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Increase in β-Ionone, a Carotenoid-Derived Volatile in Zeaxanthin-Biofortified Sweet Corn

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ABSTRACT: Carotenoids are responsible for the yellow color of sweet corn (*Zea mays* var. *saccharata*), but are also potentially the source of flavor compounds from the cleavage of carotenoid molecules. The carotenoid-derived volatile, β-ionone, was identified in both standard yellow sweet corn ('Hybrix5') and a zeaxanthin-enhanced experimental variety ('HZ') designed for sufferers of macular degeneration. As β-ionone is highly perceivable at extremely low concentration by humans, it was important to confirm if alterations in carotenoid profile may also affect flavor volatiles. The concentration of β-ionone was most strongly correlated (R^2 > 0.94) with the β-arm carotenoids, β-carotene, β-cryptoxanthin, and zeaxanthin, and to a lesser degree (R^2 = 0.90) with the α-arm carotenoid, zeinoxanthin. No correlation existed with either lutein (R^2 = 0.06) or antheraxanthin (R^2 = 0.10). Delaying harvest of cobs resulted in a significant increase of both carotenoid and β-ionone concentrations, producing a 6-fold increase of β-ionone in 'HZ' and a 2-fold increase in 'Hybrix5', reaching a maximum of 62 μg/kg FW and 24 μg/kg FW, respectively.

KEYWORDS: *β-ionone, zeaxanthin, biofortification, Zea mays, carotenoid, flavor*

INTRODUCTION

The major carotenoids present in sweet corn (*Zea mays* var. *saccharata*) are lutein and zeaxanthin, together with smaller amounts of β-cryptoxanthin, β-carotene, zeinoxanthin, α-carotene, and antheraxanthin.[1,2] Apart from providing the characteristic pigmentation of yellow corn, carotenoids may also contribute to flavor through the synthesis of carotenoid-derived volatiles formed by the action of carotenoid-cleavage enzymes (Figure 1). In tomato (*Solanum lycopersicum*), for example, lycopene and β-carotene are thought to be cleaved at the 9−10 and 9′−10′ double bonds[17] to form the volatiles 6-methyl-5-hepten-2-one and β-ionone, respectively, both of which contribute strongly to the characteristic tomato flavor.[3] In corn, Buttery and Ling[4] reported the presence of the volatiles β-ionone and α-ionone in corn tortillas, but these were seen as minor constituents relative to non-carotenoid derived volatile compounds present, and not believed to contribute significantly to flavor.

Recently, there has been considerable research into the development of high-carotenoid corn varieties, to enhance either the production of the pro-vitamin A carotenoid, β-carotene,[5,6] or the production of zeaxanthin, a carotenoid associated with protection against age-related macular degeneration (AMD).[7,8] Zeaxanthin and its isomer lutein are actively accumulated in the human macula from dietary sources[9] and are thought to protect against the degradative effects of blue light oxidation on photoreceptor cells.[10−12] Zeaxanthin, which is relatively rare in the diet, tends to be located toward the center of the macula, while lutein, its more commonly found isomer, is located toward the periphery.[13] Although sweet corn is considered a good source of zeaxanthin relative to many other food sources,[14] the amount of zeaxanthin present in a cob of corn is well below what is considered a supplementary dosage rate,[15] which would require consumption of between 4 and 11 cobs of standard yellow sweet corn per day to be achieved.

Part of the strategy to enhance zeaxanthin concentration in sweet corn has been to increase total carotenoid production and to shift the synthesis of carotenoids toward the β-arm of the carotenoid-synthesis pathway (Figure 1) where zeaxanthin is located.[7] Although such a change will alter the color of the corn kernels from yellow to an orange hue,[8] it is also possible that this change may affect the profile and synthesis of carotenoid-derived volatiles. As volatiles such as β-ionone can be perceived by humans at a very low concentration (7 ng/L),[4,16] increasing their concentration may have a significant effect on sweet corn flavor. The aim of the following investigation was to quantify changes in carotenoid-derived volatiles of a zeaxanthin-enhanced sweet corn line 'HZ' relative to the standard yellow sweet corn cultivar 'Hybrix5' over a range of harvest maturities, and to determine if these changes in volatiles are correlated to changes in carotenoid concentration and profile.

