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EVALUATION OF POTATO CULTIVARS AND BREEDING LINES FOR CAROTENOIDS CONTENT IN TUBERS

ABSTRACT

The potato is one of the most common elements of everyday human diet due to its high nutritional and culinary value. Potato tubers provide mainly energy in the form of carbohydrates and also some other nutrients, which include carotenoids as compounds linked to oxidation-preventing mechanisms. The main assimilable carotenoids present in cultivated potato are lutein and zeaxanthin. Potato cultivars rich in carotenoids are a subject of increasing consideration in breeding for improvement of the nutritional quality of tubers. The paper describes the spectrophotometric method of evaluation of total and individual carotenoids content in potato tubers.

Key words: analysis, extraction, lutein, total carotenoids (TC), zeaxanthin

INTRODUCTION

Potato tubers are an important source of many nutrients in the human diet. Among the health-promoting compounds are carotenoids, phytonutrients that have antioxidant properties. Furthermore, lutein and zeaxanthin, the main carotenoids of potato are major pigments of the yellow spot in the human retina (Bone *et al.*, 2001) and it is believed that a high dietary intake of them can protect against age-related macular degeneration (AMD) (Seddon *et al.*, 1994). Tuber carotenoids concentration is on average 38 – 175 µg/100g fresh weight (FW) (Breithaupt and Bamedi, 2002). Although carotenoids content of potato tubers is relatively small, taking into account the considerable level of potato consumption, it may be a significant source of these components in the human diet. Therefore, potatoes rich in carotenoids are the subject of increasing consideration in breeding for improvement of the nutritio-

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nal quality. In some potato breeding programs, the evaluation for tuber carotenoids content is used for the assessment of advanced breeding lines and to identify potential parental forms with an increased total carotenoids content (TC). Although the concentration of tuber carotenoids is determined by both genetic and environmental factors (Haynes *et al.*, 2010). Tatarowska *et al.* (2014) indicate the possibility of applying breeding methods for the selection of potato cultivars with a high and stable TC in tubers.

MATERIALS AND REAGENTS

1) Filtration column (A&A Biotechnology, cat. No 332-25)
2) Orange glass vials ND8 (VWR International Sp. z o. o., cat. No 548-3019)
3) Open-top-style ND8 PP caps with silicone/PTFE septa (VWR International Sp. z o. o., cat. No 548-3024)
4) Distilled water
5) Liquid nitrogen
6) Heksan (Avantor Performance Materials Poland S.A., cat. No 466310111)
7) Potassium dichromate (Avantor Performance Materials Poland S.A., cat. No BA1040119)
8) Water (HPLC) (Avantor Performance Materials Poland S.A., cat. No 885060156)
9) Ethanol 99,8% (HPLC) (Avantor Performance Materials Poland S.A., cat. No 396483150)
10) Methanol (HPLC) (Avantor Performance Materials Poland S.A., cat. No 621991154)
11) Tetrahydrofuran (HPLC) (Avantor Performance Materials Poland S.A., cat. No 278200157)
12) Lutein analytical standard ≥95.0% (HPLC) (LGC Standards, cat. No 07168 -1MG)
13) Zeaxanthin analytical standard ≥95.0% (HPLC) (LGC Standards, cat. No 14681-1MG-F)

EQUIPMENT

1) Lyophilizer (LABCONCO lyophilizer, shell freeze system, USA).
2) UV-Vis spectrophotometer
3) Centrifuge
4) Shimadzu Prominence HPLC (Shimadzu, Japan) composed of
   a. Shimadzu column oven (CTO-10AS vp)
   b. Shimadzu prominence UV/VIS detector (SPD - 20A)
   c. Shimadzu prominence liquid chromatograph (LC – 20AD)
   d. Shimadzu prominence degasser (DGU – 20A 3)
   e. Shimadzu prominence auto sampler (SIL – 20AC HT)
   f. Phenomenex Luna 4.6 µm 100A 150×4.6 mm column (shim-pol, cat. No PAX-00F-4252-E0)
5) LC solution software (Shimadzu, Japan)

PROCEDURE

A. Extraction and analysis of the total carotenoids content (TC)

1) Three tubers are chosen for each potato clone. The tubers are cut into 8 parts. The two fragments selected for an analysis are cut into small cubes and frozen in liquid nitrogen, lyophilized and milled.
2) Total carotenoids are isolated according to the procedure described in the PN-90/A-75101/12 standard with modifications. The procedure has been shortened and limited to extraction with hexane: TC are extracted from 3 g of freeze-dried tissue in a glass flask with 50 ml of hexane for 24 h in the dark.
3) Total carotenoids concentration in the solvent is evaluated by spectrophotometry with a UV-Vis spectrophotometer at 450 nm. All analyses are made with three technical replicates each.
4) The carotenoids content analysis is performed by using a standard curve prepared for potassium dichromate (colour equivalent to β-carotene according to Russell *et al.*, 1935). The calibration curve is calculated from the absorbance for six dilutions of potassium dichromate. All dilutions are made from the starting solution prepared with 360 mg of potassium dichromate dissolved in 1 l of distilled water. Then 5, 10, 20, 30, 40 and 50 ml of the starting solutions are complemented to 50 ml with distilled water and used. The β-carotene content (TC equivalent) corresponding to the absorbance obtained for these solutions at 450 nm is shown in Table 1.

