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INTACT ARCHAEOLOGIAL, WHEAT AND BARLEY GRAINS AND TIME LAPSE

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Chemical changes that occurred with lapse of time in intact barley (Hordeum vulgare) spikelets from three successive periods namely 12th Dynasty, Roman and Recent and (Triticum dicoccum) dating to 12th Dynasty and Recent were investigated. The 12th Dynasty Hordeum vulgare and Triticum dicoccum were excavated from Gabel El-Zeit area while Roman Hordeum vulgare was excavated from Abu Shaar site. Preliminary investigations using infrared spectroscopy revealed a complex mixture of organic compounds which differ in wheat and barley and change markedly with time lapse. Standard gas chromatographic procedures were used to analyze fatty acids and hydrocarbons in the investigated spikelets. The odd chain aliphatic hydrocarbons C_{25}, C_{29} and C_{31} were detected in high levels in Recent spikelets which decrease gradually with time lapse. The dominant fatty acids are C_{16}, C_{18} and $C_{18.1}$ in ancient barley and C_{16}, $C_{18.2}$ in ancient wheat. This pattern can be used to distinguish between them. Also C_{12} and C_{14} were identified from recent barley spikelets which disappeared from the 12th Dynasty spikelets and this can be used for dating archaeological barley. On the other hand, $C_{18.2}$ was detected in recent wheat and disappeared from archaeological wheat and this can be used for dating such samples.

Keywords : *Archaeochemistry; Hordeum vulgare; Triticum dicoccum; Infrared spectroscopy; Fatty acids; Hydrocarbons.*

Introduction

Grain production during the Predynastic Egypt is thought to have been elemental in the development of ancient Egyptian culture. Grain cultivation control and use during the Predynastic is however poorly understood at present (Mills, 1982). Bassett et al. (1980) suggested that storage of grains in mud bins may have provided an environment conducive to the growth of *Streptomyces*, which produce tetracyclines. These organisms prefer the alkaline soils and dry climate typical of Upper Egypt and Lower Nubia (Waksman, 1967). The conservation of archaeologic barley and wheat spikelets may be attributed to the presence of these tetracyclines (Mills, 1982). Furthermore, Farag et al. (1986) suggested that fungal attack did not seem to destroy the grains.

Identification of charred grains from archaeological sites traditionally relies on comparing a range of morphological criteria with those in modern, living, plant population using stereoscopic microscopy or scanning electron microscopy (McLaren et al., 1990). Preservation by charring can modify the morphology of ancient grains of closely related taxa such as various species of wheat and rye (Körber-Grohne, 1981; Colledge, 1988; Holden, 1990). On the other hand, McLaren et al. (1990) showed that it was possible to identify charred archaeological grains to sub-species level using infrared specectroscopic analysis.

Lipid analyses are useful in the identification of vessel contents because lipids are contained in virtually all human food, both plant and animal. Moreover, lipids in particular fatty acids are well preserved as compared with carbohydrates and proteins (Rottländer, 1990).

In the present investigations, standard chromatographic procedures are used to analyze ancient grains of *Hordeum vulgare* (12th Dynasty and Roman) and *Triticum dicoccum* (12th Dynasty), whose organic moieties, hydrocarbons and fatty acids will be identified by cross referencing with modern grains.

Materials

The investigated barley (*Hordeum vulgare*) and wheat (*Triticum dicoccum*) spikelets (Fig. 1) dating back to 12th Dynasty were excavated from Gabel El Zeit area. (Red Sea, 80 km north of Hurghada). Another sample of barley (*Hordeum vulgare*) dating back to Roman period was excavated from Abu Sháar site (Red Sea, 20 km north of Hurghada), Fig. (1). The recent *Hordeum vulgare* and *Triticum dicoccum* spikelets were provided from Crop Department, Agriculture Research Center, Giza, Egypt.