MATERIALS AND METHODS

Corn Samples. Sweet corn of a standard yellow cultivar, 'Hybrix5', and an enhanced-zeaxanthin experimental line 'HZ' were grown at Gatton Research Facility, Queensland, Australia. Both varieties have similar total carotenoid concentration, but vary in their proportion of α-arm and β-arm carotenoids. Twenty-five plants of each variety were grown in parallel rows immediately beside each other. Plants were manually self-pollinated and three cobs harvested randomly each at 16,

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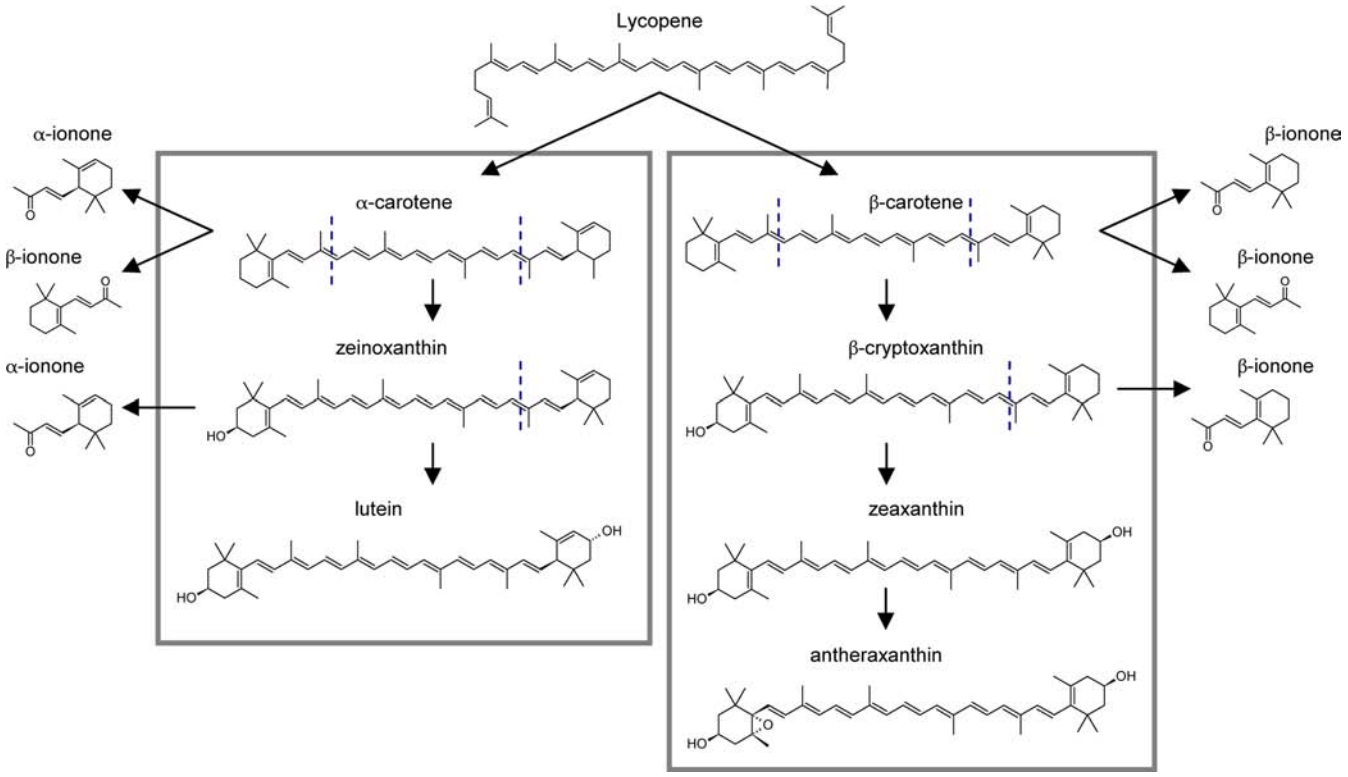

Figure 1. Structures of the principal carotenoids (boxed) of the carotenoid synthesis pathway found in sweet corn kernels (*Zea mays* var. *saccharata*) and their known carotenoid-derived volatiles formed by cleavage (dotted lines) (left box, α-arm carotenoids; right box, β-arm carotenoids).

20, and 24 days after pollination (DAP), representing early, normal, and late harvests for sweet corn. Harvested cobs were immediately stored at −20 °C for up to 15 days subsequent to carotenoid and volatile analysis. Previous analysis in our laboratory showed no significant change in carotenoids in freshly harvested kernels stored for up to 4 weeks at 4 °C. Commercially harvested cobs of a white cultivar 'Everest' were purchased from a local supermarket and stored similarly at −20 °C to serve as a nonpigmented control.

Carotenoid Extraction and HPLC Analysis. Carotenoid extraction and HPLC analysis were conducted according to the method of Fanning et al.[8] Fifteen kernels were randomly selected from each of the three cobs per harvest date. Sweet corn samples were cryogenically milled using a Retsch MM301 ball-mill (Haan, Germany), and approximately 0.6 g of sample was weighed and 6 mL of ethanol and 250 μL of β-apo-8′-carotenal (7.2 mg/L in isopropanol) as an internal standard were added. Samples were vortexed, 3 mL of deionized water and 5 mL of hexane added, and samples revortexed for 20 s before placement of capped tubes on ice. Samples were centrifuged for 2 min at 83 Hz (4 °C) to separate layers. The upper hexane layer was removed and transferred to a second tube. Fresh hexane (5 mL) was added to the non-hexane fraction containing the pellet, the mixture was vortexed, and the procedure was repeated as above 2 to 3 times until the pellet became a white color. The combined hexane fractions were dried in a centrifugal evaporator at 30 °C and the extracted carotenoids reconstituted in 2 mL of methanol/dichloromethane (50:50, v/v), containing 0.1% butylated hydroxytoluene (BHT). Samples were filtered (0.22 μm syringe filter; Grace, Sydney, Australia), placed into HPLC vials, and stored at −80 °C prior to HPLC analysis. CV was less than 5%. Authentic standards of lutein, zeaxanthin, β-carotene, and β-cryptoxanthin (Sigma-Aldrich) were prepared similarly in methanol/dichloromethane (50:50, v/v), containing 0.1% BHT. Each carotenoid standard was run by HPLC to determine peak purity. The actual concentrations of the standard solutions were then calculated by multiplying the concentration determined spectrophotometrically by the % peak area of the standard peak as determined by HPLC. Standard curves were linear over the range 0.03−10 μg/mL with R^2 values of >0.999.

