Journal of Food Composition and Analysis 23 (2010) 763–771

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Article

# Carotenoid profiling of tropical root crop chemotypes from Vanuatu, South Pacific

Antoine Champagne [a,*], Stéphane Bernillon [b,d], Annick Moing [b], Dominique Rolin [c], Laurent Legendre [a], Vincent Lebot [e]

[a] Laboratoire de Biotechnologies Végétales appliquées aux Plantes Aromatiques et Médicinales, UJM, 23 rue Dr Paul Michelon, 42023 Saint-Etienne cédex 2, France
[b] Institut National de la Recherche Agronomique, UMR619 Biologie du Fruit, 71, avenue E. Bourlaux, 33140 Villenave d'Ornon, France
[c] Université de Bordeaux 2, UMR619 Biologie du Fruit, 71, avenue E. Bourlaux, 33140 Villenave d'Ornon, France
[d] Plateforme Métabolome-Fluxome du Centre de Génomique Fonctionnelle de Bordeaux, 71, avenue E. Bourlaux, 33140 Villenave d'Ornon, France
[e] Centre de coopération Internationale en Recherche Agronomique pour le Développement - Vanuatu, UR 75, BP 946, Port-Vila, Vanuatu

ARTICLE INFO

*Article history:*
Received 5 May 2009
Received in revised form 25 March 2010
Accepted 29 March 2010

*Keywords:*
Agrobiodiversity
Aroids
Cassava
HPLC-DAD
Carotenoids
Food composition
Sweet potato
Yams

ABSTRACT

Tropical root and tuber crops are staple foods in developing countries where they are the main source of carbohydrates and a potential source of secondary metabolites such as carotenoids. Enhancing carotenoid content has considerable potential for improving human nutrition in these areas. Except for cassava and sweet potato, carotenoid content has not been evaluated in large germplasm collections. We describe a simple HPLC-DAD-based protocol for the reliable characterization of carotenoids in root crops. Less than 5% variation in carotenoid content was found among samples collected from tubers of the same plant or from clones of the same genotype. Carotenoids were evaluated in 153 accessions representing 10 different species. Each species had a specific profile of carotenoids, and accessions of the same species differed greatly in their individual content. The database assembled during this study represents a useful tool for nutritionists and breeders working on biofortification of root crops with carotenoids.



## 1. Introduction

Root and tuber crops are among the most important staple foods in developing countries, particularly in Melanesia where the yearly consumption per inhabitant is among the highest in the world. Because of fast population growth, there is an urgent need to improve the production and quality of staple foods. Tropical root and tuber crops are the major source of carbohydrates and provide all or part of the recommended daily intake of certain micronutrients such as provitamin A (Van Jaarsveld et al., 2005). In Melanesia and in particular in Vanuatu, local cultivars also play fundamental socio-cultural roles through traditional exchanges. This has resulted in a wide range of cultivars that is particularly remarkable in the two most important crops, taro (*Colocasia esculenta* Schott) and the greater yam (*Dioscorea alata* L.), but also in cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.).

In spite of their local importance, tropical root crops are under-researched with insufficient characterization of their micronutrients, especially their carotenoid composition and content. More than 750 different natural carotenoids have been identified in a wide range of bio organisms (Britton et al., 2004). Among the fraction found in food products, only cyclic carotenoids have an unsubstituted β-ring and are precursors of vitamin A. Vitamin A deficiency can lead to blindness and is a serious public health problem in developing countries, particularly in Africa. Regarding the Pacific atoll islands, serious problems of vitamin A deficiency have been underlined since lifestyle changes began (Englberger et al., 2008).

