,8,9).Analysis of mandarin and grapefruit cold-pressed oils exhibited 9 peaks from which three were not identified, while of the 8 peaks in Baladi orange only 2 were not identified "Relative percentage of the various constituents separated from citrus peel oil obtained by cold-pressing is presented in Table 14.

4.4.2.1. Terpene hydrocarbons:

The identified of individual terpene hydrocarbons in citrus peel oils expressed by cold-pressing were essentially the same as those present in oils obtained by hydro-distillation. D-limonene, was also the principle terpene hydrocarbon. Baladi orange also had highest amount (96.14%) than mandarin (94.75%) and grapefruit (93.48%). Moreover, the other identified terpene hydrocarbons exhibited a similar distribution pattern to those oils obtained by hydro-distillation . For example, mandarin and Baladi orange cold-pressed oils showed also higher content in α-pinene than B-pinene, and moreover, ocimene was present in the smallest amount. Furthermore, grapefruit oil had higher content in

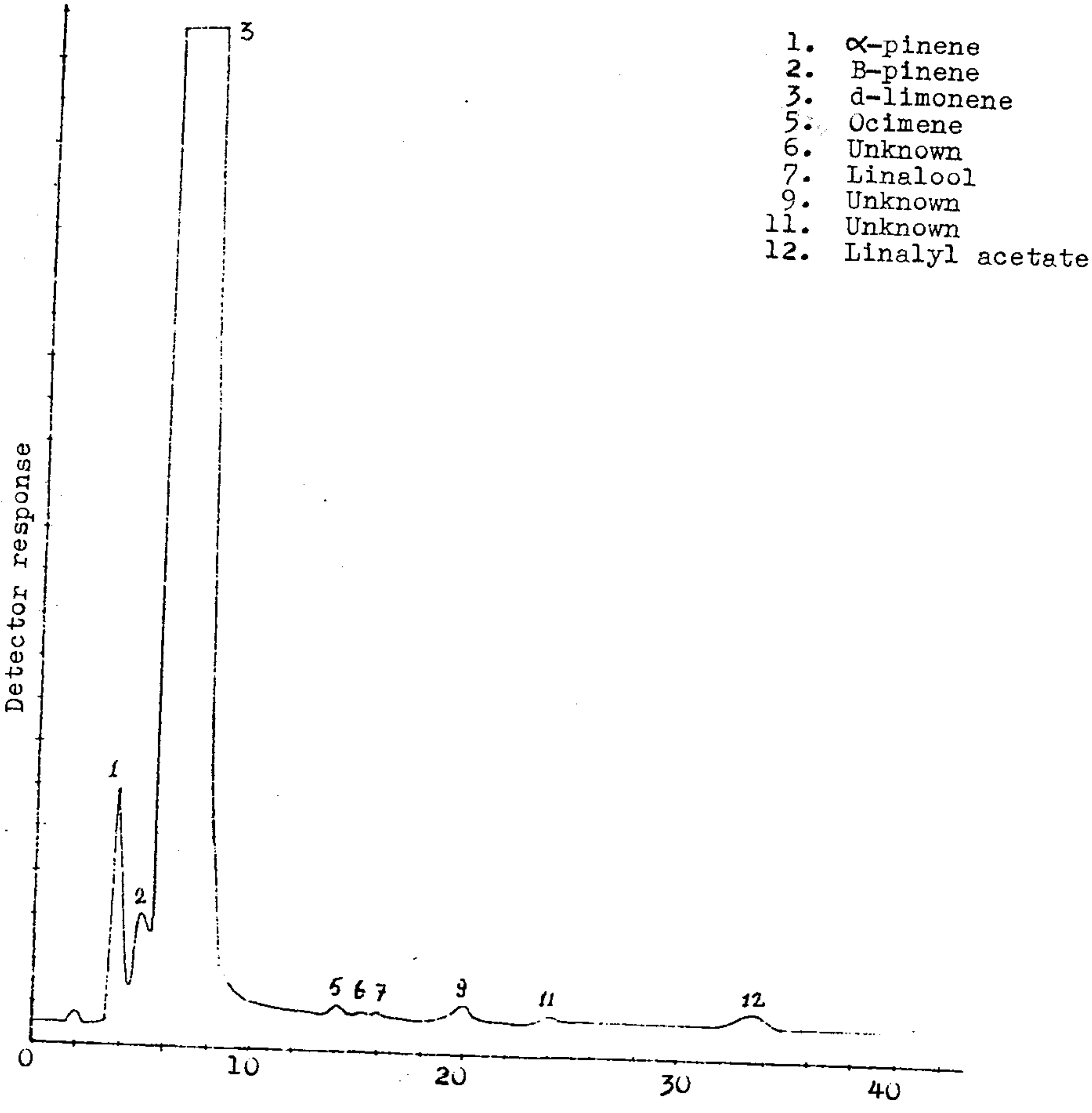

Fig. 7 - GLC separation of cold-pressed Manderin peel oil.

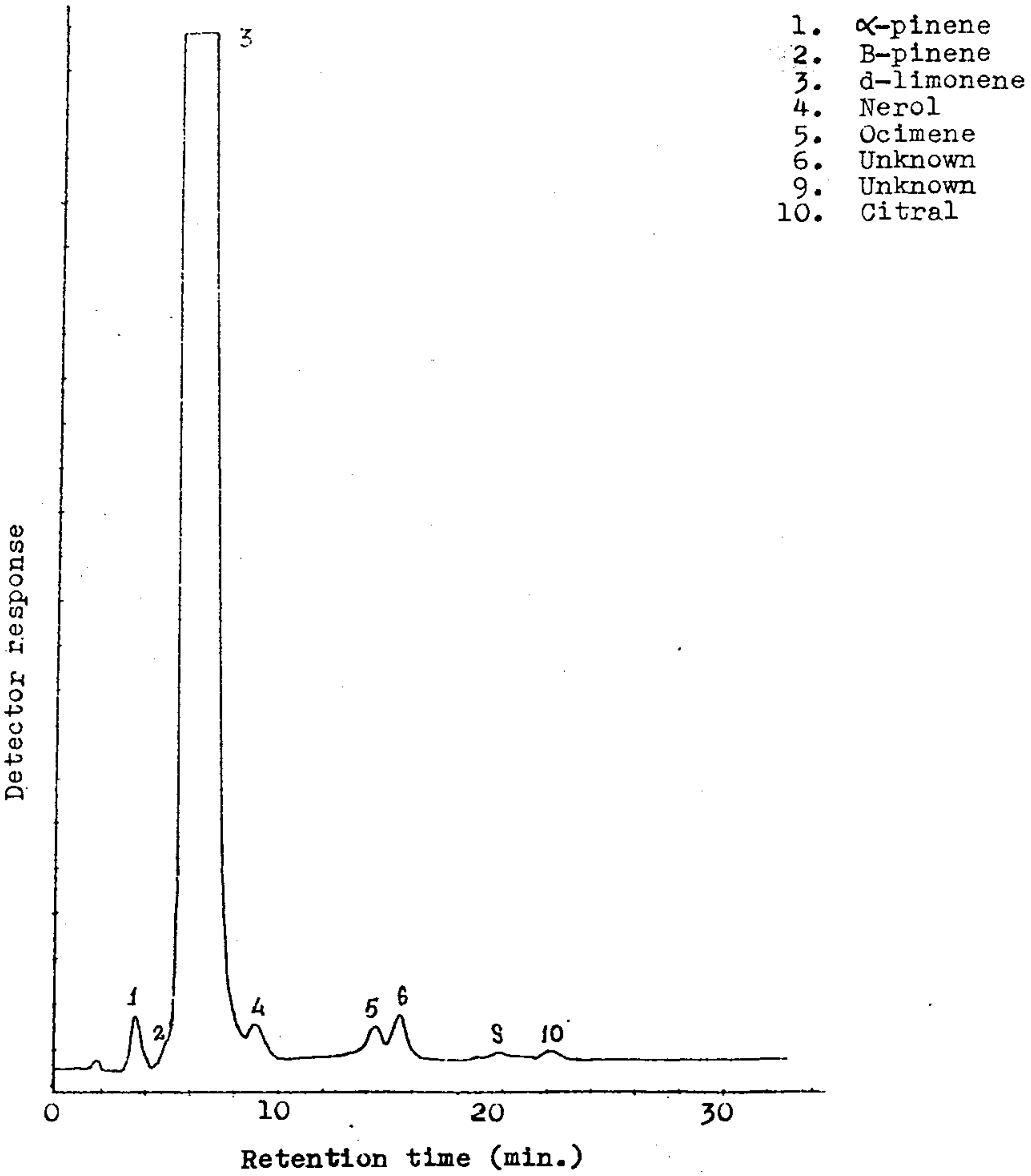

Fig. 8 - GLC separation of cold-pressed Baladi orange peel oil.

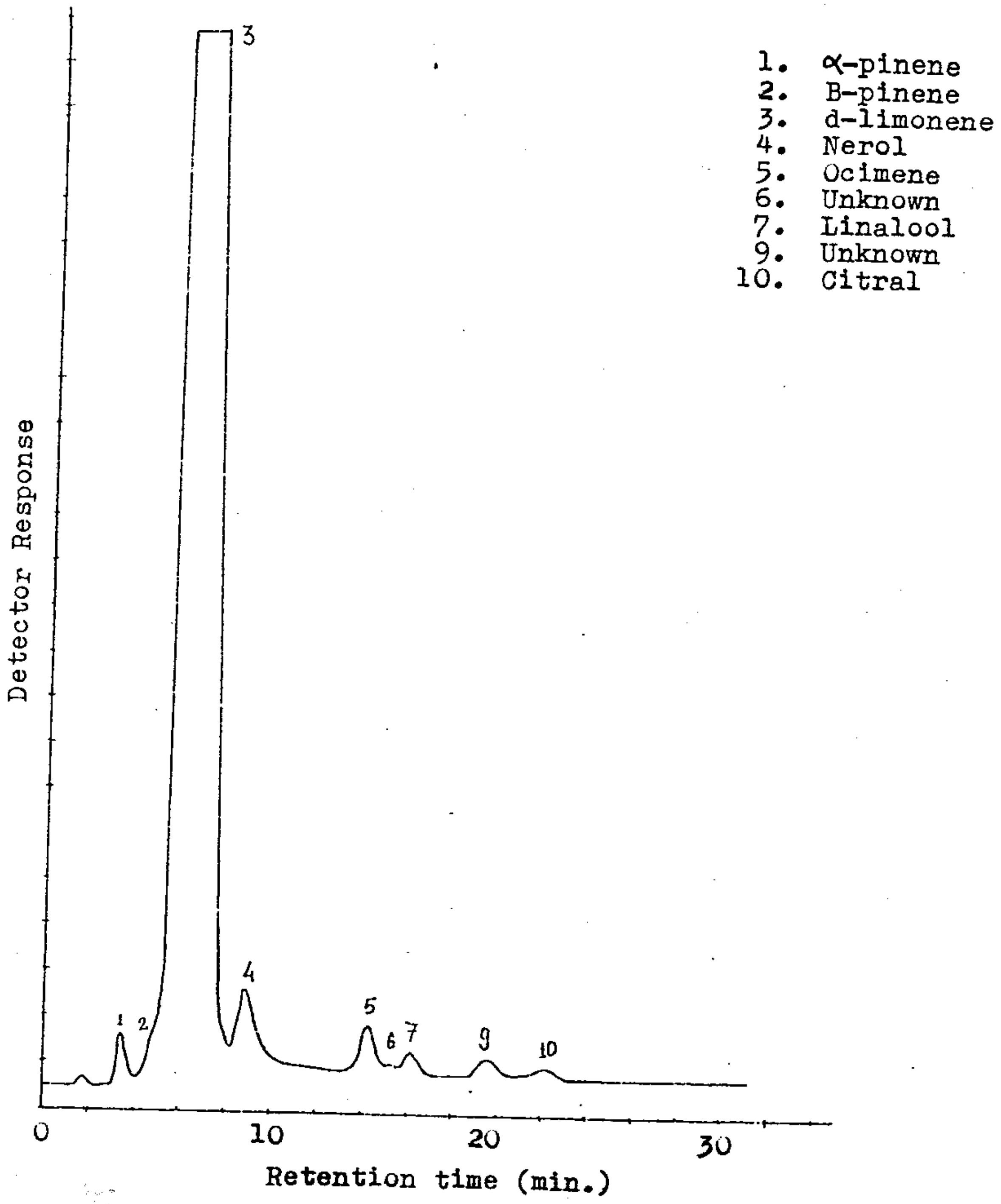

Fig. 9 - GLC separation of cold-pressed grapefruit peel oil.

Table 14 - Gas-liquid chromatographic analysis of some cold-pressed citrus peel oils

Peak No.	Components	Mandrin oil %	Baladi orange oil %	Grape fruit oil %
1	α-Pinene	2.73	1.06	0.82
2	B-Pinene	1.08	0.38	0.72
3	d-Limonene	94.75	96.14	93.48
4	Nerol	-	0.83	2.98
5	Ocimene	0.17	0.37	0.93
6	Unknown	Tr.	0.98	Tr.
7	Linalool	0.04	-	0.57
8	Unkonwn	-	-	-
9	Unknown	0.20	0.06	0.30
10	Citral	-	0.18	0.19
11	Unknown	0.25	-	-
12	Linalyl acetate	0.79	-	-

ocimene than B-pinene.

The only difference observed was that cold-pressed grapefruit oil showed low α-pinene than ocimene content.

Calculation of relative percentage of the main groups of constituents present in cold-pressed citrus peel oils (Table 15) indicate that grapefruit oil had lower terpene hydrocarbons than mandarin and Baladi orange oils, probably to its lower d-limonene content (93.5%) rather than other terpenes (2.5%), Baladi orange and mandarin oils had comparable percentage of total terpene hydrocarbons.

A comparison of the obtained results with those in literature revealed that many investigators working on cold-pressed oils from different Egyptian citrus fruits reported similar make up of terpene hydrocarbons, especially the high d-limonene content, except for the presence of small amounts of other terpenes. Comparable proportions in d-limonene α-pinene and B-pinene to those found by the present study in Baladi orange oil were reported by Ashour and El-Saadany (1981), but they did not detect ocimene which was identified in our data. On the other hand, lower d-limonene content was found in cold-pressed oils of sweet orange by Karawya et al. (1971) and El-Samahy et al.(1982). Furthermore, El-Samahy et al.(1982) found higher proportion of α-pinene (14.8 and 22.5%)in Baladi & bitter orange,respectively),but they did not

Table 15 - Relative percentages of main groups present in cold pressed peel oils of some citrus fruits as calculated from gas-liquid chromatogram.

Groups	Mandarin peel oil	Baladi orange peel oil	Grape fruit peel oil
Terpene hydrocarbons	98.73	97.95	95.95
Oxygenated compounds	0.83	1.01	3.74
Unidentified compounds	0.45	1.04	0.31
Total	100.0	100.0	100.0

identify B-pinene or ocimene.

Other terpene hydrocarbons were detected in cold-pressed oils from different Egyptian citrus varieties. In orange oils camphene, sabinene, p-cymene, and terpenene were identified by Karawya et al. (1971), camphene and Δ^3 carene by Ashour and El-Saadany (1981), and only camphene by El-Samahy et al. (1982). B-phellanderene was detected in cold-pressed bitter orange oil (Karawya et al., 1971).

The obtained results in the present study are in general agreement with those reported for cold-pressed oils obtained from various origins of citrus fruits, in respect with total terpene hydrocarbon and d-limonene proportion (Komatsu and Tanaka, 1951; Ashour and Bernhard, 1967; Lifshitz et al.; 1970, Shaw and Coleman, 1974; Moshanas and Shaw 1974; Wilson and Shaw, 1978). Moreover, Cheng and Chou (1984) found that d-limonene in different cold-pressed citrus oils constituted 69.55-96.33% of oil.

Ashour and Bernhard (1967) detected comphene Δ^3-carene, α terpenene, γ terpinene, p-cymene and α-phellandrene in cold-pressed oils of orange, mandarin and grapefruit .

With improved GLC, infra red and mass spectroscopy analysis, more terpene hydrocarbons could be separated or identified, thus citrus oils were reported to have also

Δ-cadinene, B-ylangene, B-capaene, Δ- elemene, α - elemene, α-borgamotene, D-caryophyllene, B-bisobolene, α-humulene, B-humulene, B-farnesene, valencene (Hunter and Brogden , 1965; Ashour and Bernhard, 1967; Shaw and Coleman, 1974; Moshonas and Shaw, 1974; Wilson and Shaw, 1978).

4.4.2.2. Oxygenated compounds:

Alcohols, esters and aldehyde compounds were identified in cold-pressed citrus oils. However, citrus fruits differed in kind and concentration of each of the oxygenated compounds identified in their peel oils (Table 14).

The identified alcohols were nerol and linalool, however, nerol was present in greater proportion than linalool. Nerol was detected in Baladi orange and grapefruit oils, however, grapefruit contained higher amount (2.98%) than Baladi orange oil (0.83%). Nerol was not identified in mandarin oil. Linolool was present in grapefruit and mandarin only and constituted 0.57 and 0.04% of total compounds.

The ester compound identified was linalyl acetate which was present in mandarin at proportion of 0.79%. No esters were identified in the other cold-pressed oils investigated.

