Highlights

- Color-fleshed potatoes showed a high variability in phytochemical composition.
- Introgressed potato breeding lines show high phenolic and carotenoid levels.
- Phytochemical losses after boiling were higher than those previously reported.
- Phytochemical losses highly correlated with phytochemical content in raw tubers.
- Total phenolic content correlated with dry matter in potato tubers.

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Original Research Article

Effect of boiling on the total phenolic, anthocyanin and carotenoid concentrations of potato tubers from selected cultivars and introgressed breeding lines from native potato species

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Abstract

Potato tubers are considered an important source of bioactive compounds, although the concentrations of different phytochemicals are affected by cooking and other processes.

The present study was focused on evaluating the effect of boiling on total phenolic, anthocyanin and carotenoid concentrations of ten promising introgressed breeding lines from native potato species (*Solanum tuberosum* subsp. *andigena* Hawkes and *Solanum stenotomum* Juz. & Bukasov) and three heirloom Spanish *Solanum tuberosum* L. cultivars (Jesús, Morada and Zamora). In addition, three commercial cultivars (Vitelotte, Kasta and Monalisa) with different flesh color were used as testers. Breeding lines NK-08/349 and NK-08/362 showed high total phenolic (TP) and total anthocyanin concentrations (TA), whereas breeding lines NK-08/286, NK-08/356 and NK-08/370 and the cultivar Morada were characterized by a high carotenoid concentration (TC). In general, purple cultivars and breeding lines (Vitelotte, Morada, Jesus, Kasta, NK-08/349 and NK-08/362) stood out with showed high phenolic and anthocyanin concentrations. The comparative analysis of raw and boiled tubers showed high losses of phenolics, anthocyanins and carotenoids. TP, TA and TC concentrations in boiled tubers were directly correlated with their corresponding concentration in the raw product. In addition, significant correlations ($p \leq 0.05$) between TA and TP concentrations, in both raw and boiled tubers, have been observed. The utilization of peeled and diced tubers, with the subsequent surface increase, seems to be a determinant factor explaining why phytochemical losses were so high.

Keywords: Food analysis; Food composition; Potato breeding; *Solanum tuberosum* L.; *Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes; *Solanum stenotomum* Juz. & Bukasov; Phytochemicals; Bioactive compounds; Thermal processing; Biodiversity and nutrition

1 Introduction

Potato is the fourth most important food crop after rice, wheat and maize, and it contains a wide variety of phytochemicals, including carotenoids and phenolic compounds (Brown, 2008; Ezekiel et al., 2013). The regular consumption of phytochemicals may contribute to the prevention of certain chronic diseases associated with oxidative damage (Espín et al., 2007). In particular, native potato germplasm can be considered a great source of nutritional variability in potato breeding programs for increased tuber nutritional value (Burgos et al., 2007).

Phenolic compounds are considered to be bioactive compounds as they have shown beneficial health effects. They are commonly classified into three important groups: phenolic acids, flavonoids and tannins. Substituents on the aromatic ring affect the stability and the radical-quenching ability of the phenolic acids (Rice-Evans et al., 1996). The most abundant phenolic compounds in potato tubers are caffeoyl-esters, and the major phenolic acid is chlorogenic acid, which is bioavailable in humans and constitutes about 80 to 90% of the total phenolic acids (Brown, 2005). A study of 74 Andean potato landraces revealed a wide variability for total phenolic compounds and antioxidant activity (Andre et al., 2007a).

Anthocyanins are pigments which constitute the main subclass among flavonoids. More than 600 molecular structures have been identified to date as responsible for the red-blue color of many fruits and vegetables. The de-glycosylated forms (aglycones) of anthocyanins are known as anthocyanidins, being malvidin, petunidin, delphinidin and peonidin the most common anthocyanidins found in purple-fleshed potatoes, while pelargonidin is in red-fleshed tubers (Kita et al., 2013).

Carotenoids are tetraterpenoids with a long conjugated double bond system and a near bilateral symmetry around the central double bond (Britton, 1995). There are over 750 known carotenoids with their color ranging from pale yellow to deep red (Rodriguez-Amaya, 2001). The carotenoid profiles and concentrations in tubers of *Solanum phureja* Juz. & Bukasov have been correlated with the intensity of yellow flesh color, and zeaxanthin and antheraxanthin were found as the predominant carotenoids in deep yellow-fleshed varieties while violaxanthin, antheraxanthin, lutein and zeaxanthin constituted the main carotenoid profile of yellow potatoes, and violaxanthin, lutein and β -carotene in cream-fleshed potatoes (Burgos et al., 2009).