Due to limited agricultural resources, there is a need to identify good sources of provitamin A among locally grown staple foods. Carotenoids in sweet potato (*I. batatas* L.) and cassava (*M. esculenta* Crantz) carotenoid have been documented in South America and Africa (Hagenimana et al., 1999; K'osambo et al., 1998; Iglesias et al., 1997). It has been established that several varieties, especially the orange-fleshed ones, could increase daily provitamin A intake to recommended levels (Hagenimana et al., 1999; Stahl and Sies, 2005). Human health benefits have also been linked with non-provitamin A carotenoids. Lutein and zeaxanthin are found in the macula area of the human retina and are associated with a reduced risk of age-related degeneration (Mares et al., 2006). More generally, natural carotenoids protect the skin from damaging solar radiation and reduce the risk of developing certain cancers (Stahl and Sies, 2005). Some carotenoids also exhibit antioxidant properties and regular

* Corresponding author. Tel.: +33 661 551 048; fax: +33 557 122 541.
*E-mail address:* champagne_antoine@hotmail.com (A. Champagne).

0889-1575/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2010.03.021

consumption of fresh plant products has been shown to have numerous health benefits. The particular molecular structure of carotenoids leads to physical quenching of $^1O_2$ which leaves the structure intact and requires no regenerating reaction after reduction, which is the case of other antioxidants (Miller et al., 1996; Sies and Stahl, 1995; Stanner et al., 2004). Carotenoids have such a beneficial impact on human health that biofortification of staple foods could play a key role in health improvement programs.

Worldwide, there are several breeding programs for potato, cassava and sweet potato but very limited breeding of other tropical root crops, such as aroids and yams. However, the diversity and genetic potential of these neglected species could play a crucial role in supporting rapidly increasing populations and in improving health through enriched diets. Genetic improvement is the selection of phenotypes based on their *per se* value. The identification of parents with complementary traits, their recombination and the subsequent screening of large progenies are required to identify hybrids with desirable traits. Consequently, breeders need sustainable and well-designed technologies as well as reliable data to select the most suitable progenitors and progenies. The limited knowledge available on the secondary metabolites of most tropical root crops is a serious constraint. Moreover, in some tropical countries, such as in the South Pacific, edible aroids (e.g., *C. esculenta* and *Xanthosoma sagittifolium* (L.) Schott), and yams (*Dioscorea* spp.) are more important than in other developing countries where cassava (*M. esculenta*) is usually the main root crop. The traditional selection system practiced by local farmers is based on attractive and palatable chemotypes and has generated remarkable diversity that deserves to be studied and characterized.

The aim of the present study was to assess the interspecific variability of 10 root crop species grown in Vanuatu: sweet potato (*I. batatas*), cassava (*M. esculenta*), yams (*D. alata*, *D. bulbifera* L., *D. esculenta* L., *D. cayenensis* Lam., *D. pentaphylla* L.), taro (*C. esculenta*), macabo (*X. sagittifolium*) and giant taro (*Alocasia macrorrhyza* (L.) Schott). This study is the first germplasm screening for carotenoids in Vanuatu and Melanesia. Its aims were to: (i) develop protocols for the rapid and reliable quantification of these compounds, (ii) assess the extent of variation observed in carotenoid composition and content, and (iii) establish a composition database that can be used in genetic improvement programs.

## 2. Materials and methods

### 2.1. Growing conditions and sample preparation

Cultivars were selected from various collections made during TANSAO (TAro Network for South Asia and Oceania), SPYN (South Pacific Yams Network), and RCAPV (Root Crops Agrobiodiversity Project in Vanuatu) projects, as well as from hybrid lines developed at the Vanuatu Agricultural Research and Technical Centre (VARTC, Espiritu Santo). All accessions are currently maintained in the national germplasm collection.

All varieties were grown in the same plot (VARTC, Espiritu Santo, 15°23′S 166°51′E) to minimize variations due to environmental factors. They were planted at the same time and their storage organs harvested when fully mature to avoid variation due to ontogeny. A core sample of 153 accessions was assembled to represent the full range of variation in phenotypic flesh color of the storage organs (corms, cormels, roots, tubers). Overall, roots of 33 *I. batatas* cultivars (cvs), tubery roots of 22 *M. esculenta* cvs, tubers of 20 *D. alata* cvs, tubers of 14 *D. bulbifera* cvs, aerial tubers (bulbils) of 13 *D. bulbifera* cvs, tubers of 10 *D. esculenta* cvs, tubers of 4 *D. cayenensis* cvs, tubers of 3 *D. pentaphylla* cvs, corms of 24 *C. esculenta* cvs, cormels of 7 *X. sagittifolium* cvs and corms of 3 *A. macrorrhyza* cvs were selected. Passport data characterizing each accession are presented in Table 1.