The identified aldehyde compound was citral which was present in Baladi orange and grapefruit peel oil in camparabl

Linalool was also detected by many foreign investigators in cold- pressed oils of mandarin (Moshonas and Shaw, 1974) and in grapefruit (Wilson and Shaw, 1978). Moreover, alcohols, other than linolool, were identified in cold-pressed oils of foreign citrus varieties, α-terpineol, thymol, elemol in mandarin oils (Moshonas and Shaw, 1974) and elemol in grapefruit oils (Wilson and Shaw, 1978).

In agreement to our results, linalyl acetate was not detected in cold-pressed oils of orange on bitter orange as reported by El-Samahy et al. (1982). In contrast, it was identified in cold-pressed orange oils by Karawya et al. (1971) and Ghoneim (1980). Other esters, however, were reported in cold-pressed oils of Egyptian citrus cultures. Methyl anthranilate was prsent in orange oils (Karawya et al. 1971; Ghoneim, 1980) while geranyl acetate was also detected (Karawya et al. 1971; El-Samahy et al., 1982). On the other hand, Ashour and ElSaadany (1981) did not detect any ester in cold-pressed orange oil.

In contrast to our data, many workers did not detect linalyl acetate in cold-pressed oils of foreign origin citrus fruits, neither in mandarin oil (Moshonas and Shaw, 1974) or in orange oil (shaw and Coleman, 1974). However, other esters, rather than linalyl acetate, were identified in cold-pressed grape fruit oil, e.g. octyl acetate, neryl acetate geranyl acetate, and decyl acetate (Wilson and Shaw, 1978).

With respect to aldehydes , citral was also identified in cold-pressed orange oils by Karawya et al. (1971) and Ghoneim (1980) but at higher concentration than that found by the present study. In contrast to our results, cold-pressed orange oils of different Egyptian cultivars were reported to contain citronellal (Karawya et al., 1971) nonyl aldehyde and decyl aldehyde (Ghoneim, 1980) and geranial (El-Samahy et al., 1982) Ashour and El-Saadany, 1981, did not detect any aldehyde in cold-pressed orange oil. Other aldehydes were reported cold-pressed oils of foreign citrus varieties, α sinensal, B-sinensal, decanal, dodecanal, perillaldehyde, geranial in orange oils (Shaw and Coleman, 1974), citronellal, decanal, geranial, perillaldehyde, α-sinensal in mandarin oils (Moshonas and Shaw, 1974). Nerol, in addition to these aldehydes present in mandarin, was detected in grapefruit oils by Wilson and Shaw (1978).

4.4.2.3. Unidentified compounds:

The three unidentified compounds in cold-pressing citrus peel oils constituted small proportion amounts 1.04, 0.45 and 0.3% of the total constituents in Baladi orange, mandarin and grapefruit, respectively.

As shown from Table (14) the three oils contained only one unidentified compound in common. Baladi orange and mandarin oils had two peaks, but grapefruit peel oil showed only one unidentified peak.

Part III. Characterization of pectin pomace of citrus peel

The present work investigate the potential of citrus by-products in providing a good yield of pectin pomace of good quality. The study included preparation of starting materials used for pectin extraction and their analysis from the point of their amount and as well as their content in moisture and pectic substances. Pectin pomaces were extracted by suitable procedures, characterized by simplicity and efficiency in obtaining good yield. The obtained pectins were characterized from the point of their physical and chemical properties as well as their content in neutral sugars and mineral matter.

The residual part of citrus residue, remaining after extraction of juice and removal of flavedo, for recovery of essential oils, and excluding seeds served as starting material for obtaining pectin pomace. Such starting materials, included dried grated peel and membrane (albedo and core or pulp) of Baladi orange and grapefruits as well as dried grated peels (albedo) of mandarin fruits. Citrus residue left after removal of flavedo layer and excluding seeds constituted 47.0 , 50.5 and 38.5% of the fresh weight of Baladi orange, grapefruit and mandarin fruits, respectively (Table 8), which in turn, represent 79.7, 85.6 and 77.0% of total amount of by-products obtained from the three citrus fruits, respectively. Pulps of mandarin were found difficult to separate from seeds as they usually form cake-like mass after pressing of fruits, especially if we know that pulp constitutes smaller proportion of by-product (about 10% of whole fruit) compared to other citrus fruits (Table 8) which constitutes a proportion in fresh fruits of about half that found in other citrus fruits. Separation of seeds from peels and membrane is much easier in citrus other than mandarin, due to smaller amounts of seeds, especially in Baladi orange and grapefruit, and also because of the fact that their voluminous peels retain the membrane (pulp) within it which facilitates separation of seeds through shaking. Therefore, it was decided to use the grated peels of mandarin only as a starting material for obtaining pectin pomace and not to add to it the pulp of fruits.

The aim of the present work is not to investigate optimum conditions for pectin extraction for maximum yield, which is dependent on many factors, but to characterize, in a comparative way, different citrus pectin powders prepared from dried citrus waste materials.

Pretreatment of fresh citrus by-products before its drying was a very important step, where they were washed with running water then minced and treated again with large quantity of boiled water so as to inactivate enzymes and remove sugars, colouring matter and organic acids, then washed again with cold water then dewatered. Pilnik (1970) mentioned that purity of water used in such operation is very important as it might influence pectin extraction and its further properties.

It is beleived that such extracting pectin from dried residues, after their pretreatment to inactivate pectin esterase enzymes, is more convenient and efficient practical method for extracting pectin as if would eliminates the rapid changes in pectin yield or its characteristics occurred due to shortage of time required to handle the huge amounts of fresh by-products produced daily in citrus processing plant. Kertesz (1951) indicated that processing of pectin pomace from fresh by-products should be commenced promptly so as to reduce loss in pectin due to enzyme action and not over 45 min. should be elapsed between juice extraction and heating the fresh by-product in boiled water. Moreover, extrating pectin from dried starting material would reduce cost of pectin preparation especially costs of solvents used in extraction or precipitation. Another advantage is that it an efficient mean for extending season of pectin manufacture.

Many investigators utilized dried citrus by-products as starting materials for extracting pectin and indicated that such method did not have any significant effect or yield on yield or quality of extracted pectin (Agarwal and Pruthi, 1972; Royo *et al.*, 1975; Kausar and Nomura, 1981). Forced air drying was found the best and efficient dehydration method for citrus wastes to be utilized for obtaining pectin of good quality (Agarwal and Pruthi, 1972).

In fact, various methods for citrus pectin extraction have been reported and most of them indicated that optimum conditions for extracting orange and grapefruit pectins were by using 0.3% ammonium oxalate at 90°C for 2 hr.(Abdel-Fattah,1961; Wöger et al., 1970). For mandarin and bitter orange pectins, maximum yields were obtained through extraction with 0.04 N HCl at 85° for 1 hr. as previously indicated by Tarutani and Manabe (1963).

Precipitation of pectin was performed by alcohol as ethanol at critically high concentration is the common precipitant for polymers (Walter and Sherman, 1983). In fact, precipitation with ethanol depends on the colloidal characteristics of pectinic acid and addition of Hcl was found to improve pectin precipitation (Joslyn, 1962). The use of acidified alcohol to precipitate pectin was found to increase pectin yield due to affect of acid in lowering the dissociation of pectinic acid by increasing their colloidal properties to facilitate precipitation (Joslyn and Deuel, 1963).

It was decided not to treat the precipitated pectin with any further washings with alcohol to produce a more purified pectin so as to reduce costs of preparation. Therefore,the crude precipitated pectin pomaces were dried under vacuum, then ground and analyzed for their physical and chemical properties as well as sugar and mineral content.

4.5. Proximate chemical composition of dried starting material for pectin preparation:

Dried albedo and membrane (core or pulp) of Baladi orange and grapefruit and dried albedo of mandarin were analyzed chemically for their content in moisture, crude protein, ether extract, crude fiber and ash. Results are tabulated in Table (16), with data calculated on dry weight basis.

Although moisture content of fresh raw starting materials varied with kind of fruit processed, it did not differ in their oven dried materials, where it averaged 9.7%. Such low moisture content of these materials made them suitable for long storage and consequently, have more availability for processing at any time than other sources for the production of pectic substances.

Dried by-products of Baladi orange and grapefruits exhibited comparable contents in crude protein, ether extract and ash (5.32 and 5.33; 1.75 and 1.66%; 2.95 and 2.96% ,respectively). However, dried peels of mandarin had higher content in these three nutrients (6.94, 3.39 and 4.65, respectively) compared to the other two dried materials.

Table **16** - Proximate chemical analysis of oven-dried residues of some citrus fruits after excluding seeds (g/100 g, dry weight basis).

Analysis	Balady orange	Mandrin	Grape fruit
Crude protein	5.32	6.94	5.33
Ether extract	1.75	3.39	1.66
Ash	2.95	4.65	2.96
Total **carbohydrates(by difference)**	80.43	75.25	80.32
- Crude fiber	25.37	19.39	30.96
Moisture content (as is basis)	78.35	80.03	82.79
Moisture content (oven dried residue)	9.55	9.77	9.73

The higher oil content in mandarin dried peels could be attributed to the presence of remains of flavedo, rather than the presence of any seeds, which contain high oil content, because the removal of flavedo was not so efficient, as mentioned before in Part I.

The obtained data indicated that carbohydrates constituted the major component in all citrus materials and that higher amounts of crude fiber (19.4-31%) were present. Furthermore, crude fiber were found higher in dried by-products of grapefruit (30.96%) than those of orange (25.37%) and of mandarin (19.39%). The high fiber content in dried peels and pulp of grapefruit may influence its pectin content, since fiber and pectic substances are the major components present in carbohydrates of grapefruit material.

The higher ash content of dried peels of mandarin employed the application of special extraction and precipitation procedures for obtaining mandarin pectin pomace which was different than that used with the other two citrus so as to avoid any probable binding of ash constituents with extracted pectin. Joslyn and Deuel (1963) and Aspinall <u>et al</u>. (1968) indicated that the presence of ash or (salt) in raw material created problems during extraction and precipitation of pectin.

Results of chemical composition of citrus dried peel are in agreement with those of Eaks and Sinclair (1980) for dried peels of Valancia orange with respect to crude protein, ash and crude fiber content.

4.6. Estimation of amount and yield of pectin pomace:

4.6.1. Total pectic substance and their fractions in dried citrus waste materials:

The Rouse and Atkins (1955) method for determination of pectic substances indicated that the different citrus dried by-products contained high amounts of pectin which ranged between 28.21-31.66% on dry matter basis (Table 17). However, the three fruits differed among themselves in amount of total amount of pectic substances as well as amount of each of pectin fractions, obtained by solubility in different extractants, present in their dried by-product materials. Pectic substances were present in order of decreasing abundance in grapefruit, orange and mandarin dried waste material (31.66, 28.21 and 23.19%, rspectively).

The use of water, 0.75% ammonium oxalate, 1 N sodium hydroxide provided a degree of fractionation of the pectic substances present in dried citrus waste materials. Stein and Brown (1975) indicated that the

Table (17) - Pectic substances in wastes of processing Baladi orange, grapefruit and mandarin.

Samples of type of waste	Pectin fractions							
	Water-soluble pectin (%)		Oxalate-soluble pectin (%)		Alkaline-soluble pectin (%)		Total pectic substances (%)	
	Fresh (as is) basis	Dry weight basis	Fresh (as is) basis	Dry weight basis	Fresh (as is) basis	Dry weight basis	Fresh (as is) basis	Dry weight basis
Baladi orange (peel + pulps)	0.754	3.484	1.750	8.081	3.604	16.647	6.108	28.212
Mandarin (peels only)	0.662	3.315	1.102	5.520	2.867	14.356	4.631	23.191
Grapefruit (peel + pulps)	0.782	4.544	1.752	10.183	2.915	16.935	5.449	31.662

sequence in which these extractants were used had an influence on the amount of pectic material extracted. Grapefruit material had the highest values in all pectin fractions compared to the other two materials, especially the oxalate-soluble pectin fraction.

It should be mentioned that sequence of extractants as listed in Table (17) could be taken as an arbitrary estimate to distinguish between different types of citrus pectins as well as between various sources of pectins.

Citrus peels of various origins have been reported to contain 20 to 50% pectin on dry matter basis (Kertesz, 1951). The obtained values for pectin substances content are in close agreement of those mentioned by Tressler and Joslyn (1971) and Eaks and Sinclair (1980), but much higher than those indicated by Alexander and Sulebele (1980) and El-Arnaooty (1982) who studied extraction of pectin from Egyptian citrus varieties.

It should be mentioned that kind of citrus fruit and period of maturity influence greatly amount and proportions of pectic substances as well as their fractions For example, although proportion of pectic constituents change only little during growth of grapefruit, they change more drastically in case of orange fruits where the proportion of water-soluble pectic in albedo increases rapidly during the period of rapid growth but decreases as the fruit ripens (Kertesz, 1951).

4.6.2. Yield of pectin prepared from dried citrus waste materials:

As shown in Table (18) estimation of practical yield of pectin pomace, which represents amount of pectin recovered from dried citrus starting materials, indicated that maximum yield was obtained from grapefruit dried material (27.7% of dry matter). Yield from dried orange material was slightly lower(about 24.85% of dry matter). The smallest yield was obtained upon using mandarin dried material (18% of dry matter).

Although by-products of orange and grapefruit differed in their content in total pectic substances or the theoritical yield, the efficiency of pectin recovery from these by-products remained almost indifferent (88.1 and 87.52, respectively) and was higher than that calculated for mandarin by-products (77.62). Results of theoritical and practical yields would indicate that method of extraction was a important factor in determining pectin yield together with amount of pectic substances present in starting raw material. Such conclusion is evidenced by the fact that pectins from orange and grapefruit by-products were extracted

Table 18 – Yields of pectin obtained from dried citrus waste materials as well as from the original by-product.

	Theoritical yield		Practical yield			
	Total pectic substances in fresh by-products g/ 100 g fresh waste	Total pectic substances in dried by-products g/ 100 g dry matter	Pectin pomace obtained from **fresh** by-product g/100 g fresh waste	Pectin pomace obtained from dried waste material (as is basis) g/100 g *	Pectin pomace obtained from dried waste material (on dry weight basis) g/100 g dry matter	Efficiency of pectin recovery **
Baladi orange	6.108	28.212	5.38	22.50	24.85	88.1
Mandarin	4.631	23.191	3.59	16.25	18.00	77.62
Grapefruit	5.449	31.662	4.77	25.00	27.71	87.52

* Average of 3–4 extraction and precipitation trials.

** Efficiency of pectin recovery – $\frac{\text{Pectin yield from dried matters}}{\text{Content in total pectic substances}} \times 100$

and precipitated through a common method which is completely different than that used with mandarin by-products.

The yields of pectin from different citrus sources are within the range indicated by many investigators from either fresh or dried citrus by-products. Salamon (1971) reported that pectin yield from fresh peels of Columbian oranges, grapefruit and lemons ranged from 2.26 to 7.16 g per 100 gm. Alexander and Sulebele (1980) obtained pectin yield of 15.17% of dry matter from peels of Indian citrus fruits. Royo et al. (1975) indicated that pectin yield from dried peels of Spanish citrus fruit was 26.1-30.7% from grapefruit and 12.4-22.8 for mandarin.

Although Crandall and Rouse (1977) found consistent amount of pectin yielded from peels and albedo of grapefruit (4.8 and 4.9%, respectively), he reported much higher yields from peels and albedo of oranges (7.5 and 8.0%, respectively). In contrast to our findings, Nisperos and Robertson(1982) indicated much higher yield (8.9%) from peels of New-Zealand grapefruit using special extraction and purification methods.

It should be mentioned that yield of pectin extracted from citrus by-products is influenced by kind of citrus, type of waste (i.e. peels, albedo, flavedo, core or combination of these wastes), method of preparation or extraction and also with maturity stage of fruits (Kertesz, 1951; Rouse and Knorr, 1970, 1971; Crandall and Rouse, 1977; Nisperos and Robertson, 1982). Maximum yields were reported at certain times towards early season and from albedo rather than flavedo whole peel or core of fruits.

4.7. Physical properties of citrus pectin preparations:

Physical properties of pectin include color, molecular weight, optical rotation, viscosity and gelling properties which are used to supplement data obtained by determination of chemical analysis of pectins. Physical properties of pectin preparations extracted from dried residue of different citrus as well as those of two commercial pectins are summarized in Table (19).

4.7.1. Molecular weight:

Molecular weight and molecular size of pectin are the most important factors determining dynamical properties of pectin gel. Determination of molecular weight can be done by direct procedures such as the ultracentrifuge method and osmotic pressure method by indirect procedures such as viscometery and determination values vary greatly with method and with workers. The molecular weight of pectin usually vary from 10,000 to several thousands (Whyte, 1964).

As indicated in Table (19) molecular weights of pectin preparations varied within a small range from 70,213 to 84,042 and with an average value which is higher than that for commercial pectins (51,487 and 57,446 for slow set and low methoxyl pectin, respectively) Molecular weight was much higher in pectins of orange (84,042) than those of mandarin and grapefruit (78,723 and 70,213, respectively).

Data obtained are within the range indicated by Saverborn (1940) who indicated that molecular weights of pectins from citrus albedo and apple were between 50,000 and 100,000. Moreover, a value of 89,000 was found for commercial lemon pectin by Neukom (1967) and another of 89,362 was indicated for grapefruit pectin by Nisperos and Robertson (1982). Much higher value (220,000) was early found by Schneider and Bock (1937) for citrus pectins. It should be mentioned that the softening of certain fruits upon ripening has been attributed to a decrease in molecular size of pectic substances (McCready and McComb, 1954).