However, in a recent characterization of 60 potato cultivars, including some native ones, zeaxanthin was not detected in their carotenoid profile suggesting that the storage conditions of tubers may play a modulating role on the carotenogenic pathway and subsequently in the carotenoid composition (Fernandez-Orozco et al., 2013). In addition, data presented by Fernandez-Orozco et al. (2013) suggest a direct correlation between the presence of xanthophyll esters and the ability of tubers to accumulate carotenoid pigments. Cultivated diploid potatoes derived from *Solanum stenotomum* Juz. & Bukasov and *Solanum phureja* Juz. & Bukasov have been reported to be a relevant source of zeaxanthin and lutein (Brown et al., 1993).

The concentration and stability of bioactive compounds in food can be affected by several factors such as pH, light, oxygen, enzyme activity, ascorbic acid, sugars and cooking techniques, as well as other processing conditions (Palermo et al., 2013; Patras et al., 2010). Therefore, food composition databases should contain information of foods as they are

94 finally consumed. The characterization of potato genotypes in terms of phytochemical
95 concentration has raised an increasing interest over the last two decades.

96 As noted by Ezekiel et al. (2013), some researchers have focused on the effects of thermal
97 processing on the concentration of different bioactive compounds of potatoes. Some studies
98 have shown significant decreases of total phenolic and antioxidant activity concentrations
99 in cooked tubers when compared to raw ones (Perla et al., 2012; Xu, et al., 2009); while
100 other works reported increases following cooking (Blessington et al., 2010; Burgos et al.,
101 2013). Increased total anthocyanin concentrations of tubers after different cooking
102 treatments have been recently published (Lachman et al., 2013). Lemos and Sivaramareddy
103 (2013) have also reported higher total anthocyanin concentrations in French fries when
104 compared to raw potatoes, suggesting that this enrichment can be due to the transfer of the
105 components between the frying medium (oil) and the fried product (potato). According to
106 Burgos et al. (2012), boiling significantly reduces the violaxanthin, antheraxanthin and total
107 carotenoid concentration of yellow-fleshed cultivars, whereas lutein and zeaxanthin
108 concentrations increase in some genotypes after boiling.

109 The present study aims at analyzing the total concentrations of some selected
110 phytochemicals (total phenolics, anthocyanins and carotenoids) before and after boiling of a
111 set of highly pigmented accessions that supposedly contain high levels of carotenoids and
112 phenolic compounds. The collection included three heirloom Spanish *Solanum tuberosum*
113 L. cultivars with purple flesh color (Morada, Jesus and Zamora) and ten pigmented
114 breeding lines with introgressions of native *Solanum* species (*Solanum tuberosum* subsp.
115 *andigena* (Juz. & Bukasov) Hawkes Hawkes and *Solanum stenotomum* Juz. & Bukasov),
116 using three common cultivars (Vitelotte, Kasta and Monalisa) with different flesh colors as

testers, within the context of a potato breeding program specifically addressed to increase the potato tuber nutritional value.

2 Materials and methods

2.1 Plant material

Three commercial cultivars belonging to the species *Solanum tuberosum* subsp. *tuberosum* were selected as testers on the basis of the contrasting flesh color of tubers: one deep purple (Vitelotte), one medium dark purple (Kasta), and one pale yellow (Monalisa). Three heirloom purple-fleshed Spanish cultivars (Morada, Jesus and Zamora) and ten breeding lines with introgressions from two native *Solanum* species (*Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes and *Solanum stenotomum* Juz. & Bukasov), were also selected for the objective of evaluating the effects of boiling on the phytochemical composition of highly pigmented potato tubers (Table 1). Tubers were selected from potato accessions (Potato Germplasm Collection, Neiker-Tecnalia) grown during the year 2011 in a precise field trial in Arkaute (Alava) in the north of Spain (550 m above sea level), with humid climate and annual rainfall of about 800 mm. The soil with a clay loam texture was previously subjected to conventional wheat cropping. After harvesting, mature tubers were stored at 4°C in a darkened cold room for one month.

2.2 Sample preparations of raw and boiled tubers

A total of 8 raw tubers of each accession were washed and patted dry with paper towels, and subsequently peeled and divided in eight equal parts according to the sampling diagram summarized in Fig. 1. For each tuber, four opposite parts were immediately frozen in liquid nitrogen, kept frozen at -80 °C, and later freeze dried, milled by an automatic mortar

grinder (approximately 250 g), and stored at -30 °C until analysis. Samples corresponding to the four remaining parts were placed in a stainless steel pot containing boiling water (2 L) and cooked for approximately 30 min. Boiled tubers were allowed to cool and subsequently frozen in liquid nitrogen and prepared as detailed above for raw tubers.

