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Determination of Carotenoids in Yellow Maize, the Effects of Saponification and Food Preparations

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Abstract: Maize is an important staple food consumed by millions of people in many countries. Yellow maize naturally contains carotenoids which not only provide provitamin A carotenoids but also xanthophylls, which are known to be important for eye health. This study was aimed at 1) evaluating the effect of saponification during extraction of yellow maize carotenoids, 2) determining the major carotenoids in 36 genotypes of yellow maize by high-performance liquid chromatography with a C30 column, and 3) determining the effect of cooking on the carotenoid content of yellow maize. The major carotenoids in yellow maize were identified as all-*trans* lutein, *cis*-isomers of lutein, all-*trans* zeaxanthin, α- and β-cryptoxanthin, all-*trans* β-carotene, 9-*cis* β-carotene, and 13-*cis* β-carotene. Our results indicated that carotenoid extraction without saponification showed a significantly higher yield than that obtained using saponification. Results of the current study indicate that yellow maize is a good source of provitamin A carotenoids and xanthophylls. Cooking by boiling yellow maize at 100° C for 30 minutes increased the carotenoid concentration, while baking at 450° F for 25 minutes decreased the carotenoid concentrations by almost 70% as compared to the uncooked yellow maize flour.

Key words: Carotenoids, yellow maize, saponification, vitamin A, biofortification

Introduction

Carotenoids in fruits and vegetables are believed to contribute to human health as antioxidants, as a source of vitamin A, and in providing specific functions such as protecting against age-related macular degeneration [1]. Maize is a major staple food in human diets in Africa, Latin America, and Asia and it is widely used in animal feeds in some western countries. Maize would have added nutritional value if the grain contained appreciable levels of carotenoids [2]. The carotenoid content in yellow maize can be manipulated genetically whereas white maize has little or no carotenoid content [3]. Most of the maize carotenoids are found in the hard endosperm of the kernel and only small amounts are in the germ [3]. Although the β-carotene in yellow maize could be an important source

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of vitamin A, yellow maize is not consumed by humans as much as white maize [3, 4].

Recently there have been efforts to increase the β-carotene content of yellow maize through plant breeding (biofortification) and genetic modification. Through these efforts, some yellow maize lines have been found to have very high levels of β-carotene. Maize lines with relatively high levels of pro-vitamin A carotenoids (β-carotene and β-cryptoxanthin) in the grain have been produced at the University of Illinois at Urbana [5]. This biofortified maize (high β-carotene yellow maize lines) has been shown to adequately maintain vitamin A nutriture in Mongolian gerbils, and was just as efficacious as β-carotene supplementation [6]. There are many yellow maize lines in the world markets and identifying the carotenoid content of these lines would help maize breeders, agronomists, and human nutritionists to determine which high-carotenoid lines are best suited to particular climates, soil types, and cultures for different parts of the world. Such a study would help in the documentation of carotenoid content of yellow maize in food composition tables for different maize-consuming regions of the world.

The analysis of carotenoids in foods is complicated by the complexity of carotenoid distribution and composition in various food matrices. With the development of high-performance liquid chromatography (HPLC) the separation and quantification of carotenoid isomers has been greatly improved. Some investigators [7–9] have used saponification procedures during carotenoid extraction. The saponification procedure has been often used as a step to simplify the separation by removing substances, such as chlorophylls and lipids, that could interfere with the chromatographic detection of carotenoids [10]. Therefore, saponification with potassium hydroxide has been an integral part of both vitamin A and carotenoid analyses. Numerous saponification procedures that differ in the concentration of potassium hydroxide (KOH) and the time and temperature of incubation with KOH have been used, and it has been reported that even under mild conditions the saponification step caused a reduction of the α- and β-carotene contents in vegetables [7]. Similar effects were also observed by Wilson and Bushway [10, 11]. Other investigators reported significant losses of lutein and zeaxanthin, and one study observed an increase of cryptoxanthin following saponification [7]. Since most of these studies were done in green leafy vegetables, the current study investigated if maize carotenoid values are affected by saponification. Most of the literature available on maize carotenoid analysis is outdated, and hence limited by poor sensitivity and specificity. This study aims to show the effect of saponification on the carotenoid content of yellow maize, and to help researchers decide upon effective and reliable maize carotenoid extraction procedures.

