Bulletin of the *Transilvania* University of Braşov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 10 (59) No.1 - 2017

TOTAL CAROTENOIDS CONTENT IN 20 POTATO CULTIVARS GROWN IN ROMANIA

Carmen L. BĂDĂRĂU[1,3] L. MIKE[2] F.M. ŞTEFAN[3]
Cristina M. CANJA[1] Mirabela I. LUPU[1]

Abstract: *Potato tubers with higher levels of naturally developed vitamins, could have a positive impact on the human health. In this study, ten Romanian cultivars and ten commercial potato (very appreciated by the consumers and producers), planted in two sites in Romania were evaluated for total carotenoids (pro-vitamin A) content. Significant differences in carotenoids content were observed across sites and across genotypes. This study provides information on level of important micronutrients in several potato cultivars with improved nutritional quality.*

Key words: *potato, L ascorbic acid, carotenoids.*

1. Introduction

The potato is a strategic food for our country, contributing to the national food safety system. Romania is ranked on the third position in Europe in terms of area cultivated with potatoes (after Poland and Germany) [8]. Potato is the third most consumed food, after rice and maize [8] and their tubers are recognized as a good source of carbohydrates, vitamin B1, B3 and B6, potassium, phosphorus and magnesium [7, 10]. It has a moderate content of iron, but its high L ascorbic acid levels promote iron absorption. Potato is rich in essential amino acids. It also contains pantothenic acid, folate and riboflavin [10]. While 50 years ago more than half of the global annual production output was concentrated in Russia, Poland and Germany, now, around 40% comes from China, India and Russia. China and India have seen a dramatic increase, with both countries doubling their production in the last 20 years [8]. This makes potato an important commodity in Asian developing countries, with the added advantage that food security is augmented as compared to other staple crops because potatoes are only marginally traded in international markets, making them less susceptible to price volatility.

Carotenoids are organic pigments naturally occurring in the chloroplasts of photosynthetic organisms, such as plants ant algae. All share a poly isoprenoid

[1] Faculty of Food and Tourism, *Transilvania* University of Braşov, Castelului Street, no. 148, Brasov, Romania.
[2] Station of Research and Development for Potato Targu Secuiesc, Ady Endre Street, no. 55, Covasna county, Romania.
[3] National Institute of Research and Development for Potato and Sugar Beet Brasov, Fundaturii Street, no. 2, Brasov county, Romania.
Correspondence: Carmen L. Badarau; email: badarau_carmen14@yahoo.com.

structure of 40 carbon atoms, a long conjugated chain of double bonds in the centre of the molecule and near symmetry around the central double bond. They may be split in the two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons without oxygen) [13]. Carotenoids serve two roles in plants and algae they absorb light energy for use in photosynthesis and they protect chlorophyll from photo damage [15]. In humans, beta carotene, alpha carotene and beta cryptoxanthin are the major carotenoids showing vitamin A activity (they can convert to retinal) and these and other carotenoids could also act as antioxidants [13]. In potatoes, carotenoids belong almost exclusively to xanthophyll group and are important for the yellow or orange colour of the flesh [12]. They represent minor constituents, especially beta carotene and therefore are not an important source of vitamin A in the diet [4]. However, high level in plasma of lutein and zeaxanthin have been linked to a reduction in age related macular degeneration. Lutein and zeaxanthin are the major pigments of the yellow spot in the human retina, which might protect the retina from damage by blue light and oxidizing species [1]. The main carotenoids found in potatoes are violaxanthin, antheraxanthin, lutein and zeaxanthin (Figure 1), although the ratios of these carotenoids vary among the varieties [12]. The variation among potatoes species and among the varieties in the same species is wide, with total carotenoids content up to 60 times higher in *Solanum phureja* and *Solanum tuberosum* and up to a 20-fold difference within the same species [2], [6], [11, 12].

The aim of this study was to quantify the levels of carotenoids content in 20 potato cultivars.

Potato with high increase content of carotenoids could have an important impact on human health, especially in populations where potato is the main staple food crop and therefore, would be of interest to consumers and producers.

