CAROTENOIDS OF HUNGARIAN WHEAT FLOUR

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Whether or not wheat flour contains provitamin A in amounts which could be of any importance for general nutrition is a question of considerable interest. Since Wesener and Teller (1) as well as Monier-Williams (2) considered carotene, $C_{40}H_{56}$, to be the chief fat-soluble pigment of flour, this claim has been more or less supported by numerous authors (3). It was, however, correctly pointed out in the short reviews given by Widmark and Neymark (4) and by Jörgensen (5) that the reported carotene content of flour decreased with increased progress in this field. While Ferrari and Bailey (6) considered "carotin" the chief component of the coloring matter, it is rightly stated in an important paper by Markley and Bailey (7) that only a fraction of the wheat carotenoids can consist of carotene; *e.g.*, one-third to one-seventh of the total pigment.

These figures are, however, still much too high. Malmberg and von Euler (8) were unable to detect any carotene at all in 100 gm. of wheat, and they found xanthophyll, $C_{40}H_{56}O_2$, to be the chief polyene, in accordance with the data of Bowden and Moore (9) who proved the presence of xanthophyll in wheat germ oil.

According to our new chromatographic experiments, only a small part of the carefully saponified pigment shows an epiphasic behavior, and even this fraction can easily be separated from added β-carotene in the Tswett column. Consequently we claim that the analyzed, unbleached flours are practically or absolutely free of carotene, the carotene content being less than 0.01 mg. per kilo in any case. Other samples are under investigation, but even our whole wheat flour does not contain much more carotene or xanthophyll than fine grade samples.

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As far as we know, no carotenoid has yet been isolated in crystallized form from flour, obviously because of the small amounts of the pigment present and of the very unfavorable proportion between coloring matter and colorless substances in the extracts. Earlier investigations carried out with animal fats in this laboratory showed, however, that in similar cases a sequence of several chromatographic operations may gradually improve the degree of purity, and finally allow the separation of the pigment. By applying this principle, we isolated 15 mg. of well crystallized, pure xanthophyll (lutein) from 60 kilos of flour.

EXPERIMENTAL

Chromatographic Analysis of Flour Carotenoids

Carotene—The starting material was unbleached, fine grade wheat flour from southern Hungary ("zero flour") which was harvested in July and milled in October, a few days before the experiments were begun. In the course of 4 to 5 hours 1 kilo was percolated with peroxide-free ether, whereupon the extract (1.5 liters) was kept for a night over 0.1 liter of concentrated methanolic KOH. The dark alkaline layer was then drained off, gently shaken with 0.5 liter of fresh ether, and finally, with caution, 1 liter of water was added without any further shaking. The upper layer plus the main ethereal solution (see above) was washed alkali-free, dried with sodium sulfate, and concentrated as far as possible at not over 40°, under diminished pressure, while a slow stream of CO_2 was bubbled through. In order to eliminate the last traces of the ether, it is advisable to add some petroleum ether (b.p. 70°) and to repeat the evaporation.

The next step was the chromatographic separation of the pigment. The petroleum ether solution of the residue obtained was poured onto a calcium hydroxide column (18 × 3.5 cm.) which was washed with fresh solvent until the following chromatogram was obtained, the zones of which showed blurred borders. Further washing should be avoided, as it would carry the lower layer of pigment into the filtrate. (The figures denote the width of the respective zones.)

40 mm., yellow, heterogeneous: xanthophylls
60 " colorless
2 " very pale yellow: partly carotene (?)
Filtrate: yellowish, differing from carotene but showing a similar spectrum

The two latter fractions were epiphasic; their spectra were in some cases like that of β-carotene, in other cases rather like that of α-carotene, the maxima determined in light petroleum being 482, 452 mμ or 477, 448 mμ.

Neither of these two fractions can, however, be identical with carotene, because their behavior is altered by a new treatment with methanolic alkali. The whole pigment content of the filtrate became hypophasic under this treatment, and an analogous change was observed in the larger part of the 2 mm. zone also when it was eluted in alcohol, transferred into ether, and saponified as mentioned above. As a considerable increase in the adsorbability was also observed, undoubtedly some polyenic esters which are fairly resistant to saponification had been present in the flour.

A further indication of non-identity with carotene was furnished by the so called mixed chromatogram. A solution of pure β-carotene (from red pepper) was added to the saponified fraction and the solution was passed through the Tswett column, whereupon the added carotene was more easily adsorbed, while the flour pigment passed into the filtrate. This flour pigment fraction collected from 60 kilos of flour could not be crystallized. In no case can the possible carotene content exceed 0.01 mg. per kilo of flour.