All over the world, maize is cooked prior to consumption. Common methods of processing maize are boiling, steaming, blanching, roasting, or baking. It has been found that cooking maize causes carotenoids to lose their provitamin A activity through isomer formation [12]. Results of studies on the effects of cooking on the retention of carotenoids have been contradictory [13]. In some vegetables, cooking resulted in an increase of carotenoid content while in others carotenoids decreased [13]. At the moment, there is a dearth of information on the effect of cooking on the carotenoid content of yellow maize. Results from the current study can be used in research studies on carotenoid bioavailability from cereals, in nutrition labeling, and in educating consumers on yellow maize nutrition.

Selected yellow maize lines were evaluated for carotenoid content both with and without saponification, to determine differences in carotenoid values obtained from the two extraction methods. On the basis of evaluation of carotenoid extraction methods, carotenoid contents of 36 yellow maize lines were determined by extraction without saponification. Extraction without saponification was also used to determine the effect of cooking on the carotenoid content of yellow maize.

Materials and Methods

Chemicals and standards

Standard all-*trans* β-carotene, 9-*cis* β-carotene, 13-*cis* β-carotene, cryptoxanthin, lutein, and zeaxanthin were generous gifts from the DSM Nutritional products (Basel, Switzerland), ammonium acetate, butylated hydroxytoluene (BHT), methyl-tert-butyl ether (MTBE), and potassium hydroxide were purchased from Sigma Co. (St. Louis, MO, USA). Tetrahydrofuran (THF), water, ethanol, and methanol were purchased from JT Baker. All solvents obtained were HPLC grade.

Extraction and Analysis

Maize samples were obtained from plant breeding institutes, the International Maize and Wheat Improvement Center (CIMMYT), Pioneer Hi-Bred, University of Illinois at Champaign/Urbana, and the Agricultural Research Services United States Department of Agriculture (ARS-USDA), Texas. The maize samples were received as either dry kernels or ground flour.

Extraction of carotenoids from maize without saponification was performed using the method of Riso and Porrini for vegetables [7]. 50 yellow maize kernels were

milled into a fine powder using a coffee grinder. Extraction was performed by incubating 600 mg of yellow maize flour that was randomly sampled from a thoroughly mixed powder pile, with 10 mL methanol for 2 hours at room temperature and vortexing at 30-minute intervals. Afterwards, the mixture was homogenized for 30 seconds in an ice bath. The mixture was centrifuged at 800 × g. The methanol layer was transferred into a 50-mL volumetric flask and the extraction repeated four times with 10 mL of tetrahydrofuran (THF), followed by vortexing and centrifugation. The THF layers were combined with the methanol layer and the volume brought up to 50 mL. One mL of the extract was taken, dried under nitrogen, and resuspended in 1 mL of ethanol.

Saponification used a modified method of Kurilich and Juvik [9] for the extraction of maize carotenoids. Yellow maize flour samples of 600 mg underwent a 5-minute ethanol preparation [6 mL of ethanol containing 0.1% butylated hydroxytoluene (BHT)] in an 85° C water bath before being subjected to a 10-minute saponification with 120 µL of 80% w/v KOH in water. All samples were vortexed once during saponification. Upon removal they were immediately placed in an ice bath where 5 mL of methanol was added and the mixture was homogenized for 30 seconds. The mixture was centrifuged at 800 × g. The ethanol/methanol layer was transferred into a 50 mL volumetric flask and the extraction repeated four times with 10 mL of THF, followed by vortexing and centrifugation. The THF layers were combined with the ethanol/methanol layer and the volume brought up to 50 mL. One mL of the extract was taken, dried under nitrogen, and re-suspended in 1 mL of ethanol for HPLC analysis.