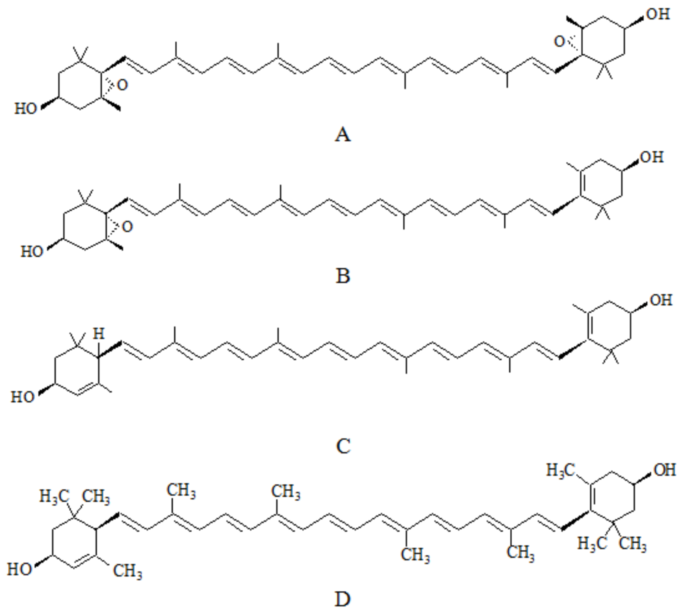

Fig. 1. *The chemical structure of the most important carotenoids found in potatoes A) Violaxanthin; B) Antheraxanthin; C) Lutein; D) Zeaxanthin*

2. Material and Methods

2.1. Biological material

The following twenty potato varieties were chosen for this study:

- Christian, Roclas, Gared, Albioana - Romanian varieties very appreciated in Romania for their nutritional quality;
- Sarmis, Marvis, Cumidava, Brasovia, Productiv, (new Romanian varieties);
- Albastru Violet Galanesti, Blue Congo, Vittelote, Salad Blue (cultivars with purple pigmentation in the flesh);
- Bellarosa, Riviera, Carrera, Jelly, Red Lady, Red Fantasy, Hermes (cultivars very appreciated by the producers, being the top 20 varieties cultivated in the Romanian area) [3].

The seed tubers of the selected varieties were obtained from the Department of Breeding and Seed Production, National Institute of Research and Development for Potato and Sugar Beet Brasov (and were cultivated in two different locations in 2016 and in one location in 2015.

Seed tubers were planted in May in Brasov (coordinates lat. 45.6744234, long. 25.539622) in 2016 and 2015 and in Târgu Secuiesc (coordinates lat. 45.984397, long. 26.0993878) in 2016, with three replicates, following an alpha block design. Similar fertilizer chemical inputs were applied in both cultivation sites. Mature tubers were harvested 160 days after planting in Brasov in 2016, 168 days in Targu Secuiesc in 2016 and 148 days in Brasov in 2015 (Table 1). Immediately after harvest, marketable tubers (of the most similar size possible, medium size and free of damage and defects) were selected, washed, prepared for analysis.

The climatic conditions at the Romanian planting sites in the 2 years Table 1

Month	Year	Site	Mean temperature [°C]	Rainfall [mm]
May	2015	Brasov	13,2	82,6
	2016	Brasov	12,4	100,4
	2016	Targu Secuiesc	12,2	118,1
June	2015	Brasov	16,3	107,7
	2016	Brasov	19,0	121,2
	2016	Targu Secuiesc	18,5	124,9
July	2015	Brasov	17,9	95,9
	2016	Brasov	19,7	28,8
	2016	Targu Secuiesc	19,7	22,7
August	2015	Brasov	17,3	78,5
	2016	Brasov	18,4	85,8
	2016	Targu Secuiesc	18,4	209
September	2015	Brasov	13,5	54,7
	2016	Brasov	15,0	38,0
	2016	Targu Secuiesc	14,4	40,6
October	2015	Brasov	8,2	42,7
	2016	Brasov	6,9	96,0
	2016	Targu Secuiesc	8,3	60,8
Total / average	2015	Brasov	14,4	462,1

2.2. Sample Preparation

Composite samples were prepared by pooling tubers. Tubers were peeled with a potato peeler, the flesh of each tuber quartered from stem to bud and one of the quarters sliced. Flesh tissues were freeze-dried, ground to a fine powder (using a coffee grinder) and stored to -20 °C until analysis.

