Effect of Storage and Domestic Processing on the Content and Composition of Flavonol Glucosides in Onion (*Allium cepa*)

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The stability of the major flavonol glucosides, quercetin 3,4′-*O*-diglucoside (QDG) and quercetin 4′-*O*-monoglucoside (QMG), was studied in two varieties of onion (Red Baron and Crossbow) that were cured and stored for 6 months under normal commercial conditons and analyzed at regular intervals. Onions were also cooked by boiling in water and by frying in oil under normal domestic conditions. Apart from a 50% loss of quercetin 4′-*O*-monoglucoside during the initial drying process, little change in content and composition was observed over 6 months of storage. Neither boiling nor frying resulted in interconversion of the quercetin conjugates or production of free quercetin, although a 25% loss overall was recorded for each process.

Keywords: *Onion; Allium cepa; flavonol; quercetin; glucoside; HPLC; storage; processing*

INTRODUCTION

Flavonoids play an important role in plant development and may act as a part of a defense mechanism against pathogens, predators, and environmental stress (Harborne, 1994). One class of flavonoids, the flavonols, are present at significant levels in many food plants and occur predominantly as glycosides (Kuhnau, 1976).

The contribution of dietary flavonols to improved health is the subject of much current debate (Huang and Ferraro, 1996), and there are two main routes that research is taking to address this concern. In the first case, epidemiological studies have indicated a relationship between a diet rich in flavonols and a reduced incidence of heart disease (Hertog, 1995). However, no such relationship was found between flavonol intakes and a range of cancers (Goldbohm, 1995), which conflicts with the evidence of a protective effect against the incidence of cancer in animal model experiments (Deschner et al., 1991). Second, using *in vitro* and *in vivo* assays, individual flavonols have been shown to have a range of protective properties such as antioxidant activity (Rice-Evans, 1994) and inhibition of mutagenic activity (Huang and Ferraro, 1996) and have also been shown to act as vasodilators (Cheng et al., 1993) and platelet disaggregators (Gryglewski et al., 1987).

To further aid these studies, basic information is required on the precise chemical nature of the flavonols in the food and the effect that normal domestic processing may have on these structures. Further information will also be necessary concerning the effect of digestion on the form and concentration of these compounds and on their bioavailability. A recent study (Hollman et al., 1995) has shown that over half of the quercetin glucosides present in onion were absorbed by the small intestine.

One of the major sources of flavonols in the European diet is the onion, which has been reported to contain a range of quercetin, isorhamnetin, and kaempferol conjugates (Leighton et al., 1992; Tsushida et al., 1995). Previous studies (Bilyk et al., 1984; Patil et al., 1995a,b; Tsushida et al., 1996) have shown a relatively small range in the variation of quercetin conjugates in the onion bulb of 110−295 mg/kg (expressed as quercetin, fresh weight) for red cultivars and 119−286 mg/kg for yellow cultivars. However, these data relate to the whole onion bulb and not the edible part, which has the outer layers rich in the flavonols removed. A recent study, which used a mild extraction technique to enable these compounds to be measured without formation of degradative artifacts (Price and Rhodes, 1996), has shown that the flavonoid composition in the edible portion of the onion bulb is made up primarily of two quercetin conjugates, quercetin 3,4′-*O*-diglucoside and the 4′-*O*-glucoside.

Since processing has been shown to affect the stability of the anthocyanins in red onion (Lin et al., 1982) and the conversion of the flavonol triglycosides in green tea to their corresponding diglycosides has been shown during the fermentation process to produce black tea (Engelhardt et al., 1992), we have studied the effect of both standard commercial storage conditions and commonly used domestic processing on the changes in the overall content and the composition of the major quercetin glucosides present in the edible portions of two varieties of onion.

MATERIALS AND METHODS

Materials. All solvents were of AnalaR grade and HPLC grade where applicable. Methanol was obtained from Fisons, Loughborough, U.K.; acetonitrile from BDH, Poole, U.K.; tetrahydrofuran from Aldrich, Milwaukee, WI; trifluoroacetic acid, *p*-anisaldehyde and borax from Sigma Chemical Co., Poole, U.K., and water was purified via a Millex Q-plus system, Millipore, Watford, U.K.; 7,8,3′,4′-tetrahydroxyflavone was purchased from Apin Chemicals Ltd., Abingdon, U.K. and quercetin dihydrate from Sigma Chemical Co., Poole, Dorset, U.K. Quercetin 3,4′-*O*-diglucoside and 4′-*O*-monoglucoside were isolated from onion tissue as described previously (Price and Rhodes, 1996).