HPLC Analysis

The extracted sample was analyzed for carotenoids using a reverse-phase, gradient HPLC system [14]. The HPLC system consisted of a Waters Alliance 2695 Separation Module LC pump, autosampler, Waters 2996 Photo Array Detector (Millipore, Milford, MA), and a C30 column (3 µm, 150 × 4.6 mm, ProntoSIL, Bischoff, Leonberg, Germany). The chromatographic separations were performed on a Waters Alliance 2695 HPLC (Millipore, Milford, MA) system using an UV detector and Waters Empower Pro software. The carotenoids were separated at a flow rate of 1 mL/minute and by a gradient elution with two mixtures of methanol, tert-butyl methyl ether, and water [mixture A: 83/15/2 (v/v/v), mixture B: 8/90/2 (v/v/v); gradient procedures were: 0 to 1 minute 100% A, 1- to 8-minute linear gradient to 70% A, 8- to 13-minute 70% A, 13- to 22-minute linear gradient to 45% A, 22- to 24-minute 45% A, 24- to 34-minute linear gradient to 5% A, 34- to 38-minute 5% A, 38- to 40-minute linear gradient to 100% A, and 40- to 50-minute 100% A]. Carotenoids were determined at 450 nm. All-*trans* β-carotene, 13-*cis* β-carotene, 9-*cis* β-carotene, cryptoxanthin, lutein, and zeaxanthin, were adequately separated and quantitated using this method by determining peak areas under the curve in the HPLC calibrated against known amounts of standards. Each peak was confirmed by the retention time and characteristic spectra of the standards. The linearity, precision, and accuracy of all-*trans* β-carotene and the isomers of carotenoids were described in the references [14,15]. The lower limit of detection for this method is 0.2 pmol for carotenoids. The injection volume of the sample was 20–50 µL.

Cooking methods

The yellow maize sample was obtained from Dr. Torbert Rocheford at the University of Illinois at Urbana-Champaign. The maize was sealed and stored at –12°C until cooking and analysis. For each cooking method, the amount of maize flour, water, other ingredients, temperature, duration of cooking, and pre-cooked and final weight of the cooked product were recorded. The cooking was done in the Metabolic Research Unit kitchen at the Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University.

1) Sadza preparation

Sadza is the Shona language name for a cooked grain meal that is the staple food in Zimbabwe. Sadza is Zimbabwe's version of the stiff porridge or dumpling common in sub-Saharan Africa [16]. Sadza in appearance is a thickened porridge. The most common form of sadza is made with white maize (Mealie-Meal). This maize meal is referred to as hupfu in Shona. Sadza is always eaten with a meat or vegetable soup or stew or sauce. The equivalent of Sadza is Ugali in east Africa, Nshima in Zambia, and pap or isitshwala in South Africa.

Sadza was prepared by adding maize flour to boiling water and stirring until a thick gel was formed. The cooking time was 30 minutes and the cooking temperatures were around 100° C. Sadza for this study was prepared using the common recipe in Zimbabwe and southern Africa [16].

2) Porridge preparation

Porridge was prepared using the sadza recipe, with modification to the water-to-maize flour ratio used. To boiling water, maize flour was added and stirred to form a semi-fluid paste and was left to boil for 30 minutes. Porridge

used the same amount of water as used for sadza but less than half the amount of maize flour [16].

3) Muffin preparation

The recipe for making corn muffins was adapted and modified using only maize flour, salt, sugar, and butter [17]. After mixing the ingredients, the dough was molded into muffin cups and baked for 25 minutes at 450°F.

4) Snack (Mangai) preparation

Dry maize kernels were boiled in water for 1 hour until the kernels were soft, and the water was drained. In this study, 200 yellow maize kernels were boiled in 500 mL.

