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*Full Length Research Paper*

# Comparing the Carotenoid Content of Mature and Immature Corn (Zea mays L.) Grains

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**This study's goal was to analyze the carotenoid content of sweet and waxy corn grains at the milk and dough stages using reversed-phase high-performance liquid chromatography using a C(30) column that was coupled to mass spectrometry and a diode array detector. All-trans-lutein, all-trans-zeaxanthin, and all-trans-α-cryptoxanthin were the three main carotenoids detected in the reverse phase. In three kinds, waxy corn grains exhibited far lower levels of total carotenoids (ranging from 1.52 to 3.68 µg/g dry weight), while sweet corn grains had higher levels (ranging from 8.42 to 39.71 µg/g dry weight). The carotenoid pattern's composition differed somewhat from data found in the literature; this could be due to environmental or genetic variations. Immature sweet corn (such as "Jingtian 5" and "Jingtian 3) may be intriguing sources of lutein and/or zeaxanthin extract that could add value to an underutilized biomass, given the use of all-trans lutein and all-trans zeaxanthin for the prevention of several pathologies, particularly age-related macular degeneration.**

**Key words:** Carotenoids, HPLC-DAD-MS/MS, Corn, Discrepancy.

## INTRODUCTION

The most widely grown and consumed cereal in the world is corn (Zea mays L.), and because of its nutritional and functional qualities, immature corn grains are becoming more and more popular as a snack or vegetable in Asia, particularly in China. A type of maize with a high sugar content, sweet corn (Zea mays L. ssp. saccharata Sturt) is harvested when it is still immature (milk stage) and cooked and consumed as a vegetable. Waxy maize (Zea mays L. var.ceratina Kulesh) tastes stickier than other corn types because it solely contains amylopectin in its endosperm rather than the amylose starch molecule.[2] Waxy corns are typically picked before they reach full maturity since they are increasingly being consumed in China as fresh foods or as raw ingredients for whole grain dishes.

It has been demonstrated that maize grains contain quantities of carotenoids, a class of lipid-soluble substances that range in hue from yellow to red.[3] Carotenoids are linked to significant physiological processes and functions in addition to their colorant qualities.[4,5] Consuming carotenoids has been linked to a lower risk of developing a number of chronic degenerative diseases, including cancer, inflammation, heart disease, cataracts, age-related macular degeneration, and more.[6] The carotenoid content of foods is known to be influenced by varietal differences. For instance, there were notable variations in carotenoid content among the kinds of tomatoes that were studied. The carotenoid quantity of the various tomatoes varied, but so did the quality distribution of some pigments like lutein, β-carotene, and lycopene.[7] Kurilich and Juvik [8] discovered that the average lutein levels across the corn genotypes ranged from 0 to 20.0 µg/g dry weight, suggesting that the genes controlling lutein metabolism vary from genotype to genotype. Recently, the impact of ripeness on the main carotenoids was assessed. The biosynthesis of carotenoids was seen during the maturation phase. Fruits with the highest carotenoid concentrations from genotype number 05 of acerola stood out among the other genotypes.[9] Although numerous studies have documented the chemical makeup of corn grains, relatively little is known about their carotenoid profile, particularly for waxy and immature corns. Thus, using high-performance liquid chromatography coupled with diode array and mass spectrometry detectors (HPLC-DAD-MS/MS), the current study compared the carotenoid composition of immature and mature corn and investigated the impact of ge0otypes on the carotenoid composition of corn grains.

## MATERIALS

### Reagents

Sigma (St. Louis, MO) provided the zeaxanthin, β-carotene, and β-cryptoxanthin. Our lab produced lutein that was more than 90% pure. We bought methanol and methyl tert-butyl ether (MTBE) from Tedia (Fairfield, USA). The suppliers of ethanol, toluene, acetone, hexane, and anhydrous sodium sulfate were Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The remaining reagents and compounds were all analytical grade.

