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A Comparison of the Levels of Lutein and Zeaxanthin in Corn Germ Oil, Corn Fiber Oil and Corn Kernel Oil

Robert A. Moreau · David B. Johnston · Kevin B. Hicks

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Abstract All commercial corn oil is obtained by pressing corn germ and/or extracting the germ with hexane. In the current study, six types of corn oil were prepared by extracting corn germ, corn fiber and ground corn, each with hexane or with ethanol. The levels of lutein, zeaxanthin and other carotenoids were quantitatively analyzed in the six corn oils. The levels of lutein + zeaxanthin in the oil ranged from 2.3 μg/g for hexane-extracted corn germ oil to 220.9 μg/g for ethanol-extracted ground corn oil. These results indicate that a diet that includes 30 g (∼2 tbsp) per day of the unrefined corn oil obtained by extracting ground corn with ethanol would provide ∼6 mg of lutein + zeaxanthin, the daily dosage that is currently considered to be necessary to slow the progression of age-related macular degeneration.

Keywords Carotenoids · Corn · Maize · Lutein · Zeaxanthin · Xanthophylls

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R. A. Moreau · D. B. Johnston · K. B. Hicks
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA 19038, USA

R. A. Moreau (✉)
Crop Conversion Science and Engineering Research Unit, ERRC, ARS, USDA, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA
e-mail: robert.moreau@ars.usda.gov

Introduction

All commercial corn oil is obtained by pressing and/or hexane-extracting corn germ [1]. Corn oil can also be obtained from ground corn kernels [2, 3] and corn fiber [4]. Previously, we have demonstrated that the oil obtained by extracting corn fiber with hexane contains high levels of phytosterols, approximately tenfold higher than the levels in commercial corn oil [4]. Recently we also demonstrated that corn oil obtained by extracting ground corn with ethanol contains higher levels of phytosterols, tocopherols, and tocotrienols, than commercial corn oil [5]. In these previous studies we observed that oil obtained by extracting corn fiber and ground corn had a much more intense yellow color than oil obtained from corn germ. Corn kernels (yellow) were previously reported to contain high levels of lutein and zeaxanthin [6–11] but surprisingly, no previous reports have examined their concentration in the various types of corn oil. Lutein and zeaxanthin have been shown to prevent age related macular degeneration [12]. The current study was undertaken to quantitatively compare the levels of carotenoids in corn germ oil, corn fiber oil and corn kernel oil extracted with hexane and with ethanol. This information will be valuable in determining whether the levels of lutein and zeaxanthin in any of these six corn oils are sufficiently high to have positive health effects.

Materials and Methods

Corn kernels (yellow dent #2, Pioneer H3361), obtained from the University of Illinois, were grown and harvested in 2005 and stored at 4 °C until used. Corn fiber and corn germ were obtained from a commercial corn wet milling company and were produced from an unspecified mixture of hybrids of yellow dent #2 corn. Corn fiber oil (hexane extracted) was prepared as previously described [4] and refined, bleached and deodorized by POS Pilot Plant, Saskatoon, SK, Canada. Ethanol extracted corn kernel oil was prepared as previously described [3] and also refined bleached and deodorized by POS. Ethanol (absolute, 200 proof) was obtained from Warner-Graham Co, Cockeysville, MD. All other solvents were HPLC grade and were obtained from Malinckrodt-Baker, Phillipsburg, NJ. Carotenoid standards (Fig. 1) were purchased from Indofine Chemical Co., Hillsborough, NJ (*β*-cryptoxanthin, lutein and zeaxanthin) and Sigma Chemical Co., St. Louis, MO (*β*-carotene). Mazola® corn oil and Carotino™ cooking oil were obtained from a local super market.

All laboratory extractions were performed in a Dionex Accelerated Solvent Extractor (ASE) Model 200 (Dionex, Sunnyvale, CA) as previously described [13]. Corn kernels and corn fiber were ground to 20 mesh (87 μm) with a Wiley Mill (Thomas Scientific, Philadelphia, PA) immediately before extraction. Wet milled corn germ samples (20 g) were ground for 1 min in a Krups model 203B coffee grinder immediately before extraction. Samples (5 g of ground corn, 5 g of corn fiber, or 1 g of corn germ) were placed in an 11 cc extraction vessel. Extractions were conducted at 50 °C and 1,000 psi with either hexane or ethanol. The extractor was programmed to extract each sample with 3×7.5 ml portions of solvent, for 10 min each. The entire extract (21.5 ml) from each sample was pooled, the solvent was evaporated under a stream of N_2, and the mass of the lipid residue was measured with an analytical balance. The lipid residue was then dissolved in hexane/isopropanol, 97/3, v/v, and HPLC analyses were conducted.

