

Original Article

Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest

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ABSTRACT

Onions are rich in different types of phenolics, mainly flavonols, and in red varieties anthocyanins are also present. This is significant because these classes of phenolics are antioxidants and hence may impart important functional properties to onions. The aim of the present work was to simultaneously determine flavonol and anthocyanin concentrations in different onion varieties, two white (Branca da Póvoa and the hybrid SK409) and three red (landrace Vermelha da Póvoa, a selected line of Vermelha da Póvoa and Red Creole). Flavonols (quercetin 7,4-diglucoside, quercetin 3,4-diglucoside, isorhamnetin 3,4-diglucoside, quercetin 3-glucoside, quercetin 4-glucoside and isorhamnetin 4-glucoside) were the predominant polyphenolic compounds. White cultivars had the lowest total flavonol content, with values of 89.3 ± 38.5 and 101.0 ± 18.9 mg quercetin/kg fresh weight for Branca da Póvoa and the hybrid SK409, respectively. The red onions had the highest levels of flavonols, especially the selected population of Vermelha da Póvoa and Red Creole, with values of 280.2 ± 41.5 and 304.3 ± 81.2 mg quercetin/kg fresh weight, respectively. Red onions are not only richer in flavonols, but also contain anthocyanins. Four anthocyanins (cyanidin 3-glucoside, cyanidin 3-laminariboside, cyanidin 3-(6"-malonylglucoside), and cyanidin 3-malonylaminariboside) were quantified in all red onions, with Red Creole presenting the highest concentration (28.6 ± 8 mg cyanidin/kg fresh weight). Red onions may be recommended for their major potential functional properties. A distinct gradient in total flavonoid content was found between the outer, central and inner edible scales and along the longitudinal axis of the bulb. Differences in flavonol levels between small- and large-sized onions were also found. All of these factors are of paramount importance for sampling and characterizing onions with regard to flavonoids.

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1. Introduction

Onions (*Allium cepa* L.) are one of the world's oldest cultivated vegetables (Fenwick and Hanley, 1985) and are the second most produced vegetable crop after tomatoes (Griffiths et al., 2002). Over the past 15 years, the total surface area dedicated to onion crops in the world has doubled, now reaching 3.07 million ha with a production of 53.6 Mt (FAOSTAT data, 2004). In Portugal, onion is the third vegetable in consumption (13 kg per capita and year), after potato and cabbage, and the landrace cultivars Branca da Póvoa and Vermelha da Póvoa are predominant in the northwestern region of the country. Onions contain high levels of non-nutrient antioxidant compounds (phenolics) which have protective effects against different degenerative pathologies such as

cardiovascular and neurological diseases, cancer and other dysfunctions based on oxidative stress (Griffiths et al., 2002).

Flavonoids (C₆–C₃–C₆) are major phenolics in onions and can be classified in different subclasses (flavones, flavanones, flavonols, isoflavones, flavanonols, flavanols, chalcones and anthocyanins) according to the degree of unsaturation and degree of oxidation of the 3-carbon skeleton. Subclasses of flavonoids can be further differentiated on the basis of the number and nature of substituent groups attached to the rings (Robards and Antolovich, 1997). Flavonols and anthocyanins are the main subclasses of flavonoids present in onions, the latter being found only in red onions. Many of these compounds are glycosylated, and some of these glycosyl derivatives are esterified with aromatic or aliphatic acids whose combinations yield a large variety of compounds (Slimestad et al., 2007). Aromatic acylation increases the stability of anthocyanins by intramolecular stacking of anthocyanins with polyphenols. Malonylation is one of the most common forms of aliphatic acylation of anthocyanins and is important for enhancing pigment solubility in

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water, protecting glycosyl moiety from enzymatic degradation, and stabilizing anthocyanin structures, what contributes to keep the colour hue. Some bioactive properties of anthocyanins, including antioxidant activity, have been shown to be strongly modulated by acylation (Suzuki et al., 2004). Within a vegetable family, the quality and quantity of the phenolic pool may change with the cultivar, growth stage, and environmental conditions.

The aim of this work is the characterization of the phenolic composition (anthocyanins and flavonols) mainly of two regional landrace cultivars of Portuguese onions (Branca da Póvoa and Vermelha da Póvoa), and to compare them with common commercial cultivars (the White Hybrid SK409 and Red Creole) and with a selected population of Vermelha da Póvoa. A methodology based on high performance liquid chromatography method coupled with diode array detection (HPLC/DAD) was developed to simultaneously determine flavonols and anthocyanins. In order to make a representative sampling, the spatial distribution of these flavonoids in an onion bulb and the correlation between onion size and flavonoid content was also studied.

