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CHANGES IN CAROTENOIDS AND TOCOPHEROLS DURING MATURATION OF *CASSIA* SEEDS

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Changes in the oil, carotenoid and total tocopherol contents of *Cassia absus; Cassia fistula* and *Cassia occidentalis* seed oils were studied during maturation between the 2nd and 12th week after the flowering of *C.absus* and *C.occidentalis* and between 4th and 24th week after the flowering of *C.fistula* seed oils. The carotenoid concentration declined from 0.68 % to 0.05 % in *C.absus*; 0.74 %-0.15 % in *C.fistula* and 0.82 %-0.11 % in *C.occidentalis* seed oils. Tocopherols, obtained after saponification of the seed oils, increased from 0.09 % to 0.475 % in *C.absus*, 0.12 %-60 % in *C.fistula* and 0.19 %-61 % in *C.occidentalis* seed oils. The oil contents increased from 0.7 % in *C.absus*; 0.48 %-4.0 % in *C.fistula* and 0.6 %-3.5 % in *C.occidentalis* seed oils. Changes in the oil, carotenoid and tocopherol contents of the three seed oils indicated that the decrease in carotenoid concentration and increase in tocopherol content was associated with increased bio-synthesis of oil and decrease in moisture content as the seeds matured.

Key words: Cassia Carotenoids, Tocopherols, Maturity.

INTRODUCTION

In earlier studies the seed oils of *Cassia* spp. have been examined with a view to elucidating their structures [1,2]. The maturing seeds of *Cassia absus, C.fistula* and *C.occidentalis* have now been studied for determining the formation of carotenoids and tocopherols in them.

Carotenoids and tocopherols are among the lipids of nutritional interest and are frequently determined in fats, oils and other foods. In general they are significant because of their vitamin activities and the important role they play in the lipid metabolism and body growth [4,5]. Since the seeds of the species studied here are used as medicines [3], it was necessary not only to know their oil compositions but also the amounts of tocopherols and carotenoids. Consequently, therefore, it has been found that tocopherol and carotenoid formation bear direct relationship with the maturity of seeds. The carotenoid concentration declined in the three varieties while maturing from the second to the twelfth week after flowering. However, the amounts of tocopherol on the contrary increased with the maturity of seeds as did the oil content.

MATERIALS AND METHODS

Plant materials. Cassia absus, C.fistula and *C. occidentalis* seed samples were harvested from the fields of PCSIR Laboratories, Lahore. Sampling commenced on the 2nd till the 12th week after flowering for *C.abusus* and *C.occidentalis* and from the 4th till the 24th week after flowering for *C.fistula* seeds.

Extraction and estimation of carotenoids. The samples were processed by the procedure used by M.E. Pattee [6]. The carotenoids and oils were extracted separately from the seeds of given maturity stage of the three species by grinding in two volumes of methanol for 2 min. using a blender. The slurry was filtered and the filtrate was saved. The filter cake was suspended in two volumes of acetone/hexane (1:1) and filtered. The residue was washed with two volumes of acetone/hexane (1:1) mixture and discarded. The combined acetone/hexane filtrates were then transferred to another separatory funnel and mixed with one volume of ethyl ether and two volumes of water by gentle swirling. After the formation of distinct phases the aqueous phase was withdrawn and discarded. The ether phase was then washed twice with an equal volume of water. The original upper hexane phase was washed by swirling several times with one volume portion of water. The ether and hexane fractions were combined, dried with sodium sulphate and distilled under pressure to leave the oil residue containing the carotenoids. This residue was weighed and then made to 25 ml with hexane to which anhydrous sodium sulphate was added to remove any traces of water in the solution and the carotenoids were measured on spectrophotometer Hitachi Model (100-20) at 450 mμ.

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An extinction coefficient of 0.25 was used to determine their concentration for the percentage of carotenoids.

Extraction of tocopherols. For extraction of tocopherols 100 mg of oil, obtained from each maturity stage, was saponified by refluxing with 0.5 N alcoholic potassium hydroxide. The unsaponified residue was dissolved in 0.05 ml distilled benzene (A.R).

