Carotenoids and β-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency

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Abstract

The present study, in line with a plant-food-based approach to address vitamin A deficiency, reports the analysis of total carotenoids, and trans- and cis-β-carotenes, in different varieties of raw and boiled orange-fleshed sweet potatoes (OFSP). Carotenoids were isolated using acetone-petroleum ether extraction followed by spectrophotometric determination. trans- and cis-β-Carotenes were analyzed by reversed-phase HPLC method using a mobile phase containing acetonitrile:methanol:2-propanol in the ratio of 85:15:33 with 0.01% ammonium acetate. Intra-varietal difference in carotenoids as well as trans- and cis-β-carotenes were noted in both the raw and boiled potatoes. Carotenoid content was found to be higher in the raw potatoes compared to the boiled samples from the same variety. Amongst the OFSP varieties, Kamalsundari (BARI SP-2) was found to contain the most carotenoids in both the raw and boiled samples. β-Carotene was significantly higher in the Kamalsundari and BARI SP-5 varieties. trans-β-Carotene was found to be the major carotenoid in all of the raw potatoes, but boiling was associated with an increase in cis-β-carotene and a decrease in the trans isomer. Kamalsundari and BARI SP-5 orange-fleshed sweet potatoes have the potential to be used as food-based supplements to reduce vitamin A deficiency.

1. Introduction

Children and pregnant women are more likely to suffer from vitamin A deficiency. The World Health Organization (WHO) reported that vitamin A deficiency (VAD) affects about 190 million preschool-aged children and 19 million pregnant women, mostly in Africa and South-East Asia (WHO, 2011). Nearly 44–50% of preschool children in South and Southeast Asia are affected by severe VAD (Akhtar et al., 2013). Among the South Asian countries, India has the highest prevalence of clinical and subclinical vitamin A deficiency, the prevalence being as much as 62% in preschool children (Suri & Kumar, 2015). In Bangladesh, the prevalence of sub-clinical vitamin A deficiency in the preschool-aged children is 20.5%, although in slum areas the prevalence is as high as 38.1% (National Micronutrients Status Survey 2011–12. icddr, UNICEF, GAIN, & Nutrition 2013).

Children begin their life with an urgent need for vitamin A. Infants (1–5 months of age) and preschool children (6–59 months of age) have increased need of vitamin A to support their rapid growth and to combat infection (WHO, 2011). Inadequate intake of vitamin A at this age can lead to vitamin A deficiency that, in turn, may cause night blindness and undermine growth and immune function. This also results in increased risk of morbidity and mortality, largely from measles, diarrhea and respiratory infections (Semba, 1999; Sommer, 2011; WHO, 2011; WHO, 2012). Children are at a higher risk of intestinal infestations and infections, which may impair absorption of vitamin A (WHO, 2014). It has also been suggested that maternal vitamin A deficiency may contribute to mother-to-child transmission of HIV (Semba et al., 1994). Neonates of vitamin A-deficient mothers are born with decreased vitamin A reserves (WHO, 2014). Breast milk is the only significant source of vitamin A for infants (Sommer, 2011) and infants fed little or no breast milk in early life are increasingly susceptible to infections (Akhtar et al., 2013).

Children in developing countries are at risk of consuming vitamin A deficient diets (Sommer, 2011). Unlike those in developed countries, who receive abundant preformed vitamin A (retinol) from animal foods (liver, eggs, milk and milk products), poor people living in third world countries rely on cheap dark green-yellow local vegetables and fruits for vitamin A. Owing to poor bioavailability (Akhtar et al., 2013; WHO, 2011), plant foods that provide β-carotene, in particular, are a substitute of preformed vitamin A.
More often than not, however, vitamin A deficiency in the preschool children and pregnant women of the third world countries is most likely attributable to diets deficient in vitamin A and/or β-carotene (Mills et al., 2009; Sommer, 2011).

Orange-fleshed sweet potato (OFSP) is an excellent source of the provitamin A β-carotene (Low et al., 2007), containing up to 276.98 μg per g (Tumuhimbise, Namutebi, & Muyonga, 2009). A 125 g serving of boiled OFSP can supply the daily requirement of vitamin A for preschool children and protect them from night blindness (Mitra, 2012; USAID, 2015). In addition to being rich in β-carotene, OFSP contains significant amounts of protein, fat, carbohydrate, dietary fibre, other micronutrients and some phytonutrients (Mills et al., 2009; Sweet potato, 2014). Therefore, orange-fleshed sweet potato is a staple food that can provide a supply of vitamin A and energy to people in resource-poor developing countries such as Bangladesh (Low et al., 2009; Mitra, 2012).