2. Materials and methods

2.1. Sample collection

Five onion (*Allium cepa* L.) cultivars were studied, two white (the landrace open pollinated Branca da Póvoa and the hybrid SK409) and three red (Vermelha da Póvoa, Red Creole and a line of Vermelha da Póvoa selected for uniform bulb morphology). The onion cultivars were grown under the same conditions in a farm located in a traditional onion-growing region in northwestern Portugal (Póvoa do Varzim, at 41°22'57"N and 8°45'46"W). Onions were harvested between July and August 2004 and left on the field to dry and cure for 10–14 days. All the cultivars were analyzed 1 day after being cured. Enzymatic autolysis and spatial distribution assays were carried out with bulbs of onion Vermelha da Póvoa from harvest 2003.

2.2. Flavonol and anthocyanin determination

A group of 30 onion bulbs from each cultivar was selected. From this initial group, 10 onion bulbs with a representative average weight were selected and individually analyzed in triplicate. Each bulb was skinned, cut longitudinally into quarters and a representative sample of each quarter (15 g) was immediately homogenized at 1200 rpm for 3 min with 25 mL of methanol:formic acid:water (MFW; 50:5:45; v:v:v) stabilized with 2 g/L of tertbutylhydroquinone (TBHQ). Homogenates were incubated for 1 h at room temperature with alternative shaking and subsequently centrifuged at 4000 rpm for 15 min at 2 °C. Two additional extractions were performed for each sample with 15 and 10 mL of MFW, respectively. The combined fractions (approximately 50 mL) were diluted to 60 or 80 mL with MFW.

An aliquot (50 µL) was then injected into HPLC/DAD (Thermo Separation Products, Madrid, Spain). The optimized instrumental parameters for the chromatographic analysis of flavonols and anthocyanins are shown in Table 1. Quantification of single anthocyanins and flavonols (mg/kg fresh weight (FW)) was achieved by calibration curves obtained using cyanidin 3-glucoside (C.A.S. 7084-24-4, from Extrasynthese, 95% pure) and quercetin (C.A.S. 6151-25-3, from Sigma–Aldrich, 98%) as quantification standards, respectively.

2.3. Flavonol and anthocyanin identification

Onion flavonoids occur mainly as glycosyl derivatives, and in the case of anthocyanins, some of these glycosyl derivatives are

Table 1

Chromatographic conditions for the quantification of flavonols and anthocyanins.

| Chromatographic conditions | |
|----------------------------|--|
| Injection | 50 µL |
| Guard column | Pelliguard LC-18 (5 mm × 4.6 mm i.d., 40 µm) (Supelco) |
| Analytical column | Water symmetry C18 (150 mm × 4.6 mm i.d., 5 µm) (Waters). |
| Mobile phase | A (formic acid in water 5% (v/v)):B (methanol) 0–5 min: 100:0 (elution step) 15–20: 85:15 (elution step) 25–40: 40:60 (elution step) 41–45: 0:100 (washing step) 46–51: 100:0 (conditioning step) |
| Flow | 1 mL min ⁻¹ |
| Detection conditions | |
| Scanning | 200–600 nm |
| Detection wavelength | Flavonols: 360 nm; anthocyanins: 520 nm |
| Scan rate | 1 Hz |
| Step | 2 nm |
| Bandwidth | 3 nm |

esterified with aromatic or aliphatic acids. Acid and alkaline hydrolysis, enzymatic autolysis and spectral analysis were performed for identification purposes. Standards used for identification purposes were: cyanidin 3-glucoside (C.A.S. 7084-24-4, from Extrasynthese, 95% pure) and quercetin (C.A.S. 6151-25-3, from Sigma–Aldrich, 98%), kaempferol (C.A.S. 520-18-3, from Sigma–Aldrich, 96%) and isorhamnetin (C.A.S. 480-19-3, from Sigma–Aldrich, 98%).

2.3.1. Acid hydrolysis

The transformation of glucosides into aglycones was carried out by acid hydrolysis of the glucoside compounds. Onion extract (10 mL) was concentrated on a rotary evaporator to eliminate methanol fraction, and the extract obtained was reconstituted with water. To eliminate the sugars and prevent browning during hydrolysis process, the solution was purified by loading in a Sep Pak Plus C₁₈ cartridge (Waters), previously activated. The loaded cartridge was washed with 10 mL water, dried with N₂ (15 min) and the flavonoids were eluted with 3 mL of 1N HCl in methanol. Then 2 mL of 2N HCl in methanol was added and the dissolution was heated at 90 °C for 2 h under N₂ atmosphere. The hydrolyzed solution was analyzed in HPLC as described.

