Original Communication

Identification of Enzymatic Cleavage Products of β-Carotene-Rich Extracts of Kale and Biofortified Maize

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Abstract: Provitamin A carotenoids in plant foods provide more than 80% of vitamin A intake for people in developing countries. Therefore, the conversion efficiency of β-carotene to vitamin A is important, as it determines the effectiveness of plant foods as sources of vitamin A in humans. The objective of this study was to determine the effect of plant food antioxidants such as α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol and total γ-oryzanol on the cleavage of β-carotene in vitro. Rat intestinal mucosa post mitochondrial fractions were incubated with β-carotene-rich extracts of kale and biofortified maize for an hour at 37°C. Rat intestinal mucosa post mitochondrial fractions were also incubated with β-carotene in the presence of either α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol or γ-oryzanol for 60 min at 37°C. The β-carotene cleavage products were extracted and analyzed by an HPLC equipped with a C18 column at 340nm and 450nm. When β-carotene alone was incubated without intestinal mucosa homogenate (control), no cleavage products were detected. When β-carotene alone was incubated with intestinal mucosa homogenate, β-apo-13-carotenone, β-apo-14-carotenal, retinal, retinol and retinoic acid were formed. However, incubation of β-carotene with either α-tocopherol, γ-tocopherol or α-tocotrienol resulted in a 10 fold inhibition of β-apo-14-carotenal and β-apo-13-carotenone formation. Antioxidant rich biofortified maize extract incubated with postmitochondrial fraction produced less β-apo-13-carotenone compared to the kale extract. These results suggest that antioxidants inhibit the cleavage of β-carotene and the formation of excentric cleavage products (β-apo-13-carotenone, β-apo-14-carotenal).

Keywords: Biofortification, kale, maize, vitamin A, BCMO1, cleavage, β-carotene, antioxidants

Introduction

Vitamin A deficiency (VAD) has always been a public health problem worldwide [1]. Since the late 1990s, many countries in south Asia and sub-Saharan Africa (SSA) had no improvement and some had deterioration in vitamin A status such that by 2013, the prevalence of VAD was 20% or higher in 56 countries, and was more than 40% in three south Asian and 40 SSA countries [2]. Vitamin A is an essential nutrient required by the body for cell differentiation, embryonic development, immune function, growth and vision [3]. VAD is characterized by increased risk of blindness, morbidity and mortality [4]. VAD is caused mainly by inadequate dietary intake of foods of animal origin (rich in preformed vitamin A) because they are expensive [5]. As a result, poor people in developing countries obtain more than 80% of their vitamin A intake from plant based foods (provitamin A carotenoids) which are easy to grow and readily available. The vitamin A value of plant food is determined by efficiency of conversion of provitamin A carotenoids to vitamin A in the body. The bioconversion of provitamin A carotenoids is affected by factors such as diet quality, age, nutritional status, vitamin A status, the food matrix, and food processing among other factors [6]. Many plant foods may not be very good sources of vitamin A for many poor people to meet their Recommended Daily Allowance (RDA) [7, 8]. Therefore, it is important to understand factors that can increase the bioconversion of plant food provitamin A carotenoids to vitamin A in humans.

It has been shown that the bioconversion of β-carotene (in oil) to vitamin A varies from 2 to 1; to 16 to 1; by weight, in humans [9]. Human studies also show wide variations in the bioconversion of β-carotene to vitamin A with conversion factors of 12 to 1 and 26 to 1 by weight observed for fruits and green vegetables respectively [10, 11]. Much better β-carotene bioconversion were observed in cereal grains with conversion factors of 3.2 to 1 by weight in biofortified maize and 3.8 to 1 by weight in golden rice [12, 13]. These studies showed that the food matrix or the constituents of a food are important in determining the bioconversion of β-carotene to vitamin A in humans. Biofortified maize and rice are known to be very high in fat soluble antioxidants such a tocopherols, tocotrienols and ferulic acid esters which may influence the bioconversion of provitamin A β-carotene to vitamin A. This may seem implausible but our previous study demonstrated that in the presence of α-tocopherol, β-carotene was converted exclusively to retinal by the 15, 15-dioxygenase enzyme and in the absence of α-tocopherol, β-apocarotenoids were formed [14]. However, the role of other forms of vitamin E such as γ-tocopherols and tocotrienols, which are abundant in many provitamin A rich foods, on β-carotene cleavage needs further investigations. The mechanisms of provitamin A β-carotene cleavage to vitamin A is of public health importance.

