

## Impact of Freezing on Flavonoids/Radical-Scavenging Activity of Two Onion Varieties

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### Abstract

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Flavonols, anthocyanins, and radical-scavenging activity of two Portuguese onion cultivars (Branca da Póvoa, white; and Vermelha da Póvoa, red) were evaluated simulating domestic freezing conditions (–18°C). Frozen portions of onions with different periods of domestic storage at ambient temperature presented increased flavonoid content when compared with the respective composition before freezing. No significant differences were observed on radical-scavenging activity. Domestic freezing of onion portions extended its shelf life. Thus, domestic freezing can be a good alternative to prevent the loss of unused fresh onions, preserving its antioxidant capacity, since frozen onions can be a useful natural antioxidant source.

**Keywords:** *Allium cepa* L.; anthocyanins; flavonols; domestic storage; frozen onions

Onions (*Allium cepa* L.) are rich in flavonoids, especially flavonols such as quercetin and its derivatives and anthocyanins. Flavonoids present radical-scavenging activity that protects cells against the damaging effects of UV radiation and hydrogen peroxide (LEE *et al.* 2008; PÉREZ-GREGORIO *et al.* 2011a,b, 2014).

Onions domestic storage is usually performed during long periods at room temperature or in a refrigerator. Post-harvest sprouting is a major physiological factor limiting their storage period (SHARMA *et al.* 2014, 2015). A few authors reported that home storage at low temperature and in low-humidity represents better conditions to preserve the flavonoid content in onions (GENNARO *et al.* 2002;

PRICE & RHODES 1997a). Additionally, according to CISNEROS-ZEVALLOS (2003), controlled time periods and temperatures of storage can affect the metabolic activity of fresh vegetables and even increase the synthesis of phytochemicals.

Nowadays, many people do not have the opportunity to eat fresh vegetables every day and use frozen vegetables. Frozen storage is a way to increase the shelf life of vegetables and reduce the wastage of unused products. Nevertheless, frozen vegetables may have a lower nutritional value than their respective commodities (ANTONIA MURCIA *et al.* 2009). Although some information on the effect of domestic storage on flavonoids and radical-scavenging activity of onions can be found (GENNARO *et al.* 2002; SHARMA

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*et al.* 2014, 2015), the effect of home freezing on nutritional quality of onions is still a parameter to be ascertained. Hence, the aim of the present work was to assess the contents of flavonols and anthocyanins by HPLC-DAD, and radical-scavenging activity by DPPH method, in two Portuguese onion varieties, one white (Branca da Póvoa) and another red (Vermelha da Póvoa), simulating domestic freezing conditions.

## MATERIAL AND METHODS

**Chemicals.** Cyanidin-3-*O*-glucoside chloride, cyanidin chloride, quercetin 3- $\beta$ -D-glucoside, all with purity higher than 95%, were purchased from Sigma-Aldrich (Steinheim, Germany). 1,1'-Diphenyl-2-picrylhydrazyl (DPPH), methanol (HPLC grade), hydrochloric acid, and formic acid p.a. were provided by Merck (Darmstadt, Germany). All standard solutions were prepared in methanol and were stored in amber vials at  $-18^{\circ}\text{C}$ .

**Sample collection.** Two Portuguese onion varieties, white (Branca da Póvoa) and red (Vermelha da Póvoa), from the Northwest region of Portugal (Póvoa do Varzim), were grown under the same conditions and harvested in August. After harvesting, onions were left on the field for 48 h until curing was complete and were then traditionally stored suspended in a natural ventilated storehouse for 3 months.

