



Coloured-fleshed potatoes after boiling: Promising sources of known antioxidant compounds



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ABSTRACT

This study aims at evaluating the effect of boiling on the anthocyanin and phenolic acid content of varieties of yellow, pink and violet flesh colored potatoes grown in central Italy. The total phenolic acid content ranged from 33.4 to 1130 mg/kg of fresh weight (FW) in uncooked tubers and from 111 to 1375 mg/kg FW in cooked potatoes, with increments obtained for six varieties (Mz012, Mz064, Vitelotte Noir, Mz032, Mz080, Mz046) after boiling. The anthocyanin content decreased in a variety-dependent mode, but an increment was evidenced after cooking in one sample Mz012. A strong correlation was observed between antioxidant capacity and total anthocyanins for pink and violet-fleshed potatoes, suggesting that these compounds are mostly responsible of the antioxidant capacity of these tubers after boiling. Overall, the yellow-fleshed cultivars have shown lower antioxidant capacity (about 3.25 times lower) than the violet-fleshed tubers. Violet Mz064 variety registered the highest anthocyanin level and a daily intake of 100 g of this tuber contains up to 200 mg of total phenols. Flesh-colored potatoes can represent an additional source of bioactive compounds, particularly of acylated anthocyanins in the human diet.

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1. Introduction

The potato (*Solanum tuberosum* L.) is a staple food in many regions of the world and it is characterized by high carbohydrate, protein and vitamin C contents. Freshly harvested potatoes contain about 20% dry matter from which the starch is about 60–80%. In addition, the potato is low in fat and it is a good source of vitamins B₁, B₃, B₆, folate, pantothenic acid, riboflavin and minerals, such as potassium, phosphorus and magnesium (Prokop and Albert, 2008).

The main phytochemicals of this tuber are solanine alkaloids, phenolic acids, anthocyanins and carotenoids; most of them are commonly in higher amount in peel (Ezekiel et al., 2013). They have attracted increasing attention in recent years due to their

health benefits; numerous studies have revealed a negative correlation between the intake of phytochemicals and chronic inflammation, cardiovascular diseases, cancer and diabetes (Acosta-Estrada et al., 2014; González-Castejón and Rodríguez-Casado, 2011). It is well known that phenolic compounds have antioxidant properties, protecting cellular constituents against oxidative damage and limiting the harmful effects of oxidative stress. Moreover, a recent study reported that purple potatoes acted as hypotensive agents, and were able to lower the risk of heart disease and stroke in hypertensive subjects without weight gain (Vinson et al., 2012). Choi et al. (2013) observed that a 20% purple-fleshed potato powder intake improved both diabetes and lipid control in diabetic rats, by significantly improving serum insulin levels and lowering blood glucose and serum cholesterol levels. Furthermore, it was hypothesized that polyphenols from pigmented potatoes would decrease oxidative stress and inflammation in humans (Kaspar et al., 2011). Nowadays, the interest toward colored-fleshed potatoes is growing also because these tubers are richer in phenolic compounds.

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Potatoes are generally cooked before consumption, and the different cooking methods are important factors affecting not only the chemical composition and physical structure of the potato, but also the intake of bioactive compounds under normal dietary conditions (Tian et al., 2016). Changes in potato chemical composition mainly occur during storage and cooking and vary depending on the cultivar and growing conditions (Blessington et al., 2010; Brown et al., 2008; Burmeister et al., 2011; Ieri et al., 2011; Lachman et al., 2012).

This study aims to evaluate the anthocyanin and phenolic acid content in several varieties of potato with yellow, pink and violet coloured flesh selected by an Italian producer farm. The effect of boiling on the whole potato was evaluated by studying changes in the concentration of chlorogenic acid, its isomers and total anthocyanins before and after cooking. The antioxidant capacity was also estimated in the processed tubers using the ABTS assay. The final aim is to valorise the pigmented-fleshed potatoes which are not yet widely consumed in the Italian market.

