CAROTENOID RETENTION IN MINIMALLY PROCESSED BIOFORTIFIED GREEN CORN STORED UNDER RETAIL MARKETING CONDITIONS

Retenção de carotenoides em milho verde biofortificado minimamente processado armazenado em condições de varejo

Natália Alves Barbosa[1], Maria Cristina Dias Paes[2], Paulo Evaristo de Oliveira Guimarães[2], Joelma Pereira[3]

ABSTRACT

Storing processed food products can cause alterations in their chemical compositions. Thus, the objective of this study was to evaluate carotenoid retention in the kernels of minimally processed normal and vitamin A precursor (proVA)-biofortified green corn ears that were packaged in polystyrene trays covered with commercial film or in multilayered polynylon packaging material and were stored. Throughout the storage period, the carotenoids were extracted from the corn kernels using organic solvents and were quantified using HPLC. A completely factorial design including three factors (cultivar, packaging and storage period) was applied for analysis. The green kernels of maize cultivars BRS1030 and BRS4104 exhibited similar carotenoid profiles, with zeaxanthin being the main carotenoid. Higher concentrations of the carotenoids lutein, β-cryptoxanthin, and β-carotene, the total carotenoids and the total vitamin A precursor carotenoids were detected in the green kernels of the biofortified BRS4104 maize. The packaging method did not affect carotenoid retention in the kernels of minimally processed green corn ears during the storage period.

Index terms: Provitamin A; biofortification; packaging; storage.

RESUMO

O armazenamento de produtos alimentícios processados pode causar alterações na sua composição química. Sendo assim, objetivou-se, com este trabalho, determinar a retenção de carotenóides em grãos de milho verde normal e biofortificado com precursores de vitamina A, em espigas minimamente processadas, embaladas em bandeja de poliestireno com cobertura de filme comercial e embalagem multicamadas nylon poli ao longo do período de estocagem do produto. Os carotenóides foram extraídos dos grãos de milho verde em esquema sequencial de solventes orgânicos e quantificados por CLAE. O experimento foi planejado em delineamento inteiramente casualizado em esquema fatorial, constituído de três fatores (cultivar, embalagem, período de armazenamento). Os grãos verdes das cultivares de milho BRS1030 e BRS4104 apresentam semelhança no perfil de carotenóides, sendo zeaxantina o principal carotenoide presente nos grãos verdes desses materiais. Maiores concentrações dos carotenóides luteína, β-criptoxantina, β-caroteno, carotenóides totais e do total de carotenóides precursores de vitamina foram identificadas nos grãos verdes do milho biofortificado BRS4104. A retenção de carotenóides em grãos verdes de milho, durante o período de estocagem das espigas minimamente processadas, não foi influenciada pelos tipos de embalagens estudadas.

Termos para indexação: Provitamina A; biofortificação; embalagem; armazenamento.

INTRODUCTION

Much of the world's population thinks that food not only nourishes but also supplies biologically active compounds or elements that provide additional health benefits (Sentanin; Rodriguez-Amaya, 2007). Thus, the concept of functional foods has emerged. Among the bioactive compounds present in food sources are carotenoids, which are natural pigments used as natural food dyes that possess biological activities.

Corn stands out as a source of carotenoids in the diets of populations for which this cereal is considered a staple. Zeaxanthin and lutein, which are xanthophylls, are the main carotenoids present in the kernels of yellow corn, which also contain significant amounts of β-cryptoxanthin and lower levels of α and β-carotenes. The industrial process by which the ears with green corn kernels usually consumed in human diet, as well as those during storage of such products, is of concern, because carotenoids exhibit instability regarding exposure to oxygen, light and oxidative enzymes, give rise to a number of degradation reactions such as the following: isomerization, oxidation and epoxidation (Rodriguez-Amaya, 2001).

