LETTERS TO THE EDITOR

NMR STUDY OF THE OIL BUILD-UP IN SUNFLOWER SEEDS

THE use of NMR to investigate the quality of the oil as a function of maturity of the seeds is demonstrated for sunflower seeds. The percentages of the saturated and individual unsaturated acids are determined as a function of time after flowering of the seeds. The percentage of saturated fatty acids is found to decrease with maturity of seeds whereas the extent of the unsaturated acids increases.

1. *Introduction*

High resolution NMR is a powerful technique for the rapid non-destructive determination of the quantity of the oil in seeds[1–4]. The quality of the oil in terms of the ratio of the saturated to unsaturated fatty acid esters and the iodine number has been investigated[5,6] using proton as well as ^{13}C NMR[7]. In the present communication, a procedure for the determination of the composition of the oil in sunflower seeds in terms of the percentages of the individual unsaturated fatty acids with respect to the saturated ones is discussed and applied to monitor the build-up of the quality of the oil as a function of maturation of the crop.

2. *Experimental*

2.1. *Materials*

The sunflower cultivar EC68415 (Armavirtsky 3497) was grown during the *Rabi* season of 1978–79 at the GKVK Farm of University of Agricultural Sciences, Bangalore, under irrigated conditions. The crop was raised under uniform fertility with an inter- and intra-row spacing of 60 and 30 cm respectively. Progressive changes in quality and quantity of oil in developing seeds was studied at ten-day intervals, after fertilization, upto forty days (full maturity). To facilitate uniform sampling, forty plants that flowered on a particular day were selected and tagged. Hand pollination was also done on these plants in addition to natural pollination to achieve good seed set. In order to achieve homogeneous sampling, capitulum of each plant was divided into four equal parts corresponding to the four sampling stages. Harvesting was done part by part after fertilization. To each sample of a known maturity period consisted of seeds from one-fourth of a capitulum from all the forty plants selected originally. The samples were dried at 50° C for 48 hours and powdered. The oil was extracted from each sample of the seed by cold percolation method using carbontetrachloride and the proton NMR spectra studied.

2.2. *NMR Measurements*

The spectra of the oil were recorded in deuterochloroform solution containing tetramethylsilane (TMS) used as the internal reference, on a Bruker WH-270 MHz Fourier Transform NMR Spectrometer. A typical spectrum is shown in Fig. 1. To facilitate the assignment of the lines due to individual acids, the proton NMR spectra of pure palmitic, stearic, oleic and linoleic acids were also recorded.

3. *Results and Discussion*

The assignments of the various groups of lines in Fig. 1 are as follows. The position of the terminal methyl group of the fatty acid chain is marked (A), that of the methylene group attached to two olefinic carbons as in linoleic acid is (B), (C) gives the position of the 4 glyceride methylene protons and the olefinic protons together with the β-glyceride group resonate around the position (D). The assignments are in conformity with those reported in literature[8]. The intensities of these bands were used to compute the percentages of linoleic, oleic and saturated acids as follows.

The linoleic acid content was determined from the integrated intensity of the band (B). This information together with the intensities of the bands (C) and (D) provided the extent of the oleic acid. The intensity of (A) was then used to obtain the saturated fatty acid content.

This procedure was used to determine the fatty acid composition of the sunflower seed oil during the course of its maturity and the results are reproduced in Table I.

TABLE I

Composition of fatty acids in sunflower seeds as a function of time after flowering

No. of days after flowering	Total oil content (%)	Total saturated acids (%)	Oleic acid (%)	Linoleic acid (%)
10	12·9	55·0	5·1	39·9
20	34·5	38·1	8·2	53·7
30	42·0	13·0	39·8	47·2
40	43·0	4·8	27·1	68·1

The oil content increases gradually with time till about thirty days after which it does not change substantially. The table shows that the percentage of the saturated acids steadily decreases while the amounts of the unsaturated ones increase. On the tenth day, the saturated acid content is 55% whereas around maturity time it reduces to nearly 5%. The results are in agreement with those predicted from the theory of biosynthesis of plant fatty acids[9]. The percentage of linoleic acid is always larger than that of the oleic acid, The oleic acid content is maximum around the

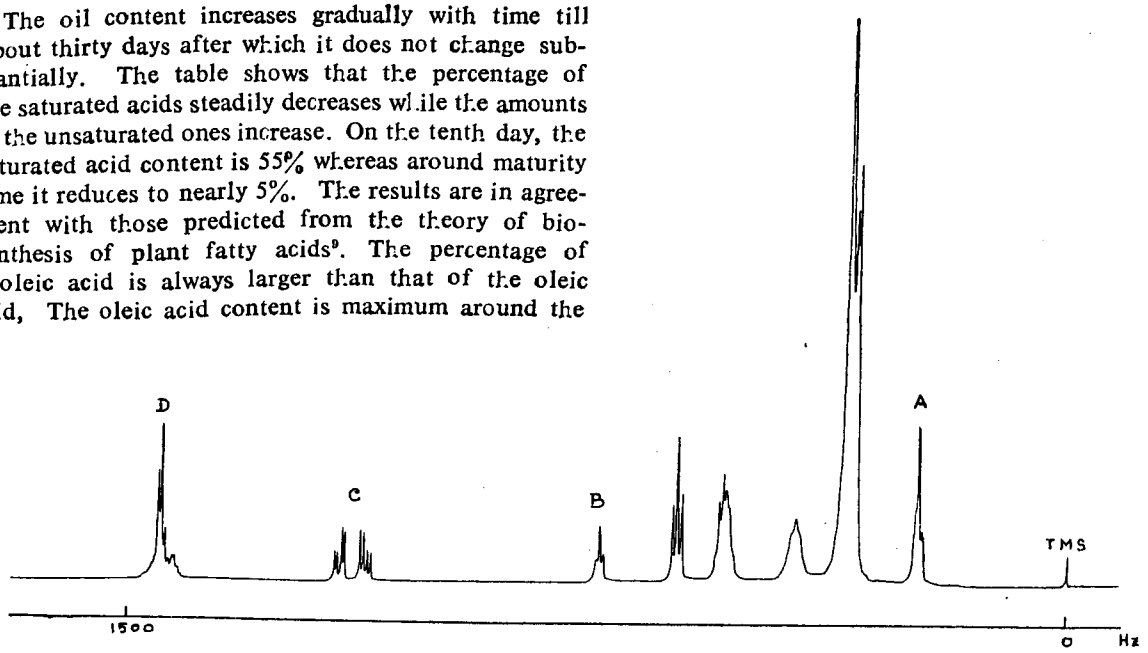

FIG. 1. 270 MHz Proton NMR spectrum of a typical sample of oil (fortieth day after flowering dissolved in deuterochloroform.

thirtieth day. On the other hand, the linoleic acid concentration is less on the thirtieth day than on the twentieth and the fortieth days. Similar trend has been observed for Rape seeds from studies involving other techniques[9]. Between thirtieth day and fortieth day though there is no accumulation of oil, there is the inter-conversion of individual fatty acids in such a way that the concentration of unsaturated acids increases at the expense of saturated ones.

The results indicate that NMR is a useful technique to investigate the quality and quantity of the oil build-up in seeds.

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December 13, 1979.

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