Bioavailability of β-carotene depends on multiple factors. Dietary fat is necessary for absorption and conversion of β-carotene to retinol (Lemmens et al., 2014; Mills et al., 2009). The retention and bioaccessibility of β-carotene determine its bioavailability (Bechoff et al., 2011). It has been documented that maceration and heat processing improve β-carotene bioaccessibility from orange-fleshed sweet potatoes, which is probably due to rupture of microstructure of plant tissue and subsequent release of nutrients from the complex food matrix (Bengtsson, Brackmann, Enejder, Alminger, & Svanberg, 2010; Tumuhimbise et al., 2009). Thus, with a view to exploring cheap plant food-based approach to combat vitamin A deficiency among the poor people of Bangladesh, the present study investigated orange-fleshed sweet potatoes for their content of total carotenoids and trans- and cis-β-carotene.

2. Materials and method

2.1. Reagents

The analytical grade acetone, petroleum ether, butylated hydroxytoluene (BHT) and HPLC grade acetonitrile and methanol were procured from Merck (Darmstadt, Germany). trans-β-Carotene and cis-β-carotene were procured from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Potato sample

Seven varieties of sweet potatoes comprising three orange-fleshed, three yellowish-cream fleshed and one white-fleshed potato variety were collected from the Tuber Crops Research Centre (TCRC) and Fisher Crops Research Centre (FRCRC) of Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh. The potatoes were harvested when they became matured at about four to five months; the average weight of each potato was 200 g. Characteristics of the potatoes are described in Table 1.

2.3. Sampling protocol

The Tuber Crops Research Centre (TCRC) developed orange-fleshed sweet potatoes for agriculture cultivation. According to the sampling protocol for analysis of nutrients in vegetables and fruits (Greenfield & Southgate, 2003; Roe, Pinchen, Church, & Finglas, 2013), six freshly harvested potatoes of each variety were collected from the cultivation field (TCRC of Bangladesh Agricultural Research Institute). After curing for at least one week, 3–4 potatoes were pooled to make three samples for each variety.

2.4. Preparation of analytical sample

2.4.1. Preparation of raw potato

The raw potatoes were cleaned with tap water before being rinsed with distilled water. After removing surface water with tissue paper, the sweet potatoes were air dried and weighed. These were peeled, quartered and cut into small cubes, which were ground in a food processor (Jaipan, JP-FM1100, Jaipan Industries Limited, Mumbai, India). The food processor was previously rinsed with sodium hypochlorite solution (2% in boiled water). Cleaning of food processor was done prior to processing of each potato variety. The sample processing operation was carried out rapidly to avoid enzymatic degradation.

2.4.2. Preparation of boiled potato

Cleaned whole sweet potatoes (3–4 in number) were put in a saucepan containing about 500 ml of boiled water (sufficient to immerse the potatoes) containing 1.5% fresh-squeezed lemon juice (to prevent oxidation), and boiled until the potatoes were almost edible. Water was drained off and the potatoes were kept at room temperature. The weight of the potatoes was recorded to calculate nutrient content under raw and boiled conditions. The boiled potatoes were then peeled to remove the skin, and cut into large chunks (5–7.5 cm) using a knife.

2.5. Analysis of carotenoids and β-carotenes

Analysis of carotenoids and β-carotenes in the sweet potatoes were carried out in the laboratory of the Institute of Nutrition and Food Science (University of Dhaka, BD) and the Nutritional Biochemistry Laboratory of International Centre for Diarrheal Disease and Research, Bangladesh (Dhaka, BD). Three analytical samples for each variety (raw and boiled) were analyzed for total carotenoids, and for trans- and cis-β-carotenes.

Table 1: Characteristic of potato variety collected from the Tuber Crops Research Centre (TCRC)*.

<table>
<thead>
<tr>
<th>Varieties of sweet potato with local name</th>
<th>Identifying colour</th>
<th>Carotene content (μg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BARI SP 1 (Tripti)OFSP</td>
<td>Skin-white, flesh-yellowish or cream</td>
<td>270</td>
</tr>
<tr>
<td>BARI SP 2 (Kamalasundari)OFSP</td>
<td>Skin-orange, flesh-deep orange</td>
<td>4500</td>
</tr>
<tr>
<td>BARI SP 3 (Daulatpurii)OFSP</td>
<td>Skin-white, flesh-deep white</td>
<td>Nil</td>
</tr>
<tr>
<td>BARI SP 4OFSP</td>
<td>Skin-orange, flesh-orange</td>
<td>630</td>
</tr>
<tr>
<td>BARI SP 5OFSP</td>
<td>Skin-deep yellow, flesh-orange</td>
<td>600</td>
</tr>
<tr>
<td>BARI SP 6OFSP</td>
<td>Skin-yellowish, flesh-cream</td>
<td>480</td>
</tr>
<tr>
<td>BARI SP 7OFSP</td>
<td>Skin-yellowish, flesh-yellowish</td>
<td>420</td>
</tr>
</tbody>
</table>

