Influence of Cooking Conditions on Carotenoid Content and Stability in Porridges Prepared from High-Carotenoid Maize

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Abstract

Maize is a staple food crop in many developing countries, hence becoming an attractive target for biofortification programs toward populations at risk of micronutrient defficiencies. A South African white endosperm maize inbred line was engineered with a carotenogenic mini-pathway to generate high-carotenoid maize, which accumulates β-carotene, lutein and zeaxanthin. As maize porridge is a traditional meal for poor populations in sub-Saharan African countries, high-carotenoid maize was used as raw material to prepare different maize meals. The objective of this work was to assess the impact of popular home-cooking techniques and different cooking parameters (temperature, time and pH) on the final carotenoid content in the cooked product, using a spectrophotometric technique based on the mean absorption of carotenoids at 450 nm.

Carotenoid levels were not only preserved, but also enhanced in high-carotenoid maize porridges. The carotenoid content was increased when temperatures ≤ 95 °C were combined with short cooking times (10-60 min). The most optimum thermal treatment was 75 °C/10 min. When treated under those conditions at pH 5, high-carotenoid maize porridges doubled the initial carotenoid content up to 88 µg/g dry weight. Regarding to cooking techniques, the highest carotenoid content was found when unfermented thin porridges were prepared (51 µg/g dry weight of high-carotenoid maize porridge). We conclude that high-carotenoid maize may

contribute to enhance the dietary status of rural populations who depend on maize as a staple food.

Keywords: Carotenoids, Processing, Fermentation, Biofortification, Metabolic engineering

Abbreviations: HC, high-carotenoid; TCC, total carotenoid content; DW, dry weight basis

Introduction

Vitamin A deficiency (VAD) is a major public health problem worldwide, and particularly in African and South-East Asian countries. A poor diet can lead to chronic insufficient vitamin A intake, the main underlying cause of VAD, which may affect vision and cellular processes. Approximately, one-third of the world’s pre-school-age children and up to 15 % of pregnant women are estimated to be affected by VAD [1]. Vitamin A is an essential nutrient which is converted into retinal in the retina of the eye, and it is also necessary for embryonic development and the maintenance of epithelial and inmune cells [2]. Meat and dairy products are common dietary sources of vitamin A, whereas dietary carotenoids (provitamin A) are obtained from colored fruits and vegetables [2]. Maize is a staple food for more than a billion people worldwide, especially for populations in Central America and Southern Africa where this crop provides almost one-third of their caloric needs [3]. In sub-Sarahan African countries, white maize is mainly used to prepare maize meals, but it is devoid of carotenoids, which may contribute to the prevalence of VAD in these populations [4].

Carotenoids are isoprenoid compounds found in fruits and vegetables, either as hydrocarbon carotenes, or as oxygen-containing xanthophylls. Some carotenoids (such as β-carotene, α-carotene and β-cryptoxanthin) can contribute to human health because of their provitamin A activity, while others act as antioxidants [5]. The intracellular location of carotenoids plays an important role in their bioavailability. In photosynthetic plant tissues, e.g. dark-green leafy vegetables and carrots, they are bound to proteins in the chloroplasts, whilst in non-photosynthetic plant tissues, e.g. yellow and orange fruits, they are mainly found in chromoplasts, dissolved in oil droplets [6]. Maize carotenoids are also present in substantial levels in amyloplasts, plastids specialized for storage of starch granules [7].

Maize is usually consumed as porridge, cooked either with a thin or thick consistency by rural populations in sub-Saharan African countries. Some maize products are fermented to facilitate grain processing and to avoid bacterial contamination, but the use of this technique depends on the geographical localization [8]. The cooking technique and cooking conditions (time and temperature) may either increase or decrease the carotenoid concentration, e.g. stewing increases it while frying reduces it [9]. Thermal treatments inactivate oxidative enzymes and disrupt some cellular structures. Thus, higher carotenoid content in food products is usually reported under mild thermal conditions. The unsaturated structure of carotenoids makes them susceptible to degradation under high temperature, low pH, light and reactive oxygen species. Processing and storage may favor the exposure to oxygen and oxidative enzymes, thus causing structural modifications in carotenoids such as geometric isomerization and oxidation [10].