It is known that in humans, the bioconversion of β-carotene to vitamin A takes place mainly in the intestine [15]. The central cleavage pathway involves the metabolism of β-carotene at the central double bond (15, 15′) to produce retinal by β-carotene 15, 15′-dioxygenase (BCMO1) [E.C.888990988] [14]. Several studies reported exclusive central cleavage of β-carotene to produce retinal, which can be converted to retinol and retinoic acid respectively [16–19]. There are conflicting reports on origins and fate of retinal in central cleavage of β-carotene with some studies reporting that it is converted to retinoic acid, thereby acting as an intermediate β-carotene cleavage product [20, 21]. The excentric cleavage of β-carotene is known to produce mostly β-apo-carotenoids which are mostly devoid of vitamin A activity [22, 23]. The formation of such products, β-apo-13-carotenone and β-apo-14-caronal, from β-carotene incubation with intestinal mucosa homogenates from human, monkey, ferret and rat was established [24, 25]. It was previously thought that apocarotenoids were formed exclusively in vitro from β-carotene auto-oxidation [26]. However, these β-carotene excentric cleavage products have been detected in humans [27]. The conclusive evidence for excentric cleavage pathway was provided by the discovery of an enzyme that cleaved β-carotene at 9, 10 double bonds to produce β-apo-10-carotenal and β-ionone [28]. Further observations from in vitro studies suggest that excentric cleavage occurs mostly under oxidative conditions especially when antioxidants are limiting as in smoking and under oxidative stress conditions [29]. Evidence from in vitro studies showed that radicals from lipoxygenase enzymes attack β-carotene excentrically forming excentric cleavage products [11, 14, 30 and 31]. One famous study showed that β-carotene supplementation increased the risk of lung cancer and total mortality among smokers. It was suggested that the pro-oxidant environment in smokers led to excentric cleavage of β-carotene forming reactive apocarotenoids [32, 33]. These findings also highlight the importance of understating mechanisms of β-carotene cleavage beyond vitamin A nutrition in developing countries.

Some evidence emerged showing beneficial effects of antioxidants on the central cleavage of β-carotene, forming vitamin A exclusively. In staple foods such as biofortified maize, rice, sweet potato and green vegetables, β-carotene co-exists with potent antioxidants such as vitamin E, phenolic compounds and γ-oryzanol, a ferulic acid ester [34–36]. Currently, there is a dearth of information on the effect of antioxidants found in these staple green vegetables and maize on the cleavage of β-carotene to vitamin A. It is worth determining how antioxidants inherent in provitamin A biofortified maize and kale affect the cleavage of their β-carotene to vitamin A. If these antioxidants increase the yield of vitamin A from provitamin A, then plants foods which are locally accepted can play a leading role towards sustainable reduction of VAD in sub-Saharan Africa. Given the limitation of using human subjects for these kinds of investigations, the use of rat intestinal mucosa homogenate model is practical and has been shown to yield interesting leads. However, the rat intestinal model has limitations because the efficiency for cleaving carotenoids is much higher in the rat intestine than in humans. Another aspect is that human subjects may have different abilities for conversion of carotenoids into retinol, due to the genetic variability in β-carotene metabolism (polymorphisms in genes for the proteins involved in the metabolism seems to be responsible for the less efficient cleavage in some individuals) [37]. However, the rat intestinal mucosa model may give valuable insights to the study of β-carotene cleavage in humans. Therefore, this study was designed to test the hypothesis that antioxidants found in plant foods inhibit excentric cleavage of β-carotene using rat intestinal mucosa homogenate to yield more centric cleavage products.

Materials and Methods

Chemicals

All HPLC solvents were HPLC grade and were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All-trans-β-carotene (≥97% purity), all-trans-retinoic acid, all-trans-retinal, dithiothreitol (DTT), Hepes, Tricine, EDTA (ethylenediamine-tetraacetic acid), Tris buffer, sodium taurocholate, α-tocopherol and γ-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Alpha-tocotrienol and γ-tocotrienol standards were purchased from Cayman Chemicals (Ann Arbor, Michigan). Gamma-oryzanol standards were purchased from Wako Chemicals (Richmond, VA, USA). The standards of β-apo-13-carotenone, β-apo-12-carotenal and β-apo-14'-carotenal were gifts from Hoffman-La Roche Inc. (Basel, Switzerland).

