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Chemical components of kernel quality in *sh2* sweet corn genotypes (*Zea mays* L. *saccharata* Sturt.) as affected by harvest date

Kemijske komponente kvalitete zrna *sh2* genotipova kukuruza šećerca (*Zea mays* L. *saccharata* Sturt.) u ovisnosti o roku berbe

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ABSTRACT

Kernel water (KW) and total soluble sugars (TSS) are major components of sweet corn taste, while total carotenoids (TC), total phenols (TP) and antioxidant activity (AA) contribute to its health benefits. Main objective was to determine the effect of genotype, harvest date and year on chemical components of kernel quality in shrunken (*sh2*) sweet corn. Nine *sh2* genotypes were harvested at 3-day intervals from 20 to 32 days after pollination (DAP) over two years. Growing season significantly affected TC and TP only. Significant differences among genotypes were found for KW (75.3 - 77.4%), TTS (282.4 - 343.2 mg/g), TC (9.3 - 15.7 µg/g), TP (243.6 - 289.4 mg GEA/100 g) and AA (74.6 - 83.6 % inhibition). Effect of harvest date and genotype by harvest date interaction was significant for all traits. Compared to the 20 DAP, KW and TSS decreased, while TC, TP and AA tended to increase with later harvest dates. Based on KW, genotypes OS 5Esh and Overland extended harvest window up to 29 DAP. In contrast, optimum harvest window for genotype OS 244sh finished at 20 DAP. Extended harvest window for genotype Overland resulted in significant increase of TC (62.8%), TP (12.1%) and AA (12.4%).

Keywords: sweet corn, soluble sugars, carotenoids, phenols, antioxidant activity

SAŽETAK

Voda u zrnu (KW) i ukupni topivi šećeri (TSS) su glavne komponente okusa kukuruza šećerca, dok ukupni karotenoidi (TC), ukupni fenoli (TP) i antioksidacijska aktivnost (AA) doprinose njegovim zdravstvenim benefitima. Glavni cilj bio je utvrditi utjecaj genotipa, rokova berbe i godine na kemijske komponenete kvalitete zrna shrunken (*sh2*) kukuruza šećerca. Devet *sh2* genotipova brano je u trodnevnim intervalima od 20. do 32. dana nakon oplodnje (DAP) kroz dvije godine. Vegetacijska sezona značajno je utjecala samo na TC i TP. Utvrđene su značajne razlike između genotipova za KW (75.3 - 77.4%), TTS (282.4 - 343.2 mg/g), TC (9.3 - 15.7 µg/g), TP (243.6 - 289.4 mg GEA/100 g) i AA (74.6 - 83.6% inhibicije). Utjecaj roka berbe i interakcije genotipa s rokom berbe, bio je značajan za sva svojstva. U odnosu na 20. DAP, KW i TSS su se smanjili, dok su TC, TP i AA imali tendenciju porasta u kasnijim rokovima berbe. Na osnovu KW, genotipovi OS 5Esh i Overland su produžili rokove berbe do 29. DAP. Nasuprot tome, optimalni rok berbe za genotip OS 244sh završio je na 20. DAP. Produženi period berbe za genotip Overland rezultirao je značajnim povećanjem TC (62.8%), TP (12.1%) i AA (12.4%).

Ključne riječi: kukuruz šećerac, topivi šećeri, karotenoidi, fenoli, antioksidacijska aktivnost