OFSP: orange fleshed sweet potato (Bhuiyan et al. (2008)).
YFSP: yellow fleshed sweet potato.
WFSP: white fleshed sweet potato.
CfSP: cream fleshed sweet potato.
2.5.1. Analysis of carotenoids content

Carotenoid content was determined using acetone-petroleum ether extraction followed by spectrophotometric analysis, as described by Rodriguez-Amaya, and Kimura (2004). Extraction of carotenoids was performed by grinding the raw or boiled potatoes in a mortar and pestle, filtration through a sintered glass filter under vacuum, and separation of the carotenoids from acetone into petroleum ether.

2.5.1.1. Carotenoids extraction. About 3 g potato was ground up with 1 g acid-washed sand in a mortar and pestle until finely mashed. Twenty ml cool distilled acetone and 100 μl BHT solution (0.01% BHT in acetone) was added to the potato and carefully mixed for about 3 min.

2.5.1.2. Filtration. The homogenate was transferred to a sintered glass filter, wrapped with aluminum foil, under vacuum. The acetone extract (orange color) was collected into the vacuum flask, also wrapped with black cloth. The mortar and pestle were rinsed with small amounts of acetone, which were added to filtrate. This was repeated until the homogenate became colourless.

2.5.1.3. Carotenoids separation. About 20 ml distilled petroleum ether was placed in a 250 ml separating funnel wrapped with black cloth. The coloured acetone extract was carefully added to the petroleum ether through a funnel, gently flowing down the wall of separating funnel. The vacuum flask was rinsed with 1–2 ml acetone. Then, 150 ml distilled water was added to the separating funnel (flowing down the funnel wall), the mixture was left undisturbed for 5–10 min to allow for separation into organic and aqueous layers. The aqueous layer, containing acetone, was discarded. This step was repeated for 3–4 times with 100 ml distilled water added each time until the residual acetone was removed. The petroleum ether extract in the separating funnel was collected, through a funnel containing small amount of anhydrous sodium sulfate on a filter paper, into a 25 ml volumetric flask covered with aluminum foil. The separating funnel was rinsed with about 2 ml petroleum ether using a pipette. The eluent volume was made up to 25 ml with petroleum ether. The flask was capped and gently mixed. For determination of the carotenoids, absorbance of the orange-coloured eluent was measured at 450 nm in a spectrophotometer (UV-1601, UV-Visible, Shimadzu, Tokyo, Japan). All the preparative and extraction procedures were performed in dim light and/or excluded light as described.

2.5.2. Analysis of carotenes

One milliliter of eluent was dried under nitrogen stream and stored at −20°C for determination of trans-β and cis-β-carotenes using reversed-phase HPLC (Shimadzu PC based Binary Gradient HPLC Prominance System with PDA Detector, SPD-M20A; Solvent delivery System, LC-20AT; LC Solution Multi Workstation Software). The nitrogen-dried carotenoids were reconstituted with mobile phase (acetoni-trile:methanol:2-propanol in the ratio of 85:15:33 with 0.01% ammonium acetate). Twenty-five microliter of reconstituted sample was injected into the VYDAC reverse phase C18 column (5 µm particle size) using the mobile phase flowing at 1.7 ml per min. The detector was set at 450 nm (SPD-M20A, Shimadzu Japan). The column was re-equilibrated with the mobile phase for at least five minutes before the next injection.

2.6. Data quality

The accuracy and precision of data were tested, in order to validate the method and determine data quality, by carrying out inter-laboratory analysis of carotenoids content in the raw Kamalasundary OFSP in the laboratories at the Institute of Nutrition and Food Science, University of Dhaka and at Nutritional Biochemistry section of the International Centre for Diarrhoeal Disease and Research, Bangladesh. No significant difference (p = 0.567) was found between the results (61.72 ± 2.27 µg per g edible, coefficient of variation = 3.68%; vs 62.51 ± 1.87 µg per g edible, coefficient of variation = 3.0%; 95% confidence level). Samples were analysed in triplicates for each OFSP.

2.7. Data analysis

SPSS (version 12.0 SPSS Inc, IL, USA) was used to analyse the data. Descriptive statistics were performed and values expressed as mean, standard deviation and percentage.