Biofortification of staple crops with organic nutrients is an alternative long-term strategy to improve nutritional health of populations at risk of micronutrient deficiencies. It can be performed at source (i.e. in the plant itself) through agronomic interventions, plant breeding or genetic engineering [11]. Several staple crops have been genetically engineered to enhance carotenoid levels and composition. Namely, high-carotenoid (HC) maize which was engineered to accumulate high levels of β-carotene, lutein and zeaxanthin [12]. The South African white maize inbred M37W, which lacks carotenoids in the endosperm due to the absence of the enzyme phytoene synthase 1 (necessary for the biosynthesis of carotenoids), was used as a basis to create the HC maize through the introduction of two genes, *Zmpsy1* (*Zea mays* phytoene synthase 1) and *PacrtI* (*Pantoea ananatis* phytoene desaturase), under the control of endosperm-specific promoters [12]. Recent studies have demonstrated a high carotenoid retention in biofortified crops after processing, either in transgenic biofortified crops such as sorghum and cassava, or in conventional biofortified crops such as maize, cassava and pumpkin [13].

Different processing methods lead to substantial changes in the nutritional composition of maize products [3]. Thus, it is important to assess the effect of processing on novel maize varieties. Our objectives were: a) to investigate the effect of temperature, cooking time and pH on carotenoid content of HC maize; b) to evaluate the effect of different home-cooking techniques on the final carotenoid content in maize porridges obtained from HC maize and its near isogenic line.

Materials and Methods

Maize. The M37W white maize and its engineered HC maize derivative were sown in a location (Lleida, Catalonia) in the Northeast of Spain. The experimental field was approved by the Spanish Ministry of Economy and Competitiveness and the Catalan Government (B/ES/13/16 and B/ES/14/04). Maize cobs were harvested, dried at low temperature (35 ºC) for 24 hours in a drying chamber, threshed and then kernels were milled (Ras® Mill, Romer® Labs Inc., MO, USA). The resulting flour was frozen at -18 °C until cooking and analysis. The fermented flour was prepared before cooking by adding 200 mL of tap water to 600 g of dry maize flour. The wet flour was allowed to spontaneously ferment in a pot with a lid at 30 °C in a heater for 48 hours.

Maize Porridge. Two independent experiments were carried out in order to assess the stability of carotenoids in HC maize under cooking conditions. Experiment 1 was performed to evaluate the effect of different processing parameters (temperature, time and pH) on the total carotenoid content (TCC) of HC maize. Results from this experiment were used to select the best processing conditions to perform Experiment 2, whose aim was to evaluate the effect of home-cooking techniques on the TCC of HC maize and its near isogenic line.

Experiment 1: Evaluation of Temperature, Time and pH During Processing. Thermal treatments from 75 to 95 °C were applied to allow gelatinization of maize starch, as this has been reported to occur at 65-76 °C [14]. Temperatures below 75 °C were discarded as gelatinization of maize starch did not occur. Maize porridges were prepared with 12.5 g of maize flour and 37.5 mL of aquous solution at different pH (4, 5 and 6). The mixture of maize flour and tap water had a pH ~5. Buffers were added to achieve pH 4 and pH 6. Citric acid 0.1 M (SAFC, Sigma-Aldrich, Madrid, Spain) and di-sodium hydrogen phosphate 0.2 M (Scharlau, Scharlab, Barcelona, Spain) were used to prepare the buffer solutions following Sigma-Aldrich guidelines. Samples were packaged in polypropylene bags (Tecnopack®, ILPRA System, Barcelona, Spain) which were heat-sealed with no headspace just before the thermal treatments. Porridge samples were treated at different temperatures (75, 85 and 95 °C) using a water thermostatic bath (Microprocessor Control MPC, Huber, Offenburg, Germany). During thermal processing, samples were taken at 10, 20, 40, 60 and 120 min, and quickly cooled in an ice bath. Maize starch gelatinization took place under any tested combination of time and temperature. All the samples were processed in duplicate and frozen at -18 °C until carotenoid analysis.

Experiment 2: Evaluation of Home-Cooking Techniques. Unfermented and fermented flours of M37W and HC maize were used to prepare thin or thick maize porridges following a similar process to the cold-water procedure described by Li et al. [15]. Taking into account the initial moisture content of maize flours, a different volume of tap water was added to each porridge in order to produce equivalent final moisture content in the unfermented and fermented porridges after cooking. Therefore, four different types of porridge (unfermented thin porridge, fermented thin porridge, unfermented thick porridge and fermented thick porridge) were prepared in duplicate. The porridges compositions are summarized in Table 1.