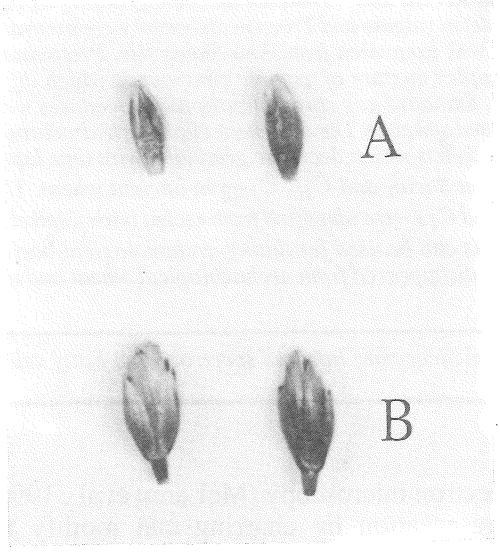

Fig. 1. Photographic view for 12th Dynasty spikelets:
A: *Hordeum vulgare* B: *Triticum dicoccum*

Methods

All the investigated samples were selected as intact spikelets. Equal weights (1 gm) of spikelets were used for anayses, and treated under the same extraction condition. The grains were initially extracted using standard soxhlet apparatus for four hours per solvent using 100 cm^3 of increasing polar solvents: hexane, chloroform and 2-propanol.

The infrared spectroscopic (IR) analysis (McLaren et al., 1990), was carried out using Perkin Elmer IR spectrometer PE 781 and Perkin Elmer 3600 data system. The extract (hexane, chloroform and 2-propanol) was spotted on the prepared potassium bromide disc, the reference disc was spotted with the same solvent used in the extraction, by this way any contaminant from the solvent was neutralized. The spectrum was measured in range 2000-600 cm^{-1} (5-17 μm).

Prior to the GC analysis, lipid fractions (n-hexane and chloroform extracts) were hydrolysed using alcoholic potassium hydroxide (10%) and each sample was refluxed for 15 minutes. After cooling, the sample was extracted three times with a small quantities of hexane; this extract contained non-saponified fraction (mainly hydrocarbons). The sample was neutralized with 2N hydrochloric acid and again extracted three times with hexane. This extract contains free fatty acids, which were methylated using ethereal solution of diazomethane and injected to gas chromatograph (GC).

Separation of hydrocarbons was carried out following the method of Rady et al., (1987). The instrument used was Perkin Elmer Sigma 2B GC, equipped with SP 4270 integrator. The column type was OV-101, 1.5x10.4 mm. glass column, oven temperature (isothermal at 250°C), injector and detector temperature (300°C), flow rates: oxygen free nitrogen 80, hydrogen 60 and air 220 ml/min.

GC separation of fatty acids were carried out using the method of Farag et al. (1990). The instrument was Perkin Elmer Sigma 2B GC, equipped with SP 4270 integrator, 1.5 mm x 0.4, glass column packed with 10% DEGS polyester on 80/100 chromosorb W-AW. Oven temperature was isothermal at 190°C, injector and detector temperatures were 300°C and 220°C respectively. Flow rates: oxygen free nitrogen 80, hydrogen 60 and air 220 ml/min.

Results and Discussion

The IR screening of Hordeum *vulgare* spikelets from three successive periods, namely 12th Dynasty, Roman and Recent, is shown in Figs. 2,3 and 4. Similarly, the IR spectra of *Triticum dicoccum* of 12th Dynasty and Recent are shown in Fig. 5.

The direct comparison of IR spectra of different solvents of *Hordeum vulgare* (Figs.2, 3 and 4) and *Triticum dicoccum* (Fig.5), recovered from different periods and sites, appear to have certain specific features which are useful for identification of such spikelets. This agrees with the concept established by McLaren et al. (1990), who identified the charred grains recovered from archaeological excavations to sub-species level.

However, some absorption bands with high intenstiy appeared in Recent *Hordeum* and *Triticum* spikelets and gave less intensity in 12th Dynasty spikelets such as the band at 1260 cm^{-1} (in chloroform extract) (Figs. 2 and 5).

