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The "Breme" Red Onion: Effects of Home-storage Methods on Quercetin and Quercetin-Glycoside Contents

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Abstract

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The "Breme" onion is a red-skinned cultivar growing in the northwest Italy. To date, its nutrient composition has not been described. In this study, we quantified the contents of quercetin (Q) and its glycosides and we studied their stability in the dependence on the local home-storage methods storage at 4°C and freezing. Quercetin-3,4'-*O*-diglycoside (3,4'-Qdg) was the most abundant form, followed by quercetin-4'-*O*-diglycoside (4'-Qmg) and Q. We observed the reduction in the contents of all the analysed flavonols after storage at 4°C and after storage in frozen state. No changes have been observed in the ratio Q/3,4'-Qdg + 4'-Qmg, as well as in 3,4'-Qdg /4'-Qmg between the fresh, stored at 4°C, and frozen onions. This could suggest an overall condition of instability, not the activation of a selective deglycosylation pathway. In conclusion, our study shows that the "Breme" onion is mainly rich in 3,4'-Qdg and that home-storage methods do not preserve the stability of some important health-promoting molecules.

Keywords: Breme onion; home-storage methods; HPLC-MS/MS; quercetin; quercetin glycosides

Onion (*Allium cepa* L.) has been recognised as an important source of valuable phytonutrients, such as flavonoids, fructo-oligosaccharides, and sulphur compounds. Among these substances, flavonoids have aroused great attention due to their role in the prevention of inflammation, cardiovascular diseases, and cancer (Arai *et al.* 2000; Middleton *et al.* 2000; Hollman *et al.* 2001; Kris-Etherton *et al.* 2002; Terao *et al.* 2008).

Quercetin (Q) is the most abundant dietary flavonol and is found in numerous fruits and vegetables. Onion represents one of the major dietary sources of Q which is contained both as aglycone, mainly in the skin, and as glycosides (Slimestad *et al.* 2007; Wach *et al.* 2007). Quercetin-3,4'-*O*-diglycoside (3,4'-Qdg) and quercetin-4'-*O*-monoglycoside (4'-Qmg) represent the two major conjugated derivates of Q in onion (Galdon *et al.* 2008; Yoo *et al.* 2010). The contents of flavonoids and Q may vary considerably between different cultivars and are related not only to the genetic factors but also to the environmental conditions, soil features, agronomic management, storage and processing conditions (Beesk *et al.* 2010; Mogren *et al.* 2006).

The heterogeneity of the environmental and climatic conditions in Italy favoured the selection of a great number of vegetables and, in particular, of different onion landraces. Today, there is a great interest to

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safeguard and characterise these local cultivars not only to highlight their nutritional properties for consumer health benefits but also to obtain protection and economic sustainability in the context of European agriculture, as suggested and discussed in a recent paper by Riggi *et al.* (2013).

The "Breme" onion is a red-skinned cultivar growing in a restricted region of the northwest Italy (Breme Lomellina, Pavia). It is characterised by a garnet red colour, soft pulp, and a very sweet taste. For these reasons, it is consumed both cooked and raw. The bulb has an elongated and flat shape, a diameter of about 12–13 cm and a weight ranging from 400 g to 600 grams. Its cultivation covers a limited territory, of about 12 ha, characterised by the alluvial soil. Only in this area the "Breme" red onion is able to grow and develop its features. This onion has only a local distribution, mainly for two reasons: (1) the production is limited and not sufficient for large scale distribution and (2) the onion is very perishable. In fact, after harvesting and drying for 10 days in the field, the "Breme" red onion may be preserved for a short time (no more than 1–2 months) in a cool, dry, and dark place. Anyway, the local practice is also to store bulbs partially consumed at 4°C or freeze them.

To our knowledge, there are no studies describing the phytonutrient composition of the "Breme" red onion nor the stability of its phytonutrients depending on the home-storage methods. For this reasons, in the present study we aimed at: (1) the quantification of the contents of Q and its glycosides and (2) the study of their stability depending on different home-storage methods (storage at 4°C and freezing).

