Carotenoids of Wheat Flour: Their Identification and Composition[1]

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ABSTRACT

Wheat flour carotenoids from two varieties of wheat were studied by chromatographic and spectrophotometric methods. The major components found were lutein (xanthophyll) and its esters, a monoester and a diester. The fatty acids, identified by gas-liquid chromatography, were a mixture of palmitic, stearic, oleic, linoleic, and linolenic acids. The esters were found mainly in Thatcher wheat flour, in which they account for 78% of total carotenoids. Free lutein, however, amounted to 85% in Mindum. There was no indication of beta-carotene in any extract.

Color is a dependable criterion for evaluating wheat flour quality. It is also a common and valuable guide to wheat breeders. Strong pigmentation in durum wheats is desired because yellow color is a quality factor in pasta. In contrast, flour for white bread or cake must be as white as possible, and minimum pigmentation is sought in wheats milled for these purposes.

Several papers have been published on the nature of carotenoids in wheat. Markley and Bailey reported in 1935 (1) that xanthophylls and their esters were the principal components of wheat pigments. Zechmeister and Cholnoky (2) showed that practically the only carotenoid occurring in Hungarian wheat flour was lutein.

This paper describes a more detailed study of wheat flour carotenoids by chromatographic and spectrophotometric methods. Application of new methods in this laboratory confirms the evidence that wheat flour is composed of free lutein (xanthophyll) and its esters, but also shows that these esters vary greatly from one variety to another. Further information on the chemical nature of the carotenoids is reported. Absorption maxima of each carotenoid, in various solvents, which permit one to select the proper wave length, are tabulated. Gas-liquid chromatographic (GLC) analyses of their fatty acids were performed to calculate the average molecular weight and the content of each carotenoid on a weight basis.

EXPERIMENTAL

Materials and Extraction

Two varieties of wheat, Mindum (durum) and Thatcher (hard red spring), were tempered to 15.5% moisture and milled to 65-70% extraction to give a straight-run flour.

Wheat flour (100 g.) was extracted with 5 volumes of water-saturated n-butanol with frequent shaking (3). The supernatant was filtered and the solids were re-extracted with the same solvent system until decoloration was complete. Extracts were pooled and brought to dryness under reduced pressure. The residue, carotenoids and lipids, was then taken up in hexane and stored in the dark under refrigeration.

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Chromatographic Procedures

In preliminary experiments, extracts were subjected to 100-transfer countercurrent distribution runs in a Craig-type apparatus; the solvent system used was hexane:benzene:87% methanol (1:1:1.5), to separate the carotenoids into (a) hydrocarbons plus monols, (b) diols, and (c) polyols, according to Curl (4).

Portions of the carotenoid extracts were fractionated on a silicic acid column. A glass column, 0.9 cm. i.d., filled to a height of 13 cm. with silicic acid (Bio-Sil HA, –325 mesh, Bio-Rad, Richmond, Calif.) was loaded with about 50 mg. of extract. Elution was started with hexane until the various components were separated into bands, then continued with 15 ml. 5% ether in hexane to obtain fraction A, with 10 ml. 50% ether in hexane for fraction B, and 5% methanol in chloroform for fraction C.

Fractions A, B, and C were further subjected to thin-layer chromatography (TLC) to test their homogeneity. Chromatoplates, 20 × 20 cm., coated with a layer of silica gel G, 250 μ thick, were used. Hexane:isopropyl ether: diethyl ether:acetone:acetic acid (85:12:1:4:1 v./v.) was the best solvent system for this purpose.

Identification Techniques

The absorption spectrum of each carotenoid was obtained with a Bausch & Lomb Spectronic 502 spectrophotometer. Quantitative measurements were made with a Bausch & Lomb 340 spectrophotometer, in water-saturated n-butanol, at 450 mμ.

Certain fractions were subjected to phase-partition between hexane and aqueous 90% (v./v.) methanol (5). Some were examined for epoxides by the hydrochloric acid-ether color test (6).