Onion (*Allium cepa*) varieties Red Baron (red skinned) and Cross Bow (brown skinned), which are commercial cultivars used in the U.K., were grown and harvested under normal commercial conditions at Colchester, Essex, U.K. Three hundred bulbs of each variety were cured by drying at 28 °C for 10 days and mechanically cleaned. They were then transferred for long-term storage at a minimum temperature of 4 °C in the dark.

Storage Experiment. Ten bulbs in the weight range 84−285 g with a mean weight of 150 g for each variety were taken at intervals between September 1995 and February 1996 of *t*

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(day) = 0 (immediately after harvest), 12 (after curing at 28 °C), 28, 56, 84, 105, 140, and 168 days (24 weeks). Each bulb was cleaned by removing outer dry skin, roots, and top. They were then cut into quarters, immediately immersed in liquid nitrogen, and subsequently freeze-dried. The dried quartered bulbs were weighed (to determine moisture content), blended in a domestic blender to a fine powder, and stored under refrigeration prior to extraction and analysis.

Cooking by Boiling. Five bulbs of each variety were cleaned, peeled, weighed, and quartered. Two opposite quarters were immersed in liquid nitrogen and freeze-dried as control material and the remaining two quarters wrapped in muslin and cooked in boiling water (700 mL) for 20 min. The onion tissue was drained, removed from the muslin, and immersed in liquid nitrogen prior to freeze-drying. The cooking water was also freeze-dried. The dry weights were used to calculate the transfer of material from onion into cooking water.

Cooking by Frying. Two samples of five bulbs of each variety (for each of the two frying times) were cleaned, peeled, weighed, and quartered. Opposite quarters of each were immersed in liquid nitrogen and freeze-dried (separate controls for each frying time) and the remaining quarters shredded for frying. Shredded onion tissue (4 mm slices) was cooked in an open frying pan with sunflower oil (20 mL) for two frying times to simulate two common domestic procedures. The first, fried for approximately 5 min, produced onion tissue which was still fleshy and translucent, while the second (approximately 15 min) produced browned and drier tissue.

The fried samples were immersed in liquid nitrogen and freeze-dried. These samples, including the controls (10 g), were defatted by homogenizing in hexane (200 mL) twice for 10 min and filtered under reduced pressure to dryness, prior to extraction and analysis by the standard method.

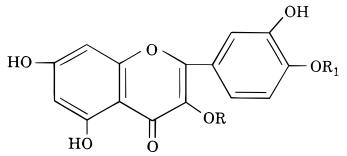
Extraction. Duplicate representative samples of each freeze-dried powder (2.00 g) was taken for extraction of the flavonol glucosides by homogenizing three times with 70% aqueous methanol (50 mL) at 1200 rpm for 1 min using a Pro400 homogenizer. The combined filtrates were evaporated to approximately 20 mL, under reduced pressure at less than 40 °C, and made to 25 mL with MeOH:DMSO (9:1 v/v).

An aliquot (1 mL) was diluted with MeOH:DMSO (9:1, 1 mL), an internal standard of 7,8,3′,4′-tetrahydroxyflavone (100 μL, 1 mg/mL solution) added, and the mixture filtered through a 0.22 μm filter for subsequent HPLC analysis.

HPLC Analysis. HPLC analysis was carried out using a model HP1050 system with autosampler and quaternary pump coupled to a diode array detector and controlled by Chemstation software. A solvent gradient of A (water:THF:TFA, 98:2:0.1) and B (acetonitrile) used in the proportion of 17% B for 2 min increasing to 25% B after 5 min, to 35% B after a further 8 min, and to 50% B after 5 min. A postrun column clean up procedure was used by increasing B to 90% after a further 5 min and finally reequilibration for 20 min at 17% B. The column was 250 mm × 4.6 mm i.d. packed with Dynamax reversed phase (ODS) silica (8 μm), the eluate was monitored at 270 nm, and 20 μL of each sample was injected in duplicate for each duplicate extract.

Quantification. Quantification of the quercetin conjugates and free quercetin using 3′,4′,7,8-tetrahydroxyflavone as internal standard gave linear responses over a range of 2−300 μg/mL extract with peak areas (mAU) in the range 50−15000 for quercetin 3,4′-*O*-diglucoside, quercetin 4′-*O*-glucoside, quercetin dihydrate, and the internal standard respectively as follows: $y = 1.892 + 14.69x$, $r^2 = 0.999$; $y = -121.29 + 62.62x$, $r^2 = 1.000$; $y = -42.34 + 23.24x$, $r^2 = 1.000$; $y = -124.50 + 50.07x$, $r^2 = 1.000$. Response factors were calculated for the four compounds as 0.234, 0.371, 0.800, and 1.000, respectively. The formula used for quantification of each conjugate was concentration X = area X/area i.s. × concentration i.s./RF.