[1]Universidade Federal de Lavras/UFLA – Departamento Ciência dos Alimentos/DCA – Câmpus Universitário – Caixa Postal 3037 – 37200-000 – Lavras – MG – Brasil – natyalvesb@gmail.com
[2]Empresa Brasileira de Pesquisa Agropecuária/EMBRAPA – Embrapa Milho e Sorgo – Sete Lagoas – MG – Brasil
[3]Universidade Federal de Lavras/UFLA – Departamento Ciência dos Alimentos/DCA – Lavras – MG – Brasil
Received in october 23, 2014 and approved in april 14, 2015

The possibility of carotenoid oxidation during processing (Rodriguez-Amaya, 1997) has made carotenoid retention a constant concern; moreover, attention has been focused not only on the effects of industrial processing but also on homemade preparation methods that might also cause high losses of the derivatives of biofortified materials (Gama Silos, 2007). However, processing has been demonstrated to have a positive effect on the availability of carotenoids by breaking cellular structures and denaturing proteins that are complexed with pigments, thus facilitating the release of these compounds from the plant-cell matrices (Saunders et al. 2000).

Nutritional and convenience factors continue to be desired by consumers. The latter, when associated with food, relates to the ease of its storage (Sgarbieri, 1986). However, the storage conditions of a product are critical to preserving its nutritional value. Thus, investigating the retention of carotenoids after the processing and during the storage of corn is essential because processed, stored corn is the form of corn product generally consumed. Given the above, the aim of this study was to evaluate the retention of carotenoids in the kernels of normal and vitamin A precursor-biofortified green corn ears that were minimally processed and packed in polystyrene trays with a polyvinyl chloride (18-mm-thick PVC) commercial film covering or in multilayered polynylon packaging under vacuum during storage under retail conditions.

MATERIAL AND METHODS

Raw material

The corn was cultivated under controlled conditions of fertilization, irrigation, and pest and disease management. The crops were harvested during the early morning hours, when the kernels were at the milky stage, known as the "green corn point." Ears with the husks were packed in polystyrene boxes containing ice and were transported to the laboratory, where minimal processing was conducted. Two corn cultivars were used for the study, the biofortified variety BRS4104 and the normal variety BRS1030.

Experimental design

The experiment was conducted using a 2x2x4 factorial design including three factors, cultivars BRS1030 and BRS4104, polystyrene packaging and multilayer polynylon, and storage for 0, 3, 6 and 9 days, and three replications were performed. The experimental unit consisted of three ears of corn.

Minimal processing

The ears were processed as shown in the flowchart in Figure 1.

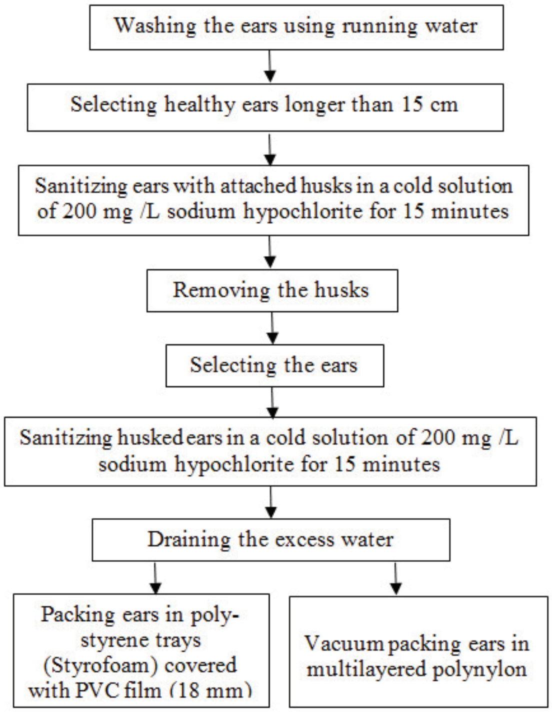

Figure 1: Flowchart of the minimal processing procedure for the corn used to evaluate carotenoid retention during the storage period.