**Sampling procedure.** Groups of twenty onion bulbs from each variety with a representative average weights (157 and 146 g, respectively, for red and white onions) were selected. Four onions of each type were randomly analysed immediately after the period of storehouse storage (coded as T0). Each bulb was cut longitudinally into two equal parts. One part was weighed and analysed, and the other was packed in a commercial airtight plastic bags (LD-PE, low density polyethylene) and immediately frozen (F) at  $-18^{\circ}\text{C}$ , simulating domestic conditions. All onions bulbs were individually analysed in quadruplicate. This sampling operation was repeated after 1 month (T1) and after 2 months (T2) of domestic storage at room temperature (RT). The portions of frozen onions were stored for different periods, 5 months for T0, 4 months for T1, and 3 months for T2, these frozen samples having been coded, respectively, as T0F, T1F, T2F, in order that all the frozen samples should be analysed after 5 months of home storage (8 months after harvest). The moisture content of the onions was evaluated before and after freezing by using a moisture analyser from Scaltec Instruments SMO-01 (Goettingen, Germany) at  $101 \pm 2^{\circ}\text{C}$ .

**Flavonoids extraction.** The samples containing 20 g of onions were ground to paste and homogenised using a mixer Moulinex 320 (France), and added to 20 ml of methanol/ hydrochloric acid 0.1% (v/v). The obtained mixture was shaken for 20 min and filtered through a  $0.45\ \mu\text{m}$  Acrodisc<sup>®</sup> Syringe filters (Pall Life Sciences, Fribourg, Switzerland). The extraction process was repeated once again, and the pooled filtrates were diluted to 50 ml with methanol.

**Chromatographic analyses.** Chromatographic analyses were carried out in an analytical HPLC unit (Jasco, Tokyo, Japan). The column was used a reverse-phase ACE<sup>®</sup> C18 (5 mm;  $250 \times 4.6\ \text{mm}$ ). Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was used for the data acquisition.

Chromatographic separation was performed using a 10 % formic acid (eluent A) and formic acid/water/methanol (10 : 40 : 50 v/v/v, eluent B). The linear gradient program used for flavonols and anthocyanins separation was 0–5 min, 10% B in A; 5–7.5 min, 10–25% B in A keeping this proportion for 5 min; 12.5–30 min, 25–75% B in A; 30–40 min, 75–10% B in A; and 40–45 min, 10% B in A for the column rinse and re-equilibration. The flow-rate was 1.0 ml/minutes.

Anthocyanins hydrolysis and anthocyanidins separation were performed as described by Pinho *et al.* (2011). Diode array detection (DAD) was set at 362 nm for flavonols and at 520 nm for anthocyanins and anthocyanidins.

**Radical-scavenging activity.** Radical-scavenging activity was determined using DPPH method (AMARO *et al.* 2013) by measuring the absorbance at 515 nm 30 min after the addition of DPPH ethanolic solution (300  $\mu\text{M}$ ).

**Statistical analyses.** *t*-Test was performed to compare the flavonoid content and radical scavenging activity of onions stored at RT conditions and the respective frozen portions. Statistical analyses were performed at 5% significance level, using SPSS software for Windows, v. 21 (IBM Corporation, New York, USA).

## RESULTS AND DISCUSSION

**Flavonols and anthocyanins in red and white onions.** The chromatographic profiles of flavonols in red (Vermelha da Póvoa) and white (Branca da Póvoa) onions were similar for the two onion va-