2.3 Reagents and gallic acid standard

All reagents were prepared using demineralized water (18 MΩ cm at 25 °C) produced with a water purification system (Simplicity 185, Millipore MA, USA). The gallic acid standard was obtained from Sigma-Aldrich Laboratories (Steinheim, Germany) with ≥ 99% % purity according to HPLC analysis by the manufacturer. Folin-Ciocalteu reagent 2 M was also supplied by Sigma-Aldrich Laboratories (Steinheim, Germany) Methanol absolute, sodium chloride, sodium carbonate, hydrochloric acid, magnesium carbonate, diethyl ether and dimethyl formamide with ≥ 99% % purity were obtained from Merck kGaA (Darmstadt, Germany).

2.4 Extraction and quantitative determination of total phenolic compounds

Phenolic compounds were extracted from lyophilized samples (0.5 g) with 10 mL methanol: H₂O (80:20, v/v). The solid was re-suspended by shaking in a vortex for 30 s, and left for 2 h in the dark at room temperature with periodic mixing. The mixture was centrifuged at 4,500 g for 15 min at 4 °C, and the supernatant containing the extracted phenolic compounds was collected. The extraction operation was repeated once again with the pellet. The methanolic extracts were pooled and the volume was reduced in a rotary evaporator to about 1 mL. The residue was taken to 5 mL with deionizer water and stored under nitrogen at -30 °C. Extractions were carried out in triplicate and the total phenolics

(TP) were determined also in triplicate for each extract by an adapted micro-scale protocol for the Folin-Ciocalteu colorimetric method (Waterhouse, 2001). Gallic acid, a phenolic acid which has been reported to be a major phenolic component in potato (Rodriguez de Sotillo et al. 2007), was used as standard. Briefly, an aliquot of 20 μL of the extract solution was taken into a cuvette, then 1.58 mL of distilled water and 100 μL of Folin-Ciocalteu reagent were added, and the cuvette was shaken thoroughly for 1 min. Finally 300 μL of sodium carbonate solution (20%, w/v) were added, and the mixture was allowed to stand for 2 h in the dark with intermittent shaking. Absorbance was measured at 765 nm using deionized water as blank. TP were calculated by interpolating the absorbance data in a calibration curve prepared with gallic acid (concentration range: 50.0-450 mg/L). Data were given as mg of gallic acid equivalents per 100 g dry sample (mg GAE 100 g⁻¹ dw).

2.5 Extraction and quantitative determination of total anthocyanins

Total anthocyanin (TA) concentration was analyzed according to Huang et al. (2006) with some modifications. Lyophilized samples were used for anthocyanin extraction. The sample (0.25 g for purple cultivars: Kasta, Morada, Jesus, Vitelotte, NK-06/130, NK-08/349, NK-08/362; and 0.50 g for light purple and yellow cultivars: Zamora, Monalisa, NK-08/284, NK-08/286, NK-08/336, NK-08/348, NK-08/355, NK-08/356, NK-08/370) was placed in a round-capped polypropylene 15 mL centrifuge tube, and extracted with acidified methanol (1% HCl) with continuous shaking for 30 min. The extraction volume ranged from 10 mL for highly-colored cultivars and 4.5 mL for medium- and low-colored cultivars. In the case of samples from boiled tubers, prior to extraction the lyophilized material was reconstituted with 2 mL of water in order to improve the anthocyanin extractability. The mixture was centrifuged at 4,500 g for 5 min at 4 °C, and the extracted

anthocyanins were collected. The extraction operation was repeated again twice, except for samples from low-colored cultivars which were extracted only once, and in the cases of samples from boiled tubers the second extraction was performed with 1% HCl - methanol: H₂O (1:1, v/v). The methanolic extracts were pooled and taken to a final volume with methanol. Final volume varied from 25 mL for highly-colored cultivars, 10 mL for medium-colored cultivars and 5 mL for low-colored cultivars. Analyses were carried out in triplicate. TA concentration was estimated by UV-visible spectrophotometry, measuring the absorbance at 530 nm and using a molar absorption coefficient of cyanidin 3-O-glucoside and acidified methanol as blank (Giusti and Wrolstad, 2001). Appropriated dilutions were prepared in most cases. According to most published data, results were given as mg cyanidin 3-O-glucoside equivalents per 100g of dry sample (mg CGE 100 g⁻¹ dw) (Brown et al., 2003, 2005; Burgos et al., 20013; Lachman et al., 2013). The following expression was used:

$$TA \text{ (mg CGE } 100 \text{ g}^{-1} \text{ dw)} = (A \times DF \times MW \times V \times 100) / (\epsilon \times W)$$

where A is the absorbance at 530 nm of the diluted sample, DF is the dilution factor, ϵ is the molar absorption coefficient for cyanidin 3-O-glucoside ($\epsilon = 34300 \text{ M}^{-1} \text{ cm}^{-1}$), MW is the molecular weight of cyanidin 3-O-glucoside ($MW = 449.2 \text{ g mol}^{-1}$), V is the final volume (mL) of the extract, and W is the dry sample weight (g) (Huang et al., 2006).