Statistical analysis

The Student's *t*-test for unpaired data with unequal variance was used to determine differences between mean carotenoid levels obtained by extraction with and without saponification. The *t*-test was also used to determine the differences in the mean values of carotenoid content of yellow maize obtained by each different cooking method compared to uncooked yellow maize flour. P values less than 0.05 were considered significantly different. SAS 9.1 (SAS Institute, NC) statistical software used for data analysis.

Results

Effect of saponification

Three yellow maize genotypes, Orange isolation 200s TTR, BC Orange DEX C17, and DE SUP C17 X BC Orange were analyzed for the effect of saponification on carotenoid content. Results showed that most carotenoid values were either significantly lower ($p < 0.05$) with saponification or no difference was detected in the two methods (Table I). In one case, lutein values were decreased with saponification, and in two cases, zeaxanthin values were decreased with saponification. Cryptoxanthin also showed lower values with saponification. All-*trans* β-carotene values were all lower with saponification. Only 13-*cis* β-carotene showed significantly higher values with saponification in two of the three samples.

Carotenoid composition and content of yellow maize

Based on the results shown in Table II, extraction without saponification was used to evaluate the carotenoid content of 46 lines of yellow maize. The major carotenoids found in these yellow maize lines were lutein, zeaxanthin, total cryptoxanthin (α+β), all-*trans* β-carotene, 9-*cis* β-carotene, and 13-*cis* β-carotene. Lutein, zeaxanthin, and all-*trans* β-carotene are the major carotenoids in yellow maize. These carotenoids were identified by the retention times and photodiode array (PDA) spectra that matched with their respective standards. All the 36 yellow maize genotypes analyzed contained lutein, zeaxanthin, and all-*trans* β-carotene (Table II). The range of lutein content was 0.88–41.85 μg/gram dry weight. For zeaxanthin content the range was 0.74–30.68 μg/gram. The range for all-*trans* β-carotene was 0.05–16.79 μg/gram.

Effect of cooking on carotenoid content of yellow maize

The effect of cooking on yellow maize carotenoid content of KUI Synthetic yellow maize line is shown in Table III. The concentrations of all of the carotenoids except cryptoxanthin were increased by cooking the yellow maize as

Table I: Effect of saponification on the carotenoid concentration in yellow maize

Maize line	Orange isolation 200s TTR		BC Orange DEX C17 (μg/g dw)		DE SUP C17 X BC Orange	
Saponification	Without	With	Without	With	Without	With
Lutein	6.19 ± 0.80	7.13 ± 1.75	7.56 ± 1.35	7.18 ± 1.80	8.69 ± 1.22*	5.75 ± 0.22
cis-zeaxanthin	0.84 ± 0.11*	0.49 ± 0.20	0.45 ± 0.29	0.50 ± 0.21	0.84 ± 0.13*	0.30 ± 0.02
Zeaxanthin	7.78 ± 1.03*	3.31 ± 0.92	3.85 ± 0.73	3.32 ± 0.95	4.51 ± 0.66*	3.33 ± 0.15
cis-lutein	0.20 ± 0.02	0.24 ± 0.02	0.25 ± 0.10	0.25 ± 0.07	0.28 ± 0.04*	0.28 ± 0.01
Cryptoxanthin	1.60 ± 0.21*	1.03 ± 0.08	0.86 ± 0.31	1.01 ± 0.06	1.49 ± 0.39*	0.58 ± 0.04
13-*cis* β-carotene	1.18 ± 0.35	1.68 ± 0.22*	1.29 ± 0.79	1.57 ± 0.20*	2.62 ± 0.49*	0.90 ± 0.08
All-*trans* β-carotene	2.68 ± 0.41*	2.27 ± 0.21	2.37 ± 0.99*	2.28 ± 0.16	3.58 ± 0.82*	1.91 ± 0.10
9-*cis* β-carotene	1.16 ± 0.23*	1.08 ± 0.08	0.93 ± 0.47*	1.08 ± 0.08	1.62 ± 1.60*	0.67 ± 0.05

Values are means of eight independent analyses ± SD
* Statistically significant at $p < 0.05$ as compared between saponification and no saponification in each maize line.

