

Carotenoid Retention in Immature Corn Ear Grains Subjected to Different Thermal Treatments

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Abstract

Processed food products may undergo changes in chemical composition during processing, leading to potential losses in nutritional value. The objective of this study was to determine carotenoid retention in immature grains of normal corn (BRS1030) and corn biofortified (BRS4104) with vitamin A precursors subjected to different thermal treatments: cooking in a microwave, cooking in a pressure cooker, cooking in a pot with a lid and cooking in a pot without a lid. The experiment had a completely randomized design in a factorial scheme (cultivar and type of cooking). The carotenoids were extracted in a sequential organic solvent scheme and quantified by high-performance liquid chromatography (HPLC). The results were submitted to analysis of variance (ANOVA), and when significant, the means were compared using the least significant difference (LSD) test ($p = 0.05$). Despite cultivars, the concentrations of carotenoid vitamin A precursors and total carotenoids in the immature corn grains were reduced after cooking the ears in a microwave or in a pressure cooker. The best treatments for preserving carotenoids according to the conditions studied are cooking in a pot with a lid and in a pot without a lid.

Keywords: biofortification, cooked corn, corn cultivars, pro-vitamin A

1. Introduction

Carotenoids are pigments found in nature and are responsible for the yellow, orange and red colors of various foods (Meléndez-Martínez et al., 2004). Corn is a prominent food source of carotenoids and is one of the most cultivated cereals in Brazil. Zeaxanthin and lutein are the major carotenoids present in this cereal, the importance of which is based on the fact that they contain so-called macular pigments, which are involved in preventing macular degeneration. The β -carotene and β -cryptoxanthin vitamin A precursors are also present in corn, but in lower concentrations (Oliveira, 2006). Special attention has been given to these compounds, as they possess properties and functions that are important to various areas of human health.

The corn cultivar BRS4104, biofortified with carotenoid vitamin A precursors, was developed in Brazil through conventional breeding and was launched in 2013 as a result of the “Harvest Plus” and “Biofortification in Brazil - developing more nutritious agricultural products” projects. This corn cultivar is an alternative product with higher nutritional value and consequently with the potential to aid in combating micronutrient deficiencies, such as vitamin A deficiency (Embrapa, 2013).

It is common that many vegetables are cooked by a simple boiling process or microwave process before use. These cooking processes would certainly bring about a number of changes in physical characteristics and chemical composition of vegetables (Sukhwant et al., 1992). Corn immature kernels are normally cooked by a boiling or microwave process before being eaten. Khachik et al. (1992) reported that various cooking procedures affected the carotenoid content in green vegetables.

The carotenoids are unstable in food in the presence of high temperatures, light, heat, acid and oxygen and during food processing may undergo changes in chemical composition, causing nutritional losses. Therefore, the nutritional value of a food can be reduced during the various steps it undergoes between harvest and consumption, a consequence of the high oxidation capacity of these compounds and the large number of

unsaturated bonds in their structures that make them susceptible to degradation (Bianchi & Antunes, 1999; Eskin, 1990).

Although the biofortified BRS4104 corn cultivar is commercially available, the chemical and physical changes resulting from the processing of immature grains of these materials have still not been determined. The present study was conducted with the main objective of determine carotenoid retention in immature corn ear grains of the common and biofortified with vitamin A carotenoids precursors cultivars after being subjected to different thermal treatments.

2. Materials and Methods

2.1 Raw Material

The corn was cultivated under controlled fertilization, irrigation and pest and disease management conditions. It was harvested when grains were at the milky stage, known as the “immature corn point”. Two corn cultivars were used in the study, the common BRS1030 cultivar and BRS4104, a cultivar biofortified with vitamin A precursors.

Fresh harvested corn ears were washed with running water and then selected by eliminating those that were or attacked by insects. After the first wash, the ears still containing husks were sanitized with 2% sodium hypochlorite solution (10 mL in 50 L of water) for 15 minutes and then husked. At this stage, a new selection of ears was made, removing those poorly granulated or attacked by caterpillars that were not detected in the previous step. The selected ears were again sanitized with 2% sodium hypochlorite solution for 15 minutes.