The HPLC system consisted of a SIL-10AD VP autoinjector, SCL-10A VP system controller, LC-10AT VC liquid chromatograph, and a SPD-M10 A VP diode array detector (Shimadzu, Kyoto, Japan). Forty microliters of each extract was injected onto a YMC C30 carotenoid column, 3 μm, 3.6 × 250 mm (Waters, Milford, MA, USA), with a mobile phase consisting of 92% methanol/8% 10 mmol/L ammonium acetate (phase A), and 100% methyl *tert*-butyl ether (phase B). The 10 mM ammonium acetate solution was made up in water and mixed with methanol in a ratio of 92:8, methanol:10 mM ammonium acetate solution (v:v). The following 40 min gradient was used:[5] 0 min, 80% phase A; 32 min, 40% phase A; 34 min, 80% phase A; 40 min, 80% phase A.

Carotenoid Identification and Quantification. Lutein, zeaxanthin, β-cryptoxanthin, and β-carotene were identified by comparison with the retention times and absorption spectra of the standards and quantified as described previously.[8] Antheraxanthin and zeinoxanthin were identified by comparison of retention time, absorption spectra, and mass data, obtained by LC−MS.[10] The standard curve of lutein was used to quantify antheraxanthin, and the standard curve of β-cryptoxanthin was used to quantify zeinoxanthin. Carotenoid concentration was expressed as $\mu g \cdot g^{-1}$ fresh weight (FW) of corn kernels.

Preparation of Kernels for Volatile Extraction. Approximately 5 g of randomly selected sweet corn kernels from the same cobs as those used above for carotenoid analysis (n = 3) was accurately weighed (± 0.01 g) directly into a Retsch MM301 ball-mill stainless steel vessel (Haan, Germany). Five milliliters of saturated sodium chloride solution containing 2-nonanone (0.06362 μg/L, Sigma-Aldrich) as an internal standard was added. The sample and extraction solution were then homogenized for 30 s at 30 cycles/s. A 10 g aliquot of the resulting homogenate was then added to a 20 mL headspace vial, which was immediately sealed using a crimp seal cap and septum. The prepared samples were stored at 4 °C prior to analysis.

Headspace GC−MS Analysis. Analysis of carotenoid-derived volatiles was performed using a Shimadzu GC-2010 gas chromatograph coupled with a Shimadzu GCMS-QP2010S mass selective detector (MSD). Headspace sampling was undertaken by solid-phase