Table II: Carotenoid concentration of 36 yellow maize genotypes

Genotype	Major carotenoids concentration (average values of two independent analysis) µg/g dry weight					
	Lutein	Zeaxanthin	Cryptoxanthin	13-*cis* β-carotene	*trans* β-carotene	9-*cis* β-carotene
1. A.T.V.C.155-2-2-1-2-1-B-B	41.85	15.10	4.96	3.76	10.18	2.11
2. A.T.Z.T.V.C.45-B-1-1-2-1-B-B-B-B	12.37	30.68	8.80	2.88	3.67	2.59
3. G9B C1 TSR-12P-1P-2P-3P-1-4-1-1-B-B-B-B	9.22	17.95	8.71	1.44	3.19	2.38
4. A.T.Z.T.V.C.58-B-1P-1-1-4-B-B-B-B	9.17	19.41	2.80	1.62	2.32	1.73
5. G9B C0 R.L.23-1P-2P-3-2P-1P-B-B-B-B-B-B	4.61	4.09	0.43	1.39	4.91	2.34
6. (G9B C1 TSR 8P-3P-2P-1-1P X (A)[1]	16.83	18.95	7.47	3.24	3.83	4.06
7. (P88 C1 F30-2-2-1 X P88 C0 F23-5-1-1) X(B)[2]	22.73	4.77	1.32	3.10	3.99	2.70
8. A.T.Z.T.V.C.82-1-3-1-1-1-B-B-B-B	16.77	20.07	5.86	2.69	2.55	1.29
9. P88ASDT C2 F83-1-2-1-2-2-B-B	6.40	8.97	3.88	1.16	2.34	1.56
10. AM.T.Z.T.V.C.48-B-3-1-1-4-B-B-B	7.75	9.46	1.97	1.52	1.17	0.30
11. 05H 291 2X292	6.35	7.63	0.65	2.06	4.29	1.20
12. CML 290-B	1.55	13.32	117	0.50	0.95	0.15
13. KUI3-B-B	0.88	14.28	1.18	0.65	0.99	0.21
14. CML 52-B	4.10	16.33	3.47	1.19	2.03	0.46
15. KUI12007-B-B	0.99	20.30	1.48	0.89	1.24	0.26
16. TZ1118-B-B	4.56	14.29	1.08	0.63	1.03	0.18
17. POB445-57-2-1-B-B-B-B	3.75	7.28	0.54	0.48	1.46	0.24
18. O5H 291 3X292	13.23	12.97	1.75	2.71	5.02	1.47
19. O5H291 5X292	3.43	4.51	0.77	1.18	1.70	0.57
20. 05H 287-4X288	27.59	3.58	ND	8.32	13.84	3.64
21. 05H 288-1X287	13.20	2.31	ND	5.92	8.87	2.31
22. 05H 288-3X287	22.86	4.28	ND	7.49	13.15	2.82
23. BC-ORANGE	4.47	4.24	0.77	1.43	1.55	0.78
24. SAPH R30	3.40	7.39	1.81	1.57	2.09	0.69
25. 05h 294-1	13.25	11.24	0.00	2.42	4.45	1.24
26. 05H 294-2	14.32	13.04	1.72	2.54	5.74	1.60
27. 05H 294-4	11.56	13.78	2.09	2.26	5.51	1.44
28. 04 157X263-1	10.24	2.15	ND	1.88	1.87	0.91
29. 05H287-6X288	19.95	2.43	ND	5.55	10.02	2.10
30. 04263x157-1	9.75	1.71	0.00	2.19	1.96	1.16
31. 04 4132x8101	2.63	6.16	1.25	1.14	1.65	0.89
32. KUI Synthetic	4.38	30.48	2.78	2.33	3.54	0.91
33. Orange Isolation 200s	6.19	7.78	1.59	1.18	2.68	1.16
34. BC orange DE exp C17	7.56	3.85	0.86	1.29	2.37	0.93
35. DE SUP C17XBC Orange	7.13	3.31	1.03	1.68	2.27	1.16
36. DEexp × C17	17.81	5.15	ND	3.48	16.79	3.55

Maize lines sources: 1–17 International Maize and Wheat Improvement Center (CIMMYT) in Mexico, 18–35 University of Illinois Urbana, 36 – ARS USDA Houston Texas – Dr. Michael Grusak.