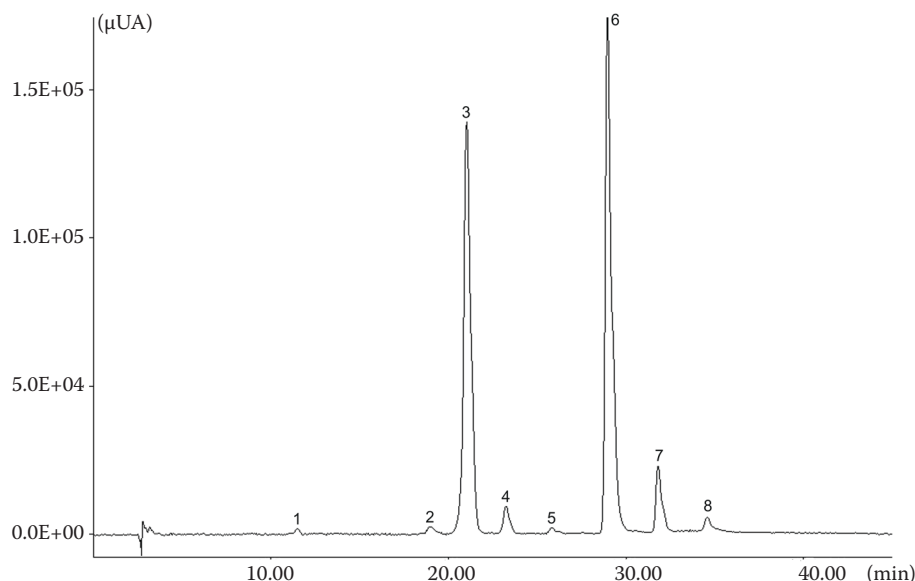


Figure 1. Fingerprint of flavonoids in white and red onions ( $\lambda = 362$  nm)

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; 8 – quercetin aglycone

rieties showing the presence of eight peaks that exhibit spectral characteristics of flavonols (Figure 1). These peaks were tentatively identified as quercetin 3,7,4'-triglucoside (peak 1), quercetin 7,4'-diglucoside (peak 2), quercetin 3,4'-diglucoside (peak 3), isorhamnetin 3,4'-diglucoside (peak 4), quercetin 3-glucoside (peak 5), quercetin 4'-glucoside (peak 6), isorhamnetin 4'-glucoside (peak 7), and quercetin aglycone

(peak 8) by comparison with available standards, the UV spectra, and the literature (PÉREZ-GREGORIO *et al.* 2010). According to PRICE and RHODES (1997b), the two main flavonols are the quercetin conjugates quercetin 3,4'-diglucoside and quercetin 4'-glucoside that represent between 85% and 95% of the total flavonols content in the onion species. In the present study, these two quercetin conjugates represented

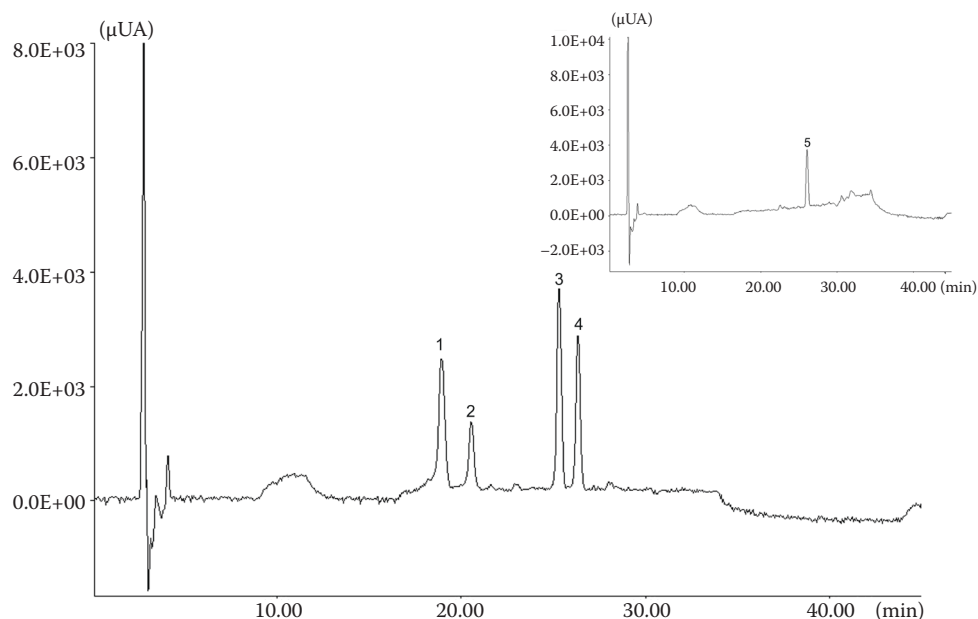


Figure 2. Chromatographic profile ( $\lambda = 520$  nm) of (a) anthocyanins and (b) anthocyanidins in red onions

1 – cyanidin 3-glucoside; 2 – cyanidin 3-laminaribioside; 3 – cyanidin 3-(6'-malonylglucoside); 4 – cyanidin 3-(6'-malonyl-laminaribioside); 5 – cyaniding