2. Material and methods

2.1. Materials

The fresh tubers were obtained from breeding lines cultivated in the same way in a conventional system in the same field in the agricultural district of Bologna (Italy). Three yellow- (Mz032, Mz080, Mz046), three violet- (Mz128, Mz064, Vitelotte Noir) and two pink-fleshed (Mz011, Mz012) varieties of potatoes were studied. The tubers were kindly provided by Pizzoli S.p.A. (Bologna-Italy), both fresh and after boiling. A summary of the morphological characteristics and the common uses of all the varieties is reported in Table 1.

Ascorbic acid (99% purity grade) was purchased from Sigma-Aldrich.

2.2. Cooking process

A representative amount of the whole tuber (about 4 kg for each variety) was boiled in a covered stainless steel pot on a moderate flame. The whole potatoes were boiled in water with NaCl (1%) and ascorbic acid for 15 min. The ratio potatoes/water was 1:4 w/v. At the end of cooking, the tubers were peeled and frozen and these were used for the extraction of phenolic compounds.

2.3. Sample preparation

The potatoes of each variety (1 g) were extracted at room temperature, by stirring twice with 30 mL of 70% EtOH adjusted to pH 2.0 by HCOOH, as already described in our previous study (Mulinacci et al., 2008). The hydroalcoholic solutions were analyzed by HPLC/DAD/MS, according to our previous work (Ieri et al., 2011) and used for the ability to scavenge the ABTS radical cation.

2.4. HPLC/DAD/MS analysis

Analysis was firstly carried out using a HP-1100 liquid chromatograph equipped with a DAD detector and a HP1100 MSD API-electrospray (Agilent-Technologies, Palo Alto, CA) operating in positive and negative ionization mode under the following conditions: gas temperature 350 °C, nitrogen flow rate 10 L/min, nebulizer pressure 35 psi, quadrupole temperature 30 °C, capillary voltage 4000 V, and applied fragmentors in the range 50–250 V.

Further identification and characterization of the phenolic compounds was performed by a TOF-MS with an ESI interface (Agilent Technologies) under the following conditions: gas temperature 300 °C, nitrogen flow rate 12 L/min, nebulizer pressure 20 psi, capillary voltage 3800 V, and applied fragmentors in the range 80–300 V.

The column was a Synergi Max RP 80 A (4 µm; 150 mm × 3 mm i.d.) from Phenomenex (Castel-Maggiore, BO, Italy). The mobile phases were: (A) acidified water (pH 2.0) and (B) acetonitrile. The following multistep linear gradient was applied: from 95% to 78% A in 8 min, 4 min to reach 74% A, then 13 min to arrive at 65% A, and finally 3 min to reach 100% B with a final plateau of 4 min. The total time of analysis was 32 min, flow rate was 0.4 mL/min, and oven temperature was 26 ± 0.5 °C, as described in our previous study (Ieri et al., 2011).

2.5. Quantitative determination of phenolic acids and anthocyanins

The phenolic acids were evaluated by HPLC/DAD using a six-point calibration curve of chlorogenic acid (3-caffeoylquinic acid-purity ≥ 99%) (Extrasynthèse, Genay, France) at 330 nm ($r^2 = 0.999$), while the anthocyanin content was determined by HPLC/DAD using a five-point calibration curve of malvin chloride (MW 691 from Extrasynthèse; purity ≥ 95%) at 520 nm ($r^2 = 0.999$).

2.6. Antioxidant capacity by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) (ABTS) method

The ABTS method (Miller et al., 1996) was used for the determination of antioxidant capacity. The hydroalcoholic extracts were diluted with distilled water (100 times) and immediately subjected to reaction with the radicals; absorbance was measured after the addition of a 200 µL sample to 2 mL of radical solution after 2 min. For the blank experiment, 200 µL of water was added. The antioxidant activity was calculated as follows by the equation:

$$\% \text{inhibition ABTS} = [(A_0 - A_t)/A_0] \times 100$$

In agreement with this study, 54.8 mg ABTS (Sigma-Aldrich, Milan, Italy) was dissolved in 20 mL phosphate buffer (pH 7.0; 5 mM) and activated to ABTS^{•+} radical by adding 1 g of MnO₂ (activation time 20 min). Then the solution was centrifuged, filtered and diluted with the buffer solution to obtain $A_{734}(t_0) = 0.800 \pm 0.02$ nm. Absorbance of the solution was measured at a wavelength of 734 nm. Values are the means of three repetitions.