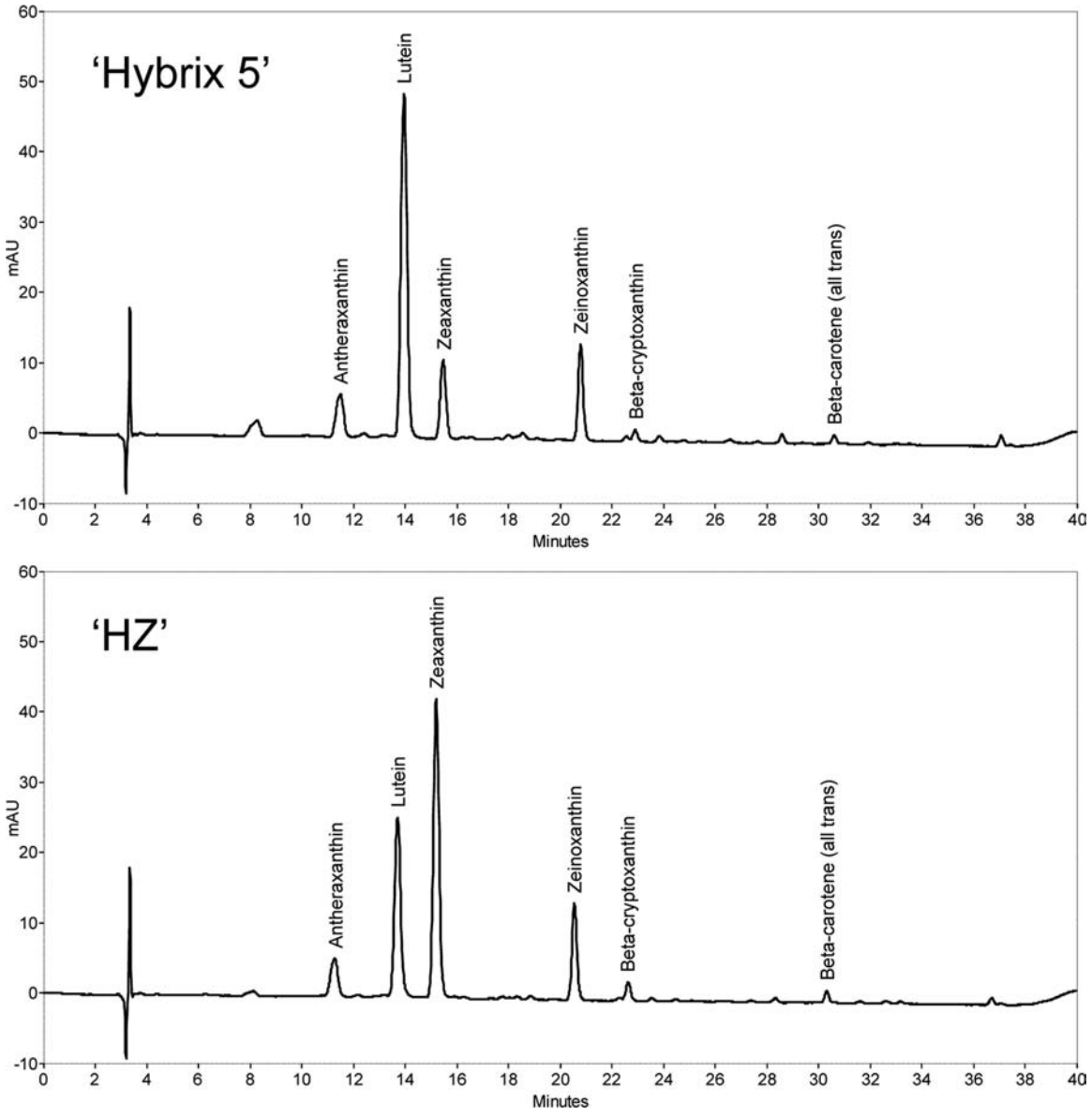

Figure 2. Principal carotenoid peaks identified in 'Hybrix5' (top) and 'HZ' sweet corn (*Zea mays* var. *saccharata*) kernels (bottom).

microextraction (SPME) using a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) controlled by Cycle Composer software (CTC Analytics, version 1.5.2). A 50/30 μm carboxen/divinylbenzene/polydimethylsiloxane (Car-DVB-PDMS StableFlex, Supelco, Bellefonte, PA) SPME fiber was used for all analyses. Prior to headspace sampling, the vials were equilibrated at 60 °C for 15 min. During extraction, the SPME fiber was exposed to the sample headspace for 30 min at 60 °C, then inserted into the heated GC inlet, and desorbed at 250 °C in splitless mode. After 2 min, a 1:50 split ratio was programmed and maintained for the duration of the analysis. The GC column oven was fitted with a DB-1 capillary column (50 m × 0.22 mm i.d., 1 μm phase; SGE, Australia). The carrier gas was helium set to a flow rate of 1.2 mL/min, linear velocity 30.6 cm/s. The initial oven temperature was 40 °C for 2 min, then ramped at 5 °C/min to 100 °C, then ramped at 20 °C/min to 220 °C, and held for 8 min. The interface temperature was set to 280 °C.

A scan of volatile compounds using an m/z range of 35 to 350 was conducted. Spectra were examined and compounds identified using Shimadzu GC−MS solutions software (version 2.53). Compounds with ionone-ring structures were tentatively identified. In addition, a search was conducted for the specific direct and indirect potential carotenoid-cleavage products, β-ionone, α-ionone, 3-hydroxy-β-ionone, 3-oxo-α-ionone, 3-oxo-β-ionone, β-damascenone, blumenol C, and metastigm-4-ene-3,9-dione.

Quantification of β-ionone was further achieved using 2-nononone (Sigma-Aldrich) as an internal standard and with the MSD in selective ion monitoring (SIM) mode. The ion source was set at 70 eV and electron multiplier at 1350 V. The target ions monitored were m/z 58 and m/z 177 for 2-nonanone and β-ionone respectively, and the ratios of their integrated area counts were used for quantification. The qualifier ions monitored for 2-nononone were m/z 57 (23.2), 71 (22.6), and 59 (22.3), and those monitored for β-ionone were m/z 91 (20.8), 93 (17.6), 135 (17.1), and 178 (13.8). Positive identification was confirmed by the presence of both target and qualifier ions at the correct retention time and with the correct ion ratios. A 6 point internal standard calibration was made by the addition of β-ionone (Sigma-Aldrich) to the white corn cultivar 'Everest' known to contain no endogenous β-ionone.