Precision. Precision of the analytical method was measured from eight extractions of each of one sample of red-skinned and one brown-skinned onion sample each in duplicate. Mean values of the two quercetin glucosides (Qdg and Qmg, μg/g dry weight) and their standard deviations for red onion extracts were 13934 ± 232 and 6722 ± 112, respectively, and for brown onion extracts were 10071 ± 333 and 3978 ± 304, respectively.



Compound	R	R_1
Quercetin	H	H
Quercetin-4′-O-glucoside	H	Glc
Quercetin-3,4′-O-diglucoside	Glc	Glc

Figure 1. Chemical structures of quercetin and its glucosides present in onion.

Statistical Analysis. Statistical analysis was carried out using Tukey's multiple analysis of variance (Minitab ver 8.21) with a family error rate of 0.05 to determine the significant differences found between the multiple sets of data.

RESULTS AND DISCUSSION

The skinned onion bulbs, used for analysis during the 24 week storage period, showed moisture contents increasing only very slightly for red- and brown-skinned varieties between 86% and 87% and 88% and 89%, respectively.

The levels of the individual quercetin glucosides (quercetin 3,4′-*O*-diglucoside and 4′-*O*-monoglucoside) and free quercetin (chemical structures are shown in Figure 1) were determined for each of the eight samples, of the brown-skinned onion variety, which had been stored over a period of 24 weeks and are given in Table 1.

The main change observed was a significant 54% reduction of quercetin monoglucoside (Qmg) content from 1004 μg/g fresh weight in freshly harvested onions to 465 μg/g following the curing process. Spectral searches of the chromatograms of extracts of onion before and after the curing process did not show the appearance of any new peaks of a flavonol character. However, due to this process the onions acquired a much thicker outer dry skin which was removed to make the sample representative of an edible part of the onion. The distribution of the quercetin conjugates in the different parts of the onion used for these two analyses is the likely reason for the gross change observed for the quercetin conjugates during this process. Thereafter, under normal storage conditions, the Qmg level increased slightly to 553 μg/g (from 465 μg/g which was not significant for a family error rate of 0.05; see Table 1) after 24 weeks and the quercetin diglucoside (Qdg) showed a similar slight increase from 1045 to 1323 μg/g over the 24 week storage period, which again was not statistically significant (see Table 1). In contrast free quercetin (Q) content was at a much lower level of 29 μg/g at $t = 0$ and was not detectable after 28 days of storage.

The total content of quercetin conjugates, expressed as free quercetin, was 1187 μg/g at harvest ($t = 0$), which then dropped to 810 μg/g after the curing process (t = 12 d) and thereafter increased to 998 μg/g (not significant) after 28 weeks of storage (t = 168 d) as shown in Table 1.

Similar data were recorded for the red-skinned variety and are shown in Table 2. The drop in Qmg level

Table 1. Changes Found in the Content of Quercetin Glucosides, Expressed as Quercetin and Free Quercetin for the Brown-Skinned Onion Stored up to 24 Weeks

	quercetin and glucosides (μg/g fr wt as quercetin)							
	Qdg		Qmg		quercetin		total quercetin and glucosides	
storage time (days)	mean	sd	mean	sd	mean	sd	mean	sd
0	504[a]	13	653	12	29	8	1187	26
12	502[a]	2	303[a]	2	5	0	810[a]	4
28	551[b]	16	335[b]	10	2[a]	1	887[b]	2
56	565[b]	5	298[a]	3	1[a]	0	864[a,b,c]	8
84	576[b]	12	312[a]	13	0		888[c]	24
105	655[c]	22	336[b]	3	0		991[d]	19
140	558[b]	4	291[a]	7	0		849[a,b,c]	3
168	638[c]	19	360	6	0		998[d]	13

[a,b,c,d] The same letter within a column denotes there was no significant difference found within a family error rate of 0.05 using Tukey's analysis of variance.