The carotenoids were analyzed using a newly developed normal phase HPLC method, with a Lichrosorb 7 Diol column, 3×100 mm, (Varian Scientific, Lake City, CA, USA) with an isocratic mobile phase consisting of hexane/isopropanol/acetic acid, 96.9/3.0/0.1, v/v/v, at a flow rate of 0.5 ml/min. HPLC analyses were conducted with an Agilent Model 1100 HPLC equipped with an auto sampler and an Agilent Model 1100 Diode Array Detector to measure the visible absorbance of the carotenoids at 450 nm. Carotenoid standards (1–50 ng) were used to construct a standard curve to calculate mass values from peak area values.

Each experiment was conducted twice with triplicate samples for each extraction condition. The results reported are the mean and standard deviation. Analysis of variance was conducted to determine statistical significance ($P < 0.05$) by the Bonferroni least significant difference method [14].

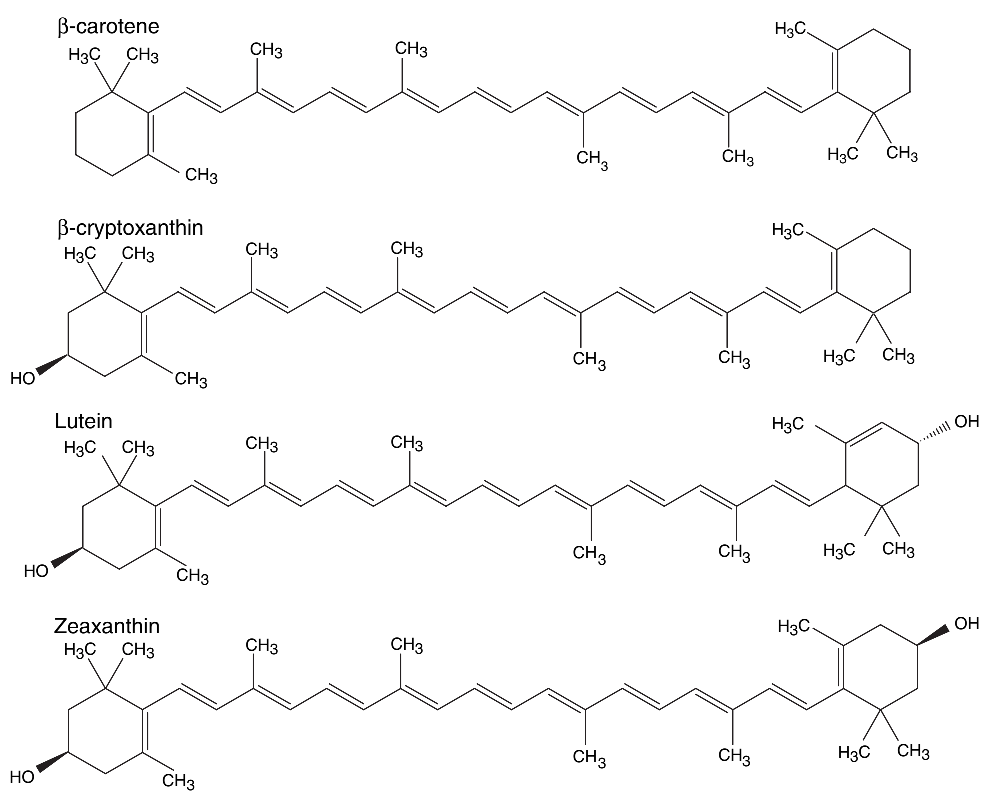

Fig. 1 Chemical structures of the major carotenoids found in corn kernels

Results and Discussion

In the current study, six types of corn oil were prepared by extracting corn germ, corn fiber and ground corn, each with hexane and with ethanol. Oil yields were lowest for the corn fiber extract (2.36 and 2.72%, for extraction by hexane and ethanol, respectively), followed by ground corn (2.60 and 3.27%, for extraction by hexane and ethanol, respectively), and highest for corn germ (34.14 and 28.07% for extraction by hexane and ethanol, respectively) (Table 1). For most plant materials (including corn fiber and ground corn in Table 1) the oil yields are higher when extracting with ethanol compared to hexane extraction. However, as observed in Table 1 and as we previously reported [3] extraction of wet milled corn germ at 50 °C with ethanol results in lower oil yields than comparable extraction with hexane. Recent studies indicate that this anomaly can be overcome by reducing the ratio of wet milled corn germ to ethanol (data not shown) or by increasing the temperature of extraction from 50 to 100 °C [3].