Tissue preparation

The use of rat models in this study protocol was approved by the Animal Care and Use Committee at Tufts University. The preparation of intestinal mucosa homogenate and incubation with β-carotene were described previously [14, 18]. Briefly, the upper half of the intestine was washed with ice-cold isotonic saline (0.85% NaCl), and the intestinal mucosa homogenate was then gently scraped off with a glass cover and homogenized on ice in a test-tube with a Brinkmann Polytron homogenizer (Westbury, NY, USA) with 50 mM HEPES buffer (weight: volume 5 1:4), pH 7.4, containing 1.15% KCl, 1 mM EDTA, and 0.1 mM DTT. A post nuclear fraction was prepared by centrifuging the intestinal homogenate at 800 x g for 30 min. The post nuclear fraction was centrifuged further at 10,000 x g for 1 h at 4°C to remove particulate matter and some organelles to obtain the post-mitochondrial fractions which were used in the experiments. The resulting supernatant solution's protein concentrations of the protein fractions were determined using the BCA (bicinchoninic acid) Protein Assay (Pierce Co.; Rockford, IL, USA).

Incubation of rat intestinal mucosa post mitochondrial fractions with β-carotene treatments

The β-carotene standard was purified by eluting it in an open column with aluminum oxide with hexane as previously published [38]. The peak purity was confirmed by an HPLC and used for the incubations immediately. The β-carotene incubation with intestinal homogenate procedure was previously described [14]. Briefly, the standard incubation mixture contained 1 mg protein, 15 μM of β-carotene, 0.1 M Tricine buffer, pH 8.0, 6 mM sodium taurocholate, and 0.5 mM DTT in a total volume of 400 μL. The protein fraction was pre-incubated with cofactors at 37°C in a shaking water bath for 5 min. After the pre-incubation, the enzyme reaction was started by adding 80 μL of β-carotene solubilized in aqueous Tween 40 to 320 μL of the incubation mixture. The 80 μL of β-carotene solubilized in aqueous Tween 40 was also added to incubation mixtures containing 0.1mM of either α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol and γ-oryzanol standards. The controls were incubated without β-carotene, or with β-carotene but without the protein fraction. Fresh kale and biofortified yellow maize samples were extracted for carotenoids and other fat soluble components such as vitamin E and γ-oryzanol as previously described [12, 39]. The biofortified yellow maize extract contained 100μg of β-carotene, 60μg α-tocopherol and 130μg γ-tocopherol and 3mg total γ-oryzanol content. The kale extract contained 100μg of β-carotene and no detectable vitamin E and γ-oryzanol compounds. The β-carotene-rich kale lipid extract and biofortified yellow maize extract were dried separately under a gentle nitrogen stream and was reconstituted in Tween40. After the pre-incubation, the enzyme reaction was started by adding 80 μL extracts of kale β-carotene and biofortified β-carotene. Control vials were run lacking either β-carotene or the protein fraction. Incubations of β-carotene with the rat intestinal mucosa homogenates were conducted in triplicates. All experimental procedures were carried out under red light.

HPLC Analysis

The HPLC analysis, mobile phase and gradient elution program were previously published [14]. Retinyl acetate was added as internal standard and the incubation mixture was extracted with 3ml of chloroform: methanol (2:1, v/v), followed by 3ml of hexane. The mixture was centrifuged for 10 min at 800 x g at 4°C. The chloroform and hexane layers were evaporated to dryness under a gentle stream of nitrogen, and the residue was reconstituted by addition of 100μL of ethanol which was then transferred to an HPLC vial. 70μL of the reconstituted sample was injected into the HPLC system. The HPLC system consisted of a Waters Corporation (Milford, MA, USA) 2596 pump, Waters 2996 Photodiode Array Detector (PDA), Waters Empower2 data acquisition and analysis software. The HPLC system was equipped with a Pecosphere-3 C18 4.6mm x 83 mm cartridge column (Perkin-Elmer, Inc.). Carotenoids, retinoids,

vitamin E and γ-oryzanol were detected at 450, 340, 292 and 325 nm wavelengths respectively. The standards of β-apo-14-carotenal, β-apo-12-carotenal, β-apo-13-carotenone, retinol, retinoic acid, and retinal, retinyl acetate were adequately separated using this method. The concentrations of β-carotene, β-apo-carotenal, β-apo-13-carotenone, retinol, retinoic acid and retinal were determined against known amounts of standards. Levels were corrected for extraction and handling losses by monitoring the recovery of the internal standards.