INTRODUCTION

Sweet corn (*Zea mays* L. *saccharata* Sturt.) is a very important fresh market and processing vegetable throughout the world. Compared to the field corn, sweet corns are based on the presence of one or more recessive genes that increase sugar and decrease starch content in the endosperm. Various endosperm mutants are known that alter the carbohydrate content but according to Boyer and Shannon (1984) the most commercially used are sugary (*su1*), shrunken (*sh2*) and sugary enhancer (*se1*). Shrunken (*sh2*) sweet corn become the dominant type in all major US sweet corn production regions and many countries in Asia, Europe, South America and Africa (Letrat and Pulam, 2007). Genotypes homozygous for a *sh2* mutation ("supersweet") have approximately three times higher kernel total soluble sugars than a standard *su1* sweet corn (Churchill and Andrew, 1984). Kernel chemical composition changes rapidly during maturation, and therefore harvesting sweet corn at the proper stage of maturity is essential to insure a high eating quality. Kernel water content is the primary quality standard for processing and ranges from about 74% towards 78% for *sh2* sweet corn. Compared to the standard sweet corn, *sh2* genotypes are characterized by slow kernel water loss, and consequently, extended harvest period (Marshall and Tracy, 2003; Szymanek et al., 2015). Kernel sugars specify flavour, which is one of the primary component of fresh quality associated with consumer preference (Azanza et al., 1996; Hale et al., 2005). Endosperm soluble sugars reach maximum concentration around 18-20 days after pollination (DAP). Because of the low conversion of endosperm sugars to starch, *sh2* genotypes can retain high sugar content longer than all other sweet corn mutants (Marshall and Tracy, 2003). Although water and sugars contents are major components of kernel quality, according to processor's requirements and consumer's preferences, bioactive compounds and their health properties have recently become the major focus of studies in sweet corn (Zhang et al., 2017; Calvo-Brenes et al., 2019; Moongngarm et al., 2019; Yang et al., 2019). The health benefits of sweet corn are mainly attributed to the high contents of bioactive phytochemicals, especially carotenoids and phenols. Health benefiting properties of carotenoids and phenolic compounds are related to high anti-oxidant and radical scavenging activities and also to anti-inflammatory, anti- cancer and neuroprotective properties (Kähkönen et al., 1999; Liu, 2004).

Carotenoids act in mechanisms related to macular degeneration prevention as well as vitamin A deficiency (Kaur and Kapoor, 2001). Significantly higher carotenoids in *sh2* sweetcorn than in other sweet corn mutants were reported (Kurilich and Juvik, 1999; Ibrahim and Juvik, 2009). The total phenols and DPPH radical scavenging activities of supersweet corn were compared with other corn types and products and were found to be higher than that of dent corn flour, flint corn, baby corn and popcorn (Midoh et al., 2010; Das et al., 2014). Study on 27 vegetables commonly consumed in the United States showed that the total phenol content of sweet corn is equivalent to that of carrot, potato and white onion, but higher than celery, lettuce and cucumber (Song et al., 2010). Also, sweet corn was shown to be the third highest total phenol provider among the 20 daily consumed vegetables (after potatoes and tomatoes) estimated from daily consumption and total phenol content (Chun et al., 2005). DPPH radical scavenging capacity was found to be higher in fresh sweet corn kernels then in spinach and peas (Bajčan et al. 2013).

Previous studies in sweet corn showed that kernel chemical composition and antioxidant activity may be affected by environmental conditions (Wong et al., 1994, Mesarović et al., 2018). Furthermore, significant harvest date (Khampas et al., 2013; O'Hare et al., 2015; Song et al., 2016; Calvo-Brenes et al., 2019) and genotype (Azanza et al., 1996; Kurilich and Juvik, 1999; Ibrahim and Juvik, 2009; Zhang et al., 2017) effects on kernel chemical composition and antioxidant activity in sweet corn were detected. However, limited information is available about changes in the content of kernel antioxidant compounds during the extended harvest period of *sh2* sweet corn genotypes.

Main objective of this research was to determine the effect of genotype, extended harvest period and

environment on the chemical components of kernel quality in *sh2* sweet corn. From a practical perspective, such information could be applied in a selection of *sh2* sweet corn genotypes with good eating quality and higher amount of health promoting compounds.

MATERIALS AND METHODS

Field experiment

Field trial was conducted at the experimental field of Agricultural Institute Osijek, Croatia (45°33'N, 18°41'E) over two years (2013 and 2014). Soybean was a previous crop in a three-year crop rotation (wheat-soybean-maize). Recommended agronomic practices and operations including spraying of pre-emergence herbicide as well as inter-row cultivation were applied during growing season. Nine *sh2* sweet corn genotypes, four commercial (Superslatki, Overland, Obsession and OS 244sh) and five experimental hybrids (OS 1Esh, OS 2Esh, OS 3Esh, OS 4Esh, OS 5Esh) developed at Agricultural Institute Osijek (OS) were grown in four-row plots, which were 6-meter-long and contained 25 plants per row (65000 plants/ha). The experiment was conducted in two replications as a randomized complete block design. Sowing was on April 24th in 2013 and on April 16th in 2014. Plants in the two central rows were self-pollinated by hand to avoid xenia effect and to ensure uniform maturity and genetic purity. Depending on tested genotype, the pollination was from July 5 to July 11 in 2013 and from July 1 to July 4 in 2014. Five consecutive hand harvests were made at a 3-day interval from 20 to 32 days after pollination (DAP).

Five random self-pollinated ears in each replication were taken as a sample. All samples were harvested early in the morning and transported directly to the processing laboratory. Weather data during the field experimentation are given in Table 1.