2.2 Processing

To study the effects of thermal treatment on carotenoid retention in immature corn ear grains, four different treatments were evaluated: 1) cooking without water in a microwave, 2) cooking in water in a pressure cooker, 3) cooking in water in a pot with a lid, and 4) cooking in water in a pot without a lid. Three replicates of 3 ears each were prepared for each treatment.

2.3 Microwave Cooking without Adding Water

Cooking was performed in an 1100 W Sharp[®] microwave. The ears were individually wrapped three times with 18-mm-thick PVC film and placed on the microwave turntable glass plate. The ears were cooked for 7 minutes at full power and then cooled to room temperature (22 °C) over 30 minutes. During these time, the corn ears were kept wrapped in PVC film until the first 5 minutes after cooking.

2.4 Cooking in Water in a Pressure Cooker

Cooking was performed in a Marcolar[®] nonstick domestic pressure cooker with a 10L capacity. The ears were immersed in 5 L of drinking water in the pressure cooker and heated on an industrial stove for 15 minutes, counted from the onset of steam emission through the valve. The corn ears were then removed from the pot and cooled to room temperature (22 °C) for 30 minutes.

2.5 Cooking in Water in a Pot with a Lid

Cooking was performed by soaking the corn ears in boiling water in a stainless steel pot with lid, containing 5 L of boiling drinking water (98 °C) for 30 minutes, counted from the onset of boiling. After cooking, the corn ears were removed from the water and cooled to room temperature (22 °C) for 30 minutes prior to obtaining samples for analysis.

2.6 Cooking in Water in a Pot without a Lid

Cooking was performed by soaking the ears in boiling water in a stainless steel pot, without a lid, containing 5 L of boiling drinking water (98 °C) for 30 minutes, counted from the onset of boiling. After cooking, the ears were removed from the water and cooled to room temperature (22°C) for 30 minutes.

2.7 Preparation of Samples for Analysis

The grains were removed from the same ear before and after processing for carotenoid and moisture analysis. The ears were weighed before and after processing to ascertain the gain or loss in weight for the carotenoid retention evaluation. The analyses were performed for both raw and cooked grains.

2.8 Determination of Carotenoid Profile

Carotenoids were extracted from the samples in a sequential organic solvent scheme, according to the protocol described by Barbosa et al. (2015).

The carotenoids were quantified using high-performance liquid chromatography (HPLC) on an Alliance Waters

e2695 liquid chromatograph equipped with a YMC C 30 polymeric column (3 μm , 4.6 \times 250 mm, Waters, Milford, MA, USA) and coupled to a diode array detector (Waters Model 2998). Gradient elution was conducted at 0.8 mL min^{-1} with a gradient of 80:20 to 20:80 methanol:methyl *tert*-butyl ether for 16 minutes, followed by a constant of 80:20 for 4 minutes, finishing with six minutes of equilibrium. The oven temperature was 30 $^{\circ}\text{C}$, the wavelength was 450 nm and the injection volume was 40 μL . The laboratory temperature was maintained at 20 $^{\circ}\text{C}$ throughout the process. To identify compounds, purified standards from carrot (α -carotene, 94.64% purity) and papaya (β -cryptoxanthin, 92.72% purity) were used, following the protocol described by Rodriguez-Amaya and Kimura (23). The standards for the carotenoids lutein (40 mg Lutein, Vision Health) (98.68% purity), zeaxanthin (Swanson, ZeaGold zeaxanthin 4 mg from paprika [97.99% purity]) and β -carotene (beta carotene [vitamin A] 25,000 IU Supplement, Swanson SW007 [94.57% purity]) were obtained from Swanson brand capsules, with each carotene from its respective capsule. Carotenoid analysis results are expressed on a fresh basis.

The total carotenoid concentration (TC) was obtained by adding the total values of all fractions being quantified: total lutein, total zeaxanthin, total β -cryptoxanthin, total α -carotene and total β -carotene, as described by Murphy et al. (1975).