A new normal phase method (Fig. 2) was used to quantitatively analyze lutein, zeaxanthin and other carotenoids in the six corn oils. Most previous HPLC analyses of carotenoids have been performed using reverse phase HPLC [6–9], but some methods have employed a normal phase Si HPLC column [10, 11]. The new method separated the three major xanthophylls in corn, lutein, zeaxanthin, and *β*-cryptoxanthin. In addition to peaks that corresponded to the four carotenoid standards, six other peaks were observed (labeled U1–U6). It should be noted however that this normal phase method would probably not separate *α*-carotene and *β*-carotene so the values reported for *β*-carotene are, in actuality, the sum of *α*-carotene and *β*-carotene. Since this study focused on the levels of lutein and zeaxanthin in corn oils, this method of quantifying the carotenes should be adequate. Based on previous reports [9, 11] and on our own observation that the peaks were initially absent in the carotenoid standards and began to appear after several days of storage at 4°C, these six peaks (labeled U1–U6) are probably *cis* isomers of the "all *trans*" natural carotenoids. The levels of total carotenoids in the oil ranged from 2.3 (for oil from corn germ extracted with hexane) to 324.5 μg/g (for oil from ground corn extracted with ethanol). Because of their importance for treating macular degeneration we focused on the levels of lutein and zeaxanthin, which ranged from 2.3 (for oil from corn germ extracted with hexane) to 220.9 μg/g (for oil from ground corn extracted with ethanol).

The data in Table 1 was used to calculate the levels of xanthophylls in each of the three corn tissues (Table 2). Interestingly, the combined levels of lutein and zeaxanthin were identical in corn germ and corn fiber (1.40 mg/kg tissue) and were about fivefold higher in ground corn (7.94 mg/kg). These results demonstrate that one tablespoon (15 ml) of corn kernel oil contains about 3.3 mg lutein and zeaxanthin. In order to obtain the same amount of xanthophylls from eating corn meal (ground corn) one would need to consume about 450 g (~1 pound) of corn meal.

Several other plant oils (refined and unrefined) were also examined to compare their levels of lutein and zeaxanthin (Table 3). Unrefined ethanol extracted corn kernel oil [3] contained high levels of all four carotenoids (similar to the values in Table 1), but conventional refining, bleaching and deodorizing (RBD), removed all of the carotenoids except a small amount of carotenes. Unrefined corn fiber oil (hexane extracted) [4] also contained lower levels of all four carotenoids and RBD also removed all of the carotenoids except a small amount of carotenes. The sample of commercial corn oil, Mazola, RBD, contained only small amounts of carotenes, but no lutein or zeaxanthin. Carotino Oil, a commercial blend of canola oil and red palm oil contained 180 μg/g of carotenes (identified as *β*-carotene by the manufacturer), but it contained no other carotenoids.

Lutein and zeaxanthin are found at high levels in the macula region of the human retina [12]. Most experts currently concur that including at least 5.8 mg per day of

Table 1 Oil yields and levels of carotenoids in wet milled corn germ oil, wet milled corn fiber oil, and whole corn kernel oil

Corn sample	Solvent	Oil yield/100 (g) sample	*β*-carotene (μg/g oil)	*β*-cryptoxanthin (μg/g oil)	Lutein (μg/g oil)	Zeaxanthin (μg/g oil)	Total carotenoids (μg/g oil)	Lutein + zeaxanthin (μg/g oil)
Corn germ (wet milled)	Hexane	34.14 ± 0.23[a]	0[e]	0[d]	1.4 ± 0.2[d]	0.9 ± 0.1[d]	2.3 ± 0.2[c]	2.3 ± 0.2[c]
	Ethanol	28.07 ± 0.03[b]	0[e]	0[d]	2.1 ± 0.3[d]	2.9 ± 0.4[cd]	5.0 ± 0.4[c]	5.0 ± 0.4[c]
Corn fiber	Hexane	2.36 ± 0.03[e]	5.6 ± 0.2[d]	6.8 ± 1.2[c]	6.3 ± 0.6[cd]	5.7 ± 0.4[cd]	24.4 ± 1.8[c]	12.0 ± 1.6[c]
	Ethanol	2.72 ± 0.10[d]	10.7 ± 0.2[c]	17.9 ± 0.4[b]	25.1 ± 1.4[b]	26.4 ± 1.9[b]	80.1 ± 3.8[b]	51.5 ± 4.0[b]
Ground corn	Hexane	2.60 ± 0.09[de]	18.6 ± 0.8[b]	15.5 ± 0.3[b]	10.4 ± 0.4[c]	16.2 ± 0.7[bc]	60.7 ± 1.8[b]	26.6 ± 0.8[bc]
	Ethanol	3.27 ± 0.03[c]	35.4 ± 1.3[a]	68.2 ± 2.2[a]	80.4 ± 5.5[a]	140.5 ± 11.7[a]	324.5 ± 24.3[a]	220.9 ± 21.2[a]

