CAROTENOIDS OF CORN AND SORGHUM

VI. Determination of Xanthophylls and Carotenes in Corn Gluten Fractions[1]

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ABSTRACT

Variation in carotenoid content of corn and of gluten fractions creates problems in blending mixed feeds to guaranteed levels of xanthophylls and carotenes. Samples of hybrid yellow dent corn, typical of those processed by wet-millers, showed a three- to fourfold variation in xanthophylls (10–30 p.p.m.) and carotenes (1–4 p.p.m.). Variation in total carotenoid content (247–379 p.p.m.) of two commercial samples of corn gluten containing 60 to 70% protein was of the same magnitude as the differences in carotenoid level (19–30 p.p.m.) of the whole corn used for processing. The total carotenoid content of gluten feed (21% protein) varied from 14 to 34 p.p.m. and that of gluten meal (41% protein) from 65 to 253 p.p.m. Presumably, the carotenoid content of the feed and meal depends to a considerable degree upon the type and amounts of materials blended during processing. The gluten fractions contained larger quantities of noncarotenoid pigments than the whole corns. Saponification of the extracts before chromatography reduced total carotenoid content in several samples. The apparent decrease may be due to conversion of hexane-soluble alkylated flavonoid compounds to water-soluble types during saponification.

Pigmentation in noodles, mayonnaise, and cake mixes must come from egg yolks of a specified color density, because present Food and Drug Administration regulations (1) prohibit artificial coloring in these food products. The yellow pigments of egg yolks are xanthophylls, which also impart color to the skin and shanks of broilers and fryers. Corn gluten products often are used to increase the level of xanthophylls in feeds, because regular poultry rations are usually prepared from materials low or deficient in these pigments. Since the manufacturer of mixed feed requires a dependable low-fiber source of xanthophylls, he is concerned about the variability of xanthophyll content in wet-milled fractions of corn. The lack of appreciable quantities of carotenoids in grain sorghum and sorghum gluten has been reported previously (2,3). As another part of this research series, xanthophylls and carotenes were determined in typical hybrid yellow dent corns and in commercial corn gluten fractions.

[1]Manuscript received January 30, 1964. Contribution from the Northern Regional Research Laboratory, Peoria, Ill. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Presented in part at the 47th annual meeting, St. Louis, Missouri, May 1962.

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Materials and Methods

Materials. Double-cross hybrid yellow corns, typical of those processed by wet-millers, were selected from various areas of the United States. Several waxy types were included in this group. Geographical locations ranged from Mississippi to Minnesota. The samples, which received no artificial drying, were stored in a refrigerator before analysis to prevent loss of carotenoids. Various gluten fractions also were obtained from the process stream in two different commercial wet-milling operations, although prior history of the corn was not available.

Analytical Method. Xanthophylls and carotenes were determined by a previously described method (2) which normally does not employ saponification. Where indicated, saponification was conducted according to Goodwin's procedure (4). All values are reported on moisture-free basis.

Results and Discussion

Commercial Hybrid Corn. Samples of commercial hybrid yellow dent corns (Fig. 1) showed three- to fourfold variation in xanthophylls

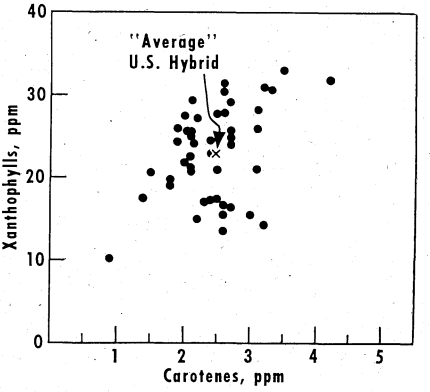

Fig. 1. Level of xanthophylls and carotenes in hybrid yellow dent corns.

(10–30 p.p.m.) and carotenes (1–4 p.p.m.). The same magnitude of variation would be expected in the carotenoid content of gluten processed from these corns. Values for individual varieties and statistical treatment of the data were reported earlier (5).

