HPLC Determination of β-Carotene Content of Sweet Potato Cultivars and Its Relationship with Color Values

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The carotenoid composition of 25 sweet potato cultivars was investigated using high performance liquid chromatography (HPLC). Although an unidentified small peak was detected which was regarded as one of the carotenoids by the UV-vis spectrum, all the orange flesh cultivars in this research showed similar carotenoid composition, which consisted mostly of β-carotene. This predominance of β-carotene throughout the cultivars characterizes the carotenoid composition of the sweet potato. The orange flesh cultivar Benihayato contained 18.7 mg (100g, fresh weight basis) of β-carotene which could be compared with the U.S. cultivars. The average retinol equivalent of representative cultivars (Resisto, Benihayato, Santo Amaro, Caromex and Red Jewel) was 2.8, which equaled the maximum value of the carrot cultivars. However, no carotenoids were detected in yellowish-white flesh cultivars. Among orange flesh cultivars, the relationships between β-carotene content and color values were also studied. Excluding the cultivars having less than five mg β-carotene, the correlation coefficients between the color value (L*, a*, b*) and the β-carotene content were, respectively, –0.852, 0.891, 0.718. These data indicated the practical use of approximating the expected β-carotene contents from the color values.

KEY WORDS: Ipomoea batatas, β-carotene, HPLC analysis, color value.

Introduction

Carotenoids in foods have been extensively investigated in cereals (HEINONEN et al., 1989a), carrots (HEINONEN, 1990), loquat fruits (KON and SHIMBA, 1988) and many other foods (HEINONEN et al., 1989b; KHACHIK et al., 1991).

In the case of the sweet potato, approximately 90% of the carotenoid content of Goldrush and Centennial was determined to be β-carotene using column chromatographic separation (PURCELL, 1962; PURCELL and WALTER, 1968). Other reports described the constituent carotenoids of sweet potato roots as only β-carotene, although the cultivar names were not mentioned (BUSHWAY, 1986; KHACHIK and BEECHER, 1987). PICH (1985) reported that Centennial contained the most total carotenoids of 9.5 mg/100g based on the colorimetric determination of crude carotenoid extracts derived from four cultivars.

In Japan, there have been very few indigenous cultivars which have orange flesh. White flesh with white skin for starch production and yellowish-white flesh with red skin for table consumption have been the major cultivars. Recently, since the demand for processing use has increased, the orange flesh variety Benihayato was released in 1985. The total carotenoid of Benihayato was 11.6 mg/100g (fresh weight basis) which exceeded Centennial having 7.4 mg/100g (KUKIMURA et al., 1988). However, this total carotenoid content of Cen-

Received February 5, 1993.
Accepted June 14, 1993.
tennial differed to the value given in Picha’s study.

In the present paper, to shed some light on varietal differences in the carotenoid composition of the sweet potato storage root, we investigated 25 sweet potato cultivars grown under possibly uniform conditions. Moreover, in order to develop a rapid and simple evaluation method for practical purposes, we studied the relationships between β-carotene content and color values.

Materials and Methods

Reagents
Phytoene and γ-carotene were generously provided by F. Hoffmann LaRoche. Lycopene, α-carotene and β-carotene were purchased from Sigma.

Sweet potato material
The vines of 22 orange flesh and three yellowish-white flesh sweet potato cultivars were provided from the Laboratory of Sweetpotato Breeding, Kyushu National Agricultural Experiment Station. The materials were transplanted on May 16 without mulching in the experimental field (thick high-humic andosols) at Nishigoshi, Kumamoto. The entries were harvested on October 7, and two roots per each cultivar were sampled.

Determination of root flesh color
On the day after the harvest, all roots were washed and longitudinally cut in two. Immediately after cutting, the color value of the cut surface was directly measured using a color difference meter (Chroma Meter CR-200, Minolta, Japan). Color measurements were taken on two areas of each root and the results were averaged. The color was described based on the values of $L^*$, $a^*$ and $b^*$. The values of $a^*$ and $b^*$ define color on a two dimensional chromatic space, $a^*$ and $b^*$ indicating the green-red and blue-yellow axis, respectively, while $L^*$ is a measure of lightness (McGuire, 1992).