Based on the preliminary results from experiment 1, a temperature of 95 °C combined with shorter cooking times was selected for this experiment. Porridges were cooked from 25 to 95 °C while stirring continously with a wood spoon. The temperature was monitored using an electronic thermometer (IKA®, Staufen, Germany). It took 9 and 12 min to cook the thick and thin porridges, respectively. After cooking, they were left to cool down at room temperature for 2-3 hours. Moisture content and pH were determined in maize flour and porridge samples. All samples were processed in duplicate and frozen at -18 °C until carotenoid analysis.

Moisture Measurement. The moisture content of the samples was determined by oven drying according to the Association of Official Analytical Chemists method [16].

pH Measurement. The pH determination was performed using a Crison pH-meter combined with an electrode 52/32 (Hach Lange Spain, Barcelona, Spain).

Carotenoids Analysis. Carotenoids extraction was carried out by mixing 0.5 g of samples with 15 mL of methanol:ethyl acetate (60:40, v/v) and heating in a double boiler at 40 °C for 40 min using a hot plate equipped with electronic contact thermometer (IKA®, Staufen, Germany). Lipophilic compounds were then partitioned into 15 mL hexane:diethyl ether (90:10, v/v) and the upper phase was collected [17]. Methanol, ethyl acetate and hexane were obtained from Scharlau Chemicals (Scharlab, Barcelona, Spain) and diethyl ether (99.5 % with BHT) was obtained from Acros Organics (Fisher Scientific, Madrid, Spain). Total carotenoids were quantified with a CECIL 2021 UV/VIS spectrophotometer (Cecil Instruments, Cambridge, UK) by measuring the absorbance at 450 nm. The TCC was calculated using the following equation [18]:

$$\text{TCC } (\mu\text{g carotenoids/g}) = \frac{\text{Abs x V x } 10^4}{A_{1\,cm}^{1\%} \text{ x W}}$$

where $A_{1\,cm}^{1\%}$= specific absorbance or extinction coefficient, defined as the theoretical absorbance of a 1 % solution (w/v) in a cuvette with a path length of 1 cm. An arbitrary $A_{1\,cm}^{1\%}$ of 2500 is used for the determination of total carotenoids. Abs= absorbance measured at 450 nm. V= total volume (mL). W= weight of the sample (g). 10^4= conversion factor to obtain the concentration in units µg/g.

Statistical analysis. ANOVA and Tukey HSD test for means comparison were used (JMP® Pro 12 SAS institute, 2015). Differences among means with $p \leq 0.05$ were accepted as representing statistically significant differences.

