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Carotenoid Bioaccessibility from Whole Grain and Degermed Maize Meal Products

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Although yellow maize (*Zea mays*) fractions and products are a source of dietary carotenoids, only limited information is available on the bioavailability of these pigments from maize-based foods. To better understand the distribution and bioavailability of carotenoid pigments from yellow maize (*Z. mays*) products, commercial milled maize fractions were screened for carotenoid content as were model foods including extruded puff, bread, and wet cooked porridge. Carotenoid content of maize fractions ranged from a low of 1.77−6.50 mg/kg in yellow maize bran (YCB) to 12.04−17.94 mg/kg in yellow corn meal (YCM). Lutein and zeaxanthin were major carotenoid species in maize milled fractions, accounting for ∼70% of total carotenoid content. Following screening, carotenoid bioaccessibility was assessed from model foods using a simulated three-stage in vitro digestion process designed to measure transfer of carotenoids from the food matrix to bile salt lipid micelles (micellarization). Micellarization efficiency of xanthophylls was similar from YCM extruded puff and bread (63 and 69%), but lower from YCM porridge (48%). Xanthophyll micellarization from whole yellow corn meal (WYCM) products was highest in bread (85%) and similar in extruded puff and porridge (46 and 47%). For extruded puffs and breads, β-carotene micellarization was 10−23%, but higher in porridge (40−63%), indicating that wet cooking may positively influence bioaccessibility of apolar carotenes. The results suggest that maize-based food products are good dietary sources of bioaccessible carotenoids and that specific food preparation methods may influence the relative bioaccessibility of individual carotenoid species.

KEYWORDS: Carotenoids; maize; lutein; carotene; bread; porridge; bioaccessibility

INTRODUCTION

Maize is one of the most widely cultivated cereals in the world, with the largest production in the United States (*1, 2*). Maize and milled maize food ingredients including meals, flours, and bran have been integral parts of the diet for populations of all socioeconomic classes worldwide. In addition to serving as a critical source of macro- and micronutrients, maize is also a rich source of many phytochemicals including phenolic acids (*3*−*5*), flavonoids, anthocynins (*3*−*6*), and carotenoid pigments (*6*−*12*). Carotenoids are a family of yellow and orange pigments abundant in plants. More than 600 carotenoid species have been identified in nature; the xanthophylls (lutein, zeaxanthin, and β-cryptoxanthin), as well as carotenes (α-carotene and β-carotene) are the most abundant in specific varieties of yellow and orange maize (*6*−*13*). Consumption of carotenoid-rich foods has been associated with a reduced risk of several chronic diseases including cancer (*14*), cardiovascular disorder (*15*), and impaired vision (*16, 17*).

The potential health-promoting properties of carotenoids have stimulated interest in the bioavailability of these pigments from whole foods including maize-based products. Bioavailability is defined as the proportion of carotenoids from a food source secreted into the circulation and made available for subsequent tissue uptake and metabolism (*18*). Carotenoid absorption from foods requires release from the food matrix through digestion and partitioning into bile salt mixed micelles in the intestinal lumen in order to be made available for uptake (*18, 19*). In this context, carotenoid bioaccessibility is often defined as the fraction of carotenoid transferred from the food matrix to mixed micelles and made available for uptake by the intestinal mucosa (*20*). Micellarization efficiency is often used as a measure of bioaccessibility and has proven to be a useful alternative for estimating carotenoid bioavailability in vivo (*21*).

Several factors may affect carotenoid absorption, including the food matrix, the presence and type of fat, the type and amount of fiber, and the degree of food processing (*19, 20, 22*−*25*). Information is available on the bioavailability of carotenoids from common dietary fruits and vegetables including spinach (*23, 26*), carrots (*23*), tomato (*26, 27*), and sweet potato (*28*). Although this information provides valuable insight, knowledge of carotenoid bioavailability from maize is limited. Furthermore, as a grain, maize is milled into various food grade fractions including meal, grits, four, flakes, and bran. Each

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Table 1. Maize Product Formulation and Carotenoid Composition before Cooking[a]

variable and unit	bread		porridge		extruded puff	
	regular	whole grain	regular	whole grain	regular	whole grain
ingredient (% w/w)						
rice milk	36.0	43.4			32.0	35.4
water			74.7	74.7		
yellow cornmeal (YCM)[b]	40.8		15.2		44.2	
whole yellow cornmeal (WYCM)[b]		32.9		15.2		41.9
vegetable shortening	10.7	10.3	10.1	10.1	10.3	10.4
egg white	9.5	10.4			9.7	9.6
baking powder and salt	3.0	2.7			2.6	2.7
total (%)	100.0	100.0	100.0	100.0	100.0	100.0
carotenoid content[c] (mg/kg of dry wt)						
Lut	2.70 ± 0.06a	2.16 ± 0.02b	1.00 ± 0.02c	1.00 ± 0.01c	2.92 ± 0.07d	2.75 ± 0.02ad
Zea	2.87 ± 0.08a	2.11 ± 0.01b	1.07 ± 0.03c	0.97 ± 0.01c	3.11 ± 0.08d	2.68 ± 0.02a
(Z)-L/Z	0.56 ± 0.01a	0.49 ± 0.02b	0.21 ± 0.00c	0.23 ± 0.01c	0.61 ± 0.01d	0.63 ± 0.03d
Zeino	0.57 ± 0.02a	0.30 ± 0.01b	0.21 ± 0.01c	0.14 ± 0.00d	0.62 ± 0.02a	0.38 ± 0.01b
β-cryp	0.32 ± 0.03a	0.25 ± 0.01a	0.12 ± 0.0b1	0.12 ± 0.01b	0.34 ± 0.03a	0.32 ± 0.02a
α-C	0.12 ± 0.04a	0.10 ± 0.01a	0.05 ± 0.01b	0.04 ± 0.01b	0.13 ± 0.04a	0.12 ± 0.02a
β-C	0.18 ± 0.04a	0.13 ± 0.02ab	0.07 ± 0.01c	0.06 ± 0.01c	0.20 ± 0.04b	0.16 ± 0.03ab
(Z)-β-C	0.12 ± 0.00a	0.10 ± 0.01a	0.04 ± 0.00b	0.05 ± 0.00b	0.13 ± 0.00a	0.13 ± 0.01a
total carotenoids	7.45 ± 0.17a	5.64 ± 0.03b	2.78 ± 0.06c	2.61 ± 0.02c	8.07 ± 0.19d	7.18 ± 0.04a