2.6 Extraction and quantitative determination of total carotenoids

The extraction procedure was carried out according to Fernandez-Orozco et al., (2013). Briefly, two grams of lyophilized sample were extracted with 20 mL of N,N-dimethylformamide (DMF) saturated with MgCO₃ by using an Ultra-Turrax homogenizer

for 1 min at the maximum speed (Ultra Turrax T25, IKA Labortechnik, Staufen, Germany). Subsequently, the mixture was centrifuged at 4,500×g for 5 min at 4 °C, and the extracted carotenoids, contained in the DMF, were collected. The extraction operation was repeated twice with the pellet, and the three DMF fractions were pooled in a separation funnel. Pigments were transferred to diethyl ether (60 mL) by adding 100 mL of 10% NaCl solution saturated with MgCO₃ at 4 °C. The mixture was vigorously shaken and allowed to stand until complete separation of phases. The lower phase (aqueous DMF) was re-extracted with diethyl ether (30 mL), and the ether fractions were pooled and washed with 100 mL 2% Na₂SO₄ solution. The extract was filtered through a solid bed of anhydrous Na₂SO₄, and the solvent was evaporated under vacuum in a rotary evaporator at 30 °C (Rotary Evaporator RV10, IKA, Labortechnik, Staufen, Germany). The pigments were dissolved in 0.5 mL of acetone and stored at 4 °C until analysis, which was carried out in the same day of the preparation of the extracts. Analyses were carried out in triplicate. All operations were performed under dimmed light to prevent isomerization and photodegradation of carotenoids.

Total carotenoid (TC) concentration was estimated spectrophotometrically by UV-visible, measuring the absorbance at 450 nm (Schiedt and Liaanen-Jensen, 1995). Appropriated dilutions were prepared in order to obtain an absorption value in the range 0.3-0.7 absorption units (AU). Data were given as µg lutein equivalents per g⁻¹ dw since lutein is a major carotenoid in potato (Lachman et al., 2003; Burgos et al., 2009; Burgos et al., 2012). The following expression was used:

$$TC (\mu g LE g^{-1} dw) = (A \times 10,000 \times DF \times V) / ((\epsilon \times 10 / MW) \times W)$$

Where A is the absorbance reading of the diluted sample at 450 nm, DF is the dilution factor, ϵ is the general molar extinction coefficient for lutein in acetone ($\epsilon = 144500 \text{ M}^{-1} \text{ cm}^{-1}$), MW is the molecular weight of lutein ($MW = 568.78 \text{ g mol}^{-1}$). V is the final volume (mL) of the extract, and W is the dry sample weight (g).

2.7 Statistical analysis

Correlation analyses between parameters were calculated by using the CORR procedure of the SAS package (SAS, 2011).

3 Results and discussion

3.1 Total phenolic compounds

Fig. 2 shows the total phenolic concentration (TP) of raw and boiled tubers among the collection of three commercial potato cultivars (used as testers), three heirloom Spanish cultivars and ten breeding lines with introgressions of native potato species. TP values ranged from 142 to 359 mg GAE 100 g⁻¹ dw in raw tubers. Overall, the highest concentrations of phenolic compounds were found in purple-fleshed cultivars, such as Vitelotte ($359 \pm 2.57 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$), Morada ($347 \pm 27.0 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$), Kasta ($332 \pm 8.36 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$) and Jesus ($251 \pm 17.6 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$), and also in the breeding lines NK-08/362 ($338 \pm 3.48 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$) and NK-08/349 ($257 \pm 5.50 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$). On the other hand, the yellow and light purple pigmented cultivars and breeding lines showed lower values (142 - 215 mg GAE 100 g⁻¹ dw). This was consistent with the results of Lachman et al. (2008), who also found lower TP concentration in yellow-fleshed potatoes (average value around 296 mg GAE 100 g⁻¹ dw), than in purple-fleshed ones (with an average value of 468 mg GAE 100 g⁻¹ dw). On the

other hand, the whole range of TP values obtained in the present work were comparable to those obtained by Andre et al. (2007a), who characterized a collection of mostly yellow, cream or white-fleshed potato accessions. However, in those cases, the analyses were carried out with the whole tuber, i.e. including the skin, which were purple in a high number of potato cultivars.

