

In Vitro Bioaccessibility of β -Carotene in Orange Fleshed Sweet Potato (*Ipomoea batatas*, Lam.)

Mark L. Failla, $*,^{\dagger,\ddagger}$ Sagar K. Thakkar,[†] and Jung Y. Kim[‡]

[†]Interdisciplinary PhD Program in Nutrition and [‡]Department of Human Nutrition, The Ohio State University, Columbus, Ohio 43210

Substitution of white with orange fleshed varieties of sweet potatoes (OFSP) was recently shown to alleviate vitamin A deficiency in children in Africa. However, the relationship between β -carotene (BC) content of different cultivars of OFSP and its bioavailability is unknown. Here, we used the three phase (oral, gastric and small intestinal) *in vitro* digestion procedure to examine the bioaccessibility of BC from eight cultivars of boiled OFSP. All-*trans* BC (all-*E*-BC) was the only isomer of BC detected in raw roots for cultivars of OFSP with amounts ranging from 112 to 281 μ g/g. Boiling OFSP decreased all-*E*-BC content by 11% with conversion to 13-*cis* BC (13-*Z*-BC). The efficiency of BC micellarization during simulated digestion of boiled OFSP was only 0.6–3%. Addition of soybean oil (2% vol/wt) to boiled OFSP prior to *in vitro* digestion more than doubled partitioning of all-*E*-BC in the micelle fraction for all cultivars. The relatively poor bioaccessibility of all-*E*-BC was not a limitation of the *in vitro* model as micellarization was proportional to amount of OFSP digested from 0.5 to 3.0 g and minimally altered by increasing bile salt content during small intestinal digestion. Moreover, micellarization of all-*E*-BC from boiled fresh OFSP and commercially processed OFSP was significantly less than from carrots processed identically. These results indicate the need for further efforts to elucidate the basis for relatively poor bioaccessibility of BC from OFSP.

KEYWORDS: β -Carotene; bioaccessibility; orange fleshed sweet potato

INTRODUCTION

Although sweet potato (SP; Ipomoea batatas Lam.) is native to South America, varieties are now grown and consumed in most tropical countries. In addition to the roots, indigenous populations in Africa and Japan also ingest cooked leaves of SP as a source of dietary protein (1). SP serves as a staple food in a number of countries, and some populations typically consume white and yellow varieties of SP that lack adequate quantities of provitamin A (pro-VA) carotenoids. Substitution of orange fleshed SP (OFSP) for white fleshed SP (WFSP) has been shown to alleviate VA deficiency (2-4). For example, liver stores of VA were markedly increased in South African children 5-10 years of age who consumed 125 g of boiled OFSP daily for two months compared to age-matched individuals consuming WFSP (2). Similarly, Low et al. (3) reported that serum retinol was five times greater in toddlers fed OFSP than in children living in households ingesting WFSP. Consequently, educational programs have been implemented with target populations to facilitate dietary behavioral change and stimulate market demand for OFSP (5). Indeed, substitution of flour prepared from OFSP for wheat flour in the preparation of "golden bread" was reported to be widely accepted by populations in central Mozambique (4).

The bioavailability of carotenoids from a particular food is dependent on various factors that include the physicochemical state of the carotenoid in the product, style of cooking, and other components in the meal (e.g., fat and fiber content), as well as the nutritional status and health of the individual (6). Such factors can affect the transfer of the carotenoid from the food matrix to mixed bile salt micelles during the small intestinal phase of digestion, uptake of the carotenoids by intestinal absorptive epithelial cells, and incorporation of carotenoids and their cleavage products into chylomicrons for secretion into lymph for delivery to tissues (6, 7). Partitioning of the ingested carotenoids into mixed micelles and their uptake into absorptive cells is often referred to as bioaccessibility, whereas absorption and transfer to tissues represents bioavailability. We recently showed that bioaccessibility of β -carotene (BC) from various cultivars of cassava is highly correlated with BC content in boiled roots (8). However, the concentration of BC in cultivars of OFSP generally exceeds 50 μ g/g wet weight (9-11), whereas that in cassava is below 10 μ g/g wet weight (8, 12, 13). The relationship between BC content in roots of OFSP and the bioaccessibility of this pro-VA carotenoid has not been previously addressed. Below, we present data from studies using the in vitro digestion method to investigate the relationship between root content of BC in eight cultivars of OFSP and its bioaccessibility.