Statistical Analysis

All statistical analysis was conducted using Statistical Analysis Software (SAS) Inc. (North Carolina) version 9.3. Descriptive statistics were conducted and difference between treatments means were analyzed by ANOVA with the level of significance set at 0.05 with, the Bonferroni method was used to adjust the level of significance for these multiple comparisons.

Results

Incubation of kale and biofortified yellow maize extracts with rat intestinal homogenate

When biofortified yellow maize extracts (containing 100μg of β-carotene, 60μg α-tocopherol and 130μg γ-tocopherol) were incubated with rat intestinal mucosa homogenate, the cleavage products formed were retinoic acid, β-apo-13-carotenone, retinal and β-apo-12-carotenal (Figure 1). Extracts of kale containing 100μg β-carotene were also incubated with rat intestinal mucosa homogenate the β-carotene cleavage product were retinoic acid and 13-apo-13-carotenone (Figure 1). There was no significant difference in the amount of retinoic acid formed from kale and biofortified yellow maize. However, there was more β-apo-13-carotenone produced in the kale incubation mixture as compared to the biofortified yellow maize mixture.

Incubation of β-carotene standard with rat intestinal homogenate in the presence of antioxidants

When β-carotene standard was incubated without the protein fraction (post mitochondrial fraction of rat intestinal mucosa homogenate), as expected there were no detectable retinoid peaks identified. When the protein fraction or the post mitochondrial fraction of rat intestinal mucosa homogenate was incubated alone without β-carotene, again as expected no β-carotene cleavage products were detected (Figure 2). When the post mitochondrial fraction of rat intestinal mucosa homogenate was incubated with β-carotene alone, it produced several peaks that were identified as β-apo-13-caroteneone, β-apo-14-carotenal, retinal, retinol, and retinoic acid (Table 1) (Figure 2). However, there were more excentric cleavage products (β-apo-13-caroteneone, β-apo-14-carotenal) than central cleavage products (retinal, retinol, and retinoic acid).

When β-carotene was incubated with rat intestinal mucosa post mitochondrial fraction homogenate in the presence of 0.01mM total γ-oryzanol, it produced several peaks that were identified as β-apo-13-caroteneone, β-apo-

Table 1. Cleavage products formed from β-carotene incubated with rat intestinal mucosa homogenate with or without an antioxidant

Treatment	β-carotene incubation cleavage products (ng)				
	Retinoic acid	β-apo-13-carotenone	Retinol	Retinal	β-apo-14-carotenal
β-carotene*	0.55 (0.03) ^{B}	30.7 (1.77) ^{C}	3.85 (0.17) ^{G}	7.48 (2.06) ^{H}	10.59 (2.01) ^{J}
BC + α-TP	0.43 (0.17) ^{B}	2.09 (0.10) ^{D}	3.56 (0.70) ^{G}		
βC + γ-TP	0.72 (0.32) ^{AB}	3.05 (0.52) ^{D}	4.02 (1.39) ^{G}		
βC + α-TT	0.79 (0.20) ^{AB}	3.9 (1.24) ^{DE}	3.27 (0.16) ^{G}		
βC + γ-TT	1.15 (0.02) ^{A}	6.0 (0.40) ^{E}	4.59 (0.51) ^{G}	3.54 (0.72) ^{I}	
βC + γ-Oryzanol	0.40 (0.16) ^{B}	16.5 (0.54) ^{F}	4.31 (0.31) ^{G}	4.55 (0.89) ^{IH}	5.28 (0.35) ^{K}

Letters in superscript show statistical differences between treatments for each cleavage product, with the same letters showing no significant differences ($p > 0.05$), and different letters showing significant difference ($p < 0.05$). The values are results of three independent analysis. In parenthesis are the standard deviations. *no antioxidant added

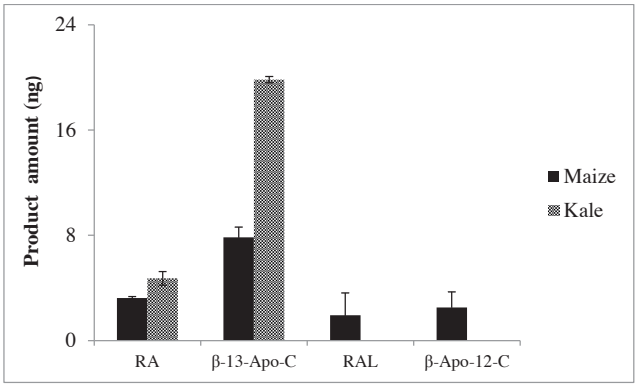

Figure 1. The cleavage products formed from β-carotene-rich extracts of kale and biofortified yellow maize incubated with rat intestinal mucosa homogenate. [Retinoic acid (RA), β-apo-13-carotenone (β-apo-13-car), Retinal (RAL) and β-apo-12-carotenal (β-apo-12-car)]

