Introduction to Colorimetric Analysis for Assessment of Carotenoid Pigments in Squash Germplasm

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Abstract : Carotenoids of squash play an important role in human health by acting as sources of provitamin A or as protective antioxidants. Among the 60 accessions of squash germplasm, fluorescent yellow and yellow types of flesh color got the highest count, followed by the orange, whitish yellow and greenish yellow. The redness and yellowness values of the flesh powder ranged from -2.45 to 86.09 and from 13.77 to 39.80, respectively. While the lightness and the total color difference values of flesh color varied from 67.64 to 86.09 and from 19.77 to 51.79, respectively. Colorimetric values of redness and yellowness showed positive correlation, and the correlation coefficient (r) was as high as 0.7386. The five accessions represented each flesh color types, IT195043 (orange), IT136696 (fluorescent yellow), IT186365 (yellow), IT137963 (whitish yellow), and IT180449 (greenish yellow). The total amount of carotenoid contents was in the order of orange color (104.64 mg/100 g), greenish yellow color (70.82 mg/100 g), fluorescent yellow color (32.41 mg/100 g), yellow color (8.73 mg/100 g), and whitish yellow color (4.73 mg/100 g). Both lutein and β-carotene were the predominant pigments of carotenoids, and lycopene was only separated and identified in the orange color flesh. According to the results, colorimetric analysis can aid breeders interested in increasing carotenoid content in squash, which could be accurately measured using a simple, reliable, and cost- and labor-efficient method for the evaluation of carotenoid pigments.

Keywords : squash germplasm, flesh color, carotenoid, calorimetric value, HPLC

INTRODUCTION

Squash is the principal ingredient of several culinary vegetable utilized at the immature and mature fruit stages. Squash provides a valuable source of carotenoids and ascorbic acid that have a major role in nutrition in the form of provitamin A and vitamin C as antioxidants, when used at repening stage or after storage (Peirce, 1987; Andres, 1990).

Pumpkins and squash (Cucurbita spp.) are excellent dietary sources of carotenoids (Wills et al., 1987; Gross, 1991) and, in 2001, ranked 11th among other vegetables produced around the world (FAOSTAT, 2008). Especially, winter squash cultivars are very good and promising sources of β-carotene (Whang et al., 1999; Chavasit et al., 2002; Gajc-Wolska et al., 2005; Murkovic et al., 2002).

Carotenoids are a class of more than 600 naturally occurring pigments synthesized de novo by plants, algae, and photosynthetic bacteria. Carotenoids are yellow, orange, and red pigments synthesized by plants. Moreover, numerous aspects of carotenoid metabolism are still unclear (Takeda, 1982; Gross, 1991; Fraser et al., 1994; Kato et al., 2004).

The most common carotenoids are α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene (Lee et al., 1984; Kim et al., 2003). Carotenoid pigments play a beneficial role in a variety of physiological mechanisms, including free radical scavenging, immune system enhancers, and precursors for vitamin A, included β-carotene, α-carotene, and β-cryptoxanthin in pumpkin (Borenslein and Bunzel, 1996; Nishino, 1998; ODS/NIH, 2006).

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(Received on June 25, 2012. Revised on November 19, 2012. Accepted on November 27, 2012)
The wide range of carotenoids in pumpkins and squash provides fertile ground for genetic improvement. When breeders have reliable information about carotenoid types and concentrations, they traditionally use a method as HPLC. HPLC is highly sensitive and reproducible, but can be expensive and time-consuming. To determine if carotenoid content of pumpkin and squash could be accurately measured using a less-expensive and simpler method, colorimetric analysis is mostly calculated as the L*, a*, b*-value system developed by Judd and Hunter, standardized in 1976. In CIE-LAB color space system, the L*-value indicates the brightness as the position of the bright/dark axis, and also the a*-and b*-value indicates the redness and yellowness as the position of the red/green axis and the position of the blue/yellow axis, respectively.

Chemical measurement of carotenoid pigments can be accomplished by spectrophotometric analysis of extracts and high pressure liquid chromatography (HPLC) using a Silica gel column. The advantage of HPLC measurement is that individual pigments can be quantified and also isomers can be separated (Gross, 1991; Emenhiser et al., 1995; Whang et al., 1999; Kim et al., 2003; Ha et al., 2009).

This study aimed to determine the correlation with colorimetric analysis and HPLC method in analyzing the carotenoid content in squash germplasm. The colorimetric analysis method could be accurately measured using a low cost, rapid and simple method. The results of this study can be used by breeders interested in increasing carotenoid content in squash germplasm.

MATERIALS AND METHODS

Plant materials
The profile of squash germplasms tested was analyzed based on the origin, the country that introduced it, and species. As previously reported, there are already information on the agronomic traits of 78 accessions of squash germplasm. From which 60 accessions were regenerated and selected in the National Agrobiodiversity Center of Korea with Genebank management program in 2009 (Kim et al., 2010). About 50 g of squash fruit flesh was harvested from 60 accessions of squash germplasm at 30 days of ripening stage and was frozen at -70°C in the deep freezer. Each frozen samples was freeze-dried and ground to a fine powder using a grinding mill for colorimetric and HPLC analysis to determine the content of carotenoid pigments.

Observation of flesh color and colorimetric analysis
The squash fruit flesh color of 60 accessions of squash germplasm after ripening stage was determined with the naked eye. The color of squash fruit flesh was categorized into the following; five types as orange, fluorescent yellow, yellow, whitish yellow and greenish yellow.

The L, a, b, and △E-system developed by Judd and Hunter, standardized in 1976 and based on sensitivity is commonly used (CIE, 1976). Colorimetric value of squash flesh powder was obtained using a Minolta CR300 colorimeter (Minolta Co., Japan) and the CIE-LAB color space (CIE, 1976). In this system, the L-value indicates the position of the bright/dark axis where in the minimum L value is zero, which represents black. The a-value indicates the position of the red/green axis wherein positive a is red and negative a is green. The b-value indicates the position of the blue/yellow axis wherein positive b is yellow and negative b is blue. The total color difference, △E, has to be calculated too. The △E is a single value which takes into account the differences between the L, a, and b of the sample and the standard. The L, a, b coordinates are directly related to the standard color values X, Y and Z.

Extraction procedure for HPLC
The efficient extraction method of carotenoids developed by Kim et al., (2003) and Ha et al. (2009) was modified. For extraction of carotenoids, 0.2 g of the freeze-dried squash flesh powder was homogenized three times with a vortex with 0.1 g MgCO3 and 5 mL of 0.2% ascorbic acid in methanol containing 0.1% BHT (butylated hydroxytoluene) as antioxidant, and was saponificated on water bath for 10 minutes at 80°C. After saponification, the sample