Journal of Food Composition and Analysis 22 (2009) 509–516

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Article

Total and individual carotenoid profiles in *Solanum phureja* cultivated potatoes: II. Development and application of near-infrared reflectance spectroscopy (NIRS) calibrations for germplasm characterization

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ARTICLE INFO

Article history:
Received 30 March 2008
Received in revised form 28 July 2008
Accepted 4 August 2008

Keywords:
Potato
Potato cultivars
Solanum phureja
Biodiversity
Carotenoids
NIRS
Food analysis
Food composition

ABSTRACT

Calibration equations to estimate total and individual carotenoid concentrations by near-infrared reflectance spectroscopy (NIRS) in *Solanum phureja* potato germplasm were developed, validated and applied to the characterization of 152 *S. phureja* germplasm accessions. The cross, external and independent validation of the calibrations indicate that total carotenoids and zeaxanthin concentrations can be estimated by NIRS with high accuracy and that NIRS can be used to differentiate accessions with low, medium and high concentrations of violaxanthin, antheraxanthin, lutein or β-carotene. Significant variability was revealed for total and all individual carotenoids measured. Two varieties with significant zeaxanthin concentrations (above 1000 μg 100 g^{-1} FW) and a group of 43 accessions with relatively high β-carotene concentrations (above 10 μg 100 g^{-1} FW) were identified. The positive relation between total carotenoids and zeaxanthin as well as their negative relation with β-carotene reported for 23 accessions in the companion manuscript was upheld. The use of NIRS in estimating and characterizing a large germplasm collection has permitted the identification of accessions with high total carotenoids and zeaxanthin and relatively high β-carotene concentrations at low cost and in reduced time compared to HPLC. This can be applied to documentation of descriptive and potentially beneficial characteristics of potato genebank materials, the assessment of the food value of present varieties, and the selection of parents in a breeding program oriented to nutritional enhancement of potato.

1. Introduction

Carotenoids are well credited with important health-promoting functions or actions such as provitamin A activity, enhancement of the immune system and reduction of the risk of degenerative diseases, e.g. cancer and cardiovascular disease (Fraser and Bramley, 2004; Rodriguez-Amaya, 2001; Van den Breg et al., 2000).

Since some studies suggest that higher consumption of carotenoids can protect consumers from several diseases, considerable interest is currently being shown in the screening and development of food crops with increased concentrations of total and individual carotenoids (Seddon et al., 1994; Stahl and Sies, 2005).

In a companion manuscript, it is reported that yellow-fleshed *Solanum phureja* (Hawkes, 1990) potatoes are an important source of zeaxanthin and lutein, the major pigments of the yellow spot in the retina of the human eye (Landrum and Bone, 2001). The total and individual carotenoid concentrations of a representative sample of 23 accessions of *S. phureja* potatoes conserved in the germplasm collection at CIP (http://research.cip.cgiar.org/genebank/) obtained by spectrophotometry and HPLC analysis, respectively were reported (Burgos et al., 2008). It was of breeders' interest to characterize the total and individual carotenoid concentration of the complete collection of this important cultivated potato germplasm source, but although spectrophotometry and HPLC are very accurate; the high costs of these methods and the time required for the analysis limits their use to small numbers of samples relative to those required in extensive screening and breeding programs.

Near-infrared reflectance spectroscopy (NIRS) is a long-established technology. Requiring only simple sample preparation methods (drying and milling) NIRS is a rapid and relatively inexpensive technique that facilitates the analysis of several traits simultaneously (Osaki et al., 2006), and is commonly use to estimate the main organic constituents like oil, protein and starch

DOI of original article: 10.1016/j.jfca.2008.08.008
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doi:10.1016/j.jfca.2008.08.009

in various agricultural products and even in the complex matrices of processed foods (Shenk and Westerhaus, 1993; Zum Felde et al., 2007). NIRS avoids problems of organic and other chemical waste disposal, and there are few if any hazards associated with the technique because it uses no toxic or corrosive reagents (Marck et al., 2002). The potential to estimate carotenoid concentrations has been demonstrated for maize (Brenna and Berardo, 2004; Berardo et al., 2004). In potatoes, NIRS has been successfully applied to estimate starch, protein, glucose, fructose and sucrose concentrations (Hartmann and Buning-Pfaue, 1998), but it has not yet been applied for carotenoid analysis.