4.7.2. Jelly grade:

The ability of pectin to form jellies or gel is the most important property of pectin, as jelling properties determine to a great extent the commercial value of pectin preparation. Pectin is usually graded according to its sugar carrying power and therefore, jelly grade is defined by Kertesz (1951) as the proportion of sugars which part of solid pectin or pectin extract is capable of turning them into a jelly with suitable characteristics.

In a decreasing order, pectins of orange, mandarin and grapefruit showed variable gelly grades (167, 162 and 112, respectively) which were lower than those of purified commercial pectins. Grapefruit, in particular, had much lower grade than the other two citrus pectins.

The found jelly grades of citrus preparation are comparable or even higher than those found for different pectins of local sources (grades of 60 to 160) as indicated by Salém et al. (1969).

Although pectin preparations were not purified, their jelly grades are comparable to those found by Crandall and Rouse (1977) and Srirangarajan and Shrikhande (1979) of about 180 for orange pomace. Moreover, Alexander and Sulebele (1980) found jelly grades of 180, 209 and 200 for pectin of sweet orange, Baladi orange and grapefruit, respectively. The gelling grade of citrus crude pectin preparation is comparable to that of purified commercial pectins from many sources which were investigated by El-Atawy (1984).

The lower gelling grade of grapefruit pectin could be attributed to its distinct lower molecular weight, together with other factors contributing to gelling properties. Kawabata (1977) indicated that pectin gels were found to be the firmer, the greater molecular weight.

From data in Table 19 and 20, one can conclude that jelly grade of citrus pectin seemed to depend mainly upon degree of polymerization of galacturonic acid and hence upon its molecular weight rather than any other pectin characteristics. These conclusions are in agreement of Lopez and Li-Hsiong (1968), Kuiper (1969) and Voragen and Pilnik (1970) who indicated that jelly grade of pectin depends mostly on its molecular weight and slightly on other pectin properties. Sarhan (1975) found that neither A.G.A. nor methoxyl content of pectin could be considered as good indices for jelly grade.

Jackman (1983) mentioned that three factors are likely to be important in determining the gelling ability of pectin, the degree of esterification, the distribution of methoxyl groups, the molecular weight or molecular weight distribution and the presence of neutral sugar in pectins.

4.7.3. Optical rotation:

Optical rotation, which is the ability of solutions of optically active substances to rotate plane of polarized light passing through them, of pectin is usually taken as indication for their purity and degree of polymerization (Kertesz, 1951).

Values for optical rotation for citrus pectins showed a narrow range of variation (+216. 50° to 232. 26°) with grapefruit pectin having the highest value while that of orange having the lowest. However, optical rotation values of different citrus pectins were higher than those for commercial pectins.

The obtained values are close to those reported by El-Arnaooty (1982) who indicated optical rotation values of+226 and +284 for crude and pure grapefruit pectin, respectively. The same investigator indicated a higher specific rotation value (+266) for purified orange pectin.

There is an evidence to indicate that optical rotation values of citrus pectin correlate with values for A.G.A. as higher purity pectin induced higher specific rotation value. On the other hand, the observed low optical rotation values could partly attributed to the presence of a high proportion of araban, associated oftenly with pectic substances, which is found to be strongly levorotatory (α) -123° to -125°C as mentioned by Saad (1985).

Turbidity of pectin solutions, which is difficult to eliminate by filteration or centrifugation could also partly explain the observed differences in optical rotation values of citrus pectin. Such observation is supported by data of appearance and absorbance of 0.2% pectin solutions, where these data correlated with those of optical rotation.

There is no evidence that all pictinic acids have the same specific optical rotation. Kertesz (1951) stated that specific optical rotation of pictinic acids is within the range of 160° to 240° and that highly purified pectins had a range of +137° to 250°. The presence of even small amount of araban with pictinic substances greatly reduces the observed optical rotation of polyuronides.

Pilnik (1970) noted that specific rotation of a pectin solution was constant over a pH range of 3.0 to 6.5 but prolonged heating with acid caused a drop in such value. Furthermore, the optical rotation decreased rapidly during hydrolysis towards that for galacturonic acid (+51.9°).

4.7.4. Color of dry pectin powder:

Although not purified, citrus pectin preparations showed lighter color than the two commercial pectins, especially orange and grapefruit pectins which possessed whiter color. These two citrus pectins had also lighter color than that of mandarin pectin, probably due to their method of extraction and precipitation which were completely different from those of mandarin pectin.

4.7.5. Physical properties of 0.2% pectin solution:

Data on Table (19) show that different citrus pectin preparations showed some variations in properties of their 0.2% pectin solutions. Citrus pectin were ranked, in order of decreasing values for flow time, as orange, mandarin and grapefruit. However, grapefruit pectin had much lower values than the other pectins. All citrus pectins had higher flow time values than those for the two commercial ones. Such variation in flow time properties could be correlated to molecular weights of these pectins and also to variable amounts of ions which might react with carboxyl groups in pectin molecule forming complex hydroxides with variable viscosities (Baker, 1948). The viscosity of pectin solution depends mainly on its molecular weight, degree of esterification, density and degree of dissociation for electrolytic groups, and content of polyvalent cations (Owens et al., 1944; Deuel and Stutz, 1958) rather than its purity. It should be mentioned that conditions of extraction could also influence flow time properties. For example, El-Atawy (1984) stated that flow time of 0.2% pectic solution was dependent on temperature of extraction were it increased by increasing temperature.

It is evident that appearance and absorbance values of pectins solutions are in,close correlation. Low absorbance and high turbidity indicate the presence of higher non-pectic substances. The observed differences in these two properties might be due to the efficiency of pectin extraction conditions. The highest turbidity (moderate turbidity) and lowest absorbance was observed in orange pectin solutions while that of mandarin pectin showed light turbidity properties. On the other hand,solution of grapefruit pectin was more clear in appearance and of higher absorption value which compared well with those properties of commercial pectins. Results of appearance and absorbance value of citrus pectin solution are parallel to data on their purity properties or A.G.A. content (Table 20), where maximum purity was found for grapefruit pectin.

Solutions of different pectins had slightly variation in their pH values. However, solutions of orange and grapefruit had lower pH values (3.4 and 4.35, respectively) while that of mandarin had slightly higher pH of 3.6 which was more comparable with pH values for commercial pectin solutions.

The observed slight increase in pH of mandarin pectin solution than those of the other citrus pectins could be attributed to method of pectin extraction as well as its precipitation, since mandarin pectin was extracted using Hcl while other pectins with ammonium oxalate solutions. Moreover, the use of acidified alcohol in precipitation pectin as adopted in case of orange and grapefruit pectin, was found to decrease pH values of resultant pectin due to the possible hydrolysis of methyl ester in pectin molecules by acid which leads to splitting of free carboxyl group (El-Atawy, 1984).

4.8. Chemical properties of citrus pectin preparations:

Evaluation of chemical properties of the tested pectins included the determination of their content of moisture, ash, anhydrogalacturonic acid or purity, methoxyl groups and acetyl group as well as their reducing power. (Table 20).

4.8.1. Moisture content:

Moisture content determines the keeping quality of pectin (Kertesz, 1951) and therefore,it is usually carried out in commercial pectins for economical consideration. Data illustrated in Table (20) indicate that moisture content of citrus pectins ranged between approximately 5.3-8.1% which was not greatly different than that of the commercial slow set pectin (7.8% moisture). However, the other commercial pectin had much lower moisture content (4.2%) which is slightly lower than that of orange pectin (5.3%).

Moisture contents of citrus pectin preparations correspond to the lower limit of moisture content for different pectins produced in USA which ranged from 7.2 to 14.9% (Joseph, 1955). Moreover, they had slightly lower values than the lower limit of the range of moisture content (8.02 to 14.89%) indicated by Kertesz (1951) for various

Table 20 - Chemical properties of pectin extracted from some citrus by-products compared to some commercial pectins.

Source of pectin	Moisture %	Ash %	Purity % (A.G.A.)	Methoxyl %	Acetyl %	Reducing power [1]
Orange	5.324	0.9957	61.667	9.098	0.592	2.515
Mandarin	8.076	3.1636	68.96	8.496	0.473	3.060
Grape fruit	7.327	1.1071	77.006	9.025	0.413	2.323
Slow set pectin [2]	7.778	1.456	66.449	7.599	0.88	4.17
Low methoxyl pectin [2]	4.197	4.377	67.33	3.641	0.95	5.7

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1. Ferricyanide number

2. Obi pectin Switzerland

pectins. In addition, moisture content of prepared citrus agree with recommendation of FAO (1978) that pectin should not contain more than 12% moisture. On the other hand, Nisperos and Robertson (1982) indicated a very low moisture content (1.3%) for pectin of grapefruit peel.

The higher moisture content in mandarin pectin (8.08%) than other citrus preparations could be attributed to method of precipitation of mandarin pectin by using non-acidified alcohol, due to the hygroscopic nature of hemicelluloses present in higher amounts in pectin precipitated by such agent. A similar explanation was given by Kertesz (1951) and Sarhan (1975).

4.8.2. <u>Ash content</u>:

Pectin preparations always show an appreciable content of ash which could influence some properties of pectin. The ash content is one of the attributes for pectin quality as it affects viscosity and jelly grade of pectins. Baker and Goodwin (1939) showed that amount and nature of pectin ash influence jelly values at certain pH values. The effect of ash is more pronounced in low-ester pectins in which high content of carboxyl groups are available to react with ash constituents forming complex hydröcides of abnormal high viscosities (Baker, 1948).

Examination of table (2o) reveals that ash content in the three citrus pectin preparations ranged from approximately 1.0 to 3.16% of dry matter. Mandarin had the highest content of 3.1636 while grapefruit and orange pectins had comparable amount (approximately 1.11 and 1.0% respectively). No great difference in ash content was observed between citrus pectin preparation and slow set commercial pectin (1.46% ash) while low methoxyl pectin had higher amount of ash (4.377%).

The distinctive higher ash content of mandarin pectin could be explained on the basis of its method of extraction and precipitation which was completely different from those of the other two citrus pectins. El-Atawy (1964) stated that pectin extracted by acids, particularly HCl, higher solvent concentration or high temperature obtained higher ash content. In the present study, mandarin was extracted using HCl solutions. Sarhan (1975) and El-Atawy (1984) reported that pectin of different sources prepared through precipitation with acidified alcohol showed less values in ash content than those precipitated with non-acidified alcohol. Precipitating pectin of orange and grapefruit in the present study was performed using acidified ethyl alcohol but not with mandarin pectin.

Moreover, it should be noticed that the increase in ash content is parallel to pH value of pectin (Table 19) where mandarin pectin showed higher pH and ash content compared to the other citrus pectins while the low methoxyl commercial had pectin of much higher values for both properties. Such observation was also indicated by El-Atawy(1984) who attributed the higher ash content to the increase of precipitation of salts present in the non-pectic substances at higher pH values during the course of preparation of pectin.

All citrus pectin preparation exhibited lower ash content than those permitted by Food Lows in many countries such as Germany and Switzerland which stated that ash content of pectin used in edible products should not to contain ash more than 12% (Kertesz, 1951).

Joseph (1955) stated that ash content of solid pectins various greatly and it may exceed 11%, which was not the case in the present study.

Amounts of ash found by the present study are comparable with the ranges found by many workers investigating citrus pectins (Schneider, 1936; Smit and Bryant, 1967; El-Arnaooty, 1982; Nisperos and Robertson, 1982, Saad, 1985) and pectin from other sources (Ibrahim 1970; Abdel-Fattah and Edrees, 1971; Sarhan, 1975; El-Atawy, 1984). In addition, ash content of grapefruit pectin is about half that indicated by Nisperos and Robertson (1982) for pectin extracted from grapefruit peels, while that for orange pectin is double that indicated by Srirangarajan and Shrikhande (1977) for pectins from peels of different orange varieties.

4.8.3. Anhydrogalacturonic acid content (A.G.A.):

Anhydrogalacturonic acid content determines percentage of pectin purity which varies greatly according to source and method of extraction, precipitation and purification of that pectin. Solid pectins contain from 44 to 86% of A.G.A. on moisture free basis and high quality pectins always exhibit higher A.G.A. content (Solms, 1960). Many associated components such as galactan, arban, ash, methoxyl and acetyl group influence greatly purity or A.G.A. of pectin.

The unpurified citrus pectins had remarkable A.G.A. content which is comparable or even exceeded those of commercial pectins, with the exception of orange pectin (61.67% A.G.A.). Mandarin pectin showed comparable or even slight higher A.G.A. content than these of commercial one. Grapefruit pectin, in particular, had the highest A.G.A. content (77.01%) which was superior than those of commercial ones which indicate that the method of pectin extraction was very successful in such respect and probably any further purification is not necessary as it will increase costs of production.

Consistent values for A.G.A. were found for citrus pectins prepared by Rouse and Knorr (1970, 1971) and Royo et al. (1975). In contrast, a high A.G.A. value was found by Saad (1985) for orange pectin 76.3% and 67% for titrametric and carbozole method, respectively). Similarly, El-Arnaooti (1982) found higher values for orange (82.3%) and grapefruit (81.1% and 85.4% for pectin extracted by oxalate and HCl, respectively). In addition, Nisperos and Robertson (1982) found that A.G.A. content of their purified grapefruit pectin preparation was 82.2%.

4.8.4. Methoxyl content:

Methoxyl content of pectin is one of the most important factors affecting pectin quality as it influences jelly formation and its properties as well as its sensitivity to

changes in hydrogen ion concentration (Woodmansee and Baker, 1954). It also influence pectin solubility in water which increases by increasing proportion of methyl ester of pectin (Myers and Baker, 1929). Furthermore, methoxyl content of a given pectin determines its ultimate use in different system.

Pectins prevailingly used for food processing are pictinic acid of which carboxyl groups are partially methoxylesterified to methoxyl groups. When pectinic acid completely methylated, the content of methoxyl group is theoretically 16.32% (Kawabata, 1977). Pectin with methoxyl group of more than 7% is called high methoxyl pectin while pectin with that of less than 7% is called low methoxyl pectin (Kertesz, 1951).

Results indicate that methoxyl content of citrus pectin preparations lied between approximately 8.5 to 9.1% which were slightly higher than that of slow-set commercial pectin. The low methoxyl commercial pectin showed 3.64% methoxyl content.

It is evident that all citrus pectins are among the high methoxyl pectins, an finding which is in close agreement to those of many investigators, in spite of the reported great variation in amount of methoxyl content which is known to vary with kind of citrus and method for extraction.

In Egypt, El-Arnaooty (1982) indicated that methoxyl content of grapefruit and orange pectins extracted by HCl were 11.9 and 11.6%, respectively, while that for grapefruit pectin extracted with ammonium oxalate solution was 10.8%. Moreover, Saad (1985) indicated a methoxyl content of 9.01% for unpurified orange pectin preparation.

Rouse and Knorr (1970, 1971) found that, methoxyl content of pectins from lemon peel and pulp was 12.0 and 12.3%, respectively. Royo et al. (1975), stated that the methoxyl content of crude pectin extracted from 30 samples of 7 Spanich orange varieties ranged from 5-7%. Kawabata (1977) found that methoxyl content of Japanese mandarin fruit was 6.14 and 6.12% as determined by gravimeteric and colorimetric method, respectively, while summer orange contained 9.09% methoxyl (gravimeterically) and 8.92% (colorimeterically). Furthermore, Nisperos and Robertson (1982) observed that the methoxyl content of pectin extracted from grapefruit peel was 9.2%.

Methoxyl content of pectin usually varies according to raw material from which pectin was extracted and also to conditions of extraction and precipitation. Methoxyl content decreased when precipitation step was carried out with acidified alcohol due to acid effect on these groups which might cause partial ester hydrolysis, especially at higher temperature.

Pectin gels can roughly be sorted into the hydrogen bonded type and ionic type gel (Kertesz, 1951). Pectins used in jams and fruit jellies belong to the hydrogen bonded type gel which is formed from high methoxyl pectin are very suitable for use as high methoxyl pectin where sugar and acid are needed to form gel and not polyvalent ion-water system as the case with low methoxyl pectin of ionic bonded gel type.

4.8.5. Acetyl group content:

Acetyl content of pectin exerts a significant effect on its jelly forming ability because the presence of these blocking groups prevents the close approach of molecule necessary for gel network formation. Pippen *et al.* (1950) and Lockwood (1976) indicated that the presence of this group on C_2 and/or C_3 renders certain pectin incapable of forming gels and reduced the solubility of pectinic acid in water as they react with secondary hydroxyl groups of pectin forming compounds with hydrophopic character. It is commonly agreeable that the presence of more than 3.5% of acetyl group would impair jelling property of pectin.

In the present investigation small amount of acetyl group were detected in all citrus pectin preparations which ranged from 0.413 to 0.592%. However, such amount are significantly (50% lower) than these present in the two commercial pectins. However, amount of this group was found to correlate well with data of jelly grade as well as appearance or absorbance of 0.2% pectin solution rather than those of purity.

Amount of acetyl group content in the prepared citrus pectins are slightly higher than these reported by McComb and McGready (1957) for lemon peel pectin (0.37%), Lockwood (1976) for different citrus peel pectins (0.2%) and Saad (1985) for crude orange peel pectins.