Sample preparation

Samples from the field were placed in a refrigerator at 4 °C and within half an hour ears were husked, a portion of the tip and butt of each ear was discarded and kernels were manually cut from the cob. The 100 g of kernels were pre-frozen, then freeze- dried (ALPHA 1-2 LD, Christ, Germany) and stored at 20 °C. Lyophilized samples were used for bioactive components extraction and determination. Remainder of the fresh kernels was blended and used for sugar extraction and determination.

Kernel water content

The 5 g samples of homogenized fresh sweet corn kernels were dried in an oven at 105 °C to assure full removal of heat volatile substances until the weight loss between measurements was <0.05 g. The difference between the fresh and dry weights was used to calculate the percentage of water content of the kernel.

Total soluble sugars

High-performance liquid chromatography (HPLC) was used for separation, identification and quantification of sugars according to method of Colaric at al. (2006) with some modification. Extraction of sugars was performed on 2 g of fresh blended and homogenized sweet corn kernels with the 30 mL of ultrapure water at 60 °C for 15 minutes and thereafter transferred into 100 mL volumetric flask and filled to the mark after cooling. After filtration,

Table 1. Average monthly air temperatures and precipitation sums during the growing seasons of 2013 and 2014

	Year	April	May	June	July	August	Average
Temperature	2013	13.1	16.7	20.0	22.9	26.9	19.9
(°C)	2014	13.2	16.1	20.4	21.8	22.5	18.8
Precipitation	2013	44.7	118.9	63.2	36.5	0.0	263.3
(mm)	2014	81.3	159.4	91.0	66.4	12.4	410.5

extracts were passed through 0.45 µm syringe filter, just before analyses. Galactose was added as internal standard. Sucrose, glucose and fructose were analyzed by HPLC system series 200 equipped with degasser, isocratic pump, refractive index detector and TotalChrom Navigator software (Perkin-Elmer, Massachusetts, USA). The separation was performed on MetaCharb Ca Plus column (300×7.8), at 90 °C. Twenty µL aliquots were injected into the column and eluted isocratically with degassed water at flow rate of 0.5 mL/min. Standard solution was composed of sucrose, glucose, galactose and fructose at concentrations of 5, 10 and 15 mg/mL. Sugars from aqueous sample extract were identified by their retention time and quantified by peak area using galactose as internal standard. Total soluble sugars were represented as the sum of sucrose, glucose and fructose and were expressed as mg/g of dried weight (DW) of sweet corn kernel.

Total carotenoids

Total carotenoids were analyzed according to the method of Luterotti and Kljak (2010). All samples were analyzed in duplicate. Homogenized lyophilized sample (0.6 g) was hydrated with 2 mL of water for 1 hour. Acetone (4 mL) was added and mixture was ultrasonificated (Sonorex RK510 H, Bandelin, Germany) for 5 min.

Hexane (2 mL) was added and mixture was vortexed (Vibromix 204 EV, Tehtnica, Slovenia) for 30 sec. Hexane layer was collected after centrifugation (Universal 320 R, Hettich, Germany; 4000 rpm, 5 min). The procedure without sonification was repeated until hexane layer became colorless. Hexane supernatants were combined and total volume was filled up to 10 mL. A UV-Vis spectrum of hexane extracts was recorded in range 380-520 nm with pure hexane used as a probe (Specord 200, Analytic Jena, Germany). Absorbance in maximum was used and total carotenoid content was calculated as β-carotene equivalents (µg/g of sample on a dry weight basis) according to β-carotene calibration curve in range of 0.2-10 mg/L.

Total phenols

Extraction of phenolic constituents was performed on 1 g of homogenized lyophilized sample with 10 mL of acidified methanol (1% HCl) in an ultrasonic bath for 60 minutes. After the extraction, samples were centrifuged for 10 minutes at 4000 rpm. Supernatant was collected and used for total phenols and antioxidant activity determination. All samples were further analyzed in duplicate with two independent reaction mixtures per duplicate.

The amount of total phenols was determined using the Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965) with some modification. The 0.1 mL of sample was mixed with 0.9 mL of dH_2O and 5 mL of Folin-Ciocalteu reagent (1:10; v/v diluted with water) followed by adding 4 mL of sodium carbonate solution (7.5%; w/v diluted with water). Homogenized reaction mixture was left for 2 hours in dark at room temperature, after which absorbance reading at 765 nm were taken with a spectrophotometer (Specord 200, Analytic Jenna, Germany) and water as a blank. The content of total phenols was expressed as mg of gallic acid equivalents per 100 g of lyophilized sweet corn (mgGAE/100 g DW), based on a gallic acid calibration curve in range from 0.05-1 mg/mL.