2.3.2. Alkaline hydrolysis

To confirm the identity of acylated anthocyanins, alkaline hydrolysis (Hong and Wrolstad, 1990) was performed by adding 10 mL of 10% aqueous KOH to 5 mL of onion extract, in sealed vessels under N₂, and maintaining in the dark for 8 min at room temperature. The pigments were converted to their red oxonium salt form by the addition of 2N HCl. The solution was then purified in a Sep Pak Plus C₁₈ cartridge, previously activated. The loaded cartridge was washed with 5 mL of 0.001% HCl in distilled water, and the elution of flavonoids was made with 5 mL of 0.001% HCl in methanol. The methanol fraction was concentrated to 1 mL in a rotary evaporator, and the extract obtained was reconstituted with 5% formic acid in water to a final volume of 5 mL and separated by HPLC, to identify the deacylated anthocyanins.

2.3.3. Enzymatic autolysis

For the autolysis experiment, an onion bulb of the red cultivar Vermelha da Póvoa was skinned and chopped to obtain a homogenous sample. Four portions of 15 g each were placed in Petri dishes and maintained at 40 °C for 0 (control sample), 1, 3 and 24 h. All samples were analyzed following the protocol for flavonoid quantification as described. This procedure was repeated for three bulbs ($n = 3$).

2.3.4. UV/vis spectroscopy

UV/vis spectra of the anthocyanins and flavonols were measured at 200–600 nm. The ratio of $A_{440}/A_{\lambda_{\max}}$ was calculated for each anthocyanin to determine C-3 or C-5 hydroxyl substitution in the flavilium ring (Giusti et al., 1999). To estimate the identity of acyl substituents, the search for the presence of a shoulder in the range 310–360 nm (Hong and Wrolstad, 1990) was performed.

2.4. Distribution of flavonoids in bulb scales

In order to make a representative sampling, the spatial and tissue distribution of flavonoids in the onion bulb was studied. To verify the distribution from outer to inner part, an onion bulb of the red cultivar Vermelha da Póvoa was skinned and divided into three parts: the outer two edible scales, the two next middle scales, and the rest of inner scales. To verify the distribution from the top to the base of the scales, the first edible scale was divided into three equal parts. These procedures were repeated for three bulbs ($n = 3$).

2.5. Onion size and flavonoid content

The correlation between the bulb weight and flavonoid levels was studied for the cultivar Vermelha da Póvoa. Ten small (70 ± 16 g) and 10 large (210 ± 30 g) bulbs were selected and individually analyzed as described in Section 2.2.

2.6. Statistical analysis

Data were subjected to one-way analysis of variance at the 95% level to assess the differences in flavonoid concentration among cultivars. When significant effects were observed, means were separated by the least significant difference multiple range test. All statistical analyses were performed with the Statgraphics Plus statistical software (v. 5.1, Statistical Graphics Corp., Herndon, VA 20171, USA).

3. Results and discussion

3.1. Performance of the analytical procedure

Flavonols and anthocyanins are soluble in polar solvents, with the glucosides more soluble in water and the aglycones more soluble in alcohols. These phenolics are commonly extracted from plant materials with water–alcohol (methanol or ethanol) mixtures acidified with mineral acids (Harborne et al., 1986; Hertog et al., 1992; Fossen et al., 1996; Robards and Antolovich, 1997; García-Viguera et al., 1998; Revilla et al., 1998; Da Costa et al., 2000; Mozetic and Trebse, 2004) to prevent the degradation of

the non-acylated anthocyanin pigments. Additionally, acid solutions ($\text{pH} < 2$) keep the anthocyanins in their stable flavilium form, increasing the intensity of red hue, facilitating the visual control of extraction.

Different mixtures of methanol:water, containing 5% of formic acid to lower pH, were tested for their efficiency as onion extraction solvent. The results obtained show that methanol proportions higher than 60% are not compatible with chromatographic mobile phase, since the higher elution power of the extraction solvent in relation to the mobile phase gives place to asymmetric and tailed peaks for the first compounds eluted from the column (this problem disappears when the proportion of MeOH is $< 60\%$). To avoid flavonoid degradation, TBHQ (2 g/L) was incorporated as an antioxidant. The best extraction solvent was found to be methanol:formic acid:water (50:5:45 vv) with TBHQ (2 g/L), which allowed a complete extraction of the tested flavonoids and remained stable during extraction and analysis, including after 1 month at -20°C .

Completeness of extraction was monitored by measuring flavonoid levels in successive solvent fractions of onion samples extracted multiple times (3 times). For this study, we have analyzed in one red onion sample, for sixfold, the percentage of flavonoids that remain in the solid matrix, after two successive extractions. Near complete extraction of anthocyanins was achieved with two extractions (a negligible 1–3% of total anthocyanins were detected in the residue remaining after the second extraction). Instead, the complete extraction of flavonols required a third extraction, because the remaining residue after the second extraction contained about 25–30% of total flavonols. Adding a third extraction for flavonols made it possible to obtain recoveries of 92–99% for all flavonols (Table 2).