Corn Gluten Fractions. Xanthophylls and carotenes were determined in two commercial samples of corn and the gluten fractions processed from them (Table I). Corn A was used to prepare the gluten fractions marked "A," and corn B, those marked "B." Protein content

TABLE I

CAROTENOID CONTENT OF WHOLE CORN AND ITS COMMERCIAL GLUTEN FRACTIONS

SAMPLE	SOURCE	TREATMENT	CAROTENES		XANTHOPHYLLS		RATIO OF XANTHOPHYLLS TO CAROTENES	TOTAL CAROTENOIDS		OTHER PIGMENTS
			Concentration	Percent of Total Carotenoids	Concentration	Percent of Total Carotenoids		Concentration	Percent of Total Pigments	
			p.p.m.		*p.p.m.*			*p.p.m.*		*p.p.m.*
Whole corn	A	Nonsaponified	2.2	9	24	91	11	26	93	2
	A	Saponified	1.4	7	18	93	13	19	70	8
	B	Nonsaponified	3.0	10	27	90	9	30	91	3
	B	Saponified	2.5	8	27	92	11	30	91	3
Gluten feed	A	Nonsaponified	2.9	19	12	81	4	15	39	24
	A	Saponified	0.8	6	13	94	17	14	36	25
	B	Nonsaponified	2.2	6	35	94	16	37	58	27
	B	Saponified	1.8	5	32	95	18	34	53	30
Gluten meal	A	Nonsaponified	8.5	10	75	90	9	83	73	31
	A	Saponified	2.6	4	62	96	24	65	57	49
	B	Nonsaponified	20.9	9	221	91	11	242	83	51
	B	Saponified	18.7	7	234	93	12	253	86	40
Gluten	A	Nonsaponified	20.6	8	234	92	11	255	87	37
	A	Saponified	15.2	6	232	94	15	247	85	45
	B	Nonsaponified	23.8	6	382	94	16	406	91	39
	B	Saponified	30.7	8	348	92	11	379	85	66

of corn A was 11%; corn B, 9%; gluten feed, 21%; gluten meal, 41%; gluten A, 60%; and gluten B, 70%.

All values in Table I are the average of at least two determinations. The total pigment values (2) are identical for each saponified and nonsaponified sample. These values are based on the total absorbance at 445 mμ of all the compounds (carotenes, xanthophylls, flavonoids, and chlorophyll-like compounds) contained in the ethanol extract. The "other pigment" value (2) is, therefore, an indication of the relative amount of noncarotenoid pigment in the extract. Total carotenoids accounted for approximately 90% of the total pigments in the nonsaponified extracts from the two whole corns. Previous work (2) indicated that saponification had no appreciable effect on total carotenoid content of corn extracts. However, saponification of the extract from whole corn A reduced apparent carotenoid content 30%. Possible causes for this reduction will be discussed later.

Total carotenoid content increased with increasing protein content (compare values for gluten feed, gluten meal, and gluten) and accounted for a progressively greater portion of the total pigments. The gluten fractions contained considerable amounts of other pigments or noncarotenoid pigments. A reduction in the apparent level of carotenes of the whole corn and gluten fractions upon saponification may indicate the presence of xanthophyll esters in the nonsaponified samples (2). The reduction in carotenes was not reflected by a comparable increase in xanthophyll level in several samples. Upon saponification, one sample, gluten B, increased in carotenes from 23.8 to 30.7 p.p.m. This unusual behavior may be due to physical and chemical changes which occurred during the wet-milling process. Saponification might have released additional carotenes from an alcohol-soluble lipoprotein complex (2). Further work is planned in this area.

Upon saponification, xanthophylls and total carotenoids decreased in several samples, notably corn A, gluten meal A, and gluten B. The loss may be due to the presence of alkylated flavonoid-type compounds. Although not investigated, such compounds should behave similarly to carotenoids in nonsaponified samples but would be removed in the alkaline aqueous phase during saponification.

