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Comparative Study the Effects of Normal Heat and Microwave Heat on Different Edible Oils by ^{1}H Nuclear Magnetic Resonance

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Abstract:

Studying the effects of heating, reheating and exposure to microwave radiation on the structure of five types of edible oils such as olive oil, corn oil, cotton seeds oil, supply oil and sunflower oils in which used two kilograms for each sample and were collected from the origin of production in the local market and considered as fresh oils. Studying the change in the structure of edible oils by nuclear magnetic resonance (NMR) spectra, describe ,in general, a number of regions which can be considered as a base line of comparison to differentiate the properties of fresh oils from that exposed to normal heating or exposure to microwave once or several times. The NMR results revealed changes in the chemical structures of the considered edible oils. The changes occurred in the function groups of the oils especially; the alylic region of the oil (between 1.93 – 2.13 ppm), the bis-alylic region (2.72 – 2.84 ppm), olefeinic region (5.2 – 5.4 ppm), hydroproxide region (5.9 – 7.2 ppm), and the aldehyde region (9.5 – 9.7 ppm). The changes in these regions, indicate degradation and the formation of new complex chemical structures as a result of normal heating. Negligible changes in the chemical structures of the considered oils occurred by microwave heating, this give an impression that microwave heating even with more exposure frequency does not induce any hazards i.e., the absence of aldehyde groups.

Key words:
Edible oils, microwave, nuclear magnetic resonance

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1. Introduction:

Edible oils from either plant or animal sources are of special interest in various food and industrial applications. Edible oils from plant sources act as carrier of a number of fat soluble vitamins, such as, vitamin A, D, E, and K. They also provide energy and essential linoleic and linolenic acids which are acids they also provide energy and essential linoleic and linolenic acids responsible for growth responsible for growth. (1)
Fatty acids, esterified to glycerol, are the main constituents of oils and fats. The industrial exploitation of oils and fats, both for food and oleochemical products, is based on chemical modification of both the carboxyl and unsaturated groups present in fatty acids. Although the most reactive sites in fatty acids are the carboxyl group and double bonds, methylenes adjacent to them are activated, increasing their reactivity. Only rarely do saturated chains show reactivity. Carboxyl groups and unsaturated centers usually react independently, but when in close proximity, both may react through neighboring group participation. In enzymatic reactions, the reactivity of the carboxyl group can be influenced by the presence of a nearby double bond. (2)
In homes and restaurants, vegetable oils used for cooking are usually heated to very high temperatures, and may be reused several times to save costs. The recycling process involves repetitive heating of the oils. Successive heating of oils several times may be bad for health. Microwave heating of roasted seeds and beans shows a better retention of flavor and antioxidant compounds without any significant chemical changes of the lipids. (3-5) With respect to lipid components, microwave heating was studied to verify eventual heat induced effects on different oils and fats. (6-8) for this purpose, peroxide value, carbonyl value and conjugated diene and triene levels were assessed.
Few studies, however, have been published concerning the comparison of the effects of microwave heating with those of conventional heating systems (9). An experimental investigation ascertained the variation of the contents of saturated, unsaturated and polyenoic fatty acids, as well as of the trans-isomers of unsaturated fatty acids, in different vegetable oils (virgin olive oil, refined sunflower, refined peanut) submitted to either conventional or microwave heating. The results obtained showed that heat treatment causes a worsening of the nutritional quality of the fatty fraction. As a consequence, the contents of unsaturated and polyenoic fatty acids decreased, with greater variations in the oils heated by microwave than by a conventional oven, while the saturated fatty acid contents did not change substantially (10). The heat treatments also caused an increase in the trans-isomers of unsaturated fatty acids and this was more evident after microwave treatment**.** H^1 nuclear magnetic resonance has proved to be a very valuable tool which allows one to follow not only the degradation of acyl groups but also, at the same time, the formation and degradation of primary oxidation compounds as well as the formation and evolution of secondary oxidation products throughout the oxidation process. This technique has proved the formation of the genotoxic and citotoxic 4-hydroperoxy-, 4-hydroxy- and 4, 5-epoxy-trans-2-alkenals in the oxidation process undergone by sesame oil and oils rich in linolenic acyl groups at 70

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^{o}C with aeration. [11, 6] The fact that these harmful compounds, detected in cells and tissues, and known to be responsible for numerous diseases [12-15], can be present in foods and can be ingested, introduces a new perspective in relation to the safety of both oxidation processes and oils. [16, 11]

2. Materials and methods

2.1 Materials:

2.1.1 Edible oil samples:

In the present work samples of edible oils; olive oil, cottonseeds oil, sunflower oil, supply oil (sunflower 75% and soybean oil 25%) and corn oil were used, to integrate the effect of temperature on some of their physical and chemical properties. The mass of the sample from each type was 2Kg. The oils were collected from the origin of production in the local market and considered as fresh oils.

2.2 Methods:

Each sample was divided into three main parts as follows:

Part I: Was used as control part for determining the biophysical and biochemical properties before heating, re-heating or microwave exposure.

Part II: Was used to study the effect of normal heating on the physical and chemical properties of the considered oils. This part was divided into three sub-parts for studying the effect of normal heating (N-heating), reheating (once and more). Each heating period was 25 min. Heating and reheating once and /or more was considered to resemble the conditions in daily life as can as possible.

2.2.1 Normal Heating (N-heating):

To investigation the effect of normal heating on all studied oil samples, the samples were subjected to heating using flame oven. Each sample was heated for a single period of 25 min/day for 4 dayes. Unheated samples of oil were used as control (corresponding to zero min). Afterwards, the samples were kept in Falcon tubes and refrigerated until analysis.

Part III: Was used to study the effects of exposure of edible oils to microwave radiation, on structure of oils by NMR spectrum. The exposure time, is a heating period of 20 min each time, for four times.

2.2.2 Microwave oven (MW):

Five hundred ml (500 mL) of each oil was individually placed in a Petri dish (15cm in hight and 22cm in diameter) and subjected to heating in a microwave oven at maximum potency (800 Watt) for 20 min/day for 4 dayes. The exposed samples were kept in

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Falcon tubes and refrigerated until analysis. [17] The microwave oven used was Sharp model R-241R(S), serial No: 10075380 with 800 W effective power and 2450 MHZ frequency.

Nuclear Magnetic Resonance (NMR):
The ^{1}H NMR spectra were recorded on a JEOL JNM ECA 500 Plus spectrometer operating at 500 MHz, made in Japan, Fig.4-11. Each oil sample, weighing 20 mg was mixed with 600 µl of deuterated chloroform. The mixture was introduced into a 5 mm diameter tube. The acquisition parameters of ^{1}H NMR were: spectral width 5000 Hz, relaxation delay 3 s, number of scans 32, acquisition time 3.744 s and pulse width 90, with a total acquisition time of 3.37 min. The experiment was carried out at 25oC. Spectra were acquired periodically throughout the oxidation process.
The area of the signals was determined by using the equipment software and the integrations. All figures of ^{1}H NMR spectra or of expanded ^{1}H NMR spectra regions were plotted at a fixed value of absolute intensity to be valid for comparative purposes. [18]

3. Results:

^{1}H nuclear magnetic resonance spectrum:
Intense heating of oils may cause changes in the chemical structure of these compounds. The evaluation of these changes was carried out by ^{1}H nuclear magnetic resonance. Fig 1.
Normal and microwave heating for fresh corn oil show no change in the region from 0.8-0.9 ppm of ^{1}H nuclear magnetic resonance spectra after heating the oil normally, there was a major change in the regions (1.9-2.1, 2.7-2.8, 5.2-5.4 ppm) as shawan in Fig 2 (A-C). In addition to the appearance of two regions at (5.9-7.2) and (9.5-9.7) ppm as shawan in Fig 3(D-E). For oil heated by microwave, there was no changes in these regions, normal heating with the appearance of only one region at (5.9-6.5ppm) that was weak signal and weak intensity.
The same results were obtained for all oils used at the same condition, except for olive oils that show stable spectra at all conditions (intense heating and microwave heating).
At the regions which are changed after processing, the degradation percentage of most oils was varied, olive oil showed stable with no degradation rate at the three observed regions.

4. Discussion:

Studying the nuclear magnetic resonance spectrum for oils, as a biological material, describe ,in general, a number of regions which can be considered as a base line of comparison to differentiate the properties of fresh oils from that exposed to any physical or chemical factors, e.g., exposure to day light, or any other physical or chemical factors.

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So, the aim was to use this technique to introduce an evaluation of the biochemical and/or the biophysical changes occurred as a result of either exposure of the oils to normal heating or exposure to microwave once or several times.
The base line regions are as follows: [18,19]

- Region 1, is known as the methyl group region. It ranges between 0.8 – 0.9 ppm.
- Region 2, is known as the allyl region. It ranges between 1.9 – 2.1 ppm.
- Region 3, is known as the bis allyl CH_2. It ranges between 2.7 – 2.8 ppm.
- Region 4, is known as the olefeinic, .i.e, CH=CH. It ranges between 5.2 – 5.4 ppm.
- Region 5, is known as the hydroperoxide. It ranges between 5.9 – 7.2 ppm.
- Region 6, is known as the aldehyde group, CHO. It ranges between 9.5 – 9.7 ppm.