Statistical Analysis. The trial was conducted as a completely randomized design. Differences in carotenoid and β-ionone concentrations were evaluated using one-way analysis of variance (ANOVA) and means separated using least significant difference (LSD) at $P < 0.05$. Carotenoid and β-ionone concentrations were correlated using linear regression analysis and coefficient of determination (R^2)

Table 1. Carotenoid Concentrations (± SE) of Standard Yellow Hybrid, 'Hybrix5', and Enhanced β:α Ratio Hybrid 'HZ' Sweet Corn (*Zea mays* var. *saccharata*) at Increasing Harvest Maturities (DAP)

Hybrid		Carotenoid ($\mu g.g^{-1}$ FW)	Days after pollination (DAP)		
			16	20	24
'Hybrix 5'	β-arm	β-carotene[A]	0.24±0.01 a	0.41±0.05 ab	0.51±0.02 b
		β-cryptoxanthin	0.31±0.04 a	0.34±0.03 a	0.41±0.03 a
		zeaxanthin	2.09±0.14 a	2.80±0.23 a	2.92±0.10 a
		antheraxanthin	1.30±0.02 a	1.64±0.09 b	1.74±0.29 b
		Total β-arm	3.95±0.21 a	5.21±0.39 b	5.60±0.16 b
	α-arm	zeinoxanthin	1.54±0.29 a	1.81±0.19 a	2.68±0.16 a
		lutein	2.38±0.09 a	5.52±0.38 b	6.21±0.23 b
		Total α-arm	3.39±0.35 a	7.34±0.54 b	8.90±0.23 c
'HZ'	β-arm	β-carotene	0.28±0.01 a	0.49±0.06 a	1.07±0.12 b
		β-cryptoxanthin	0.19±0.02 a	0.45±0.03 b	0.87±0.06 c
		zeaxanthin	2.62±0.05 a	4.90±0.27 b	8.27±0.97 c
		antheraxanthin	0.94±0.01 a	1.18±0.04 ab	1.49±0.15 b
		Total β-arm	4.05±0.06 a	7.04±0.38 b	11.72±0.95 c
	α-arm	zeinoxanthin	1.20±0.08 a	2.17±0.54 a	2.68±0.55 b
		lutein	1.60±0.05 a	2.75±0.05 b	3.97±0.24 c
		Total α-arm	2.81±0.09 a	4.93±0.50 b	7.67±0.79 c

[a]Data (n = 3) within rows followed by different letters are significantly different (P < 0.05).

determined. Both ANOVA and linear regression analyses were performed using Genstat software (version 11.1).

RESULTS

Effect of Variety and Harvest Maturity on Carotenoid Accumulation. The principal carotenoids identified in both 'Hybrix5' and 'HZ' cultivars were the α-arm carotenoids, lutein and zeinoxanthin, and the β-arm carotenoids, zeaxanthin, β-cryptoxanthin, β-carotene, and antheraxanthin (Figure 2; Table 1). α-Carotene was detected in only trace amounts. Zeaxanthin was the predominant carotenoid in 'HZ', and lutein the predominant carotenoid in 'Hybrix5'. Zeaxanthin and lutein reached maximum concentrations of 8.3 and 4.0 μg/g FW in 'HZ', and 2.9 and 6.2 μg/g FW, respectively, in 'Hybrix5' (Table 1). Carotenoid levels in the white cultivar 'Everest' were extremely low (data not presented).

As expected, the ratio of β-arm carotenoids to α-arm carotenoids differed markedly between the two yellow cultivars, with 'Hybrix5' accumulating more α-arm carotenoids than 'HZ', and 'HZ' accumulating more β-arm carotenoids than 'Hybrix5' (Table 1). This was reflected by 'Hybrix5' averaging a β:α ratio of 0.78 and 'HZ' a β:α ratio of 1.48 (Table 2). Generally, carotenoid concentrations increased with increasing harvest maturity (Table 1), although in 'Hybrix5', zeaxanthin, β-cryptoxanthin, and zeinoxanthin did not significantly increase (P < 0.05) after the initial harvest time at 16 DAP. Consequently, the β:α ratio of 'Hybrix5' was observed to decline with increasing harvest maturity, while that for 'HZ' remained relatively constant (Table 2).

Identification and Quantification of Carotenoid-Derived Volatile Compounds. Potential carotenoid-derived volatiles identified with an ionone-like ring structure eluted at 24.56 and 25.53 min (Figure 3). These peaks were tentatively identified as either α-ionone, 6-methyl-α-ionone, or γ-ionone at 24.56 min, and β-ionone at 25.53 min. There was no spectral evidence for the presence of the potential primary and secondary cleavage products, 3-hydroxy-β-ionone, β-damascenone, 3-oxo-α-ionone, 3-oxo-β-ionone, blumenol C, or metastigm-4-ene-3,9-dione. The presence of β-ionone was confirmed by selected ion monitoring mode (SIM), and although common ions (e.g., m/z 177) were found at a retention time expected for α-ionone, the identity of α-ionone was not confirmed.