Antioxidant activity

The free radical scavenging activity was determined in accordance with the method of Brand-Williams et al. (1995) using 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical. For the analysis, the same methanolic extract as for total phenols determination was used. The initial absorbance of the DPPH solution (0.8 mM) in methanol was measured at 517 nm and did not change throughout the period of assay (DPPH blank solution). An aliquot (0.2 mL) of each sample was added to 1.0 mL of methanolic DPPH solution and 2 mL of methanol. Discolorations were measured at 517 nm (Specord 200, Analytic Jenna, Germany) against methanol after incubation for 30 min at room temperature in the dark. DPPH-radical scavenging activity was expressed as % of neutralized free radicals (inhibition):

$$\% \text{ inhibition} = [(A_{DPPH} - A_{sample})/A_{DPPH}] \times 100,$$

where A_{DPPH} is the absorbance of the DPPH blank solution, and A_{sample} is absorbance value of the sample solution.

Statistics

All collected data were analyzed using the proc mixed procedure in SAS 9.4 (SAS Institute, 2013) to perform the repeated measures ANOVA. Genotype, year and harvest were considered fixed factors and replications a random factor. When significant F-tests ($P<0.05$) were observed, mean separation was obtained using a LSD test at the 0.05 probability level.

RESULTS AND DISCUSSION

Effect of growing season on kernel chemical components

The growing season of 2013 was warm and dry with an average of 12.6 heat units per day (data not shown), and 10.8 mm of rainfall from pollination to the first harvest day (20 DAP). The growing season of 2014 was cooler and wetter (Table 1) with 11.7 heat units per day and 50.2 mm of rainfall for the same growing period. Year significantly affected total carotenoids and total phenols, but had no effect on water content, total sugar content and DPPH radical scavenging activity (Table 2). Despite variations in growing conditions over two years, kernel moisture content was only slightly lower in the drier and hotter growing season of 2013 compared to normal growing season of 2014 (Table 3). Similarly to kernel water content, total soluble sugars showed small variations over two years. However, it is well known that environmental conditions may affect kernel quality in sweet corn. For example, warmer years were associated with higher sucrose level in a research by Michaels and Andrew (1986). The results in the present study indicated that basic parameters of eating quality (kernel water and total soluble sugars content) were relatively stable over contrasting growing conditions.

Total carotenoids were highly affected by growing conditions and averaged 15.7 µg/g DW in 2013 and 19.7 µg/g DW in 2014. In general, the accumulation of carotenoids may be favoured by warm and dry conditions. However, Ortiz-Covarrubias et al. (2019) reported the decrease in the zeaxanthin (5.6%) and β-carotene (16.5%) under drought stress in field corn. Nemeskéri et al. (2019) obtained significantly higher carotenoids content in dry growing conditions. Total phenols had absolutely small, but statistically higher values in the growing season of 2014 (276 mg GEA/100g DW) when compared to the

Table 2. Mean squares from the ANOVA for the chemical components of kernel quality in *sh2* sweet corn genotypes

Source of variability	n-1	Kernel water	Total soluble sugars	Total carotenoids	Total phenols	DPPH
Year (Y)	1	14.09	72.17	714.06*	31721.7*	275.91
Error a	2	1.46	15.63	14.11	616.4	26.17
Genotype (G)	8	11.67**	61.83**	188.47**	4945.2**	20.98*
Y x G	8	1.09	9.11	13.39**	1309.4**	17.25
Error b	16	1.00	5.40	1.80	329.7	7.97
Harvest (DAP)	4	184.05**	814.44**	448.36**	11436.8**	112.51**
Y x DAP	4	0.64	3.62	3.99	1361.5**	10.8
Error c	8	0.49	3.82	2.56	110.6	4.51
DAP x G	32	3.34**	8.45**	8.14**	896.4**	12.06**
Y x DAP x G	32	1.34	5.42	5.34**	773.1**	7.71**
Error d	64	1.08	3.68	1.74	275	3.82

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

growing conditions of 2013 (262 mg GEA/100 g DW). Similarly to the results in the present study, Mesarović et al. (2018) reported a significant effect of growing season on total phenols in the grain of three sweet corn hybrids. Total phenol content was lower in drier growing season for all hybrids.