The results concerning the use of nuclear magnetic resonance, and using the considered five types of edible oils submitted to normal heating,
showed no changes in the methyl region in the range from 0.8 – 0.9 ppm.
This behavior is attributed to the fact that this region, i.e., the methyl group represents a non active group.
However, microwave exposure resulted in negligible changes in all the mentioned six regions. [18] As a result of normal heating, four types of oils, namely; sunflower oil, supply oil, corn oil and cotton oil suffered changes in the remaining five regions.
Referring to Tables (supply oil, sunflower oil, corn oil, cotton oil and olive oil) it is clear that the percentage changes in the number of protons ,i.e., changes occurred in the allyl region in case of normal heating was in the order :sunflower oil = supply oil (40%) > corn oil (22.2%). This is due to the reduced number of protons detected by the NMR integration, Table (1), (2), (3), (4), (5).
The bis allyl region suffered reduced number of protons as a result of normal heating in four types of oils in the order : sunflower (100%) > supply oil (75%) > corn oil (50%) > cotton oil (33.3).
The olefeinic region decreased in the number of protons with reduction percent in the order: sunflower = supply oil (60%) > cotton oil (45.5%) >corn oil (40%). This means that these oils are shifted towards increase of saturation (i.e., reduced number of double bonds, as judged by the lower of iodine number), which leads to increase of oil viscosity.
The appearance of the hydro-peroxide group in the NMR spectrum indicates increase in the peroxide values as obtained in the biochemical change. This also indicates that the probability of oil rancidity is increased, specially after the fourth frequency of frying. [20]
The peroxide group and the aldehyde group , that ranges between 5.9 – 7.2 ppm and 9.5 – 9.7 ppm respectively are of more interest. The formation of this group means relative increase in oil toxicity. It must be mentioned that, these groups are new and appear in the four a fore-said oils after normal heating.
It must be mentioned that, according to table (6). The olive oil did not show any changes in all these regions due to normal heating. Also, according to Tables (1), (2), (3), (4), (5),

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the exposure to microwave resulted in weak intensity signals in hydroperoxide region which indicate very low concentrations of peroxide in the four mentioned edible oils.

In fact, ^{1}H NMR spectroscopy has proved to be very useful in evaluating the oxidative status of oils and fats, determining their oxidative stability, monitoring oil degradation processes, as well as in providing information on the nature and proportions of the aldehydes generated in these processes which are present in the oil liquid phase. For all oils under investigation, the evaluation of the changes due to degradative conditions by normal and microwave heating was carried out by ^{1}H nuclear magnetic resonance.

Taking Table 7 into consideration, the content of sunflower, corn oil, soybean and cotton oil of polyunsaturated fatty acids are 70%, 59%, 54%, and 54% respectively. The rates of oxidation of these oils are high and similar behavior is obtained in this work. Also, the olive oil content of polyunsaturated fatty acid is 8% this indicates that olive oil is the least ability to be auto-oxidized than the other mentioned oils. This expression is coinciding with our results in this work.

Conclusion

The results of this work can be summarized into the following:

1- The normal heating for four times induced biophysical and biochemical changes that must be taken into consideration.
2- The olive oil showed the lowest level of changes. This may be due to its high content of mono-unsaturated fatty acids, which may be the reason of its least auto-oxidation and rancidity.
3- The use of the nuclear magnetic resonance proved its efficiency in giving interpretation of the biophysical and biochemical changes due to the two methods of heating, i.e., normal heating, and microwave exposure.
4- It is advisable to use microwave for heating oils or using normal heating once or two times maximum.

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Table (1): Illustrate the change in structure of Supply oil after normal heating and microwave exposure by NMR spectrum.

Chemical shift		Number of proton ^1H			Percentage of change on normal heating	Percentage of change on microwave heating
Region	Function group	Fresh oil	Microwave heating oil	Normal heating oil		
0.8- 0.9 ppm	CH_3	9 H	9 H	9 H	Zero	Zero
1.9- 2.1 ppm	Allyl H	10 H	10 H	6 H	40	Zero
2.7- 2.8 ppm	bis allyl CH_2	4 H	4 H	1 H	75	Zero
5.2- 5.4 ppm	Olefeinic H (CH=CH)	10 H	10 H	4 H	60	Zero
5.9- 7.2 ppm	Hydroperoxide		Weak intensity	Weak intensity	100	100
9.5- 9.7 ppm	Aldehydic			Weak intensity	100	Zero

Table (2): Illustrate the change in structure of sunflower oil after normal heating and microwave exposure by NMR spectrum.

Chemical shift		Number of proton ^1H			Percentage of change on normal heating	Percentage of change on microwave heating
Region	Function group	Fresh oil	Microwave heating oil	Normal heating oil		
0.8- 0.9 ppm	CH_3	9 H	9 H	9 H	Zero	Zero
1.9- 2.1 ppm	Allyl H	10H	10H	6H	40	Zero
2.7- 2.8 ppm	bis allyl CH_2	4H	4H	zero	100	Zero
5.2- 5.4 ppm	Olefeinic H (CH=CH)	10 H	10 H	4 H	60	Zero
5.9- 7.2 ppm	Hydroperoxide		Weak intensity	Weak intensity	100	100
9.5- 9.7 ppm	Aldehydic			Weak intensity	100	Zero

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Table (3): Illustrate the change in structure of corn oil after normal heating and microwave exposure by NMR spectrum.

Chemical shift		Number of proton ^1H			Percentage of change on normal heating	Percentage of change on microwave heating
Region	Function group	Fresh oil	Microwave heating oil	Normal heating oil		
0.8- 0.9 ppm	CH_3	9 H	9 H	9 H	Zero	Zero
1.9- 2.1 ppm	Allyl H	9 H	9 H	7 H	22.2	Zero
2.7- 2.8 ppm	bis allyl CH_2	4 H	4 H	2 H	50	Zero
5.2- 5.4 ppm	Olefeinic H (CH=CH)	10 H	10 H	6 H	40	Zero
5.9- 7.2 ppm	Hydroperoxide		Weak intensity	Weak intensity	100	100
9.5- 9.7 ppm	Aldehydic			Weak intensity	100	Zero

Table (4): Illustrate the change in structure of cotton oil after normal heating and microwave exposure by NMR spectrum.

Chemical shift		Number of proton ^1H			Percentage of change on normal heating	Percentage of change on microwave heating
Region	Function group	Fresh oil	Microwave heating oil	Normal heating oil		
0.8- 0.9 ppm	CH_3	9 H	9 H	9 H	Zero	Zero
1.9- 2.1 ppm	Allyl H	9 H	9 H	9 H	Zero	Zero
2.7- 2.8 ppm	bis allyl CH_2	3 H	3 H	1 H	33.3	Zero
5.2- 5.4 ppm	Olefeinic H (CH=CH)	9 H	9 H	5 H	45.5	Zero
5.9- 7.2 ppm	Hydroperoxide		Weak intensity	Weak intensity	100	100

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Table (5): Illustrate the change in structure of olive oil after normal heating and microwave exposure by NMR spectrum.

Chemical shift		Number of proton ^1H			Percentage of change on normal heating	Percentage of change on microwave heating
Region	Function group	Fresh oil	Microwave heating oil	Normal heating oil		
0.8- 0.9 ppm	CH_3	9 H	9 H	9 H	Zero	Zero
1.9- 2.1 ppm	Allyl H	10 H	10 H	10 H	Zero	Zero
2.7- 2.8 ppm	bis allyl CH_2	1 H	1 H	1 H	zero	Zero
5.2- 5.4 ppm	Olefeinic H (CH=CH)	6 H	6 H	6 H	zero	Zero

Table (6): Percent of change in edible oils by normal heating using NMR spectrum:

Type of oil	Percent of change at 1.9 – 2.1 ppm	Percent of change at 2.7 – 2.8 ppm	Percent of change at 5.2 – 5.4 ppm	Percent of change at 5.9- 7.2 ppm	Percent of change at 9.5- 9.7 ppm
Sunflower	40	100	60	100	100
Supply	40	75	60	100	100
Cotton	Zero	33.3	45.5	100	zero
Corn	22.2	50	60	100	100
Olive	Zero	Zero	Zero	zero	zero

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Table (7) Chemical composition of some oils according to literatures. [21-22]

Oils	Saturated fatty acid		Monounsaturated fatty acid	Polyunsaturated fatty acid	
	C16:0	C18:0	C18:1	C18:2	C18:3
Sunflower	6	4	18	70	
Soybean	11	4	24	54	7
Olive	11	2	73	8	<1
Corn	11	2	27	59	1
Cotton	22	3	19	54	1