Table 2. Ratio of β-Arm to α-Arm Carotenoids (± SE) in 'Hybrix5' and 'HZ' Sweet Corn Hybrids (*Zea mays* var. *saccharata*) at Different Harvest Maturities (DAP)

hybrid	days after pollination (DAP)		
	16[a]	20	24
'Hybrix5'[b]	1.01 ± 0.05 Aa	0.71 ± 0.01 Ba	0.63 ± 0.02 Ba
'HZ'	1.45 ± 0.05 Ab	1.44 ± 0.06 Ab	1.54 ± 0.07 Ab

[a]Data (n = 3) within columns followed by different lowercase letters are significantly different (P < 0.05). [b]Data (n = 3) within rows followed by different uppercase letters are significantly different (P < 0.05).

β-Ionone increased with increasing harvest maturity in both 'Hybrix5' and 'HZ' but was absent in the white cultivar 'Everest' (Figure 4A). β-Ionone reached a maximum concentration of 0.063 μg/g FW in 'HZ' at 24 DAP, approximately 2.5 times higher than that observed in 'Hybrix5' (0.024 μg/g FW).

Correlation between β-Ionone and Carotenoid Concentration. β-Ionone was found to be strongly correlated with the β-arm carotenoids, β-carotene (R^2 = 0.94), β-cryptoxanthin (R^2 = 0.95), and zeaxanthin (R^2 = 0.95), but not antheraxanthin (R^2 = 0.10). β-Ionone was also positively correlated with the α-arm carotenoid, zeinoxanthin (R^2 = 0.90), but not lutein (R^2 = 0.06) (Table 3).

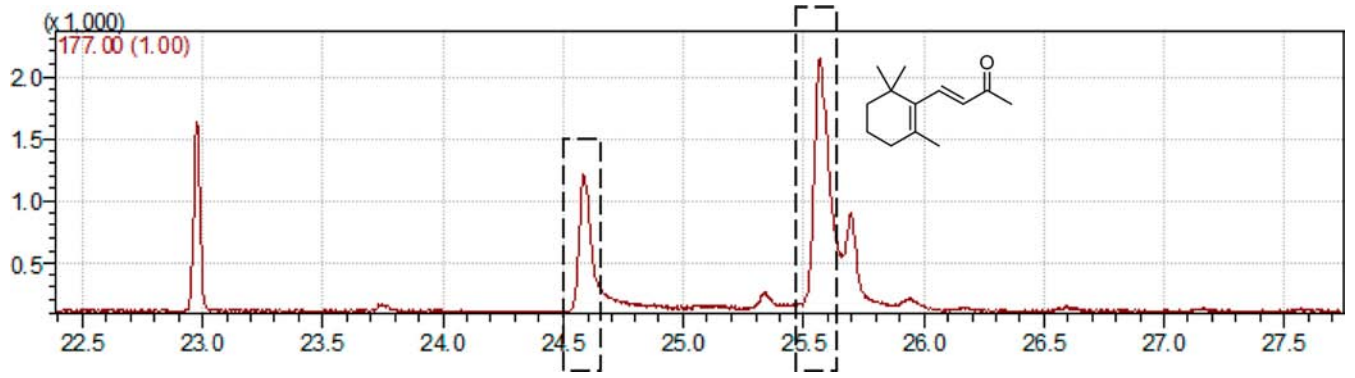

Figure 3. Boxed peaks detected in 'Hybrix5' and 'HZ' sweet corn with an ionone-like ring structure. The boxed peak at right was identified as *β*-ionone.