However, they also obtained significant year effect on DPPH radical scavenging activity that is not corroborating in the present research.

Effect of harvest date on kernel chemical components

As expected, harvest date significantly affected all measured traits (Table 2). Kernel water content averaged 76.2% at first harvest date (20 DAP), and then consistently declined at later harvesting dates (Table 3), averaging 70.3% at the last harvest date (32 DAP). Marshal and Tracy (2003) reported that at 76% moisture, the supersweet corn loses about 0.25% moisture per 24-hour period. In the present experiment, kernel water content loss was higher and averaged 0.5% per day for the full harvest period (20-32 DAP). Total soluble sugars showed a similar pattern of response as kernel water content. At the first

Table 3. Chemical components of kernel quality in *sh2* sweet corn averaged over genotypes and harvest dates in two growing seasons (2013 and 2014)

	Kernel water (%)		Total sugars (mg/g DW)		Total carotenoids (µg/g DW)		Total phenols (mg GAE/100g DW)		DPPH (% inhibition)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Genotype										
OS 1Esh	72.4	73.4	218.3	231.4	18.6 ^{d}	23.7 ^{a1}	280.1 ^{bc}	309.4 ^{a1}	81.6	79.8
OS 2Esh	72.5	72.4	240.8	231.7	17.4 ^{de}	24.5 ^{a}	237.4 ^{de}	277.6 ^{bc}	80.6	79.5
OS 3Esh	71.8	72.6	241.1	243.3	15.9 ^{e}	20.4 ^{cd}	225.8 ^{e}	283.0 ^{bc}	84.7	80.3
OS 4Esh	71.7	72,6	229.2	255.2	17.6 ^{de}	20.7 ^{bc}	283.1 ^{bc}	294.7 ^{ab}	80.3	78.9
OS 5Esh	73.7	73.9	243.8	252.6	12.8 ^{g}	15.5 ^{ef}	285.9 ^{bc}	291.6 ^{ab}	81.4	80.5
Overland	74.0	74.0	247.1	247.7	14.1 ^{fg}	19.6 ^{cd}	249.8 ^{d}	283.8 ^{bc}	81.4	80.6
Superslatki	72.8	74.0	211.7	241.5	16.3 ^{e}	18.9 ^{cd}	254.9 ^{d}	265.8 ^{cd}	85.9	79.6
Obsession	73.5	74.0	278.1	292.3	10.7 ^{h}	13.1 ^{g}	274.2 ^{bc}	300.5 ^{ab}	82.5	80.1
OS 244sh	72.0	72.5	226.2	254.4	18.1 ^{d}	21.1 ^{bc}	269.8 ^{cd}	293.7 ^{ab}	82.2	79.3
Harvest date										
20 DAP	75.8	76.6	300.6	304.8	11.4	14.7	252.5 ^{eg}	281.6 ^{c2}	78.5	77.8
23 DAP	73.8	74.5	267.2	277.0	13.9	17.6	246.4 ^{eg}	280.4 ^{c}	81.8	79.3
26 DAP	72.4	72.9	240.3	252.0	15.4	20.4	245.4 ^{g}	280.6 ^{c}	83.9	80.3
29 DAP	71.6	71.7	209.1	226.3	17.6	21.9	264.9 ^{d}	294.2 ^{b}	83.8	80.7
32 DAP	70.0	70.6	169.6	190.0	20.3	24.0	302.6 ^{ab}	307.7 ^{a}	83.5	80.9
Average	72.7	73.3	237.4	250.0	15.7	19.7*	262.4	288.9*	82.3	79.8

* Significant differences between years at P<0.05

[1] Significant genotype x year interaction, different lowercase letters indicate significant difference among genotypes across growing seasons at P<0.05

[2] Significant harvest date x year interaction, different lowercase letters indicate significant difference among harvest dates across growing seasons at P<0.05

harvest date, total soluble sugars averaged 302.7 mg/g DW and then decreased to 179.8 mg/g DW at 32 DAP, a total loss of 41% over a 12-day period, or 3.1% per day. The absence of year × harvest date interaction for kernel moisture content and total soluble sugars indicated that harvest date effect was relatively consistent in both years.

Contrary to responses for kernel water and total soluble sugars, total carotenoids consistently increased at later harvesting dates (Table 3). Averaged over harvest date, the DPPH also tended to increase in early harvest dates and then levelled off (Table 3), and this response was consistent in both growing seasons. However, total phenols had various responses to harvest date over two growing seasons, as indicated by a significant year x harvest date interaction (Table 2).