Table 2. Changes Found in the Content of Quercetin Glucosides, Expressed as Quercetin and Free Quercetin for the Red-Skinned Onion Stored up to 24 Weeks

	quercetin and glucosides (μg/g fr wt as quercetin)							
	Qdg		Qmg		quercetin		total as quercetin and glucosides	
storage time (days)	mean	sd	mean	sd	mean	sd	mean	sd
0	1001[a]	47	899	47	17[a]	1	1917	96
12	786[b]	9	563[a]	14	41	14	1390[a]	36
28	950[a,c]	7	542[a]	8	20	2	1512[b]	16
56	844[d]	12	477	7	16[a]	1	1337[a]	9
84	817[b,d]	38	373	6	0		1191[c]	45
105	993[a]	8	526[a]	4	0		1519[b]	12
140	901[c]	16	424[b]	7	0		1326[a,d]	23
168	812[b,d]	7	428[b]	3	0		1239[c,d]	10

[a,b,c,d] The same letter within a column denotes there was no significant difference found within a family error rate of 0.05 using Tukey's analysis of variance.

for the red-skinned variety after the initial curing stage was less than that observed for the brown-skinned variety but still fell by 37% (1381 to 865 μg/g). The Qmg content showed an overall decrease for the subsequent storage period dropping from 865 to 657 μg/g after 24 weeks. The level of Qdg showed no obvious trend, varying between 2076 and 1630 μg/g while free quercetin reached a maximum of 41 μg/g after the drying stage and then dropped until it was not detectable after 84 days of storage.

Total quercetin conjugate levels expressed as free quercetin for the red-skinned variety are shown in Table 2. Levels decreased from a maximum of 1917 μg/g at harvest to 1239 μg/g after 24 weeks of storage, demonstrating a similar trend to that shown by the brown-skinned variety. Again, no significant loss was observed after the curing stage (1390−1239 μg/g).

Changes in the quercetin conjugates due to boiling onion tissue in water are shown in Figure 2, which gives their distribution in the raw and cooked onion tissue together with that in the cooking water for both brown- and red-skinned varieties.

There was a loss of dry matter during the cooking process of 5.4% and 1.6% for red- and brown-skinned onions, respectively, while a transfer of total quercetin conjugates into the cooking water was calculated at 14.3% and 21.9%, respectively.

For the brown-skinned onion, there was an overall loss for Qdg and Qmg during cooking of 11.3% and 2.6%, respectively, but these losses were not translated into production of free quercetin. A significant proportion of each conjugate was leached unchanged from the onion tissue into the cooking water, 18% of Qdg and 19% of Qmg. The ratio of Qdg:Qmg was found to be constant in both the raw and the boiled tissue as well as in the cooking water, again confirming that no preferential leaching or degradation of either conjugate occurred.

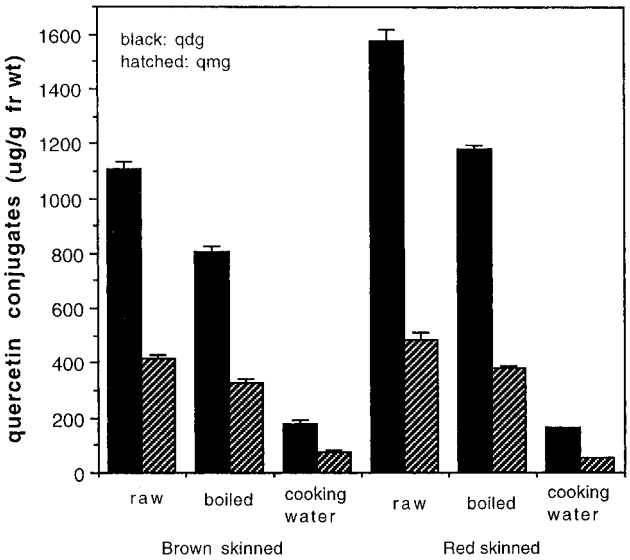


Figure 2. Effect of boiling in water for 15 min on the composition of quercetin glucosides in red- and brown-skinned onions.

A similar pattern was observed for the red-skinned onion, although larger overall losses of Qdg and Qmg during the process were recorded, 14.6% and 10.3%, respectively. Once again production of free quercetin was not found. Slightly less leaching into the cooking water was observed, 12% of Qdg and 13% of Qmg. As in the case of the brown-skinned onion, the ratio of Qdg:Qmg was the same in raw and boiled tissue, and in the cooking water.

Changes in the composition of the quercetin conjugates due to frying are shown in Figure 3. Both the brown- and red-skinned onion showed small increases of 3−14% in both Qdg and Qmg content after a frying

