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Carotenoids From Marigold *(Tagetes erecta)* Petals and Their Esterified Fatty Acids

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The main carotenoid in *Tagetes erecta* petals is lutein, wich is found either free or esterified to one or two fatty acids. Column and thin-layer chromatographic methods are described for the separation of the different lutein species, and compared to other, previously published, techniques. In addition, fatty acid distributions are given for whole marigold plants, crude carotenoid extracts and purified mono and diesterified luteins obtained from fresh petals as well as from commercial powdered petal preparations.

The powdered petals of marigold *(Tagetes erecta)* constitute a good source of carotenoid pigments. This cheap preparation has found considerable application in aviculture where it is used as an additive in order to give a yellow-orange colour to the poultry eggs and fat (2). Up to seventeen different carotenoids have been isolated from marigold petals, although lutein constitutes about 90 % by weight (10). Lutein in it is found esterified to one or two fatty acids (1). These ester bonds are unusually alkali-stable, and relatively strong conditions are required for their hydrolysis (9).

The study of the different forms of lutein, free and esterified, is relevant from the physiological point of view since the pigment can only be absorbed after hydrolysis of the esterified forms (5). Thus, a convenient method for separating the free, mono and diesterified forms of lutein is essential for the study of carotenoid absorption.

With respect to the nature of the fatty acids esterified to lutein, previously published results appear inconsistent (1, 10). This could be due to the fact that fatty acids are altered by auto-oxidation (12) during the commercial preparation of marigold petal powders.

The purpose of this paper is, then, to describe preparative and analytical methods for the separation of free, mono and diesterified luteins having some advantages over previously published techniques as well as to establish the fatty acid distribution of the different marigold petal powder preparations.

Materials and Methods

Marigold *(Tagetes erecta)* plants were purchased from commercial sources, where they are sold for ornamental purposes. The commercial marigold petal powder was a kind gift from Coronado, S. A., Bilbao (Spain). Total lipids were extracted fom the whole plant according to BLIGH and DYER (3). Carotenoids were extracted either from the plant or the powdered preparation by the method of QUACKENBUSH (8) with the modification of QUACKENBUSH and MILLER (10).

Column chromatography was performed by a modification of the method of QUACKENBUSH in columns 20 mm I.D. Silicagel H, 3 g, and diatomaceous earth, 3 g, were mixed and transferred to the column without any solvent. The stationary phase was dry packed under reduced pressure until the effective column height was of about 5 cm. Anhydrous sodium sulfate was then added until the total column height was of about 7 cm. The carotenoid mixture was applied in a minimum volume of hexane. The column was eluted with the following solvent mixtures: *a*) hexane-acetone (96:4), 10 ml; *b*) hexane-acetone (90:10), 20 ml; *c*) hexane-acetone (80:20), 25 ml; *d*) hexane-acetone-methanol (80:10:10), 20 ml. The different fractions contained respectively: *a*) the diesterified fraction; *b*) the monoesterified lutein; *c*) the unesterified lutein; *d*) polyoxypigments. The whole experiment took place under reduced pressure in 4-5 minutes. The column was easily regenerated by eluting first with acetone (10 ml) and then with pure hexane (10 ml).

Thin-layer chromatography was carried out in silicagel G plates, 0.25 mm thick. Three different solvent systems were succesful in separating the free, mono and diesterified lutein, namely: *a*) petroleum ether-benzene-acetone (2:2:1); *b*) ethyl ether-hexane-formic acid (25:74:1); *c*) ethyl ether-hexane (7:3). The carotenoids were easily identified by their bright yellow colour; the Rf decreased in the order: diesterified > monoesterified > free lutein. Luteins for fatty acid analysis were purified by thin-layer chromatography using solvent system C. The contamination by sterol esters or glycerides in the lutein preparations was discarded after rechromatography of the eluted yellow spots in various solvents.