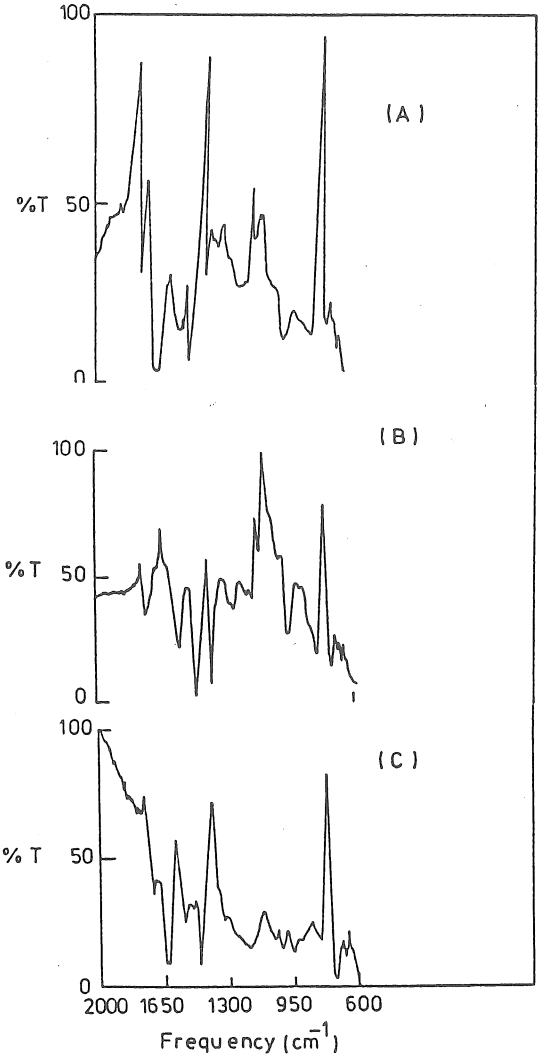

Fig.2. IR adsorption spectra for *Hordeum vulgare* extracted with Hexane:
12th Dynasty (A), Roman (B) and Recent (C)

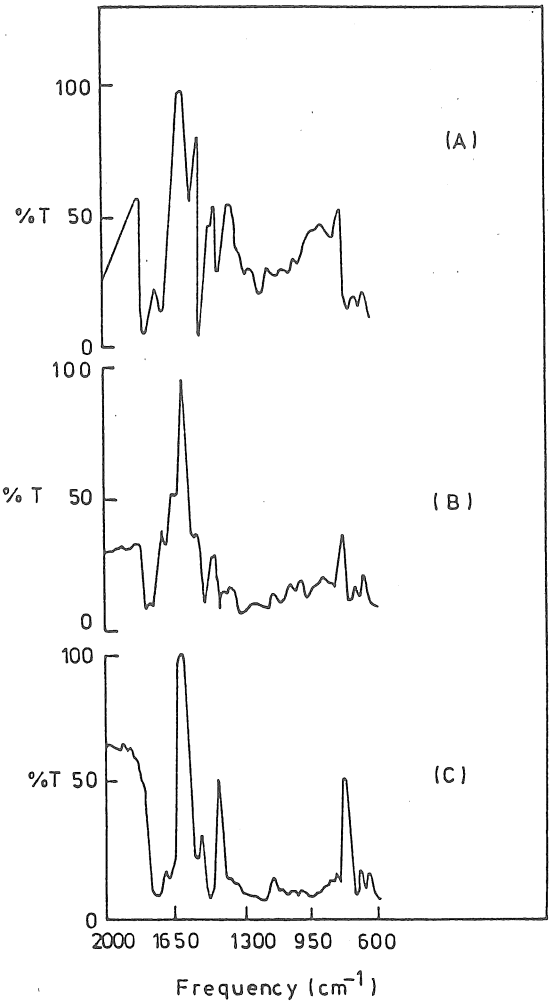

Fig.3. IR absorption spectra for *Hordeum vulgare* extracted with Chloroform:
12th Dynasty (A), Roman (B) and Recent (C)

This can be attributed to the changes that occurred in the organic groups with time lapse.

Fatty acids

The comparison of fat composition of ancient spikelets of *Hordeum vulgare* (12th Dynasty and Roman) and *Triticum dicoccum* (Roman period) with modern species is shown in Figs. 6 and 7.

For modern wheat grains the major fat constituent is linoleic acid which represent about 38%. It is also dominant in ancient wheat (29%). Myristic, palmitic, oleic and lauric acids constitute about 20, 15, 15 and 11% respectively in modern grains and 5, 25, 7 and 2% in ancient grains. It may be noticed that stearic acid was a minor component (4%) in modern grains, and was accumulating (20 %) in ancient one. On the other hand, the level of myristic and lauric acids decrease with lapse of time. Concerning modern barley grains, the major