MATERIAL AND METHODS

Source of Sweet Potatoes. Eight cultivars of orange fleshed sweet potatoes (OFSP) and two cultivars of white fleshed sweet potatoes

^{*}Corresponding author. Mailing address: Department of Human Nutrition, The Ohio State University, 325 Campbell Hall, 1787 Neil Ave., Columbus, OH 43210. Tel: 614-247-2412. Fax: 614-247-5048. E-mail: failla.3@osu.edu.

Table 1. BC Content in Raw and Boiled Roots of Cultivars of Sweet Potatoes^a

| cultivar | µg/g wet weight | | | | |
|----------|---------------------------|-------------------|--------------------------|------------------|-----------------------|
| | raw SP, all- <i>E-</i> BC | all- <i>E-</i> BC | boiled SP, 13-Z-BC | 13-Z-BC, % total | apparent retention, % |
| | | Wh | ite Fleshed Sweet Potato | | |
| 1 | 0.80 ± 0.0 | 0.5 ± 0.0 | ND^b | | 62.5 |
| 2 | 0.88 ± 0.0 | 0.4 ± 0.0 | ND | | 45.5 |
| | | Orar | nge Fleshed Sweet Potato | | |
| 3 | 112.1 ± 1.6 | 99.6 ± 3.1 | 15.6 ± 0.3 | 13.5 | 102.8 |
| 4 | 126.0 ± 2.4 | 103.3 ± 2.7 | 17.2 ± 0.7 | 14.3 | 100.1 |
| 5 | 222.6 ± 5.0 | 200.7 ± 3.8 | 28.1 ± 0.3 | 12.3 | 102.7 |
| 6 | 223.6 ± 2.9 | 199.4 ± 1.6 | 25.1 ± 0.6 | 11.2 | 100.4 |
| 7 | 243.9 ± 11.2 | 226.8 ± 13.7 | 17.3 ± 1.2 | 7.1 | 100.8 |
| 8 | 250.3 ± 6.0 | 225.9 ± 4.3 | 32.5 ± 0.9 | 12.6 | 103.2 |
| 9 | 260.2 ± 4.5 | 243.8 ± 5.3 | 33.9 ± 1.0 | 12.2 | 106.5 |
| 10 | 280.8 ± 1.5 | 265.1 ± 4.3 | 24.1 ± 0.4 | 8.3 | 103.2 |

^a Data are means \pm SEM from six independent extractions from each cultivar. ^bND = not detected.

(WFSP) were received from Centro Internacional de la Papa (CIP) (International Potato Center), Peru. Upon arrival, roots were stored overnight at 4 °C and processed on the following day. Accession numbers of the different cultivars along with arbitrary numbers assigned in our laboratory for ease of labeling (listed in parentheses) were as follows: DLP 3163420269 [1]; 103082.43 [2]; 194568.1 [3]; 194512.7 [4]; 192033.50 [5]; 194583.2 [6], 189531.2 [7]; 102025.3 [8]; 189123.5 [9]; and, 190094.2 [10].

Preparation of Sweet Potato. SP were prepared in a manner similar to that described by van Jaarsveld et al. (2). Briefly, two roots of each variety were washed and cut longitudinally and the exposed flesh of halves was covered with aluminum foil before submerging in 10 parts (approximately 1 L) deionized (DI) water in a stainless steel pot. The pot was covered with a stainless steel plate to prevent evaporation, and samples were boiled for 30 min. Roots were cooled at room temperature for 10 min before distal and proximal tips (1 cm) were removed and boiled flesh was mashed. Representative samples were placed in 50 mL polypropylene tubes, blanketed with nitrogen gas, sealed and stored at -80 °C until analysis.