The concentration of carotenoids with pro-vitamin A activity (proVA) was obtained using the following formula: total β -carotene + $\frac{1}{2}$ total α -carotene + $\frac{1}{2}$ total β -cryptoxanthin ($\mu\text{g g}^{-1}$), as described by Murphy et al. (1975). The percent true retention was calculated according to the formula proposed by Murphy et al. (1975) for each variable. This calculation is based on the chemical characteristics of each of these molecules. Chemical structures having at least one β -ionone ring and one carbon-11 chain have pro-vitamin A activity. The molecular structure of β -carotene is formed by two β -ionone rings that give rise to two retinol molecules, which therefore assigns 100% pro-vitamin activity to this carotenoid. To a lesser extent, β -cryptoxanthin and α -carotene have approximately 50% activity, as there is only one β -ionone ring in each of their chemical structures. The *cis* isomers were not quantified, as they presented no significant biological activity. The percent true retention was calculated according to the formula proposed by Murphy et al. (1975).

$$\% \text{ true retention} = \frac{(\text{Carotenoid concentration / g of processed food}) \times \text{g of food after processing}}{(\text{Carotenoid concentration / g of raw food}) \times \text{g food before processing}} \times 100 \quad (1)$$

2.9 Experimental Design

The experiment had a completely randomized statistical design, in a factorial scheme consisting of two factors: cultivar \times type of cooking, totaling 9 treatments (T): (T1 = BRS1030/cooking in a microwave; T2 = BRS1030/cooking in a pressure cooker; T3 = BRS1030/cooking in a pot with a lid; T4 = BRS1030/cooking in a pot without a lid; T5 = BRS4104/cooking in a microwave; T6 = BRS4104/cooking in a pressure cooker; T7 = BRS4104/cooking in a pot with a lid; T8 = BRS4104/cooking in a pot without a lid; T9 = unprocessed).

The experimental unit was made up of three ears of corn. All statistical analyses were performed with the help of the statistical program SISVAR, version 5.3 (Build. 77) (FERREIRA, 2000).

3. Results and Discussion

Significant difference was observed when compared carotenoids profiles of normal and proVA biofortified immature corn grains ($p < 0.05$). Table 1 shows the average quantified contents ($\mu\text{g g}^{-1}$) of lutein, zeaxanthin, β -cryptoxanthin, β -carotene, total carotenoids and proVA carotenoids for the BRS1030 (normal) and BRS4104 (biofortified) corn cultivars.

Table 1. Carotenoid concentration expressed in $\mu\text{g g}^{-1}$ (fresh weight basis) in normal and biofortified immature corn grains

Carotenoids	¹ Mean carotenoid concentration \pm ² SD	
	BRS1030	BRS4104
Lutein	0.91 \pm 0.24 ^b	1.74 \pm 0.35 ^a
Zeaxanthin	7.18 \pm 3.59 ^a	7.22 \pm 2.15 ^a
β -cryptoxanthin	1.12 \pm 0.59 ^b	2.22 \pm 0.67 ^a
β -carotene	0.71 \pm 0.34 ^b	1.03 \pm 0.54 ^a
Total carotenoids	9.91 \pm 4.62 ^b	12.20 \pm 3.37 ^a
ProVA carotenoids	1.27 \pm 0.62 ^b	2.13 \pm 0.79 ^a

Note. ¹Means followed by the same lowercase letter in the row do not differ statistically according to the least significant difference (LSD) test at 5% probability ($p < 0.05$). ²SD: Standard deviation.

The carotenoid concentration of immature corn grain expressed in $\mu\text{g g}^{-1}$ (fresh weight) was considered as 100% for the retention analysis (unprocessed corn grains).

The BRS4104 immature corn grains had higher carotenoid concentrations than those of the BRS1030 ($p < 0.05$), except for the zeaxanthin ($p > 0.05$).

Figure 1 shows the chromatogram carotenoid concentration of immature corn grain cultivars.