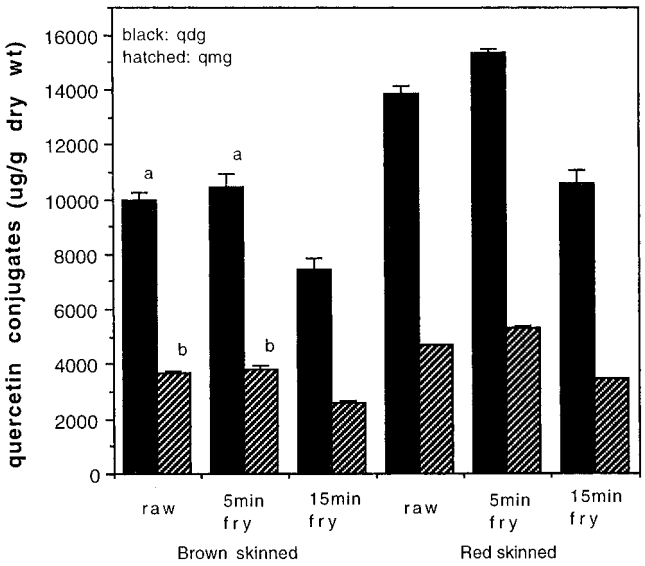


Figure 3. Effect of frying time on the composition of quercetin glucosides in red- and brown-skinned onions. a and b denote no significant differences between columns with a family error rate of 0.05 using Tukey's analysis of variance.

time of 5 min; from 9942 to 10470 μg/g and 3701 to 3796 μg/g dry weight, respectively, for the brown-skinned onion. These differences were not significant within the family error rate of 0.05 (Figure 3). However, the increase observed in the red-skinned onion for Qdg and Qmg of 13832−15329 and 4694−5342 μg/g, respectively, were significant.

After a total of 15 min frying, however, the levels of the quercetin conjugates were reduced by between 23% and 29%, and the loss of water during this longer frying time was 11.4% of the total moisture content for the red-skinned onion and 8.4% for the brown-skinned onion. Qdg and Qmg were reduced significantly from 9942 to 7418 and 3701 to 2617 μg/g, and 13 832 to 10 601 and 4694 to 3495 μg/g dry weight for brown- and red-skinned onions, respectively.

As in the case of the onion cooked by boiling, the frying process did not alter the ratio of Qdg:Qmg present in the tissue after either 5 or 15 min frying time for either variety and there was no increase in free quercetin observed. However, an overall loss of flavonol derivatives was observed in the tissue from the longer frying time which also showed extensive caramelization.

The main conclusion to be drawn from these results is that the quercetin conjugates present in the onion bulb are remarkably resistant to degradation during normal processing operations and that there is only small amounts of free quercetin present in the edible parts of either raw or cooked onion. This is in contrast to other biologically active compounds in onion such as the sulfur compounds. This diverse class of compounds, which in contrast to the flavonol conjugates, are unstable to processing and breakdown to a complex mixture of mostly volatile components. Even frying shredded onion tissue for 15 min which resulted in significant charring and caramelization failed to degrade more than 20−25% of the conjugates. The HPLC of extracts of fried (15 min) onion from both brown- and red-skinned onions contained an extra early running peak which was not present in the raw onion or fried for 5 min extracts. Due to the relative retention time of this peak it would appear to be more polar than quercetin 3,4′-di-*O*-glucoside and from its UV spectrum (λ_{max} = 285 nm) to have a structure similar to either a flavanone or flavanol; further work is in hand to isolate and identify this component and will be reported elsewhere. However, there was no evidence that the quercetin diglucoside was converted to its monoglucoside analogue or broken down further to the aglycon, quercetin.

In the case of cooking by boiling, there was a similar overall loss of quercetin glucosides of 20−25% which arose mainly from the leaching action of the cooking water rather than from chemical or thermal degradation.

During storage, the onions showed little physiological change until the storage time reached 140 days, at which time dormancy was broken and growth of new shoots in the bulbs occurred. This was much more evident in the last sample (t = 168 days). However no gross changes in the quercetin glucoside levels were observed even though significant physiological changes in the onion were evident. Since only the edible portions of the onion bulbs were used for extraction and analysis, any changes that may have occurred in the outer dry skin of the onion bulb were not observed in this study.

Mucosal uptake of simple phenolic acids has been shown to be dependent on the existence of a Na^+ dependent saturable transport mechanism (Wolfram et al., 1995), but the conjugates of quercetin, naturally present in fried onion, have also been demonstrated to be preferentially absorbed in the human small intestine and in contrast to the lower level of absorption observed for their aglycon, quercetin (Hollman et al., 1995). There is little information regarding the mechanism of bioavailability, in terms of absorption in the small intestine, of these conjugated compounds, and their transport across the intestinal wall is not currently understood.

This work demonstrates that there is little gross change in either the overall level or the composition of quercetin glucosides during normal commercial storage. Both forms of cooking commonly used in the U.K. for mature onion bulbs also do not result in gross changes in quercetin glucoside composition, although an overall loss of up to 25% is found for both processes, in the former by leaching into the cooking water and in the latter by thermal degradation into products which are, as yet, unidentified.

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