Simulated Digestion. Samples (0.5-3.0 g, but generally 1.0 g) of cooked SP, highly processed (pureed baby food) OFSP, and boiled and highly processed carrots were subjected to simulated oral, gastric and small intestinal phases of digestion as described by Thakkar et al. (8). Final concentrations of porcine bile extract, pancreatin, and lipase were 2.4, 0.4, and 0.2 mg/mL, respectively, during the small intestinal phase of digestion unless otherwise indicated. Aliquots of digesta collected after completion of the small intestinal phase of digestion and the filtered (0.22 μ m pores) aqueous fraction collected after centrifugation of digesta at 5000g for 45 min at 4 °C, and stored under nitrogen gas at -80 °C until analysis. Recovery of carotenoids after digestion is calculated by dividing the amount of carotenoids in digesta by the amount of carotenoids in boiled SP and multiplying the ratio by 100%. The efficiency of micellarization is calculated by determining the percentage of carotenoids transferred from the SP matrix into the filtered aqueous fraction. Such partitioning of BC is dependent on the presence of bile salts and therefore represents incorporation of the carotenoid into mixed micelles during the small intestinal phase of digestion (14).

Extraction and Analysis of Carotenoids. A representative sample of mashed raw or cooked SP was weighed (0.3 g wet weight) and placed in a 15 mL polypropylene tube. After addition of 75 μ g of Sudan I in absolute ethanol as recovery standard (*15*), 3 mL of ice cold (4 °C) tetrahydrofuran (THF) containing 0.1% butylated-hydroxytoluene (BHT) was added. Tubes were placed in ice bath and samples sonicated (probe sonicator, Vibra Cell, model VC 130, Sonic and Materials Inc., Newton, CT) for 1 min under a stream of nitrogen gas. Tubes were then centrifuged at 2000g for 5 min at room temperature, and the supernatant was transferred to a glass vial. Samples were identically extracted at least two additional times until the remaining residue was colorless. The pooled solvent was evaporated under a stream of nitrogen gas, and the film was reconstituted

in 5 mL of mobile phase (methyl *tert*-butyl ether:methanol, 1:1) with 1 mL aliquoted for HPLC analysis. Pilot experiments demonstrated that this miniextraction procedure yielded results that were not significantly different from the larger scale method of Kimura et al. (*16*). Extraction efficiency for raw and cooked SP was $98.1 \pm 0.27\%$ as assessed by recovery of Sudan I.

Aliquots (1 mL) of digesta and micelle fraction were mixed with 4 mL of THF:hexane (1:1) mixture containing 0.1% BHT after addition of 2 μ g of Sudan I in absolute ethanol. The mixture was processed identically as described above for raw and cooked SP. Extraction efficiency for digesta and micelle fractions was 95.9 ± 0.45%.

A Waters 2695 separation module and a Waters 2996 photodiode array detector were used to separate and quantify carotenoids. Separation of carotenoids was achieved at 25 °C using a Waters YMC Carotenoid S-5 C₃₀ reversed-phase column (4.6 mm × 250 mm; particle size, 5 μ m). The mobile phase consisted of methanol and ammonium acetate 1 mol/L (98:2) [solvent A] and methyl *tert*-butyl ether [solvent B] with a gradient flow rate of 0.8 mL/min to 1.0 mL/min. The injection volume was 20 μ L, and carotenoids were eluted using the following solvent gradient: 0–10 min, 80% A; 11–20 min, 60% A; 21–25 min, 40% A; 26–30 min, 80% A. Compounds in eluate were identified by their retention time and spectral profiles compared to published characteristics and AUC for all*trans* BC (all-*E*-BC) standard. Quantification of carotenoids was achieved by comparison of AUC of carotenoids in a sample with a standard curve obtained from a pure all-*E*-BC.