14-carotenal, retinal, retinol, and retinoic acid (Table 1). More excentric cleavage products as β-apo-13-carotenone, β-apo-14-carotenal were produced compared to the central cleavage products retinal, retinol, and retinoic acid. These were the same products formed when β-carotene was incubated alone without antioxidants (Table 1); however, the difference was the concentrations or amounts of cleavage products formed. The presence of total γ-oryzanol only slowed the overall cleavage of β-carotene.

When β-carotene was incubated with rat intestinal mucosa post mitochondrial fraction homogenate in the presence of either α-tocopherol (0.01mM), γ-tocopherol (0.01mM) or α-tocotrienol (0.01mM) it produced mainly retinoic acid, retinol and β-apo-13-carotenone (Table 1).

However, amounts of β-apo-13-carotenone formed in the presence of α-tocopherol, γ-tocopherol or α-tocotrienol was nearly 10 fold low er than in cu bation of β-carotene without antioxidants (Table 1). This indicated significant inhibition of β-apo-13-carotenone formation by α-tocopherol, γ-tocopherol and α-tocotrienol respectively (Table 1). There were no significant differences in the amount of retinol formed between all the groups (Table 1). Retinal was only detected in β-carotene incubation in the presence of total γ-oryzanol, γ-tocotrienol and in the control β-carotene incubation with no antioxidants present (Table 1).

Discussion

A review of the published literature shows this is the first study in which extracts of plant foods have been incubated with intestinal homogenate to study β-carotene cleavage products. Methanol and THF solvent system were used to extract both amphiphilic and lipophilic antioxidants from the kale and biofortified yellow maize. When kale extracts were incubated with rat intestinal post mitochondrial fraction homogenate, the β-carotene cleavage products detected were retinoic acid and β-apo-13-carotenone (Figure 1). This can be explained by the fact that kale extracts did not contain any detectable levels of antioxidants such as vitamin E. Therefore, in the absence of potent antioxidants, β-apo-13-carotenone, an excentric cleavage product, was the major product formed. In contrast, when biofortified yellow maize extracts which contained significant concentrations of α-tocopherol, γ-tocopherol and γ-oryzanol were incubated with rat intestinal post mitochondrial fraction homogenate, the β-carotene cleavage products detected were retinol, retinoic acid, retinal and β-apo-13-carotenone and β-apo-12-carotenal. The excentric cleavage product β-apo-13-carotenone formed from maize extracts was less than that from kale (Figure 1). This may suggest that antioxidants in maize extract were able to inhibit excentric cleavage of β-carotene. This study finding suggests that antioxidants rich foods when consumed with β-carotene may prevent the excessive formation of excentric cleavage products. However, efforts such as biofortification may be required to boost the antioxidants in provitamin A carotenoid rich staple foods.

When β-carotene standard alone was incubated with rat intestinal post mitochondrial fraction homogenate as a control, the most abundant cleavage products formed were β-apo-13-carotenone and β-apo-14-carotenal as observed in other studies [14, 24]. These excentric cleavage products were confirmed *in vitro* from β-carotene incubation with intestinal mucosa homogenates from rat, ferret, monkey and humans [24]. Studies that used pure BCMO1 enzyme or purified post-mitochondrial fractions of intestinal homogenate for β-carotene incubation reported retinal as the only β-carotene cleavage product [16–19]. In our study, crude or unpurified rat intestinal post-mitochondrial homogenates were used which produced a variety of cleavage products including retinal, retinol and retinoic (Table 1) [21, 23 and 24]. This is not surprising because crude intestinal homogenate still contained enzymes such as aldehyde dehydrogenases, NAD+, and alcohol dehydrogenases that convert the retinal to retinol and retinoic acid [16–18]. Crude or unpurified rat intestinal post-mitochondrial homogenates better reflect the intestinal cell cytosolic conditions where β-carotene cleavage takes place in the presence of these redox compounds. We can therefore imply that under cytosolic conditions the cleavage of β-carotene produces a variety of products which may include retinol, retinal, retinoic acid, and apocarotenoids. Therefore, increasing the physiological concentration of antioxidants such as α-tocopherol may lead to more central cleavage products of β-carotene to yield more vitamin A active compounds.