When the potatoes were boiled, the TP concentration diminished for all cultivars with values in the range from 78.0 to 221 mg GAE 100 g⁻¹ dw. The highest values were again found in the cultivars Vitelotte (221 ± 1.39), Kasta (202 ± 5.72), Morada (164 ± 1.50), as well as in the breeding line NK-08/362 (198 ± 4.38). After thermal processing, from 45 to 74% of the TP concentration was retained for all cultivars, with the exception of the pale yellow-fleshed commercial Monalisa, in which the retention was very much higher (93.5%) (Table 2). Also, after boiling, this cultivar retained a similar amount of TP (144 mg GAE 100 g⁻¹ dw) to that of Morada (164 mg GAE 100 g⁻¹ dw), and even higher than Jesus and the breeding line NK-08/349 (127 and 114 mg GAE 100 g⁻¹ dw, respectively), despite being all of them cultivars with high levels of TP in raw tubers. Besides Monalisa, the accessions which have shown higher TPR values were, in decreasing order, NK-08/284 (73.9%), Zamora (65.4%), Kasta (61.9%), NK-08/286 (62.59%) and Vitelotte (61.6%).

Polyphenolic compounds including anthocyanins and proanthocyanidins are not completely stable during processing (Talcott et al., 2003). Physical and biological factors such as temperature increase and enzymatic activity may result in destruction of phenolics such as phenolic acids and anthocyanins (Kader et al. 2003). But recent research has now established that food processing also has some positive effects that improve the quality and health benefits of foods (Nicoli et al. 1999). Several authors have studied the effect of

different thermal treatments on TP in different potato genotypes, having also found for some TP reductions after cooking (Xu et al., 2009; Perla et al., 2012). In this sense, Xu et al. (2009) reported a TP concentration drop after boiling, baking, and microwave cooking in eight commercial potato varieties, also being the effect of the different processes cultivar dependent. Similar results were obtained in other five potato cultivars and nine breeding lines with different skin and flesh colors, with approximately 40 – 60% of TP reduction during cooking (Perla et al., 2012). However, Blessington et al. (2010) found an increase of TP in potato eight breeding lines when tubers were baked, microwaved and fried, and no loss when were boiled, just like it has been previously reported for two pigmented potato cultivars (Mulinacci et al., 2008), whereas Burgos et al. (2013) also quantified higher amounts of TP in boiled tubers than in raw tubers of four accessions, although these authors point out that the differences may be attributed to a more efficient extraction obtained in cooked samples.

Therefore, great differences can be found among the published data. As Blessington et al. (2010) state, these discrepancies may be related to multiple factors such as the potato genotype and the amount of physical processing of the vegetable before cooking, as well as the conditions of the cooking process, among others. In our study, peeled and sliced potatoes were boiled, while in other studies whole potatoes were roasted, steamed or baked. The unmodified concentration of TP after boiling and microwave heating reported by Mulinacci et al. (2008) using whole unpeeled tubers suggests that tuber peels contribute to a high retention of phenolic compounds. On the other hand, it has been reported that F-C assay suffers from a number of interfering substances, such as ascorbic acid, sugars, aromatic amines, sulfur dioxide, organic acids and iron, but can include at least 50

additional compounds naturally found in fruits and vegetables (Prior et al., 2005). In this sense, a significant proportion of TP losses could be explained by variations in the concentration of non-phenolic interfering substances.

3.2 Total anthocyanins

Total anthocyanins concentration (TA) of the raw tuber samples ranged from 2.46 in the purple-fleshed Zamora cultivar to 253 mg CGE 100 g⁻¹ dw in the dark purple pigmented Vitelotte, and after boiling them from 0.673 to 208 mg CGE 100 g⁻¹ dw (Fig. 3). Anthocyanins were not detected in the pale yellow accessions Monalisa, NK-08/356 and NK-08/370. These results were in agreement with data presented by Andre et al. (2007b). In this study, anthocyanins were not found in most of the white, cream or yellow-fleshed potatoes, being only present in those with purple color in the flesh and/or the skin. Data showed a wide variation range due to differences in flesh and skin color because the analyses were carried out with the whole unpeeled tubers, with a dark purple skinned and-fleshed tuber containing remarkably high amounts of total anthocyanins (1633 mg petanin equivalents 100 g⁻¹ dw). In the present work, Vitelotte and NK-08/362 samples stood out with very much higher TA values, both fresh (253 ± 1.88 and 175 ± 0.771 mg CGE 100 g⁻¹ dw, respectively) and boiled (208 ± 3.33 and 107 ± 1.17 mg CGE 100 g⁻¹ dw, respectively), followed by Morada (49.7 ± 0.160 mg CGE 100 g⁻¹ dw). Moreover, although boiling reduced the TA concentrations in all cultivars and breeding lines, losses were lower for the anthocyanin high-producing cultivars, particularly for the dark purple cultivar Vitelotte, which retained 82.4 % of TA, followed by NK-08/362, Morada, Jesus and Kasta (TAR around 60%) (Table 2). For the other accessions, TA concentrations were retained in lower amounts after

boiling the sliced peeled tubers (TAR from 27.2 to 52.9%). Since phenolics are water-soluble compounds, some degree of leaching into the cooking water may be expected. In the case of cooking recipes and practices in which the resulting liquid phase is consumed together with the tubers, this apparent negative effect would be rather a benefit, due to the increasing bioaccessibility of water soluble phytochemicals. Nevertheless, further experiments should be conducted in order to evaluate the chemical degradation of the anthocyanins under different cooking conditions.