Mean ± standard deviation ($n = 3$). Mean in the same column with no letter in common are significantly different ($P < 0.05$) by the Bonferroni least significant difference method [14]

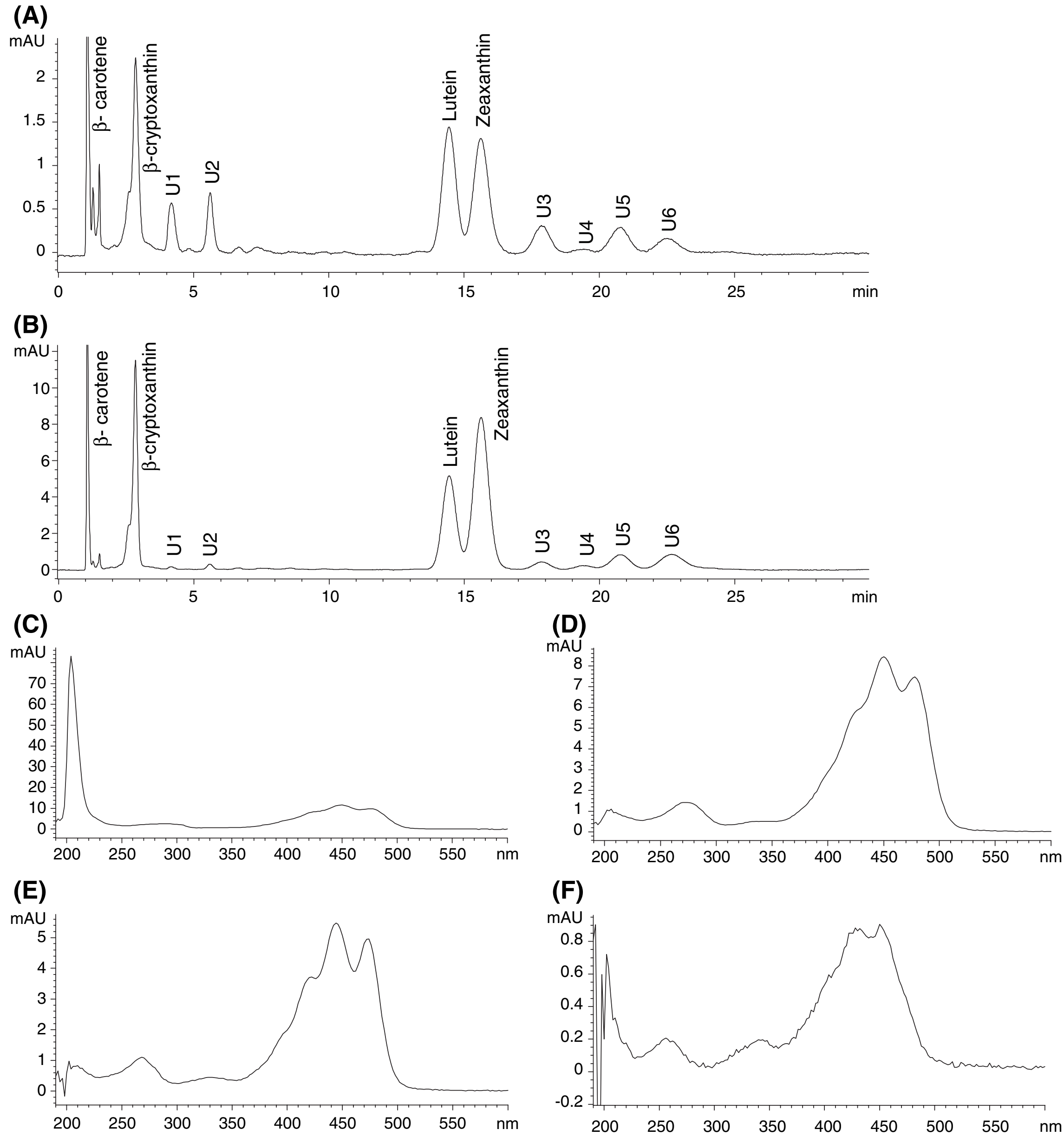

Fig. 2 Normal phase HPLC chromatogram of the carotenoids in corn oil obtained by **a** extracting corn fiber with ethanol samples and **b** extracting ground whole corn kernels with ethanol. **c** Absorption spectra of the β-cryptoxanthin peak from **b**, **d** absorption spectrum of the lutein peak from **b**, **e** absorption spectrum of the zeaxanthin peak from **b** and **f** absorption spectrum of U6 from **b** *U* unknown peaks

Table 2 The levels of carotenoids in wet milled corn germ, wet milled corn fiber, and whole corn kernels (calculated from the ethanol extraction values in Table 1)

Corn sample	β-carotene (mg/kg)	β-cryptoxanthin (mg/kg)	Lutein (mg/kg)	Zeaxanthin (mg/kg)	Total carotenoids (mg/kg)	Lutein + zeaxanthin (mg/kg)
Corn germ (wet milled)	0^{c}	0^{c}	0.59 ± 0.08^{b}	0.81 ± 0.11^{b}	1.40 ± 0.12^{b}	1.40 ± 0.12^{b}
Corn fiber	0.29 ± 0.01^{b}	0.49 ± 0.01^{b}	0.68 ± 0.04^{b}	0.72 ± 0.05^{b}	2.18 ± 0.17^{b}	1.40 ± 0.11^{b}
Ground corn	1.16 ± 0.04^{a}	2.23 ± 0.07^{a}	2.63 ± 0.18^{a}	4.59 ± 0.38^{a}	10.61 ± 0.79^{a}	7.94 ± 0.76^{a}