## METHODS

### Sample Treatment

Luhe Animal Science Base, Jiangsu Academy of Agricultural Sciences (Nanjing, China), located at 32.08°N 118.40°E, provided the tested corn varieties, which included the sweet corn varieties "Jingtian 3" and "Jingtian 5," as well as the waxy corn varieties "Suyunuo 11," "Jingnuo 8," and "Jingtianzihuanuo 2." The kernels were promptly brought to our lab after being harvested at the milk (immature) and dough (mature) stages, respectively. Twelve kernels of each harvested sample that were uniform in size and shape and free of physical damage were chosen in order to provide trustworthy samples. Before analysis, the samples were kept at -80°C and instantly frozen in liquid N2 to stop the enzymatic activity. At least three replicates were used.

### Carotenoid Extraction

A constant weight with a 2.6% water content was achieved by lyophilizing corn grains (FD-1A-50, Beijing Boyikang Laboratory Instruments Co., Ltd., China) at –50°C (vacuum degree of 15 Pa) prior to analysis. Following total drying, the kernel samples were crushed into a powder using a Tianjin Taisite Instrument Co., Ltd., China, FW100 grinder, and sieved through 40 standard meshes to guarantee particle size symmetry.

In a 100 mL volumetric flask, three grams of maize grain powder were treated with 30 mL of a hexane-ethanol-acetone-toluene (10:6:7:7, v/v) mixture, and the mixture was agitated for one hour. For saponification, 2 mL of 40% methanolic potassium hydroxide was added to the contents and left at 25°C for 16 hours in the dark with nitrogen gas. 30 mL of hexane was added for the carotenoids' partition after saponification, agitated for one minute, and then 10% sodium sulfate solution was added and diluted to volume. The mixture was left to stand until it became evident that two phases had separated. For HPLC analysis, the lutein-containing top layer was gathered, dried by evaporation, redissolved in 10 milliliters of methanol, and then passed through a 0.45 μm membrane filter. To prevent isomerization or carotenoid degradation, the entire extraction process was conducted in low light, and nitrogen gas was pushed into vials.

### HPLC-DAD-MS/MS Analysis

With a few adjustments, carotenoid analysis was performed using the previously published methodology.[10,11] An analytical scale C30 reversed phase column (250 mm × 4.6 mm i.d.) with a particle size of 5 μm was used for the HPLC analysis (YMC, Wilmington, MA, USA). Eluent B was made by combining MTBE/methanol/water (85: 10:5, v/v), while eluent A was made up of methanol/MTBE/water (70: 25: 5, v/v). Using a gradient elution condition (Table 1), separation was carried out in 24 minutes at a flow rate of 0.6 mL/min at a column temperature of 25°C. For HPLC, 20 μL aliquots were utilized. In order to conduct studies, an Agilent 1290 Infinity LC/Agilent Technologies 6460 MS was used. With a total ion current (TIC) scanning range of 80–1000 m/z, a corona current of 4 μA, a capillary voltage of 2500 V, nitrogen as the nebulizer gas (purity 99.9% and flow rate 4 L/min), and a vaporizer temperature of 350°C, the positive ion mode (APCI) was employed to detect carotenoids. After creating a five-point external standard calibration curve for every available standard, the external standards method was used to do the quantification; the standard calibration curve's R2 values fell between 0.9991 and 0.9995.

### Statistical Analysis

Carotenoid content was expressed as mean ± standard deviation (SD) of three independent observa- tions. Data were analyzed using SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA).

## RESULTS AND DISCUSSION

### Carotenoid Identification

Information regarding the existence and kind of xanthophyll esterification was left out of the current work because analyses were conducted on saponified extracts. Compared to C18, the use of C30 column technology has yielded a more precise profile of the geometrical isomers of lutein and zeaxanthin in fruits and vegetables.[12,13] Fig. 1 displays the chromatogram of carotenoid extracts from both mature and immature corn grains (Jingtian 5 variety). Comparing the retention time and ultraviolet (UV) spectra of genuine standards allowed for the identification of the primary carotenoids, after which the quantitative data were computed.