Effect of genotype on kernel chemical components

Genotype and genotype by harvest date interaction significantly affected all evaluated chemical components of kernel quality (Table 2). Kernel water content at 20 DAP ranged among *sh2* genotypes from 75.3% to 77.4% (Table 4). Marshal and Tracy (2003) stated that for freezing and canning of *sh2* genotypes, the optimum water content at harvest is between 74% and 78%.The results in the present study suggest that all genotypes could be harvested before 20 DAP which is in accordance with previous findings that *sh2* sweet corn reaches optimum kernel water content for harvesting at about 18-20 days after pollination (Wong et al., 1994). Kernel water content declined with each consecutive harvest date in all genotypes. According to Marshal and Tracy (2003), harvest period for supersweet corn is approximately four times longer than for standard sweet corn (*su1*). This increased length of the harvest period offers flexibility to a processing line. In the present research, based on the above given optimum kernel water range, genotype OS 244sh which had the smallest kernel moisture content at 20 DAP didn't extend harvest window beyond this harvest. Most tested genotypes (OS 1Esh, OS 2Esh, OS 3Esh, OS 4Esh and Superslatki) extended harvest window up to second harvest date (23 DAP). Genotype Obsession had satisfactory kernel water content also at third harvest date (26 DAP). Only genotypes OS 5Esh and Overland were suitable for harvesting at 29 DAP. None of the genotypes was suitable for harvest at 32 DAP, based on their kernel water content.

Table 4. Average kernel water content (%) in nine *sh2* sweet corn genotypes at five harvest dates (20-32 DAP)

	Kernel water (%)				
Genotype	20 DAP	23 DAP	26 DAP	29 DAP	32 DAP
OS 1Esh	77.4	74.7	72.3	70.6	69.4
OS 2Esh	76.3	73.4	71.1	70.9	70.4
OS 3Esh	75.8	73.9	72.1	71.0	68.3
OS 4Esh	76.5	74.4	72.7	69.8	67.4
OS 5Esh	76.2	73.9	73.8	73.5	71.6
Overland	75.8	74.8	73.8	73.5	72.2
Superslatki	75.8	74.5	72.8	72.4	71.5
Obsession	76.4	74.5	73.6	72.9	71.6
OS 244sh	75.3	72.9	72.1	70.7	70.3

LSD (0.05) = 1.42% for comparing means within the same genotype
LSD (0.05) = 1.45% for comparing means within the same harvest date
LSD (0.05) = 1.41% for comparing means across genotypes and harvest dates

Interestingly, non-significant year x harvest date x genotype interaction for kernel water content indicated that genotype-specific responses were consistent in both growing seasons (Table 2). However, it is well-known that at late harvest date other kernel quality traits such as kernel tenderness might be a limiting factor for sweet corn quality, as found by Szymanek (2009).

Similarly to kernel moisture content responses, genotype differences in total soluble sugars were consistent in both years, but depended upon harvest date (Table 2). At 20 DAP, the highest total soluble sugars were detected for genotype Obsession, and the lowest for genotype OS 2Esh (Table 5). Study by Wong et al. (1994) showed much greater variability in the kernel sugar content (133 – 455 mg/g DW) among 24 *sh2* sweet corn hybrids harvested at 20 DAP. All genotypes decreased total soluble sugars at later harvesting dates but the rate of change differed among genotypes.

From 20 DAP to 32 DAP, the largest reduction in total soluble sugars had genotype Superslatki, and the smallest genotype Obsession. Within the optimum harvest window based on water content (74-78%), the largest loss in total soluble sugars was detected for the two genotypes with the longest harvest window (OS 5Esh and Overland). This decrease in total soluble sugars averaged 23.4% (67 mg/g DW) for genotype OS 5Esh and 31.4% (88 mg/g DW) for genotype Overland. The smallest decline in total soluble sugars had genotype with a short harvest window OS 4Esh, which averaged 5.3% (16 mg/g DW). The absence of year x harvest day x genotype interaction indicated that these genotype specific responses for total soluble sugars were consistent in both growing seasons (Table 2). Regardless of the sugar loss at later harvesting dates, all examined genotypes have maintained a satisfying kernel sugar content that was at least two times higher than in standard *su1* sweet corn (Wong et al., 1994; Hale et al., 2005).