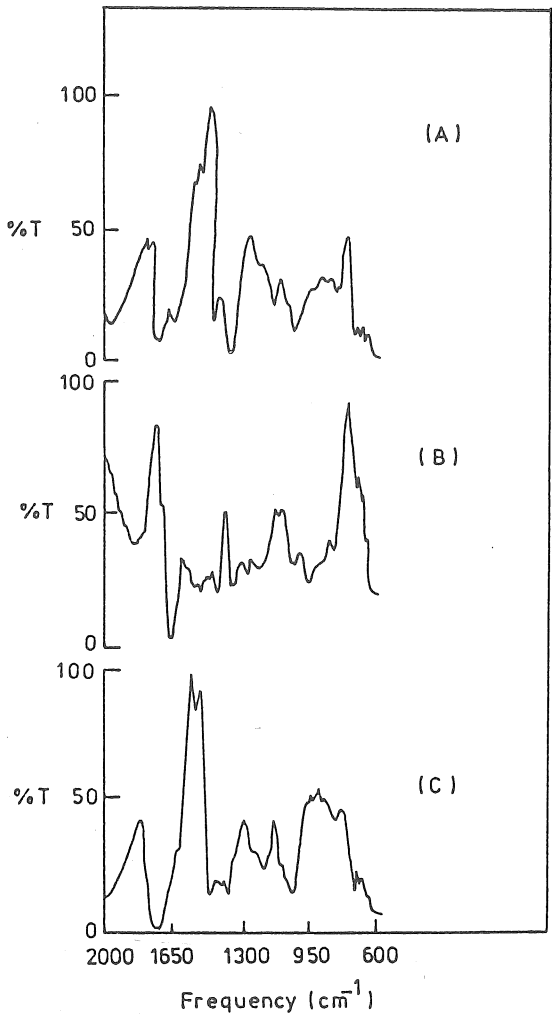

Fig.4. IR absorption spectra for *Hordeum vulgare* extracted with 2-Propanol: 12th Dynasty (A), Roman (B) and Recent (C)

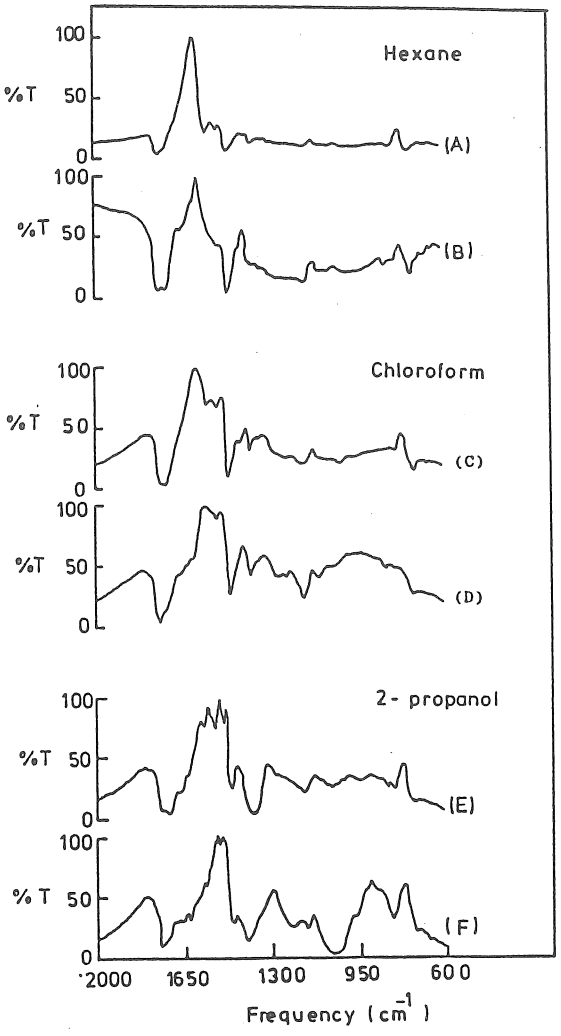

Fig. 5. IR absorption spectra for *Triticum dicoccum* of 12th Dynasty, (A, C and E) and Recent (B, D and F) spikletes extracted with different solvents

fatty acid constituents were almost of the same level (myristic, palmitic, oleic, myristoleic and linoleic 22, 21, 17, 15 and 12% respectively). It may be noticed that palmitic and oleic acids are the major constituents in both ancient grains and constitute about 50 %, 22 % for 12th Dynasty and 35% for both acids in Roman grains.

The levels of myristic in both *Triticum* and *Hordeum* decrease with time lapse and disappear completely from 12th Dynasty barley. Accordingly, it can be used as a measure for dating the average age of archaeological grains.