From their linear calibration curves under analysis conditions. Micrograms per gram on a dry matter basis were used to express the results for the primary carotenoids. All-trans-lutein, all-trans-zeaxanthin, all-trans-α-cryptoxanthin, all-trans-β-cryptoxanthin, and violaxanthin were the primary carotenoid molecules, as can be observed. Based on the combined data of electron ionization, chemical ionization mass spectroscopy (EIMS and CIMS) fragmentation patterns, HPLC retention time, and UV-visible absorption maxima, a few peaks were found (Table 2). All 16 of the 21 carotenoids found in the corn grains examined in this study are being reported for the first time in corn grains, while all-trans-lutein, all-trans-zeaxanthin, all-trans-β-cryptoxanthin, all-trans-α-carotene, and all-trans-β-carotene were previously reported by other researchers [10,14,15]. Mature had an impact on the carotenoid profiles, which varied among the maize genotypes.

**Quantitative Composition of Different Genotypes**

Although the carotenoid profiles varied among the genotypes of corn, the most prevalent carotenoids for both immature and mature samples were lutein (range from 0.14 to 9.80 μg/g) and zeaxanthin (0.83 to 10.86 μg/g), which is consistent with a prior study by Lozano-Alejo et al.[16] It is noteworthy that both the overall carotenoid profile and the lutein:zeaxanthin ratio differ among genotypes.[17] The main carotenoids for each species of sweet corn at each stage were all-trans-α-cryptoxanthin, zeaxanthin, and lutein (ranging from 1.80 to 6.38 μg/g). With the exception of mature sweet corn "Jingtian 3," which showed a lower carotenoid content (approximately 8.42 μg/g dry weight [DW]), all of the sweet corn types examined generally seemed to be good sources of carotenoids. Waxy corn types had the lowest carotenoid concentrations, ranging from 1.52 to 3.68 μg/g DW, while sweet corn varieties "Jingtian 5" had the highest total carotenoids content in the milky stage (39.71 μg/g DW) (Table 3).

While the types of carotenoids in waxy corn at the dough stage grew, the species of carotenoids in sweet corn and waxy corn at the succulent stage of maturation (milky stage) significantly declined. In addition to "Jingtianzihuanuo 2," the total carotenoids concentration in all types of maize varieties fell during the dough stage. All-trans-lutein, all-trans-zeaxanthin, violaxanthin, and all-trans-α-cryptoxanthin were the main carotenoids in sweet corn at the milk stage. All-trans zeaxanthin, violaxanthin, and neoxanthin were present in waxy corn during the milk stage. Additionally, more than half of the total carotenoids were made up of all-trans-lutein and all-trans-zeaxanthin. Major carotenoids gradually declined in sweet corn from the milk stage to the dough stage, but new minor carotenoids, including the cis-isomers 9-cis-β-cryptoxanthin and 9-cis-β-carotene, produced. The epoxide product, which contained 13-cis-lutein-5,6-epoxide, also rose. Waxy maize also had an increase in 13-cis-Neoxanthin and freshly produced all-trans-α-cryptoxanthin. Newilah et al. [18] reported the same occurrence, which may be caused by the breakdown of trans carotenoids, which promotes the production of additional carotenoid isomers during maturity. According to Steenbock, the carotenoid content of yellow maize grains determines their hue; the more carotenoids present, the deeper the yellow color is in comparison. While there was a difference in the color of the corn grains that caught our attention throughout the maturity of yellow corns, perhaps due to variations in the amount of moisture and other media at different phases of maturation, the conclusion from the varieties of corn is consistent with the findings of this study.

## CONCLUSION

Zeaxanthin, α-cryptoxanthin, and lutein were the main carotenoids in maize grains that may provide health benefits for people. The primary carotenoids in corn grains were separated and quantified, demonstrating a distinct difference between those of various types. Variations in corn crop kinds and ripening stages are probably the cause of the quantitative variance in carotenoid makeup. The results imply that, in comparison to other corn grains, immature sweet corn can be a better or comparable dietary source of carotenoids.