Other studies have also found TA losses in cooked potatoes; however, retention values were high in most of them. Thus, Mulinacci et al. (2008) published TA losses ranging from 16 to 29% with respect to the fresh samples (i.e. retention values from 71 to 84%), in the purple and red-fleshed cultivars Vitelotte Noir and Highland Burgundy Red, respectively, after boiling and microwave heating. Burgos et al. (2013) did not find significant differences between the TA concentrations determined in raw and boiled tubers from three purple-fleshed cultivars but they quantified losses of around 23% (data calculated from the work) in the case of the Guincho cultivar, a deep purple-fleshed accession. On the opposite, Lachman et al. (2013) reported increases of TA concentration in five red or purple-fleshed potato cultivars after different thermal processing methods, including boiling peeled potatoes.

The stability of anthocyanins depends on many factors such as temperature, pH, light intensity, presence of other pigments, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide, etc (Mazza and Minitati, 1993). Factors that may affect the retention of anthocyanins in boiled tubers include the area to volume ratio, the presence or absence of peel, the temperature and quantity of water for boiling, and the

specific anthocyanin composition. In fact, in this respect, Baublis et al. (1994) compared the stabilities of structurally different anthocyanins from concord grapes, red cabbage and leaves of tradescantia and ajuga, having found that anthocyanins from tradescantia were much more stable than the other extracts, probably due to the high degree of acylation and substitution in the B ring of the chromophore.

3.3 Total carotenoids

Total carotenoid concentrations (TC) of raw and boiled tubers are shown in Fig. 4, with values ranging from 1.12 to 11.9 $\mu\text{g LE g}^{-1}$ dw, and from 0.605 to 4.60 $\mu\text{g LE g}^{-1}$ dw, respectively. In general, with the exception of Monalisa cultivar ($3.82 \pm 0.283 \mu\text{g LE g}^{-1}$ dw), the carotenoid concentration in raw tubers was very much higher in the pale yellow-fleshed samples — the breeding lines with introgressions of native species NK-08/356 (*Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes) ($11.9 \pm 0.407 \mu\text{g LE g}^{-1}$ dw), NK-08/370 (*Solanum stenotomum* Juz. & Bukasov) ($11.4 \pm 0.114 \mu\text{g LE g}^{-1}$ dw), and NK-08/286 (*Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes) ($10.2 \pm 0.643 \mu\text{g LE g}^{-1}$ dw) — than in the others, with different purple colors, excluding the Morada cultivar ($10.9 \pm 0.618 \mu\text{g LE g}^{-1}$ dw) which also showed similar a TA concentration to the pale yellow-fleshed accessions. However, Morada cultivar also showed high TA values which masked the yellow pigments. The absence of anthocyanins in most of the pale yellow potatoes, Monalisa and the breeding lines NK-08/356 and NK-08/370, or the small amounts contained in the breeding line NK-08/286, allowed them to be mainly yellow. Previous studies have reported that there is a direct and positive relationship between intensity of the yellow color of the flesh and the TC values (Burgos et al., 2009), and a negative correlation between TC and TA (Brown et al., 2007).

After boiling, the TC diminished for all tubers except for the dark purple Vitelotte, which stood out showing the same value of yellow pigments before and after the thermal processing (TCR = 99.6%). For the other accessions, different TCR values were obtained (Table 2), being lower for those samples with higher TC concentration, i.e., the accessions NK-08/356 (32.2%), NK-08/286 (32.7%), NK-08/370 (40.3%), and Morada (39.5%). Nevertheless, retention values below 50% were also quantified in the breeding lines NK-08/355 (37.7%), NK-08/336 (43.6%) and NK-06/130 (44.7%). The medium-dark potato cultivar Kasta stood out, after Vitelotte, because 83.6% of TC was retained after boiling, followed by the breeding line NK 08/348 (75.4%) and Monalisa cultivar (73.7%). For the other breeding lines and cultivars, (NK 08/284, NK 08/349, NK 08/362, Zamora and Jesus), an average TCR of 62% (ranging from 57.9 to 67.7%). Nevertheless, although losses of carotenoids were detected after boiling potatoes, it is known that in many cases, carotenoids become more bioavailable after cooking, which probably facilitates the releasing of them from the food matrix (Clevidence et al., 2000).