Results and Discussion

Influence of pH and Temperature-Time Processing Conditions on Carotenoid Content. The moisture content of HC maize porridges was 79.32 % and the pH did not change during thermal processing time. The TCC in HC untreated maize porridges was 39 ± 1.3 µg/g DW (dry weight basis). The carotenoid content was higher in HC maize porridges after thermal processing than in uncooked porridges, varying according to the conditions used, although the increase was lower when long cooking times were applied (Fig. 1). There were statistically significant differences ($p < 0.05$) among treatments, as well as among temperature-time and temperature-pH interactions. The highest TCC was found when the lowest temperature was used (75 ºC), but there were no significant differences among samples treated at 85 and 95 °C. At pH 5, the carotenoid content of HC maize porridges heated at 75, 85 and 95 ºC for 10 min, was increased by 2.3-, 1.4- and 1.3-fold, respectively. At high temperatures (> 80 °C), most maize proteins are denaturated and unsolubilized [19]. Under those circumstances, carotenoids could not be easily released from the carotenoid-protein complexes, which may explain the differences found in carotenoid content between samples treated at 75 °C and 85-95 °C. With respect to time, the highest TCC was found in samples treated only for 10 min, with the prolongation of the treatment, the increase in TCC was consecutively lower. A slight decrease (0.5 µg/g DW) was only observed in HC porridge samples treated at 95 °C for 120 min. At pH 5, the carotenoid content of HC maize porridges heated at 75 ºC for 10, 20, 40, 60 and 120 min was increased by 2.25-, 1.93-, 1.77-, 1.32- and 1.34-fold, respectively. The best results were obtained in porridges treated at 75 °C during 10-40 min. When samples were treated for more than 40 min, no significant differences in carotenoid content were observed among treatments with respect to the temperature/time binomious. This may be explained by an increase in oxidation processes as longer cooking times increase oxidation due to excessive exposure to heat conditions [9]. Oxidation is the main factor accounting for carotenoid losses, and it is stimulated by light and heat, thus depending on available oxygen and the type of carotenoid [20]. Despite pro-vitamin A carotenoids were not individually analyzed, it has been previously reported that their retention decreases with long times and high temperatures regardless of the cooking technique used [21]. For instance, β-carotene was generally reduced in orange-fleshed sweet potato after thermal treatment (e.g. boiling, frying, roasting and steaming), although there were slight differences among cultivars [22]. As other biofortified crops, HC maize may prevent the loss of carotenoids during cooking more effectively than standard fortification, probably due to food matrix related effects [13]. Temperature and processing time seem to be more important factors than pH in relation to the TCC in HC maize porridges. HC maize porridges heated at 75 ºC for 10 min had 1.3–fold higher carotenoid levels at pH 5 than at pH 6 and pH 4. This is a relevant isssue as the highest contents were obtained when tap water was used instead of buffer solutions. The lower TCC in samples at pH 6 may be related to a higher lipoxygenase activity during the first minutes of heat treatment. It has been previously reported that this enzyme exhibits its highest activity at pH 6 and it has a relatively high thermal stability, as it is inactivated at 93°C/4-9 min depending on the maize cultivar [23, 24]. On the other hand, the lower TCC in samples at pH 4 could be explained by protein denaturation and/or production of ion-pairs, as carotenoids exposed to acids are protonated, undergoing cis–trans isomerization and additional degradation reactions [25].

Effect of Home-Cooking Techniques on Carotenoids Content. After performing the first experiment, a temperature of 95 °C and a cooking time below 20 minutes were selected for processing the maize under home conditions. The moisture content in thin porridges was significantly higher than in thick porridges ($p < 0.05$). A slightly higher moisture content was observed in fermented porridges regardless the amount of added water ($p < 0.05$). The differences found among porridges obtained from M37W and HC maize were not related to the type of maize (Table 2). The TCC was measured in the different porridges before and after cooking (Fig. 2). The TCC in the M37W and HC untreated maize porridges was 1.5 ± 0.5 and 39.5 ± 2.6 µg/g DW, respectively, in the unfermented porridges, and 2.7 ± 0.0 and 40 ± 0.6 µg/g DW, respectively, in the fermented porridges. The great difference in carotenoid content among

M37W and HC maize porridges still existed after processing ($p < 0.001$). Thus, statistical analyses were performed separately depending on the type of maize. When unfermented flour was used, thin and thick porridges elaborated with HC maize had 9.2- and 15.6-fold more carotenoids, respectively, than the same porridges based on M37W maize. This difference was higher when fermented flour was used. Thin and thick porridges elaborated with fermented flour from HC maize had 20.9- and 30-fold more carotenoids, respectively, than the same porridges based on M37W maize. The results obtained under laboratory conditions were confirmed, a significantly higher carotenoid content was found when HC maize flour (unfermented and fermented) was cooked into thin and thick porridges ($p < 0.05$). Our results are in accordance with those reported by Muzhingi et al. [26] who found an increase in carotenoid concentration when yellow maize was cooked into porridge (either with a thin or thick consistency) at 100 °C for 30 min. Thin and thick porridges showed a higher retention of provitamin A carotenoids (106 to 131 %) compared to *samp* porridge (53 to 98 %), which uses dehulled maize kernels [27]. Other authors found that *phutu* (a very thick porridge) and *samp* had a higher retention of provitamin A carotenoids (78 to 118 %) compared to thin porridge (63 to 78 %) [28]. In both studies, the carotenoid retention was high in most cases (>75 %) [27, 28]. In our experiment, the highest increase in the final carotenoid content was found in thin porridges elaborated with unfermented flour for both maize types ($p < 0.05$) (Fig. 2). Thus, this technique would be the most adequate to cook maize into porridge in order to preserve its initial carotenoid content. In contrast, yellow maize porridge retained only 52 % of total carotenoids [29] and β-carotene retention losses were found when a biofortified β-carotene maize was cooked into porridge (24.8 and 24.5 % of cumulative losses for unfermented and fermented porridges, respectively) [15]. Fermentation did not affect carotenoid content of HC maize porridges, whereas M37W maize porridges doubled the initial carotenoid content after fermentation, which could be attributed to the enzimatic degradation of subcellular compartments. However, it seems that carotenoids were more negatively affected by heating in fermented flours, as carotenoid content was not so much increased (HC porridge) or even reduced (M37W porridge) when fermented flour was used. The wet flour was allowed to spontaneously ferment, resulting in the expected reduction of pH in both thin and think porridges, with significant differences among porridges ($p < 0.05$). The pH reduction was less prominent in HC fermented porridges than in M37W fermented porridges (Table 3), which may explain why carotenoid levels were not decreased when HC fermented flour was used. Li et al. [15] showed that fermentation significantly affected the retention of β-carotene (10.2 % of losses), although they did not find differences between unfermented and fermented porridges after cooking. The pH of their fermented maize flour was 4, whereas the minimum pH found in our porridge samples was ~ 5.