Table 1
Morphological characteristics of the analyzed varieties.

variety or code	country of origin	maturity	skin colour	flesh colour	tuber form	% dry matter	cooking use
Mz011	Italy	late	pink	pink	long oval	16–19	boiled, mashed
Mz012	Netherland	medium late	dark pink/red	dark pink/red	long oval	17	boiled, mashed
Mz128	Netherland	late	brown/violet	light violet	round oval	16–18	boiled, mashed
Mz064	Netherland	medium late	brown/violet	blue/violet	small long	20–23	boiled, mashed and chips
Vitelotte Noir	France	very late	dark violet	dark violet	long irregular	23	boiled, mashed and chips
Mz032	United Kingdom	medium early	dark yellow and purple	dark yellow	small long	>23	boiled, mashed
Mz046	United Kingdom	medium early	dark yellow	dark yellow	small long	>23	boiled, mashed
Mz080	Netherland	medium late	yellow with red eyes	yellow	oval	18–22	multiuse

2.7. Statistical analysis

Data were not normally distributed (Kolmogorov-Smirnov one sample test) and were analyzed by the non-parametric Kruskal-Wallis ANOVA, followed by the Mann-Whitney *U* test for multiple comparisons using SYSTAT 12.0 software (Systat Software Inc., Richmond, CA). Differences were accepted as significant at the 5% level.

3. Results and discussion

3.1. Phenolic acid and anthocyanin content

The chromatographic profiles obtained according to our previous works (Ieri et al., 2011; Mulinacci et al., 2008), demonstrated the same compounds in both raw and cooked potatoes, (Tables 2 and 3). The same phenolic acid pattern was highlighted, and the main phenolic acids detected were 3-caffeoylquinic, 5-caffeoylquinic, 4-caffeoylquinic and ferulic acids. The identified anthocyanins were acylated glycosides of pelargonidin, malvidin, petunidin, peonidin and delphinidin; the rutinosides in C3 were the dominant forms, acylated with a cinnamoyl residue in C4 of the rhamnose unit.

For all of the varieties, the total levels of anthocyanins and phenolic acids varied significantly between raw and cooked tubers, as shown in Tables 2 and 3. Violet potatoes were rich in anthocyanins, and the Mz064 cultivar in particular registered the highest levels (691 mg/kg FW; Fig. 1a). The boiling was responsible of a significant decrease in the amount of anthocyanins when compared to raw potatoes (Fig. 1a) with the highest loss showed for Mz128 and Mz064 (66.1% and 36.7% respectively). In only one case, for Mz012 cultivar, a statistically significant increase in the amount of total anthocyanins was observed after cooking, with an increment of more than a double (from 88.9 in fresh to 182 mg/kg in cooked potato).

Contradictory results were found in the literature relating to the effect of cooking on the polyphenol content of potatoes. Our data are partly in accordance with the study performed by Brown et al. that reported a significant decrease in the total anthocyanins in five pigmented varieties after boiling, microwaving and baking (Brown et al., 2008). According to Lachman et al., the increase in anthocyanin content after cooking is strongly variety-dependent (Lachman et al., 2013). Various factors influence the composition of anthocyanins in fresh tubers, such as the cultivar and climatic conditions including altitude and storage conditions (Lachman et al., 2012).

As reported in Table 2, a significant decrease in the level of all the anthocyanins was detected between raw and cooked potatoes, particularly for the varieties Mz011, Mz064 and Vitelotte Noir. In the variety Mz128, the amount of malvidin 3-*O*-*p*-coum-rut-5-*O*-glu did not vary significantly after cooking, while the statistical Mann-Whitney *U* test showed significant variations for all of the other anthocyanins present. Significant increases were shown in the Mz012 variety for pelargonidin 3-*O*-rut-5-*O*-glu, pelargonidin 3-*O*-rut, pelargonidin derivative 1, pelargonidin 3-*O*-caf-rut-5-*O*-glu, pelargonidin 3-*O*-*cis*-*p*-coum-rut-5-*O*-glu, pelargonidinderivative 2 and pelargonidin 3-*O*-*p*-coum-rut-5-*O*-Glu. Pelargonidin 3-*O*-*p*-coum-rut-5-*O*-glu was the major anthocyanin in the pink varieties (Mz011, Mz012) and in Vitelotte Noir; the same anthocyanin pattern was found in these three varieties. The violet varieties Mz128 and Mz064 showed high concentrations of petunidin 3-*O*-*p*-coum-rut-5-*O*-Glu.