Xanthophyll—The 40 mm. zone was eluted with alcohol and this solution was mixed with petroleum ether. Sufficient (but no more) water was then cautiously added just to remove the pigment to the upper layer. Too much water would transfer an unusually large amount of the colorless impurities as well. The separated and dried pigment solution was adsorbed on a calcium carbonate column (18 × 4.5 cm.) prepared from a mixture of 1 part of calcium carbonate precip. and 3 parts of calcium carbonate leviss. It is advantageous to develop the chromatogram by means of a benzene-petroleum ether mixture (1:20) and finally to eliminate from the column traces of benzene by washing it with petroleum ether. The developed column showed the following zones.

70 mm., colorless

2 " brownish yellow
0.5 " yellow
} (neoluteins, in light petroleum 473, 444 mμ; when treated with traces of iodine, they are partially converted into lutein)

5 " colorless

10 " yellow: xanthophyll (lutein), in light petroleum 477.5, 447 mμ

The pigmented layers were eluted with alcohol, transferred into light petroleum, and measured with the photometer. The values obtained vary between 1 and 2.5 mg. of xanthophyll per kilo of flour. As xanthophyll is practically the only carotenoid present, for the purpose of an approximate estimation of the whole polyene content it is satisfactory to exhaust 100 gm. of the flour with light petroleum, to concentrate the solution to 100 cc., and to measure the extract photometrically after it has been cleared up by standing.

Isolation of Xanthophyll (Lutein)

We extracted 60 kilos of flour in 5 kilo portions with ether. The first 5 liters of each percolate were worked up directly, as described below, while the following pale solution was used for the extraction of the next 5 kilo portion. The whole liquid was concentrated under a vacuum from 60 to 15 liters. Further concentration was prevented by frothing, which was rapidly getting worse. The saponification with methanolic KOH and the transfer of the coloring matter into ether were carried out as described in the foregoing section, whereupon the solution (3 liters) was washed alkali-free, dried, and evaporated under diminished pressure in a slow stream of CO_2. It was found advisable to dissolve the resulting dark oil in petroleum ether and to evaporate the liquid again.

The chromatogram, obtained on calcium hydroxide (20 × 6 cm.), showed a comparatively well fixed, broad, blurred, but on the whole apparently homogeneous zone, while it was easy to wash the traces of other pigments into the filtrate. The xanthophyll was eluted with alcohol and cautiously transferred into light petroleum with as little water as possible. Finally the whole adsorption procedure was repeated twice more. In the course of such operations it is noticed that the chromatogram gradually improves; *i.e.*, the last column shows a sharply bordered main zone. When this point is reached, the main bulk of the sterols is eliminated and may be easily crystallized from the filtrates.

Nevertheless, further purifying processes are necessary. We eluted the pigment with alcohol and carried out three successive chromatographic separations by using petroleum ether and calcium carbonate. Finally the column was washed with a mixture of benzene and petroleum ether (1:20), and the colored layer was

eluted and transferred into benzene-light petroleum, which was dried and completely evaporated. It left a partially crystallized residue behind, which was dissolved in very little benzene and mixed with light petroleum. There appeared microscopic yellow needles, wart-like grouped, and typical for xanthophyll crystallized in the way described. These were filtered and rapidly dried at 60° under 0.1 mm. pressure.

Yield, 15 mg. of pure lutein, m. p. 193° (corrected). Calculated for $C_{40}H_{56}O_2$, C 84.44, H 9.93; found, C 84.34, H 9.97. Absorption maxima in carbon disulfide, 507.5, 474.5 mμ; in benzene, 489, 457 mμ; in light petroleum, 477, 447 mμ; in alcohol, 477.5, 446.5 mμ. A mixture of a sample of the isolated pigment with lutein (xanthophyll) was inseparable in the Tswett column.

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SUMMARY

1. Unbleached wheat flour from southern Hungary contains no more than 0.01 mg. of carotene per kilo, if any. Since it is free also from cryptoxanthin, it is valueless as a provitamin A source.

2. Practically the only polyene occurring in the flours investigated is xanthophyll (lutein). Some of the other pigments are isomerization products (10), while the nature of the others is unknown.

3. Through repeated application of the chromatographic method, 15 mg. of pure xanthophyll crystals have been isolated from 60 kilos of flour.

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