The presence of linoleic acid as a major component of modern *Triticum* and in less amounts in ancient one, and its gradual decrease from *Hordeum vulgare* with time lapse till complate disappearance from 12th Dynasty, led us to assume that this fatty acid can be used to distinguish between archaeological samples of barley and wheat.

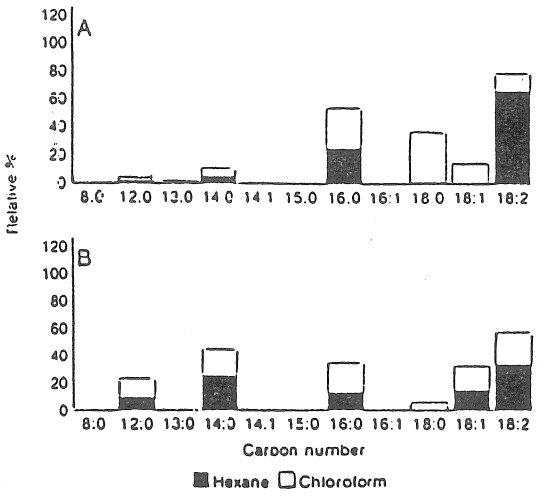

Fig.6. Fatty acids distribution for *Triticum dicoccum* at 12th Dynasty (A) and Recent (B).

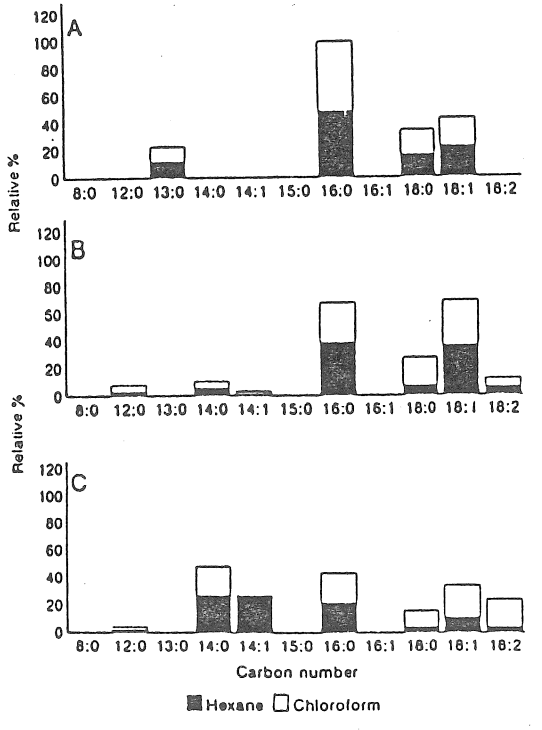

Fig.7. Fatty acids distribution for *Hordeum vulgare* at 12th Dynasty (A) Roman (B) and Recent (C).

The sequential oxidation and decarboxylation of free fatty acid to a fatty acid containing one less carbon atom is confirmed by Artman (1969). Palmitic and stearic acids which are major constituents of *Hordeum vulgare*, and also present in detectable amounts in *Triticum dicoccum* are common constituents of animal fats and vegatable oils (Gerhardt et al., 1990). Oxidation combined with the action of soil microflora results in very similar mixtures of fatty acids comprised mainly of palmitic and stearic acids (Rottländer and Schlichtherle, 1983), which obliterate any evidence of the original composition of fat and oil.

Hydrocarbons

Hydrocarbons distribution in ancient *Triticum dicoccum* (12th Dynasty), *Hordeum vulgare* (12th Dynasty and Roman) spikelets and modern grains of the same species are shown in Figs. 8 and 9.

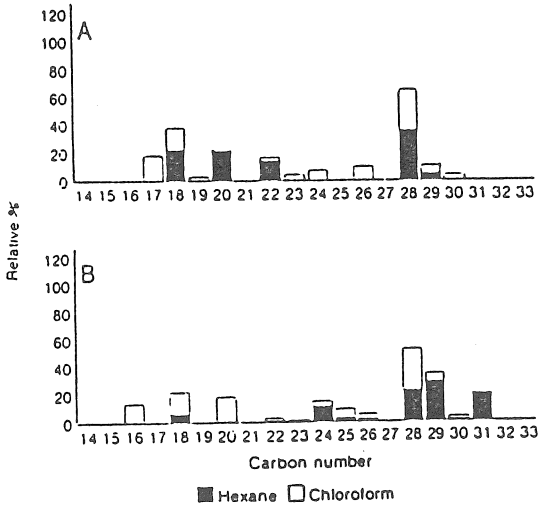

Fig. 8. Hydrocarbon distributions for Triticum dicoccum at 12th Dynasty (A) and Recent (B)