When β-carotene standard alone was incubated with rat intestinal mucosa post mitochondrial fraction homogenate more cleavage products were formed and in higher quantities (Table 1) (Figure 2). When β-carotene was incubated with rat intestinal mucosa post mitochondrial fraction homogenate in the presence of antioxidants such as α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol and γ-oryzanol standards, the amounts of excentric cleavage products (β-apo-13-carotenone and β-apo-14-carotenal) formed was significantly reduced compared to the β-carotene incubation alone. This result confirms our previous findings that the efficiency of the conversion of β-carotene to retinal was reduced to 1/10 by omitting α-tocopherol in the β-carotene incubation mixture [14]. In this study the absence of antioxidant α-tocopherol resulted in excentric cleavage of β-carotene by free radicals formed by lipoxygenase enzymes to form β-apo-carotenoids [14] and this was also confirmed by other similar studies [30, 31].

There were no significant differences in the amount of retinol formed between different β-carotene incubations with or without antioxidants ($p<0.05$) (Table 1). This showed that the amount of retinol formed was homeostatically controlled. This suggests that the amount of substrate (β-carotene) was not a limiting factor during the enzymatic cleavage of β-carotene. Also retinal was not detected in β-carotene incubation with rat intestinal mucosa homogenate in the presence of α-tocopherol, γ-tocopherol and α-tocotrienol. This suggests that retinal, an intermediate product of β-carotene cleavage, was converted to retinoic acid faster in the presence of α-tocopherol, γ-tocopherol and α-tocotrienol. Also we observed that in the absence of vitamin E isoforms, β-apo-14-carotenal was detected, maybe as an intermediate product of excentric β-carotene cleavage. We also observed that the amounts and variety of β-carotene cleavage products was dependent on the type of antioxidant (Table 1). The largest β-carotene ex-

