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Quercetin and Isorhamnetin in Sweet and Red Cultivars of Onion (*Allium cepa* L.) at Harvest, after Field Curing, Heat Treatment, and Storage

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Effects of heat treatment and storage on quercetin and isorhamnetin content, major and minor components of isorhamnetin, and quercetin glucosides and aglycone, were investigated in onion (*Allium cepa* L.). The sweet onion 'Recorra' and red onions 'Hyred' and 'Red Baron' were cultivated in the south part of Norway and thereafter stored for eight months. The onions were either not field dried, but stored directly, or field dried and then stored, or field dried and then heat treated before storage. Neither storage nor heat treatment caused any major differences in total flavonol content in the investigated sweet onion as well as in the red onion cultivars. The two major quercetin glucosides differed in their changes in content during storage; quercetin-4′-glucoside did not show any consistent changes during storage in the two red cultivars, independent of treatment, whereas quercetin-3,4′-diglucoside increased significantly by 30 or 51%, respectively, during storage in 'Hyred' and 'Red Baron' in the 24 h heat treated onions. Isorhamnetin-4′-glucoside, which might possibly be of special interest from a human health point of view, was present at 2−3 times higher amount in the sweet onion cultivar than in the two red cultivars. Some of the quercetin glucosides present at lower concentrations, isorhamnetin-3,4′-diglucoside, quercetin-3,7,4′-triglucoside, and quercetin-7,4′-diglucoside, increased during storage in all treatments in both 'Hyred' and 'Red Baron', though sometimes a decrease was found at the end of storage.

KEYWORDS: Flavonol; quercetin; isorhamnetin; flavonoid; onion; heat treatment; curing; storage; *Allium cepa*; sweet onion; red onion; quercetin glucosides; drying; postharvest

INTRODUCTION

Onion (*Allium cepa* L.) is an important crop, grown in tropical, subtropical, and temperate climates. Epidemiological data have shown that a diet rich in fruits and vegetables decreases the risk of cardiovascular disease, diabetes, some forms of cancer, as well as other diseases (*1*). Onion contains a variety of phytochemicals, and as it is commonly consumed, it could be a valuable source of health promoting substances. Onion has been found to be associated with a lower risk for lung cancer and for esophageal and stomach cancer (*2*, *3*). Also in several animal models, onion has been shown to have beneficial health effects. In an experiment using pigs as a biomedical model, onion was found to reduce total blood cholesterol, low density lipoprotein, and triglycerides, which have been used as indicators of cardiovascular disease risk (*4*). Further, onion showed antithrombotic activity both *in vitro* and *in vivo* in mice (*5*) and an antithrombotic effect in streptozotocin-induced diabetic rats (*6*).

Onions contain several groups of substances that have been suggested to have health promoting effects, though much attention has been focused on flavonoids and sulfur compounds, such as alk(en)yl cysteine sulphoxides.

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Flavonoids in onions, especially flavonols and anthocyanins (in red varieties), have been suggested to be important dietary antioxidants, as well as to have a role in prevention of cardiovascular disease and some forms of cancer (*7*). They have also been associated with age-related diseases and to be potential neuroprotectants (*8*). *In vitro* they are powerful antioxidants, acting to scavenge a wide range of free radicals, though their beneficial medical effects may also originate from other physiological activities (*9*).

Onions are one of the most important sources of dietary flavonoids, and the estimates of total intake of these antioxidants vary between 190 mg and 1 g/day (*10*, *11*). Quercetin glucosides are the most common forms found in onion, and they have been found in varying amounts and composition in yellow, white, and red onion cultivars (*12*). A range of flavonoids—flavonols, anthocyanins, and dihydroflavonols—have been found to be present in onion (*12*). The range of flavonols has been reported to be somewhat higher in red onion (415−1917 mg per kilogram of FW) than in yellow cultivars (270−1187 mg per kilogram FW) (*12*). In recent years more interest has been focused on sweet onion cultivars, due to their appealing sweeter and less pungent taste. The sweetest varieties of 15 cultivars of yellow onion were found to contain flavonols in the same range as that for the less

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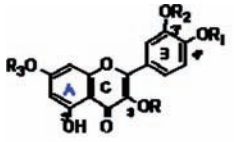

	R	R_1	R_2	R_3
1. Quercetin 3,7,4'-triglucoside	Glu	Glu	H	Glu
2. Quercetin 7,4'-diglucoside	H	Glu	H	Glu
3. Quercetin 3,4'-diglucoside	Glu	Glu	H	H
4. Isorhamnetin 3,4'-diglucoside	Glu	Glu	CH_3	H
5. Quercetin 3-glucoside	Glu	H	H	H
6. Quercetin 4'-glucoside	H	Glu	H	H
7. Isorhamnetin 4'-glucoside	H	Glu	CH_3	H

Figure 1. Structures of flavonols found in red and sweet onion.

sweet cultivars (*13*). Sweet onion is considered to have a shorter shelf life than nonsweet yellow and red cultivars due to higher water content, though methods for short-term storage have been developed (*14*). Heat treatment has been suggested to increase the storage potential in sweet onion cultivars (*15*, *16*). Different methods for curing and heat treatments have been tested (*17*), but so far no investigations of how such postharvest treatments and subsequent storage affect the content of flavonoids have been reported.

The content of flavonols in yellow onion (*18*, *19*) and in red onion cultivars (*20*, *21*) during field curing and storage of onion has been investigated. Storage was found to have no major effects on the flavonol content or composition in the onions, while the content increased during curing in the field (*18*). Though there is an increasing interest in both sweet and red cultivars, most investigations have so far concerned yellow cultivars. To the best of our knowledge, no previous investigation has presented more detailed compositional changes in red and sweet onion cultivars during harvest and after storage.