Estimation of tocopherols. 10-20 μl of the benzene solution were injected into a stoppered test tube which contained 1.5 ml of absolute ethanol and 0.25 ml of 6×10^{-3} M bipyridine in absolute ethanol. Exactly 0.25 ml of 1×10^{-3} M ferric chloride in absolute ethanol was added and the test tube was shaken well. Exactly 2 min. after adding the ferric chloride, 0.25 ml of 4×10^{-2} M orthophosphoric acid in absolute ethanol was added, the test tube was shaken and the contents were transferred to a 1.5 cm spectrophotometric cell. The absorbance was read at 520 mμ against a reference cell containing absolute ethanol. The same procedure was followed omitting the tocopherol solution for the blank. Net extinction was equal to absorbance of the sample minus the absorbance of blank [7].

RESULTS AND DISCUSSION

Data for changes in concentration of carotenoids and tocopherols in the mature and developing seeds of *C.absus,*

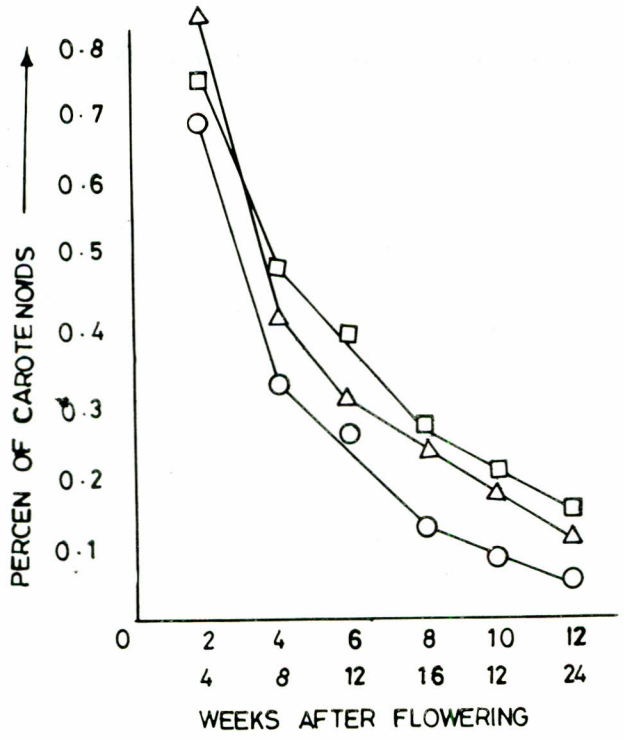

Fig. 1. Influence of maturity on the carotenoid percentage of *Cassia absus* ○, *Cassia fistula* □ and *Cassia occidentalis* △ Seed oils.

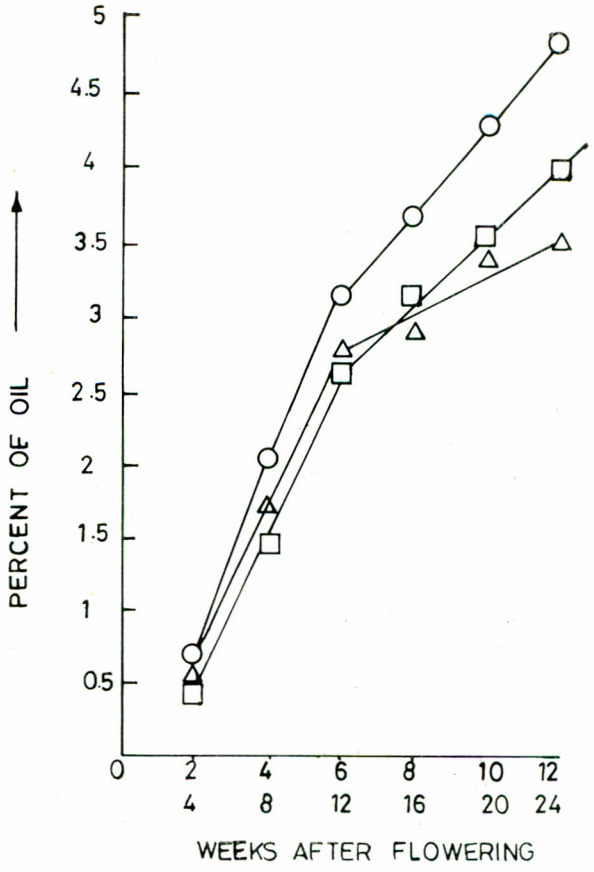

Fig. 2. Influence of maturity on the tocopherols percentage of *Cassia absus* ○. *Cassia fistus* □ and *Cassia occidentalis* △ Seed oils.