MATERIALS AND METHODS

Chemicals and reagents. 3,4'-Qdg (≥ 98.5%) and 4'-Qmg (≥ 95%) standards were purchased from Extrasynthese (Genay Cedex, France). Q (≥ 95%) and the certified reference material deuterium-labelled ethyl glucuronide (D5-EtG), used as internal standard, were obtained from Sigma-Aldrich (Milan, Italy). Solutions of Q (1 mg/ml, 100 µg/ml, and 10 µg/ml) and 4'-Qmg (10 µg/ml) were prepared in methanol. 3,4'-Qdg and D5-EtG were dissolved in distilled water in the amounts 50 and 100 µg/ml, respectively. The solvents used were distilled water, methanol (99.9%), glacial acetic acid, sodium acetate (99%), formic acid (99%), ammonium formate (≥ 99.0%), and acetonitrile (> 99.9%) (Sigma-Aldrich, Milan, Italy).

Samples. Onions were grown under idential agricultural conditions in open fields in Breme Lomellina (Pavia, Italy) and were harvested in June 2012. The inedible outer layers and the neck and basal parts of the onions were removed. Onions were subjected to 3 different treatments under simulated domestic conditions. Treatment 1: onions were harvested, cut, homogenised, and then immediately extracted. Treatment 2: onions were harvested, cut in a half, stored in a closed box at 4°C for 7 days and then homogenised and extracted. Treatment 3: onions were harvested, cut, stored in a closed box at –20°C for 7 days and then homogenised and extracted. For each treatment group, five bulbs (each of about 400–500 g and free from visible blemishes or defects) were selected from the harvested onions.

Extraction method. The onion samples of about 10 g were homogenised to a puree with a mincer and centrifuged at 17 864 g for 10 minutes. Extraction was then performed on both the liquid and solid phases obtained. Two ml of a buffered solution (pH 4, 0.1 mM acetic acid – sodium acetate) and 1 ml of a hydro-alcoholic solution (water : methanol – 80 : 20 v/v) were added to 1 g of the solid phase. After 2 h incubation in an ultrasonic bath at room temperature, 1 ml of the supernatant was diluted in the ratio 1 : 2 with acetonitrile. In relation to the liquid phase, the samples were immediately diluted 1 : 4 with acetonitrile.

After centrifugation at 17 864 g for 15 min, 10 µl of the solution and 10 µl of D5-EtG were added to 980 µl of the mobile phase. The final sample dilution was 1 : 600 for the solid phase and 1 : 400 for the liquid phase.

Calibration curves. Standard calibration curves made based on the following concentrations in water: 0–1–5–10–20–50 µg/ml. The standards were then diluted 1 : 2 with acetonitrile. After centrifugation at 17 864 g for 15 min, 10 µl of the solution and 10 µl of D5-EtG were added to 980 µl of the mobile phase. The final dilution was 1 : 200. Quantitative analysis was performed by creating the standard curve according to the model $y = mx + b$. where: x – concentration of the analyte of interest; y – ratio between the peak area of the analyte and that of the internal standard.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis. The samples were analysed on a ABSciex 3200 LC-MS/MS system coupled with a HPLC DIONEX 3000 Ultimate pump. The column used was a Phenomenex GEMINI C18 (3 µm; 2 ×

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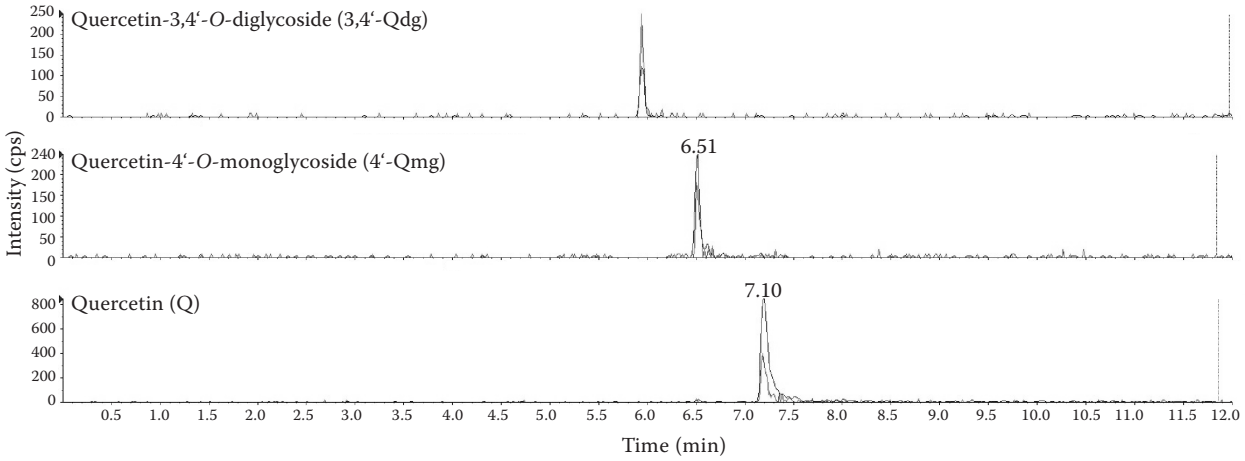