The following were used to package the ears: polystyrene trays (Styrofoam) that were 23.5 cm long x 18.2 cm wide, wrapped in 18-mm-thick polyvinyl chloride (PVC) film (Figure 2A) and multilayered polynylon packaging (20 x 25 cm; TecMaqbrand) suitable for food (Figure 2B). A TM model 150 vacuum sealer was used to seal the latter under vacuum. The packed ears of corn were stored in a BOD at a fixed temperature of 5 °C, with light for nine hours daily, for a period of nine days. The products were distributed randomly within the BOD. To evaluate carotenoid retention, the ears were threshed using a Mecapau brand (model 032 D) autothresher, and the kernels were quartered and then were triturated using a model 1119 Toastmaster coffee grinder for 20 seconds. The carotenoid retention analysis was conducted on days 3, 6 and 9 of storage.

Determination of the carotenoid profile

The carotenoids were extracted from the samples using a series of organic solvents according to the protocol of Rodriguez-Amaya, Kimura and Mieko (2004), with some modifications. The modifications concerned the volume of ethanol used (7 mL), the volume of deionized water used (4 mL), not adding an internal standard, the volume of hexane used (4 mL), replacing dichloroethane with methanol, using acetone (2 mL) to reconstitute the extract for HPLC analysis (sample injected = 40 µL) and not injecting the standard β-apo-8'-carotenal for HPLC analysis.

The carotenoid contents were quantified using high-performance liquid chromatography (HPLC) using an Alliance Waters model e2695 liquid chromatography system equipped with a polymeric YMC C 30 column (3 µm, 4.6 x 250 mm, Waters, Milford, MA, USA) coupled to a diode array detector (Waters Model 2998). Gradient elution was conducted at a flow rate of 0.8 mL/min under gradient conditions that ranged from 80:20 to 20:80 of methanol:tert-butyl methyl ether over 16 minutes, followed by a constant flow of an 80:20 solution for 4 minutes, and finishing with 6 minutes of equilibration. The oven temperature was 30 °C, the detection wavelength was 450 nm, and the injection volume was 40 µL. The laboratory temperature was maintained at 20 °C throughout the process. For compound identification, standards purified from carrots (α-carotene, 94.64% purity) and papayas (β-cryptoxanthin, 92.72% purity) were used, following the protocol of Rodriguez-Amaya, Kimura and Mieko (2004). The carotenoid standards 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 the respective Swanson-brand capsules. The results of the carotenoid analysis were expressed on a fresh basis.

The concentration of total carotenoids was obtained by summing the values for all of the quantified fractions, including the total lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene fractions, as described by Murphy, Criner and Gray (1975).

The concentration of carotenoids with provitamin A activity (proVA) was obtained using the following formula: total β-carotene + ½ total α-carotene + ½ total β-cryptoxanthin (mg/g), as described by Murphy, Criner and Gray (1975). The apparent percentage of retention percentage for each variable was calculated using the formula proposed by Murphy, Criner and Gray (1975). This calculation was based on the inherent chemical characteristics of each of these molecules. The chemicals with structures that have at least one β-ionone ring and a chain of eleven carbons exhibit provitamin A activity. β-carotene is formed by two β-ionone rings that give rise to two molecules of retinol, and therefore, this carotenoid has 100% provitamin A activity. β-cryptoxanthin and α-carotene exhibit approximately 50% provitamin A activity because they have only one β-ionone ring. The content of *cis* isomers was not determined because they do not have significant biological activity. The apparent percentage of retention was calculated according to the formula proposed by Murphy, Criner and Gray (1975), as follows:

$$\%\ apparent\ retention = \frac{carotenoid\ content / g\ of\ processed\ food\,(fresh\ basis)\ x\ 100}{carotenoid\ content / g\ of\ unprocessed\ food\ (fresh\ basis)}$$

Figure 2: Image of green corn ears packed in polystyrene (Styrofoam) trays and wrapped with 18-mm-thick polyvinyl chloride (PVC) film (A). Image of green corn ears vacuum-packed in multilayered polynylon (B).