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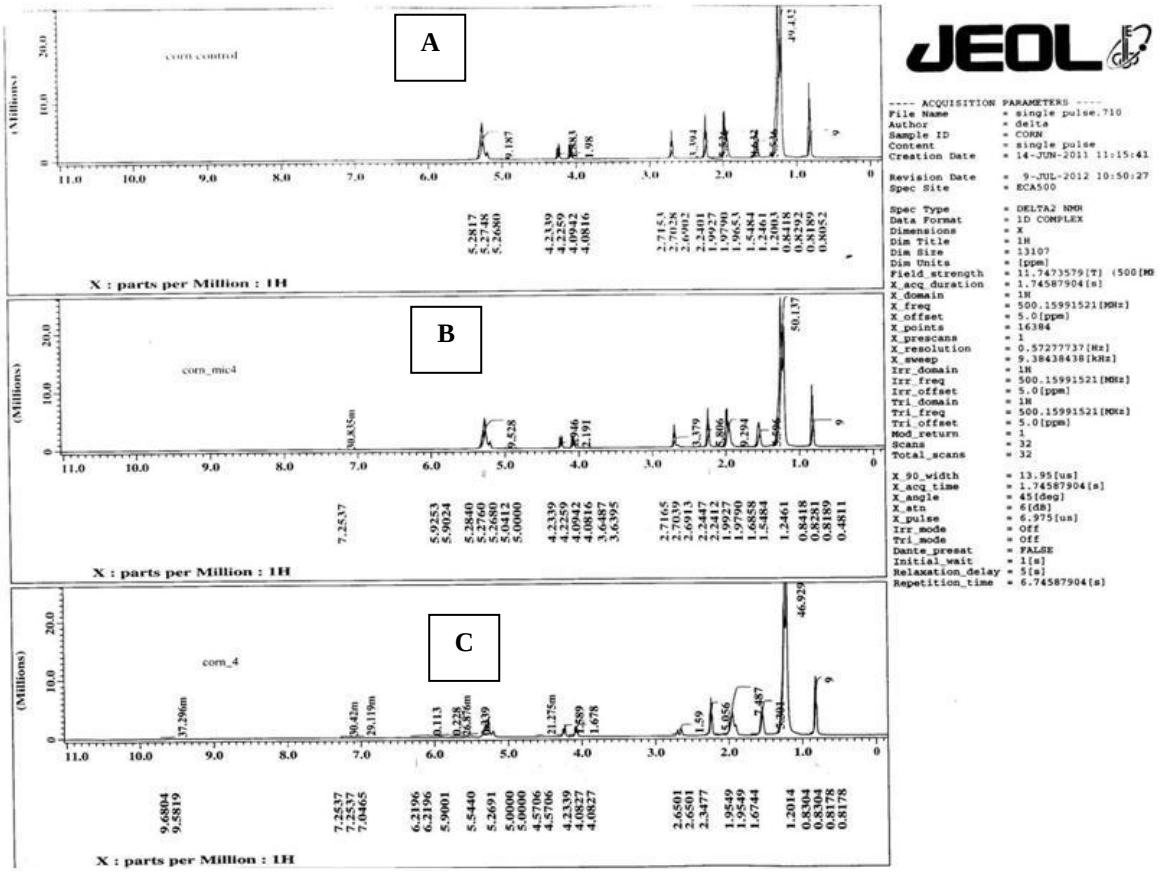

Fig. (1): Full ^{1}H NMR spectra of corn oils at different times during microwave and normal heating.

Where:
(A) Fresh corn oil.
(B) Corn oil after fourth microwave heating.
(C) Corn oil after fourth normal heating.

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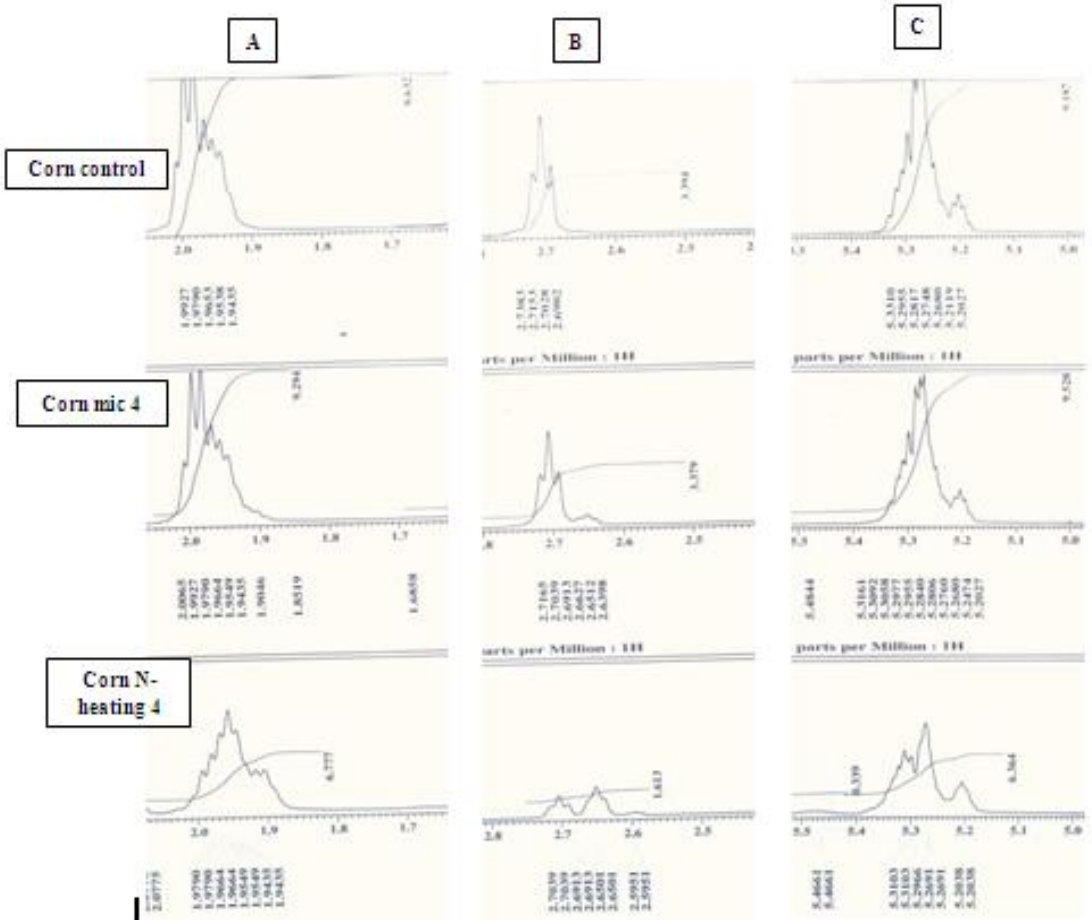

Fig. (2): (A). Expansions of the allylic region, between 1.93 and 2.13 ppm, (B) Expansions of the bis-allylic region, between 2.72 and 2.84 ppm, (C) Expansions of the Olefeinic region, between 5.2 and 5.4 ppm, of the ^{1}H NMR spectra of corn oils at different times during microwave and normal heating.