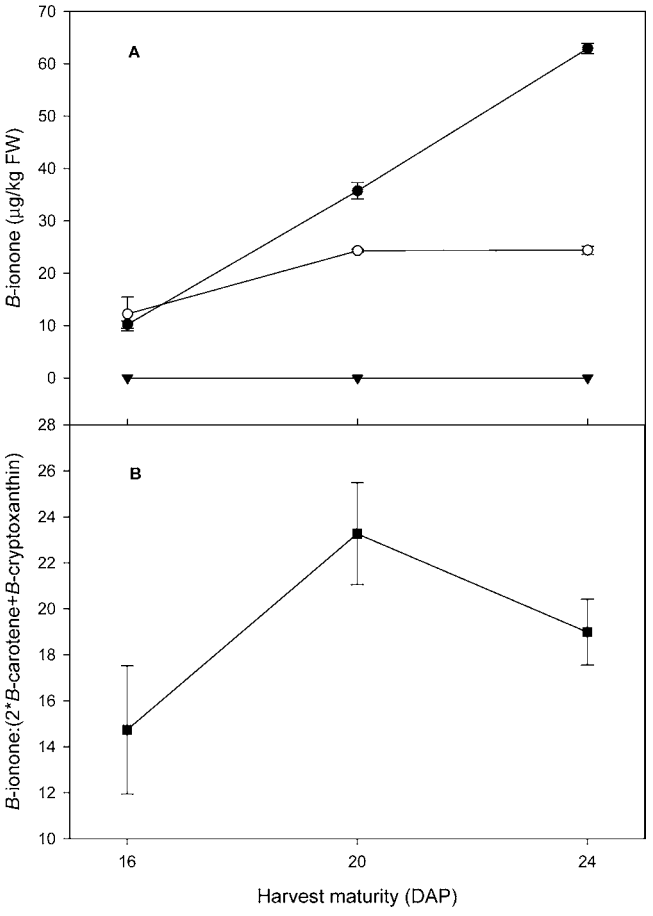

Figure 4. (A) Accumulation of *β*-ionone in 'HZ'(●), 'Hybrix5' (○), and 'Everest' (▼) sweet corn (*Zea mays* var. *saccharata*) kernels with increasing harvest maturity (n = 3). (B) Ratio of *β*-ionone to its precursor carotenoids at different harvest maturity stages (n = 6; data for 'HZ' and 'Hybrix5' were pooled due to nil significant varietal difference at each harvest point).

The carotenoids that correlated well with *β*-ionone also correlated highly with each other. For example, *β*-carotene was highly correlated with the *β*-arm carotenoids, *β*-cryptoxanthin (R^2 = 0.95) and zeaxanthin (R^2 = 0.90), as well as the *α*-arm carotenoid, zeinoxanthin (R^2 = 0.90) (Table 3). However, as with *β*-ionone, *β*-carotene was poorly correlated with lutein (R^2 = 0.09) and antheraxanthin (R^2 = 0.13).

Table 3. Coefficient of Determination (R^2) of Linear Correlations between the Volatile, *β*-Ionone, and *β*-Arm and *α*-Arm Carotenoids and between *β*-Carotene (a Known *β*-Ionone Precursor) and Other Carotenoids Present in Sweet Corn (*Zea mays* var. *saccharata*)

Compound	β-arm carotenoids	α-arm carotenoids	R^2
β-ionone	β-carotene		0.94
	β-cryptoxanthin		0.95
	zeaxanthin		0.95
	antheraxanthin		0.10
		zeinoxanthin	0.84
		lutein	0.06
β-carotene	β-cryptoxanthin		0.95
	zeaxanthin		0.90
	antheraxanthin		0.13
		zeinoxanthin	0.90
		lutein	0.09

Change in *β*-Ionone Concentration Relative to Potential Precursor Carotenoids. The ratio of *β*-ionone produced relative to the concentration of its potential precursor carotenoids, *β*-carotene and *β*-cryptoxanthin, was observed to change with harvest maturity in the present trial (Figure 4B). Both varieties behaved similarly, with no significant difference ($P < 0.05$) detected between 'Hybrix5' and 'HZ' at each harvest maturity. The ratio of *β*-ionone to *β*-carotene (×2) + *β*-cryptoxanthin significantly increased from 16 to 20 DAP, and then declined slightly from 20 to 24 DAP (Figure 4B).

DISCUSSION

The current study indicated that altering the carotenoid profile of sweet corn, specifically the ratio of the *β*-arm to the *α*-arm of the carotenoid synthesis pathway, can significantly impact the production of carotenoid-derived volatile compounds. The underlying reason for increasing zeaxanthin concentration in sweet corn was to provide a more concentrated source of zeaxanthin in the diet, as a means of potentially ameliorating the progression of AMD. However, increasing this compound by increasing the ratio of *β*-arm carotenoids may potentially influence the synthesis of carotenoid-derived volatiles, some of which are strongly perceived at extremely low concentrations., and may therefore affect the flavor of the kernels, either favorably or otherwise. In the current study, *β*-ionone and a second compound with an ionone-like ring structure

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(potentially α-ionone) were the only carotenoid-derived volatiles detected by GC−MS. Although α-ionone, 6-methyl-α-ionone, and γ-ionone were potential candidates for the unknown second compound, the latter two compounds were considered unlikely, as both are relatively rare or formed via a different pathway.[18] Both β-ionone and α-ionone have been previously detected in corn tortillas,[4] but have not been reported in fresh or cooked corn.[19] The presence of β-ionone was confirmed in the present trial, but the presence of α-ionone remains to be confirmed. In the variety 'HZ', increasing the proportion of β-arm carotenoids was found to increase β-ionone concentration up to 2.5 times relative to the standard yellow variety 'H5', with the effect becoming more pronounced with increased harvest maturity (Figure 4A). Whether this increase impacts flavor significantly remains to be assessed, but is potentially possible, as β-ionone can be perceived by humans at extremely low concentrations.[4,16]

β-Ionone is thought to be a direct cleavage product from β-carotene and potentially the nonhydroxylated end of β-cryptoxanthin,[20,21] while α-ionone is a cleavage product of α-carotene and zeinoxanthin[22,23] (Figure 1). Other potential carotenoid cleavage volatiles such as 3-hydroxy-β-ionone, which has been shown in vitro to be a cleavage product of zeaxanthin in bacterial cultures,[24] were not detected, despite large concentrations of zeaxanthin being present in samples. Interestingly, α-carotene was detected in only trace amounts in 'Hybrix5' and 'HZ', while zeinoxanthin was detected in significant amounts. Li et al.[2] reported a similar observation recently with 'B73' field-corn.