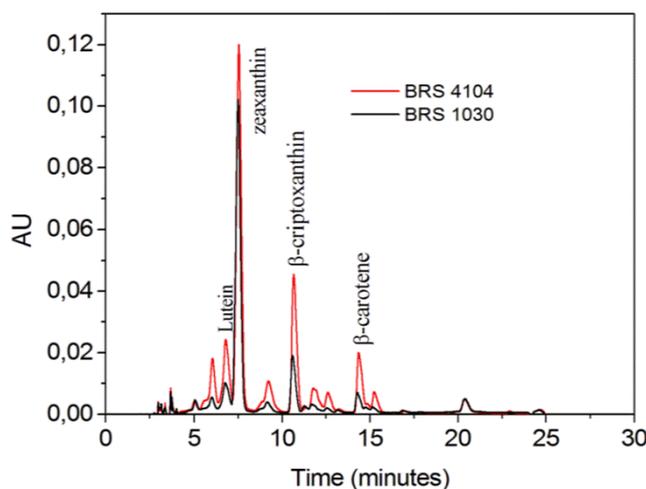


Figure 1. Chromatogram of carotenoids extracts from immature corn grain cultivars

Tables 2 and 3 show the mean quantified contents, in $\mu\text{g g}^{-1}$, of the lutein, zeaxanthin, β -cryptoxanthin, β -carotene, total carotenoids and proVA carotenoids for the BRS1030 and BRS4104 cultivars.

Table 2. Carotenoid concentration expressed in $\mu\text{g g}^{-1}$ (fresh weight basis) in normal corn grains after thermal treatments

Cooking type	Carotenoids					
	Lutein	Zeaxanthin	β -cryptoxanthin	β -caroteno	Total carotenoids	Total proVA
Without water in a microwave	0.63	3.81	0.65	0.34	4.57	0.58
With water in a pressure cooker	0.72	2.51	0.49	0.38	3.97	0.71
With water in a pot with a lid	0.93	8.08	1.13	0.72	11.99	1.18
With water in a pot without a lid	0.95	9.06	1.39	0.84	13.76	1.40

Table 3. Carotenoid concentration expressed in $\mu\text{g g}^{-1}$ (fresh weight basis) in biofortified corn grains after thermal treatments

Cooking type	Carotenoids					
	Lutein	Zeaxanthin	β -cryptoxanthin	β -carotene	Total carotenoids	Total proVA
Without water in a microwave	1.15	4.83	1.12	0.58	8.17	1.19
With water in a pressure cooker	1.71	5.29	1.72	0.72	9.27	1.37
With water in a pot with a lid	2.00	6.22	2.13	0.91	10.75	2.21
With water in a pot without a lid	2.21	7.63	1.99	1.07	12.48	2.58

Table 4 shows the analysis of variance (ANOVA) F test values for the studied variables. Significance was detected for factor cultivar and cooking type for the lutein variable. Significant effects ($p < 0.05$) were detected for the zeaxanthin, β -cryptoxanthin, β -carotene and total carotenoids variables for the interaction cultivar x cooking type. A significant effect was detected for the proVA carotenoids variable in the factor cooking type.

Table 4. Summary of ANOVA results of the effect of cultivar and cooking methods on the studied variables

SV	DF	Mean square					
		Lutein	Zeaxanthin	β -cryptoxanthin	β -carotene	Total carotenoids	Total proVA
Cultivar	1	10114.76*	4.98 ^{ns}	1482.13*	24.73 ^{ns}	4.65 ^{ns}	380.63 ^{ns}
Cooking type	1	3582.67*	9927.55*	8199.72*	5460.20*	7928.77*	6622.76*
Cultivar x Cooking type	4	334.58 ^{ns}	911.41*	2076.92*	2530.01*	1075.35*	557.10 ^{ns}

Note. * significant at 5% probability ($p < 0.05$); ns: not significant.

After establishing the association of the cultivar and processing factors, the LSD test was applied at 5% probability for the factors cultivar and cooking type. There were differences in the response of cultivars to the different thermal treatments for lutein retention. Table 5 shows the mean lutein retention in the immature corn grains of the cultivars studied before thermal treatment.