[1] (G9B C1 TSR 8P-3P-2P-1-1P XG9B C1 TSR 12P-1P-2P-1-1P-6) 47-1-1-1-2-2-B
[2] [(P88 C1 F30-2-2-1 X P88 C0 F23-5-1-1) X (P88 C1 F30-2-2-1 X P88 C1 F30-2-2-1)] 1-3-1-1-1-1-5-B
[5] ND = not detected

sadza, whereas only lutein, *cis*-lutein, cryptoxanthin, all-*trans*-β-carotene and 9-*cis* β-carotene were increased by making porridge. When mangai was prepared, *cis*-lutein and the isomers of β-carotene were increased, while zeaxanthin and cryptoxanthin decreased. The baked muffin preparation resulted in losses up to 70% in all-*trans* β-carotene, 13-*cis* β-carotene, and 9-*cis* β-carotene zeaxanthin. Cryptoxanthin loses were around 50%, but an increase in *cis*-lutein. The concentration of lutein, *cis*-lutein, zeaxanthin, 13-*cis* β-carotene, all-*trans* β-carotene, and 9-*cis* β-carotene in cooked sadza were significantly higher as compared to those in raw uncooked maize flour ($p < 0.05$). There was a 31% increase in lutein, 12 % for zeaxanthin and 22.6% for all-*trans* β-carotene content. There was no significant change in the levels of cryptoxanthin between cooked (sadza) and uncooked maize flour ($p > 0.05$). The same pattern was observed in cooked porridge in which there were significantly higher concentrations of lutein, *cis*-lutein, and cryptoxanthin, all-*trans* β-carotene, and 9-*cis* β-carotene ($p < 0.05$). There were significantly

Table III: Effect of cooking on carotenoid contents in KUI Synthetic yellow maize line

			Carotenoid content µg/g of dried corn product						
Product	Temp °C	% corn/ gram	Lutein	*Cis*-Lutein	Zeaxan-thin	Cryptoxan-thin	13-*cis* B-C	t-B-C	9-*cis* B-C
Uncooked	20	100	3.96 ± 0.12	1.65 ± 0.08	32.85 ± 1.17	3.22 ± 0.14	2.08 ± 0.12	3.35 ± 0.10	0.62 ± 0.04
Sadza	100	34	5.16 ± 0.43*	2.39 ± 0.32*	36.63 ± 3.68*	3.24 ± 0.35	2.87 ± 0.32*	4.11 ± 0.43*	0.93 ± .010*
Porridge	100	20	4.46 ± 0.37*	2.55 ± 0.19*	32.94 ± 2.50	2.86 ± 0.23*	2.19 ± 0.24	3.77 ± 0.26*	0.77 ± 0.05*
Mangai	100	62	4.57 ± 1.52	3.20 ± 0.2*	29.35 ± 1.67*	2.97 ± 0.20*	2.48 ± 0.25*	3.67 ± 0.30*	0.86 ± 0.08*
Muffin	450	34	3.83 ± 0.27	2.62 ± 0.27*	25.78 ± 2.38*	1.65 ± 0.24*	0.52 ± 0.24*	0.92 ± 0.39*	0.19 ± 0.09*

Values are means of eight independent analyses ± SD.
Each maize product value was compared with those in uncooked maize flour.
* $p < 0.05$ results significantly different from uncooked yellow maize flour using one-factor ANOVA with Dunnett's multiple-comparison test.