Statistical Analysis of Data. A minimum of three independent extractions and digestions were made for each cultivar in an experiment, and each experiment was replicated once to generate six independent observations for each cultivar of SP. Statistical analyses were performed using SPSS (version 14.0, SPSS Inc., Chicago, IL). Means were compared using one way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test. Linear regression analysis was done to compare the total amounts of BC partitioned into micelle fraction when OFSP was digested with and without oil. Pearson's coefficients were calculated for comparison of BC incorporated into micelle fraction when digested at 0.5, 1.0, and 2.0 g with 2% soybean oil. All data are expressed as mean \pm SEM, and statistical significance was set at the level of P < 0.05.

RESULTS

Carotenoid Composition of Cultivars of SP. WFSP contained $< 1 \mu g$ of BC/g wet weight, whereas BC content of roots from the eight cultivars of OFSP ranged from 112 to 281 $\mu g/g$ wet weight (**Table 1**). All-*E*-BC was the only isomer of BC detected in raw cultivars of SP (**Figure 1**). Although several minor peaks having retention times and spectral characteristics indicative of *Z* isomers of zeaxanthin and zeaxanthin epoxides were detected, lack of pure standards prevented unequivocal identification. Moisture



Figure 1. A representative chromatogram of carotenoid extracted from raw and boiled cultivars of OFSP (cultivar 3) is shown. All-E-BC by comparison to elution profile and spectrum of a pure standard. Sudan I was added to OFSP prior to extraction as a recovery standard.

Table 2. Quantity of Total BC and Isomers Partitioned into Micelles during Simulated Digestion of 1.0 g of Boiled OFSP without and with 2% Soybean Oil^a

| cultivar | bioaccessible BC: μg of micellarized BC/g wet weight | | | | | | | | |
|----------|--|------------------------|------------------------------|------------------------|-----------------------|-----------------------|--|--|--|
| | total BC | | boiled SP, all- <i>E</i> -BC | | boiled SP, 13-Z-BC | | | | |
| | without oil | with 2% oil | without oil | with 2% oil | without oil | with 2% oil | | | |
| 3 | $6.4\pm0.9\mathrm{b}$ | $13.9\pm1.5\mathrm{a}$ | $3.0\pm0.2b$ | 9.8 ± 1.2 a | 3.4 ± 0.7 | 4.1 ± 0.3 | | | |
| 4 | $5.5\pm1.4\mathrm{b}$ | $8.8\pm1.5a$ | $3.1\pm0.4\mathrm{b}$ | $6.4\pm0.9\mathrm{a}$ | 2.4 ± 0.8 | 2.4 ± 0.5 | | | |
| 5 | $5.8\pm0.9\mathrm{b}$ | $11.5 \pm 1.5 a$ | $3.2\pm0.5\mathrm{b}$ | $7.8\pm0.6a$ | 2.6 ± 0.4 | 3.7 ± 0.8 | | | |
| 6 | $7.8\pm1.2\mathrm{b}$ | $16.3\pm1.5\mathrm{a}$ | $3.3\pm0.3\mathrm{b}$ | $10.5\pm0.7\mathrm{a}$ | 4.5 ± 0.9 | 5.8 ± 0.7 | | | |
| 7 | 6.3 ± 0.5 b | $12.4\pm0.8\mathrm{a}$ | $3.1\pm0.1\mathrm{b}$ | $8.5\pm0.7\mathrm{a}$ | 3.2 ± 0.4 | 3.9 ± 0.1 | | | |
| 8 | $11.9\pm1.2\mathrm{b}$ | $20.7 \pm 1.1 a$ | 5.1 ± 0.6 b | $12.4\pm0.5\mathrm{a}$ | $6.8\pm0.5\mathrm{b}$ | $8.3\pm0.5\mathrm{a}$ | | | |
| 9 | $3.2\pm1.3\mathrm{b}$ | $19.8\pm0.4\mathrm{a}$ | $1.4\pm0.1\mathrm{b}$ | $12.5 \pm 0.1 a$ | 1.8 ± 0.3 b | $7.3\pm0.3\mathrm{a}$ | | | |
| 10 | $9.1\pm0.2\text{b}$ | $13.8\pm0.9\text{a}$ | $3.2\pm0.1b$ | $7.6\pm0.4a$ | 5.9 ± 0.1 | 6.2 ± 0.5 | | | |