Table 5. Mean true lutein retention (%) for immature grains of the BRS1030 and BRS4104 corn cultivars

¹ True lutein retention (%) \pm ² SD	
Cultivar	Processing
BRS1030	86.80 \pm 2.10 ^b
BRS4104	124.91 \pm 3.15 ^a

Note. ¹Means followed by the same uppercase letter in the same column do not differ statistically according to the LSD test at 5% probability ($p < 0.05$). ²SD: Standard deviation.

The BRS4104 cultivar showed an increase in true lutein retention. It is possible that the changes experienced by the material as a result of processing facilitated the extraction of lutein.

Some studies evaluating carotenoid increasing content of foods have reported enhancement of carotenoid extraction after thermal processing, leading to an increase in the total concentration of pro-vitamin A carotenoids compared to fresh vegetables. This difference is most likely due to more efficient denaturation (breakdown) of the carotenoid-protein complex after cooking (Khachik et al., 1992; Pinheiro-Santana et al., 1998; Campos & Rosado, 2005).

Another factor that may favor carotenoid concentration after thermal processing is the deactivation of oxidative enzymes present in the raw tissue, which stimulates isomerization and oxidative degradation of carotenoids (Aman et al., 2005; Rodriguez-Amaya, 2001), such as lipoxigenase, which is known for indirectly catalyzing the

oxidative breakdown of carotenoids (Het Hof et al., 2000). However, these enzymes can be deactivated during thermal processing.

Furthermore, it is clear that processing type affected the true lutein retention in immature corn grains after thermal treatment. Table 6 shows the mean values of lutein retention (%) for the different thermal processing types.

Table 6. Mean true lutein retention (%) of immature corn grains according to the different thermal processing types applied to corn ears

Cooking type	¹ True lutein retention (%) \pm ² SD	
	BRS1030	BRS4104
Without water in a microwave	73.86 \pm 5.12 ^c	50.79 \pm 7.81 ^b
With water in a pressure cooker	92.08 \pm 2.41 ^{bc}	53.78 \pm 7.97 ^b
With water in a pot with a lid	116.96 \pm 1.56 ^{ab}	82.68 \pm 5.01 ^a
With water in a pot without a lid	138.06 \pm 3.48 ^a	120.87 \pm 7.18 ^a
Unprocessed	100.00 ^{ab}	100.00 ^a

Note. ¹Means followed by the same uppercase letter in the column in the row do not differ statistically according to the LSD test at 5% probability ($p < 0.05$). ²SD: Standard deviation.

Immature corn ear grains cooked with different thermal treatments had less apparent lutein retention after cooking in the microwave, with no difference between this response and the true retention obtained for ears cooked in a pressure cooker. However, the means for lutein retention of corn grains cooked in a pressure cooker, in a pot with a lid and in a pot without a lid did not differ, indicating the similar effects of these treatments on lutein retention. These three processes have in common the immersion of the ears in water during cooking.

The most carotenoid bioavailability from food is achieved when cooking is performed in water (Chandler & Schwartz, 1987; Lessin, 1997). However, the loss of soluble components to cooking water can increase the efficiency of carotenoid extraction (Khachik et al., 1992; Het Hof et al., 2000), which may have influenced the extraction of the immature grains cooked in the microwave because water was not used in this cooking process.

For the zeaxanthin variable, the associations of the cultivar and processing type factors were significant. Table 7 presents the mean values of zeaxanthin retention of the immature corn grain cultivars after treatments thermal.