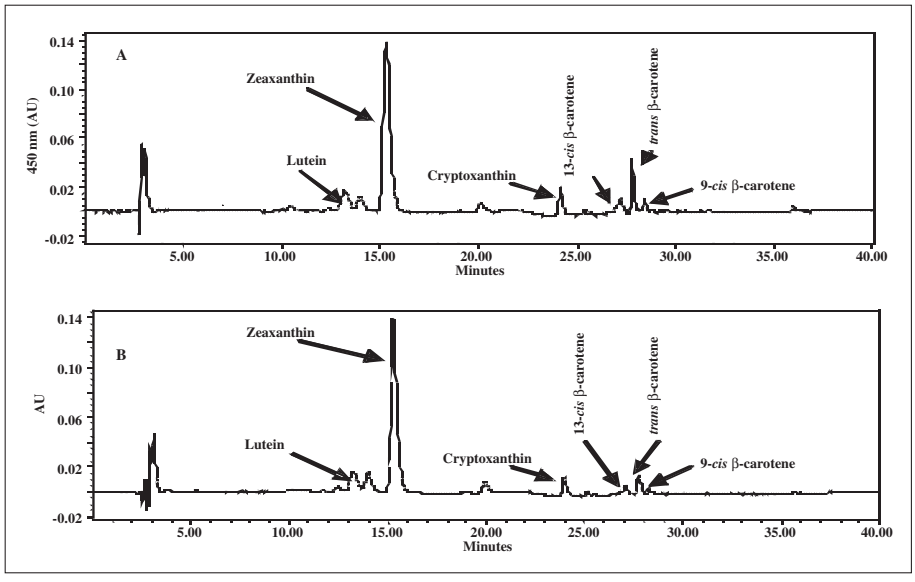

Figure 1: HPLC chromatogram of uncooked yellow maize (A) and baked yellow maize muffin (B) (KUI Synthetic)

higher concentrations of zeaxanthin and 13-*cis* β-carotene. In maize snack (mangai) there were also significantly higher concentrations of *cis*-lutein, 13-*cis* β-carotene, all-*trans* β-carotene and 9-*cis* β-carotene. Baked muffin contained the least amount of carotenoids with losses of up to 70% for β-carotene and its isomers (Figure 1).

Discussion

The current study indicates that extraction saponification yields lower carotenoid values compared to those extracted with without saponification. Other investigators reported that the direct solvent extraction method presents an alternative technique to saponification for analysis of vitamins and β-carotene [18]. Loss of total carotenoid content during saponification has been reported previously in the literature [19, 20]. In the current study the mean difference between β-carotene values obtained with saponification and without saponification was about 0.97 µg/gram. The most sensitive compounds to alkaline treatments are xanthophylls, particularly the epoxycarotenoids [20]; other studies showed that α-carotene, β-carotene, and β-cryptoxanthin resist saponification damage [21, 22]. In this study saponification resulted in significantly lower values in all-*trans* β-carotene, 9-*cis* β-carotene, and cryptoxanthin. The β-carotene values were at least 30% higher without saponification than with saponification. High alkali concentration during saponification may produce greater losses of carotenoids because of emulsion and soap formation [19]. Another study confirmed our results where numerous saponification procedures differing in the concentration of KOH, times, and temperatures found that under mild conditions the saponification step

caused only slight modification of α- and β-carotene, but lutein was significantly lost [7, 20]. Alkali saponification and enzymatic hydrolysis effects on the total carotenoid concentration of Costa Rican crude palm oil was evaluated and findings showed greater concentrations of carotenoids using enzymatic hydrolysis [19]. Our study results confirm that lutein, zeaxanthin, and other dihydroxycarotenoids are also reduced by the saponification process due to destruction. According to Riso and Porrin, carotenoid extraction with saponification is the most efficient procedure for removing chlorophylls and unwanted lipids and for hydrolyzing carotenol esters [7]. In our study we observed different effects of saponification in the three yellow maize lines. That is, DE SUP C17 X BC Orange maize line had exclusively less carotenoid values with saponification compared to Orange isolation 200s TTR and BC Orange DEX C17 yellow maize lines, and this may be because they are different genotypes. Since no ester forms of some of the carotenoids were detected, it can be speculated that these differences could be due to the food matrix of these maize genotypes. Other factors such as moisture content, protein content, and oil content could have played a role in the differences in the saponification values. Further research is required in this area.