The phenolic acid content was determined for the eight varieties, and ranged from 33.4 (Mz080) to 1130 mg/kg FW (Vitelotte Noir) in fresh potatoes, and from 111 (Mz080) to 1375 mg/kg FW (Mz012) in cooked potatoes (Table 3). In the

Table 2
Amount of anthocyanins in fresh and cooked tubers expressed as $\mu\text{g/g}$ FW; mean data are from three replicates.

Compounds	Mz011			Mz012			Mz128			Mz064			Vitelotte Noir								
	fresh $\mu\text{g/g}$	SD	χ^2	fresh $\mu\text{g/g}$	SD	χ^2	fresh $\mu\text{g/g}$	SD	χ^2	fresh $\mu\text{g/g}$	SD	χ^2	fresh $\mu\text{g/g}$	SD	χ^2						
pet 3- <i>O</i> -rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	6.93	0.0	3.72	0.1	*	3.9	8.26	0.4	11.3	0.2	*	3.9	ND	-	-
pet 3- <i>O</i> -caf-rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	13.6	3.2	9.48	0.3	*	3.9	37.0	0.9	68.8	1.7	*	3.9	ND	-	-
delp 3- <i>O</i> - <i>p</i> -coum-rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	ND	-	ND	-	-	-	7.92	0.1	ND	-	-	-	ND	-	-
mal 3- <i>O</i> -caf-rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	ND	-	ND	-	-	-	4.10	0.2	1.24	0.1	*	3.9	ND	-	-
pet 3- <i>O</i> - <i>p</i> -coum-rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	148	0.8	40.5	1.1	*	3.9	537	11.2	297	6.1	*	3.9	ND	-	-
pet 3- <i>O</i> -ferul rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	15.3	0.0	5.84	0.0	*	3.9	18.7	0.4	9.19	0.0	*	3.9	ND	-	-
mal 3- <i>O</i> - <i>p</i> -coum-rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	3.33	0.2	3.33	0.1	NS	-	74.2	0.9	47.5	0.9	*	3.9	ND	-	-
mal 3- <i>O</i> -ferul-rut-5- <i>O</i> -glu	ND	-	-	ND	-	-	1.71	0.1	1.34	0.0	*	3.9	4.52	0.2	2.11	0.1	*	3.9	ND	-	-
pet 3- <i>O</i> -rut-5- <i>O</i> -glu	17.5	0.9	13.6	0.4	*	3.9	4.67	0.1	7.70	0.1	*	3.9	ND	-	ND	-	-	ND	-	-	
pet 3- <i>O</i> -rut	11.9	0.8	14.5	0.5	*	3.9	ND	-	2.18	0.0	*	4.4	ND	-	ND	-	-	ND	-	-	
pel derivative1	4.28	0.4	2.03	0.1	*	3.9	ND	-	1.29	0.0	*	4.4	ND	-	ND	-	-	ND	-	-	
pel 3- <i>O</i> -caf-rut-5- <i>O</i> -glu	19.4	2.7	8.77	0.3	*	3.9	ND	-	2.18	0.1	*	4.4	ND	-	ND	-	-	ND	-	-	
pel 3- <i>O</i> - <i>cis</i> - <i>p</i> -coum-rut-5- <i>O</i> -glu	4.28	0.2	6.61	0.2	*	3.9	9.20	0.5	16.8	0.5	*	3.9	ND	-	ND	-	-	ND	-	-	
pel derivative2	4.60	0.5	3.34	0.1	*	3.9	1.18	0.1	2.95	0.1	*	3.9	ND	-	ND	-	-	ND	-	-	
pel 3- <i>O</i> - <i>p</i> -coum-rut-5- <i>O</i> -glu	97.4	10.4	63.3	2.1	*	3.9	59.7	2.9	134	3.5	*	3.9	ND	-	ND	-	-	ND	-	-	
pet 3- <i>O</i> -ferul rut-5- <i>O</i> -glu	35.4	4.1	22.3	0.7	*	3.9	11.8	0.8	12.2	0.7	NS	-	ND	-	ND	-	-	ND	-	-	
pel 3- <i>O</i> - <i>p</i> -coum-rut	2.88	0.2	3.27	0.1	*	3.9	2.36	0.2	2.31	0.0	NS	-	ND	-	ND	-	-	ND	-	-	