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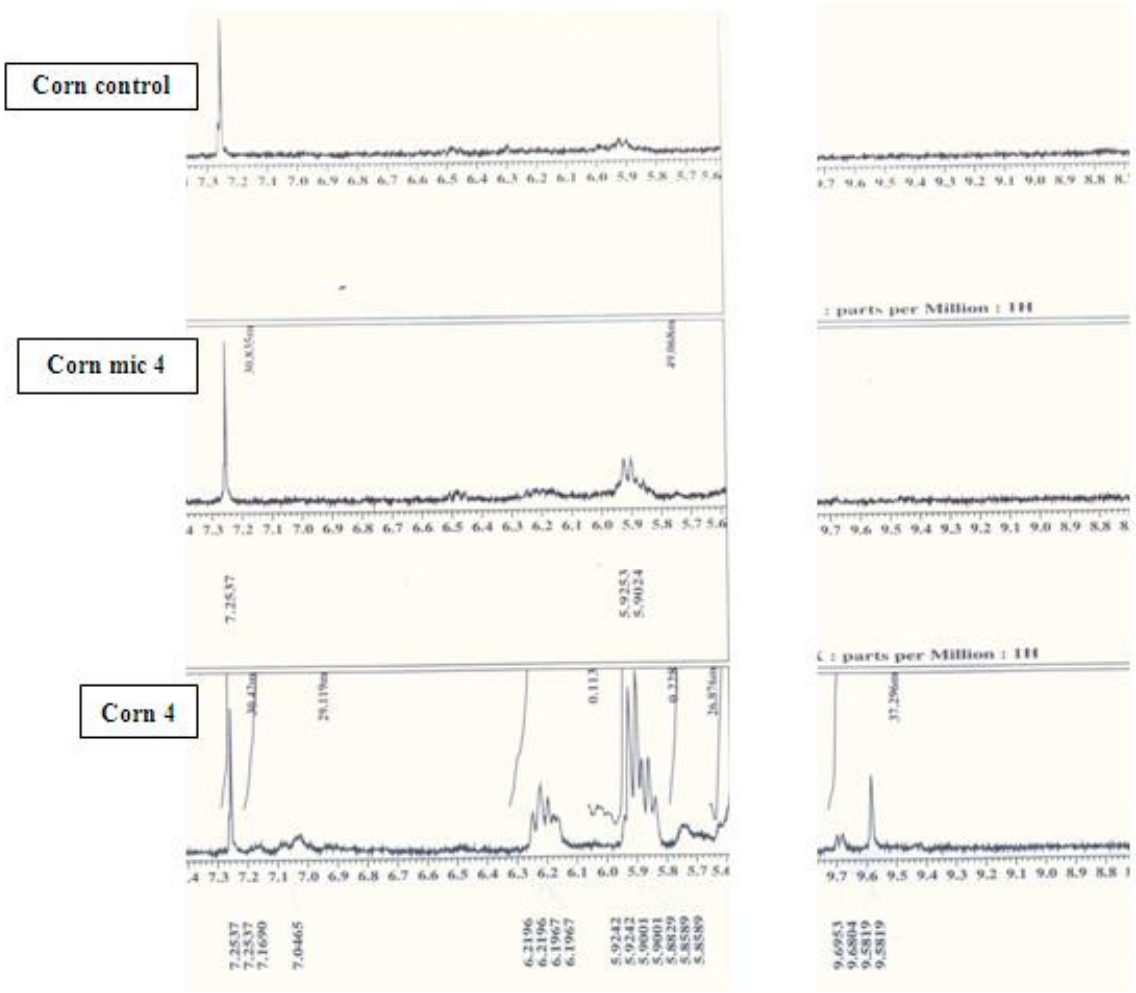

Fig. (3): (D) Expanded regions between 5.4 and 7.2 ppm, (E) Expanded regions between 8.8 and 10.3 ppm of the ^{1}H NMR spectra of corn oils at different times during microwave and normal heating.

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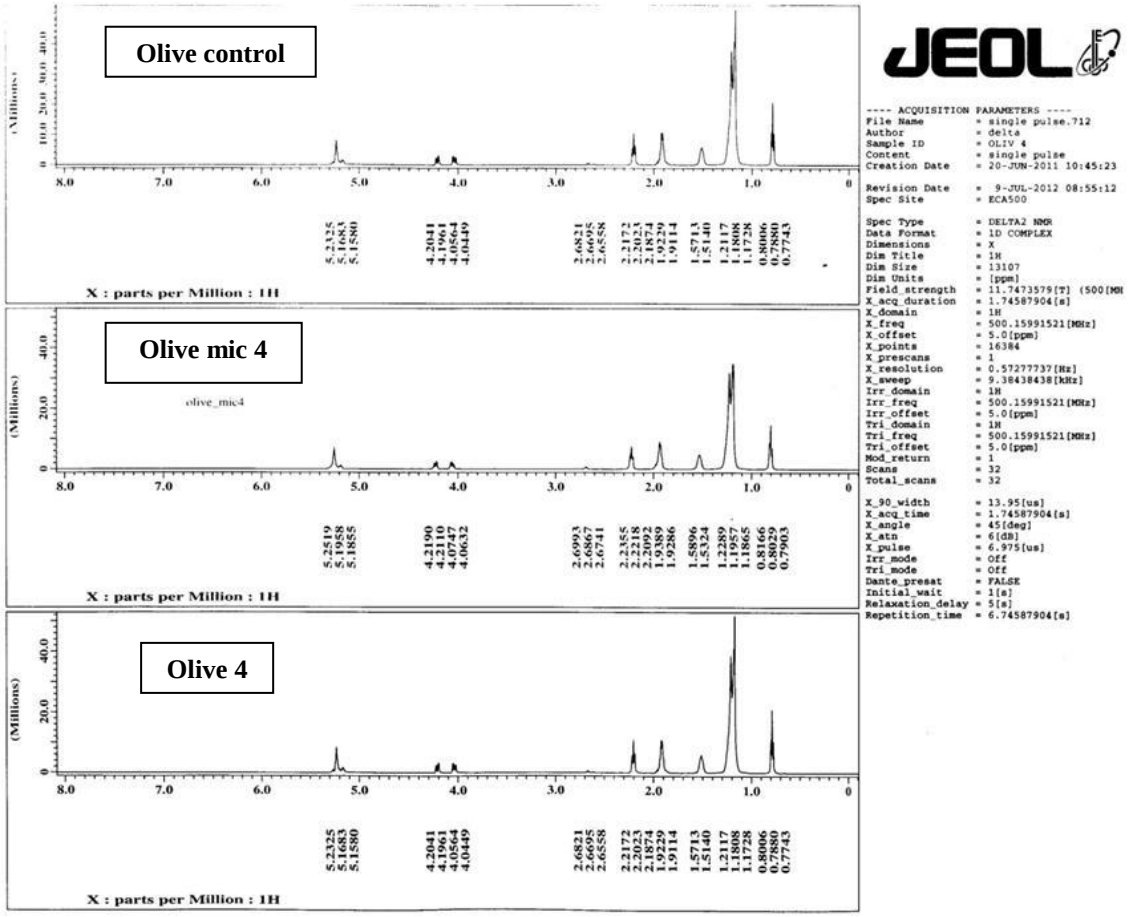

Fig. (4): Full ^{1}H NMR spectra of olive oils at different times during microwave and normal heating.

Where:
(A) Fresh olive oil.
(B) Olive oil after fourth microwave heating.
(C) Olive oil after fourth normal heating.

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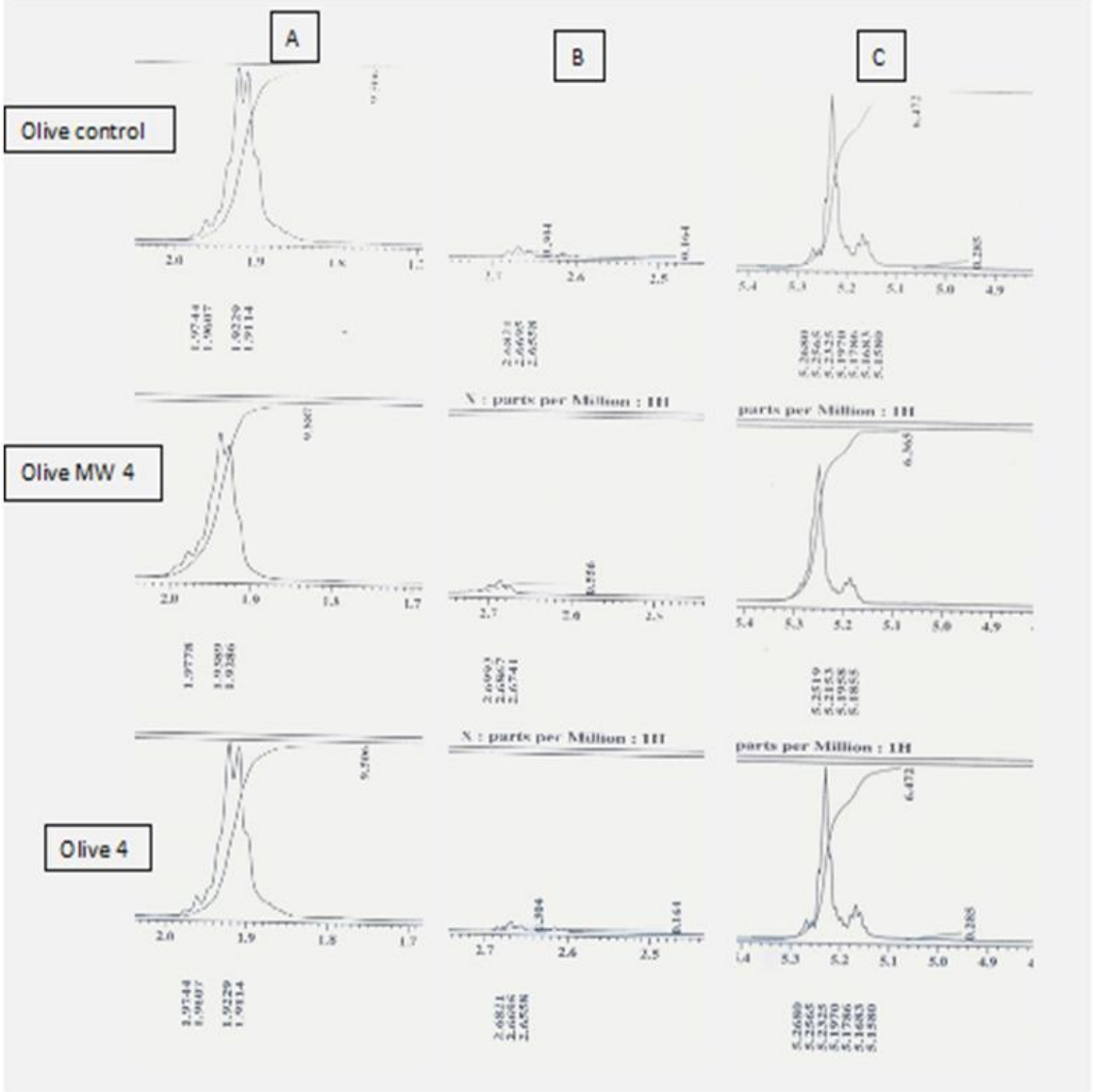

Fig. (5): (A). Expansions of the allylic region, between 1.93 and 2.13 ppm, (B) Expansions of the bis-allylic region, between 2.72 and 2.84 ppm, (C) Expansions of the Olefeinic region, between 5.2 and 5.4 ppm, of the 1H NMR spectra of olive oils at different times during microwave and normal heating.