Acetyl content of pectin is influenced by extraction and precipitation step in pectin preparation. Pippen _et al._ (1950) indicated that acid used in extraction or in precipitation of pectin could influence the acetylated hydroxyl group in pectin which may cause partial ester hydrolysis. Sarhan (1975) mentioned that such influence is more pronounced with higher temperature of extraction.

According to the aforementioned results, one can conclude that the prepared unpurified citrus pectins are of good technological properties from the point of their lower acetyl content.

4.8.6. <u>Reducing power</u>:

Reducing power or ferricyanide number is an estimate of degree of pectin hydrolysis during its extraction precipitation since the degraded pectin would have aldehydic groups and hence have more reducing power. More reducing power was found in mandarin pectin (3.06) than the orange and grapefruit pectin which did not differ greatly in such respect (2.51 and 2.32, respectively). The commercial pectins had much higher ferricyanide number (4.17 and 5.7) than those of citrus pectins.

El-Arnaooty (1982) reported slight higher reducing power values of 4.38 and 4.61 for grapefruit and orange pectins extracted by Hcl and of 5.45 for grapefruit pectin.

Such result would indicate that method for citrus pectin preparation, especially temperature, did not influence greatly their aldehyde groups as using HCl in extraction of mandarin pectin did not influence greatly its reducing power.

The difference in pectin reducing power is related to method of their preparation, especially temperature of extraction which might cause some thermal degradation on polygalacturonic acid leading to rupture of some glycosidic linkages which influence the number of aldehyde groups. Moreover, partial acid hydrolysis may occur during pectin precipitation with acidified alcohol as concluded by Albersheim et al. (1960) and Abdel-Fattah and Edrees (1968).

It should be mentioned that reducing power determinations is not on accurate measurement of degree of pectin hydrolysis since some non-pectic materials, such as arbans and galactans could interfere in determination of such property.

4.9. Constituent neutral sugars of pectin substances of citrus:

Pectin consists mainly of galacturonic acid polymers but also contains a small proportion of other sugars, where some of them are covalently attached to the main galacturonic chain as accompanying polysaccharide impurities or linked as side groups to the acid chain while others are associated with the non pectic (or neutral) polymer (Kertesz, 1951). The acidic molecule is thought to be responsible for the gelling power of pectin, while the associated neutral fraction acts as diluent of specific gelling ability (Speiser *et al*., 1945). Certain proportion of covalently attached neutral sugars would be advantages to gelling ability of pectin (Saad, 1985). Furthermore, proportion of neutral sugar in pectin seems to vary with source, maturity and method of extraction (Saad, 1985).

Identification of neutral sugars of crude citrus pectins was performed using paper chromatography separation of sugars from acid hydrolysates of these pectins. Chromatograms of sugars liberated upon acid hydrolysis indicated the presence of galacturonic acid, galactose, arabinose and rhamnose in all citrus pectins analyzed (Fig. 10). Qualitative estimation of these neutral sugar fractions revealed that quantity of rhamnose was lower than other sugars present. (Table 21).

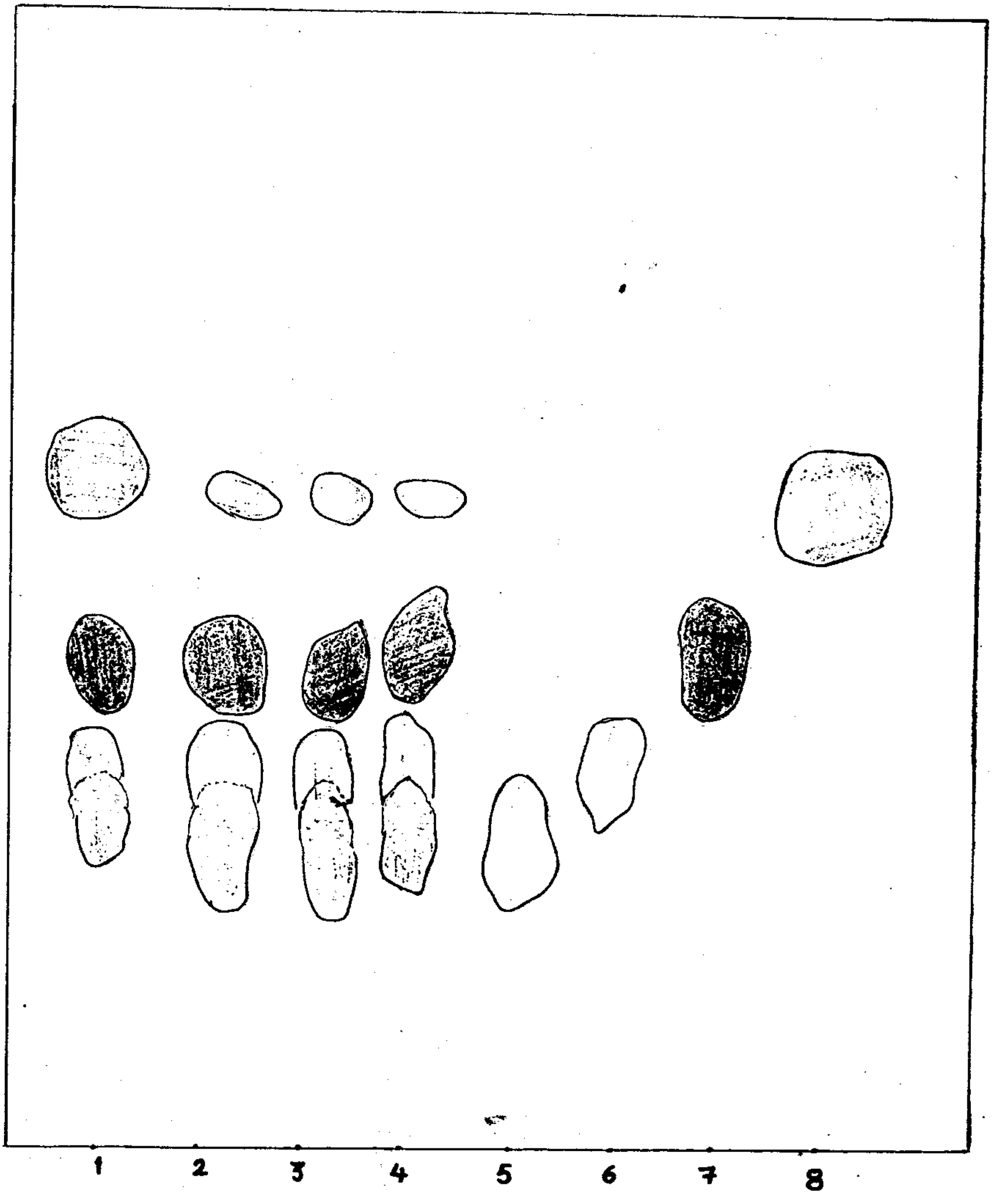

Fig. 10 - Chromatogram of sugars liberated upon acid hydrolysis of citrus pectins prepared from by-products of different citrus fruits.

(1, mixture of standards; 2, Baladi orange pectin pomace; 3, grapefruit pectins; 4, Mandarin pectins; 5, galacturonic acid; 6, galactose, 7; arabinose and 8, rhamnose).

Table 21 - Qualitative estimation of sugars separated by paper chromatography technique from acid hydrolyzates of pectin extracted from by-products of different citrus fruits.

Source	Galacturonic acid	Galactose	Arabinose	Rhamnose
Baladi orange	+++	+++	+++	+
Mandarin	+++	+++	+++	+
Grapefruit	+++	+++	+++	+
Standard	++++	++++	++++	++++

++++ = Excess
+++ = High
++ = Moderate
+ = Low

In agreement with our results, El-Arnaooty (1982) also detected galacturonic acid, galactose, arabinose, and rhamnose in partial acid hydrolysates of pectins extracted from Egyptian cultivars of orange and grapefruit using paper chromatography technique. Furthermore, Kawabata (1977) using high performance liquid chromatography indicated that neutral sugars (4-16%) of different citrus pectins contained arabinose and galactose in higher proportions, and also rhamnose in all citrus pectins, while glucose, xylose, mannose and ribose were not present in pectins of grapefruit and orange. However, they found xylose and glucose in mandarin pectin but at very low concentration. Acidic sugars comprised about 50-74% as anhydrogalacturonic acid.

Earlier reports of Abdel-Fattah(1961) using paper chromatography found that sugars of different citrus pectin contained tetra-galacturonic acid, tri-galacturonic acid, di-galacturonic acid, mono-galacturonic acid, arabinose and traces of galactose and rhamnose. Aspinall et al. (1968) also indicated that neutral sugars such as arabinose, galactose and rhamnose were presented in lemon pectin. Galactose was reported to be found usually in the form of galactan, which was observed to be a linear polymer of B-D-galactose units linked to the 1 and 4 positions while arabinose was present in the form of arabinan found to be a branched chain of L-arabinofuranose (Solms and Deuel 1951).

According to Pilnik(1970) & Gonzalez Charrière et Petit (1971), neutral sugars are always found in pectic substances and rhamnose exists between the main chains of galacturonic acids, but in case of arabinose, galactose and xylose some of them are coordinated to the side chains of galacturonic acids, and others are mingled with acids. Using ion exchange chromatographic technique, Baig <u>et al</u>. (1982) stated that grapefruit pectin composed of galacturonic acid 76%, rhamnose 7.13%, arabinose 2.65%, xylose 0.75%, mannose 0.42%, galactose 11.5% and glucose 1.2%.

4.10. Mineral content:

The determination of individual minerals in citrus by-products as well as in prepared crude pectins included the spectrophotometeric determination of phosphorus and atomic absorption spectroscopic methods for sodium, potassium, cupper, zinc, manganese and iron.

4.10.1. Mineral content of dried citrus waste materials:

As indicated in table (22) calcium was the principal element in ash of dried citrus waste materials used

Table (22) - Chemical analysis of mineral content in citrus by-products used for the preparation of pectins compared to those present in the prepared pectins and as two commercial types of pectins (reported in mg per 100 g of dry weight)[1]

Element	Source of pectin			Prepared pectins			Commercial pectins	
	Peels and pulp of baladi orange	Peels of mandarin	Peels and pulp of grape-fruit	from baladi orange	from mandarin	from grape-fruit	T_2[2]	T_3[3]
Macroelement								
Ca	1491.1	1792.1	1311.8	243.4	1617.3	100.2	220.7	76.7
Na	96.2	146.3	154.1	102.6	44.2	56.9	148.4	3833.8
Mg	89.9	180.4	118.6	26.8	63.7	23.1	59.6	2.8
K	169.9	140.5	205.2	22.0	8.1	2.3	292.5	85.8
P	0.16	0.04	0.04	0.02	0.02	0.01	0.02	0.06
Microelement								
Cu	0.99	0.63	0.64	1.6	3.8	0.81	0.92	0.60
Mn	1.6	10.2	6.3	2.9	5.8	2.1	1.1	0.47
Zn	1.3	4.3	1.8	4.6	10.3	3.4	1.3	1.1
Fe	31.0	36.2	30.5	15.4	22.7	16.5	-	-

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1. Average of three determinations
2. T_2 , slow set pectin,
3. T_3 , low methoxyl pectin.

for preparation of pectins. Despite of kind of fruit, minerals present in the different ashes were, in amounts of decreasing order, Ca, K, Na, Mg, Fe, Mn, Zn, Cu and P. Orange dry waste material, in particular, contained Na and Mg, Mn in much lower amount as compared to other citrus. Except for Cu, K and P, dried peels of mandarin had higher amounts of most minerals especially Ca and Mg than other citrus materials. Such higher amount could be attributed to the higher ash content in mandarin waste material (Table 16).

The presence of high mineral matter has been reported to create problems during extraction and subsequent precipitation of pectins (Joslyn and Deuel 1963); Aspinall et al. (1968). It should be mentioned that water used in preliminary steps, such as washing of fresh citrus peels, may contribute to the mineral matter of the dried citrus materials.

4.10.2. Mineral content of pectin preparations:

Amount and nature of ash as well as constituent minerals present in pectin influence many properties of that pectin. Metallic ions present in pectins exert an pronounced effect on viscosity of pectin solution at pH above 2.75

where abnormal viscosities resulted, impart, by formation of complex hydroxides and any excess amount of heavy metals ions would cause coagulation and precipitation at certain specific pH values (Baker, 1948). High level of some mineral constituents, especially the polyvalent ions, tends to facilitate local precipitation (no gelation) and consequently synersis (Kohn and Furda, 1967).

The comparative analysis of mineral matter of the prepared citrus and two commercial pectins is presented in Table (22). The different citrus pectins as well as commercial ones showed a great variation in amounts of each individual elements, especially Ca and Mg. Analysis showed that citrus pectins kept much of element present in this starting material although every individual pectin differed from the others in extent of such trend. In fact, all citrus pectins have the trend of having calcium as the dominant element among inorganic ions bonded to pectin where it was present in higher concentrations compared to others. Magnesium ranked the second, from the point of abundance, in mandarin pectins and the third in pectins of orange and grapefruit. The content of Mg were approximately equal in orange and grapefruit pectins. Except for Ca, Mg and Na, all citrus pectin preparations had lower amounts of other minerals.

The commercial pectin had the same trend, however, they contained K, in addition to Ca, Na, Mg in higher amounts.

The two commercial pectins showed also differences between themselves with respect to mineral content. The predominant element in the slow set pectin (T_2) was K while the low methoxyl one (T_3) had sodium, which was present in very high concentrations, and K was in the second order of abundance. Calcium ranked in the second and the third position in T_2 and T_3, respectively, in respect with order of abundance. Furthermore, the low methoxyl pectin T_3 contained distinguished high Na and low Mg compared to the other commercial one as well as all the citrus pectin preparations. In addition, T_3 had lower amounts of all minerals than others, except that of Na and P in T_2, and those of Na, K and P in citrus preparations.

Mandarin pectin, in particular, exhibited higher amounts of Ca and Mg, Cu, Mn, Zn and Fe than other citrus pectins as well as commercial ones. The extra ordinary high amount of Ca (8-10 times than of others) and high Mg (three times that of others) could be attributed to the effect of method of extraction rather than the higher content in these two minerals in starting material. Orange and grapefruit pectins contained much lower amounts of Ca and Mg, although their starting materials contained appreciable amounts of them. Moreover, the higher concentrations of many elements in mandarin pectin is related to its high ash content compared to other pectins (Table 20). The same relation of predominant inorganic elements bound to pectin is noticed for T_3 commercial pectin.

Pectin extraction and its precipitation influence greatly mineral matter of the resultant pectin. Sarhan (1975) stated that precipitation step was the most effective variable which determined mineral matter of extracted pectins, and when precipitation of pectin was conducted with acidified alcohol, considerable amounts of all elements were dissolved and removed in alcohol solution which resulted in lower mineral matter in pectin, especially calcium. Probably such observations could explain the lower concentration of minerals in orange and grapefruit pectin preparations in the present study than that of mandarin pectin, as both of them were obtained through precipitation with acidified alcohol. Moreover, Worth (1967) indicated that ash content and its constituents especially Ca, were dissolved and removed with alcoholic solutions upon precipitating pectin with acidified alcohol which certainly influence amount and distribution of minerals in the resultant pectin.

The content of elements of all citrus preparations are within the range found in some American commercial pectins as indicated by Joseph (1955) which contained (in mg/ 100 g) the following, calcium (30-2800), Mg (60-1800); Na (100-3000); K (1-3900); Mn (1-5) and Cu (4-100).

The observation that Ca and Mg were the predominant inorganic elements bound to pectin is previously reported by many investigators (Joseph 1940, 1955; Kawamura, 1955; Sarhan, 1975, Kawabata, 1977, El-Arnaooty 1982 and Saad, 1985).

Furthermore, the found element content of orange pectin preparation is consistent to that of orange pectin as reported by Saad (1985). The amount of Ca, Mg, Cu and Zn are in agreement of those of crude and purified pectin from peels and pulps of Egyptian cultivars of orange and grapefruit as indicated by El-Arnaooty (1982). In addition, the same investigator did not found any significant difference in mineral content between crude and pure citrus pectins. The found concentrations of Ca and Mg are also close to those present in crude or pure pectins of Japanese citrus detected by Kawabata (1977).

The higher amounts of Ca and Mg in pectins could be explained by the fact that carboxyl groups of galacturonic acid, the main heteropolysaccharide in pectin, are partically esterificated by methyl group and some of them link with ions such as Ca and Mg (Kawamura, 1955; Kawabata, 1977). Baker and Goodwin (1941) found that Ca, Mg, Cu and Al present in pectin did not appreciably affect its viscosity property, at pH values below 2.75. Moreover, pectin have reported to have a higher binding ability of polyvalent cations (Vidal-Valverde et al., 1982) which make them usuable in many applications as a detoxicant (Kerstez, 1951) and in heavy metal industry (Surikova et al., 1977).

As far, as the mineral content of citrus pectin preparations is concerned, those pectins could be safely employed in different edible foods without no further problem. However, amounts of Cu and Zn were slightly higher than those indicated by FAO (1978) who stated that pectin should not contain more than 5 and 2.5 mg per 100 g of Cu and Zn respectively. Probably purificatio would certainly decrease amount of in such crude preparations as a resu of metals present in the non-pectic fractions.