Mean ± standard deviation ($n = 3$). Mean in the same column with no letter in common are significantly different ($P < 0.05$) by the Bonferroni least significant difference method [14]

Table 3 The effect of conventional refining, bleaching and deodorizing (RBD) on the levels of carotenoids in oils from corn and other plant materials

	β-carotene (μg/g oil)	*β*-cryptoxanthin (μg/g oil)	Lutein (μg/g oil)	Zeaxanthin (μg/g oil)	Total carotenoids (μg/g oil)
Corn fiber oil (hexane extracted, unrefined)	20.3 ± 0.3[c]	45.7 ± 0.1[b]	25.0 ± 0.4[b]	27.3 ± 0.1[b]	118.3 ± 3.4[c]
Corn fiber oil (hexane extracted, RBD)	2.2 ± 0.0[d]	0 [c]	0[c]	0[c]	2.2 ± 0.0[d]
Ethanol-extracted corn kernel oil (unrefined)	36.4 ± 1.6[b]	69.0 ± 0.8[a]	123.8 ± 4.3[a]	78.3 ± 6.6[a]	307.5 ± 13.5[a]
Ethanol-extracted corn kernel oil (RBD)	2.3 ± 0.2[d]	0[c]	0[c]	0[c]	2.3 ± 0.2[d]
Commercial corn oil, Mazola® (RBD)	3.4 ± 0.1[d]	0[c]	0[c]	0[c]	3.4 ± 0.1[d]
Carotino™ cooking oil (RBD)	182 ± 2.9[a]	0[c]	0[c]	0[c]	182 ± 2.9[b]

Mean ± standard deviation ($n = 3$). Mean in the same column with no letter in common are significantly different ($P < 0.05$) by the Bonferroni least significant difference method [14]