The fatty acyl residues were converted to the corresponding methyl esters with 14 % BF_3 in methanol, as described by MORRISON and SMITH (7). Fatty acid methyl esters were then analyzed by gas-liquid chromatography in a 5120A Hewlett-Packard chomatograph. The apparatus was equipped with a dual flame-ionization detector. Stainless steel columns (1.80 m × 3 mm) packed with 5 % PEGA were used. Carrier gas was N_2, at 40 ml/min. The oven temperature was 180°, isothermal.

Results and Discussion

The elution process of the chromatographic column took about five minutes, when working under reduced pressure, which is less than half the time required by the procedure of QUACKENBUSCH *et al.* (11), although the resolution was at least as good. Moreover the use of relatively wide (2 cm I.D.) dry-packed columns, as proposed by us, makes it easier to obtain perfectly horizontal bands, which in turn improve resolution and prevent remixing

Table I. *Rf values for free, mono and diesterified lutein separated on silicagel G with the solvent systems A, petroleum ether-benzene-acetone (2:2:1); B, ethyl ether-hexane-formic acid (25:74:1), and C, ethyl-ether-hexane (7:3)*

	Rf values		
	A	B	C
Free lutein	0.91	0.77	0.95
Monoesterified lutein	0.56	0.23	0.70
Diesterified lutein	0.23	0.10	0.30

Table II. *Percent distribution of fatty acids in the whole lipid contents of marigold* (Tagetes erecta) *and in various carotenoid preparations obtained thereof.*

A. Total lipid extract.

B. Carotenoid extracts from fresh marigold petals: B_1, total carotenoid extract; B_2, pure monoesterified lutein; B_3, pure diesterified lutein.

C. Carotenoid extracts from commercial petal powders: C_1, total carotenoid extract; C_2, pure esterified lutein; C_3, pure diesterified lutein.

Fatty acid	N.° of carbon atoms	Mole %						
		A	B1	B2	B3	C1	C2	C3
Lauric	12:0	trace	trace	7.8	8.3	3.0	4.3	3,0
Myristic	14:0	7.1	12.0	12.0	12.7	14.4	10.2	6.3
Pentadecanoic	15:0	—	trace	trace	1.0	—	—	—
Palmitic	16:0	38.2	53.7	34.0	36.7	37.7	41.4	40.9
Stearic	18:0	12.6	17.1	38.1	32.2	21.4	41.4	42.3
Oleic	18:1	17.1	9.5	8.1	6.3	18.8	3.0	7.4
Linoleic	18:2	25.7	7.6	—	2.3	4.4	—	—
Total unsaturated	—	42.8	17.1	8.1	8.6	23.2	3.0	7.4

of the different components. Finally, the possibility of regenerating the column did not exist in the previously published method (11), but was very feasible in ours, as it constitutes an additional advantage.

Table I lists the Rf values for the free, mono and diesterified lutein in the three chromatographic systems mentioned under «Materials and Methods» as A, B and C. In all three cases the plates were developed in 35-40 minutes. No particular advantage was associated with any of these three procedures, but all of them proved very superior in resolution to the systems published previously by BOLLIGER and KÖNIG (4).

The fatty acid distribution of several lipid preparations from *Tagetes erecta* is shown in Table II. When examining the total lipids of the whole plant (column A), it can be seen that palmitic is the most abundant of the fatty acids but considerable amounts of unsaturated, oleic and linoleic, fatty acids are also found, as expected in plant tissues. The results shown in column B correspond to the fatty acids esterified to carotenoids extracted from fresh intact marigold petals. The fatty acid distribution of total carotenoid extract (B1), pure monoesterified (B2) and pure diesterified (B3) lutein are included. In general it can be said that the degree of unsaturation is much lower than in the total lipid extract. This is especially true in the case of the purified luteins. The mono and diesterified luteins do not differ considerably in their fatty acid content, except for the small amounts of pentadecanoic and linoleic acids in the diesterified form, which are not found in the monoesterified lutein. In both cases, palmitic and stearic acids are present in nearly equal proportions. The total carotenoid extract differs from the purified luteins mainly because of the lower relative content of lauric and stearic acid and the higher content of palmitic and linoleic acid. These differences can be due to the influence of fatty acids esterified to carotenoids other than lutein, or else to the presence of triglycerides in the carotenoid extract.