[a] Carotenoid data are mean ± SEM; $n = 5$. Different letters indicate significant ($p < 0.05$) differences in carotenoid content between products within individual species and total carotenoids. [b] YCM, yellow maize meal; WYCM, whole grain yellow maize meal. [c] Carotenoid species: Lut, lutein; Zea, zeaxanthin; (*Z*)-L/Z, lutein + zeaxanthin cis isomers; Zeino, zeinoxanthin; β-cryp, β-cryptoxanthin; α-C, α-carotene; β-C, β-carotene; (Z)-β-C, β-carotene cis isomer.

fraction varies in composition and functional characteristics and results in the distribution of macronutrients, fiber, enzymatic activity, and pigments within the maize kernel (*29*). Total carotenoid levels in maize can be as high as 30 mg/kg of dry weight, mostly as lutein and zeaxanthin (*9, 11, 13*), offering raw materials suitable for the development of carotenoid-rich maize milled fractions and foods.

Although promising, only limited information is currently available on the bioavailability of these pigments from maize-based foods (*30*−*32*). Furthermore, the specific impact of common milling process and the food matrix on carotenoid content and bioaccessibility from maize foods is not clearly understood. To determine the extent to which food products based on milled maize fractions could serve as dietary sources of bioactive carotenoids, the objectives of this study were to assess the carotenoid profile of select maize fractions generated through commercial dry milling processes and to determine the bioaccessibility of carotenoids from model foods based on yellow maize meals.

MATERIALS AND METHODS

Chemicals and Standards. Extraction and HPLC solvents, acetone, ethyl acetate, methanol, and petroleum ether (Sigma-Aldrich, St. Louis, MO), were all of certified HPLC and ACS-grade. Ammonium acetate (Sigma-Aldrich) was dissolved in double-distilled water and adjusted to pH 4.6 with glacial acetic acid to make a 1.0 mol L^{-1} solution. α-Amylase, pepsin (porcine), pancreatin (porcine), bile extract (porcine), and all other reagents used for in vitro digestion were purchased from Sigma-Aldrich. Analytical standards of lutein, β-carotene (Sigma-Aldrich), zeaxanthin, β-cryptoxanthin (Indofine Chemical, Hillsborough NJ), and α-carotene (Chromadex, Irvine, CA) were obtained for HPLC calibration. Commercial milled maize fractions were obtained from three commercial maize dry milling operations in the midwestern United States identified as suppliers A, B, and C. Food ingredients utilized to produce model food systems including egg whites, vegetable shortening, rice milk, salt, and baking powder were purchased from a local market in West Lafayette, IN.

Carotenoid Profiling of Commercial Maize Fractions. All sample handling, extraction, and analysis were conducted under yellow lights to minimize photo-oxidative reactions. Representative samples of maize milled fractions and whole maize kernels were stored at refrigerated temperature upon arrival. Subsamples were ground for approximately 3 min at the highest setting on a ball mill (Retsch Vibratory Mill, type MM-2, Brinkmann). Afterward, representative samples of each were ground with a mortar and pestle for ∼10 min. Ground samples were placed in 50 mL centrifuge tubes, blanketed with nitrogen, sealed, and stored at −80 °C immediately after grinding until further analysis. The moisture content for each subsample was determined following American Association of Cereal Chemists (AACC) method 44-15a (*33*).

Carotenoid extractions were completed as described by Kean et al. (*34*) with minor modification. Approximately 1 g of ground maize sample was dispersed in 4 mL of double-distilled water, and 200 μL of stock solution of β-apo-8-carotenal (internal standard) was added and mixed by vortex. The resulting slurry was then placed in an 85 °C water bath in the dark for 10 min; it was removed, 2 mL of cold distilled water was added, and then the samples were placed on ice. Two milliliters of porcine pancreatin (160 mg/mL) prepared in 0.1 N $NaHCO_3$ was added to the slurry, and the samples were incubated at 37 °C in a shaking water bath in the dark for 15 min. Following incubation the slurry was saponified with 30% methanolic NaOH for 30 min at 37 °C. Carotenoids were extracted with 4 mL of petroleum ether/acetone (3:1), containing 0.1% BHT. Samples were vortex mixed for 30 s and then centrifuged at 2500*g* for 3 min to facilitate phase separation. The petroleum ether layer was collected, and the residue was re-extracted a total of five times. Combined petroleum ether fractions were dried under a stream of nitrogen, resolubilized in 50:50 MeOH/ethyl acetate, and then filtered through a 0.45 μm filter in preparation for LC analysis. Percent recovery of β-apo-8-carotenal following extraction was determined to be 94 ± 2.7% from maize fractions.

Production of Maize Model Foods. Formulations for maize model foods can be seen in **Table 1**. Yellow corn meal (YCM) and whole grain yellow corn meal (WYCM) were chosen as ingredients on the basis of preliminary assessment of their carotenoid content and their common use in the preparation/manufacture of many maize-based food products including corn bread, wet cooked porridge (grits), and extruded snacks.

Maize bread was formulated with vegetable shortening as lipid source and with egg white to minimize background carotenoid levels. All ingredients were combined and mixed manually at room temperature under amber light for ∼5 min to produce dough. Dough (∼3.5 kg) was divided into 700 g loaves and baked at 250 °C for 25 min. Baked bread was cooled for about 1 h at room temperature, and representative samples were taken for moisture analysis.

Maize-based porridge was produced using a cold-boiling-water procedure (*35*) with modification. Maize meals were mixed with double-

distilled water to form a slurry. The slurry was then poured into boiling water and stirred during cooking for ~3 min; afterward, lipid in the form of vegetable shortening was added to mixture. The resulting slurry with lipid was cooked with continuous stirring at 95 °C for a further 17 min. Preparation was completed with the addition of salt. Porridge was allowed to cool at room temperature for about ~1 h, after which representative aliquots were taken and immediately analyzed for moisture content.