Our results are consistent with previous studies suggesting that carotenoids are released from the food matrix when thermal treatments are applied. Thermal processing can produce structural changes in food matrices, softening the plant tissue and disrupting the carotenoid-protein complexes, which leads to increased carotenoid levels in foods [30]. In addition to thermal processing, food structure and dietary components (e.g. lipids) are other important factors to be considered in the carotenoid bioaccesibility [31]. On the other hand, processing may affect the stability of carotenoids as they are prone to isomerization and oxidation [21]. Isomerization is one of the major consequences of food thermal processing on carotenoids, consequently, carotenoids bioavailability and physiological activity are affected [30]. Therefore, it would be interesting to study the isomerization as well as the oxidation of HC maize after cooking in future studies. It must be kept in mind that carotenoid analysis has been performed using spectrophotometry instead of high-performance liquid chromatography (HPLC), which allows individual carotenoid detection and quantification. Spectrophotometry has been generally used to quantify total carotenoids in maize [32]. When different spectrophotometric methods were compared to HPLC, the method based on the mean absorption of carotenoids at 450 nm was highly correlated with HPLC [33]. Thus, it was selected as the most appropriate technique to screen, for the first time, the effect of cooking on HC maize. However, it has some limitations such as food with an unbalanced carotenoid profile, overestimation due to minor compounds and degradation products or underestimation due to colorless carotenoids [33]. Further studies using HPLC will be necessary to assess the effect on provitamin A carotenoids and other nutritionally important carotenoids, and consequently, their role in human health.

Furthermore, white maize is preferred by many African consumers, as yellow maize is commonly associated with food aid and animal feed [34]. Nevertheless, it has been demonstrated that existing preferences for white maize or negative connotations associated with yellow maize do not adversely affect the acceptance of orange maize (provitamin A-biofortified maize) [35]. Moreover, recent studies have shown that rural consumers are willing to pay for biofortified crops with visible traits (e.g. orange maize) as much as, if not more, than conventional varieties [34]. Therefore, education programs and information campaigns are necessary to promote the nutritional qualities of biofortified maize.

Conclusions

High-carotenoid maize can be a cost-effective and sustainable source of carotenoids, which may contribute to improve health status of populations who depend on maize as a staple crop. Our results show that final carotenoid content in maize meals varies depending on the processing conditions and cooking method applied, even it can be enhanced due to the increase in carotenoid extractability from the food matrix. The optimum thermal treatment for HC maize was 75 °C/10 min, followed by 75 °C/20 min and 75 °C/40 min. Higher temperatures are used when maize is cooked into porridge (95 °C), but they would also be acceptable, as carotenoid content was only decreased when cooking was prolonged during 2 hours. The carotenoid levels in maize meals may be affected by low pH values, thus, it would be useful to control pH levels in fermented products. Further studies are necessary to evaluate structural changes in carotenoids in HC maize after cooking, as well as, carotenoid bioavailability after consumption of meals based on HC maize.