^a Data are means \pm SEM from six independent simulated digestions of each cultivar of boiled OFSP. The presence of different letters in parentheses within the respective columns indicates significant (P < 0.05) differences between either total amounts of BC or each of the isomers in micelles generated during digestion of the cultivars of boiled OFSP without and with oil.

content in raw cultivars of SP averaged $73.9 \pm 1.37\%$ with a range of 69–80%. Boiling for 30 min did not significantly (P > 0.05) affect either total content of BC or moisture content of OFSP (**Table 1**). However, boiling OFSP for 30 min decreased the content of all-*E*-BC by 11.0 ± 0.27% with the majority of the lost isomer converted to 13-*Z*-BC. As the primary purpose of this study was to investigate the bioaccessibility of BC from SP containing different amounts of BC, the remainder of the study focused on the OFSP cultivars.

Recovery of BC during Simulated Digestion of Boiled OFSP. Apparent recovery of all-*E*-BC after simulated digestion of OFSP was 79.3 \pm 0.23% (range, 65–91%). Recovery of all-*E*-BC in digested OFSP was not significantly altered when soybean oil (2% vol/wt) was added to samples before digestion, although the apparent mean recovery of 13-*Z*-BC was significantly higher (*P* < 0.05) for samples containing soybean oil. It is unclear if this increase in the content of 13-*Z*-BC in digesta was due to greater stability and/or enhanced isomerization of all-*E*-BC during digestion of OFSP containing oil.

Micellarization of BC during Simulated Digestion. When 1.0 g of OFSP was digested without added oil, total amount of BC (all-*E*-BC + 13-*Z*-BC) in the micelle fraction ranged from 3.2 to $11.9 \,\mu$ g/g wet weight for the eight cultivars of OFSP (**Table 2**). The quantities of all-*E*-BC partitioning in micelles generated during simulated digestion of the boiled roots ranged from 1.4 to 5.1 μ g/g wet weight for the cultivars, whereas that for 13-*Z*-BC ranged

from 1.8 to 6.8 μ g/g wet weight. This represented 0.6–3.0% and 5.3–24.5% of the quantities of all-*E*-BC and 13-*Z*-BC in the boiled cultivars, respectively. The efficiency of micellarization, *i.e.*, the relative amount of BC transferred from the food matrix to micelles during digestion, was relatively independent of the amount of BC in boiled OFSP (y = 0.0097x + 4.87) (Figure 3). There was an inverse relationship between the percentage of total all-*E*-BC in the boiled OFSP cultivars transferred to micelles generated during simulated digestion ($R^2 = -0.74$). In contrast, the percentage of 13-*Z*-BC that partitioned in micelles during digestion of the various cultivars was not correlated with content of this isomer in boiled OFSP ($R^2 = 0.04$).

The quantity of total BC incorporated into micelles during digestion approximately doubled when soybean oil was added (2% vol/wt) to boiled OFSP prior to digestion (y = 0.0339x + 7.2) (**Table 2; Figure 2**). The promoting effect of oil was independent of cultivar. The increase was largely due to the more efficient micellarization of all-*E*-BC as micellarization of 13-*Z*-BC was only slightly increased when samples contained oil.

We considered the possibility that the seemingly poor micellarization efficiency of BC during simulated digestion of OFSP was due to inherent limitations of the *in vitro* model. The relationship between the amount of OFSP digested and the amount of BC partitioning in micelles with constant concentrations of enzymes and bile extract was tested. The quantity of BC micellarized was directly proportional to the amount of boiled OFSP (cultivar 3)



Figure 2. Quantity of total BC partitioned into micelle fraction during simulated digestion of 1.0 g of boiled OFSP without and with 2% soybean oil. The numbers within circles and triangles indicate assigned numbers of OFSP cultivars listed in **Tables 1** and **2**. Data are means from six independent digestions of each cultivar of boiled OFSP without and with oil.

when 0.5–2.0 g with 2% soybean oil was digested. Correlation coefficients for total BC, all-*E*-BC and 13-*Z*-BC were 0.96, 0.95 and 0.97, respectively. Next, the amount of bile extract present during the small intestinal phase of digestion was increased from the standard amount of 2.4 to either 3.6 or 4.8 mg/mL. Doubling the concentration of bile extract increased transfer of all-*E*-BC and 13-*Z*-BC into the micelle fraction during simulated digestion by less than 20%. Collectively, these data indicate that the standard concentrations of enzymes and bile extract used during the small intestinal phase of simulated digestion of 0.3 g of OFSP from the different cultivars were not limiting factors for transfer of BC from boiled OFSP to micelles.