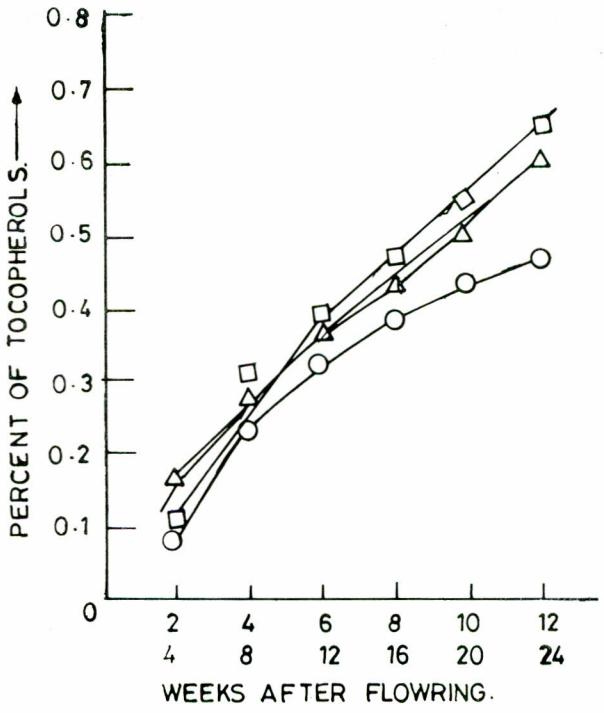

Fig. 3. Influence of maturity on the oil percentage of *Cassia absus* ○. *Cassia fistula* □ and *Cassia occidentalis* △ Seed oils.

C.fistula and *C.occidentalis* are presented in Fig. 1 and 2 respectively. The pattern of changes in carotenoids and tocopherols were about the same for the three species. The reduction of carotenoids was more rapid upto the sixth week after flowering in *C.absus* and *C.occidentalis* seed oils and upto 12th week after flowering in *C.fistula* seed oil. Throughout the remaining maturation period the data are generally alike and the carotenoid concentration decreased 60-70% between the 6th to 12th week after flowering. One of the most rapidly increasing fractions of the seed oils during development and maturation is the lipid fraction. The oil fraction increased rapidly in relation to other constituents from the 4th to the 7th week after flowering but after wards this increase is not as rapid (Fig. 3).

Tocopherols increased gradually throughout the maturation period from 2nd to the 12th week after flowering in the seed oils of *C.absus* and *C.occidentalis* and 4th to 24th week after flowering in the seed oil of *C.fistula*. The increase of tocopherol in *C.absus, C.fistula* and *C.occidentalis* was 4,5 and 3 times respectively and was associated with the increased bio-synthesis of oil.

The results of the present study are similar to those reported earlier concerning seed oils of almonds and Leguminoseae family [9]. Similar studies on a variety of other plants also show the same trend towards the tocopherol and carotenoid formation [10-12].

In view of this it is assumed that the decrease of carotenoids and increase in tocopherols is directly related to the formation of lipids in the developing/maturing seeds. As a consequence of this phenomenon lipids would increase and thus cause a dilution of the carotenoids and this is what has exactly been observed.

REFERENCES

1. Shahina Zaka, Waheed Akhtar, Shafiq Ahmad Khan and M.K. Bhatty, submitted to the Proc. Acad. Sci.
2. Shahina Zaka, Waheed Akhtar and Shafiq Ahmad Khan submitted to the Proc. Acad. Sci.
3. William Dymock, *Pharmacographia India* (printed by the Inst. Health and Tibbi Res., 1972), Pakistan p. 511-513.
4. Willy Lange, J. Am. Oil Chem. Soc., **27**, 414 (1950).
5. Meyer Freed, *Methods of Vitamin Assay* (Inter Science Publishers, New York,1966), p. 98.
6. M.E. Pattee, A.E. Purcell and Elizabeth B. Johns, J. Am. Oil Chem. Soc., **46**, 629 (1969).
7. S. Sharen Bnatcher, A.R. Kemmerer and D.D. Rubis, J. Am. Oil Chem. Soc., **46**, 174 (1969).
8. A.A. Rikhter and V.I. Kriventsov, Fiziol Rast, **27**, 729 (1980).
9. V.K. Men'Kim and A.D. Tumriev, IZV. Timiryazevsk S. Kh. Akad., **2**, 175 (1978).
10. G. Okombi, J. Billot and C. Hartmann, Fruits, **35**, 313 (1980).
11. Gross and Jeana, Gartenbauwissen Schaft, **47** (4), 162 (1982).
12. Gross, Jeana and Habera, Sci. Horticul., **20**(3), 251-71 (1983).