Lutein is the predominant carotenoid in leaves, green vegetables, and some yellow flowers. While zeaxanthin is a minor carotenoid in most fruits and vegetables, it is the major pigment in yellow maize [24]. A similar carotenoid composition as the current study was observed in other studies [25, 26]. The carotenoid composition and content of yellow maize genotypes analyzed in this study are similar to the range of the major carotenoid content observed in yellow maize genotypes in Illinois and in fourteen Hungarian yellow maize landraces [5, 26]. Lutein and zeaxanthin may be protective in eye diseases because they absorb damaging blue light that enters the eye [27], thus protecting against diseases such as age-related macular degeneration [1]. The high β-carotene yellow maize lines can be used in interventions aimed at alleviating vitamin A deficiency (VAD) in developing countries where it is a public health problem. An estimated 2.8 million preschool-age children are at risk of blindness from VAD, and the health and survival of 251 million others are seriously compromised [28]. In some countries with a high incidence of VAD, the *per capita* maize consumption is very high with 411 g/day in Zambia, 135 g/day in Nepal, and 293 g/day in South Africa [3]. In these countries maize can be a very good vehicle to supply daily doses of vitamin A. Biofortified maize adequately maintained vitamin A status in Mongolian gerbils and was as efficacious as β-carotene supplementation; therefore in populations consuming maize as a staple food, using yellow instead of white maize could dramatically improve vitamin A status [6].

In general, the stability of dietary carotenoid is a concern due to the instability of carotenoids with multi-unsaturated double bonds. To understand the stability that may affect the biopotency of the maize carotenoids, the effect of various means of cooking on the levels of carotenoids in raw and cooked (microwaved, boiled, steamed, stewed) green vegetables and tomatoes has been extensively studied. Our study confirmed previous reported results that boiling did not alter the carotenoid profile in vegetables, but the amounts of carotenoids quantified were higher when compared to those in raw samples [13, 29]. In our study we observed an increase in the concentration of carotenoid content in all the cooking methods except baking compared to raw uncooked maize flour. Khachik *et al* showed that conventional blanching and cooking resulted in a significant ($p < 0.05$) increase in the concentration of carotenoids in the cowpea, peanut, and pumpkin leaf [12]. In another study they showed that while the epoxycarotenoids were somewhat sensitive to heat treatment, lutein and hydrocarbon carotenoid survived the heat treatments [12]. Young evaluated the effects of freezing, thawing, cooking, and drying on carotene retention of carrots, broccoli, and spinach by comparing the relative differences in carotene retention among these vegetables [29]. It has been shown that cooking does not affect ?? carotene content and cooked samples contained 2.0, 2.9, and 1.2 times more carotene than the respective dehydrated vegetables and the destruction of carotene was relatively lower when initial concentrations were low [31]. This could be a result of easy extraction of carotenoids due to the breakdown of the food matrix [30]. The losses of carotenes in the muffin could be due to heating effect at 450° F. Therefore, to prevent losses of carotenoids, avoiding high temperatures such as those used in baking should be recommended.

The current study indicated that 1) yellow maize is an important source of provitamin A carotenoids, 2) extraction of carotenoids without saponification yields higher values compared to saponification, and 3) boiling does not decrease the carotenoid concentration in yellow maize but baking does. This information can help researchers in choosing proper cooking methods to increase the biopotency of carotenoids from yellow maize, and in educating consumers on the best cooking practices towards retention of yellow maize nutritional value.

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