Extraction of carotenoids
Each cut storage root used for color determination was peeled and cut into small pieces for freeze-drying. The freeze-dried materials were milled until they passed through a 500 μm mesh sieve. The flour samples were stored at $-30^\circ$C until used for analysis. A 500 mg sample of the flour was extracted with 50 ml of an acetone-hexane solution (50:50, containing 0.1% BHT) in a volumetric flask and allowed to stand overnight in the dark. A twenty-ml aliquot was evaporated to dryness and redissolved with 1 ml of chloroform and filtered through a 0.50 μm membrane before HPLC determination. The saponification procedure was omitted because the procedure produced no change in the chromatographic profile.

HPLC determination
HPLC was performed on a JASCO (Japan Spectroscopic Co., Ltd) 800 PU series equipped with a UV-vis diode array detector MD-980. The system included a JASCO Finepak SIL C18 T-25 (4.6 mm × 25 cm) column. Solvent system A consisting of acetonitrile/methanol/THF (58:35:7) was pumped at a flow rate of 1 ml/min (Bushway, 1985). Solvent system B consisting of acetonitrile/dichloromethane/methanol (70:20:10) was pumped at a flow rate of 1 ml/min (Heinonen, 1990). The detection was set at 460 nm for lycopene, α, β-, and γ-carotene and 260 nm for phytoene. The running temperature was 30°C for both solvent sys-
\( \beta \)-Carotene content of sweet potato cultivars

Ten \( \mu l \) portions of both samples and the standard solution were injected. Identification of the peaks was performed with authentic standards based on their retention times and UV-vis spectra.

Results

Figures 1a and b show the typical chromatograms of standard solution (a) and sweet potato extract (b, Benihayato) using solvent system A. As shown in Fig. 1b, a small peak appeared which was likely to correspond to the retention time of lycopene. When used solvent system B for the identification of the small peak (data not shown), this peak did not correspond to lycopene. However, the UV-vis spectrum of this peak was distinctive of carotenoids which had absorption maxima at about 450 and 480 nm. Thus, this peak was not \( \alpha \)-, \( \gamma \)- carotene nor lycopene but was regarded as an unidentified carotene. Phytoene was not detected in either solvent systems.

Table 1 shows the \( \beta \)-carotene content using solvent system A. In our experiment, which was performed under uniform growth conditions and determination procedures, the \( \beta \)-carotene contents of 25 cultivars ranged from N.D. to 26.5 (mg/100g, fresh weight basis). The highest content of \( \beta \)-carotene was seen in SPV-61; Resisto, UC700, Benihayato, L-2-116 and L-4-89 had relatively high levels. The \( \beta \)-carotene content of Benihayato was higher than those of Red Jewel and Caromex but lower than that of Resisto. Thus, Benihayato is almost equivalent in \( \beta \)-carotene content to the U.S. cultivars. To the contrary, no carotenoids were de-
Table 1. β-Carotene content of 25 sweet potato cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Source</th>
<th>β-Carotene content¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPV-61</td>
<td>U.S.A.</td>
<td>26.5 (17.3, 35.6)</td>
</tr>
<tr>
<td>Resisto</td>
<td>U.S.A.</td>
<td>20.3 (11.9, 28.7)</td>
</tr>
<tr>
<td>UC700</td>
<td>Venezuela</td>
<td>18.9 (13.3, 24.5)</td>
</tr>
<tr>
<td>Benihayato</td>
<td>Japan</td>
<td>18.7 (14.2, 23.2)</td>
</tr>
<tr>
<td>L-2-116</td>
<td>U.S.A.</td>
<td>18.5 (17.3, 19.8)</td>
</tr>
<tr>
<td>L-4-89</td>
<td>U.S.A.</td>
<td>18.5 (14.1, 22.8)</td>
</tr>
<tr>
<td>Santo Amaro</td>
<td>Brazil</td>
<td>15.5 (12.9, 18.2)</td>
</tr>
<tr>
<td>Caromex</td>
<td>U.S.A.</td>
<td>14.9 (14.2, 15.5)</td>
</tr>
<tr>
<td>Red Jewel</td>
<td>U.S.A.</td>
<td>13.8 (13.2, 14.4)</td>
</tr>
<tr>
<td>Benihayato (mutant)</td>
<td>Japan</td>
<td>11.7 (8.3, 15.2)</td>
</tr>
<tr>
<td>Heart Gold</td>
<td>U.S.A.</td>
<td>9.9 (9.5, 10.4)</td>
</tr>
<tr>
<td>SPV-67</td>
<td>U.S.A.</td>
<td>9.8²)</td>
</tr>
<tr>
<td>AIP-587</td>
<td>Papua New Guinea</td>
<td>9.8 (5.3, 14.2)</td>
</tr>
<tr>
<td>AIP-326</td>
<td>Papua New Guinea</td>
<td>8.0 (4.0, 12.1)</td>
</tr>
<tr>
<td>F411</td>
<td>Indonesia</td>
<td>7.7 (6.8, 8.7)</td>
</tr>
<tr>
<td>Kyukeni114</td>
<td>Japan</td>
<td>7.5 (2.8, 12.2)</td>
</tr>
<tr>
<td>Unit 1 Porto Rico</td>
<td>U.S.A.</td>
<td>6.9 (5.4, 8.4)</td>
</tr>
<tr>
<td>Georgia Jet</td>
<td>U.S.A.</td>
<td>6.8 (4.9, 8.8)</td>
</tr>
<tr>
<td>W-36</td>
<td>U.S.A.</td>
<td>5.5 (–11.4, 22.4)</td>
</tr>
<tr>
<td>Okinawa100 (mutant)</td>
<td>Japan</td>
<td>2.3 (–5.5, 10.1)</td>
</tr>
<tr>
<td>SPV-43</td>
<td>U.S.A.</td>
<td>1.3 (0.5, 2.2)</td>
</tr>
<tr>
<td>PI208886</td>
<td>U.S.A.</td>
<td>1.1 (–2.1, 4.2)</td>
</tr>
<tr>
<td>Koganesengan</td>
<td>Japan</td>
<td>N.D.³)</td>
</tr>
<tr>
<td>Beniazuma</td>
<td>Japan</td>
<td>N.D.</td>
</tr>
<tr>
<td>Beniotome</td>
<td>Japan</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