The possibility that the matrix of OFSP roots limits the bioaccessibility of BC also was examined by comparing the micellarization of BC from OFSP and carrot, another root food with similar concentration of BC. Fresh OFSP and carrots purchased at a local market were boiled for 30 min and mashed. Total BC content for the boiled OFSP and carrot were 156.1 \pm 9.12 μ g/g wet weight and 126.5 \pm 1.05 μ g/g wet weight, respectively. The carrot also contained 111.8 \pm 1.04 μ g of α -carotene (AC)/g and 15.1 \pm 0.22 μ g/g Z-BC. We and others have previously reported that the efficiencies of micellarization of BC and AC are similar during simulated digestion (14, 17). The efficiency of micellarization of all-E-BC during simulated digestion of boiled fresh OFSP with 2% soybean oil was $2.9 \pm 0.07\%$ and significantly less than micellarization of all-E-BC from carrots $(8.3 \pm 0.01\%, P < 0.05)$ (Figure 3). We also purchased highly processed OFSP and carrot (baby foods) that contained $257.0 \pm 3.75 \,\mu g$ of BC/g and $294.6 \pm 6.56 \,\mu g/g$ carotenes (67%) BC, 33% AC), respectively. The efficiency of micellarization of BC during digestion of baby food OFSP with 2% soybean oil was $7.2 \pm 0.65\%$, which was significantly (P < 0.05) less than that for baby food carrot (17.4 \pm 2.04% total carotenes; 18.3 \pm 0.97% all-*E*-BC; $28.4 \pm 0.39 \ Z$ isomers of BC; $15.9 \pm 1.29\%$ all-*E*-AC, respectively) (Figure 3). These data support the likelihood that the properties of the matrix of OFSP may limit the bioaccessibility of BC.

DISCUSSION

It has been shown that substitution of OFSP for WFSP in several African countries where SP is a dietary staple improves VA status (2-4). Transfer of carotenoids into mixed micelles



Figure 3. Efficiency of micellarization of all-*E*-BC on digestion of boiled fresh and highly processed OFSP and carrot in the presence of 2% vol/wt soybean oil. Data are means \pm SEM for three independent simulated digestions. Significant differences between the two respective sources of BC are indicated by the presence of different letters above errors.

during small intestinal digestion is required for delivery of these lipophilic compounds to the absorptive epithelium. The objective of our study was to investigate the relationship between the pro-VA content of various cultivars of OFSP and the micellarization of BC during simulated digestion. The simulated digestion model provides a cost-effective tool for screening the relative bioaccessibility of carotenoids and other fat soluble compounds in foods. Indeed, results obtained using the two phase (gastric plus small intestinal phases) model of simulated digestion model have been shown to be highly correlated (r = 0.90) to the more technically challenging and expensive studies for assessing carotenoid bioavailability in humans (17).