Maize-based snack puffs were produced by combining dry ingredients and minimal water to produce dough suitable for extrusion through a Krupp Werner and Pfleiderer ZSK-25 (WP-25) twin-screw extruder (Ramsey, NJ). Extrusion conditions were as follows: water input flow rate, 5 g/min; feed input flow rate, 150 g/min; and zone temperatures, 40, 60, 80, 120, and 130 °C, at 269 rpm. The feed rate was set and measured by using the weight loss feeder (Ktronsoder K1-71430; Pitman, NJ). These conditions were predetermined to facilitate extrudate expansion of the formulated ingredients. The extruder was allowed to run and stabilize for 15 min using "warm up dough". Samples of extruded puffs were collected after 5 min of processing to ensure equilibrium, cooled at room temperature, and analyzed for moisture immediately.

In Vitro Digestion. Cooked maize bread (~3 g), extruded puffs (~3 g), and porridge (~10 g) were subjected to a three-stage simulated digestion including oral, gastric, and small intestinal phases as described by Thakkar et al. (*36*), with minor modifications. Modifications included increased lipase, pancreatin, and bile extract concentrations to 0.40, 0.80, and 1.80 g/L, in the small intestinal phase, respectively. This increase in digestive enzymes was made following pilot studies supporting the notion that increased enzymes were required to adequately digest the specific maize food matrix used in these studies (data not shown). Following digestion, samples were centrifuged (10000*g*, 4 °C) for 1 h and filtered (0.22 μm pore size) to isolate the aqueous micellar fractions from digesta. Aliquots of undigested maize products, final digesta, and aqueous micellar fractions were collected, blanked with nitrogen, and stored at −80 °C until analysis.

Extraction of Carotenoids from Product, Digesta, and Micelle Fraction. Carotenoids were extracted from digesta and aqueous micellar fractions, as described by Ferruzzi et al. (*37*). The extraction of carotenoids from 3 g aliquots of maize foods was accomplished as described earlier for maize fractions. Percent recovery of β-apo-8-carotenal following extraction was found to be 96 ± 1.9% from maize-based food products.

Instrumentation and Chromatography. Carotenoid analysis was completed with a Hewlett-Packard model 1090A HPLC system equipped with a model 79880A diode array detector. Carotenoid separations were achieved using a YMC carotenoid reversed phase (2.0 × 250 mm) polymeric C30 column with a guard column containing the same stationary phase (Waters Corp., Milford, MA). A gradient elution profile based on a binary mobile phase system consisting of methanol/1 M ammonium acetate (98:2 v/v) in phase A and ethyl acetate in phase B was used. A flow rate of 0.37 mL/min was utilized with initial conditions set at 100% A with a linear gradient to 80:20 A/B over 6 min. The gradient was held for 2 min followed by a 3 min linear gradient back to 100% A and equilibration at initial conditions for 3 min for a total analysis time of 14 min. Detection and tentative identification of all carotenoids was accomplished using inline diode array data between 250 and 600 nm. Quantification of carotenoids was accomplished using multilevel response curves constructed at 450 nm with authentic carotenoid standards for lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene. Due to a lack of authentic standards, (*Z*)-isomers of lutein, zeaxanthin, and β-carotene were tentatively identified on the basis of comparison of electronic absorption spectra and elution profiles to previous results using similar C30 chromatography (*9*). Zeinoxanthin was similarly tentatively identified by comparison to previous separations by our group and others (*6, 12, 34*). Levels of these carotenoids in test foods and grains were estimated on the basis of response curves for corresponding *all-E*-isomer (for *Z*-isomers) and lutein (for zeinoxanthin). Intraday coefficients of variation (CV) for extraction and analysis were 3.1, 2.7, 5.1, 3.9, and 5.7% for (*all-E*)-lutein, (*all-E*)-zeaxanthin, (*Z*)-lutein + (*Z*)-zeaxanthin, zeinoxanthin, and β-carotene, respectively.

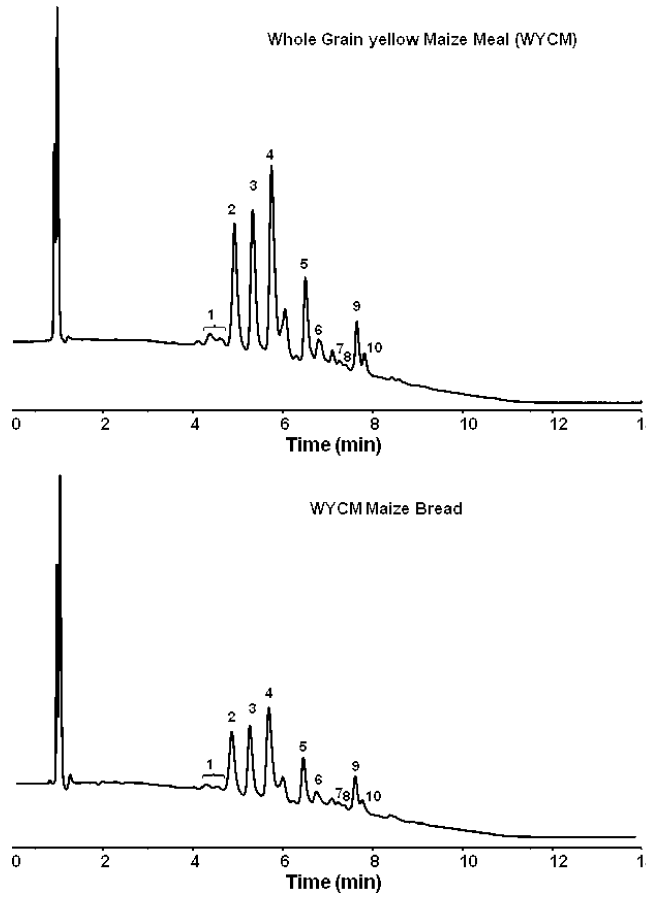

Figure 1. HPLC separation of carotenoids from extracts of (top) whole grain yellow maize meal (WYCM) and (bottom) bread made from WYCM. Carotenoid analysis was completed as described under Materials and Methods using a polymeric C30 column (2.0 × 250 mm). Peaks: 1, (*Z*)-lutein + zeaxanthin; 2, (*all-E*)-lutein; 3, (*all-E*)-zeaxanthin; 4, β-apo-8-carotenal (internal standard); 5, (*all-E*)-zeinoxanthin; 6, (*all-E*)-β-cryptoxanthin; 7, (13*Z*)-β-carotene; 8, (*all-E*)-α-carotene; 9, (*all-E*)-β-carotene; 10, (9*Z*)-β-carotene.