The aforementioned data would indicate the suitability of the adopted simple methods for extraction and preparation of pectin from dried residue of citrus in providing pectin in reasonable yield and of good physical and chemical characteristics and suitable from safely, technological and economic points of view. Costs of production could be reduced greatly as any further purification seems to be unnecessary as indicated data of acetyl and AGA content. About 500 kg of pectin could be produced daily from by-products of single plant processing citrus fruits, which would produce as much as 11 ton/day of residues. Such amount of pectin production would improve economy of citrus manufacture as save much of hard currency used for importing pectin storage of pectin in Egyptian market.

Table 23 - Weight and relative percentage of seeds present in processing by-products of different citrus fruits as well as analysis of seeds in moisture and oil content.

	Baladi orange	Bitter orange	Mandarin
Total residue left after pressing fruits, % as is basis.[1]	59.0	65.0	50
Weight of seeds per kg of fresh fruits, g.[1]	20.0	68.0	45
Percentage of seeds in fruits, %.[1]	2.0	6.8	4.5
Percentage of seeds in by-products, %[1]	3.4	10.5	9.0
Weight of 100 seeds (air-dried), g.[1]	14.52	12.20	12.52
Moisture content of seeds, (%)[2].	56.30	56.10	52.30
Seed content in ether extract, % oven dry basis[2].	36.00	38.19	27.02

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[1] Average of ten determination.

[2] Average of triplicate determination.

higher weight compared to those of the other two citrus fruits, as indicated data on weight of 100 seeds when calculated using the air dried seeds (Table 23). Seeds of mandarin and bitter orange were of comparable weight.

Chemical analysis indicated that the different citrus seeds investigated had comparable moisture content and high ether extract content per dry weight of seeds. Bitter orange seeds had the highest ether extract content (38.19%), compared to Baladi orange and mandarin seed (36 and 27.02%, respectively). It could be noticed that although Baladi orange contained lower amount of seed (2%), it showed a good amount of oils in its seeds, in a quantity even more than that of mandarin seeds.

The obtained data on oil content of different seeds are in agreement with the findings of the few workers who investigated seeds oils of Egyptian citrus cultivas as well as foreign varieties. In Egypt, Youssef (1980) found that bitter orange, sweet orange and mandarin seed contained 38.6, 30.1 and 28.2% of oils, respectively. Moreover, Metwally and Khafagy (1975) found oil content in seeds of Baladi orange and mandarin to be 37.1 and 29%, respectively. In addition, seeds of citrus varieties of foreign origin were reported to contain oils in amount of 25-30% (Parekh et al., 1959), 35.9% (Kamel et al., 1982); 32.3-38.4% (Tsuyuki et al., 1984).

On a dry weight basis,the oil content of seeds from different citrus fruits is relatively higher as compared with that of seeds from other fruits, such as figs (23.5%), pears (14.1%), apples (18-23%), grapes (12-22%) and is comparable to that of soybean (35%) (Jacobs, 1962).

Based on data of daily production of wastes from processing citrus fruits in factories of El-Nasr for Preserved Foods (Kaha), Egypt,(Anon, 1985), one would expect that 11 ton/day of citrus residue would contain about 887 kg of seeds which, in turn can produce 133 kg of seed oil per day. These calculation are based on an estimation of average percentage of seeds in total wastes of 4.4% and average seed oil content of 15 %.

4.12. Physical and chemical properties of citrus seed oils:

Analysis of oils and fats is concerned not, as in other food nutrient, with percentage composition but rather with this physical and chemical properties which serve as a basis for identification and assay and also with suitability of a given oil or fat for a given purpose physical and chemical properties of citrus seed oils varied greatly with kind of citrus fruit as illustrated in Table (24).

Table 24 - Physical and chemical properties of oils obtained from some citrus seeds.

Properties	Balady orange	Mandarin	Bitter orange
Refractive index at 25°C	1.4680	1.4692	1.4667
Acid number	1.29	2.55	1.61
Peroxide number (MEq/k)	6.31	11.89	8.36
Iodine number (Hanus)	99.07	95.91	95.11
Saponification value	201.15	201.58	201.44
Unsaponifiable matter %	0.97	0.81	0.67

4.12.1. Refractive index:

From results in table (24), refractive index values (25°C) of different citrus seed oils ranged between 1.4667 to 1.4692. Refractive index value of bitter orange seed oil was lower (1.4667) than mandarin and Baladi orange seed oils (1.4692 and 1.4680, respectively). On the other hand, the low refractive index in bitter orange seed oil than other seed oils could be as a result of the presence of lower amount of di-unsaturated fatty acid glycerides. These indications were supported by the GIC analysis of fatty acids (Table 25).

The obtained values were within the range found by Shābana (1969) and Youssef (1980) for seed oils of Egyptian citrus fruits and Van Atta and Dietrich (1944) of foreign citrus varieties.

4.12.2. Acid number:

Data presented in table (24) indicated that acid value of different citrus seed oils ranged between 1.29 to 2.55. A high acid value was found for mandarin seed oil (2.55) than those of Baladi and bitter orange seed oils (1.29 and 1.61, respectively). The high acid number in mandarin seed oils could be attributed to the presence of increased amount of oleic acid. Such indication was supported by GIC analysis of fatty acids (Table 25). However, acidity calculated as oleic acid was 1.28, 0.65 and 0.81 for mandarin, Baladi orange and bitter orange seed oils, respectively. These results coincided with those stated by Hafez (1964) and Van Atta and Dietrich (1944) with respect to acid value of Baladi orange and mandarin seed oils. In contrast, Shabana (1969) reported higher acid value for bitter orange seed oil.

4.12.3. Peroxide number:

Peroxide number is not a constant for any oil, it is rather a reflection of its tendency towards oxidation. It showed a range of 6.31-11.89 in the different citrus seed oils. The peroxide number of mandarin seed oil was found higher (11.89) than those for Baladi and bitter orange seed oils (6.31 and 8.36, respectively). The found values for Baladi orange seed oil coincided with those reported by Hafez (1964). The found peroxide value could indicate that no considerable oxidation was taking place in extracted crude citrus seed oil.

4.12.4. Iodine number:

Iodine number in oils reflects the degree of unsaturation, especially when measured for untreated oils. All citrus seed oils showed higher iodine values (95.11-99.07) which indicate that oils were relatively high in unsaturated oils. Baladi orange seed oil had higher iodine number (99.07) than those of mandarin and bitter orange seed oils (95.93 and 95.11, respectively). The higher iodine value in Baladi orange seed oil could be taken as an indication of increased unsaturated fatty acids in oil composition. Data obtained agreed with those reported by Hafez (1964), Shoeb (1970) and Youssef (1980) for seed oils of different Egyptian varieties of citrus fruits. Moreover, in agreement of our results, Youssef (1980) reported comparable iodine number values for mandarin and bitter orange seed oils.

In general, edible oils are semi-drying ones with iodine value ranging from 90-130 (Rady, 1976). Therefore, citrus seed oils could be used as edible oils especially if we know that cotton seed oil had an iodine value of 103-113 (Rady, 1976).

4.12.5. Saponification value:

The saponification value is a reflection of the average molecular weight of fatty acids. There was no considerable differences in saponification values of seed oils of the different citrus varieties.

The saponification values of Baladi orange, bitter orange and mandarin seed oils were 201.15, 201.44, and 201.58, respectively. These values are similar to those indicated by Shabana (1969) and Shoeb (1970) for seed oils of Egyptian citrus varieties. Many Egyptian investigators (Hafez, 1964; Youssef, 1980) and foreign workers (Jamieson, 1932; Murgo et al., 1943; Swift, 1949; Van Atta and Dietrich, 1944) reported slight lower values for orange seed oil (192-197).

The saponification number (201) indicate that all citrus seed oils had very close chain length of fatty acids which on the average, is slightly higher or comparable to that in oils from other sources such as soybean (192) cotton seed (195), Sesame (192) and olive seed (192) (Codd et al., 1975).

4.12.6. Unsaponifiable matter:

All edible fats or oils contain unsaponifiable matter in varying amounts ranging from a few tenths of one percent up to two percent (Mehilenbracher, 1960). Unsaponifiable matter was reported to exist an effect on oil stability (Kulta, 1972).

All citrus seed oil were found to contain unsaponifiable matter of less than 1% of oil constituents. However great variation in unsaponifiable matter content was observed among the seed oil tested. Baladi orange seed oil was shown to contain higher unsaponifiable matter (0.97%) than bitter orange and mandarin seed oils (0.81 and 0.67, respectively).

Consistent amounts of unsaponifiable matter were found in different seed oils of Egyptian citrus varieties (Shoeb, 1970; Youssef, 1980).

4.13. Fatty acidscomposition of citrus seed oils as determined by GLC analysis:

GIC analysis helped in separation of 11 methyl esters of fatty acids present in citrus seed oils investigated as shown in chromatograms presented in Figs. 11, 12 and 13).

As indicated in table 25, the major fatty acids, detected in citrus seed oils, in order of decreasing abundance were linoleic acid, palmitic acid, oleic acid, linolenic acid, stearic acid. Small amount of myristic, caprylic, pentadecenoic ($C_{15:1}$), palmitoleic ($C_{16:1}$) and margaric (C_{17}) acids were also found. Traces of one unidentified fatty acid was also found which amounted to about 0.12 and 0.24% of the total fatty acids in Baladi orange and bitter orange seed oils, respectively.

All citrus seed oilshad the same trend in higher unsaturated fatty acids and lower saturated fatty acids. However, they differed among themselves in total amount of these two groups as well as in the percentage contributing of each individual unsaturated or saturated fatty acids. The main unsaturated fatty acid in all seed oils was linoleic (50.12; 42.68, and 40.06%) in seed oils of Baladi orange, mandarin and bitter orange, respectively. On the other hand palmitic acid represented the major saturated fatty acid in all citrus seed oils.

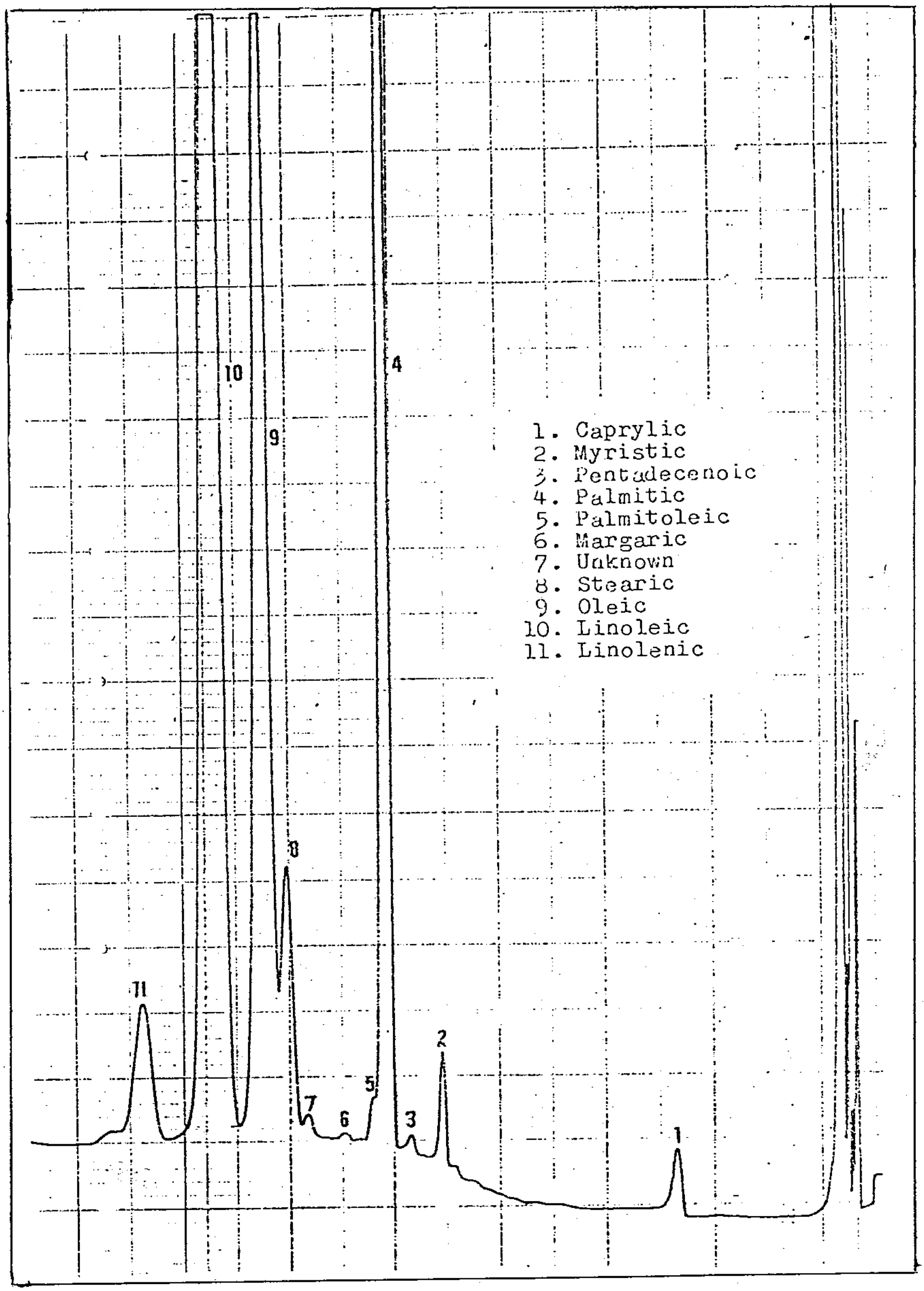

Fig. 11 – Chromatogram of GLC analysis of fatty acid methyl esters of Baladi orange seed oils.

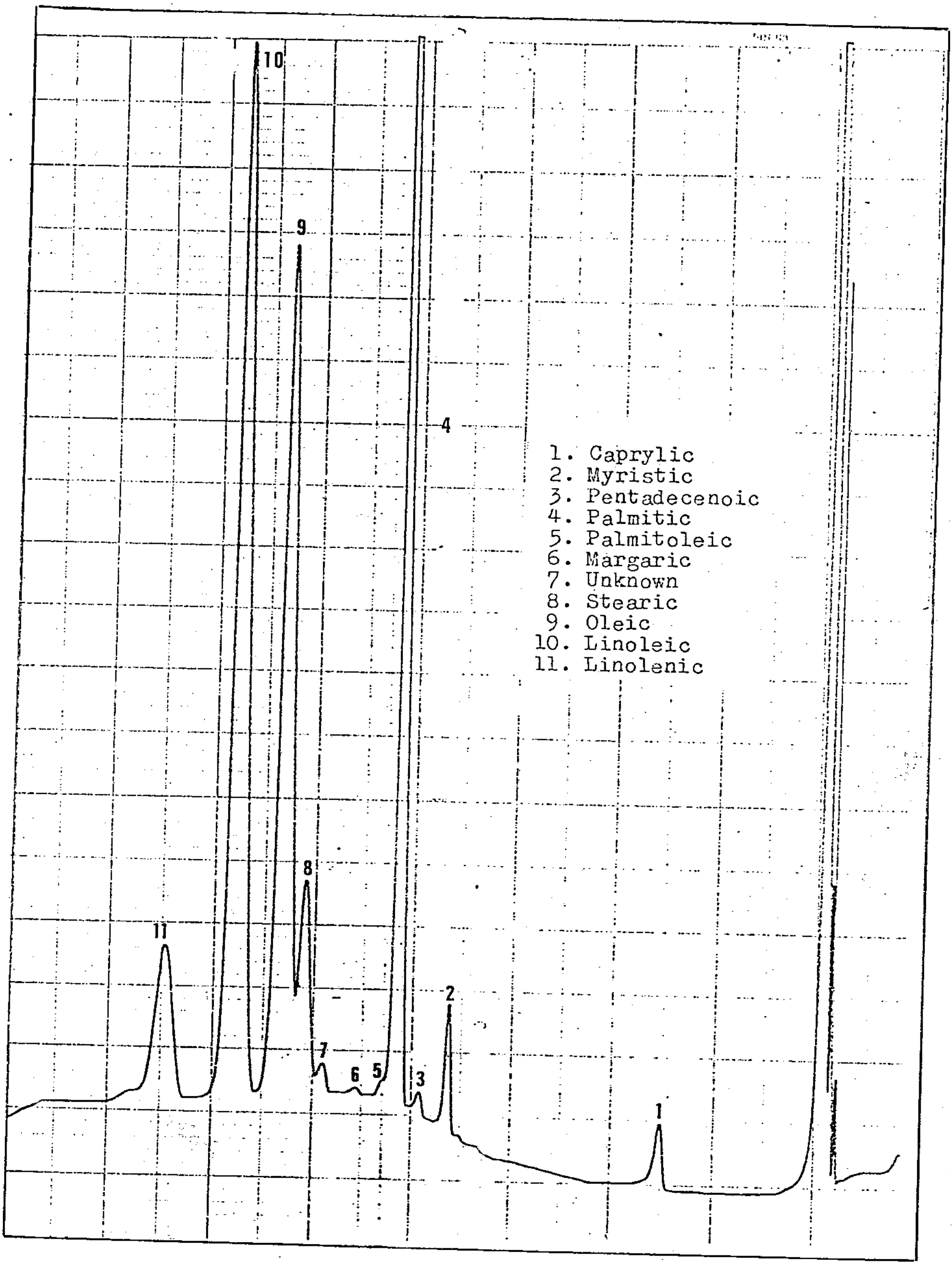

Fig. 12 - Chromatogram of methyl esters of fatty acids present in Bitter orange seed oils.