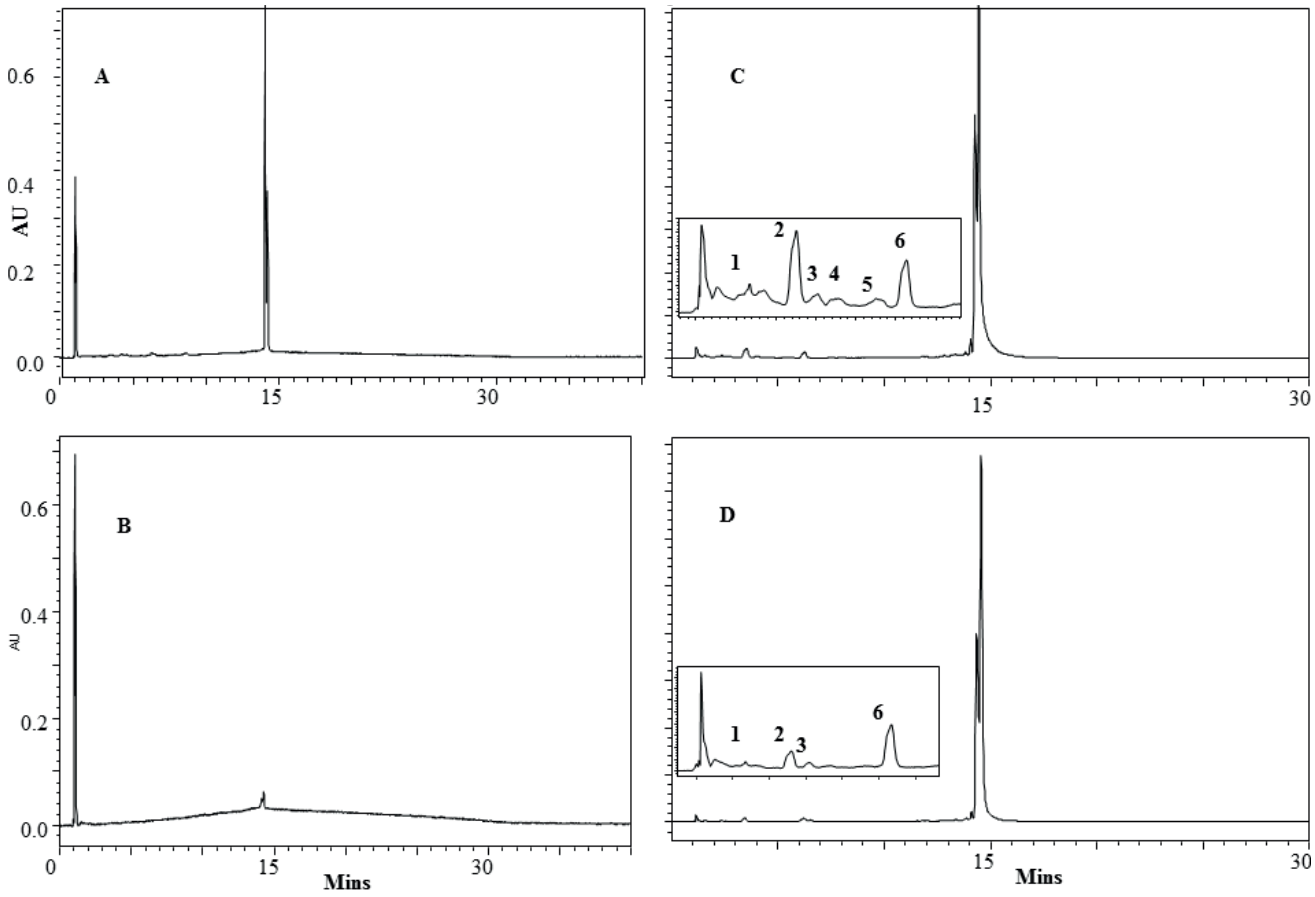

Figure 2. HPLC chromatograms at 340 nm
Panel A : β-carotene incubated without rat intestinal homogenate (control A)
Panel B: Rat intestinal homogenate without β-carotene (control B).
Panel C: β-carotene incubation products with rat intestinal mucosal homogenate in the absence of an antioxidant. Insert C: Peak1 = retinoic acid; Peak 2 = β-apo-13-carotenone; Peak 3 = retinol; Peak 4 = retinal;
Peak 5 = β-apo14-carotenal; Peak 6 = internal standard (retinal acetate).
Panel D Cleavage products from β-carotene incubated with rat intestinal homogenate in the presence of an antioxidant (γ-tocopherol). Insert D: Peak 1 = retinoic acid; Peak 2 = β-apo-13-carotenone; Peak 3 = retinol; Peak 6 = internal standard (retinyl acetate).

centric cleavage inhibition was observed with α-tocopherol followed by γ-tocopherol and α-tocotrienol in line with published observations on the antioxidant potencies of vitamin E forms [40]. These observations may be significant for human health because in vitro incubations of β-carotene with post-nuclear fractions of lung tissue from cigarette smoke exposed-ferret led to more excentric cleavage of β-carotene forming an abundance of β-apo-carotenals because of the free radical rich atmosphere [41]. A diet rich in antioxidants typical of fruit and vegetable diet may reduce β-carotene cleavage and β-apo-carotenoids formation among smokers [42, 43]. Recent research suggests that β-apo-carotenoids may exert powerful biological effects on mammalian cells by possible disruption of normal signaling through multiple ligand-activated nuclear receptors [44]. Some studies show that carotenoids act by blocking the translocation of nuclear factor κ B to the nucleus thus carotenoids thus inhibit the downstream production of inflammatory cytokines, such as interleukin-8 or prostaglandin E2 [45]. However, more research is required because the health implications of these signaling disruptions are not yet clear. Results from this study show the need to conduct in vivo studies to validate the effects of antioxidants on the cleavage of β-carotene at physiological concentrations. The limitation of this study is that it is based on an in vitro model using rat intestinal post mitochondrial fraction, the cleavage of β-carotene by rats is different from humans. Therefore, more research is necessary using human subjects.

Conclusion

This study found that extracts of biofortified yellow maize contain antioxidants that also inhibit excentric cleavage of β-carotene. Our study also showed that vitamin E isoforms inhibit excentric cleavage of β-carotene to form β-apo-13-carotenone and β-apo-14-carotenal. This finding is of significance to people in developing countries who depend on plant food β-carotene for their vitamin A requirements. Dietary messages can be tailored to promote intake of antioxidant rich foods such as nuts and peanut butters together with provitamin A carotenoid rich foods such as kale or biofortified maize in order to get the most vitamin A.

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