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Table 1. Flavonoid content and radical-scavenging activity of red and white onions stored at domestic room temperature conditions

| Flavonoids                              | Red onion   |             |             | White onion |             |             |
|---|-------------|-------------|-------------|-------------|-------------|-------------|
|   | T0          | T1          | T2          | T0          | T1          | T2          |
| Quercetin 3,7,4'-triglucoside           | nd          | 0.04 ± 0.01 | nq          | nd          | nd          | 0.04 ± 0.00 |
| Quercetin 7,4'-diglucoside              | 0.13 ± 0.02 | 0.13 ± 0.03 | 0.12 ± 0.04 | 0.10 ± 0.04 | 0.10 ± 0.03 | 0.10 ± 0.03 |
| Quercetin 3,4'-diglucoside              | 11.7 ± 2.8  | 11.5 ± 2.4  | 10.6 ± 1.50 | 8.21 ± 2.3  | 10.4 ± 2.7  | 9.80 ± 1.8  |
| Isorhamnetin 3,4'-diglucoside           | 0.50 ± 0.1  | 0.40 ± 0.2  | 0.38 ± 0.06 | 0.31 ± 0.1  | 0.30 ± 0.1  | 0.33 ± 0.08 |
| Quercetin 3-glucoside                   | 0.07 ± 0.05 | 0.04 ± 0.05 | nd          | 0.10 ± 0.1  | nd          | nd          |
| Quercetin 4'-glucoside                  | 14.2 ± 3.3  | 13.4 ± 2.9  | 11.1 ± 2.2  | 9.0 ± 3.2   | 8.41 ± 0.9  | 9.11 ± 1.1  |
| Isorhamnetin 4'-glucoside               | 1.60 ± 0.7  | 0.81 ± 0.8  | 1.20 ± 0.2  | 0.82 ± 0.1  | 0.61 ± 0.3  | 0.90 ± 0.2  |
| Quercetin aglycone                      | 0.10 ± 0.1  | 0.10 ± 0.1  | 0.06 ± 0.07 | 0.21 ± 0.2  | 0.04 ± 0.01 | 0.13 ± 0.04 |
| Total flavonols                         | 28.3        | 26.4        | 23.5        | 18.7        | 20.6        | 20.4        |
| Cyanidin 3-glucoside                    | 0.10 ± 0.06 | 0.08 ± 0.02 | 0.05 ± 0.01 | nd          | nd          | nd          |
| Cyanidin 3-laminaribioside              | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.05 ± 0.06 | nd          | nd          | nd          |
| Cyanidin 3-(6'-malonyl-glucoside)       | 0.11 ± 0.05 | 0.10 ± 0.03 | 0.07 ± 0.03 | nd          | nd          | nd          |
| Cyanidin 3-(6'-malonyl-laminaribioside) | 0.09 ± 0.03 | 0.06 ± 0.01 | 0.04 ± 0.02 | nd          | nd          | nd          |
| Total anthocyanins                      | 0.40        | 0.29        | 0.21        | –           | –           | –           |
| EC <sub>50</sub>                        | 66.3 ± 11.6 | 58.0 ± 18.9 | 60.0 ± 14.4 | 73.4 ± 18.6 | 89.3 ± 15.2 | 63.4 ± 16.2 |

Flavonol concentrations expressed as mg of quercetin 3-β-D-glucoside/100 g of onion fresh weight (fw); anthocyanin concentrations expressed as mg of cyanidin-3-O-glucoside equivalent/100 g of onion fresh weight (fw); EC<sub>50</sub> expressed as mg/ml; values represent mean ± standard deviation; nd – not detected (below 0.02 mg/100 g of Quercetin-gl and below 0.01 mg/100 g of Cy 3-gl); nq – not quantified (below 0.04 mg/100 g of Quercetin-gl or Cy 3-gl)

around 90% of the total flavonols, which is in agreement with the literature. PÉREZ-GREGORIO *et al.* (2010) identified flavonoids in the same Portuguese cultivars of red and white onions through acid and alkaline hydrolysis, enzymatic autolysis, and spectral analysis and obtained the same chromatographic profile.