Data Analysis. Carotenoid content in grain fractions, model foods, and digestive fractions was expressed as mean ± standard error of mean (SEM) of five independent extractions. Micellarization efficiency was calculated as the percentage of carotenoid transferred from the maize food digesta to the aqueous micellar fraction following simulated digestion. Significant differences in carotenoid contents of the various maize fractions, products, digestates, micellar fractions, and micellarization efficiency were determined by analysis of variance (SAS software 9.1; SAS Institute, NC) with the Tukey−Kramer Honestly Significant Difference post hoc test ($\alpha < 0.05$).

RESULTS AND DISCUSSION

HPLC Separation and Profile of Carotenoids in Milled Maize Fractions. HPLC chromatograms of carotenoids in representative samples of both WYCM and WYCM breads are shown in **Figure 1**. Yellow maize and its milled fractions are known to contain several carotenoid species including both xanthophylls and carotenes (*6−9, 11, 13*). Resolution of lutein, zeaxanthin, β-apo-8-carotenal (internal standard, peak 4), zeinoxanthin, β-cryptoxanthin, and α- and β-carotenes and several (*Z*)-isomers of each carotenoid species was achieved with a polymeric RP-C30 column within 15 min. Tentative identification of carotenoids was achieved by comparing retention time and in-line electronic absorption spectra collected between 250 and 600 nm with those from authentic standards and previously published values (*6, 12, 34*). Carotenoid (*Z*)-isomers were

Table 2. Total Carotenoid Content of Selected Yellow-Endosperm Commercial Maize Fractions[a]

supplier and maize fraction[b]	carotenoid (mg/kg of dry wt)									% moisture[c]
	Lut	Zea	(Z)-L/Z	Zeino	β-cryp	α-C	β-C	(Z)-β-C	total	
supplier A										
YC	6.88 ± 0.21a	4.69 ± 0.20a	0.87 ± 0.07a	0.86 ± 0.09a	0.46 ± 0.02a	0.15 ± 0.02a	0.27 ± 0.01a	0.10 ± 0.01a	14.27 ± 0.51a	12.22 ± 0.25a
YCM	8.39 ± 0.08b	5.45 ± 0.09b	1.29 ± 0.06b	1.47 ± 0.07b	0.64 ± 0.02b	0.15 ± 0.02a	0.44 ± 0.05b	0.13 ± 0.01a	17.94 ± 0.22b	10.99 ± 0.22b
WYCM	6.55 ± 0.05a	6.40 ± 0.04c	1.50 ± 0.06bc	0.90 ± 0.03a	0.77 ± 0.04c	0.29 ± 0.04b	0.39 ± 0.07b	0.31 ± 0.03b	17.11 ± 0.09b	10.88 ± 0.12b
YCF	6.80 ± 0.20a	4.47 ± 0.09a	1.10 ± 0.05ab	1.00 ± 0.03c	0.55 ± 0.04d	0.11 ± 0.01a	0.24 ± 0.03a	0.10 ± 0.01a	14.37 ± 0.29a	12.61 ± 0.24a
YCB	2.40 ± 0.01c	1.40 ± 0.01d	0.44 ± 0.00d	0.28 ± 0.01d	0.22 ± 0.03e	0.11 ± 0.02a	0.20 ± 0.01a	0.09 ± 0.00a	5.13 ± 0.04c	10.51 ± 0.21b
supplier B										
YC	4.26 ± 0.11d	3.83 ± 0.12e	0.65 ± 0.02e	0.46 ± 0.01e	0.31 ± 0.03f	0.12 ± 0.03a	0.12 ± 0.01c	0.06 ± 0.00c	9.81 ± 0.20d	13.58 ± 0.28ac
YCM	5.32 ± 0.12e	5.79 ± 0.13b	0.88 ± 0.06a	0.50 ± 0.02e	0.55 ± 0.03d	0.15 ± 0.02a	0.39 ± 0.03b	0.11 ± 0.01a	13.68 ± 0.03ae	13.71 ± 0.29c
YCF	3.32 ± 0.06f	3.43 ± 0.03e	0.59 ± 0.03e	0.39 ± 0.03f	0.34 ± 0.03f	0.08 ± 0.02a	0.33 ± 0.02b	0.08 ± 0.00ac	8.57 ± 0.03df	13.07 ± 0.29ac
YCB	0.52 ± 0.01g	0.59 ± 0.05f	ND[d]	0.21 ± 0.01g	0.17 ± 0.04e	0.12 ± 0.04a	0.16 ± 0.03c	ND	1.77 ± 0.02g	10.44 ± 0.22b
WCM	0.03 ± 0.00h	0.03 ± 0.00g	ND	ND	ND	ND	0.01 ± 0.00d	ND	0.09 ± 0.00h	10.44 ± 0.25b
supplier C										
YC	6.44 ± 0.17a	4.42 ± 0.09a	0.74 ± 0.03f	0.71 ± 0.02h	0.41 ± 0.01f	0.10 ± 0.01a	0.19 ± 0.01a	0.09 ± 0.01a	13.09 ± 0.28ae	13.48 ± 0.24ac
YCM	5.00 ± 0.16de	2.66 ± 0.09h	0.84 ± 0.02a	0.82 ± 0.04a	0.39 ± 0.04f	0.87 ± 0.07c	1.21 ± 0.09e	0.27 ± 0.02b	12.04 ± 0.02e	12.01 ± 0.22a
YCF	3.74 ± 0.10f	2.00 ± 0.06h	0.50 ± 0.11e	0.72 ± 0.09h	0.18 ± 0.01e	0.13 ± 0.04a	0.22 ± 0.05a	0.09 ± 0.01a	7.58 ± 0.1i	13.25 ± 0.25ax
YCB	3.08 ± 0.17f	1.65 ± 0.04d	0.53 ± 0.08e	0.58 ± 0.04e	0.17 ± 0.02e	0.14 ± 0.05a	0.24 ± 0.07a	0.11 ± 0.02a	6.50 ± 0.39j	10.37 ± 0.19b
WCM	0.07 ± 0.01i	0.09 ± 0.00i	ND	0.02 ± 0.00i	0.03 ± 0.00g	0.01 ± 0.00d	0.01 ± 0.00d	ND	0.24 ± 0.01k	12.57 ± 0.23a