Gluten, the endosperm protein fraction separated from the starch during the wet-milling process, should reflect the differences in carotenoid level of grain for wet-milling. To test this hypothesis, the ratios of carotenoids were compared in the fractions from two different wet-milling operations (Table II). Saponification increased the ratio of carotenes (B to A) threefold in the gluten feed and gluten meal. The

TABLE II

COMPARISON OF RATIOS OF CAROTENOIDS IN GLUTEN FRACTIONS FROM TWO WET-MILLING OPERATIONS

SAMPLE	TREATMENT	RATIO OF CAROTENES: B/A	RATIO OF XANTHOPHYLLS: B/A	RATIO OF TOTAL CAROTENOIDS: B/A
Whole corn	Nonsaponified	1.4	1.1	1.2
	Saponified	1.8	1.5	1.6
Gluten feed	Nonsaponified	0.8	2.8	2.4
	Saponified	2.2	2.4	2.4
Gluten meal	Nonsaponified	2.5	3.0	2.9
	Saponified	7.2	3.8	3.9
Gluten	Nonsaponified	1.2	1.6	1.6
	Saponified	2.0	1.5	1.5

increase is due to reduction of carotene levels in gluten feed A and gluten meal A following saponification (Table I). Saponification had little effect on the other ratios. Variation in total carotenoid content of the samples of "pure" gluten was of the same magnitude (approximately 1.5) as differences in total carotenoid levels of the whole corn used for processing. However, the ratio of total carotenoid content in the gluten feed of 2.4 and gluten meal of 3.4 deviates from the pure gluten ratio. The carotenoid content of the feed and meal fractions presumably depends to a considerable degree upon type and amounts of materials blended to obtain certain protein levels. Therefore, both natural variations and processing differences contribute to the over-all differences in carotenoid levels of wet-milled products. Such variations create problems in blending mixed feeds to a guaranteed analysis for carotenes and xanthophylls, since protein content does not indicate any definite carotenoid level.

TABLE III

ESTIMATION OF CAROTENOID CONTENT OF GLUTEN FRACTIONS

CAROTENES			XANTHOPHYLLS			TOTAL CAROTENOIDS		
Actual	Calculated	(Actual/calc.) × 100	Actual	Calculated	(Actual/calc.) × 100	Actual	Calculated	(Actual/calc.) × 100
p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*	
			Gluten source A, nonsaponified					
20.6	33.9	61	234	362	65	255	396	64
			Gluten source A, saponified					
15.2	21.6	70	232	273	85	247	294	84
			Gluten source B, nonsaponified					
23.8	46.2	52	382	411	93	406	457	89
			Gluten source B, saponified					
30.7	38.5	80	348	420	83	379	459	83

An estimation of the percentage recovery of corn carotenoids in gluten from corn wet-milling was based on a gluten yield of 6.5% (3). On this basis, only 50 to 80% of the theoretical yield of total carotenes was obtained in the gluten fraction (Table III). A greater portion (65–95%) of the total xanthophylls remained with the gluten. Data on total carotenoids were similar to data on total xanthophylls.

Some of the carotenoids are located in parts of the corn kernel other than the endosperm (6). Therefore, some of the carotenoids undoubtedly remained with wet-milled fractions (especially the germ) other than the gluten. These nongluten fractions should account, at least in part, for a portion of the carotenoids not recovered in the gluten fractions.

Acknowledgment

Commercial corn gluten fractions were supplied by Clinton Corn Processing Co. and Corn Products Co. and hybrid corn samples, by the Cereal Crops Research Branch of USDA's Crops Research Division.

Literature Cited

1. ANONYMOUS. More traffic. Feedstuffs **34** (10): 2 (1962).
2. BLESSIN, C. W. Carotenoids of corn and sorghum. I. Analytical procedure. Cereal Chem. **39:** 236–242 (1962).
3. WATSON, S. A. What the wet-milling industry sees in grain sorghum. Chemurgic Dig. **19:** 4–7 (1960).
4. GOODWIN, T. W. Carotenoids. *In* Methods of plant analysis, ed. by K. Peach and M. V. Tracey; vol. 3, pp. 272–311. Springer-Verlag: Berlin (1955).
5. BLESSIN, C. W., BRECHER, J. D., DIMLER, R. J., GROGAN, C. O., and CAMPBELL, C. M. Carotenoids of corn and sorghum. III. Variation in xanthophylls and carotenes in hybrid, inbred, and exotic corn lines. Cereal Chem. **40:** 436–442 (1963).
6. BLESSIN, C. W., BRECHER, J. D., and DIMLER, R. J. Carotenoids of corn and sorghum. V. Distribution of xanthophylls and carotenes in hand-dissected and dry-milled fractions of yellow dent corn. Cereal Chem. **40:** 582–586 (1963).