Table 1

The β-carotene content corresponding to the absorbance values obtained for the potassium dichromate solutions.

Dilution of the potassium dichromate solution	The β-carotene content [µg/ml] corresponding to the absorbance obtained for the solution (TC equivalent)	Example absorbance
5	0.208	0.047
10	0.416	0.100
20	0.832	0.203
30	1.248	0.316
40	1.664	0.420
50	2.080	0.529

Calculations

The total carotenoids content corresponding to the absorbance value of the analysed sample is calculated with using the standard curve method. The reference curve is a linear function with the formula:

$$y = a \times x + b$$

where:
y – absorbance value
x – content of the analysed TC
a and *b* – equation coefficients, calculated according to the formula:

$$TC_S = \frac{y - b}{a}$$

where:
TC_S – TC in 1ml analysed sample
y – absorbance value for the analysed sample
a and *b* – equation coefficients calculated from the formulas:

$$a = \frac{n \times \sum(x \times y) - \sum x \times \sum y}{n \times \sum x^2 - (\sum x)^2}$$

$$b = \frac{\sum y - a \times \sum x}{n}$$

where:
x – TC equivalent in 1ml dilution of the potassium dichromate solution (Table 1)
y – absorbance value for the potassium dichromate solution (exemplary absorbance values shown in Table 1)
n – the number of used dilutions of the potassium dichromate solution (for us n=6)
TC_{FW} – TC in µg/100g FW for the tested potato clone is calculated according to the formula:

$$TC_{FW} = TC_S \times \frac{a}{b} \times 25$$

where:
TC_S – TC in 1ml analysed sample
a – the amount of hexane used for carotenoids isolation [ml]
b – the amount of freeze-dried tissue used for extraction [g]
The standard error of the measurement (SEM) for this method was 0.8.

B. Evaluation of lutein and zeaxanthin content

1) Carotenoids are extracted from 2 g of freeze-dried tissue in the 50 ml falcon tube with 5 ml of ethanol (for HPLC) in 24 h in the dark. Then 2 ml of the eluent is centrifuged on a filtration column (A&A Biotechnology, Poland) at 600 RPM for 30 seconds.
2) 8 µl of the filtered sample is separated on a Shimadzu Prominence

HPLC (Shimadzu, Japan) using a reversed-phase Phenomenex Luna 4.6 µm 100A 150×4.6 mm HPLC column at temperature 50oC. The flow rate is 1 ml/min using mobile phase A: Methanol/ Tetrahydrofuran/ Water = 45/10/45 (v/v/v) and B: Tetrahydrofuran. The gradient is as follows: 0% B increasing to 50% over 25 min, equilibrated to 0% B for 5 min and held at 0% for 5 min. The UV/Vis absorbance detector collects data at 452 nm. Lutein and zeaxanthin quantification is performed by using a standard curve ranging from 0.5 to 4 µg/ml. The standard curve is prepared for lutein and zeaxanthin standards (LGC Standards, UK). Quantification is performed using LCsolution software (Shimadzu, Japan). All analyses are conducted with three technical replicates each. An example of the analysis of lutein and zeaxanthin is shown in Figure 1. The standard error of measurement (SEM) for this method was 0.1.

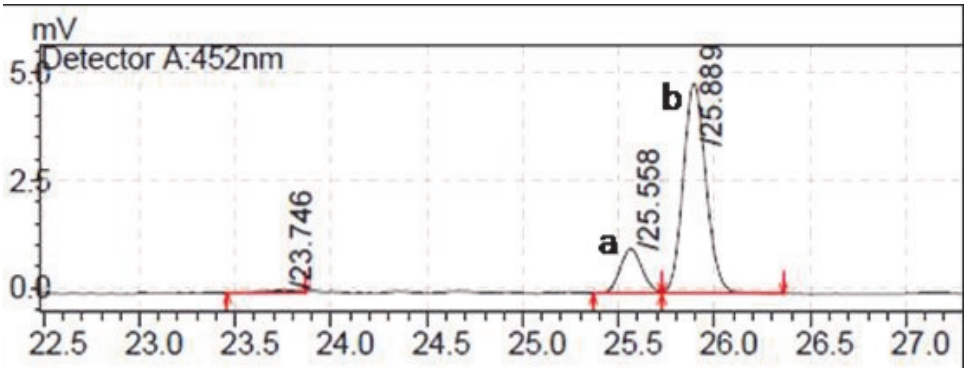

Fig. 1. Chromatogram of zeaxanthin (a) and lutein (b) standard solution

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