2.3. Total Carotenoids Analysis (TC)

Total carotenoids was determined according to Burgos et al. (2009) [4] without alkaline hydrolysis. Extraction of TC from 0.5g of powdered skin or 2g of powdered flesh was sequentially carried out in triplicate with acetone (Sigma, prod. no. 34850) using 10.7 and 5 ml volumes shaking in 50 ml polypropylene tubes at 10 000 rot/min for 15 minutes. The supernatants were combined and 5 ml of petroleum ether (Sigma, prod. No 77379) and 20 ml of pure water added. The tubes were shaken vigorously by hand and centrifuged at 10 000 rot/min for 1 minute to separate the aqueous and organic phases. The top organic phase was removed using a Pasteur glass pipette and washed with 40 ml pure water, separating both phases as described above. The top organic phase was again removed and a tip of spatula of sodium sulphate anhydrous (Sigma, prod no S9627) added to absorb minor quantities of water at the bottom of the tubes. The extracts were transferred to tarred polypropylene tubes (50ml), washing the sodium sulphate precipitate with around 0.5ml of petroleum ether in triplicate. The tubes containing the extract were weighed and the absorbance of an aliquot was measured at 450 nm against petroleum ether using a UV VIS spectrophotometer Spectronic Genesys 5 (Milton Roy). TC content was calculated as follow:

$$C_s\,(mg/g) = A \times 10 \times (0.65 \times 2500)^{-1} \quad (1)$$

Cs is the concentration of carotenoids in the extract, A the absorbance measured, 10 the concentration of a solution 1% (mg/ml), 0.65 is the density of petroleum ether (g/ml) and 2500 is the absorbance of a 1% solution;

$$TC\,(mg/kg\;DW) = C_s \times 1000 \times W_e \times W_s^{-1} \quad (2)$$

where:

TC is the total carotenoids content;
DW – the dry weight;
C_s – concentration in solution calculated above [mg/g];
1000 – the conversion factor from grams to kilograms;
W_e – the weight of the extract calculated by difference between the tubers with and without the extract [g];
W_s – the initial sample weight [g].

2.4. Statistical Interpretation

Each set of comparable assay was conducted with the same bulk sample. Analysis of variance (ANOVA) and Duncan’s multiple range test were used to analyze the data.

3. Results and Discussions

The trials conducted in Brasov in 2015 and 2016 show that cv ‘Roclas' had the highest mean TC value in both skin and flesh. In Targu Secuiesc, the highest values were found for cv. ‘Red Fantasy’ in the skin and cv. ‘Roclas’ in the flesh. Varieties with the lowest quantified values were ‘Albioana’ in Brasov in 2015 and 2016 for both tissues, flesh and skin respectively (Table 2 and Figure 2).

TC of twenty potato cultivars grown in two Romanian locations over two years Table 2