ND not detected (LOD 0.7 $\mu\text{g/g}$ FW); SD standard deviation; * significant differences ($p < 0.05$); NS not significant. Degrees of freedom (d.f.) = 1. Abbreviations used: pel, pelargonidin; pet, petunidin; mal, malvidin; delp, delphinidin; rut, rutinoside; glu, glucoside; ferul, feruloyl; *p*-coum, *p*-coumaroyl; caff, caffeoyl.

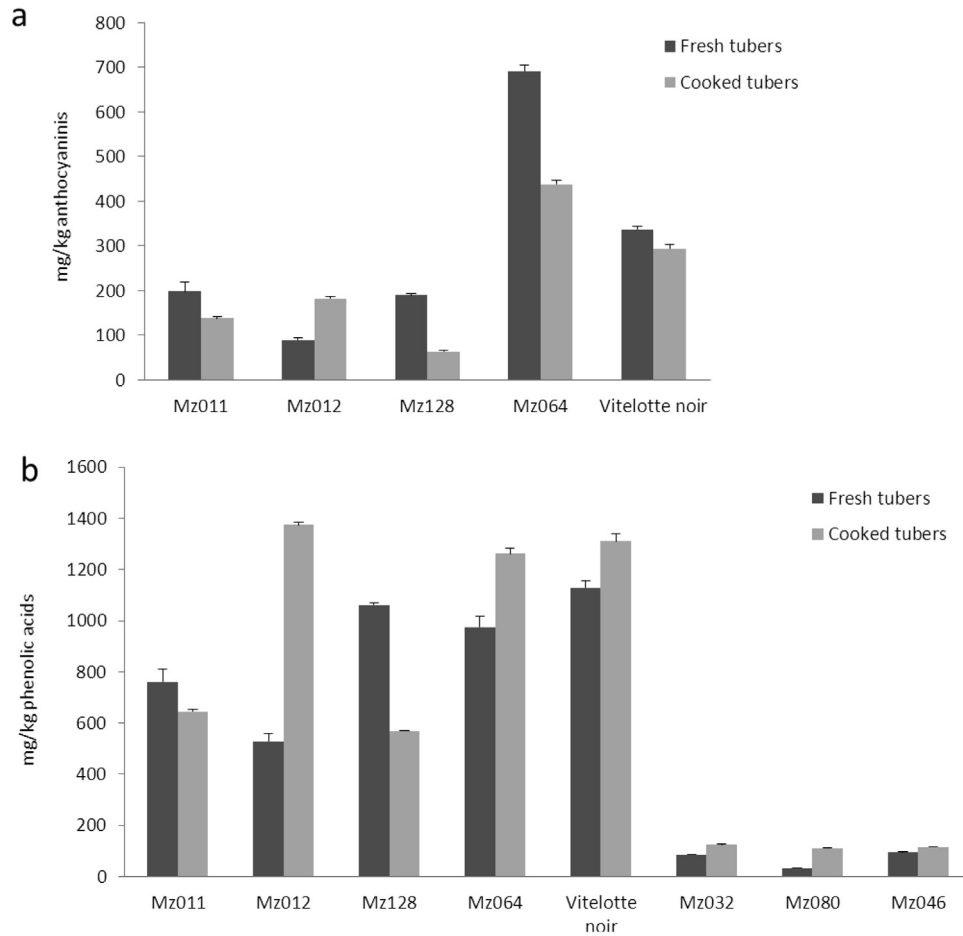


Fig. 1. Total anthocyanins (a) and total phenolic acids (b) in fresh and cooked pink-, violet- and yellow-fleshed cultivars of *Solanum tuberosum* L.; data are expressed as mg/kg FW (fresh weight). The differences between fresh and cooked tubers are significant for all the varieties (χ^2 3.9; degrees of freedom (d.f.) 1).