Table 7. Mean true zeaxanthin retention (%) of immature corn grains in the ears of the studied cultivars after treatments thermal

Cooking type	¹ True zeaxanthin retention (%) \pm ² SD	
	BRS1030	BRS4104
Without water in a microwave	26.70 \pm 2.01 ^b	50.79 \pm 7.81 ^b
With water in a pressure cooker	24.31 \pm 1.81 ^b	53.78 \pm 7.97 ^b
With water in a pot with a lid	107.63 \pm 1.50 ^a	82.68 \pm 5.01 ^a
With water in a pot without a lid	124.89 \pm 2.16 ^a	120.87 \pm 7.18 ^a
Unprocessed	100.00 ^a	100.00 ^a

Note. ¹Means followed by the same uppercase letter in the column, within the cultivar factor, do not differ according to the t test (LSD) at the 5% significance level. ²SD: Standard deviation.

After cooking in either microwave or pressure cooker, zeaxanthin concentrations in the immature corn ear grains were reduced, regardless of the cultivar. In contrast, treatment in a pot without a lid did not affect the zeaxanthin concentration in the immature corn grains.

For the β -cryptoxanthin variable, the associations of the cultivar and cooking type factors were significant. Table 8 presents the mean values of β -cryptoxanthin retention in the immature grains of the corn cultivars after treatments thermal.

Table 8. Mean true β -cryptoxanthin retention (%) of immature corn grains in the ears of the studied cultivars after treatments thermal

Cooking type	¹ True β -cryptoxanthin retention (%) \pm ² SD	
	BRS1030	BRS4104
Without water in a microwave	32.47 \pm 4.34 ^b	37.01 \pm 2.11 ^c
With water in a pressure cooker	27.78 \pm 1.70 ^b	67.08 \pm 1.59 ^b
With water in a pot with a lid	101.98 \pm 4.21 ^a	96.79 \pm 1.63 ^a
With water in a pot without a lid	125.49 \pm 3.82 ^a	95.47 \pm 1.45 ^a
Unprocessed	100.00 ^b	100.00 ^a

Note. ¹Means followed by the same uppercase letter in the column, within the cultivar factor, do not differ according to the t test (LSD) at the 5% significance level. ²SD: Standard deviation.

Regarding the zeaxanthin, cooking the immature corn ears in the microwave and pressure cooker resulted in β -cryptoxanthin losses in the grains of the BRS1030 and BRS4104 cultivars. Processing in a pot with and without a lid did not change the β -cryptoxanthin concentration in the immature corn ear grains of both cultivars.

Regarding the β -carotene variable, the associations of the cultivar and cooking type factors were also significant. Table 9 shows the mean β -carotene retention values for the immature corn ear grains of the studied cultivars after being subjected to the different cooking methods.

Table 9. Mean true β -carotene retention (%) of immature grains in the ears of the studied corn cultivars

Cooking type	¹ True β -carotene retention (%) \pm ² SD	
	BRS1030	BRS4104
Without water in a microwave	29.41 \pm 2.01 ^b	44.65 \pm 2.87 ^b
With water in a pressure cooker	32.88 \pm 4.07 ^b	58.54 \pm 5.43 ^b
With water in a pot with a lid	105.27 \pm 3.01 ^a	97.95 \pm 3.21 ^a
With water in a pot without a lid	121.62 \pm 5.76 ^a	110.99 \pm 4.47 ^a
Unprocessed	100.00 ^a	100.00 ^a

Note. ¹Means followed by the same uppercase letter in the column, within the cultivar factor, do not differ according to the t test (LSD) at the 5% significance level. ²SD: Standard deviation.

Regarding the zeaxanthin and β -cryptoxanthin variables, after cooking the BRS1030 and BRS4104 cultivar immature corn ear grains in either the microwave or the pressure cooker, a reduction in β -carotene was observed, while there was no reduction when the cultivars were cooked in a pot with or without a lid.

Studies have reported β -carotene loss in leaves prepared by cooking in boiling water for 10 minutes or for 7 minutes in a microwave oven. The β -carotene losses were 21 and 20% for sow thistle and 11 and 26% for celery cooked conventionally and in a microwave, respectively (Muradian et al., 2000). These losses are minor compared to the losses obtained after the same processes applied to the ears of normal and biofortified corn. However, the cooking time in boiling water was shorter because the materials studied by those authors required less cooking time than immature kernels in the corn ears. Furthermore, water was used in microwave cooking, unlike what occurred in the present study.

