

\*Proceedings\*

# Postharvest Storage of Reddish-Purple Sweetcorn: Changes in Anthocyanin, Starch and Sugar Content during Ambient and Refrigerated Storage <sup>†</sup>

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**Abstract:** Reddish-purple sweetcorn has recently been developed by our laboratory through the inclusion of an active anthocyanin biosynthesis pathway in white sweetcorn. Anthocyanin is not only responsible for the reddish-purple colour of the kernels, but has also been associated with a number of health benefits. As this product is novel, there is currently no information on changes in anthocyanin, starch or sugar concentration during postharvest storage. The current study reports the effect of a common domestic storage temperature on these key quality characteristics of reddish-purple sweetcorn over a two-week period. Storage of reddish-purple sweetcorn for 14 days at 23 °C significantly increased ( $p < 0.05$ ) kernel anthocyanin concentration in half of the cobs from 36.4 mg/100 g at day 0 to 62.8 mg/100 g FW, and concurrently decreased sugar from 116.6 mg/g to 27.0 mg/g FW. By contrast, at 4 °C, no significant change in anthocyanin was observed, and the rate of decline in sugar concentration was significantly reduced, declining 20% after 14 days. Although storage at ambient temperature appears to be able to induce a significant increase in anthocyanin concentration, this would have to be manipulated carefully so as not to cause a decline in sugars that would significantly affect sweetness, an important attribute of sweetcorn.

**Keywords:** storage; anthocyanin; sugars; starch; pigmented sweetcorn

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## 1. Introduction

Sweetcorn (*Zea mays var. rugosa*) is very closely related to starchy maize (*Zea mays var. indentata*), with the major genetic difference being a supersweet mutation, such as *brittle1* or *shrunk2*. [1] These mutations significantly reduce conversion of sugar to starch, both as kernels mature on the plant, and during postharvest storage. [2] In contrast to maize, sweetcorn is harvested when kernels are tender and physiologically immature. [3,4] Consequently, sweetcorn is actively respiring when it is harvested, with sugar being utilised as a substrate for metabolism. [5] Reducing the rate of respiration during postharvest storage consequently minimises sugar loss and maintains kernel sweetness. [6] Although most sweetcorn is yellow or white, we have recently developed reddish-purple sweetcorn by the inclusion of an active anthocyanin biosynthesis pathway. [4]

The effect of ambient and refrigerated postharvest storage on the two most important attributes of reddish-purple sweetcorn, anthocyanin content (responsible for the reddish-purple pigment) and sweetness (starch and sugar content) are currently unknown. Therefore, this study determined the effect of postharvest storage at common domestic refrigeration temperature (4 °C) and ambient temperature (23 °C) on these key factors affecting sweetcorn visual and organoleptic quality.

## 2. Materials and Method

### 2.1. Plant Materials

Reddish-purple sweetcorn cobs were harvested at 26 days after pollination (DAP) in autumn 2018 at the Gatton Research Facility, The University of Queensland, QLD, Australia. Cobs were immediately transported to the laboratory at the Health and Food Sciences Precinct, Coopers Plains, where they were randomly divided into two batches for storage at 4 °C and 23 °C, respectively. Two rows of kernels from cobs were removed at day 0, 1, 3, 7 and day 14 at 4 °C and 23 °C and immediately snap frozen by immersion into liquid nitrogen. Samples were subsequently stored at −35 °C prior to analysis. The remaining cobs were placed in loosely-sealed plastic bags at a relative humidity of 90% ± 3% and stored at 4 °C (batch-1) and 23 °C (batch-2). The frozen kernels were cryo-milled and frozen powdered samples were used to determine total anthocyanin, starch, individual sugars, moisture content.

### 2.2. Anthocyanin and Sugar Analysis

Anthocyanin extraction and determination was conducted following the method of Hong, Netzel [7]. Sugar analysis was performed following the method as reported previously by Rego, Jesus [8], with some modifications.