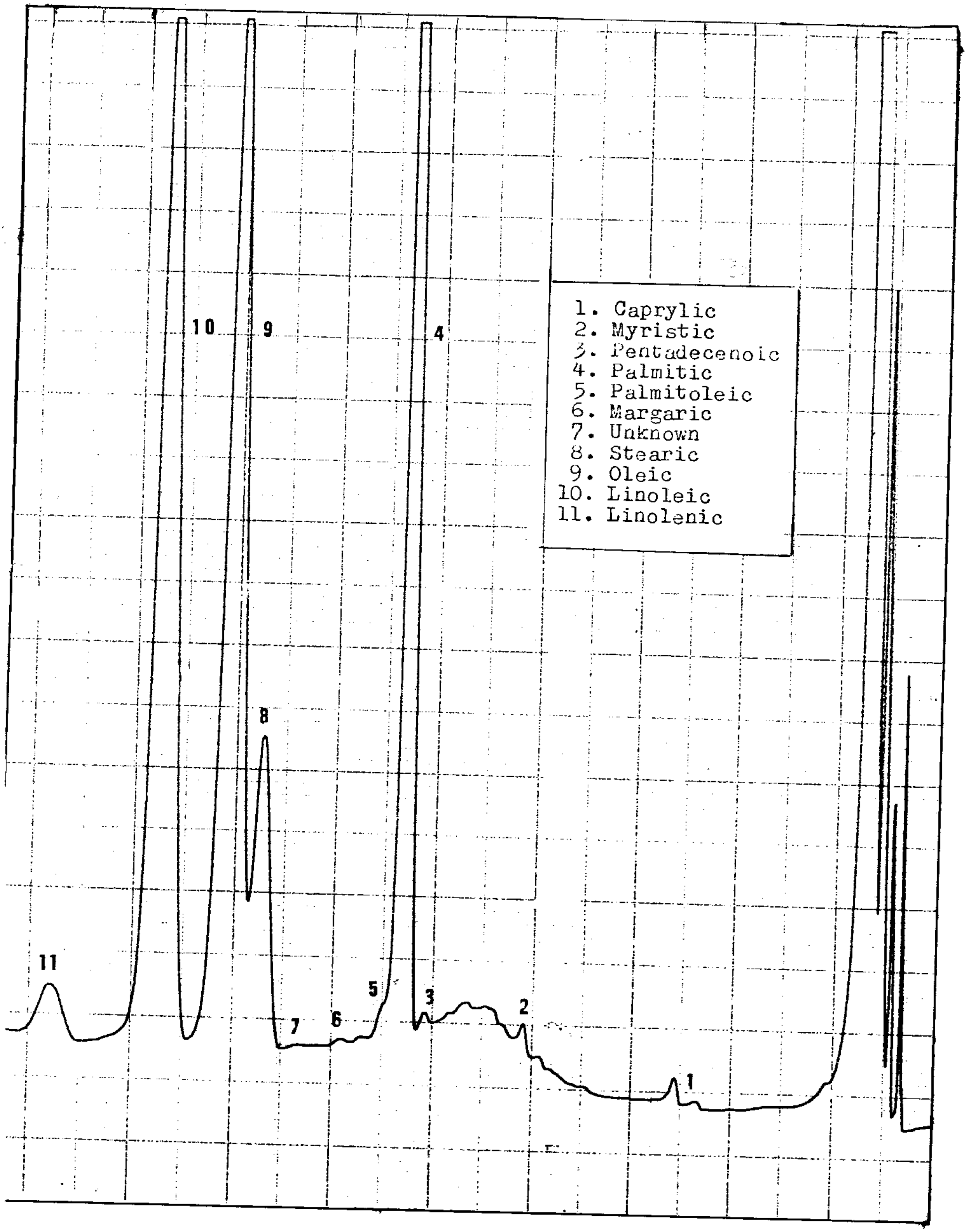

Fig. 13 - Chromatogram of GLC analysis of fatty acid methyl ester of mandarin seed oil .

Table 25 - Relative percentages of fatty acids components of some citrus seed oils as determined by GLC analysis.

Peak No.	Components		RRT*	Baladi orange seed oils		Bitter orange seed oils		Mandarin seed oils	
				Peak area (cm^2)	Area %	Peak area (cm2)	Area %	Peak area (cm^2)	Area %
1	Caprylic	(C_8)	0.2656	0.195	0.59	0.18	0.86	0.015	0.05
2	Myristic	(C_{14})	0.5145	0.275	0.83	0.255	1.22	0.041	0.13
3	Pentadecenoic	($C_{15:1}$)	0.6888	0.03	0.09	0.045	0.22	0.023	0.07
4	Palmitic	(C_{16})	0.7178	7.595	22.80	6.64	31.82	8.498	25.96
5	Palmitoleic	($C_{16:1}$)	0.7469	0.02	0.06	0.01	0.05	0.018	0.06
6	Margaric	(C_{17})	0.7925	0.01	0.03	0.01	0.05	0.008	0.02
7	Unknown		0.8499	0.04	0.12	0.05	0.24	Tr	Tr **
8	Stearic	(C_{18})	0.8797	0.495	1.48	0.49	2.34	1.373	4.20
9	Oleic	($C_{18:1}$)	0.9212	7.175	21.54	3.78	18.11	8.4	25.66
10	Linoleic	($C_{18:2}$)	1.00	16.695	50.12	8.36	40.06	13.97	42.68
11	Linolenic	($C_{18:3}$)	1.1203	0.78	2.34	1.05	5.03	0.385	1.18

* Relative retention time for linoleic was given a value of 1.00

** Tr = traces

Baladi orange seed oil had the highest amount of total unsaturated fatty acids (74.15%), due to its higher linoleic (50.12%), and the lowest total saturated fatty acids (25.73), due to reduced palmitic acid content, compared with other citrus seed oil (Table 25 and 26).

Bitter orange seed oil showed the highest amount of total saturated fatty acids, due to its higher content of each individual saturated fatty acid, pelmitic (31.82), stearic (2.34%), myristic (1.22%), caprylic (0.86%) and margaric (0.05%), Moreover, bitter orange seed oil, showed the lowest content in total unsaturated fatty acids, and in all individual mono and di-unsaturated fatty acid, except in linolenic acid.

Mandarin seed oil lied inbetween the other two seed oils in respect with amount of total unsaturated and saturated fatty acids. However, it had the highest content in oleic acid (25.66%) compared to others.

The fatty acid composition as determined by the present study is entirely comparable to literature values, from the point of relative percentage of main groups as well as amount of major fatty acids, despite of method of chromatographic analysis applied (Nofel et al., 1977; Youssef, 1980; Kamel, 1982; Tsuyuki et al. 1984).

Table 26 - Relative percentages of main groups of fatty acids persent in some citrus seed oils as calculated from gas-liquid chromatograms.

Components	Baladi orange seed oil %	Bitter orange seed oil %	Mandarin seed oil %
Total saturated fatty acids	25.73	36.29	30.35
Total mono-unsaturated fatty acids	21.69	18.38	25.79
Total di-unsaturated fatty acids	50.12	40.06	42.68
Total tri-unsaturated fatty acids	2.34	5.03	1.18
Total unsaturated fatty acids	74.15	63.47	69.65
Unidentified fatty acids	0.12	0.24	Tr.

Analysis of fatty acids in citrus seed oils provided an evidence that they could be classed among those oils with high content in total unsaturated fatty acids; and more precisely, they could be allocated in the oleic/linoleic group of the high unsaturated fatty acids oils, as distinguished from the linoleic/linolenic oils. In this respect, citrus seed oils contain a higher linoleic acid (40.06-54.82) and lower oleic (18.11-31.64), thus resembling cotton seed oil (of 42-54 linoleic and 23-35% oleic), corn oil (34-62 linoleic and 19-49 oleic) and sunflower Oil (44-75 linoleic and 14-43 oleic) (Codd et al. 1975). Moreover, citrus seed oils differ from olive, peanut, sesame oils as these oils contain a higher proportion of oleic than linoleic acid (Codd et al., 1975).

The amount of linolenic acid in citrus seed oils, especially that of Baladi orange (2.34%), is low, even half, that of soybean oil (5-11%). Such low linolenic acid could be considered as an advantage, in view of the reports that the difficulties in oil refining and the inability of complete elimination of taste, as in case of soybean oil, is associated with high linolenic acid content of oil (Wolf and Cowan, 1975).

Unsaponifiable matter:

Unsaponifiable matter as defined in the official and tentative methods of the American Oil Chemists Society is

4.14. Composition in unsaponifiable matter as determined by GLC analysis:

Unsaponifiable matter as defined in the official and tentative methods of the American Oil Chemists Society is "those substances frequently found dissolved naturally in fats and oils which cannot be saponifiable by the caustic alkaline, but are soluble in the ordinary fat solvents. Included are the higher aliphatic alcohols, sterols, pigments and hydrocarbons (Mehilenbrácher, 1960).

The unsaponifiable matter of citrus seed oils under investigation were identified using gas chromatographic technique and relative percentage of each compound separated was calculated as illustrated in table (27). Different number of peaks were present in chromatograms of each of the citrus seed oils, 33 peaks in that for bitter orange seed oils, 31 peaks in that for mandarin seed oil and 27 peaks in that for Baladi orange seed oil (Fig. 14, 15, 16). However, only 18 compounds were identified in all chromatograms according to their relative retention time. On the other hand, some peaks were not identified which were 16 unknown compounds in chromatograms of bitter orange seed oil, 13 in that for mandarin seed oil, and only 9 in that of Baladi orange seed oil. Total unknown compounds constituted about 7.72, 4.29 and 18.56% of total unsaponifiable matter in oils of Baladi, bitter orange mandarin seeds, respectively. The percentage of any individual unknown compound did not exceed 0.66% of total constituents of bitter orange seed oil. However, with the other two seed oils, some unknown compounds constituted a higher percentage than those unknowns in bitter orange seed oil.

Table **27** - Relative percentage of unsaponifiable matter components of some citrus seed oils-as determined by GLC analysis

Peak No.	Baladi orange seed oils				Bitter orange seed oils				Mandarin seed oils			
	Components	RRT *	Peak area (cm^2)	Area %	Components	RRT	Peak area (cm^2)	Area %	Components	RRT	Peak area (cm^2)	Area %
1	Unknown	0.0575	0.08	0.59	Unknown	0.0663	0.02	0.11	Unknown	0.0650	0.17	1.05
2	C_{14}	0.1899	0.04	0.29	Unknown	0.2107	0.04	0.22	C_{12}	0.0875	0.02	0.12
3	Unknown	0.2364	0.15	1.10	C_{16}	0.2671	0.03	0.16	C_{14}	0.1812	0.04	0.25
4	C_{16}	0.2722	0.28	2.06	C_{18}	0.3447	0.12	0.66	Unknown	0.2291	0.21	1.29
5	Unknown	0.3131	0.34	2.50	Unknown	0.3774	0.03	0.16	C_{16}	0.2688	0.50	3.08
6	C_{18}	0.3418	0.45	3.31	Unknown	0.3868	0.03	0.16	Unknown	0.2786	0.06	0.37
7	Unknown	0.3866	0.26	1.91	n-eicosane (C_{20})	0.4099	0.01	0.05	Unknown	0.3003	0.63	3.87
8	n-eicosane (C_{20})	0.4114	0.32	2.35	Unknown	0.4119	0.08	0.44	C_{18}	0.3438	0.94	5.78
9	n-hencosane (C_{21})	0.4430	0.54	3.97	Unknown	0.4465	0.12	0.66	Unknown	0.3715	0.56	3.44
10	n-docosane (C_{22})	0.4747	0.82	6.03	n-hencosane (C_{21})	0.4441	1.05	5.77	n-eicosane (C_{20})	0.4094	0.62	3.81
11	n-tricosane (C_{23})	0.5032	4.4	32.35	n-docosane (C_{22})	0.4720	1.34	7.37	Unknown	0.4241	0.04	0.25
12	n-tetracosane (C_{24})	0.5285	0.20	1.47	n-tricosane (C_{23})	0.5000	6.0	32.99	Unknown	0.4334	0.95	5.84
13	n-pentacosane (C_{25})	0.5570	0.20	1.47	n-tetracosane (C_{24})	0.5280	0.18	0.99	n-hencosane (C_{21})	0.4469	0.02	0.12
14	C_{26}	0.5791	0.19	1.40	n-pentacosane (C_{25})	0.5528	0.11	0.05	n-docosane (C_{22})	0.4750	0.99	6.09
15	Unknown	0.6198	0.13	0.96	Unknown	0.5597	0.09	0.49	n-tricosane (C_{23})	0.5000	1.25	7.69
16	n-octacosane (C_{28})	0.6329	0.02	0.15	Unknown	0.5786	0.05	0.27	n-tetracosane (C_{24})	0.5281	0.32	2.03
17	Unknown	0.6422	0.01	0.07	C_{26}	0.5807	0.04	0.22	n-pentacosane (C_{25})	0.5563	0.31	1.91
18	Unknown	0.6581	0.02	0.15	Unknown	0.6101	0.07	0.38	C_{26}	0.5813	0.22	1.35
19	Squalene	0.6709	0.06	0.44	n-octacosane (C_{28})	0.6273	0.03	0.16	Unknown	0.5944	0.14	0.86
20	C_{30} (C_{30})	0.6835	0.32	2.35	Unknown	0.6321	0.01	0.05	Unknown	0.6130	0.11	0.68
21	Unknown	0.7284	0.03	0.22	Unknown	0.6447	0.03	0.16	Unknown	0.6192	0.03	0.18
22	n-dotriacontane (C_{32})	0.7595	0.01	0.07	Unknown	0.6572	0.05	0.27	n-octacosane (C_{28})	0.6281	0.03	0.18
23	Unknown	0.7923	0.03	0.22	Squalene	0.6615	0.23	1.26	Unknown	0.6440	0.07	0.43
24	Cholesterol	0.8450	Tr.**	Tr.	C_{30}	0.6770	0.02	0.11	Squalene	0.6625	0.35	2.15
25	Campsterol	0.9304	0.26	1.91	Unknown	0.7170	0.04	0.22	Unknown	0.7059	0.02	0.12
26	Stigmasterol	0.9557	0.03	0.22	Unknown	0.7453	0.01	0.05	Unknown	0.7368	0.03	0.18
27	B-sitosterol	1.00	4.41	32.42	n-dotriacontanel (C_{32})	0.7578	0.02	0.11	n-dotriacontanel (C_{32})	0.7625	0.05	0.31
28					Unknown	0.7830	0.11	0.60	Cholesterol	0.8375	Tr.	Tr.
29					Unknown	0.8113	0.01	0.05	Campsterol	0.9125	0.5	3.08
30					Cholesterol	0.8385	0.08	0.44	Stigmasterol	0.9438	0.15	0.92
31					Campsterol	0.9193	0.84	4.62	B-sitosterol	1.00	6.93	42.62
32					Stigmasterol	0.9441	0.05	0.27				
33					B-sitosterol	1.00	7.40	40.68				
	TOTAL HYDROCARBONS			65.4				54				53.4
	TOTAL STEROLS			34.6				46				46.6

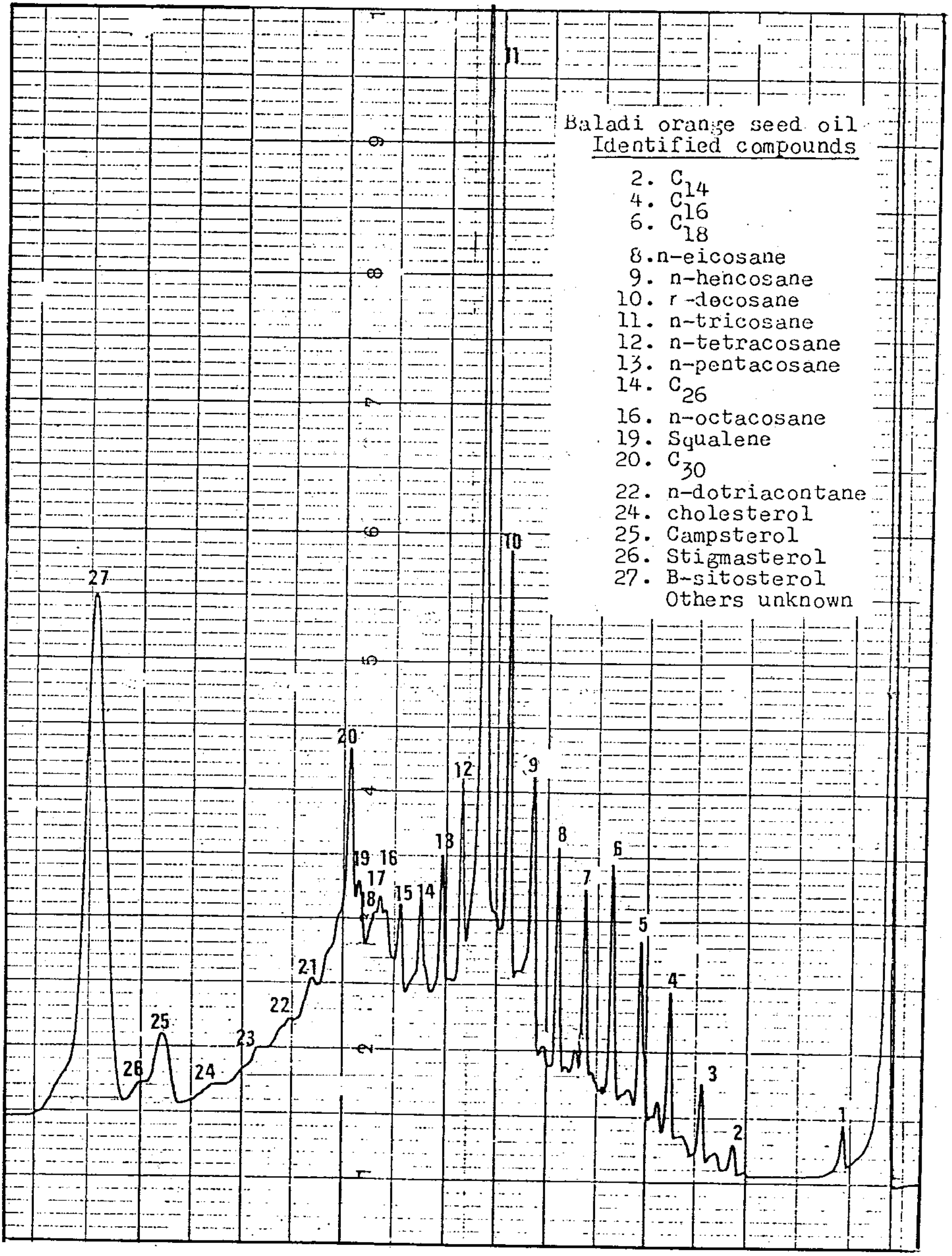

Fig. 14 - Chromatograms of GIC separation of unsaponifiable matters in Baladi orange seed oil.