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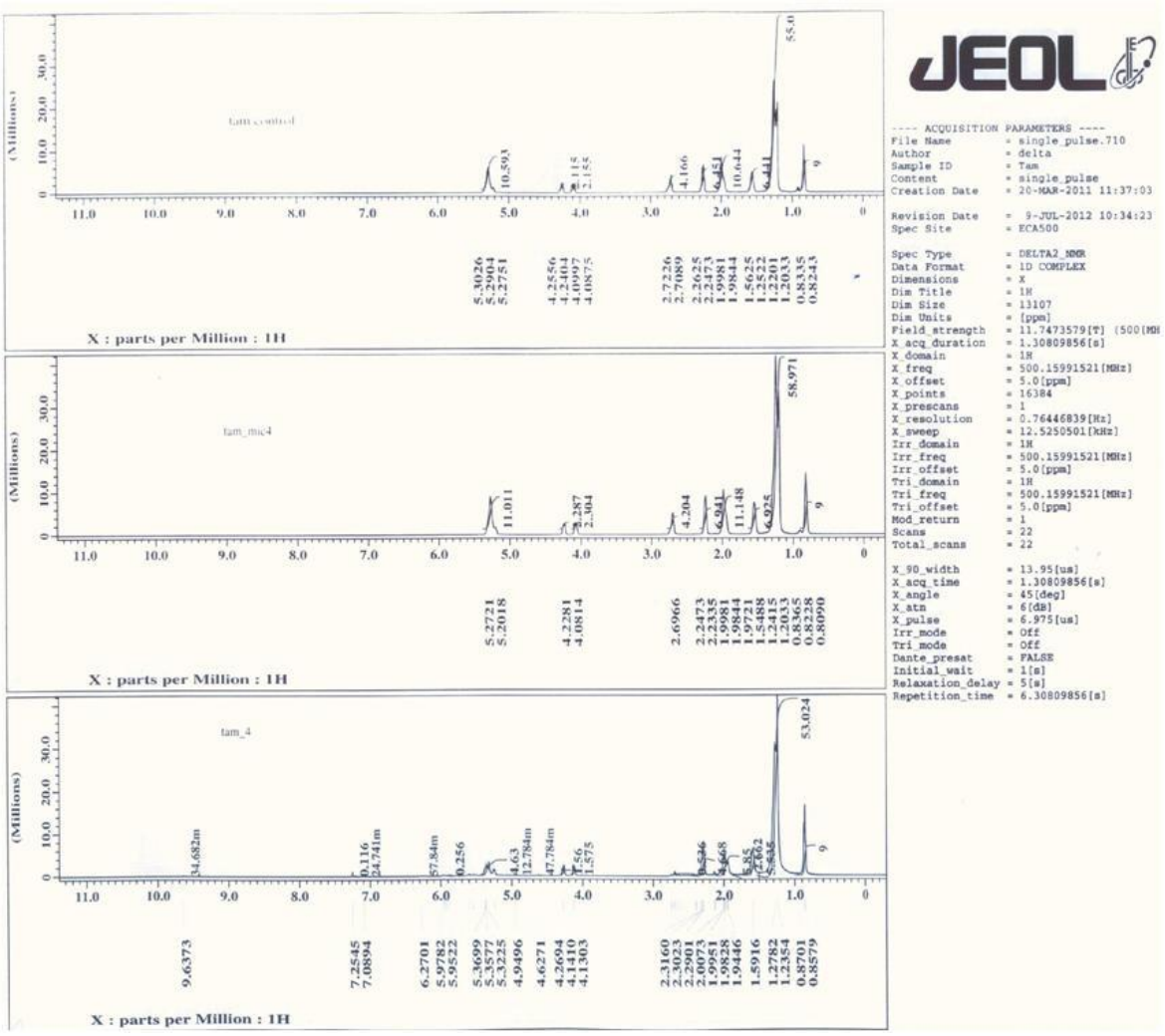

Fig. (6): Full 1H NMR spectra of supply oils at different times during microwave and normal heating.

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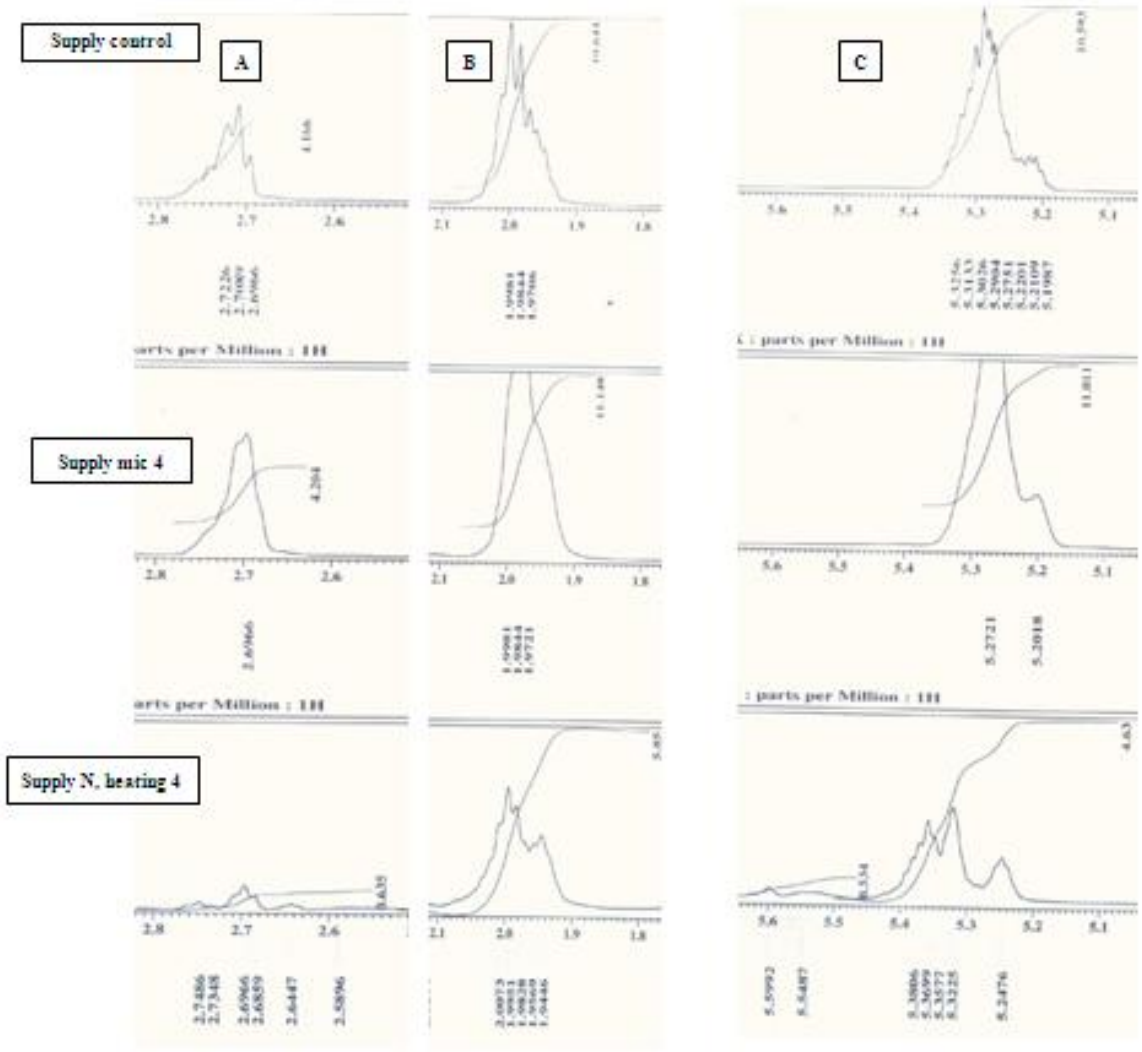

Fig. (7). (A). Expansions of the allylic region, between 1.93 and 2.13 ppm, (B) Expansions of the bis-allylic region, between 2.72 and 2.84 ppm, (C) Expansions of the Olefeinic region, between 5.2 and 5.4 ppm, of the 1H NMR spectra of supply oils at different times during microwave and normal heating.