lutein + zeaxanthin in the diet is a sufficient daily dosage to slow the progression of age-related macular degeneration [12, 15]. Spinach and other green leafy vegetables have been shown to contain the highest levels of lutein + zeaxanthin [16]. According to the USDA Nutrient database [16], 1/4 cup (~25 g) of cooked spinach contains 7–8 mg of lutein + zeaxanthin, so it is possible to consume the recommended 6 mg of lutein + zeaxanthin by consuming a reasonable portion of spinach. One cup of canned sweet corn or one cup of corn meal contain about 1.7 and 2.1 mg of lutein and zeaxanthin, respectively [16], so it is probably not practical to consume the recommended 6 mg of lutein + zeaxanthin by eating sweet corn or cornmeal. The results of the current study indicate that a diet that includes 30 g (~2 tbsp) per day of corn oil obtained by extracting ground corn with ethanol will provide ~6 mg of lutein + zeaxanthin. It should also be noted that there is disagreement among experts about the bioavailability of lutein + zeaxanthin in various foods [12, 15]. There is evidence that the lutein + zeaxanthin in egg yolks is highly bioavailable [17] whereas, the lutein + zeaxanthin in spinach and other green leafy vegetable has limited bioavailability [15].

Almost all edible plant oils contain free fatty acids and other minor components that need to be removed before the oils are palatable and stable [1]. Most oils are purified by three steps, refining, bleaching and deodorizing (RBD). Unfortunately, much of the lutein and zeaxanthin in edible oils is removed and/or destroyed during conventional refining, bleaching and deodorizing (Table 2). Several gentle refining techniques have been developed [18]. In future studies we will evaluate some of these methods to try to retain most of the lutein + zeaxanthin in ethanol-extracted corn oil. In our previous report [5] we noted that unrefined corn oil obtained by the ethanol extraction of corn kernels contained high levels of two compounds whose toxicity has not been established (diferuloylputrescine and *p*-coumaroylferululoylputrescine). We confirmed that both compounds were undetectable in ethanol-extracted corn kernel oil after conventional RBD (data not shown), but it will also be necessary to establish their complete removal or their safety before a suitable gentle refining method is established to retain high levels of carotenoids in the oil.

It is also likely that the extraction of ground corn with ethanol will also result in the extraction of other polar compounds such as sugars and amino acids and additional refining steps may be needed to remove these non-oil components. Obtaining corn oil by the extraction of corn oil from corn kernels may be more costly than obtaining corn oil by extracting corn germ with hexane, but the economics of the former process may be improved if it is incorporated as part of a zein extraction biorefinery and/or if it is conducted on-site at an ethanol plant [3]. Finally, it should be noted that it was recently reported that the lutein in corn kernels is tightly bound to the protein zein, at a ratio of three lutein molecules per zein molecule [19], so future studies of lutein extraction from corn kernels should consider this association.

If a suitable gentle refining method can be identified and shown to retain most of the carotenoids, then a diet that includes 30 g (~2 tbsp) per day of corn oil obtained by extracting ground corn with ethanol will provide ~6 mg of lutein + zeaxanthin. An average serving of salad oil is about 30 g, so it is feasible to propose the daily consumption of 30 g (~240 calories) ethanol-extracted corn kernel oil for those at risk for age-related macular degeneration. In contrast, the data in Table 2 indicate that to obtain ~6 mg per day of lutein + zeaxanthin from corn meal (ground corn) one would need to eat about 0.75 kg per day, an amount that is impractical.

After the experiments in this manuscript were conducted, a new publication reported a process to co-extract zein and xanthophylls (with 70% ethanol) from ground corn and separate them via preparative size exclusion chromatography [20]. The authors estimated that if their 70% ethanol extraction-chromatography process was incorporated into a typical dry grind ethanol plant producing 50 million gallons (189 million l) of ethanol per year, then the plant could also produce 13 million kg of zein and 7,500 kg of xanthophylls per year. For comparison, the results of our current manuscript can be used to estimate that if an ethanol extraction process was incorporated into the front end of a typical dry grind ethanol plant producing 189 million l (50 million gallons) of ethanol per year, the ethanol pre-extraction (100% ethanol) of 463 million kg of corn before fermentation would produce 19 million kg of corn oil (amber-colored) that would contain about 0.03% xanthophylls. The total amount of xanthophylls in the corn oil would be 5,700 kg or 5.7 metric tonnes. A comparison of the xanthophyll yields in the two studies may lead some readers to conclude that less xanthophylls are extracted with 100% ethanol than with 70% ethanol. This may be true, but it is also possible that differences in the levels of xanthophylls in various corn hybrids as reported previously [7] and/or differences in cultural practices or climate may also account for this small (~24%) difference in xanthophyll yields. As stated above, we believe that the enhanced bioavailability of the natural levels of xanthophylls in ethanol-extracted corn kernel oil may make this new type of healthy edible oil a good delivery system for xanthophylls.

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