Variety	F / S	Brasov 2015		Brasov 2016		Tg Secuiesc 2016	
		Flesh	Skin	Flesh	Skin	Flesh	Skin
Roclas	Y / Y	3.87 *a**	6.17 *a*	4.69 *a*	8.38 a	3.75 *a*	6.68 ab
Christian	Y / R	1.26 *cd*	2.44 *e*	2.15 *d*	5.30 *b*	1.88 *c*	4.27 *d*
Productiv	LY / Y	0.57 *defg*	1.22 *ghi*	1.06 *fg*	2.43 d	0.68 *fg*	2.32 *e*
Castrum	LY / Y	1.09 *cdef*	1.45 *gh*	1.36 *e*	2.34 *de*	-	-
Cosiana	WY/ R	0.76 *defg*	1.15 *ghi*	1.47 *de*	3.70 *c*	-	-
Marvis	WY/ Y	0.51 *efg*	1.26 *gh*	0.63 *h*	1.53 *fg*	-	-
Brasovia	WY/ Y	0.28 *g*	1.64 *fg*	0.30 *i*	1.83 *efg*	-	-
Gared	C / R	0.65 *defg*	1.55 *g*	1.04 *fg*	1.32 *gh*	0.84 *f*	1.25 *f*
Albioana	W/ LY	0.34 *g*	0.71 *hi*	0.28 *i*	0.90 *i*	0.33 *h*	1.19 *f*
AVG	B/B	1.17 *cde*	2.37 *ef*	1.15 *f*	1.73 *efg*	1.06 *ef*	nd
Jelly	Y / Y	3.41 *a*	5.40 *b*	4.14 *ab*	8.15 a	3.09 *b*	6.25 *bc*
Carrera	Y / Y	0.30 *g*	2.36 *ef*	0.48 *hi*	2.89 *d*	0.42 *gh*	3.09 *e*
Riviera	Y / Y	0.62 *defg*	1.48 *gh*	0.59 *h*	2.26 *def*	0.90 *ef*	2.47 e
Red Fantasy	Y / R	2.11 *b*	4.34 *c*	3.63 *b*	7.65 *a*	3.34 *b*	7.40 a
Bellarosa	Y / R	0.41 *fg*	1.74 *efg*	0.96 *fg*	2.39 *de*	1.29 *de*	2.61 *e*
Red Lady	Y / R	1.70 *bc*	3.44 *d*	2.83 c	4.23 *c*	2.17 *c*	5.53 c
Hermes	LY / C	0.82 *defg*	1.21 *ghi*	1.61 *d*	2.46 *def*	1.48 *d*	2.67 *e*
Salad Blue	B / B	0.47 *fg*	0.69 *i*	0.86 *gh*	1.59 *fgh*	-	-
Vittelote	B / B	0.74 *defg*	1.75 *efg*	0.91 *gh*	2.26 *ef*	-	-
Blue Congo	PB / B	0.31 *g*	1.30 *gh*	0.57 *h*	2.43 *def*	-	-
Mean		**0.97**	**2.18**	**1.54**	**3.29**	**1.63**	**3.52**

* Means with different lowercases letters are significantly different at $p<0.05$ in each column. Values reported for the two Romanian sites are the mean of three filed replicates; TC= Total carotenoids content expressed in milligrams over kg DW; AVG = Albastru Violet Galanesti; DW=dray weight; F=flesh; S=skin; W=white; C=cream; LY=light yellow; WY= white yellow; Y= yellow; R= red; B= blue; PB= partial blue; nd= not detected; - no sample available.

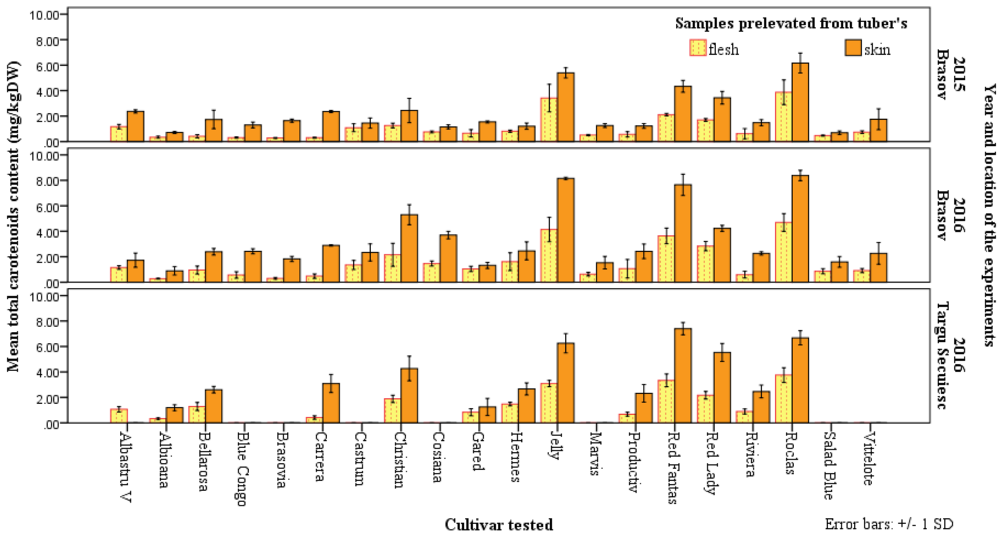

Fig. 2. *Total carotenoids content in flesh and skin of several potato cultivars grown in two locations in Romania in 2015 and 2016*