The chromatographic conditions were optimized to obtain chromatograms with good resolution. The incorporation of formic acid to the mobile phase is important to reach a pH below 2 and to keep the anthocyanins in the red flavilium cation form (absorbing in 520 nm) (Hong and Wrolstad, 1990; Da Costa et al., 2000). There is 96% of anthocyanin in the flavilium form at pH 1.5, but only 67% in that form at pH 2.5. Above pH 2 severe peak broadening results from the slow interconversion between species, the red flavilium cation, the blue quinoidal base, the carbinol pseudobase and the chalcone pseudobase (Neill, 2002; Kosir et al., 2004).

To evaluate the recovery and repeatability of the analytical method, the levels of flavonoids that remain in the onion matrix after three successive extractions were determined. Recovery percentages were higher than 92% with relative standard deviations lower than 4% (Table 2). Phenolic compounds were quantified using external standards and subsequent regression of the peak areas for the different analytes against their concentrations. Flavonol and anthocyanin concentrations were expressed in mg kg^{-1} quercetin and cyanidin 3-glucoside, respectively. Table 2

Table 2
Quality parameters of the optimized HPLC/DAD for flavonol and anthocyanin quantification ($n=6$).

| Flavonols | % Recovery | % RSD | Quercetin calibration range (mg/L) | r^2 |
|--|------------|-------|---|-------|
| (1) Quercetin 7,4-diglucoside | 97 | 3 | 0.25–8 | 0.995 |
| (2) Quercetin 3,4-diglucoside | 97 | 2 | | |
| (3) Isorhamnetin 3,4-diglucoside | 98 | 2 | | |
| (4) Quercetin 3-glucoside | 98 | 2 | | |
| (5) Quercetin 4-glucoside | 92 | 4 | | |
| (6) Isorhamnetin 4-glucoside | 96 | 2 | | |
| Anthocyanins | % Recovery | % RSD | Cyanidin 3-glucoside calibration range (mg/L) | r^2 |
| (1) Cyanidin 3-glucoside | 99 | 1 | 0.04–10 | 0.999 |
| (2) Cyanidin 3-laminaribioside | 99 | 2 | | |
| (3) Cyanidin 3-(6''-malonyl)glucoside | 99 | 1 | | |
| (4) Cyanidin 3-malonyl-laminaribioside | 99 | 1 | | |
| (5) Cyanidin 3-dimalonylaminaribioside | 93 | 1 | | |

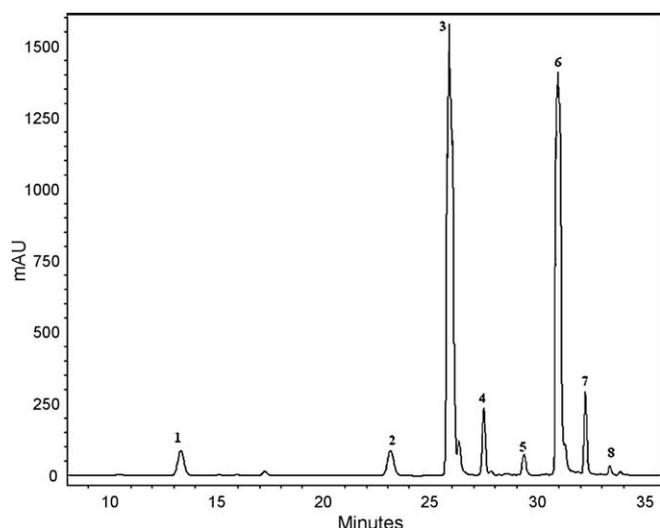


Fig. 1. Red Creole onion chromatogram ($\lambda = 360$ nm). Peak: (1) quercetin 3,7,4'-triglucoside, (2) quercetin 7,4'-diglucoside, (3) quercetin 3,4'-diglucoside, (4) isorhamnetin 3,4'-diglucoside, (5) quercetin 3-glucoside, (6) quercetin 4'-glucoside, (7) isorhamnetin 4'-glucoside and (8) quercetin.