Genotype performance for kernel total carotenoid content was significantly affected by growing year (Table 2). Although all tested genotypes significantly increased total carotenoids in 2014 compared to 2013, they differed among themselves in the rate of change (Table 3). The largest difference in carotenoids content between two years had genotype OS 2Esh (17.4 and 24.5 µg/g DW in 2013 and 2014, respectively) while the smallest had genotype Superslatki (16.3 and 18.9 µg/g

Table 5. Average kernel total soluble sugars content (mg/g DW) in nine *sh2* sweet corn genotypes at five harvest dates (20-32 DAP)

	Total soluble sugars (mg/g DW)				
Genotype	20 DAP	23 DAP	26 DAP	29 DAP	32 DAP
OS 1Esh	289.2	258.7	198.7	201.9	175.7
OS 2Esh	282.4	265.2	243.6	216.2	173.8
OS 3Esh	287.1	262.8	242.7	229.4	188.8
OS 4Esh	304.2	287.9	262.0	195.1	161.9
OS 5Esh	309.2	275.4	254.9	242.4	159.1
Overland	308.9	272.7	260.2	220.6	174.6
Superslatki	305.6	265.2	219.4	188.4	154.4
Obsession	343.2	292.6	280.5	262.5	247.2
OS 244sh	294.3	268.5	253.2	203.2	182.6

LSD (0.05) = 27.1 mg/g DW for comparing means within the same genotype
LSD (0.05) = 28.3 mg/g DW for comparing means within the same harvest date
LSD (0.05) = 28.3 mg/g DW for comparing means across genotypes and harvest dates

DW in 2013 and 2014, respectively). In average, total kernel carotenoid content was the lowest at 20 DAP and ranged among genotypes from 9.3 -15.6 µg/g DW (Table 6). Khampas et al. (2013) reported the total carotenoid content of 9.6 and 20.2 µg/g DW in two *sh2* sweet corn hybrids. Although all genotypes tended to increase total carotenoids with later harvests, a significant genotype x harvest date interaction indicated various rates of change. Within the optimum harvest window based on kernel moisture content, the highest increase in total carotenoids (62.8%) was found in genotype Overland while there was no change in genotype OS 4Esh. The results indicated genotype specific responses for total carotenoid content as well as for their rate of increase at later harvest dates. Therefore, both variables are important in breeding programs for high amount of carotenoids in *sh2* sweet corn. Potential conflict between maximizing carotenoids concentration and keeping kernel eating quality can be avoided by well- defined genotype-specific harvest window.

Genotype differences in kernel total phenols content were significantly affected by growing season and harvest date (Table 2). All tested genotypes had higher total phenols in 2014 compared to the previous growing season (Table 3). Similarly, increased phenols content in colder and wetter growing season compared to the dry one was also reported by Mesarović et al. (2018). However, genotypes OS 4Esh, OS 5Esh and Superslatki showed only slight increase in total phenols while all other genotypes showed significant improvements in the second year of the experimentation. In average, kernel total phenols at first harvest date (20 DAP) ranged from 243.6 mg GEA/100 g DW for genotype Superslatki to 289.9 mg GEA/100 g DW for genotype Obsession (Table 7). Based on a fresh weight, total phenol content ranged from 59.6 to 70.1 mg GEA/100 g FW among tested genotypes. Zhang et al. (2017) reported the total phenol content from 38 to 57 mg GAE/100g FW in eight *sh2* sweet corn hybrids harvested between 18 to 20 DAP. Most evaluated genotypes increased total phenol content at the last harvest date (32 DAP). However, within the optimum harvest window, only genotype Overland significantly increased total phenols by an average of 12% (31.2 mg GEA/100 g DW). For all other genotypes there was no significant change in total phenols within the optimum harvest window. The results of the present research indicated relatively small potential for increase kernel total phenol with later harvest. However, a much better estimate could be made by studying a larger number of genotypes.

Table 6. Average kernel total carotenoid content (µg/g DW) in nine *sh2* sweet corn genotypes at five harvest dates (20-32 DAP)

	Total carotenoids (µg/g DW)				
Genotype	20 DAP	23 DAP	26 DAP	29 DAP	32 DAP
OS 1Esh	13.6	18.4	22.8	24.2	26.7
OS 2Esh	14.9	19.6	22.2	23.1	25.0
OS 3Esh	13.5	15.4	17.6	21.0	23.3
OS 4Esh	15.7	15.4	19.2	21.6	23.7
OS 5Esh	11.2	12.4	14.1	14.9	18.1
Overland	12.1	15.0	14.6	19.7	22.8
Superslatki	11.3	15.9	17.5	19.5	23.9
Obsession	9.3	10.7	12.9	13.2	13.3
OS 244sh	15.6	18.9	20.4	20.6	22.5