Anthocyanic pigments in unpeeled red onions analysed immediately after the period of storehouse storage (T0) presented four peaks of anthocyanins (Figure 2a), identified as cyanidin 3-glucoside (peak 1), cyanidin 3-laminaribioside (peak 2), cyanidin 3-(6'-malonylglucoside) (peak 3) and cyanidin 3-(6'-malonyl-laminaribioside) (peak 4) by analysis of the UV spectra and standards as well as comparison with the literature (PÉREZ-GREGORIO *et al.* 2010). After acid hydrolysis of methanolic extract of onions, the anthocyanins glycosides were reduced to their aglycone form (anthocyanidin). HPLC-DAD analysis of the hydrolysed anthocyanins revealed only one peak, which was identified as cyanidin by comparison with the retention time and spectra of the standard (Figure 2b), thus, the four anthocyanins previously

assessed contain cyanidin as aglycone. Anthocyanic content in white onions was negligible.

Table 1 shows the mean results obtained for the individual and total flavonols and anthocyanins studied immediately after storehouse storage (T0) and after 1 and 2 months of domestic RT conditions (T1 and T2). The higher flavonols content in red onion than in white onion is in agreement with those given by other authors (PÉREZ-GREGORIO *et al.* 2010). No significant changes were observed during RT storage. According to RODRIGUES *et al.* (2010) total flavonols increased during the first 3 months of storage at RT, whereas in the period between 3 and 7 months the flavonol content was not significantly modified.

Cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-(6'-malonylglucoside), and cyanidin 3-(6'-malonyl-laminaribioside) were quantified in all red onions. These compounds were not detected in white onions except traces of cyanidin 3-laminaribioside and cyanidin 3-(6'-malonylglucoside) that were quantified in T2. In general, lower EC<sub>50</sub> values were observed in red onions than in white onions.

Table 2. Flavonoid content and radical-scavenging activity of frozen red and white onions after 5 months of simulating home storage

| Flavonoids                              | Red onion   |             |             | White onion |             |             |
|---|-------------|-------------|-------------|-------------|-------------|-------------|
|   | T0F         | T1F         | T2F         | T0F         | T1F         | T2F         |
| Quercetin 3,7,4'-triglucoside           | 0.08 ± 0.05 | 0.07 ± 0.07 | 0.22 ± 0.02 | 0.07 ± 0.04 | 0.20 ± 0.06 | 0.12 ± 0.10 |
| Quercetin 7,4'-diglucoside              | 0.33 ± 0.09 | 0.30 ± 0.10 | 0.25 ± 0.07 | 0.20 ± 0.04 | 0.21 ± 0.02 | 0.20 ± 0.01 |
| Quercetin 3,4'-diglucoside              | 20.8 ± 4.4  | 12.5 ± 1.4  | 17.0 ± 2.4  | 13.4 ± 4.2  | 17.2 ± 5.1  | 14.7 ± 8.00 |
| Isorhamnetin 3,4'-diglucoside           | 0.82 ± 0.20 | 0.41 ± 0.30 | 0.70 ± 0.21 | 0.51 ± 0.30 | 0.50 ± 0.30 | 0.51 ± 0.20 |
| Quercetin 3-glucoside                   | 0.21 ± 0.08 | 0.08 ± 0.07 | nq          | 0.11 ± 0.03 | nq          | nq          |
| Quercetin 4'-glucoside                  | 23.0 ± 6.0  | 16.4 ± 5.9  | 15.8 ± 2.2  | 11.7 ± 1.9  | 14.2 ± 3.5  | 12.9 ± 5.7  |
| Isorhamnetin 4'-glucoside               | 2.4 ± 1.0   | 1.0 ± 0.8   | 1.7 ± 0.4   | 1.1 ± 0.4   | 0.9 ± 0.5   | 1.1 ± 0.5   |
| Quercetin aglycone                      | 0.2 ± 0.2   | 0.2 ± 0.2   | 0.04 ± 0.04 | n.q         | nd          | 0.05 ± 0.05 |
| Total flavonols                         | 47.9        | 31.2        | 35.9        | 28.6        | 33.5        | 29.7        |
| Cyanidin 3-glucoside                    | 0.19 ± 0.13 | 0.12 ± 0.02 | 0.12 ± 0.04 | nd          | nq          | nq          |
| Cyanidin 3-laminaribioside              | 0.11 ± 0.04 | 0.05 ± 0.01 | 0.11 ± 0.10 | nd          | nq          | nd          |
| Cyanidin 3-(6'-malonylglucoside)        | 0.23 ± 0.11 | 0.14 ± 0.06 | 0.12 ± 0.06 | nq          | nq          | nq          |
| Cyanidin 3-(6'-malonyl-laminaribioside) | 0.18 ± 0.07 | 0.10 ± 0.03 | 0.06 ± 0.01 | nq          | nq          | nq          |
| Total anthocyanins                      | 0.73        | 0.43        | 0.42        | -           | -           | -           |
| EC <sub>50</sub>                        | 58.1 ± 18.3 | 49.9 ± 22.1 | 76.5 ± 18.0 | 87.8 ± 12.4 | 82.8 ± 29.0 | 82.4 ± 25.4 |