[a] All data are expressed as mg of carotenoids per kg of maize fraction (dry weight). Data represent mean ± SEM; $n = 5$. Different letters indicate significant ($p < 0.05$) differences in carotenoid content between products within individual species and total carotenoids. [b] Maize fractions: YC, yellow corn; YCM, yellow corn meal; WYCM, whole yellow corn meal; YCF, yellow corn flour; YCB, yellow corn bran; WCM, white corn meal (commercial suppliers). [c] Moisture represents mean ± SEM; $n = 3$. [d] ND, not detected.

tentatively identified by comparison to *all-E* spectra and the presence of a strong absorption at ~350 nm consistent with carotenoid (*Z*)-isomers (*12, 32, 38, 39*). Due to the coelution of several (*Z*)-isomers of lutein, speciation was not possible and those peaks were collectively labeled as lutein + zeaxanthin (*Z*)-isomers. These isomers are known to be present naturally in maize grains and have been observed to form through processing (*12, 13, 34, 39*).

Carotenoid content expressed as milligrams per kilogram of maize fraction analyzed from maize dry milled fractions from commercial suppliers can be seen in **Table 2**. In general, (*all-E*)-lutein and (*all-E*)-zeaxanthin were determined to be the predominant carotenoid species for all maize fractions, accounting for >70% of the total carotenoid content in each fraction. Highest carotenoid levels were observed in yellow maize meal (YCM) (12.04−17.94 mg/kg) followed by yellow maize flour (YCF) (8.57−14.37), with yellow maize bran (YCB) (1.77−6.50) having the lowest carotenoid content among yellow maize milled products. White endosperm maize meal (WCM) was determined to have only trace levels of total carotenoids (<0.25 mg/kg). Overall, these levels are comparable to carotenoid contents reported previously for both yellow and white maize fractions (*6−13, 34*).

Whereas (*all-E*)-lutein and (*all-E*)-zeaxanthin were the predominant carotenoid species in maize and maize milled fractions, appreciable levels of zeinoxanthin and β-cryptoxanthin were also detected in all milled yellow maize fractions, ranging from 0.17 to 1.47 mg/kg. Carotene levels similarly ranged from 0.10 to 1.21 mg/kg, making both carotenes and cryptoxanthins relatively minor components of the maize and milled fractions analyzed in this study. Lutein and zeaxanthin are well-known to be the most abundant carotenoids in cereal grains, with published ratios of lutein to zeaxanthin ranging from 10:1 in durum wheat (*40, 41*) to ~2:1 in barley (*41*) and ~1:1− 3:1 for maize and sorghum (*6, 7, 13, 34*). Our results indicated a similar ratio for lutein to zeaxanthin observed in grain factions consistent with typical maize kernels (**Table 2**). (*Z*)-Isomers of lutein + zeaxanthin and β-carotene in milled maize samples accounted for ~1−7% of the total carotenoid content of these fractions, consistent with previous reports of carotenoid isomers in fresh and processed maize products and fractions (*6, 9−11, 34*).

Differences in the carotenoid contents of specific maize fractions were observed, with carotenoid levels in maize meal (YCM) generally exceeding that of flour (YCF), and bran (YCB) has the lowest amount of carotenoids of all milled fractions (**Table 2**). These differences may be attributed to fractionation of the kernel through the milling process. Blessin et al. (*7*) reported that the majority of the carotenoids are concentrated in the horny (74−86%) and floury endosperm fractions (9−23%), whereas germ and bran have lower carotenoid contents. The lower carotenoid content of maize bran fractions (1.77−6.50 mg/kg) compared to fractions rich in endosperm (7.58−17.94 mg/kg) observed in this study is in agreement with those findings.

Whereas quantitative differences were observed between different milled fractions, qualitative differences in carotenoid profiles within the same supplier were minor, although a tendency for a higher percentage of (*Z*)-isomers in both bran and whole grain fractions compared to fractions without bran was observed. Observed differences in carotenoid profiles from maize and maize fractions originating from different suppliers may be a reflection of differences in starting material and specific milling practices utilized to separate bran from endosperm fractions.

Overall, these qualitative and quantitative carotenoid profiles of yellow maize and milled fractions are comparable to previous results (*6, 7, 9−13, 34*) and compared well to other grains and select vegetables including durum wheat (6.65 mg/kg), pumpkins (21.20 mg/kg), and green beans (16.50 mg/kg) (*41*) but remains lower than other commonly consumed carotenoid-rich fruits and vegetables such as sweet potato (389.3 mg/kg), spinach (150.00 mg/kg), and mango (49.00 mg/kg) (*41−44*).

Carotenoid Stability during Food Preparation/Processing. Although yellow maize and its milled products are good carotenoid sources, the extent to which these carotenoids are available for absorption (bioaccessible) from common maize-based food products is poorly understood. Carotenoid-rich YCM and WYCM maize fractions were selected to produce model food systems (bread, porridge, and extruded puff) on the basis of the common use of maize meal in processed maize for foods. Inclusion of a whole grain meal also allowed for assessment of the impact maize bran and germ fractions would have on

Table 3. Carotenoid and Moisture Contents of Maize Products after Cooking[a]

	bread		porridge		extruded puff	
carotenoids and moisture	YCM	WYCM	YCM	WYCM	YCM	WYCM
carotenoid (mg/kg of dry wt)						
Lut	1.52 ± 0.05a	1.34 ± 0.05b	0.60 ± 0.02c	0.52 ± 0.02c	1.66 ± 0.08ad	1.70 ± 0.03d
Zea	1.71 ± 0.07a	1.50 ± 0.06a	0.69 ± 0.03b	0.53 ± 0.02b	1.72 ± 0.06a	1.72 ± 0.06a
(Z)- L/Z	0.73 ± 0.01a	0.64 ± 0.01b	0.10 ± 0.00c	0.09 ± 0.00c	0.39 ± 0.01d	0.44 ± 0.01e
Zeino	0.34 ± 0.01a	0.30 ± 0.01b	0.11 ± 0.00c	0.08 ± 0.00d	0.25 ± 0.00e	0.32 ± 0.01ab
β-cryp	0.32 ± 0.01a	0.28 ± 0.01b	0.09 ± 0.00c	0.08 ± 0.00c	0.22 ± 0.00d	0.27 ± 0.00b
α-C	0.06 ± 0.00a	0.05 ± 0.00ab	0.01 ± 0.00c	0.01 ± 0.00c	0.05 ± 0.00ab	0.04 ± 0.00b
β-C	0.09 ± 0.01a	0.08 ± 0.01a	0.04 ± 0.00b	0.02 ± 0.00b	0.12 ± 0.00c	0.12 ± 0.00c
(Z)-β-C	0.05 ± 0.00a	0.05 ± 0.01a	0.02 ± 0.00b	0.04 ± 0.00b	0.06 ± 0.00ac	0.07 ± 0.00ac
total carotenoids	4.83 ± 0.10a	4.25 ± 0.17b	1.66 ± 0.07c	1.37 ± 0.05c	4.49 ± 0.11b	4.67 ± 0.08ab
product % moisture	37.90 ± 0.26a	39.54 ± 0.30a	74.48 ± 0.47b	75.09 ± 0.44b	37.16 ± 0.24a	36.98 ± 0.11a