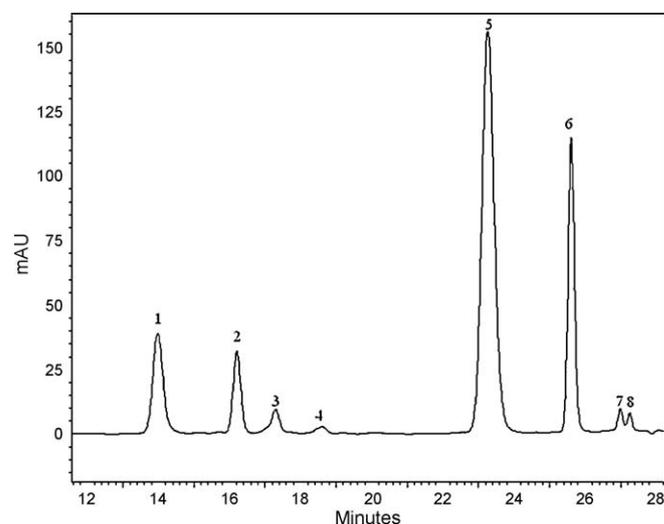


Fig. 2. Red Creole onion chromatogram ($\lambda = 520$ nm). Peaks: (1) cyanidin 3-glucoside, (2) cyanidin 3-laminaribioside, (3) cyanidin 3-(3''-malonylglucoside), (4) pedonidin 3-glucoside, (5) cyanidin 3-(6''-malonylglucoside), (6) cyanidin 3-(6''-malonyl-laminaribioside), (7) peonidin 3-malonylglucoside and (8) cyanidin 3-dimalonyllaminaribioside.

shows the linearity parameters for these compounds. Detection and quantitation limits (American Chemical Society, 1980) were lower than 0.5 mg kg^{-1} quercetin for flavonols, and lower than 0.1 mg kg^{-1} cyanidin 3-glucoside for anthocyanins.

3.2. Identification of flavonols and anthocyanins

Anthocyanins have high absorbance at 240–280 nm and at 465–560 nm, while absorbance maxima in the 240–280 and 300–380 nm range are characteristic of flavonols (Merken and Beecher, 2000). Eight of the peaks isolated from the edible part of all onion varieties tested exhibit spectral characteristics of flavonols (Fig. 1). Eight peaks were also isolated in red onions with spectral properties of anthocyanins (Fig. 2).

The UV–vis spectra of the anthocyanins yield information with regard to the nature of the aglycon and, although the nature of the sugar substitution had no effect on spectra, the $A_{440}/A_{\lambda_{\text{max}}}$ ratio can give information regarding glycosidation substitution pattern. So, the 3-glucosides and 3,5-diglucosides have similar spectra maxima, but show differences in the 400–460 nm region, with the $A_{440}/A_{\lambda_{\text{max}}}$ ratio of the 3-glucosides being twice as large as for 3,5-diglucosides or 5-glucosides (Giusti et al., 1999). $A_{440}/A_{\lambda_{\text{max}}}$ ratio values calculated for each anthocyanin in the different onion varieties studied are similar, ranging from 28 to 35%. These percentages indicated the substitution in the C-3 position of the flavilium ring.

HPLC analysis of the acid hydrolyzed onion extract produced two peaks at 520 nm, identified as cyanidin and peonidin, in comparison with pure compound. Anthocyanin peaks 1, 2 and 4 resulted from alkaline hydrolysis whereas peaks 3, 5–8 disappeared, indicating that the latter are acylated forms of the former peaks, identified in acid hydrolysis as cyanidin and peonidin derivatives. Information regarding the presence of acylation by hydroxylated organic acids was also obtained from spectra. The presence of an absorbance band/shoulder in the range 310–360 nm indicated that the pigment is acylated with aromatic acids (Hong and Wrolstad, 1990). No shoulders were found in the range 310–360 nm in anthocyanin spectra of the different onion varieties studied.

Based on the results and the scientific literature, the identities of these eight compounds were determined as cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-(3''-malonylglu-

coside), peonidin 3-glucoside, cyanidin 3-(6''-malonylglucoside), cyanidin 3-(6''-malonyl-laminaribioside), peonidin 3-malonylglucoside and cyanidin 3-dimalonyllaminaribioside. These identities were in agreement with those found by Terehara et al. (1994), Fossen et al. (1996) and Donner et al. (1997). Other authors have identified delphinidin or petunidin derivatives, although they were found at very low levels as regards to cyanidin (Gennaro et al., 2002). Ferreres et al. (1996) have identified the 3-arabinoside and 3-malonylarabinoside of cyanidin among the main anthocyanins of Spanish red onion. Fossen et al. (2003) have reported the presence of several C-4 substituted anthocyanins isolated in minor amounts from pigmented scales of red onion.

Based on acid hydrolyses, spectral analysis and published data (Bonaccorsi et al., 2005), the eight flavonols present in the onion samples were identified as quercetin 3,7,4-triglucoside, quercetin 7,4-diglucoside, quercetin 3,4-diglucoside, isorhamnetin 3,4-diglucoside, quercetin 3-glucoside, quercetin 4-glucoside and isorhamnetin 4-glucoside and quercetin aglycone. We found no structures based on kaempferol on the onion cultivars studied, unlike other authors (Ferreres et al., 1996; Mian and Mohamed, 2001; Lombard et al., 2002; Sellappan and Akoh, 2002).