Determination of the moisture content of the samples

The moisture content of the samples was determined using 2 g of ground sample that was dried at 60 °C for 72 hours in a ShelLab SL Model 1350FX oven. The moisture content was calculated according to method 44-15 of the AACC (2000).

Statistical analyzes

The statistical analyses were performed using SISVAR version 5.3 (Build 77) statistical software (Ferreira, 2000).

The data were subjected to an analysis of variance (ANOVA), and the mean values were compared using the Least Significant Difference test (LSD) ($p = 0.05$) when evaluating the significance of the differences using the F test.

RESULTS AND DISCUSSION

The average concentrations of the studied carotenoids in the green corn kernels of the two cultivars differed significantly. The concentrations (µg/g) of lutein, zeaxanthin, β-cryptoxanthin, β-carotene, total carotenoids and vitamin A precursor carotenoids in the kernels of minimally processed green corn are shown in Table 1.

The kernels of the BRS4104 corn cultivar had higher concentrations of carotenoids than did those of cultivar BRS1030 ($p<0.05$), except for the zeaxanthin concentration ($p>05$). In addition to the average concentration of total carotenoids in the green corn kernels of cultivar BRS4104 (15.96 µg/g) being higher than that of cultivar BRS1030 (11.47 µg/g), this value was higher than the corresponding values for the dried yellow kernels of other commercial cultivars of Brazilian QPM (Quality Protein Maize), such as Assum Preto (11.7 µg/g) and BR 473 (9.17 µg/g), which were determined by Kimura, Rodriguez-Amaya and Nestel (2007).

Cândido (2010) found that the average total carotenoid content of dried kernels of synthetic corn that was also biofortified with provitamin A was 34.49 µg/g in grains, which was higher than that found in the corn biofortified with provitamin A that was studied in the present investigation. This result can be explained by the carotenoid synthesis of the latter being interrupted soon after harvesting by the minimal processing procedure. In this study, the ears were harvested at the milky stage, which is the ideal stage at which to harvest corn and differs from the time of harvest of the cited study. Moreover, the cited study examined dried kernels. Moisture content is another factor that could have affected the results because dried kernels contain an average of 13% moisture, whereas fresh kernels contain 70% to 80% moisture (Nunes, 2013). Therefore, the carotenoids would presumably be more concentrated in the dried corn kernels.

Carotenoid biosynthesis can continue after harvesting, increasing the carotenoid contents of fruits, vegetables and tubers that are maintained fully intact, thereby preserving the enzymatic system responsible for carotenogenesis (Rodriguez-Amaya; Kimura; Amaya-Farfan, 2008). However, minimal processing, even if performed with the recommended care, does not maintain harvested material fully intact.

Table 1: Concentration of carotenoids on a fresh basis, expressed in µg/g of kernel samples of minimally processed green corn ears from normal and biofortified cultivars.

Carotenoids	[1]Mean concentration of carotenoids (µg/g) ± [2]SD	
	BRS 1030	BRS4104
Lutein	1.03±0.28b	1.88±0.52a
Zeaxanthin	8.38±1.60a	9.26±2.00a
β-cryptoxanthin	1.36±0.22b	2.95±0.94a
β-carotene	0.71±0.16b	1.86±0.58a
Total carotenoids	11.47±2.01b	15.96±3.23a
proVA carotenoids	1.38±0.22b	3.33±0.94a

[1]Mean values followed by the same letter in the rows did not differ significantly according to the LSD test at the 5% probability level ($p<0,05$) – [2]SD (standard deviation).

The kernels of the green corn ears of cultivars BRS1030 and BRS4104 exhibited a linear rate of moisture reduction throughout the storage period (Figures 3 and 4), regardless of the packaging used. For cultivar BRS1030, the moisture content of the kernels was higher from the 3rd day of storing the ears in the multilayered polynylon packaging rather than in the wrapped polystyrene trays. However, the moisture content of the kernels of cultivar BRS4104 green corn ears stored in polystyrene trays was higher throughout the storage period.