This study had 2 objectives. First, to study the potential of NIRS to estimate total and individual carotenoid concentrations in cultivated potatoes using *S. phureja accessions* as an example. The second objective was to characterize an extensive germplasm collection of *S. phureja* potatoes to estimate total and individual carotenoid concentrations by NIRS and to validate patterns of accumulation of different carotenoids that had been documented in genotypes of a sample of the same collection analyzed by HPLC.

2. Materials and methods

2.1. Plant material and sample preparation

Fig. 1 illustrates the sources and distribution of samples used for this research: (A) Development of NIRS calibrations to estimate total and individual carotenoid concentrations (consisting of calibration model development and external validation), (B) independent validation, and (C) application of NIRS to germplasm characterization.

2.2. Development and validation of NIRS calibrations

Potato tubers harvested from 37 and 42 *S. phureja* accessions grown in 3 replications in the highlands of Peru: Comas (3335 m.a.s.l) and Huancayo (3315 m.a.s.l), Junin, Peru, in November 2006 and February 2007, respectively were used for the development of NIRS calibrations and external validation (Fig. 1A). One to three samples of five to ten unblemished medium sized tubers (1 per plot replication) were collected at random from different plants of each accession. Potato tubers from 26 *S. phureja* accessions grown in 1 replication in Huancayo and harvested in November 2007 were used for independent validation (Fig. 1B). One sample of five to ten unblemished medium sized tubers was collected at random from different plants of each accession.

A total of 215 samples were prepared for calibration development and external and independent validation of the calibrations. The tubers of each sample were washed thoroughly with tap water, rinsed with deionized, distilled water, patted dry with paper towels and peeled. Tubers were quartered longitudinally, from stem to bud end and 3 quarters were used for spectrophotometry and HPLC analysis and 1 quarter was used for NIRS analysis. The 3 quarters were cut into small pieces, homogenized in a food processor and the homogenized samples were analyzed immediately in fresh condition for total and individual carotenoid concentrations. From the other quarter, slices were taken until a sample of approximately 50 g was obtained. The sample was

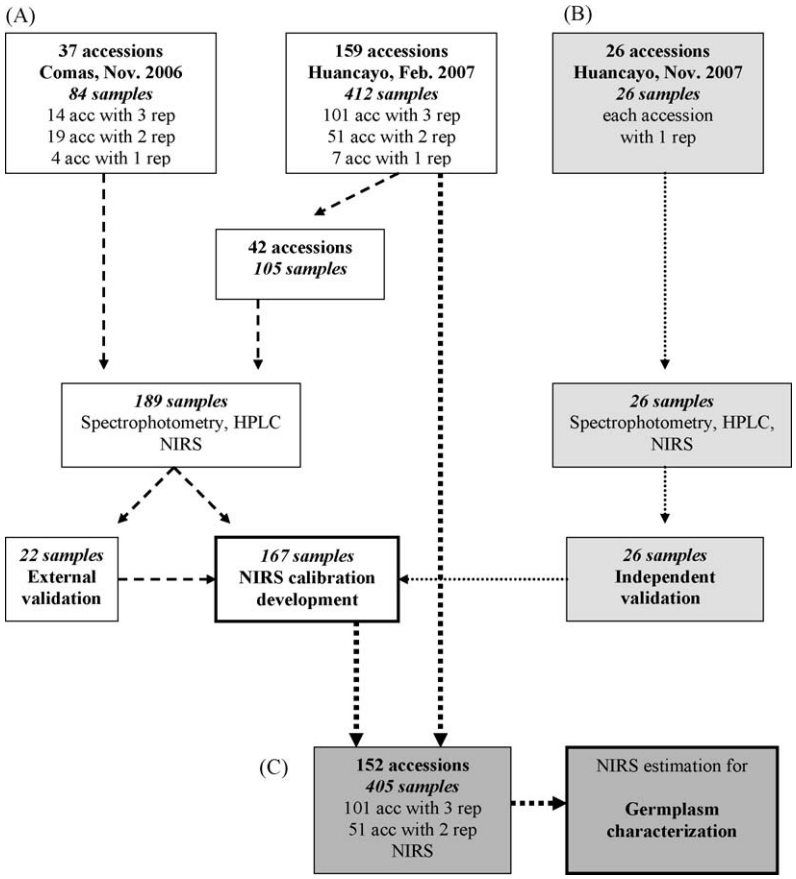

Fig. 1. Sample distribution: (A) Development of NIRS calibrations for estimation of total and individual carotenoid concentrations, (B) Independent validation, and (C) Application of NIRS for germplasm characterization.