The interactions of the three studied factors (cultivar and cooking type) also influenced the responses of the total carotenoids variable. Table 10 shows the mean values of total carotenoid retention of the immature corn grain cultivars after thermal treatments.

Table 10. Mean true total carotenoid retention (%) of immature corn grain in the ears of the studied cultivars after treatments thermal

Cooking type	¹ True total carotenoid retention (%) \pm ² SD	
	BRS1030	BRS4104
Without water in a microwave	29.41 \pm 2.01 ^c	51.77 \pm 4.36 ^d
With water in a pressure cooker	28.24 \pm 2.18 ^c	63.26 \pm 6.92 ^{cd}
With water in a pot with a lid	106.33 \pm 2.80 ^b	82.83 \pm 5.92 ^{bc}
With water in a pot without a lid	137.77 \pm 4.33 ^a	113.45 \pm 6.43 ^a
Unprocessed	100.00 ^b	100.00 ^{ab}

Note. ¹Means followed by the same uppercase letter in the column, within the cultivar factor, do not differ according to the t test (LSD) at the 5% significance level. ²SD: Standard deviation.

After cooking BRS1030 corn ears without water in the microwave and with water in the pressure cooker, a reduction in total carotenoids was observed in the grains. Conversely, the corn grains cooked in water in a pot without a lid exhibited higher retention, indicating possible carotenoid concentration. Increases in carotenoid retention as a result of cooking in a pot common have been reported previously (Muzhingi et al., 2008; Khachik et al., 1992; Granado et al., 1992), who studied the effect of cooking on the retention of carotenoids in vegetables.

For BRS4104 cultivar, loss in carotenoid concentration was detected after cooking the ears without water in the microwave and with water in the pressure cooker. There was no reduction when the cultivar was cooked in a pot with or without a lid.

The true total carotenoid retention for BRS4104 cultivar immature corn ear grains cooked in a pot with or without a lid and for BRS1030 cultivar immature ear grains cooked in a pot with or without a lid were higher than the value reported by Sant'Ana et al. (1998) for carrot (*Daucus carota* L.) after cooking with water without a pressure cooker (60.13%). However, considering total carotenoids the immature grains of the corn cultivars, the best treatment for carotenoid retention was cooking in water without pressure, with or without a lid.

Table 11 shows the mean proVA carotenoid percent retention observed for the immature corn grains according to processing type, after thermal processing.

Table 11. Mean true proVA carotenoid retention (%) of immature corn grains in the ears of the studied cultivars

Cooking type	¹ True proVA carotenoid retention (%) \pm ² SD
Without water in a microwave	36.42 \pm 8.09 ^b
With water in a pressure cooker	50.92 \pm 4.34 ^b
With water in a pot with a lid	104.37 \pm 6.60 ^a
With water in a pot without a lid	108.60 \pm 7.13 ^a
Unprocessed	100.00 ^a

Note. ¹Means followed by the same uppercase letter in the column and lowercase letter in the row, within the cultivar factor, do not differ statistically according to the LSD test at 5% probability ($p < 0.05$). ²SD: Standard deviation.

Cooking the immature corn ears in either a microwave without water or immersed in water in a pressure cooker resulted in lower proVA carotenoid retention. However, the other cooking processes did not affect the retention of these compounds in the the same material. Therefore, the the cooking method of immersion the corn ears in boiling water in a pot with or without a lid is, the best for preventing losses of proVA carotenoids.

4. Conclusion

The biofortified BRS4104 and normal BRS1030 corn cultivars have similar zeaxanthin concentration in the immature kernels but the biofortified corn grains have higher lutein, β -cryptoxanthin, β -carotene, total

carotenoids and pro VA carotenoid than the normal corn grains.

In general, irrespective regardless of the cultivar, the best thermal processing method for carotenoid retention in immature corn grains is cooking in boiling water in a pot with or without a lid, since significant losses of carotenoids occurs when the immature corn grains are submitted to thermal processing in either a microwave without water or in pressure cooker with water.

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