Comparing with some of the few works that can be found in the literature, our results are in accordance with those of Burmeister et al. (2011). Those authors found higher amounts of TC in raw *Solanum* (*Solanum tuberosum* L. and *Solanum phureja* Juz. & Bukasov) tubers than in heat processed ones, being quantified TC reductions ranging from 7 to 50% after boiling. Moreover, carotenoid changes from all-*trans* to a 9-*cis* and 13-*cis* isomeric forms were reported. Recently, Burgos et al. (2012) obtained a significant variability among potato accessions for the changes in carotenoid concentrations due to boiling. Authors mentioned above did not find significant differences in TC concentrations of raw and boiled tubers in 5 of 7 native Andean (*Solanum tuberosum* L. and *Solanum phureja* Juz. &

Bukasov) potato accessions with different flesh color, while they quantified significant losses of 3 and 16% after boiling, for the two remaining accessions. Previously, Blessington et al. (2010) had studied the effect of various cooking methods on the carotenoid concentrations of eight different potato genotypes, and they found that boiled tubers contained less carotenoids than the raw ones, while baking, frying and microwaving processes did not modify carotenoid concentrations.

3.4 Correlation results among TP, TA and TC

Strong positive correlations ($p \leq 0.001$) were obtained for each type of compounds (TP, TA and TC) among values of raw and boiled tubers ($r = 0.869$, 0.987 and 0.918 for TP, TA and TC, respectively) (Table 3), despite of the different degrees of retention (Table 2) obtained for each one among the various accessions analyzed in the present study. As expected, the statistical analysis also revealed very high correlations ($p \leq 0.001$) among the TP and TA concentration both in raw ($r = 0.815$) and boiled tubers ($r = 0.757$), but neither of those compounds showed correlation with TC. However, other authors have found a negative correlation between TC and TA ($r = -0.41$, $p \leq 0.05$) in 38 native potato cultivars of different taxonomic groups from South America (Brown et al., 2007). On the other hand, TP and TA concentration in boiled potatoes also showed significant correlations with the dry matter (DM) of tubers ($r = 0.522$ and 0.678 , respectively), using a confidence levels higher than 95%.

4 Conclusions

The analyses of total phenolics, anthocyanins and carotenoids among the collection of cultivars and breeding lines with introgressions of native germplasm have shown promising

results. Cultivars and breeding lines presenting high TP, TA and TC values have been recorded because of their importance in potato breeding for nutritional quality. The dark purple Vitelotte tubers, both fresh and boiled, showed the highest TP and TA concentrations and the lower TC, whereas the pale yellow breeding line NK-08/356 was the one with higher amounts of TC but lesser TP and no anthocyanins. However, the purple Morada cultivar stood out overall, showing high quantities of all the bioactive secondary metabolites studied in the present work, in both the raw and boiled tubers. In most cases, the comparative analysis of raw and boiled tubers showed high losses for phenolics, anthocyanins and carotenoids. TP, TA and TC concentrations in boiled tubers were directly correlated with their corresponding concentration in the raw product. In addition, significant correlations ($p \leq 0.05$) between TA and TP concentrations, in both raw and boiled tubers, have been observed.

Factors affecting phytochemical retention in processed food need to be considered in future works. In our opinion, the utilization of diced peeled tubers has probably facilitated the migration and degradation of the studied phytochemicals (phenolic compounds and carotenoids) during boiling. Therefore, the presence of peel could have counteracted this effect to a certain point, which explains the reduction of phytochemical losses in other similar works using unpeeled tubers.

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Figure captions

Figure 1. Sampling scheme and experimental design.

Figure 2. Total phenolic (TP) concentration (mg gallic acid equivalents per 100 g⁻¹ dw) in a collection of six potato cultivars and ten breeding lines with introgressions of *Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes and *Solanum stenotomum* Juz. & Bukasov. Bars represent means and standard deviations (n = 3). Cultivars and breeding lines were arranged in decreasing order of TP concentration in the raw tubers.

Figure 3. Total anthocyanin (TA) concentration (mg cyanidin 3-O-glucoside equivalents per 100 g⁻¹ dw) in a collection of six potato cultivars and ten breeding lines with introgressions of *Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes and *Solanum stenotomum* Juz. & Bukasov. Bars represent means and standard deviations (n = 3). Cultivars and breeding lines were arranged in decreasing order of TA concentration in the raw tubers.

Figure 4. Total carotenoid (TC) concentration (µg lutein equivalents per g⁻¹ dw) in a collection of six potato cultivars and ten breeding lines with introgressions of *Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes and *Solanum stenotomum* Juz. & Bukasov. Bars represent means and standard deviations (n = 3). Cultivars and breeding lines were arranged in decreasing order of TC concentration in the raw tubers.