3.3. Changes in composition resulting from autolysis

The identity of the two major flavonols (quercetin 3,4-diglucoside and quercetin 4-glucoside) was confirmed by an autolysis test over a 24 h period. Compositional changes during autolysis of onion Vermelha da Póvoa are shown in Fig. 3. After 24 h of incubation, very little loss in total flavonols was observed (20%). However, within this period there were significant qualitative changes in major flavonol composition. The loss of quercetin 3,4-diglucoside was associated with an initial increase and then a decrease after 3 h in quercetin 4-glucoside, accompanied by a steady increase of the aglycone quercetin. After 24 h almost no quercetin 3,4-diglucoside could be detected. This variability in flavonol concentration is due to the conversion of quercetin 3,4-diglucoside into quercetin 4-glucoside by the activity of the quercetin 3-O- β -glucosidase, and to the conversion of quercetin 4-glucoside in quercetin by the activity of quercetin 4'-O- β -glucosidase (Price and Rhodes, 1996; Tsushida and Suzuki, 1996; Rhodes and Price, 1997). The sudden conversion (after just

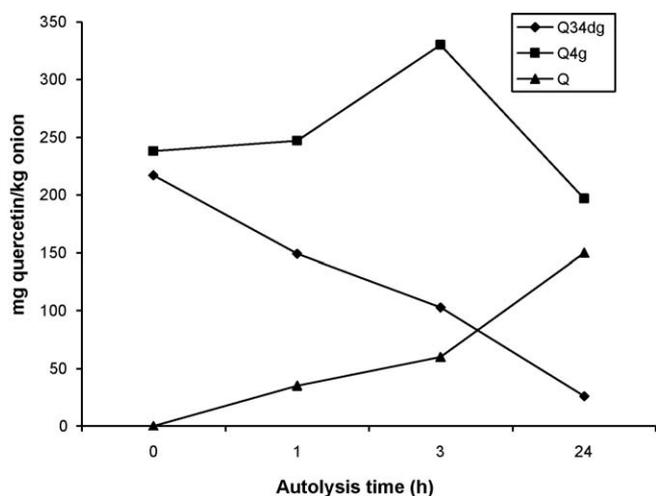


Fig. 3. Compositional changes during autolysis for the Vermelha da Póvoa variety. Relative standard deviations are always lower than 10%.

3 h) of quercetin 4-glucoside into quercetin aglycone can be explained by competitive inhibition of quercetin 4'-O- β -glucosidase by quercetin 3,4-diglucoside, a mechanism that explains how the conversion rate of quercetin 4-glucoside to quercetin aglycone is kept low while the concentration of quercetin 3,4-diglucoside remains high (Wegh and Luyten, 1997).

3.4. Distribution of flavonoids in scales

Fig. 4 shows changes in levels of the two major flavonols from the inner to the outer and from the top to the bottom of scales for the cultivar Vermelha da Póvoa. Levels of flavonols decreased from the outer to the inner scales and from the top to the base. The molar ratios of quercetin 3,4-diglucoside/quercetin 4-glucoside decreased from the inner (1.58 ± 0.07) to the outer (1.00 ± 0.04) of the scales, and increased from the top (1.00 ± 0.1) to the base (1.4 ± 0.03). Quercetin aglycone was found in outer scales and in the top of the bulb.

Flavonol distribution has been studied in a number of white, yellow and red onion cultivars (Bilyk et al., 1984; Mizuno et al., 1992; Leighton et al., 1993; Patil and Pike, 1995; Tsushida and Suzuki, 1996; Hirota et al., 1998, 1999; Mogren et al., 2007), and the results reported herein are in agreement with the published information. It is suggested that flavonol content increases during aging of cells that constitute scales of onion bulbs, because it is known that cells of the outer scales are more aged than those of the inner scales in a bulb, and that cells in the upper position are older than those in the lower position in a scale. It has also been discussed that flavonols can protect plant cells from UV light. This is based on the observation that flavonoids, which absorb UV light, are present in the epidermis of leaves. Taking into account that the onions studied were collected and sun-cured, flavonol distribution from outer scales to inner ones agrees with our observation in this study.

Table 3

Flavonol content (mg quercetin/kg fresh weight \pm SD ($n = 10$)) of the onion varieties studied.