### 2.3. Starch and Moisture Analysis

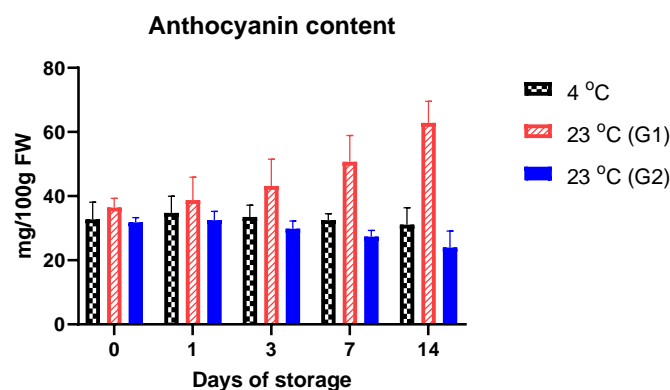
Starch content in reddish-purple sweetcorn kernels was assayed by enzymatic degradation of the starch to glucose with  $\alpha$ -amylase and amyloglucosidase, using the total starch assay procedure from Megazyme (Megazyme 2016) according to AOAC-Association of Analytical Communities (Official Method 996.11), using a starch assay kit (Megazyme International, Ireland). Moisture content of sweetcorn kernels was determined in triplicate, using AOAC method 934.01 (AOAC, 1990).

## 3. Results and Discussion

### 3.1. The Effect of Storage Time and Temperature on Anthocyanin Content of Reddish-Purple Sweetcorn

Storage temperature was observed to have a significant effect on total anthocyanin content in reddish-purple sweetcorn (Figure 1). While kernels of reddish-purple sweetcorn had no significant ( $p > 0.05$ ) change in total anthocyanin content at 4 °C, storage at 23 °C affected anthocyanin concentration of different cobs in a divergent manner. One half of the cobs were observed to increase significantly ( $p < 0.05$ ), while the other half had a significant ( $p < 0.05$ ) decrease of anthocyanin content during two-weeks storage at 23 °C (Figure 1). The lack of change at 4 °C for two weeks indicates that this common refrigeration temperature is sufficient to maintain anthocyanin concentration and appearance, which averaged approximately 32 mg/100g FW. By contrast, for the half of the cobs stored at 23 °C which increased in anthocyanin, the reddish-purple pigment gradually spread from the stigma end of the kernel towards the base of the kernel, eventually covering the entire kernel surface. Anthocyanin content in these cobs also increased significantly from 36.4 mg/100g FW to 62.8 mg/100g FW over the 14-day period. The concurrent increase in anthocyanin concentration with an expansion of the pigment cross the kernel surface, indicated that anthocyanins were continuing to be biosynthesized and accumulated during storage at 23 °C. The findings indicated that although reddish-purple sweetcorn was harvested at an immature stage, the accumulation of anthocyanin in its kernels behaved similarly to postharvest anthocyanin accumulation in strawberries [9] and blood orange [10], both of which have can continue to accumulate anthocyanin after harvest when held at non-refrigerated temperatures. Surprisingly, the pigmentation of the other half of the cobs stored changed from a reddish-purple to a brownish-purple, which accompanied the significant decline in anthocyanin content from 31.9 mg/100g FW to 23.9 mg/100g FW at day 14. Currently, it is uncertain why this decrease in concentration of anthocyanin occurred in some cobs stored at 23 °C, and not in others. However, a decrease in anthocyanin during ambient storage is not unknown, and is observed

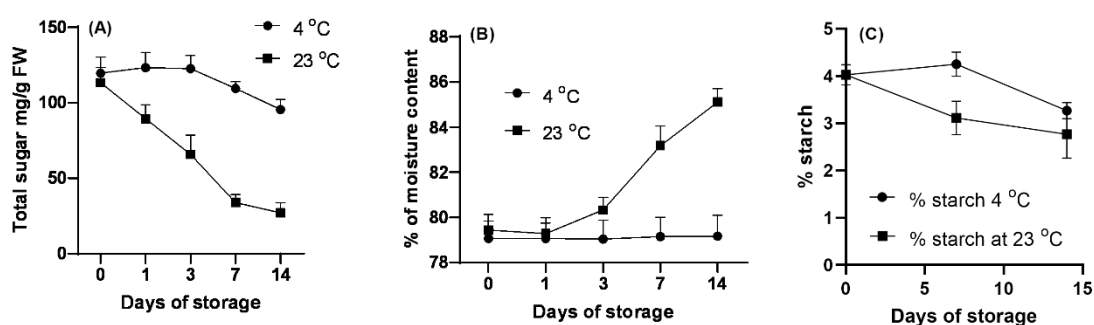
in red onions [11] and red lettuce, [12,13]. The fact that the current changes in anthocyanin were divergent could indicate genetic differences between the cobs, as this breeding line is not inbred.