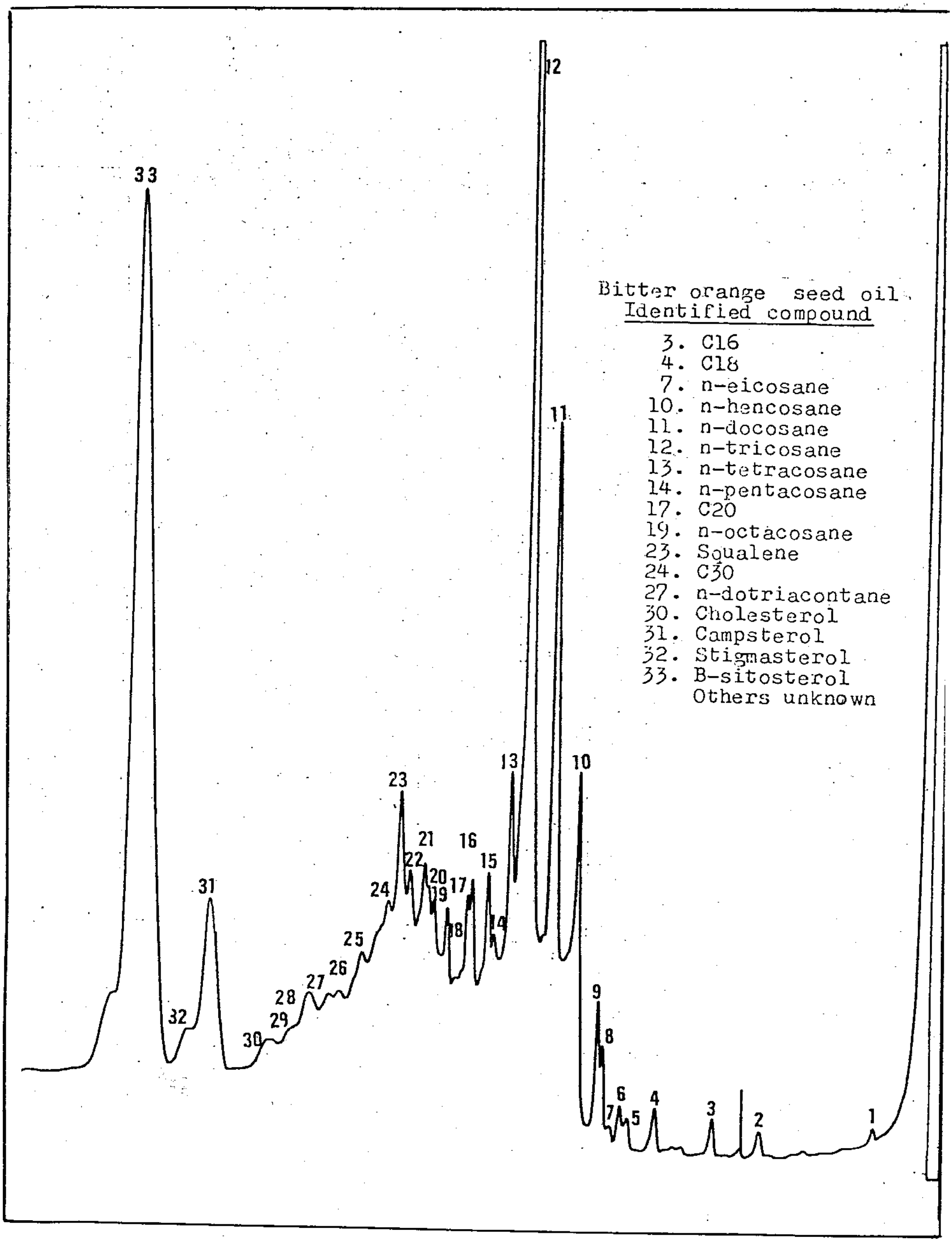

Fig. 15 - GLC analysis of unsaponifiable matters in bitter orange seed oil.

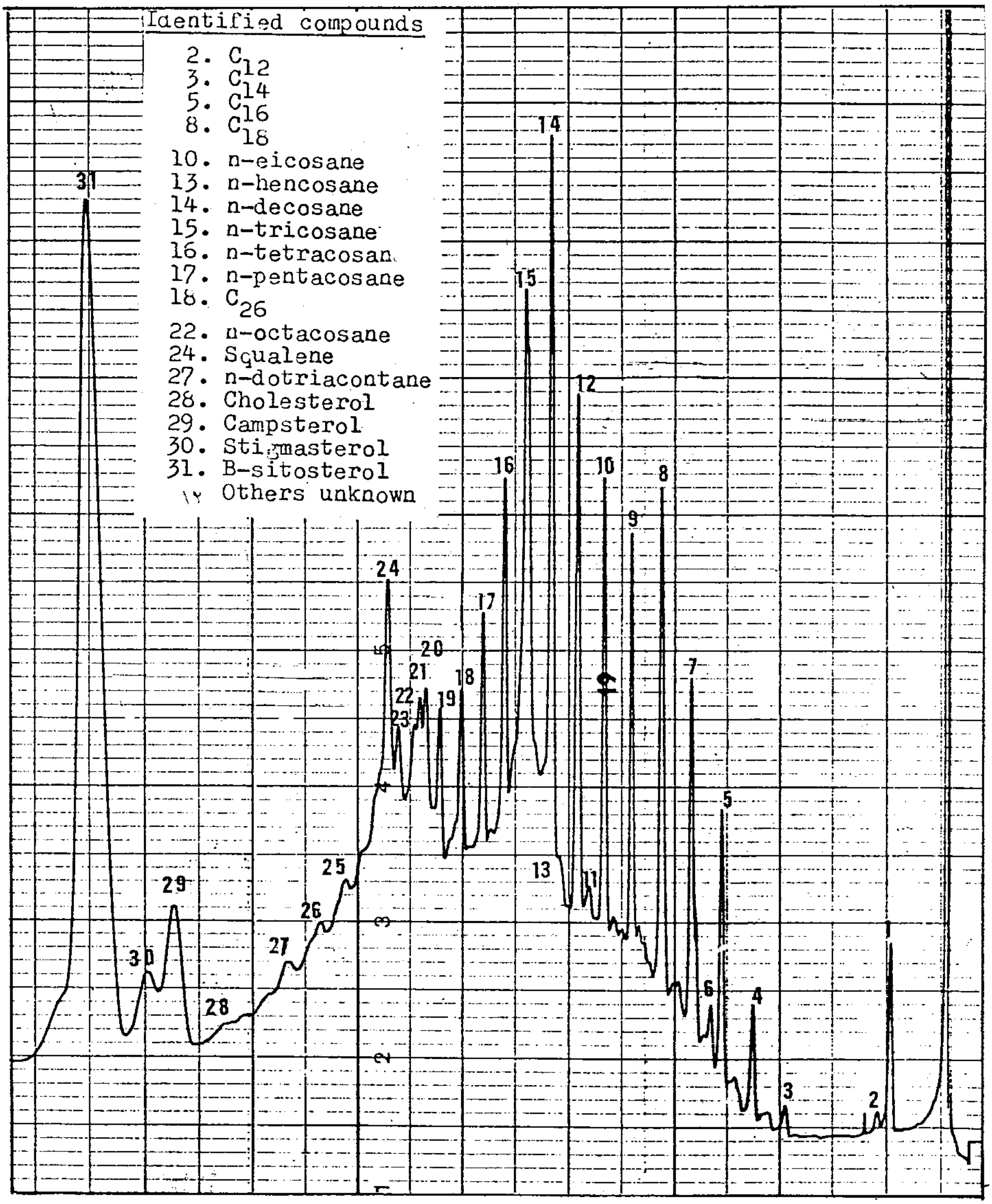

Fig. 16 - Chromatograms of separation of unsaponifiable matters in mandarin seed oils by GLC analysis.

Total hydrocarbons constituted a range of 53.4–65.4% of total components with Baladi orange seed oil having the highest margin while mandarin and bitter orange oils had comparable content of an average of 53.5%. The major hydrocarbon compound in all oils was n-tricosane (C_{23}) which constituted 32.3% in Baladi orange, 32.99% in bitter orange, and only 7.69% in mandarin seed oils. The other identified hydrocarbons were present in smaller concentrations. In all oils, n-docosane (C_{27}) was in second order of abundance constituting, 6.03%, 7.37 and 6.09% in oils of Baladi orange, bitter orange and mandarin seeds. Moreover, squalene, a major hydrocarbon in many edible oils, was present in amounts of 0.44, 1.26 and 2.15% in oils of Baladi, bitter orange and mandarin seeds, respectively. It should be noted that nearly all oils contain squalene, and olive oil has been reported to contain higher content of it than other oils (Jacobs, 1962).

Amount of other hydrocarbons, did not exceed more than 2 % of components, except C_{16}, C_{18}, C_{20}, C_{21}, C_{30} in Baladi orange, C_{21} in bitter orange and C_{16}, C_{18}, C_{20} and C_{24} in mandarin seed oil.

Expressed in terms of peak area, sterols were present in proportion of 34.6–46.6% of the total unsaponifiable matter of citrus seed oil. Sterols which are found in all edible fats and oils were present in comparable concentration in bitter orange and mandarin seeds oils (46 and 46.6%, respectively) while Baladi orange oil had lower proportion (34.6% of total unsaponifiable matter). In all citrus seed oils, sterols were fractionated into four main constituents which were, in order of decreasing abundance, B-sitosterol, campsterol, stigmasterol and cholesterol. B-sitosterol was the major sterol compound, and amounted 42.6, 40.68 and 32.42% of unsaponifiable matter in seed oils of mandarin, bitter orange and Baladi orange, respectively.

Although citrus seed oils showed variation in content of B-sitosterol, especially Baladi orange oil compared with the other two seed oils, they showed less variation with respect to the relative contribution of B-sitosterol to total sterol compounds, with the highest proportion in Baladi orange seed oil (93.9%) as compared to the others (91.4 and 88.5% of sterol separated from oils of mandarin and bitter orange seeds, respectively) (Table 28). Campsterol constituted higher proportion of sterols of bitter orange seed oil (10%) compared to those of mandarin and Baladi orange (6.6 and 5.5% of total sterols). Stigmasterol constituted 2% of sterols of seed oil mandarin and only 0.6% in both seed oils of Baladi and bitter orange.

Table 28 - Total and relative percentage of sterol compounds present in some citrus seed oils.

Compounds	Baladi orange seed oil %	Bitter orange seed oil %	Mandarin seed oil %
Cholesterol	Traces	0.9	Traces
Campsterol	5.5	10.0	6.6
Stigmasterol	0.6	0.6	2.0
B-sitosterol	93.9	88.5	91.4
Total phytosterols	100.0	99.10	100.0

Cholesterol was only detected in very small proportion in oil of bitter orange seeds constituting 0.44% of unsaponifiable matter and about 0.9% of total sterol compounds in oils. However, it was found as traces in the other two seed oils. Cholesterol is assumed to be a characteristic of animal fat while phytosterol, mainly sitosterol, is the sterol characteristic of vegetable oils and fats (Mehlenbacher 1960; Jacobs, 1962).

Results of the present study are comparable to these of Barroso et al. (1972) who found also the three sterol detected in our studies in grapefruit seed oil where B-sitosterol, campsterol and stigmasterol constituted 90.1, 7.4 and 2.5%, respectively.

Moreovers, the found result are in close agreement with those of Tortosa et al. (1977) who found the sterol fraction of citrus seed oil to contain 79-86% B-sitosterol in addition to campsterol, stigmasterol and cholesterol.

Analysis of sterols present in other seed oils indicate that B-sitosterol constitute an average values of 72.9, 81.9 and 96.2% of sterol compounds of oils of soybean, safflower and cotton seed. (Barroso et al., 1972). Cotton seed oil was found not to contain stigmasterol while safflower and soybean oils contained as average of 5.6 and 12.5%.

It should be mentioned that γ-sitosterol was reported in edible oils but it was not detected in the present study. Moreover, tocopherol was not detected although Tortosa _et_ _al_. (1977) reported a low value for them in citrus seed oils.

All data collected in the present study lead to conclude that citrus seed oils could be considered edible ones, as previously suggested by Braverman (1949) Parekh _et_ _al_. (1959). Moreover, its rather high linoleic acid content, similar to corn oil and sunflower oil adovocate it as a suitable dietary fat, especially in conditions of heart disease and high blood cholesterol levels. However, another uses can be created as they may serve as a potential in drying oil industries i.e. in paints, varnishes, linoleum, printing ink, etc. Moreover, their fatty acids could be used for manufacturing soap, as previously suggested by Srinivasara and Gogte (1976).

Part V. Characterization of citrus seed meal:

In view of exploring the potential of citrus in providing high protein material, the present work was planned analyse different kinds of citrus seeds, after and before fixed oil extraction, to be utilized as safe source for nutritious food or feed. For such purpose, seeds were collected from by-products, washed, air dried and ground and prepared to provide whole seed meal, defatted seed meal, dehulled seed meal (kernels) and seed hulls.

Evaluation of citrus seed meals and seed components included the determination of proximate chemical composition,crude fiber and mineral content and soluble protein classes or fractions.

4.15. Amount of seed recovered from citrus by-products:

As illustrated in table 29, amount of seed differed with kind of citrus fruits and ranged between 0.5-6.8% of fresh weight. The greatest amount was found in bitter orange,by-products (6.8% of fresh weight). Mandarin contained higher amounts than oranges (4.5 vs. 2%) while grapefruit showed very small seed content (0.53% of fresh weight). Ammerman et al. (1963) reported comparable seed contents (1.8-8.3% seeds).

Table 29- Weight and percentage composition of seeds and their components obtained from some citrus fruits.

	Balady orange	Mandarin	Bitter orange	Grape-fruit
Weight of seeds per kg of fruits	20	45	68	5.3
Percentage of seeds	2	4.5	6.8	0.53
Moisture content % of seeds (as is basis)	56.30	52.30	56.10	—
Percentage of kernels in seeds (air dry basis)	70.73	72.76	69	71.62
Percentage of hulls in seeds (air dry basis)	29.27	27.24	31	28.38
Weight of 100 seeds (air dried)	14.52	12.52	12.20	16.237
Weight of kernels in 100 seeds	10.27	9.11	8.42	11.629
Weight of hulls in 100 seeds	4.25	3.41	3.78	4.608

Despite kind of fruit, seed had comparable values for moisture content. Kernel constituted about 69.0-72.8% while hulls constituted 27.24-31% of seed weight. These proportions are slightly different than those reported by Youssef (1980) that hulls of seeds of various Egyptian citrus cultures formed 38-43% while kernels constituted 58-62%.

Mandarin seeds showed slightly higher kernels and lower hulls than other citrus seeds. Data of weight of 100 seeds or their components indicated that different fruit were arranged on a decreasing order from the point of seed weight as grapefruit, Baladi orange, mandarin and finelly bitter orange. The presence of small proportion of seeds in grapefruit variety tested, inspite of their bigger size, would render their separation from by-products not feasible under practical conditions, and therefore, they were not analyzed or prepared into meals as practiced with seeds of other citrus.