The levels of TC ranged from negligible quantities to 8.84 and 4.54 $mg \cdot kg^{-1}$ DW in the skin and flesh, respectively, with flesh and skin contents showing a significant difference for both sites and years (Table 3). On average, the skin of the potatoes analysed contained between two and half and three times more TC than in the flesh.

*Effects of same variables on TC** Table 3

Variables /interactions between variables	ANOVA p values
Site (location)	0.218
Year	< 0.01
Variety	< 0.01
Tissue	< 0.01
Replicate (site)	0.348
Replicate (year)	0.269
Site x variety	< 0.01
Site x tissue	< 0.01
Year x variety	< 0.01
Year x tissue	0. 504
Variety x tissue	< 0.01

** TC = total carotenoids content*

TC content in both tissues was positively correlated, with a Pearson's coefficient of 0.953 ($p<0.01$).

These data are in agreement with other studies: it has been reported [4] that the total quantity of the four main carotenoids analysed in eight commercial potato varieties was between 0.38 and 1.75 $mg \cdot Kg^{-1}$, which would be equivalent to 1.90-8.75 $mg \cdot kg^{-1}$ DW assuming 80% of water in the fresh samples. Other authors [11, 12] reported that for varieties 'Pentland Javelin' (white flesh), TC was 1.60 $mg \cdot kg^{-1}$ DW and for variety 'Yukon Gold' (yellow flesh) and 'Superior' (white flesh) were 1.11 and 0.64 $mg \cdot kg^{-1}$ FW, respectively (equivalent to approximately 5.55 and 3.20 $mg \cdot kg^{-1}$ DW). The varieties included in this work had low content of TC in the flesh, with values below 1$mg \cdot kg^{-1}$ DW (table 2). It had been established that higher quantities of carotenoids are accumulated in the flesh of yellow tubers [2, 4, 6, 11, 12]. This is in agreement with our results. Statistical analyses showed that darker yellow fleshed varieties had the highest mean TC content, which was not significantly different to the second highest mean, corresponding to light yellow fleshed varieties. Cream, blue and white flesh coloured varieties contained significantly lower TC levels. This was confirmed by the results on the skin, with blue skinned varieties being no different to varieties with red, white and yellow skins. Only yellow skinned tubers showed higher TC content than the white counterparts. In potatoes, as in many other vegetables, colours red and blue are produced by phenolic compounds (anthocyanin pigments). TC presented a very weak positive correlation with the phenolic compounds, with a Pearson coefficient of 0.311 ($p<0.001$) (data not shown). Other studies with whole tubers report no correlation between total phenolic and TC [14], independence between total anthocyanin and TC [2] or even a negative correlation between the latter [5].

Tuber grown in Brasov in 2016 had an average higher TC contents than those grown in Brasov in 2015, but no significant difference was found between potatoes from Brasov and Târgu Secuiesc in the same year. 2016. Significant interactions between site and variety and variety and year were also found (Table 3).

This suggest that evaluation of TC across years may be more important than evaluation across sites, which should be confirmed by further filed trials considering a wider variety of locations and over more than 2 years. A variety of factors can influence variation between trial sites and structure to climatic conditions or pressure from pests or pathogens. Climate data for the growing season in Brasov (Table 1) show that average temperatures in 2016 were slightly higher than in 2015. This difference was accentuated in June, July and August and was accompanied also by increased rainfall. TC content seems to be higher in early developing tubers [12] so these climatic conditions during the summer months could contributed to difference observed. Studies looking at variations between sites with different climates [9], [12], [14] report a significant effect of the site of cultivation. Although interaction between site and variety was not significant differences between trials taking place on the same site in two consecutive years (Table 3) and that tubers planted in the location with higher average temperature and increased rainfall contained higher levels of carotenoids (Table 1).