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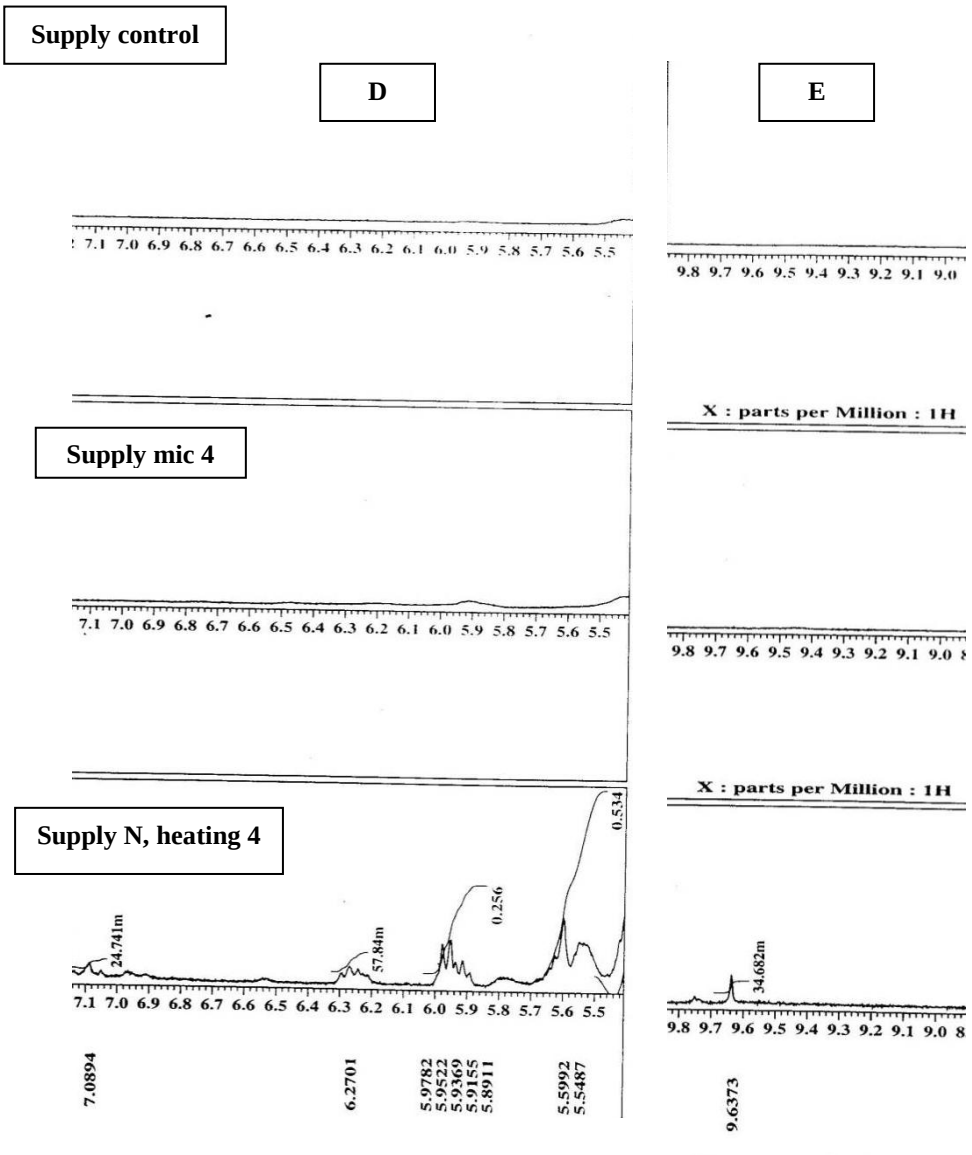

Fig. (8): (D) Expanded regions between 5.4 and 7.2 ppm, (E) Expanded regions between 8.8 and 10.3 ppm of the ^{1}H NMR spectra of suplly oils at different times during microwave and normal heating.

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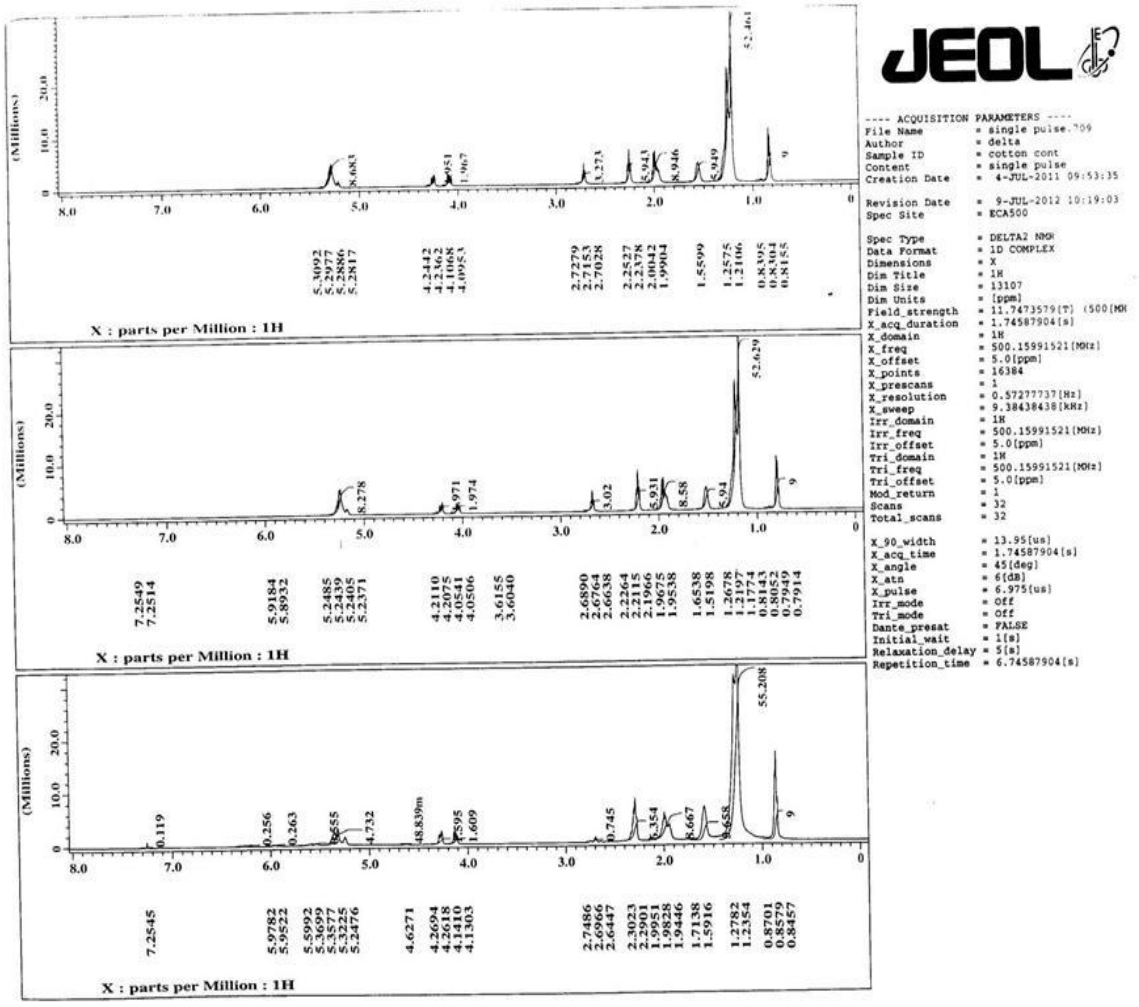

Fig. (9): Full ^{1}H NMR spectra of cotton oils at different times during microwave and normal heating.