To evaluate the apparent retention rate, the average carotenoid content of the kernels of the green corn ears at Day zero expressed in µg/g (wet weight) was considered to be 100%.

Tables 2 and 3 show the levels of retained carotenoids expressed in µg/g (wet weight).

A significant effect ($p < 0.05$) of the interaction of the cultivar x the storage period was detected for the variable lutein. The effect of this interaction was not significant ($p > 0.05$) for the variables zeaxanthin, β-cryptoxanthin, β-carotene, total carotenoids or proVA carotenoids. However, we found that the storage period significantly affected the variable zeaxanthin and that the cultivar type significantly affected the variable β-cryptoxanthin (Table 4).

The concentration of α-carotene was below the limit of detection, so this variable was not analyzed.

After the ANOVA was performed regression analysis was applied to the storage period factor for the variable lutein. Regardless of the type of packaging used, the green corn kernels of cultivar BRS1030 retained 67.92% of the lutein on the 3rd day of storage, whereas the green corn kernels of cultivar BRS4104 retained 105.67% of the lutein, showing no apparent loss of this carotenoid during this period. However, the green

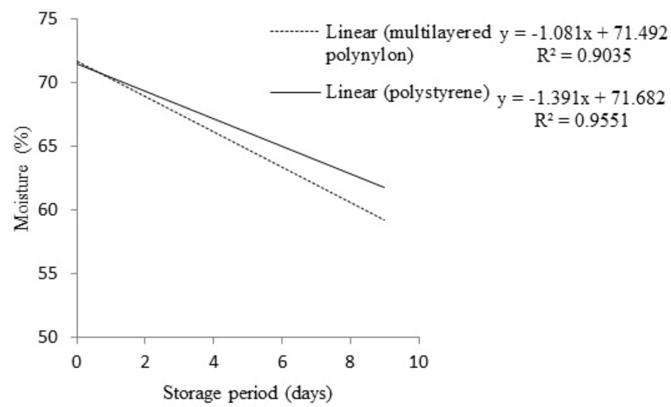

Figure 3: Percentage of moisture in the kernels of green corn ears of cultivar BRS1030 throughout storage

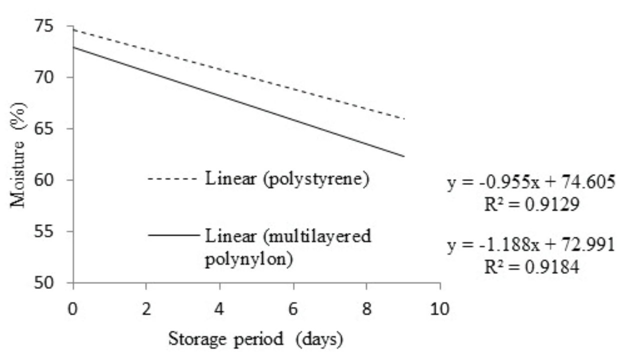

Figure 4: Percentage of moisture in the kernels of green corn ears of cultivar BRS4104 throughout storage

Table 2: Mean concentrations of carotenoids in µg/g of kernels (wet weight) at 0, 3, 6 and 9 days of storage of minimally processed corn ears packed in polystyrene trays covered with polyvinyl chloride.

Carotenoids	Mean concentration of carotenoids (µg/g)							
	Day 0		Day 3		Day 6		Day 9	
	BRS1030	BRS4104	BRS 1030	BRS4104	BRS1030	BR4104	BRS1030	BR4104
Lutein	1.12	1.94	0.74	2.07	0.92	1.39	1.19	1.36
Zeaxathin	8.42	8.82	7.84	8.17	6.89	7.74	9.47	11.47
β-cryptoxanthin	1.27	2.76	1.20	2.12	1.43	2.35	1.22	2.12
β-carotene	0.79	1.38	0.59	1.69	0.85	1.13	0.59	1.67
Total Carotenoids	11.60	14.90	10.36	14.04	10.09	12.60	12.47	16.61
ProVA Carotenoids	2.77	1.43	2.75	1.19	2.30	1.56	2.73	1.20

Table 3: Mean concentrations of carotenoids in µg/g of kernels (wet weight) at 0, 3, 6 and 9 days of storage of minimally processed corn ears packed in multilayered polynylon packaging.