The fatty acid distribution of carotenoids extracted from commercial marigold petal powders are shown in Table II, column C. Again the results corresponding

to total carotenoid extract, and purified mono and diesterified lutein are given. The commercial powder is prepared from marigold flowers which are dried in the sun and then powdered. Apparently, not only petals, but also other parts of the flower are dried and powdered so that a direct comparison of the total carotenoid extracts from column B and C is not feasible.

However, the fatty acid distribution of the purified luteins can be compared. In both cases, the nearly equal proportion of palmitic and stearic acids is maintained, but there is an increase of the relative amounts of saturated over unsaturated fatty acids. This can be easily explained on account of fatty acid auto-oxydation (12) in the process of drying and storage of the commercial preparation.

Thus, it can be stated that palmitic acid is the main fatty acid in the whole of marigold lipids, as well as in the total carotenoid extracts of fresh petals and dried powders. However, in the pure luteins there are equal amounts of palmitic and stearic acids. Appreciable amounts of unsaturated acids are also found. The significance of the fatty acids with respect to the absorption of carotenoids is discussed in a further paper (5).

Resumen

El principal carotenoide de los pétalos de *Tagetes erecta* es la luteína, que se presenta libre o esterificada a uno o dos ácidos grasos. Se describen métodos de cromatografía en columna y en capa delgada para separar las distintas formas de luteína, y se comparan con técnicas publicadas con anterioridad. Además, se describen la distribución porcentual de ácidos grasos en las plantas enteras, extractos crudos de carotenoides y luteínas mono y diesterificada obtenidas de pétalos frescos y de preparaciones comerciales pulverizadas.

References

1. ALAM, A. V., COUCH, J. R. and CREGER, G. R.: *Lipids*, 3, 183-185, 1968.
2. BAUERFEIND, J. C., BROBACHER, G. B., KUALI, H. M. and MARUSICH, W. L.: In «Carotenoids» (O. Isler, ed.), Birkhauser, Berlín, 1971, pp. 743-767.
3. BLIGH, E. G. and DYER, W. J.: *Can. J. Biochem. Physiol.*, 37, 911-918, 1959.
4. BOLLIGER, H. R. and KÖNIG, A.: In «Thin-Layer Chromatography» (E. Stahl, ed.), Springer, Berlín, 1969, pp. 259-311.
5. GÓMEZ, R., ALONSO, A. and MARTÍN, M.: *Rev. esp. Fisiol.*, 34, 257-260. 1978.
6. MARUSICH, W. L. and BAUERFEIND, J. C.: *Poultry Sci.*, 49, 1555-1557, 1970.
7. MORRISON, W. R. and SMITH, L. M.: *J. Lipid Res.*, 5, 600-605, 1964.
8. QUACKENBUSH, F. W.: *J. Ass. Offic. Anal. Chem.*, 33, 647-650, 1950.
9. QUACKENBUSH, F. W.: *J. Ass. Offic. Anal. Chem.*, 56, 748-753, 1973.
10. QUACKENBUSH, F. W. and MILLER, S. L.: *J. Ass. Offic. Anal Chem.*, 55, 3-6, 1972.
11. QUACKENBUSH, F. W., DYER, M. A. and SMALLIDGE, R. L.: *J. Ass. Offic. Anal. Chem.*, 53, 181-183, 1970.
12. URI, N.: In «Autoxydation and Autoxydants» (W. O. Lundberg, ed.), vol. I, Interscience, New York, 1962, pp. 122-165.