4. Conclusions

TC content in skin and flesh tissues were investigated in twenty cultivars of potato grown under uniform cultural conditions. Values reported for total carotenoids content were not different to those found in the literature. Significant differences were observed between varieties for TC. Higher contents of total carotenoids were found in intense yellow fleshed tubers than in white counterparts, which should allow visual selection of varieties with enhanced levels of these compounds.

The effect of environment on TC was significant. Higher rainfall seemed to increase the accumulation of TC in tubers. Higher temperatures produced higher TC content in tubers. Furthermore, significant interactions between variety and site and year of cultivation were found for TC (curiously site of cultivation was not found significant for TC) which mean that the action and extent of the environmental effects are different depending on the variety. However, it must be considered that the results presented in this paper arise from field trials over two years and two years and in two sites, so extended filed trials would be necessary to confirm these results.

Acknowledgements

This work was partially supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, PN-II-PT-PCCA-2013-4-0452, project number 178/2014.

References

1. Ahmed S.S., Lott M.N., Marcus, O.M., 2005. The macular xanthophylls. In: Survey Ophthalmology, vol. 50(2), pp. 183-193.
2. Andre C.M., Ghislain M., Bertin P. et al., 2006. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. In: Journal of Agriculture and Food Chemistry, vol. 55(2), pp. 366-378.
3. Bădărău C.L., Damşa F., Olteanu Gh. et al., 2015. Necrotic stalks of Potato Y virus – a constant challenge for farmers and producers (in Romanian). In: Cartoful în Romania, vol. 24(1), pp. 105-114.
4. Breithaupt D.E., Barnedi A., 2002. Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): new insights into an ancient vegetable. In: Journal of Agriculture and Food Chemistry, vol. 50, pp. 7175-7181.
5. Brown C.R., Culley O., Bonierbale M. et al., 2007. Anthocyanin, carotenoid content and antioxidant values in native South American potato cultivars. In: HortScience, vol. 47(7), pp. 1733-1736.

6. Burgos G., Salas E., Amoros W. et al., 2009. Total and individual profiles in *Solanum phureja* of cultivated potatoes. I. Concentration and relationships as determined by spectrophotometry and HPLC. In: Journal of Food Composition and Analysis, vol. 22(6), pp. 503-508.
7. Camire M.E., Kubow S., Donnelly D.J., 2009. Potatoes and human health. In: Critical Reviews in Food Science Nutrition, vol. 49(10), pp. 821- 840.
8. FAO Statistical Pocketbook World Food and Agriculture 2015. Availavle at: http://www.fao.org/3/a-i469le.pdf. Accessed on May, 2017.
9. Haynes K.G., Sieczka J.B., Henninger M.R. et al., 1996. Clone X environment interactions for yellow-flesh intensity in tetraploid potatoes. In: Journal of the American Society for Horticultural Sciences, vol. 121(2), pp. 175-177.
10. Kolbe H., Stephan-Beckmann S., 1997. Development, growth and chemical composition of the potato crop (*Solanum tuberosum* L.). II. Tuber and whole plant. In: Potato Research, vol. 40(2), pp. 135-153.
11. Lu W.H., Haynes K., Wiley E. et al., 2001. Carotenoid content and color in diploid potatoes. In: Journal of the American Society for Horticultural Sciences, vol. 126(6), pp. 722-726.
12. Morris W.L., Ducreux L., Griffiths D.W. et al., 2004. Carotenoids genesis during tuber development and storage in potato. In: Journal of Experimental Botany, vol. 55, pp. 975-982.
13. Rao A.V., Rao L.G., 2007. Carotenoids and human health. In: Pharmacology Research, vol. 55(3), pp. 207-216.
14. Reddivari L., Hale A.L., Miller J.C., 2007. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. In: Journal of Agriculture and Food Chemistry, vol. 55(7), pp. 8073-8079.
15. Young A.J., 1991. The photo protective role of carotenoids in higher plants. In: Physiology Plant, vol. 83(4), pp. 702-708.