Carotenoids	Mean concentration of carotenoids (µg/g)							
	Day 0		Day 3		Day 6		Day 9	
	BRS1030	BRS4104	BRS 1030	BRS4104	BRS1030	BR4104	BRS1030	BR4104
Lutein	1.12	2.00	0.72	1.92	0.92	1.98	1.52	2.39
Zeaxathin	8.42	9.65	6.94	9.50	7.91	9.01	11.16	9.75
β-cryptoxanthin	1.27	3.85	1.42	2.96	1.31	4.38	1.71	3.08
β-carotene	0.79	1.93	0.62	1.97	0.68	2.41	0.78	2.67
Total Carotenoids	11.60	17.42	9.69	16.34	10.82	17.78	15.15	17.93
ProVA Carotenoids	3.85	1.43	3.45	1.33	4.60	1.34	4.21	1.63

Table 4: Summary of results of an analysis of variance (ANOVA) of the effects of the treatments on the variables.

SV	DF	Mean square					
		Lutein	Zeaxanthin	β-cryptoxanthin	β-carotene	Total carotenoids	proVA carotenoids
Cultivar	1	202.66ns	0.39ns	4541.38*	2709.16ns	11.77ns	0.69ns
Packaging	1	1326.89ns	6.19ns	1317.86ns	624.17ns	254.52ns	995.54ns
Storage period	3	1636.70ns	1895.17*	422.21ns	523.77ns	1154.83ns	281.79ns
Cultivar x packaging	1	236.34ns	239.59ns	4.52ns	260.45ns	16.21ns	67.21ns
Cultivar x storage period	3	2000.89*	209.93ns	931.94ns	1065.99ns	280.11ns	16.81ns
Packaging x storage period	3	888.62ns	89.14ns	213.79ns	465.85ns	141.98ns	286.36ns
Cultivar x packaging x storage period	3	183.75ns	591.26ns	784.67ns	1253.39ns	303.69ns	916.27ns

*significant at 5% probability (p<0.05); ns (not significant); SV (variation factors); DF (degrees of freedom).

corn kernels of cultivar BRS1030 exhibited a higher apparent retention of lutein (124.07%) on the 9th day of storage, which was significantly higher than that of the synthetic cultivar BRS4104, with a difference of 26% greater retention.

The 2nd-degree polynomial model of the variation in lutein retention in green corn kernels during the storage of ears of the BRS1030 cultivar is shown in Figure 5. Lutein loss apparently occurred by Days 3 (67.92%) and 6 (85.85%), although the loss of moisture may have favored the lutein concentration at the end of the storage period.

For the synthetic BRS4104 cultivar, the apparent lutein retention rate found on the 9th day of storage was 98.97%, which remained constant throughout storage.

The effect of minimal processing and storage for five days at 5-7 °C on collard led to reduced levels of retained lutein (27%), violaxanthin (20%) and neoxanthin (31%) and an even greater loss of β-carotene. The loss was greatest from Day 0 to Day 1, with no change observed from Day 1 to Day 5 (Azevedo; Rodriguez-Amaya; 2005).

The zeaxanthin retained in the immature kernels of minimally processed corn ears stored for nine days significantly increased at the end of the storage period, regardless of the packaging applied to the ears. As previously reported for lutein, an increase in the concentration of this carotenoid was possibly due to moisture loss.