Flavonol concentrations expressed as mg of quercetin 3-β-D-glucoside/100 g of onion fresh weight (fw); anthocyanin concentrations expressed as mg of cyanidin-3-O-glucoside equivalent/100 g of onion fresh weight (fw); EC<sub>50</sub> expressed as mg/ml; values represent mean ± standard deviation; nd – not detected (below 0.02 mg/100 g of Quercetin-gl and below 0.01 mg/100 g of Cy 3-gl); nq – not quantified (below 0.04 mg/100g of Quercetin-gl or Cy 3-gl)

EC<sub>50</sub> of onions with different RT storage oscillated without any increasing or decreasing trend.

It should be highlighted that after 2 months under domestic RT storage (thus, 5 months after harvest) onions bulbs began to sprout, thus alternative methods of domestic storage to increase the shelf life of these vegetables and reduce the waste are needed.

**Influence of domestic freezing on flavonoids composition and radical-scavenging activity.** All frozen samples presented an increase of flavonoids content when compared with the respective contents before freezing (Table 2). The major positive increment for flavonols and anthocyanins was obtained for red onions portions kept frozen for 5 months (T0F) ( $P < 0.05$ ,  $t$ -test, for all flavonols and anthocyanins except quercetin aglycone and cyanidin 3-glucoside), which were from the same onions analysed immediately after the period of storehouse storage. The increase of flavonoid content in red onion samples previously stored at RT 1 and 2 months and frozen, respectively, for 4 months (T1F) and 3 months (T2F), although it was lower, was statistically significant ( $P < 0.05$ ,  $t$ -test) for most flavonols and antocyanins. As regards the white

onions, a significant increase of flavonols content in onion portions ( $P < 0.05$ ) was also observed after the freezing process. Furthermore, the moisture content of the onion samples did not change significantly, being  $92.01 \pm 0.01$  and  $92.2 \pm 0.1\%$ , respectively, before and after freezing. These results suggest that frozen storage of onion pieces positively affected flavonol and anthocyanin metabolisms. Vegetable wounding due to the alteration of the structural integrity activates the synthesis and accumulation of phenolic compounds (CISNEROS-ZEVALLOS 2003).

$t$ -Test was performed to compare the radical-scavenging activity expressed as EC<sub>50</sub> of onions stored under RT conditions and the respective frozen portions (Table 2), and no significant differences were observed ( $P > 0.05$ ,  $t$ -test) except between T2 and T2F of red onions ( $P = 0.006$ ,  $t$ -test). These results are in agreement with those reported by ANTONIA MURCIA *et al.* (2009), which reported that onions maintain their antioxidant activity after freezing. Thus, the increase of flavonols and anthocyanins contents in frozen onions did not lead to an increment of radical scavenging activity, probably due



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to the loss of other antioxidant compounds during frozen storage, such as vitamin C. Onions contain this vitamin that is prone to be lost during storage (Antonia Murcia *et al.* 2009).

## CONCLUSIONS

Domestic freezing of onion extends its shelf life and maintains the antioxidant capacity, thus it can be a good alternative to prevent the loss of unused fresh onions.

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