## FUNDING

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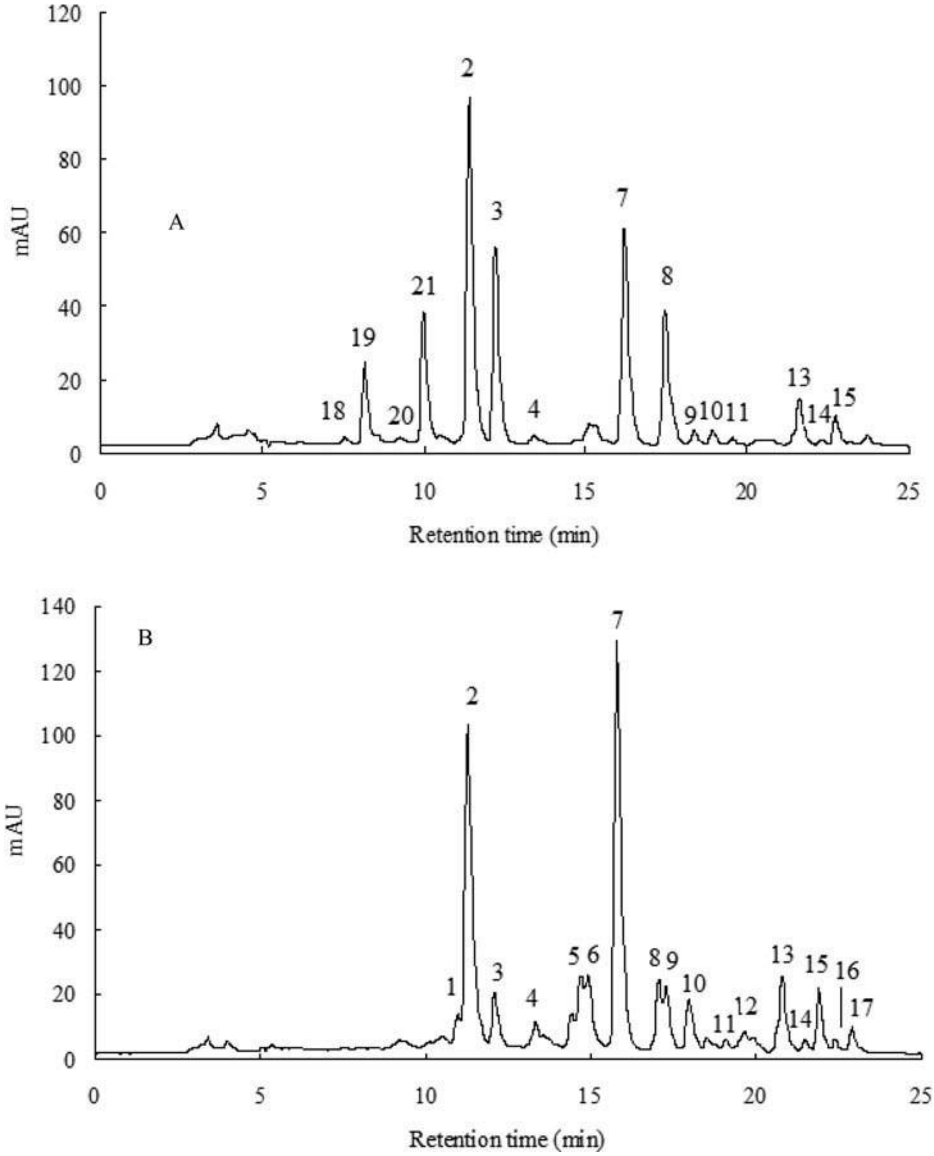
TABLE 1
The HPLC gradient elution conditions

| *Time (min)* | *A (%)* | *B (%)* |
|---|---|---|
| 0 | 95 | 5 |
| 4.5 | 80 | |
| 12.5 | 50 | |
| 18 | 25 | |
| 24 | 5 | |