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Table 1 Composition of porridges

Fermentation	Type of porridge	Maize flour (g)	Water (mL)
Unfermented	Thin	76.8	250
	Thick	125	150
Fermented	Thin	125 [*]	275
	Thick	125 [*]	80

[*] Fermented flour

Table 2 Moisture content (%) in cooked porridge samples

	M37W	HC
Thin, unfermented	74.66 ± 0.38 ^{b}	76.64 ± 0.36 ^{ab}
Thin, fermented	78.26 ± 0.32 ^{a}	77.52 ± 0.62 ^{ab}
Thick, unfermented	60.61 ± 0.33 ^{c}	59.96 ± 0.70 ^{c}
Thick, fermented	61.52 ± 1.25 ^{c}	62.41 ± 1.02 ^{c}

Values are expressed as mean ± standard error (n=4). Means with no letter in common are significantly different ($p < 0.05$)

Table 3 pH values in cooked porridge samples

	M37W	HC
Thin, unfermented	6.20 ± 0.01 ^{a}	6.16 ± 0.02 ^{a}
Thin, fermented	4.98 ± 0.12 ^{b}	5.84 ± 0.06 ^{a}
Thick, unfermented	5.97 ± 0.01 ^{a}	5.97 ± 0,01 ^{a}
Thick, fermented	5.23 ± 0.23 ^{b}	5.79 ± 0.08 ^{a}

Values are expressed as mean ± standard error (n=4). Means with no letter in common are significantly different ($p < 0.05$)

Fig. 1 Total carotenoid content (TCC, in µg/g DW –dry weight basis–) in HC maize porridge treated at different processing conditions. Values are expressed as mean ± standard error (n=2). a) pH 6; b) pH 5; c) pH 4

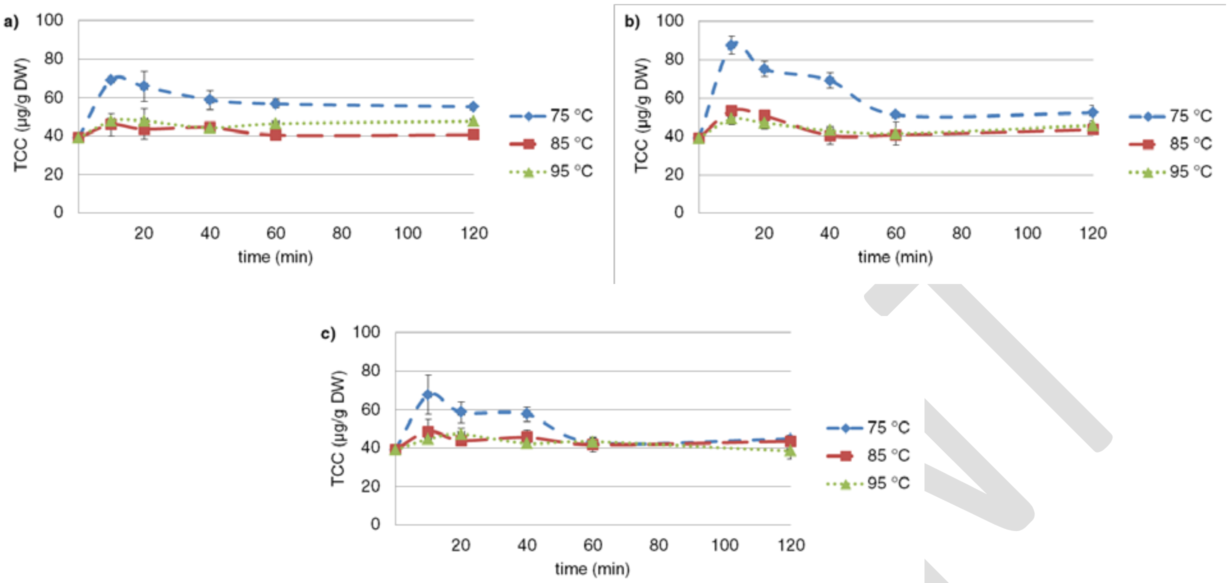

Fig. 2 Total carotenoid content (TCC, in µg/g DW –dry weight basis–) in M37W and HC maize porridges measured before cooking (n=2) and after cooking (n=4). Thick and thin porridges were cooked at 95 °C during 9 and 12 min, respectively. Values are expressed as mean ± standard error. Means with no letter in common (within each type of maize) are significantly different (*p* < 0.05)