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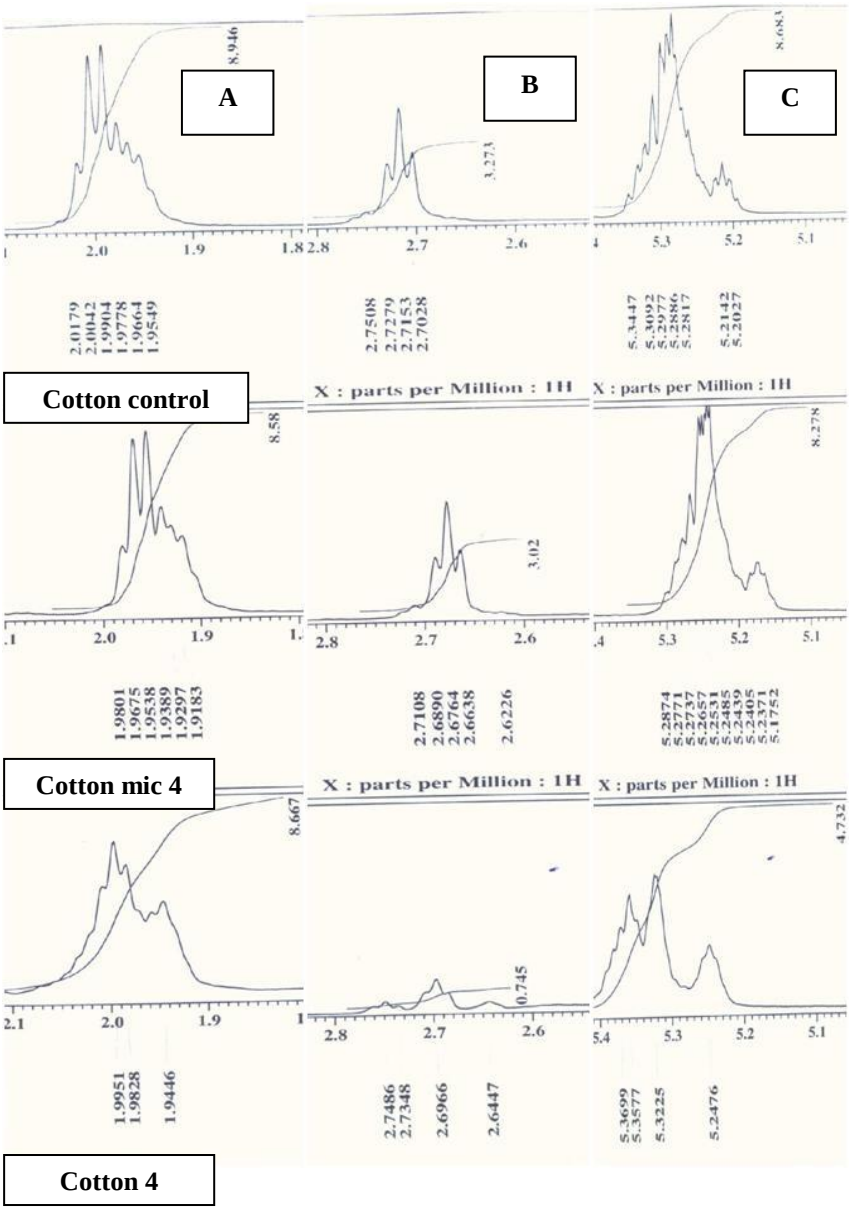

Fig. (10): (A). Expansions of the allylic region, between 1.93 and 2.13 ppm, (B) Expansions of the bis-allylic region, between 2.72 and 2.84 ppm, (C) Expansions of the Olefeinic region, between 5.2 and 5.4 ppm, of the ^{1}H NMR spectra of cotton oils at different times during microwave and normal heating.

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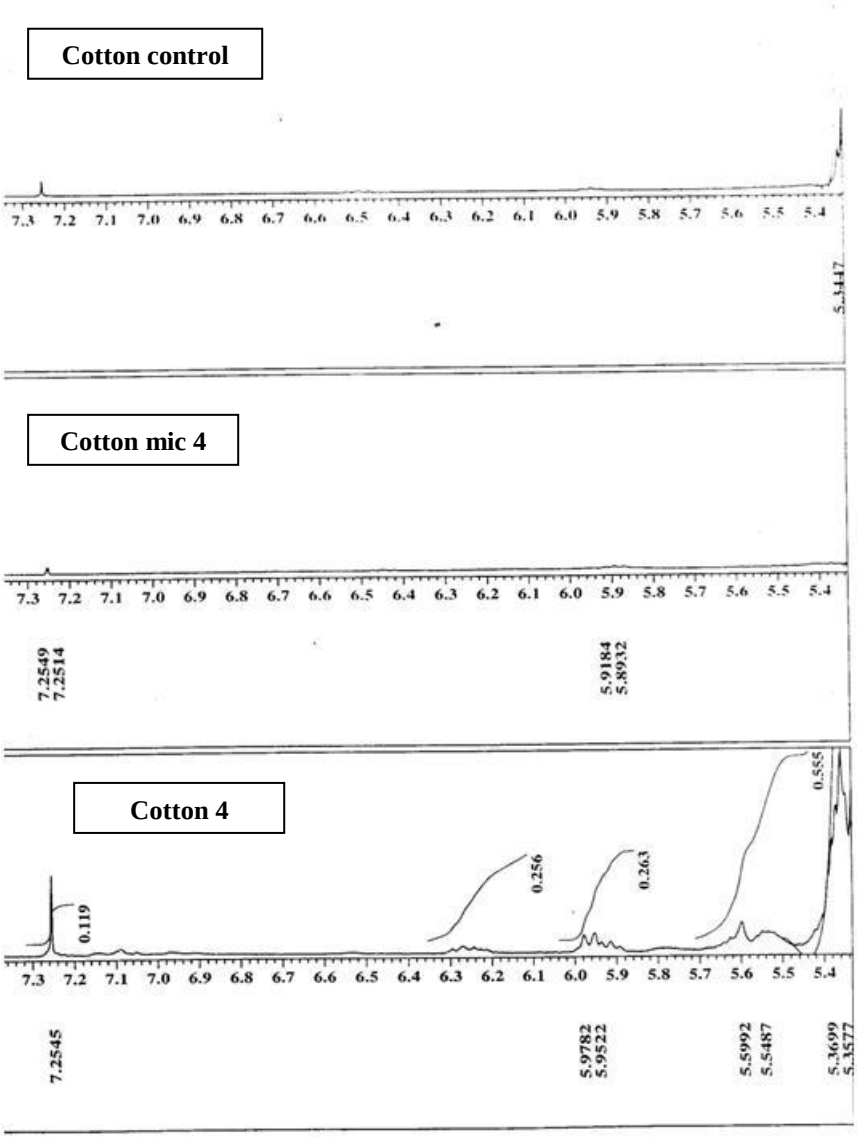

Fig. (11): (D) Expanded regions between 5.4 and 7.2 ppm of 1H NMR spectra of cotton oils at different times during microwave and normal heating.

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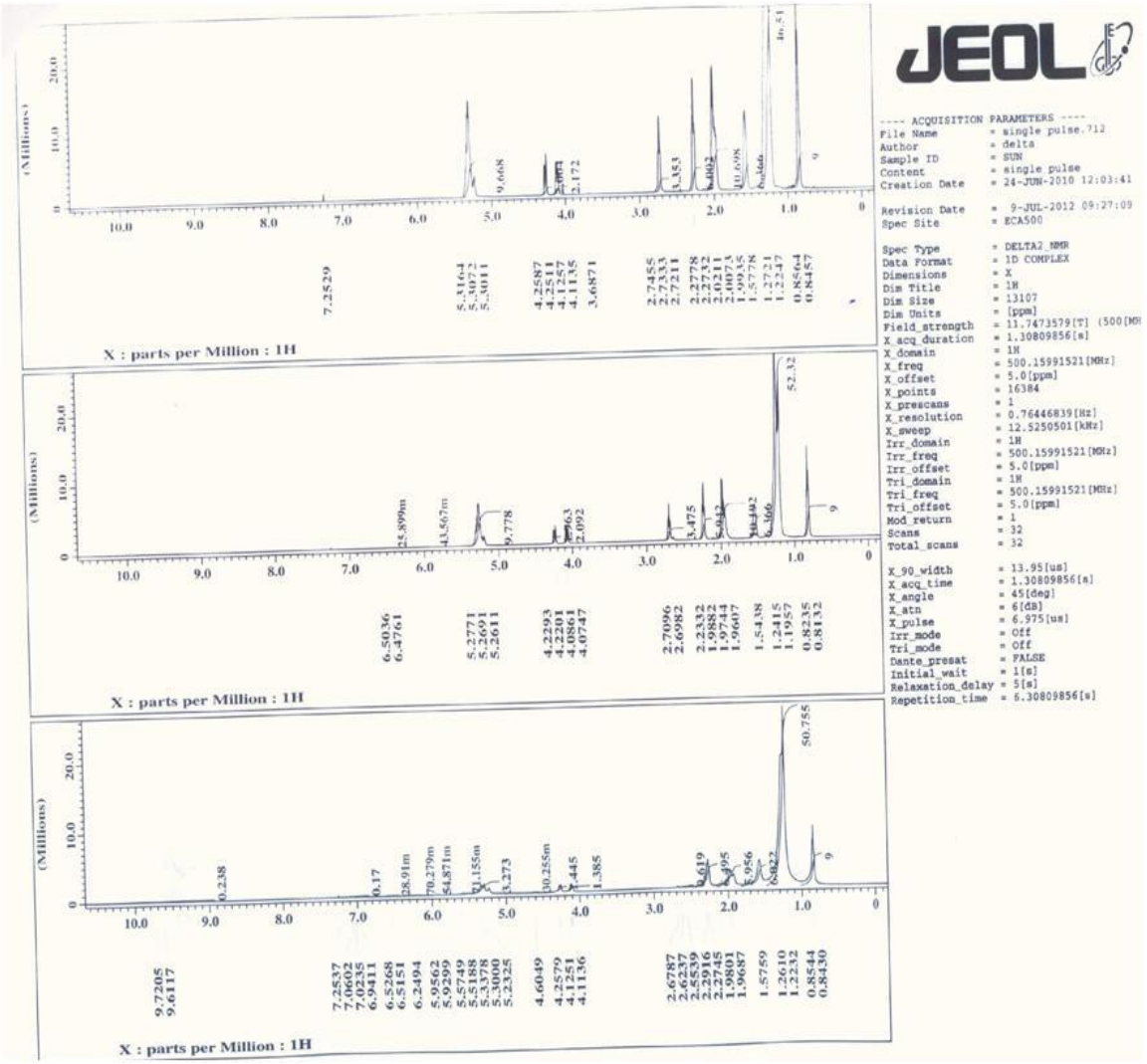