3. Results and discussion

Table 2 shows significant intravarietal difference in both total carotenoid content and percentages of trans- and cis-β-carotenes in the different varieties of sweet potato. Intravarietal differences were also documented by Liu, Lin, and Yang (2009).

3.1. Carotenoids in sweet potato

Total carotenoid content was higher in all the raw samples compared with corresponding boiled samples (Table 2). Similar finding have also been reported in a previous study by Tumuhimbise et al. (2009), where raw samples of OFSP were found to contain significantly more carotenoids than corresponding boiled samples. Lower concentrations in boiled sample might due to the loss of carotenoids

<table>
<thead>
<tr>
<th>Variety of sweet potato with local name</th>
<th>Raw sweet potato</th>
<th>Boiled sweet potato</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Carotenoid (µg/g)</td>
<td>Trans-β-carotene</td>
</tr>
<tr>
<td>BARI SP 1 (Tripi)</td>
<td>5.64 ± 0.56</td>
<td>83.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.89 ± 0.08</td>
</tr>
<tr>
<td>BARI SP 2 (Kamalasundari)</td>
<td>61.94 ± 2.37</td>
<td>86.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58.32 ± 0.47</td>
</tr>
<tr>
<td>BARI SP 3 (Daulatpuri)</td>
<td>1.02 ± 0.05</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>BARI SP 4</td>
<td>36.23 ± 1.67</td>
<td>76.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.29 ± 0.93</td>
</tr>
<tr>
<td>BARI SP 5</td>
<td>19.31 ± 0.78</td>
<td>96.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.24 ± 0.32</td>
</tr>
<tr>
<td>BARI SP 6</td>
<td>3.78 ± 0.17</td>
<td>84.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.41 ± 0.11</td>
</tr>
<tr>
<td>BARI SP 7</td>
<td>3.28 ± 0.27</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.62 ± 0.19</td>
</tr>
</tbody>
</table>

| ND: not done.                     |                                 |                                  |                                 |

VALUES: were expressed in mean ± standard deviation, and percentage.
during boiling, which was also reported by van Jaarsveld, Marais, Harmse, Nestel, and Rodriguez-Amaya (2006).

Among the seven varieties of sweet potatoes, total carotenoid content, in both raw and boiled samples, was highest in Kamalasundari (BARI SP-2OSP) (61.94 ± 2.37 μg per g vs 58.32 ± 0.47 μg per g, respectively), followed by BARI SP-4OSP (36.23 ± 1.67 μg per g vs 32.29 ± 0.93 μg per g respectively) and BARI SP-5OSP (19.31 ± 0.78 μg per g vs 18.24 ± 0.32 μg per g, respectively). The other varieties were found to have relatively low amounts of carotenoids. The Kamalasundari variety has also been reported to be rich in carotenoids by other investigators (Bhuiyan et al., 2008; Tumwegamire et al., 2014).

3.2. trans- and cis-β-carotene

The percentage of trans-β-carotene was noted as much higher than cis-β-carotene in all the raw potato samples. The boiled samples were found to contain more cis-β-carotene than trans isomer, which is in agreement with Tumuhimbise et al. (2009). In the raw sweet potatoes, the percentage of trans- and cis-β-carotene ranged from 76.56–96.49% and 3.50–23.44%, respectively, while in the boiled samples, percents of trans- and cis-β-carotene were in the range from 61.28 to 95.29 and 4.71 to 38.72, respectively (Table 2). These results indicate that boiling sweet potato decreases trans-β-carotene and increases cis-β-carotene. Previously, Thakkar, Kim, and Failla (2009) reported the boiling loss of trans-β-carotene to be 11% with concomitant generation of 13-cis-β-carotene in orange-fleshed sweet potato. It has also been documented elsewhere that boiling of sweet potato isomerizes trans-β-carotene to cis-β-carotene (Tumuhimbise et al., 2009), meaning the cis isomer is comparatively greater in boiled samples than in the raw potato. In general, cis-β-carotene content is very low or insignificant in sweet potato, although white- or yellowish-fleshed varieties have much more cis-β-carotene than the orange-fleshed variety (Liu et al., 2009).

4. Conclusion

The present study identified that BARI SP-2 (Kamalasundari) and BARI SP-5 orange-fleshed sweet potatoes contained significant content of β-carotene. Thus, these varieties could be used as a food-based supplement to combat vitamin A deficiency among the poor and nutrient-starved people of Bangladesh. The data generated in this study will be included in the food composition database for Bangladesh.

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References