Figure 1

Figure 2

Figure 3

Figure 4

Table 1. Breeding lines with introgressions from two native *Solanum* species (*Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes and *Solanum stenotomum* Juz. & Bukasov).

Clones	Genitor (♂)	Genitor (♀)	Skin/Flesh Type ¹
NK-08/284	Socco huaccoto (ADG)	Matador (TBR)	P/P
NK-08/286	Socco huaccoto (ADG)	Matador (TBR)	Y/Y
NK-08/336	Lutetia (TBR)	Puca Quitish (ADG)	P/P
NK-08/348	Morada (TBR)	Puca Quitish (ADG)	P/P
NK-08/349	Morada (TBR)	Puca Quitish (ADG)	P/YP
NK-08/355	Heidrun (TBR)	Puca Quitish (ADG)	P/WP
NK-08/356	Heidrun (TBR)	Puca Quitish (ADG)	Y/Y
NK-08/362	Jesus (TBR)	Puca Quitish (ADG)	P/P
NK-08/370	Panda (TBR)	Morada Turuna (STN)	Y/Y
NK-06/130	Jesus (TBR)	N-180 (TBR)	P/P

ADG: *S. tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes; TBR: *Solanum tuberosum* L.; STN: *S. stenotomum* Juz. & Bukasov.

¹Key to Skin and flesh types: P = Purple; Y = Yellow; W = White.

Table 2. Dry matter content and TP, TA and TC retentions after boiling (TPR, TAR and TCR) of tubers from a collection of three heirloom Spanish cultivars (Jesús, Morada and Zamora), ten pigmented breeding lines with introgressions of *Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes and *Solanum stenotomum* Juz. & Bukasov and three commercial cultivars (Vitelotte, Kasta and Monalisa). Dry matter content (DM) is expressed as percentage of total fresh weight. Mean and standard deviations of TPR, TAR and TCR are expressed as percentage of total phytochemical values in raw tubers (n = 3).

Genotypes	DM (%)	TPR (%)	TAR (%)	TCR (%)
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Vitelotte	34.3	61.6 ± 0.826	82.4 ± 1.92	99.6 ± 5.97
Kasta	25.1	61.2 ± 3.19	59.5 ± 1.64	83.6 ± 9.52
Monalisa	20.3	93.5 ± 4.32	-	73.7 ± 6.04
Jesús	27.9	50.6 ± 3.53	61.1 ± 1.05	64.6 ± 9.47
Morada	27.6	47.4 ± 3.82	60.4 ± 1.01	39.5 ± 4.05
Zamora	22.5	65.4 ± 3.08	27.2 ± 2.67	59.0 ± 7.49
NK-08/284	18.8	73.9 ± 3.94	42.4 ± 2.26	67.7 ± 6.22
NK-08/286	17.4	62.6 ± 2.02	52.9 ± 1.00	32.7 ± 2.58
NK-08/336	19.3	50.8 ± 2.95	38.8 ± 1.11	43.6 ± 4.60
NK-08/348	18.0	50.6 ± 0.851	51.7 ± 1.70	75.4 ± 3.97
NK-08/349	24.1	44.6 ± 2.00	51.8 ± 1.95	57.9 ± 3.36
NK-08/355	21.8	56.6 ± 2.04	37.9 ± 1.68	37.7 ± 5.25
NK-08/356	19.5	55.0 ± 3.29	-	32.2 ± 2.50
NK-08/362	20.3	58.5 ± 1.88	61.4 ± 0.932	59.7 ± 4.87
NK-08/370	20.2	54.7 ± 3.59	-	40.3 ± 3.40
NK-06/130	28.2	48.3 ± 1.75	52.7 ± 1.78	44.7 ± 6.55

Table 3. Matrix for correlation coefficients (r) showing the simple linear relationship among total phenolic content (TP), total anthocyanin content (TA), and total carotenoid content (TC) in raw and boiled tubers, and dry matter (DM).

	TP (Raw)	TA (Raw)	TC (Raw)	TP (Boiled)	TA (Boiled)	TC (Boiled)	DM
TP (Raw)	1.00						
TA (Raw)	0.815***	1.00					
TC (Raw)	-0.193	-0.260	1.00				
TP (Boiled)	0.869***	0.782***	-0.235	1.00			
TA (Boiled)	0.760***	0.987***	-0.255	0.757***	1.00		
TC (Boiled)	-0.108	-0.202	0.918***	-0.0431	-0.204	1.00	
DM	0.663**	0.653**	-0.342	0.522*	0.678**	-0.360	1.00

*, **, *** Significant at $p \leq 0.05$, 0.01, 0.001, respectively.