Extracts and fractions obtained by column chromatography were deacetylated with 0.2*N* methanolic potassium hydroxide at 37°C. for 20 min. The products were extracted with hexane and separated by column chromatography as above. Their absorption spectra were compared with those of intact carotenoids and with those of authentic lutein obtained through the courtesy of Eastman Kodak, Rochester, N. Y.

To test for the presence of free hydroxyl groups, certain fractions were acetylated with acetic anhydride in pyridine.

Fatty acids were converted to their methyl esters (7) and analyzed by GLC (8); a column of 5% DEGS on Gas Chrom. P (Applied Science, State College, Pa.) was used.

RESULTS

Flour from the wheat varieties Mindum and Thatcher contained 3.7 and 2.8 p.p.m. of total carotenoids, respectively.

In preliminary experiments with countercurrent distribution techniques, flour carotenoids separated into two major fractions, with N_{100} values of 71 (tube number in a 100-transfer extraction) for compounds containing two free hydroxyl groups and 95 for those containing one free hydroxyl group or none, as indicated in Table I. The first consisted of a diol, or lutein. It predominated in Mindum but was a minor constituent of Thatcher. The

TABLE I

COUNTERCURRENT DISTRIBUTION OF WHEAT FLOUR CAROTENOIDS SYSTEM, HEXANE: BENZENE: 87% METHANOL[a]

WHEAT FLOUR	N_{100}[b] DIOLS Found	DIOLS Reported[c]	HYDROCARBONS AND MONOLS Found	HYDROCARBONS AND MONOLS Reported[c]
Mindum	71	70	95	95
Thatcher	68	70	91/95	95

[a] 100 transfers.
[b] N_{100} = tube number of peak for the 100-transfer run.
[c] See ref. 6.

constituents of the second fraction were minor components in Mindum and major components in Thatcher. In the latter, it showed a double peak with maxima at 91 and 95, indicating the presence of more than one component.

Chromatographic adsorption on silicic acid produced three zones, as expected from the above results. Fractions A and B, which first separated

TABLE II

COLUMN CHROMATOGRAPHIC SEPARATION OF WHEAT FLOUR CAROTENOIDS

FRACTION	ELUTING SOLVENT	VOLUME	EMERGENCE	ESTER GROUP	EPOXY GROUP	CAROTENOIDS
		ml.	*ml.*			
A	5% ether in hexane	15	12–14	+	—	Diol diester
B	50% ether in hexane	10	7–10	+	—	Diol monoester
C	5% methanol in chloroform	25	17–23	—	—	Free lutein

in hexane, were obtained quantitatively with 5% ether in hexane and 50% ether in hexane, as given in Table II. Fraction C, which was more strongly adsorbed, was eluted with 5% methanol in chloroform. This fraction, corresponding to fraction N_{100} 71, had chromatographic properties similar to those of authentic *trans*-lutein. None of these fractions had the same retention volume as beta-carotene, however.

Separation of the same extracts on TLC revealed the same components, with R_f values of 0.50, 0.16, and 0.03 respectively in the solvent system hexane:isopropyl ether:diethyl ether:acetone:acetic acid (Fig. 1). Beta-carotene, which was not detected in the flour extracts, had a R_f value of 0.75 under these conditions. As carotenoids were present in relatively small quantities, it was essential to fractionate and concentrate them on a column prior to TLC.

Partition of each carotenoid between hexane and 90% methanol clearly indicated that fraction C was hypophasic and was consequently a free xanthophyll. On the other hand, fractions A and B were epiphasic. The hydrochloric acid-ether color test was negative and demonstrated that they were not epoxides.