¹) mg/100g (fresh weight basis), figures in parenthesis indicate the confidence interval at 95% level.
²) No replication
³) Not detected (<0.005 mg/100g)

Note: Each datum is a mean of at least two replicate HPLC injections. HPLC injection was performed on two roots each.

The percentages of the small peak area relative to the total carotenoid peak area (β-carotene peak plus the unidentified peak) were less than 10% for every cultivar (data not shown). Therefore, although we have not yet identified the carotenoid for the small peak, these data indicate that the carotenoid composition of the orange flesh sweet potato, consisting mostly of β-carotene, does not differ among cultivars. In the case of other vegetables, the carotenoid composition of carrots was found to be a mixture of α-, β- and

Discussion

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\(\beta\)-Carotene content of sweet potato cultivars

\(\gamma\)-carotenes and proportion of the three carotenes varied among cultivars. \(\beta\)-Carotene accounted for 44~79\% of the total carotene found in carrot cultivars (Heinonen, 1990). The most predominant carotenoid of tomato fruits was lycopene, and the carotenoid compositions also ranged widely among cultivars (Hirola, 1975; Lee and Robinson, 1980; Wann et al., 1985). In the present study, although the levels of \(\beta\)-carotene content ranged widely among cultivars, the carotenoid found in the storage roots was almost exclusively \(\beta\)-carotene. This simple carotenoid composition throughout all the orange flesh cultivars is noteworthy.

\(\beta\)-Carotene is well known as a most excellent carotenoid having an important biological function as pro-vitamin A. In carrot cultivars, the retinol equivalents (RE; a unit which indicates the nutritional efficiency of vitamin A, 0.167 × \(\beta\)-carotene + 0.083 × (\(\alpha\)+\(\gamma\)-carotene)) ranged from 1.2~2.3 (mg/100g) (Heinonen, 1990). On the other hand, the RE of sweet potato cultivars in this study ranged from 0.0~4.4, and the average RE of representative cultivars (Resisto, Benihayato, Santo Amaro, Caromex and Red Jewel) was 2.8, which equals the maximum value of the carrot cultivars. Furthermore, \(\beta\)-carotene contained in foods has been reported as an anti-oxidant (Burton and Ingold, 1984) and an anti-carcinogenic compound (Peto et al., 1981).

Therefore, some sweet potato cultivars which contain more \(\beta\)-carotene are judged to be superior to the other crops containing other types of carotenes. Considering the convenient culturing trait of the sweet potato, we conclude that the orange flesh cultivars provide a greater contribution to human health regarding these functions.