LSD (0.05) = 1.91 µg/g DW for comparing means within the same genotype
LSD (0.05) = 1.86 µg/g DW for comparing means within the same harvest date
LSD (0.05) = 1.91 µg/g DW for comparing means across genotypes and harvest dates

Table 7. Average kernel total phenol content (mg GEA/100 g DW) in nine *sh2* sweet corn genotypes at five harvest dates (20-32 DAP)

	Total phenols (mg GEA/100 g DW)				
Genotype	20 DAP	23 DAP	26 DAP	29 DAP	32 DAP
OS 1Esh	263.9	263.1	285.8	329.4	331.4
OS 2Esh	252.4	251.1	233.5	266.0	284.6
OS 3Esh	257.6	245.5	240.4	243.0	275.6
OS 4Esh	276.1	271.8	278.0	286.1	332.6
OS 5Esh	275.2	276.6	274.8	269.2	348.0
Overland	251.4	249.2	248.6	282.6	302.2
Superslatki	243.6	247.0	244.6	281.2	285.5
Obsession	289.4	283.1	278.7	280.5	305.2
OS 244sh	283.9	283.0	282.4	278.2	281.3

LSD (0.05) = 22.6 mg GEA/100 g DW for comparing means within the same genotype
LSD (0.05) = 23.8 mg GEA/100 g DW for comparing means within the same harvest date
LSD (0.05) = 23.0 mg GEA/100 g DW for comparing means across genotypes and harvest dates

The DDPH-radical scavenging activity differed significantly among genotypes with different harvest dates (Table 2). At the earliest harvest date (20 DAP), the DPPH ranged from 74.6% for genotype OS 1Esh to 83.6% for genotype Superslatki (Table 8).

Similarly, significant variation in DDPH activity among eight *sh2* sweet corn hybrids harvested at the same maturity stage was obtained by Zhang et al. (2017). However, reported values for DPPH in sweet corn vary considerably among different studies.

Table 8. Average kernel DPPH-radical scavenging activity (% inhibition) in nine *sh2* sweet corn genotypes at five harvest dates (20-32 DAP)

	DPPH (% inhibition)				
Genotype	20 DAP	23 DAP	26 DAP	29 DAP	32 DAP
OS 1Esh	74.6	81.3	84.5	82.7	80.4
OS 2Esh	74.8	78.5	81.2	82.5	83.5
OS 3Esh	80.4	80.5	84.5	84.6	82.7
OS 4Esh	77.8	81.1	80.1	79.9	79.8
OS 5Esh	77.5	80.8	79.3	83.6	83.5
Overland	79.5	79.8	80.4	82.6	82.5
Superslatki	83.6	82.0	84.0	82.6	81.6
Obsession	77.5	81.1	82.5	80.7	84.6
OS 244sh	78.7	79.9	82.7	81.0	81.4

LSD (0.05) = 2.78% for comparing means within the same genotype
LSD (0.05) = 3.04% for comparing means within the same harvest date
LSD (0.05) = 3.06% for comparing means across genotypes and harvest dates

Khampas et al. (2013) reported 29.1 and 34.5% inhibition in two supersweet corn hybrids. Those differences could be due to a genetic background, stage of maturity or wide range of environmental factors which affect antioxidant activity.

During maturation genotypes performed differently, showing no identifiable trend. There was either continuous increase, or increase followed by decrease or no change in DPPH scavenging activity. However, within the harvest window, most genotypes slightly increased DPPH at later harvest dates.

CONCLUSIONS

Tested *sh2* sweet corn genotypes significantly differed for all chemical components of kernel quality. Based on the kernel water content, harvest window may be extended up to 29 DAP for genotypes OS 5Esh and Overland. In contrast, optimum harvest window for genotype OS 244sh finished at 20 DAP.

Growing season significantly affected total carotenoids and total phenols, but had no effect on kernel water and total soluble sugars content as well as on DDPH-radical scavenging activity. Moreover, genotype-specific responses for major components of kernel eating quality were consistent over two years. Sweet corn *sh2* genotypes with extended harvest period significantly increased total carotenoids at later harvest dates, while no consistent pattern of response was found for total phenols and DPPH-radical scavenging activity. Considering significant variation among genotypes, both the absolute amounts of kernel chemicals and their rates of change during maturation should be evaluated in breeding programs designed to improve sweet corn eating quality and health benefits. Genotype-specific harvest window plays a key role for obtaining high kernel quality in *sh2* sweet corn production.

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