The 2nd-degree polynomial model of the variation in zeaxanthin retention during storage is presented in Figure 6. The apparent rates of retention of β-cryptoxanthin in the green corn kernels of both cultivars differed significantly. The green kernels of cultivar BRS4104 had a lower average β-cryptoxanthin retention rate, 19.46% less than that of the green corn kernels of the ears of cultivar BRS1030. The apparent β-cryptoxanthin retention rate of cultivar BRS1030 was 109.22%, whereas that of cultivar BRS4104 was 89.76%.

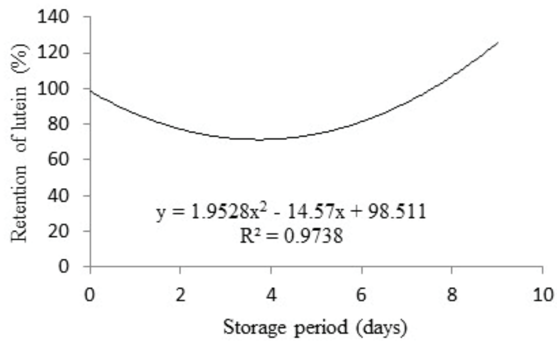

Figure 5: Variation of the apparent retention (%) of lutein during the storage of cultivar BRS1030 green corn at 5 °C, with light for 9 h/day.

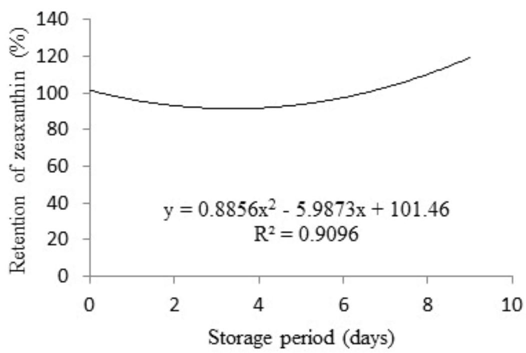

Figure 6: Retention of zeaxanthin in green kernels during the storage of minimally processed corn ears

None of the factors and none of their interactions affected the remaining variables. Thus, there was no significant difference in the apparent retention rates of β-carotene, the total carotenoids and the proVA carotenoids regardless of the cultivar and the types of packaging during storage. The average apparent retention rate of the β-carotene was 106.53%, that of the total carotenoids was 100.86% and that of the proVA carotenoids was 101.62%.

Processing and storing foods may significantly alter the qualitative and quantitative carotenoid composition. However, the retention of proVA carotenoids during storage of processed foods is favored by a low temperature, protection from light and antioxidants that are naturally present or are added to preserve food (Rodriguez-Amaya, 1997). A previous study showed that reducing the processing period and temperature and the interval between peeling, cutting or chopping and processing can significantly improve the retention of carotenoids (Rodriguez-Amaya, 1993).

Hussein et al. (2000) studied the effect of two different containers (squeeze and vacuum) on the retention of β-carotene in minimally processed broccoli that was stored for the retail period of this product (10 days) and found that there was no significant difference due to the packaging. The same result was observed in the present study. The type of packaging (polystyrene or multilayered polynylon) did not affect the retention of any of the carotenoids studied, regardless of the cultivar.

CONCLUSIONS

The green corn kernels of cultivars BRS1030 and synthetic BRS4104 had similar carotenoid profiles, with zeaxanthin being the main carotenoid present. The concentrations of lutein, β-cryptoxanthin, β-carotene, the total carotenoids and the total vitamin A precursor carotenoids in the green corn kernels of the synthetic cultivar BRS4104 were higher than those of cultivar BRS1030. Storing minimally processed normal and vitamin A precursor-biofortified ears of green corn for nine days caused a reduction in the moisture content of the kernels but did not affect the apparent retention of β-carotene, the total carotenoids and the proVA carotenoids.

ACKNOWLEDGMENTS

The authors would like to thank the Coordination of Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES), the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq) and the Arthur Bernardes Foundation (Fundação Arthur Bernardes – FUNARBE) for their financial support.

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