In contrast, yellowish-white flesh cultivars such as Koganesengan and Beniazuma did not have any significant amount of carotenes (lower than 0.005 mg/100g, fresh weight basis). Ac-
cording to the Standard Tables of Food Composition in Japan, it is recorded that white and yellowish-white flesh sweet potatoes contain 0.01 and 0.05 mg carotene contents, respectively. However, cultivar names and storage length of the materials were obscure. At all events, we can conclude that yellowish-white flesh cultivars immediately after harvest have only a negligible amount of β-carotene.

Regarding the relationships between the color values and β-carotene content among orange flesh cultivars (Figure 2; n = 22), the correlation coefficients between the color value \(L^*, a^*, b^*\) and β-carotene content were \(-0.885, 0.897,\) and \(0.810\), respectively. Excluding the cultivars having a β-carotene content of less than 5 mg (n = 19), the above correlation coefficients changed to \(-0.852, 0.891,\) and \(0.718\), respectively. Furthermore, regression coefficient of the color value \(a^*\) showed the largest variance ratio among three color values used in regression analysis (data not shown). The color value \(a^*\) had the closest relationship to β-carotene content. Hence, we can propose usefulness of colorvalue \(a^*\), as a rapid method of estimating the β-carotene content in breeding and as a quality control method in processing procedures.

Acknowledgement

We are grateful to F. Hoffmann LaRoche for their gift of carotene standards. We also thank the staff members of the Sweetpotato Breeding Laboratory of the Station for their generous supply of materials. This study was partially supported by a Grant-in-Aid from the Ministry of Agriculture, Forestry and Fisheries, Japan.

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Kanesho品種のβ-カロテン含量のHPLC分析および色彩値との関係

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Kaneshoは白色・黄色の肉色をした品種・系統が一般的であるが, 橙色の品種・系統もあり, それらはカロテノイドが豊富といわれている。カロテノイドは自然界に600種以上存在するといわれ, その中でもβ-カロテンは最も高いビタミンA活性を有する。また, カロテノイドは, 発ガンや老化と関係する活性酸素やフリーラジカルを消去し, その作用を制御することが, 近年報告されており, 注目される成分である。Kaneshoのカロテノイド組成についての品種間差は不明の部分が多いため, これを明らかにするために本実験を行った。また, 同時にその肉色を色彩色差計によって測定し, カロテノイド含量との関係を調査した。

橙肉色品種・系統22種および黄肉色品種3種を用いた。色彩測定は, 測定直前に塊根を半分に切断し, 切断面中央部2ケ所を色彩差計 (ミノルタCR-200) により行い, L*, a*, b*値で表示した。カロテノイド組成の測定は, 冷蔵乾燥カンナシ塊根粉末を溶媒 (ヘキサン: アセトン=1:1, 酸化防止剤としてBHT 0.1%) 抽出し, 乾燥しクロホルムで再溶解した試料を用いてHPLCによりおこなった。

全ての橙肉色品種・系統でβ-カロテノンピークが検出され, それ以外にはごくわずかな1つのピークが見られるだけであった（Fig. 1）。カロテノイド含量の品種間変異はきわめて大きかったが（Table 1）, 橙肉色カンナシ塊根のカロテノイド組成は, 全品種・系統を通じて大部分がβ-カロテノンによって占められていることが明らかとなった。これらは他作物（ニンジン・カボチャ・トマト）と比較した場合, 特筆すべき単純な成分組成であった。また, 主要な橙肉色カンナシ品種のβ-カロテノン含量はニンジン・カボチャの含量の数～十数倍に達した。さらにビタミンA効力を示すレチノール当量としては, 高レチノール当量のニンジンと比較して同等以上であった。また, 国内唯一の高カロテノイド品種であるベニハヤトのβ-カロテノン含量は18.7 mg/100 g生で, 米国種とは同等の含量を示した。一方, 黄肉色品種3種は, HPLCの検出限界 (0.005 mg/100 g) 以下であった。四訂食品成分表ではカンナシのカロテノン含量は黄色肉種で0.01, 黄色肉種で0.05 mgと記載されているが, 品種, 記載日数・条件が不明である。今回, 結果から, 現在一般に市販されている黄褐色系の肉色のカンナシには, 収穫直後の場合, カロテノンはほとんど含まれていないと考えられた。橙肉色品種・系統（n=22）における各色彩値とβ-カロテノン含量との相関係数は, L*値で-0.885, a*値で0.897, b*値で0.810となった。β-カロテノン含量が5 mg以下のものを除外した場合（n=19）には, それぞれ, -0.852, 0.891, 0.718となり, b*値に比べてa*値との相関関係がより鮮明となっ

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