The HPLC chromatogram for a saponified carotenoid extract of immature corn grains (A) and mature corn grains (B) (Jingtian 5 variety) is shown in Figure 1. Peak identities: 1: 13-cis-lutein-5,6-epoxide; 2: all-trans-lutein; 3: all-trans-zeaxanthin; 4: 9- or 9'-cis-lutein; 5: 15-cis-β-cryptoxanthin; 6: 13-or 13'-cis-β-cryptoxanthin; 7: all-trans-α-cryptoxanthin; 8: all- trans-β- cryptoxanthin; 9: 9-cis-α- cryptoxanthin; 10: 9-cis-β-cryptoxanthin; 11: 9'-cis-β- cryptoxanthin; 12: 13-cis-β- carotene; 13: all-trans-α-carotene; 14: 9-cis-α-carotene; 15: all-trans-β-carotene; 16: 9'-cis-α-carotene; 17: 9-cis-β- carotene; 18: 13-cis-neoxanthin; 19: neoxanthin; 20: neochrome; 21: violaxanthin.

TABLE 2
Chromatographic, UV-Vis, and mass spectrometry characteristics of carotenoids from corn grain obtained by HPLC-DAD-MS/MS

| *Peak number* | *Carotenoid* | *Retention time (min)* | *Λmax (nm)* | *m/z* |
|---|---|---|---|---|
| 18 | 13-cis-neoxanthin | 7.562 | 420,442,470 | $C_{40}H_{56}O_4$ 601.4 |
| 19 | Neoxanthin | 8.174 | 330,416,442,470 | $C_{40}H_{56}O_4$ 601.4 |
| 20 | Neochrome | 9.266 | 298,398,422,448 | $C_{40}H_{56}O_4$ 601.4 |
| 21 | Violaxanthin | 9.990 | 420,444,472 | $C_{40}H_{56}O_4$ 601.4 |
| 1 | 13-cis-lutein-5,6-epoxide | 10.962 | 332,412,440,466 | $C_{40}H_{56}O_3$(585.4, 586.4, 587.4) |
| 2 | All-trans-lutein | 11.262 | 330,420,446,472 | $C_{40}H_{56}O_2$551.4, 569.4 |
| 3 | All-trans- zeaxanthin | 12.080 | 338,420,450,476 | $C_{40}H_{56}O_2$ 569.4, 586.4$(M+NH_4)^+$ |
| 4 | 9- or 9'-cis-lutein | 13.317 | 418,442,468 | |
| 5 | 15-cis-β-cryptoxanthin | 14.696 | 332,410,440,468 | $C_{40}H_{56}O$ 553.4, 554.4, 555.4 |
| 6 | 13- or 13'-cis-β-Cryptoxanthin | 14.910 | 444,470 | $C_{40}H_{56}O$ 553.4, 554.4, 555.4 |
| 7 | All-trans-α- cryptoxanthin | 15.783 | 336,420,446,474 | $C_{40}H_{56}O$ 553.4 |
| 8 | All-trans-β- cryptoxanthin | 17.042 | 414,450,476 | $C_{40}H_{56}O$ 553.4 |
| 9 | 9-cis-α- cryptoxanthin | 17.261 | 416,440,468 | $C_{40}H_{56}O$ 553.4 |
| 10 | 9-cis-β- cryptoxanthin | 17.953 | 418,442,468 | $C_{40}H_{56}O$ 553.4 |
| 11 | 9'-cis-β- cryptoxanthin | 19.065 | 446,472 | $C_{40}H_{56}O$ 553.4 |
| 12 | 13-cis-β-carotene | 19.649 | 416,440,470 | $C_{40}H_{56}$ 537.4 |
| 13 | All-trans-α-carotene | 20.782 | 338,418,446,472 | $C_{40}H_{56}$ 37.4,603.5 |
| 14 | 9-cis-α-carotene | 21.460 | 440,468 | |
| 15 | All-trans-β-carotene | 21.891 | 422,452,476 | $C_{40}H_{56}$ 537.4 |
| 16 | 9'-cis-α- carotene | 22.377 | 330,416,446,470 | |
| 17 | 9-cis-β-carotene | 22.889 | 446,448,472 | |