All-E-BC is the predominant isomer found in unprocessed carotene-rich plant foods such as OFSP. Processing can lead to degradation and isomerization of all-E to Z isomers of BC (12, 18, 19). We observed that boiling of OFSP for 30 min resulted in minimal loss and conversion of approximately 11% of all-E isomer of BC to 13-Z-BC. The stability of BC exceeded 80% when OFSP was digested in vitro, as previously reported for other vegetables (8, 20-23). We observed that efficiency of micellarization of all-E-BC during simulated small intestinal digestion of boiled and highly processed OFSP was rather poor (0.6-3.0%) in comparison to that of carrot (Figure 3) and other plant foods in the absence of exogenous lipid (8, 14, 23-26). As previously noted (27), micellarization of 13-Z-BC exceeded that of all-E-BC and the amount of the two isomers present in the aqueous fraction after digestion was actually similar. However, addition of a small amount of soybean oil (2% vol/wt) to OFSP before simulated digestion markedly increased the quantity of all-E-BC incorporated into micelles. The observation that micellarization is enhanced by the presence of minimal quantities of exogenous lipid during simulated small intestinal digestion is supported by other in vitro studies (21, 22, 28, 29). Moreover, dietary fat increases bioavailability of carotenoids and their metabolites by stimulating assembly and secretion of chylomicrons, the vehicle for transfer of carotenoids from enterocytes to peripheral tissues (30, 31).

The relatively poor *in vitro* bioaccessibility of BC in OFSP appears to be associated with the matrix of SP, since doubling the concentration of digestive enzymes and bile extract during the small intestinal phase did not markedly increase transfer of carotenoids to micelles. It is well established that multiple factors influence the bioavailability of carotenoids from foods: physico-chemical state (e.g., crystalline vs solubilized vs protein-bound),

subcellular localization (chloroplasts vs chromoplasts), tissue type (flower vs leaf vs root), processing (particle size, style of cooking), and other chemical components. Pectin, guar and wheat bran also decreased carotenoid bioavailability *in vivo* (*32*, *33*) and post-absorptive conversion and utilization of BC in chicks and Mongolian gerbils (*34*, *35*). However, Mills et al. (*31*) recently found that increasing the dietary content of soluble fiber from OFSP from 0.24 to 0.80% did not significantly affect bioefficacy of BC in Mongolian gerbils. Thus, the basis for the relatively poor efficiency of micellarization of BC during digestion of OFSP remains unclear. Determination of the physical form and subcellular localization of the pro-A carotenoid in SP merits investigation.

Three independent human intervention trials have demonstrated that OFSP serves as a good source of pro-VA to alleviate VA deficiency in developing countries (2-4). Despite the poor bioaccessibility of BC in our *in vitro* study, sufficient amounts of the carotenoid are bioavailable as VA status was improved in children fed OFSP instead of an equivalent amount of WFSP. As the amount of all-*E*-BC partitioning in micelles was not correlated with the amount in the eight cultivars of OFSP, we suggest that further efforts are needed to elucidate the basis for the poor bioaccessibility of BC in OFSP rather than increasing BC content.

In summary, BC content in cultivars of OFSP was not significantly altered by boiling, although there was partial isomerization all-E-BC to 13-Z-BC. The extent of micellarization of all-E-BC during simulated small intestinal digestion of boiled OFSP and pureed OFSP baby food was significantly less than that during digestion of similarly processed carrots. Addition of a small amount of soybean oil (2% vol/wt) to OFSP before simulated digestion markedly increased the quantity of all-E-BC incorporated into micelles. The lack of proportional increases in quantity of BC micellarized during digestion of boiled cultivars of OFSP with increased BC content is not due to limitations in digestive enzyme activity or quantity of bile extract during simulated digestion. Further investigation is needed to elucidate the basis for the relatively poor bioaccessibility of BC in OFSP and the lack of a positive relationship between the BC content of OFSP and the quantity of BC transferred to micelles during simulated digestion. Nevertheless, the high content of BC in OFSP is sufficient to offset the relatively poor bioaccessibility as evidenced by the efficacy of this staple food for maintaining adequate vitamin A status.

ACKNOWLEDGMENT

We thank Drs. Magnolia Ariza-Nieto and Ray Glahn at USDA/Cornell University for their assistance with transfer of the cultivars of sweet potatoes to The Ohio State University. We also thank Drs. Carolyn Gunther and Chureeporn Chitchumroonchokchai for critical review of the manuscript.

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Received February 5, 2009. Revised manuscript received September 21, 2009. Accepted September 25, 2009. Financial support for this project was provided by HarvestPlus and the Ohio Agricultural and Research Development Center (OARDC).