Figure 1. A representative chromatogram of quercetin-3,4'-*O*-diglycoside (3,4'-Qdg), quercetin-4'-*O*-monoglycoside (4'-Qmg), and quercetin (Q) in the "Breme" red onion

100 mm), conditioned at 30°C. The mobile phase consisted of (A) distilled water with 0.1% formic acid (20 mM ammonium formate) and (B) acetonitrile. The flow rate was 0.4 ml/min and the injection volume was 2 µl. The binary gradient used was as follows: 95 : 5 A : B for 2 min, 50 : 50 A : B from min 2 to 6, 5 : 95 A : B from min 6 to 8, 5 : 95 A:B from min 8 to 10, 95 : 5 A : B from min 10 to 12.

The compounds were ionised in the negative ion mode. The ionisation source conditions were as follows: spray voltage –4500 V, source temperature 150°C, Gas 1 (nitrogen) pressure 45 psi, Gas 2 (nitrogen) pressure 55 psi, declustering potential –60 V. The precursors and transition fragment ions monitored were: Q parent ion of *m*/*z* 301.1 with a retention time of 7.2 min and first-generation product masses of *m*/*z* 179.0 and 151.1; 3,4'-Qdg parent ion of *m/z* 625.4 with a retention time of 5.9 min and first-generation product masses of *m/z* 463.4 and 301.1; 4'-Qmg parent ion of *m*/*z* 463.4 with a retention time of 6.5 min and first-generation product masses of *m/z* 301.1 and 151.1; D5-EtG parent ion of *m/z* 226.0 with a retention time of 1.3 min and first-generation product masses of *m/z* 85.0 and 75. Collision energy was –30 eV for Q, –40 for 3,4'-Qdg, –50 for 4'-Qdg, and –20 for D5-EtG.

Statistical analysis. The data were expressed as mean ± standard deviation (SD). The results obtained on liquid and solid phases were combined and expressed as mg per kg of fresh vegetable. The normality of data distribution was assessed by the Kolmogorov-Smirnoff test. Differences across groups were compared by one way analysis of variance (ANOVA) followed by Tukey post-hoc test. Statistical analysis was performed using GraphPad Prism 5.0 biochemical statistical package (GraphPad Software, Inc., San Diego, USA). All analyses were performed in triplicate. The *P*-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The phytonutrient composition of the Breme red onion has never been analysed till now and this

Table 1. Quercetin (Q), quercetin-4'-O-monoglucoside (4'-Qmg) and quercetin-3,4'-O-diglucoside (3,4'-Qdg) content in fresh, 4°C-stored, and frozen "Breme" red onion

	Fresh (mg/kg)	4°C		Frozen	
		(mg/kg)	% (↑↓)	(mg/kg)	% (↑↓)
Q	6.85 ± 1.90	4.29 ± 1.28	–37.37	3.13 ± 1.36*	–54.31
4'-Qmg	40.42 ± 7.44	23.28 ± 9.90*	–42.40	20.78 ± 4.52**	–48.60
3,4'-Qdg	71.88 ± 15.72	45.59 ± 9.56	–36.57	27.68 ± 7.73**	–61.49

Data are expressed as mean ± SD (mg/kg of fresh weight) and percent of variation compared to fresh vegetable; each treatment group consisted of five samples and a triplicate analysis of each one has been carried out; *$P < 0.05$, **$P < 0.001$ vs. fresh onion

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Table 2. Variation in Q/4'-Qmg + 3-4'-Qdg and 4'-Qmg/3-4'-Qdg ratio in fresh, 4°C-stored, and frozen "Breme" red onion