Fig. (12): Full ^{1}H NMR spectra of sunflower oils at different times during microwave and normal heating.

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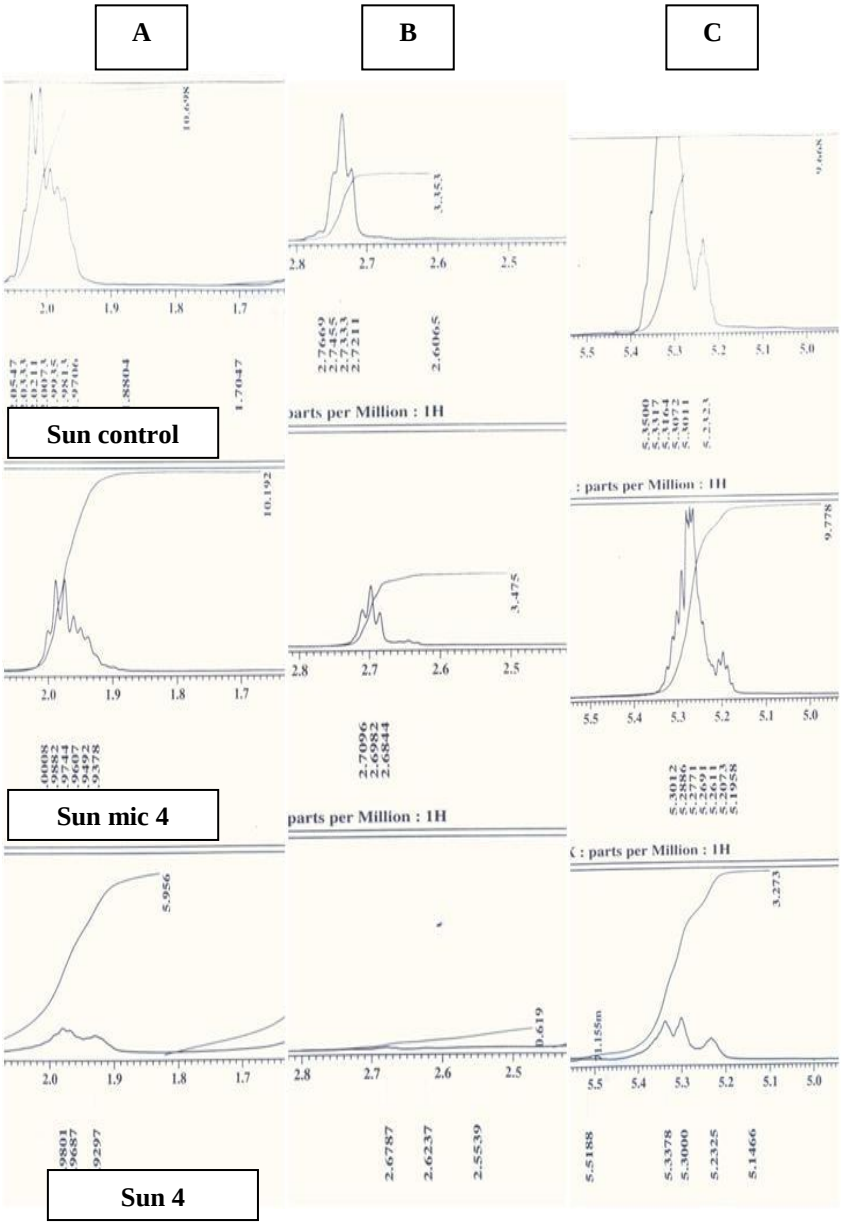

Fig. (13): (A). Expansions of the allylic region, between 1.93 and 2.13 ppm, (B) Expansions of the bis-allylic region, between 2.72 and 2.84 ppm, (C) Expansions of the Olefeinic region, between 5.2 and 5.4 ppm, of the ^{1}H NMR spectra of sunflower oils at different times during microwave and normal heating.

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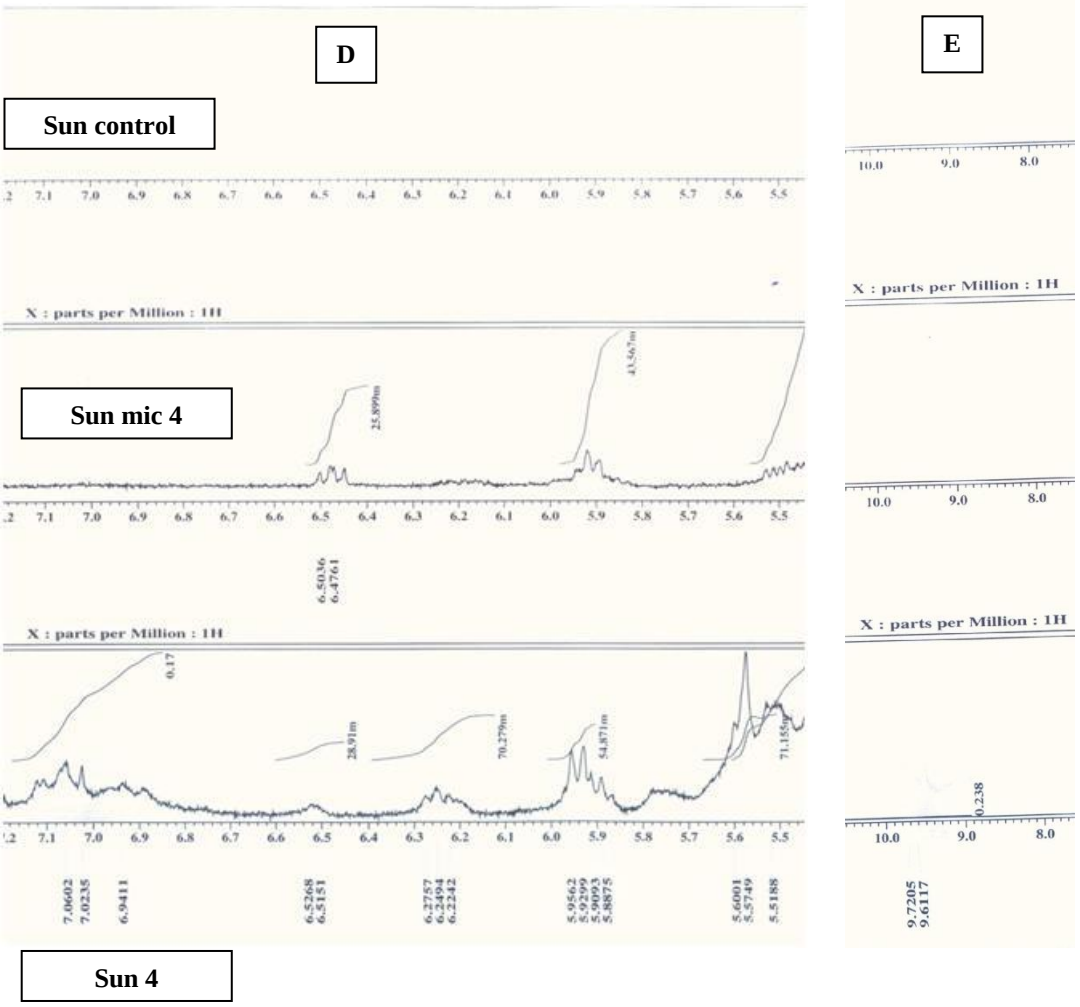

Fig. (14): (D) Expanded regions between 5.4 and 7.2 ppm, (E) Expanded regions between 8.8 and 10.3 ppm of the ^{1}H NMR spectra of sunflower oils at different times during microwave and normal heating.