The storage organs were washed peeled under water and their surface quickly dried on a towel. Roots were cut longitudinally and then transversally into two equal parts of about 200 g fresh weight (FW). The material was grated using a cheese grater. Half was sealed in zip-lock plastic bags and stored at −20 °C overnight. Frozen material was freeze-dried with a TELSTAR Cryodos-50 (Terrassa, Spain) for two days. Dried material was kept in paper bags enclosed in black polyethylene sealed bags at −20 °C until analysis. Every step of the procedure took place in a dark room to prevent photo-oxidation.

The moisture content of the samples was determined on the other half of the sample (100 g) by drying it in a ventilated oven at 60 °C until constant weight (about 7 days).

### 2.2. Reagents and standards

Acetone, methanol and hydrochloric acid were purchased from VWR Int. (Fontenay-Sous-Bois, France). *tert*-Butyl-methyl ether, ammonium acetate, sodium hydroxide, anhydrous sodium sulphate, ethyl acetate and trishydroxymethylaminomethane (Tris) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Ethyl ether was purchased from Cooper (Melun, France), and chloroform from SDS (Peypin, France).

All-*trans*-β-carotene and lutein were purchased from Carotenature GmbH (Lupsingen, Switzerland) and lycopene from Sigma–Aldrich Co. (St. Louis, MO). δ-Carotene, ε-carotene, neurosporene, phytoene and zeaxanthin were obtained from *Escherichia coli* harboring the plasmids pAC-DELTA, pAC-EPSILON, pAC-NEUR, pAC-PHYT and pAC-ZEAX kindly provided by Dr. F.X. Cunningham Jr. (University of Maryland, USA). Carotenoids were extracted from bacteria cultures using ethyl ether. α-Carotene was extracted from carrots (*Daucus carota* L.) as described in Section 2.3.

### 2.3. Extraction methods

The extraction method was adapted from the procedure described by Rodriguez-Amaya and Kimura (2000). For each step of the protocols, samples were protected from light and maintained at 4 °C. A 2–4 g freeze-dried powder sample was homogenized in 10 mL acetone using a polytron Biotrona 6403 (Küssnacht, Switzerland). To ensure full recovery of analytes, knifes were rinsed with 5 mL of acetone and the 5 mL was then pooled with the first 10 mL. The sedimentation of the powder was achieved by centrifugation at 4 °C at 3000 × *g* for 10 min. The supernatant was recovered with a Pasteur pipette and the extraction process was repeated on the pellets. In order to guarantee optimal extraction conditions, the process was performed on highly concentrated samples of each species using a High-Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD). The process was optimized by setting the DAD at 460 and 290 nm – for colored and non-colored carotenoids. Generally two to four extractions were needed for optimal results. Each extract was evaporated to dryness under a nitrogen stream.

To simplify chromatogram of some species, saponification was adapted from the procedure described by Pérez-Gálvez and Mínguez-Mosquera (2001). Only *D. alata*, *D. bulbifera*, *D. pentaphylla* and *D. cayenensis* samples were saponified, because saponification did not modify the chromatographic profiles of the other species. After carotenoid extraction, the dried residue was dissolved in 1 mL of acetone. The solution was placed in a glass tube before adding 3 mL of ethyl ether and 4 mL of 10% (w/v) sodium chloride in water. The tube was vigorously shaken to transfer pigments to the organic phase. The process was repeated once. The organic phases were recovered with a Pasteur pipette, and pooled. One milliliter of 10% KOH in methanol was added to the solution and stirred. Optimization was assisted by HLPC-DAD. Pasteur pipette was used as a