A series of 11 hydrocarbons in the C_{17-30} range were identified in ancient *Triticum* while it ranged from C_{16-31} in modern one. Concerning ancient *Hordeum* (12th Dynasty), a series of 10 saturated chain hydrocarbons in the C_{18-30} range were identified. Also 13 and 16 hydrocarbons in a range of C_{17-30} and C_{14-31} were detected in Roman and Recent *Hordeum* samples.

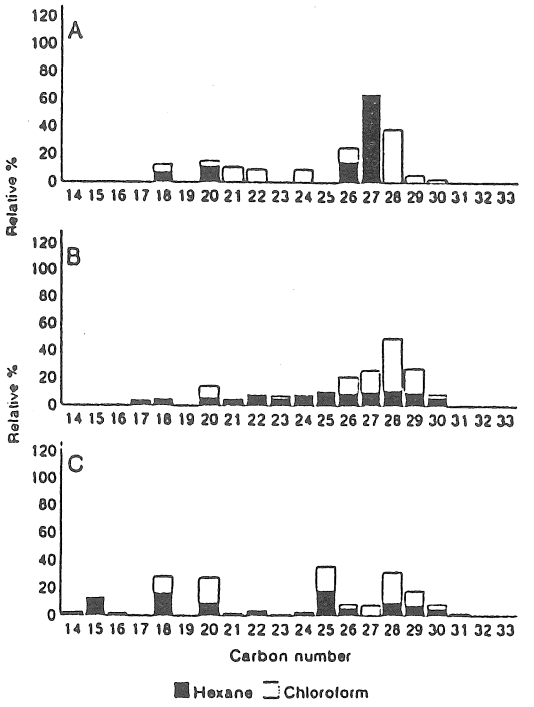

Fig. 9. Hydrocarbon distributions for Hordeum vulgare at 12th Dynasty (A) Roman (B) and Recent (C)

It may be noticed that the hydrocarbon pattern in ancient grain in both *Hordeum* and *Triticum* is similar to the reference standard of the modern grains. Recent *Hordeum* shows the dominance of C_{25}, C_{28}, C_{18} and C_{20}. The same pattern was shown in ancient spikelets except C_{25} which was detected in trace amounts in Roman sample and cannot be detected in more older (12th Dynasty) ones while C_{14}-C_{16} appear only in recent spikelets.

Comparing ancient *Triticum* with modern material shows close similarity, C_{28}, C_{18} and C_{20} were dominant, while C_{31}, C_{16} and C_{25} appear only in modern grains.

Hydrocarbons are currently of considerable interest as biological markers in the study of food cycle and in organic geochemistry (Swain, 1966).

Gerhadt et al. (1990) identified a series of 10 saturated straight chain hydrocarbons in the $C_{14\text{-}27}$ range using Corinthian of "figure vases" of the sixth century B.C. Such mixtures are very characteristic of flowers (though found in other waxy parts of plants also).

The accumulation of C_{27} and C_{28} in 12th Dynasty *Hordeum* and *Triticum* respectively and their marked decrease with time lapse can help in determining the age of archaeological grains.

The odd straight chain aliphatic hydrocarbons (C_{31}, C_{29}, C_{25} and C_{31}) in barley spikelets were detected in higher concentrations in recent grains and decrease gradually with time lapse (Figs. 8 and 9). This phenomena was explained by Kelley (1959) who claimed that the odd hydrocarbons do not fit together as that of even carbon chain. Accordingly these compounds are susceptible for oxidation and consequently form a shorter chain hydrocarbons. This was supported by Oudemans and Boon (1991) who claimed that the pyrolysis of long chain aliphatic alkane, that were bound to some larger structure would result in homologous series of alkane with carbon number of the longest chain minus one. Figs. 8 and 9 show that the short carbon chain hydrocarbons up to C_{16} are present in higher percentages in recent barley and wheat spikelets than that in ancient one. This may be attributed to their low melting points and low molecular weight (Cram and Hammond, 1964) which enhanced their evaporation.

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