[a] Carotenoid data are mean ± SEM; $n = 5$. Different letters indicate significant ($p < 0.05$) differences in carotenoid content between products within individual species and total carotenoids.

Table 4. Carotenoid Contents (Milligrams per Kilogram of Wet Weight) of Maize Products Following Simulated Digestions[a]

	bread		porridge		extruded puff	
carotenoid	YCM	WYCM	YCM	WYCM	YCM	WYCM
Lut	1.79 ± 0.07a	0.97 ± 0.07b	0.67 ± 0.06bc	0.57 ± 0.02c	2.19 ± 0.01d	1.64 ± 0.09a
Zea	2.10 ± 0.08a	1.04 ± 0.04b	0.80 ± 0.06bc	0.63 ± 0.03c	2.09 ± 0.06a	1.76 ± 0.09d
(Z)-L/Z	0.49 ± 0.03a	0.38 ± 0.04a	0.14 ± 0.01b	0.15 ± 0.02b	0.53 ± 0.02a	0.41 ± 0.02a
Zeino	0.41 ± 0.03a	0.28 ± 0.03b	0.12 ± 0.01c	0.09 ± 0.01c	0.42 ± 0.02a	0.32 ± 0.02b
β-cryp	0.28 ± 0.04a	0.20 ± 0.04b	0.10 ± 0.01c	0.08 ± 0.00c	0.38 ± 0.02d	0.26 ± 0.02a
α-C	0.06 ± 0.01a	0.09 ± 0.01a	0.02 ± 0.00b	0.02 ± 0.00b	0.08 ± 0.01a	0.09 ± 0.01a
β-C	0.18 ± 0.01a	0.14 ± 0.01a	0.05 ± 0.01b	0.06 ± 0.00b	0.08 ± 0.00bc	0.11 ± 0.01c
(Z)-β-C	0.08 ± 0.01a	0.08 ± 0.01a	0.03 ± 0.00b	0.06 ± 0.00ab	0.05 ± 0.01ab	0.07 ± 0.00ab
total carotenoids	5.39 ± 0.10ab	3.18 ± 0.17b	1.93 ± 0.07c	1.66 ± 0.05c	5.82 ± 0.11a	4.66 ± 0.08b

[a] All data are expressed as mg of carotenoids per kg of maize food (wet weight). Carotenoid data are mean ± SEM; $n = 5$. Different letters indicate significant ($p < 0.05$) differences in carotenoid content between products within individual species and total carotenoids.

carotenoid bioaccessibility as fiber is known to affect carotenoid bioavailability in vivo (*19, 22, 25*). Carotenoid stability and subsequent bioaccessibility were then assessed in vitro using a simulated digestion previously described (*36*). Formulations and carotenoid contributions by maize fractions to model food systems are shown in **Table 1**. Carotenoid stability to processing of maize-based foods was determined by comparing saponified extracts of finished products (**Table 3**) to the initial content in maize milled fractions in product formulation (**Table 1**). Carotenoid retention to processing ranged from 75% in WYCM bread to 52% in WYCM porridge, with an average retention through processing of ~62% for total carotenoids among maize food products. These results are comparable to carotenoid stability to processing in wheat breads (74 to 55%) reported by Leenhardt et al. (*45*) and carotene retention in predigested cassava subjected to baking (71.9%) and boiling (55.7%) (*46*).

All products had significant reductions in α- and β-carotene contents relative to precooked food content. Lutein and zeaxanthin were sensitive to thermal treatment during preparation of test foods, with losses of ~29−50% in both WYCM and YCM products. Some loss of (*all-E*)-lutein and zeaxanthin is often explained by isomerization during thermal processing. With the exception of bread products, which had a substantial increase (~23%) in lutein + zeaxanthin (*Z*)-isomers, lutein and zeaxanthin (*Z*)-isomer content was found to decrease following cooking (**Table 3**). These observations from porridge and extruded puffs are not consistent with previous results characterizing moderate formation of (*Z*)-isomers in thermally processed maize products (*39, 47*). However, these previous studies focused primarily on processed (retorted/sterilized) whole kernel maize products rather than products from milled maize fractions. Differences in ingredient interactions and type of process may explain, in part, these observed differences. Interestingly, a modest reduction in (*Z*)-β-carotene was also observed in all products. Li et al. (*13*) found a similar reduction of (*Z*)-β-carotene in cooked β-carotene-rich wet milled maize flour.

Carotenoid Bioaccessibility from Processed Maize Products. Following preparation, representative samples of each food were subjected to a three-stage in vitro digestion to determine digestive stability and bioaccessibility of carotenoids from maize products. Digestive stability signifies the percentage of the carotenoids in maize products recovered in the digesta following simulated digestion of maize-based foods. The percentage of carotenoids transferred from the digesta to the filtered aqueous fraction is defined as the micellarization efficiency and is used as a measure of relative bioaccessibility. In vitro micellarization efficiency is a useful measure for comparative assessment of bioaccessibility as it is a positive predictor of carotenoid bioavailability in vivo (*18, 21*).