cooking, except in the Mz011, Mz128, Mz064, Vitelotte Noir and Mz046 varieties. We can hypothesize a partial inter-conversion among the isomers in these varieties, particularly from 5-caffeoylquinic acid to 3-caffeoylquinic acid. According to the peculiarities of the different varieties, gentisic acid monoglucoside was identified and quantified only in the Mz032 sample.

3.2. Antioxidant capacity

Potatoes are a source of phenolic acids but also of anthocyanins and carotenoids, which can contribute to increase the antioxidant intake in the human diet (Ezekiel et al., 2013; Hamouz et al., 2011; Lachman and Hamouz, 2005; Reyes et al., 2005). Considering that

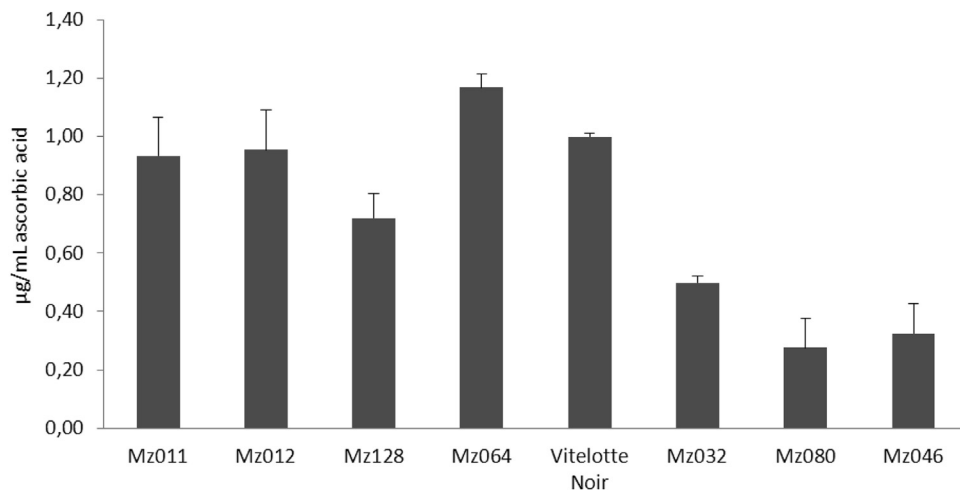


Fig. 2. Antioxidant capacity (expressed as µg/mL ascorbic acid) measured for each cooked sample of potatoes.

the human consumption of the tuber occurs after cooking, the antioxidant capacity of our samples was assayed by the ABTS method only after boiling. As reported in Fig. 2, the lowest antioxidant capacity was observed in the yellow fleshed varieties, averaging $0.37 \mu\text{g}/\text{mL}$ of ascorbic acid. In agreement with other studies (Külen et al., 2013; López-Cobo et al., 2014) that reported blue and purple potatoes possessing a higher antioxidant capacity compared to the white-yellow tubers, the blue-violet fleshed potato (Mz064) showed the highest antioxidant capacity in the ABTS assay.