**Figure 1.** Total anthocyanin concentrations of reddish-purple sweetcorn (26 DAP) during 14 days storage at 23 °C and 4 °C. Data were means of three technical replicates each from six replicate cobs ( $n = 6$ ) at 4 °C and 23 °C, (G1) the cob group continued accumulating anthocyanin and (G2) the cob group declining in anthocyanin concentration.

### 3.2. The Effect of Storage Time and Temperature on Sugar, Starch and Moisture Content

Total sugar concentration was significantly affected ( $p < 0.05$ ) at both storage temperatures, although the decline was more rapid at 23 °C (Figure 2A). At 23°C, total sugar concentration decreased significantly ( $p < 0.05$ ) rapidly from approximately 117 mg/g FW to 90 mg/g FW after 1-day storage and eventually to 27 mg/g FW at 14 days. By contrast, the total sugar concentration of cobs stored at 4 °C remained unchanged (120 mg/100 gFW) for at least 7 days, and only declined to approximately 96 mg/100 gFW after 14 days. The use of sugar as a respiratory substrate during storage is the main reason for significant decrease of total sugar content in sweetcorn kernels [14]. The current results are similar to previous findings of post-harvest yellow sweetcorn (*Zea mays*) cv [15].



**Figure 2.** Total sugar content (A), moisture content (B) and starch content (C) of reddish-purple sweetcorn (26 DAP) during 14-day storage at 23 °C and 4 °C ( $n = 3$ ).

In addition to change in anthocyanin and sugar concentration, ambient storage temperature was also observed to significantly affect the moisture content of reddish-purple sweetcorn kernels (Figure 1B). While storage at 4 °C resulted in no significant change in moisture content over a 14-day storage, storage at 23 °C resulted in a significant increase in moisture content, from 79.4% at day 0 to 85.1% at day 14. This increase of moisture content was concurrent with the observed decrease of total sugar concentration at 23 °C, and is the consequence of sweetcorn kernels using sugar as a respiratory substrate. Respiratory activity utilizing 1 unit of glucose produces 6 units of water) [16], which appears to account directly for the observed increase in kernel moisture content. Despite this increase in moisture content at 23 °C, total anthocyanin concentration still increased, indicating that synthesis

and accumulation were active at ambient temperature, at least in half of the replicate cobs of reddish-purple sweetcorn.

Storage temperature was also observed to significantly affect total starch content (Figure 2C), even though starch is typically low in sweetcorn. Reddish-purple sweetcorn cobs had no change in starch content at 4 °C, remaining at approximately 4%. By 14 days. However, this decline was faster at 23 °C, with starch content reducing from 4% to about 3% at day 7, and to below 2.8% at day 14. The above response is typical of what is observed with supersweet sweetcorn. The shrunken2 (*sh2*) mutation generally minimises starch synthesis during storage at both 4 °C and 23 °C [15], which is in contrast to standard sweetcorns based on the *sugary-1* mutation (*su-1*), where starch is observed to increase with duration of storage [14].

#### 4. Conclusions

The current study showed that the biosynthetic pathway of anthocyanin was not operative during post-harvest storage of reddish-purple sweetcorn cobs held at 4 °C. However, this biosynthetic pathway appeared to be operational in at least half of the cobs stored at 23 °C. The reason for this divergent effect of ambient temperature on anthocyanin accumulation is uncertain at this stage, although it is possible that genetic differences may exist within the current breeding line, which could affect this response. Storage at the common domestic refrigeration of 4 °C was effective at slowing sugar loss, and was similar to that observed in yellow supersweet sweetcorn. Although temperature was found to significantly affect both anthocyanin and sugar content, the divergent results of the current trial would indicate that these parameters are not directly linked.

**Author Contributions:** A short paragraph specifying their individual contributions must be provided. The following statements should be used “X.X. and Y.Y. conceived and designed the experiments; X.X. performed the experiments; X.X. and Y.Y. analyzed the data; W.W. contributed reagents/materials/analysis tools; Y.Y. wrote the paper.” Authorship must be limited to those who have contributed substantially to the work reported.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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