4.16. Chemical composition of whole seed meals and components of seeds:

Chemical analysis of whole and dehulled seed meals (Table 30) indicated that crude oil constituted the major component which ranged between 36-38.19% of dry weight of whole seed meals where bitter orange seed had the highest amount over those of other citrus. Crude protein constituted the second component with respect to amount as well as importance where it ranged from 11.83-16.22% of dry weight. In order of decreasing abundance, amount of crude protein in whole seed meal, dehulled meal or kernels and hulls, were present in mandarin, Baladi orange and bitter orange. Dehulled meals or kernels contained higher content

Table 30 - Proximate chemical composition of seed components of some citrus fruits (g/100 g dry weight basi

Analysis	Baladi orange			Mandarin			Bitter orange		
	whole	kernels	hulls	whole	kernels	hulls	whole	kernels	hul
Crude protein	15.66	19.68	6.58	16.22	19.31	5.33	11.83	18.73	4.
Ether extract	36	39.77	4.05	27.02	31.56	1.80	38.19	39.98	3.
Ash	3.08	3.29	2.95	3.03	3.32	2.68	2.24	2.64	1.
Total carbohydrates(by difference)	12.88	8.18	66.85	19.01	10.58	71.5	27.03	19.59	75.
- Crude fiber	11.91	2.09	30.40	14.63	2.56	38.12	15.87	4.64	36.
Moisture	32.38	29.08	19.57	34.72	35.23	18.69	20.71	19.06	14.

of oil (31.56-39.98%) and also crude protein (18.73-19.68%) due to the small content of these components in hulls.

Carbohydrates, mainly crude fiber, represented the component in the third order from the point of . amount. In whole meals carbohydrates ranged from 12.88-27.03, while crude fiber ranged from 11.91-15.87%. Bitter orange whole seed had the highest amount of both components while that of Baladi orange contained the lowest amount . Dehulling of seeds resulted in decreasing crude fiber content in dehulled meals(or kernels) which ranged from 2.09 to 4.64% of dry weight.

Ash content in whole seed meals ranged from 2.24 to 3.08%, whole seeds of Baladi orange and mandarin contained comparable amount of ash (3.08 vs. 3.03%) but ash was much lower in bitter orange seed (2.24% dry weight). Slight higher amounts of ash were present in kernels,mostly due lower ash content in hulls.

With regard to hulls left after preparation of dehulled meals, they contained higher amounts of crude fiber (30.40-38.12%) fair amount of crude protein (4.50-6.58%) and ash (1.71-2.95%) but low oil content (1.80-4.05). Hulls of Baladi orange seeds, in particular,contained the highest content of crude protein, ether extract and ash, but the lowest content in fiber compared to other hulls.

Comparison of the obtained data with those in literature reveals close agreement with results of Ammermann et al. (1963) who indicated that dried citrus seeds contained (on 90% dry matter basis) 40.5% ether extract, 14.6% crude protein, 19.7% carbohydrates, 12.1% crude fiber and 3.1% ash. In contrast to our results, Youssef (1980) reported much lower values in most constituents of citrus seed (protein, 4.0-4.4% ,ash 2.0-2.8% crude fiber 4.0-4.4%). Furthermore, he reported comparable content of oil (24.3-38.6%) and higher content in carbohydrates (51.2-65.1%). However, he did not indicate whether these obtained values were calculated on as is basis or dry matter basis.

4.17. Chemical analysis of defatted seed meals:

Removal of fixed oils from seed press-cake or whole seed meal resulted in increasing concentration of other nutritents,except moisture (Table 31). Defatted meals contained 7.35-8.94% moisture instead of 20.71-34.72% moisture in whole meals of air dried seeds. In mandarin, Baladi orange and bitter orange defatted meals crude protein content reached 18.76, 17.55 and 15.33%, respectively, ash represented 4.18, 3.88 and 3.2% of dry weight, respectively. while crude fiber of defatted meals of whole seeds constituted 18.17, 16.01 and 19.86%, respectively. The lower protein content in bitter orange meal is related to its higher content of total carbohydrates, especially crude fiber.

Table 31 - Proximate chemical analysis of defatted citrus seeds (g/100 dry weight basis).

Analysis	seeds of Balady orange	seeds of Mandarin	seeds of Bitter orange
Crude protein	17.55	18.76	15.33
Ash	3.88	4.18	3.21
Total carbohydrates(by difference)	70.4	68.12	74.11
- Crude fiber	16.01	18.17	19.86
Moisture	8.17	8.94	7.35

4.18. Mineral content of whole citrus seed meals and components of seeds:

Mineral analysis included the spectrophotometeric determination of phosphorus and determination of Na, Ca, Mg, K, Cu, Mn and Zn by flame atomic abosorption spectroscopy (Table 32).

From the point of availability of mineral elements in all whole seed meals they were ranked, in a decreasing order of abundance, as K, Ca, Na, Mg, Zn, Mn, Cu and P. The only exception for such order was found in mandarin whole seed meal and also its seed kernels, where Mg ranked the third instead of Na, which ranked, in turn, the fourth from the point of abundance. Seed kernels and hulls of all fruit seeds exhibited the same trend of the order of mineral concentration, except that, sometimes Na was found either lower or higher than Mg. Hulls from all seeds showed distinctive higher concentration in Ca than dehulled meals or kernels.

In general, mineral matter in seeds of Baladi orange or mandarin were almost higher than those present in bitter orange seeds. Data of mineral analysis correlated with those of ash content (Table 30 and 31). Although the three citrus seeds did not differ in the general gradual order of mineral distribution, they varied greatly with regard to concentration of each of these minerals. Such variation

Table (32) - Chemical analysis of mineral content of different citrus seeds and their components (hulls and kernels) reported in mg% of dry weight.

Element[1]	Baladi orange			Mandarin			Bitter orange		
	Whole	Kernels	Hulls	Whole	Kernels	Hulls	Whole	Kernels	Hulls
Macro element:									
Calcium	454.265	432.558	593.177	401.454	200.405	603.002	212.421	258.712	481.928
Magnesium	192.485	182.771	152.302	157.173	139.676	96.034	129.384	168.955	134.085
Sodium	249.461	152.309	207.772	95.440	69.636	117.920	133.632	119.3 4	121.259
Potassium	808.439	810.284	596.383	1022.572	991.903	576.202	473.119	580.780	660.707
Phosphorus	0.42	0.24	0.11	0.24	0.33	0.07	0.24	0.33	0.23
Micro element:									
Copper	1.925	1.340	2.437	1.060	0.891	0.670	1.043	1.214	1.399
Manganese	2.002	1.706	1.539	1.212	1.377	0.983	1.081	1.056	1.166
Zinc	2.926	2.681	1.924	2.500	1.700	0.849	1.892	2.376	3.964

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[1] Average of two analysis.

might reflect the difference in mineral uptake of different citrus fruits during growth period which is certainly influenced by location, environmental conditions and different agricultural practices. Among these factors, fertilization, especially nitrogen and calcium or phosphate fertilizers, have been reported to influence greatly the mineral content (Harris and Karmas, 1975). Literature relating to such effect and in relation to effect of soil are voluminous and generally contradictory.

There is lack of literature concerning reliable quantitative determination of mineral matter of citrus seed meals so as to be compared with our data. However, Gallarati and Lanza (1973), Kamel et al. (1982) mentioned that they contain appreciable amounts of Ca, Mg, K, Mn, Cu, Zn and P. Result of the present study indicate also such trend, except for content of P which appeared to be very low. The observed higher Ca content in seed hulls than kernels could be explained on similar basis to the findings of Verma et al. (1964) that seed coats of various plant seeds contain high Ca content which is in the pectate form.

The recommended daily allowance for humans is established for only five minerals (NAS, 1974) while mineral requirement are established for Ca, Mg, Fe, Zn, Cu and I

(FAO,1974). It seem that consumption as small as about 100 g of each citrus seed meal would satisfy the daily recommended allowance and, in turn, requirements for Mg (4-100 mg/day), Cu (1.3-2 mg/day), Ca (400-500 mg/day) for infants) while probably 250-300 g of these meals per day would be sufficient to provide requirements for Zn (6 mg/day).

These results would prove that citrus seed meals contain appreciable amounts of minerals and that could be used as a good source for these minerals in human foods as well as animal feeds (Glasscock *et al.*, 1950).

4.19. Classification of citrus seed proteins:

The classical Osborne classification of unheated whole flour proteins into general classes, on the basis of solubility, (Osborne and Mendel, 1914) was carried out to provide information of nature of proteins present in citrus seed meals. Such information is valuable upon further extraction of these proteins to prepare protein products and to know and estimate the nutritional value of these proteins from the point of amino acid make-up as each protein fraction is known to be characterized with special amino acid composition.

Results of fractionating proteins of defatted citrus meals based on their solubility in different extraction systems are presented in Tables 33, 34, and 35. The majority of soluble proteins in citrus seeds were globulins, which represented 43.54 and 35.38 of total proteins of orange and mandarin seeds, respectively. Albumins constituted the second major class (18.19 and 24.04, respectively) while glutelins were the third fraction from the point of abundance and amounted to 8.98 and 11.55, of total proteins, respectively. In contrast the major class in bitter orange seed protein was glutelins while globulins and albumins were in the second and the third position. Prolamins were the least fraction in all the three kinds of seeds which was present in trace amount in orange seeds and reached 4.72, 3.62% and traces total proteins in those of mandarin and bitter orange and Baladi orange, respectively.

The observed variation in percentage contribution of different protein classes in proteins of different seeds in function to kind of fruit (Fig. 17) would contribute to the difference in amino acid composition of seeds of different kind of citrus as previously indicated by Braddock and Kesterson (1972).

It should be mentioned that the albumin fraction does not contain only the water soluble proteins, since salt, naturally present in seeds would solublize globulins.

Table 33 - Classification of **Baladi orange** seed protein based on Osborne classical solubility, fractionation.

Fraction	Condition of extraction	Supernatant volume ml	Total Nitrogen g N/100 ml	Total nitrogen mg N/total extract	Extraction yield %
Water soluble albumin	Distilled water	79	0.030	23.46	18.19
Globulin	1 M NaCl, pH = 7	95	0.59	56.42	43.74
Prolamin	70% aqueous ethanol	80	Traces	Traces	Traces
Glutelin	0.1 N NaoH pH = 11	91	0.13	11.58	8.98
	Residue		0.113*		25.03
Non-protein nitrogen	10% Tri-chloro acetic acid	82	0.011	4.36	16.92
	Residue		2.39*	-	92.81

* g per 100 g of dry seed meal.

Table 34 - Classification of Bitter orange seed protein based on Osborne classical solubility, fractionation.

Fraction	Condition of extraction	Supernatant volume ml	Total Nitrogen g N/100 ml	Total nitrogen mg N/total extract	Extraction yield %
Water soluble albumin	Distilled water	82	0.028	22.96	20.22
Globulin	1 M NaCl, pH = 7	89	0.034	29.90	26.33
Prolamin	70% aqueous ethanol	74	0.006	4.14	3.65
Glutelin	0.1 N NaoH pH = 11	88	0.039	39.50	30.37
	Residue		0.112*		23.79
Non-protein nitrogen	10% Tri-chloro acetic acid	86	0.003	1.14	5.04
	Residue		2.1945*		96.61

* g per 100 g of dry seed meal.

Table 35 - Classification of **Mandarin seed** protein based on Osborne classical solubility, fractionation.

Fraction	Condition of extraction	Supernatant volume ml	Total Nitrogen g N/100 ml	Total nitrogen mg N/total extract	Extraction yield %
Water soluble albumin	Distilled water	83	0.040	32.86	24.04
Globulin	1 M NaCl, pH = 7	95	0.050	48.36	35.38
Prolamin	70% aqueous ethanol	76	0.008	6.45	4.72
Glutelin	0.1 N NaoH pH = 11	93	0.017	15.78	11.55
	Residue		0.113*	-	24.34
Non-protein nitrogen	10% Tri-chloro acetic acid	84	0.008	3.3516	12.26
	Residue	-	2.527*	-	92.45

* g per 100 g of dry seed meal.

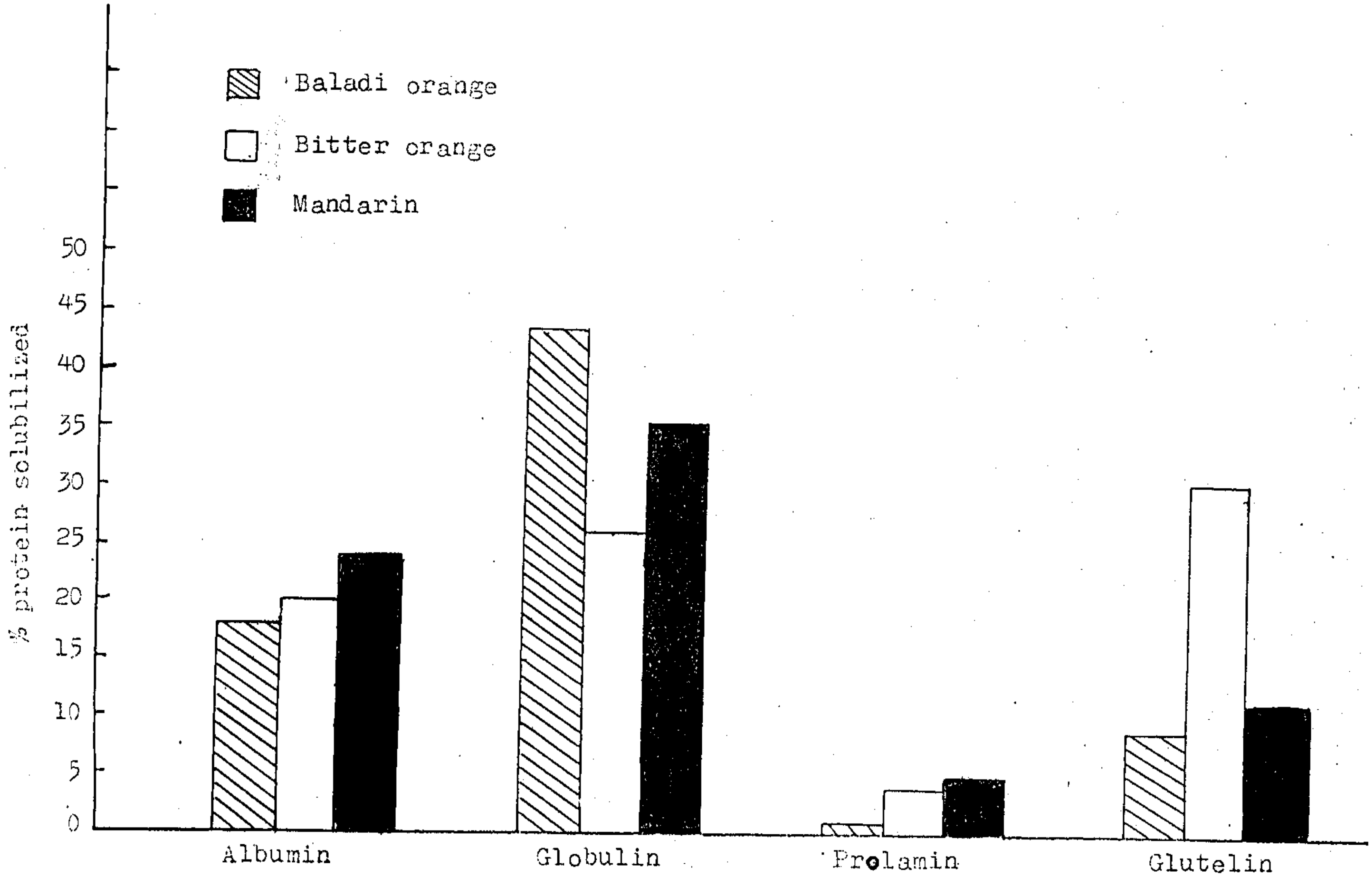

Fig.17 - Protein fractionation based on Osborne solubility classifications of proteins from Baladi orange, bitter orange and mandarin defatted seed meal.

Besides, this fraction contained also the non-protein nitrogenous constituents which amounted 16.92, 12.26 and 5.04% of total seed proteins of oranges, mandarin and bitter orange, respectively. Non-protein nitrogenous substances include free amino acid, peptides, nitrogen bases which do not precipitate upon using 10% TCA solution.

Results of protein fractionation into classes indicate that about 75% of proteins of citrus seed meals are readily extractable as were solublized in the various solvents utilized. However, about 23-25% of proteins were found unsoluble and were present in residue after the successive extraction, probably as a result of physical phenomena caused by protein-starch matrix configuration of seed (Skoch <u>et al</u>., 1970). Moreover, of these residual proteins in meals are those that are denatured or conjugated with different non-protein groups constituting glucoproteins,lipoproteins, chromoproteins and others. Furthermore, high content of fiber bodies, as shown in Tables 30 and 31 could, by occluding proteins, prevent their extraction.

For the best of our knowledge, any data concerning nature or classification of proteins of citrus seed meals are lacking. Therefore, the present results would encourage research in this field so as to provide further information of protein of citrus seed to be utilized in many edible uses by humans as well as by animals as feed.

Results of the present part indicate that possible nutritious meals could be prepared in different form (whole, defatted or dehulled) as processing facilities are available in citrus manufacture plants. About 887 kg of whole seed meal would be produced daily while if oil is extracted about 754 kg of defatted meal will be obtained daily from by-products of Kaha Company Egypt (11 ton of by-products are produced from 20 ton citrus processed daily in the season). Methods for separation and milling of citrus seeds have been developed (Nolte and Von Loesecke 1940, Eckey, 1954, Teles et al., 1972). Deriggers et al. (1951) reported a procedure for removal of bitter factor present in citrus seed meals. Citrus seed meals were found safe and of high nutritional value in animal rations (Glasscock et al., 1950; Deriggers et al., 1951, Ammermann et al., 1963).