	Fresh ratio	4°C		Frozen	
		ratio	*P*	ratio	*P*
Q/4'-Qmg + 3,4'-Qdg	0.061 ± 0.009	0.062 ± 0.020	0.92	0.065 ± 0.026	0.83
4'-Qmg/3,4'-Qdg	1.77 ± 0.13	1.73 ± 0.02	0.76	1.73 ± 0.47	0.96

Each treatment group consisted of five samples and a triplicate analysis of each one has been carried out; Q – quercetin; 4'-Qmg – quercetin-4'-*O*-diglucoside; 3,4'-Qdg – quercetin-3,4'-*O*-diglucoside

paper represents the first study describing the contents of Q, 4'-Qmg, 3,4'-Qdg and their stability in the dependence on the home-storage procedures. A representative chromatogram of Q, 4'-Qmg, 3,4'-Qdg content in the Breme red onion is shown in Figure 1.

The 3,4'-Qdg was the main form detected in the fresh "Breme" red onion (71.88 ± 15.72 mg/kg), followed by 4'-Qmg (40.42 ± 7.44 mg/kg) and Q (6.85 ± 1.90 mg/kg) (Table 1). The quantitative comparison of Q and Q glycoside contents between "Breme" red onion and other previously described cultivars must be performed carefully both due to the different methodological procedures used for the analyses and also because the data could be reported as related to the fresh or dried weights and to the whole or specific parts of the vegetable. Among different red cultivars characterised till now (reviewed in Slimestad *et al.* 2013), the edible part of the "Breme" onion seems to have a specific and unique profile with a quite high 3,4'-Qdg content.

The "Breme" red onions are immediately consumed after harvesting or, according to local habits, conserved lifted and cropped at 4°C for a few days or kept frozen for a longer storage. However, these home-storage procedures, especially the freezing one which is carried out in a way different from the industrial practice, could compromise the stability of the different phytonutrients contained in the vegetable. For these reasons we explored how these storage methods may affect the stability of Q and its glycosides. We observed a reduction in the content of each molecule after storage at 4°C and after freezing (Table 1). In particular, after storage at 4°C, the contents of 4'-Qdg and Q decreased by about 37% and that of 4'-Qmg by about 42% ($P < 0.05$) (Table1). Although frozen onions may be stored for different numbers of months, in this study we performed the analysis after a short-term storage with the aim to evaluate the immediate effects of this procedure on the stability of Q and its glycosides. We observed that after freezing, the contents of 3,4'-Qdg, 4'-Qmg and Q decreased of about 61% ($P < 0.001$), 49% ($P < 0.001$) and 37% ($P < 0.05$), respectively (Table 1). Because each molecule was quantified both in the liquid and solid phases and its content was expressed as the sum of these two fractions, we may confirm that the reductions observed are due to the processing procedure and not to the loss of water during the thawing cycle. In our opinion, the greater loss of flavonols observed with the frozen onions could be related to a more severe cellular disruption of the vegetables stored in this condition. In fact, since onions were frozen after having been to cut in small pieces, it is possible that this processing led to oxidative degradation of polyphenols as a result of the cellular decompartmentation and contact between cytoplasmic polyphenol oxidase and phenolic substrates present in the vacuoles.

Previous works which evaluated the stability of both Q and its glycosides in different onion cultivars stored according to industrial procedures reported the general stability of these molecules (Perez-Gregorio *et al.* 2011). On the contrary, other studies considering the domestic storage methods indicated a decrease in the content of diglycosides and an increase in monoglycosides and aglycones (Rhodes *et al.* 1996; Price *et al.*1997). As reported in Table 2, we did not observe any difference in the ratio of 3,4'-Qdg/4'-Qmg as well as of Q/3,4'-Qdg + 4'-Qmg between the fresh, stored at 4°C, and frozen onions. These data thus suggest that the home-storage procedures utilised to preserve the "Breme" red onion strongly affect the stability of all the analysed phytonutrients and are not specifically associated with the activation of a selective deglycosilation pathway.

CONCLUSION

The "Breme" red onion has never been studied till now and the data reported here represent the first results obtained in the characterisation of the phytonutrient composition of this cultivar. The "Breme" red

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onion is rich mainly in 3,4'-Qdg, followed by 4'-Qmg and Q. Although the edibility of the "Breme" onion may be preserved by home-storage procedures, these methods do not maintain the stability of Q and its glycosides.

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