In general, qualitative and quantitative carotenoid profiles in bread, extruded puff, and porridge remained relatively unchanged following cooking and simulated digestions (**Tables 3** and **4**). Digestive recoveries for individual carotenoid species ranged from 90 to 120%. Observed recoveries >100% were most likely due to differences in extraction recoveries from the digesta and raw food samples. Although percent recovery of β-apo-8-carotenal was found to be >95%, it is likely that differences exist between recoveries of specific carotenoid species within the matrix itself. The maize endosperm contains ~75% of the total kernel's proteins, which are associated in a proteinaceous complex with the carotenoids (*10*). Such complex microstructure has major implications for their behavior during processing, extraction, digestion, and analysis. In preliminary experiments, it was determined that a short-term (15 min) treatment with pancreatin prior to solvent extraction increased the recovery of both carotenes and xanthophylls from both maize

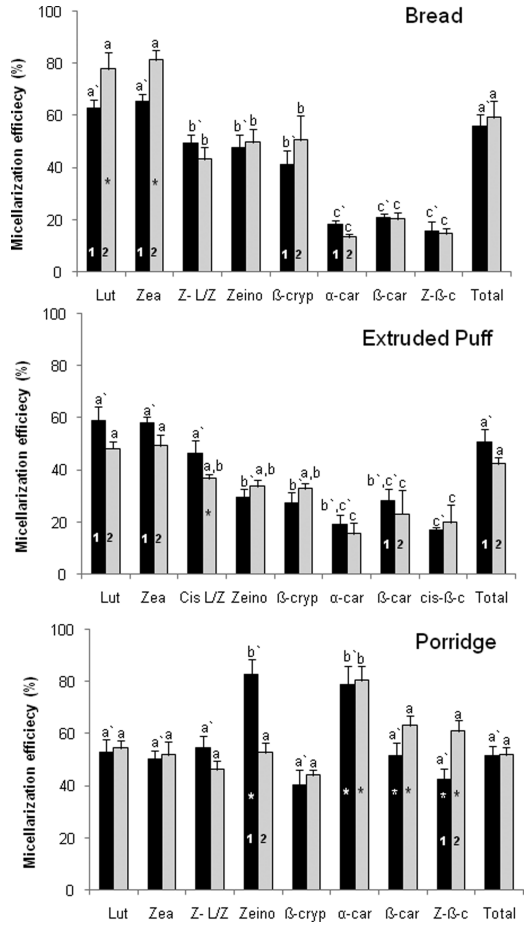

Figure 2. Micellarization efficiency (relative bioaccessibility) of individual carotenoid species from (black bars) YCM-based and (gray bars) WYCM-based bread, porridge, and extruded puff. Values are expressed as mean ± SEM for five independent observations. Different letters above bars indicate significant differences in micellarization efficiency between individual carotenoids within a test meal ($p < 0.05$). The presence of different numbers in the bars indicates significant differences in micellarization efficiency between test meals for individual carotenoids ($p < 0.05$).

milled fractions meal materials and predigested foods (data not shown). Although providing significant improvement in recovery, further breakdown of the maize/food matrix appears to be required to fully liberate the carotenoids. Despite these differences, overall these data support the notion that maize carotenoids are stable to the simulated digestive environment, in agreement with previous results indicating digestive stability of carotenoids in similar models (*26, 36, 37, 48*). Because of the stability of carotenoids to digestive conditions, pretreatment by in vitro digestion merits future consideration as a strategy to improve carotenoid extraction efficiencies from whole foods.

Due to the apparent higher recovery of carotenoids from maize food digesta and the documented stability of carotenoids to in vitro digestion (*21, 23, 37, 49, 50*), micellarization efficiency was calculated as the fraction of carotenoids transferred from digesta to aqueous fractions. The mean efficiency of micellarization for carotenoids from digested maize foods varied between carotenoid species and products. In general, carotenoid micellarization ranged from 59 to 67% for bread, from 51 to 53% for porridge, and from 45 to 50% for extruded puffs (**Figure 2**). With the exception of porridge (57−68%), carotene micellarization efficiencies were lower than those of xanthophylls. For breads, micellarization of (*all-E*)-zeaxanthin and (*all-E*)-lutein (∼62−81%) was greater than that of β-cryptoxanthin and zeinoxanthin (∼41−50%) or α- and β-carotene (∼13−20%). Micellarization efficiency was significantly ($P < 0.05$) higher for (*all-E*)-lutein, (*all-E*)-zeaxanthin, and (*all-E*)-β-cryptoxanthin from WYCM compared to YCM bread. In contrast, micellarization of (*all-E*)-α-carotene was higher ($P < 0.05$) in YCM compared to WYCM breads.

In extruded puffs (*all-E*)-zeaxanthin, (*all-E*)-lutein, and their (*Z*)-isomers had micellarization efficiencies between 48 and 58%, compared to ∼15−28% for the carotenes. In contrast to bread products, YCM extruded puffs had significantly ($P < 0.05$) higher micellarization efficiencies for lutein, zeaxanthin, and (*all-E*)-β-carotenes compared to WYCM products.

In contrast to both bread and extruded puffs, carotenoid micellarization from wet cooked porridge samples was similar for both xanthophylls and carotenes. With the exception of zeinoxanthin (∼82%) and α-carotene (∼78−80%), micellarization of all carotenoids ranged between 38 and 63%. No significant differences were found between lutein (∼52−54%), zeaxanthin (∼50−52%), and β-carotene (∼51−63%) micellarization efficiencies. Carotene micellarization efficiency was significantly ($P < 0.05$) higher from porridge than from bread or extruded puffs.

Overall, these data are consistent with previous studies that found preferential incorporation of xanthophylls into mixed micelles compared to carotenes during in vitro digestion (*23, 26, 48*). Most interestingly, it is apparent from these data that the incorporation of individual carotenoid species into mixed micelles during digestion was dependent on the makeup of the food matrix and type of preparation. In both bread and extruded puffs, xanthophyll micellarization predictably exceeded carotene micellarization efficiency. However, in porridge samples carotene micellarization was dramatically higher than from bread or extruded puffs (**Figure 2**). This enhanced micellarization of carotenes in porridge may be due to several factors including carotenoid content and processed induced extraction. Wet cooked porridge foods were lowest in total carotenoid content (**Table 3**). With lipid levels similar among all products, the lower amount of carotenoid relative to lipid present in porridge samples may have favored more efficient micellarization of apolar carotenes. Micellarization of apolar carotenes but not xanthophylls has previously been shown to be affected by lipid quantity (*51*). It is also possible that the wet cooking process itself facilitated extraction of carotenes into the bulk lipid during cooking itself. The higher moisture content of porridge would have facilitated such a process, whereas lower moisture bread and extruded puffs would have minimized such mobility. Carotenes present in the free lipid would be free of the food matrix and more available for association with mixed micelles. Some displacement of xanthophylls by carotenes in mixed micelles may explain the slight reduction in xanthophylls micellarization in porridge compared to bread (**Figure 2**).