The obtained correlation coefficients are very similar considering phenolic acids (Fig. 3a), anthocyanins (Fig. 3b) and the total phenols (Fig. 3c), with r^2 values from 0.83 to 0.89. A better correlation ($r^2 = 0.98$) was found between the antioxidant capacity and total phenolic acid amount in the yellow-fleshed cultivars selected. The pink and violet-fleshed potatoes did not show a correlation between antioxidant capacity and total phenolic acids ($r^2 = 0.5$), even if they contain considerably higher levels of caffeoylquinic acid isomers than the yellow varieties (Ieri et al., 2011). As expected and according to previous results (Brown, 2005; Brown et al., 2005; Lachman et al., 2012, 2009), these pigmented

tubers showed a higher and positive correlation between antioxidant capacity and total anthocyanins (Fig. 3b), with anthocyanins being mainly responsible for the antioxidant properties. The ORAC and FRAP assays revealed that antioxidant levels in red or purple-fleshed potatoes were two or three times higher than in white or yellow-fleshed potatoes (Teow et al., 2007). Overall, these varieties are a natural source of acylated anthocyanins and cinnamoyl acids and can positively contribute to increased antioxidant intake in the diet.

4. Conclusions

In this study, we investigated the effect of boiling on the total levels of phenolic compounds in different varieties cultivated in the same experimental conditions. Significant positive and negative variations were found in the concentration of phenolic acids and anthocyanins in the cooked tubers, highlighting a variety-dependent correlation. The antioxidant capacity estimated by the ABTS assay on the cooked tubers demonstrated a similar positive correlation with the content of phenolic acids, total anthocyanins and total phenols.

The boiled pink and violet-fleshed potatoes showed the highest efficacy as radical scavengers in the ABTS test. Chlorogenic acid provided the greatest contribution to antioxidant capacity in yellow fleshed potatoes, while anthocyanins were the major contributors as antioxidants in pink and violet-fleshed tubers. Our results indicate that pink and violet-fleshed potatoes show significantly higher antioxidant potency than yellow-fleshed varieties. Thus, the use of violet-fleshed potatoes in cuisine should be recommended because it can help to increase the daily intake of these interesting acylated anthocyanins.

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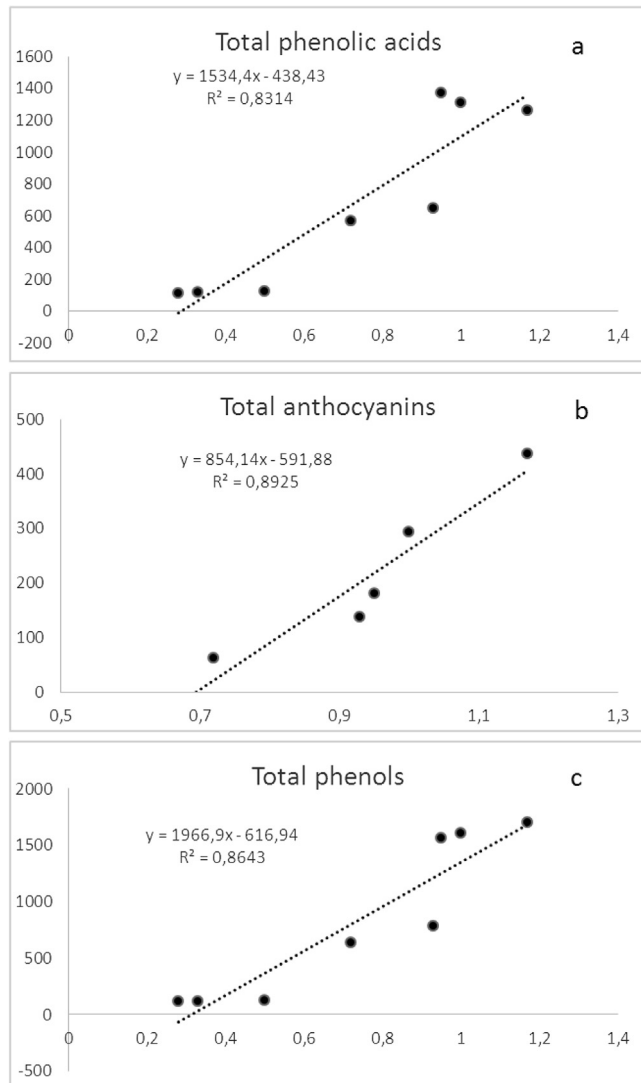


Fig. 3. Correlation between total phenolic acid (a), anthocyanin (b) and total phenol (c) contents expressed as mg/kg FW and antioxidant capacity expressed as $\mu\text{g}/\text{mL}$ ascorbic acid.

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