Increased carotenoid concentration, particularly of the β-arm carotenoids, zeaxanthin, β-cryptoxanthin, and β-carotene, was significantly correlated with an increased production of β-ionone. As would be expected, the white sweet corn 'Everest', which had minimal carotenoids present, had no detectable β-ionone, owing to a lack of suitable precursors. β-Ionone can be directly formed following enzymatic cleavage of β-carotene and β-cryptoxanthin (nonhydroxylated end) by carotenoid cleavage dioxygenase enzymes (CCDs).[25] Although β-ionone is not reported to be formed directly from zeaxanthin, β-ionone was observed to be strongly correlated with zeaxanthin concentration (R^2 = 0.95). Zeaxanthin is not an obvious precursor of β-ionone, and would be theoretically cleaved to form 3-hydroxy-β-ionone, which was not detected in the present trial. Whether secondary conversion of 3-hydroxy-β-ionone to β-ionone is possible is not currently known, although secondary conversion of initial cleavage elements is not unusual in carotenoid-derived volatile formation, and accounts for the majority of carotenoid-derived volatiles found in plants.[26] However, considering that 3-hydroxy-β-ionone is also a potential cleavage product of lutein and antheraxanthin, and yet there was no correlation between these compounds and β-ionone, this would make conversion of 3-hydroxy-β-ionone to β-ionone appear unlikely. If zeaxanthin is subject to similar cleavage mechanisms (i.e., CCD1) as β-carotene, it is possible that a cleavage product such as 3-hydroxy-β-ionone may undergo rapid conversion to other compounds such as C13 cyclohexenone, a carotenoid-cleavage product that has been detected in maize roots.[27]

Although zeaxanthin, β-cryptoxanthin, and β-carotene concentrations were highly correlated with β-ionone production (and zeinoxanthin to a lesser degree), it was interesting that the carotenoids lutein and antheraxanthin were poorly correlated with β-ionone (Table 3). In in vitro systems, CCD1 will accept a wide range of C-40 carotenoid substrates,[28] cleaving at the 9−10 and 9′−10′ double bonds.[24] Among the carotenoids present, this would result in an initial cleavage product of β-ionone for only β-carotene, β-cryptoxanthin, and α-carotene (Figure 1). By contrast, lutein would be cleaved to form 3-hydroxy-β-ionone and 3-hydroxy-α-ionone, while antheraxanthin would form 3-hydroxy-β-ionone and 3-hydroxy-5,6-epoxy-β-ionone. As 3-hydroxy-β-ionone is a potential cleavage product of both lutein and antheraxanthin, but also of zeaxanthin (which was highly correlated to β-ionone), this would support the proposal that conversion of 3-hydroxy-β-ionone to β-ionone is unlikely in sweet corn, and that the strong correlation between zeaxanthin and β-ionone is simply because zeaxanthin is strongly correlated (R^2 = 0.90) with its precursor carotenoids, β-carotene and β-cryptoxanthin (Table 3).

Both cultivar and harvest maturity were observed to impact β-ionone production. Within the current trial, β-ionone concentration reached a maximum of 63 μg/kg FW in 'HZ' at the late harvest maturity (24 DAP), relative to 24 μg/kg FW in the standard yellow hybrid, 'Hybrix5'. Later harvest also corresponded to maximum concentrations of the potential β-ionone precursor carotenoids, β-carotene and β-cryptoxanthin, in both varieties, which is likely to explain the differences observed. Interestingly, the ratio of β-ionone to these carotenoids increased between 16 and 20 DAP, before declining again at 24 DAP. This may indicate that activity of the carotenoid-cleavage enzyme, CCD1, changes with harvest maturity, first increasing and then decreasing again as sweet corn kernels become increasingly mature.

Although β-ionone dissolved in water has been reported to be perceptible by humans at concentrations as low as 7 ng/L,[4,16] there was no obvious taste difference between 'Everest' (white sweet corn with no detectable β-ionone) and either uncooked 'Hybrix5' or 'HZ' sweet corn when tasted by the authors. It is consequently possible that any flavor differences brought about by an increased β-ionone concentration either are subtle or may be masked by other volatile compounds not derived from carotenoids, such as dimethyl sulfide or 2-acetyl-1-pyrroline, which have been identified as major contributors to sweet corn aroma.[4,19] Despite the potential advantages of a high-zeaxanthin sweet corn for sufferers of AMD, further study using a trained taste panel would be required to determine if any significant difference in flavor exists in either cooked or uncooked zeaxanthin-biofortified sweet corn, in order to provide evidence that an increase in carotenoid-derived volatiles would not make the product unpalatable.

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Notes

The authors declare no competing financial interest.

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