| Flavonols | WH | BP | VP | I VP | RC |
|------------------------------|------------------|-----------------|------------------|------------------|------------------|
| Quercetin 7,4-diglucoside | 1.0 \pm 0.4 | 0.9 \pm 0.6 | 3.0 \pm 0.4 | 3.8 \pm 0.8 | 3.1 \pm 1.1 |
| Quercetin 3,4-diglucoside | 44.7 \pm 9.4 | 43.2 \pm 17.6 | 97.1 \pm 4.4 | 129.9 \pm 16.2 | 146.9 \pm 34.5 |
| Isorhamnetin 3,4-diglucoside | 1.5 \pm 0.5 | 1.9 \pm 1.2 | 4.2 \pm 0.8 | 5.7 \pm 2.0 | 4.9 \pm 2.4 |
| Quercetin 3-glucoside | 2.7 \pm 1.2 | 0.8 \pm 0.4 | 2.6 \pm 0.5 | 5.1 \pm 1.4 | 4.7 \pm 2.1 |
| Quercetin 4-glucoside | 41.7 \pm 6.4 | 36.6 \pm 14.9 | 91.7 \pm 6.8 | 115.2 \pm 15.4 | 127.2 \pm 32.6 |
| Isorhamnetin 4-glucoside | 9.3 \pm 1.04 | 5.9 \pm 3.9 | 13.1 \pm 3.0 | 20.5 \pm 5.7 | 17.6 \pm 8.5 |
| Σ Flavonols | 101.0 \pm 18.9 | 89.3 \pm 38.5 | 211.7 \pm 15.9 | 280.2 \pm 41.5 | 304.3 \pm 81.2 |

WH, White Hybrid SK409; BP, Branca da Póvoa; VP, Vermelha da Póvoa; I VP, improved Vermelha da Póvoa; RC, Red Creole.

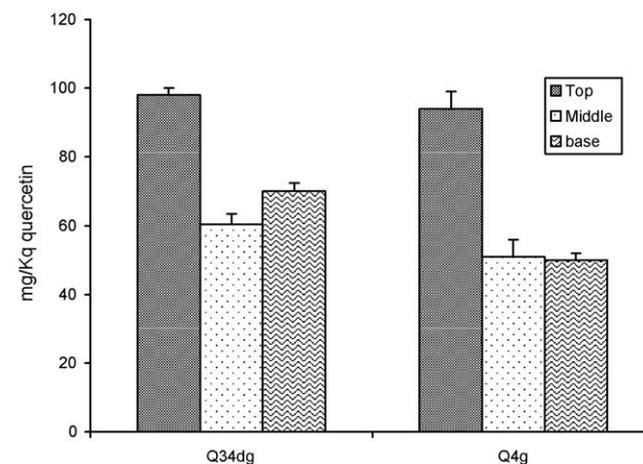
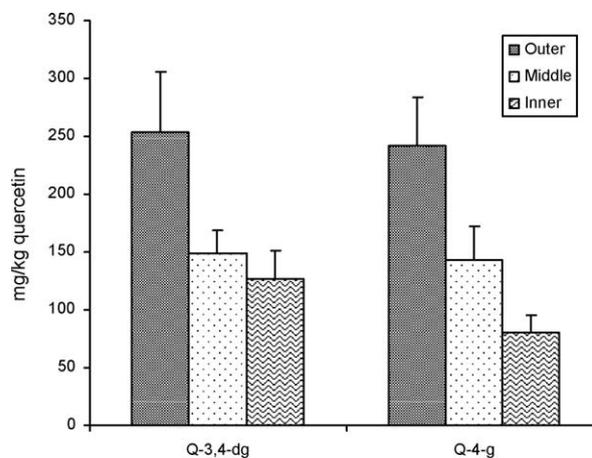


Fig. 4. Changes in levels of the two major flavonols from the inner to the outer side and from the top to the bottom of scales for the Vermelha da Póvoa variety ($n = 3$).

Unlike flavonols, the distribution pattern of anthocyanins has not been performed in onions before. In Vermelha da Póvoa, no changes were observed in anthocyanin concentration from outer to inner scales (outer: 5.15 ± 1.24 mg/kg of cyanidin 3-glucoside; central: 5.02 ± 1.39 mg/kg of cyanidin 3-glucoside; inner: 5.30 ± 1.80 mg/kg of cyanidin 3-glucoside). Total anthocyanin levels also showed a non-significant decrease from the top (7.20 ± 1.39) to the bottom (5.25 ± 1.10) of the bulb.

3.5. Onion size and flavonoid content

Small-sized Vermelha da Póvoa onions had a higher content of total flavonols and anthocyanins than large onions (419 ± 80 vs. 274 ± 64 mg quercetin/kg, and 5 ± 2 vs. 4 ± 1 mg/kg of cyanidin 3-glucoside, respectively). These findings show the need for sampling onion bulbs with a typical average size when the intention is to characterize onions as regards to their levels of flavonols. However, no

significant differences in quercetin glucoside content between small, medium and large onions were found in other studies (Patil and Pike, 1995; Mogren et al., 2007).