It is important to point out that the extremely high levels of micellarization observed for α-carotene (∼78−80%) and zeinoxanthin (∼82%) from porridge could be due to several factors. Thermal processing is known to facilitate carotenoid degradation reactions, including isomerization and oxidation (*13, 38, 39, 47*). These reactions may be more pronounced in a slow wet cooking process with oil compared to extrusion or baking, resulting in the generation of products that may coelute with zeinoxanthin and α-carotene. Whereas the analytical methods employed in this study offered some degree of isomer resolution, the lower levels of these carotenoids in the porridge and resulting digesta (compared to lutein and zeaxanthin) could have been affected

Table 5. Bioaccessible Carotenoid Contents (Milligrams per Kilogram of Wet Weight) of Maize Products Transferred to Micellar Fractions[a]

	bread		porridge		extruded puff	
carotenoid	YCM	WYCM	YCM	WYCM	YCM	WYCM
Lut	1.12 ± 0.05a	0.75 ± 0.03b	0.35 ± 0.02c	0.31 ± 0.02c	1.27 ± 0.09a	0.79 ± 0.05b
Zea	1.36 ± 0.04a	0.84 ± 0.03b	0.39 ± 0.02c	0.32 ± 0.02c	1.20 ± 0.03a	0.87 ± 0.09b
(Z)- L/Z	0.24 ± 0.00a	0.16 ± 0.00b	0.08 ± 0.00c	0.07 ± 0.00c	0.24 ± 0.01a	0.15 ± 0.01b
Zeino	0.19 ± 0.01a	0.14 ± 0.00b	0.10 ± 0.00c	0.05 ± 0.00d	0.12 ± 0.01bc	0.11 ± 0.01bc
β-cryp	0.11 ± 0.00a	0.08 ± 0.00a	0.04 ± 0.00b	0.04 ± 0.00b	0.10 ± 0.01a	0.09 ± 0.01a
α-C	0.01 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a	0.01 ± 0.00a
β-C	0.04 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a
(Z)-β-C	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00b	0.04 ± 0.00b	0.01 ± 0.00a	0.01 ± 0.00a
total carotenoids	3.07 ± 0.10a	2.02 ± 0.17b	1.02 ± 0.07c	0.89 ± 0.05c	2.98 ± 0.11a	2.05 ± 0.08b

[a] All data are expressed as mg of carotenoids per kg of maize food (wet weight). Carotenoid data are mean ± SEM; $n = 5$. Different letters indicate significant ($p < 0.05$) differences in carotenoid content between products within individual species and total carotenoids.

by small amounts of lutein and zeaxanthin and/or β-carotene isomerization or oxidation products artificially increasing digesta contents and, by extension, apparent bioaccessibility.

Another factor believed to influence carotenoid bioaccessibility is the presence of dietary fiber. Soluble fiber in particular is known to interfere with the absorption of carotenoids and lipolysis in vivo (*17, 19, 22, 25*). With few exceptions, carotenoid micellarization did not differ between products made with YCM and WYCM, although significant differences ($P < 0.05$) were noted for micellarization of specific carotenoid species from bread and extruded puffs in **Figure 2**. Considering that maize bran is predominantly insoluble fiber (*52*), it is possible that slight variations observed between YCM and WYCM in bread and extruded puff products are more likely due to food-processing effects on structural properties of the food itself. Extrusion processing has been shown to increase the soluble fiber content of grains through the release of soluble polysaccharide components (*53*). Furthermore, complexation between lipids and fiber can occur during extrusion (*54*), which may limit the availability of lipids for solubilization of carotenoids. The extent to which these effects and/or physical changes in food structure through extrusion may specifically affect carotenoid bioaccessibility merits further exploration. However, although minor differences do exist, these data support the notion that whole grain (WYCM) and regular (YCM) maize fractions are both good sources of bioaccessible carotenoids and that added fiber in the form of maize bran does not negatively affect the initial stages (digestive release and micellarization) of carotenoid absorption. However, whereas relative bioaccessibilities were similar, the total amounts of bioaccessible carotenoids did differ between WYCM and YCM bread and extruded puff products (**Table 5**). These differences can be explained by combining the minor differences in relative bioaccessibility (**Figure 2**) with the reduced amounts of WYCM utilized in product formulation (**Table 1**). Lower doses of WYCM were used to obtain close rheological properties of dough and finished products to optimize processing. As a result, slightly lower carotenoid levels were observed in finished products (**Table 3**) and by extension in the final digesta and aqueous bioaccessible fraction (**Table 5**).

The 2005 Dietary Guidelines for Americans encourages consumption of at least 44 g of whole grains per day (*55*). Whole grain maize can be used to achieve this recommendation and serve as a rich source of bioactive and health-promoting carotenoid pigments. Moreover, the use of different maize fractions as ingredients in ready-to-eat breakfast cereals, bakery, and other products has increased in the United States and abroad (*56*), providing additional carotenoid-rich consumer products. The results of the current study indicate that the type of food product and processes may affect relative carotenoid bioaccessibility, whereas selection of a specific maize milled fraction may affect initial carotenoid levels and by extension the amount of bioaccessible carotenoid in the final food product. Interestingly, relative bioaccessibilities of carotenoids from maize foods are similar to those observed for common lutein and zeaxanthin-rich fruits and vegetables including carrots, spinach, and peppers (37−88%) (*26, 50*) and higher than those of foods rich in poorly bioaccessible carotenoid such as lycopene from tomatoes (<11%) (*21, 51, 57*). When combined with data on carotenoid content of maize (*6, 7, 9*−*13*), these data support the notion that maize-rich foods can provide appreciable amounts of bioactive carotenoids in a readily bioaccessible form.

ABBREVIATIONS USED

RP-HPLC, reversed phase high-performance liquid chromatography; AMD, age-related macular degeneration; SEM, standard error mean; AACC, American Association of Cereal Chemists; WYCM, whole yellow corn meal; YCM, yellow corn meal; WCM, white corn meal.

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