Absorption spectra of all fractions contained identical maxima at 424, 445, and 473 mμ in hexane, and at 424, 449, and 477 mμ in water-saturated n-butanol (Table III). These maxima are in close agreement with those re-

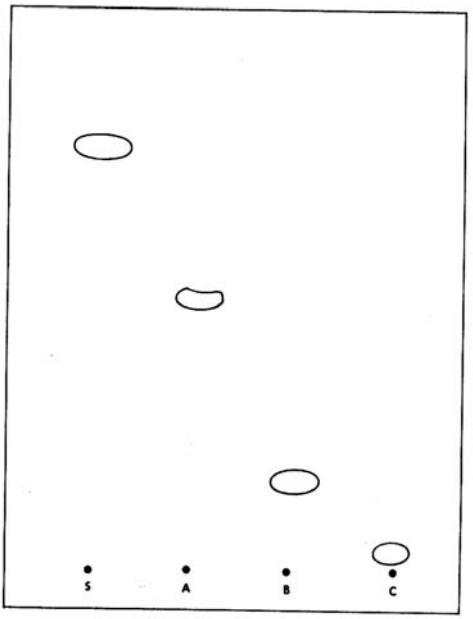

Fig. 1. Tracing of a thin-layer chromatogram of wheat flour carotenoids. Solvent system, hexane:-isopropyl ether:-diethyl ether:-acetone:-acetic acid (85:12:1:4:1). S, pure beta-carotene; A, B, and C, fractions eluted from silicic acid columns.

TABLE III

ABSORPTION MAXIMA FOR EACH CAROTENOID ELUTED FROM SILICIC ACID COLUMNS

FRACTION	SOLVENT	MEASURED SPECTRA (MAX.)			REFERENCE SPECTRA (MAX.)			CAROTENOID
		mμ	mμ	mμ	mμ	mμ	mμ	
A	Hexane	424	445	473	....	446	474[a]	Lutein diester
	WSB[b]	424	449	477				
B	Hexane	420	445	473				Lutein monoester
	WSB[b]	424	449	477				
C	Hexane	424	445	473	420	447	477[c]	Free lutein
	WSB	424	448	476				
	Chloroform	430	454	483	428	456	487[a]	

[a] See ref. 9.
[b] WSB = water-saturated n-butanol.
[c] See ref. 10.

ported for *trans*-lutein (9) and lutein diester (10), but differ from those of free beta-carotene (9), which absorbs at higher wave lengths.

Table IV gives percentages of carotenoids in Mindum and Thatcher wheat. Fractions were read at 450 mμ, in water-saturated n-butanol, and percentages were calculated with the same molecular absorption coefficient. It is noted that Mindum is composed of 84.8% free lutein, whereas in Thatcher, fractions A and B account for 78.4% of the total carotenoids.

Fractions A and B, when hydrolyzed with 0.2*N* methanolic potassium

TABLE IV

SPECTROPHOTOMETRIC DETERMINATION OF WHEAT FLOUR CAROTENOIDS IN WATER-SATURATED n-BUTANOL AT 450 mμ

FRACTION	CAROTENOIDS	AVERAGE MOLECULAR WEIGHT	MOLECULAR WEIGHT RATIOS	RELATIVE WEIGHT PERCENT			
				Mindum Wheat		Thatcher Wheat	
				OD	%	*OD*	%
A	Lutein diester	1,084	1.91	0.022	5.3	0.181	31.9
B	Lutein monoester	826	1.45	0.054	9.8	0.347	46.5
C	Free lutein	568	1.00	0.674	84.8	0.233	21.6

hydroxide, yielded a product which cochromatographed with fraction C or with free lutein. Acetylation of fractions B and C gave rise to a product of very similar properties to fraction A, which strongly suggested that fractions A and B were lutein esters, a diester and a monoester respectively.

To confirm the presence of esters, to determine their fatty acid composition, and to calculate their average molecular weights, carotenoids were separated on a preparative scale from Thatcher wheat flour by column chromatography. Fraction A was further purified by TLC, transmethylated, and analyzed by GLC. The main fatty acids were palmitic (22.7%), stearic (4.4%), oleic (33.9%), linoleic (32.2%), and linolenic acids (2.1%). These percentages permit calculation of the average molecular weight of each pigment (Table IV) and thus the conversion of absorbances into percentages by weight.

The practical application of these data is described in a companion publication.

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