3.6. Flavonol and anthocyanin content in different onion varieties

Flavonol contents, expressed as mg quercetin/kg FW, are shown in Table 3. The percent moisture of the five onion cultivars studied ranged from 82 to 91%. None of the tested samples had measurable levels of quercetin 3,7,4-triglucoside or quercetin aglycone. Significant differences in total flavonol concentration were observed among the red and white cultivars, but were not observed between the cultivars originally from Póvoa do Varzím or the others that were not originally from the region. White onions showed the lowest total flavonol content, with values of 89.3 ± 38.5 and 101.0 ± 18.9 mg/kg FW for Branca da Póvoa and hybrid SK409, respectively. In contrast, the red onions showed the highest levels, especially the selected line of Vermelha da Póvoa and Red Creole, with values of 280.2 ± 41.5 and 304.3 ± 81.2 mg/kg FW. The composition of all onion varieties tested showed a predominance of two flavonols, namely quercetin 3,4-diglucoside and quercetin 4-glucoside, which constitute over 44–48% and 41–43% of the total fraction, respectively, depending on the cultivar. In general, this profile is similar to those reported in other studies (Andlauer et al., 1999; Lombard et al., 2002; Slimestad et al., 2007). The impact of field curing on flavonoid levels was already reported by us (Rodrigues et al., 2009).

Data reported in the literature for onion phenolic composition, vary due to normal biological variations related to cultivar, growing season, environmental and agronomic conditions and, in some instances, the region of the bulb analyzed. However, our results are in good agreement with those from Gennaro et al. (2002), who found higher levels of flavonols in red onions than in white onions. In contrast, Crozier et al. (1997) reported the opposite. In general, we found a total flavonol content lower than that reported by Ferreres et al. (1996) (red onions: 943 mg/kg FW); Crozier et al. (1997) (white: 185–634 mg/kg FW); Sellappan and Akoh (2002) (yellow: 120–520 mg/kg FW); Lombard et al. (2002) (yellow: 251–479 mg/kg FW); but higher than the values reported by Marotti and Piccaglia (2002) in white varieties (1.8 mg/kg FW).

The total content of anthocyanins in red onions ranged from 5.7 to 28.6 mg/kg FW. Significant differences in total anthocyanin content were observed among the red varieties. The higher content was obtained for these compounds in Red Creole. Although eight anthocyanins were identified in the samples, only four were found at quantifiable levels (Table 4). In general, we found total anthocyanins considerably lower than those reported by Ferreres et al. (1996) (233 mg/kg FW); Donner et al. (1997) (1090–2190 mg/kg DW); or Gennaro et al. (2002) (90 mg/kg FW) for Spanish, North American and Italian red onions, respectively. In all of these cases, it is not specified whether the contents refer to the edible fraction or to the whole bulb, including also the most external layers that are rich in these pigments. HPLC chromatograms revealed one major anthocyanin peak (cyanidin 3-(6''-malonylglucoside)) (43–55%) in all the red varieties studied;

Table 4

Anthocyanin content (mg cyanidin 3-glucoside/kg fresh weight \pm SD ($n = 10$)) of the onion varieties studied.

| Anthocyanins | VP | I VP | RC |
|--------------------------------------|---------------|----------------|----------------|
| Cyanidin 3-glucoside | 1.6 ± 0.5 | 4.6 ± 1.8 | 3.6 ± 1.6 |
| Cyanidin 3-lamarinaribioside | 0.2 ± 0.1 | 0.4 ± 0.2 | 2.1 ± 1.4 |
| Cyanidin 3-(6''-malonylglucoside) | 2.8 ± 1.3 | 5.5 ± 1.8 | 16.1 ± 5.2 |
| Cyanidin 3-malonyl-lamarinaribioside | 1.1 ± 0.4 | 2.3 ± 1.1 | 6.8 ± 2.1 |
| Σ Anthocyanins | 5.7 ± 1.8 | 12.8 ± 4.6 | 28.6 ± 8.0 |

VP, Vermelha da Póvoa; I VP, improved Vermelha da Póvoa; RC, Red Creole.

another acyl-derivative (cyanidin 3-malonyl-lamarinaribioside) is also an important component in concentration. These acyl-derivatives can maintain pigmentation during storage because they are more stable than the non-acyl ones (Ferreres et al., 1996). Similar results for this anthocyanin profile were found by Fossen et al. (1996) and Donner et al. (1997) in cultivars of red onion.

4. Conclusions

Eight flavonols and eight anthocyanins were isolated from the edible part of the onion varieties tested. Major flavonols were identified as quercetin 3,4-diglucoside and quercetin 4-glucoside, and the major anthocyanins as cyanidin 3-(6''-malonylglucoside) and cyanidin 3-glucoside. Significant differences in total flavonoid concentrations were observed among the red and white varieties, but were not observed between native and foreign varieties. The content of total flavonoids is considerably higher in red onions, which are thus recommended for their major health benefits.

A distinct gradient in total flavonoid content was found between the outer, central and inner edible scales and along the longitudinal axis of the bulb. Differences in flavonol levels between small- and large-sized onions were also found. All of these factors are of paramount importance for sampling and characterizing onions with regard to flavonoids.

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