The aim of the present work was to investigate the effects of storage of onions after different field curing durations, with or without heat treatment, on the flavonol content and composition in two different red cultivars and one sweet onion cultivar.

MATERIALS AND METHODS

Location of Field Study and Cultivation. The field study was performed in 2006 at the Norwegian Institute for Agricultural and Environmental Research, Bioforsk Arable Crops Division Landvik, in Grimstad (58° 20′N) in the southernmost part of Norway. The onions were grown on a coarse sandy soil field (10% silt, 3% clay, 5% soil organic matter). Two red onion cultivars—'Hyred' and 'Red Baron'—and one sweet onion cultivar—'Recorra F1'—were used in the experiments. However, under the growing conditions at the experimental site in Norway, the cultivar 'Recorra' may be considered as semisweet (*25*). The field experiments had three replications, which each were planted as separate but adjacent blocks, with free randomization of cultivars within each block. The onions were planted from sets size 3 (15−21 mm), obtained from NORGRO, Norway. The setting date was May 22nd. The onions were set in rows, on beds set 1.6 m apart, with 3 rows on each bed, giving a plant density of 40 onions per m^2. The harvest was started at August 30th. The timing of lifting the onions from the ground was determined by the approximate number of onions with fallen leaves. The onions were lifted when 50% of the plants in the stands had fallen leaves.

A basal dressing of 89 kg N ha^{-1}, 55 kg P ha^{-1}, and 123 kg K ha^{-1} was applied before planting. In the beginning of June, there was an application of 39 kg N ha^{-1}, to make up for potential N losses due to heavy rainfall during the last part of May. In mid June, another 39 kg N ha^{-1} was applied, and finally, 14 kg N ha^{-1}, 12 kg P ha^{-1}, and 50 kg K ha^{-1} was applied during the first week of July. The first application at planting was broadcasted and incorporated into the soil, while the following ones were broadcasted as surface applications.

The mean daily temperature of the experimental period was 16.4 °C, and the average daily global radiation was 19.8 MJ m^{-2}. Soil moisture was kept at a nonlimiting level throughout the growing periods, as the crops were sprinkler irrigated when the estimated soil−water deficit reached about 0 mm.

Curing and Heat Treatment. After lifting, the onions were subjected to four different treatments. Treatment 1 (T1): the onions were harvested directly after lifting, dried at room temperature for 2 weeks, and stored at 2 °C, 70% RH. Treatment 2 (T2): after lifting the onions were field cured for 2 weeks, dried at room temperature for 2 weeks, and stored at 2 °C, 70% RH. Treatment 3 (T3): after lifting the onions were field cured for 2 weeks, dried at room temperature for 2 weeks, subjected to heat treatment at 36 °C 24 h, and stored at 2 °C, 70% RH. Treatment 4 (T4): after lifting, the onions were field cured for 2 weeks, dried at room temperature for 2 weeks, subjected to heat treatment at 36 °C 96 h, and stored at 2 °C, 70% RH.

Field curing for approximately 2 weeks after lifting is common practice in many countries. After this curing time, the onion bulbs had got papery outer scales, and the necks were partly dry and tight. Both the onions harvested directly after lifting and the onions left for field curing (T1 and T2) were transported to the site for analysis as fast as possible, and an aliquot of ten onions from each treatment 1 and 2 was extracted for further analysis as described below. The remaining onions from T1 and T2 were then left at room temperature for 2 weeks, before storage at 2 °C and 70% relative humidity. Aliquots of onions from T2 (field cured) of ten onions each were taken after the storage at room temperature to heat treatment either at 36 °C for 24 h (treatment 3; T3) or at 36 °C for 96 h (treatment 4; T4).

Chemicals. All solvents were of HPLC grade, Lichrosolv, and purchased from Merck, Germany, except for ethanol 99.7%, pa, which was from SOLVECO, Sweden. The external standards used for identification and quantification were quercetin (Sigma-Aldrich Chemie Gmbh, Germany), quercetin 4′-glucoside, quercetin-3-glucoside, and quercetin, 3,4′-diglucoside (Spiraeoside) (Extrasynthese, France), and other chemicals were purchased from Merck, Germany.

Extraction Method. Each onion sample consisted of 6−7 onions from each experimental plot. To mimic domestic peeling, the roots, the leaves, and the outer dry skins were removed. Each onion was divided longitudinally from the top to the base into 4 pieces. Two opposite pieces from each onion were chopped and homogenized in a Waring blender (Waring Products, Inc., USA). Four portions of each onion sample, each comprising 5.00 g of homogenized onion tissue, were extracted for 2 weeks at −20 °C in 20 mL of acidified (150 mM HCl) 99.7% ethanol. The extracts were centrifuged at 16 500*g* for 10 min at 4 °C and put into Eppendorf tubes and stored at −20 °C until analysis. Before HPLC analysis, the Eppendorf tubes were thawed and centrifuged at 16 500*g* for 5 min at 20 °C.

HPLC Analysis Method. The analyses of the onion extracts were performed as in ref *30*. In short, the samples were analyzed on an Agilent 1100 HPLC system. The column used was a Phenomenex Luna 5 μm C18 (2) (150 mm × 4.6 mm, 5 μm). The mobile phase consisted of (A) 50 mM acetic acid (HAc) in Millipore Ultrapure water with 5% acetonitrile and (B) acetonitrile with 5% methanol. The flow rate was 1.0 mL min^{-1}, and the injection volume was 10 μL. The binary gradient used was as follows: 0−2 min, 0% eluent B; 2−17 min, 0−45% B; 17−20 min, 45−80% B; 20−21 min, 80% B; 21−23 min, 80−0% B; 23−25 min, 0% B. The absorbance was measured at 370 nm using an Agilent 1100 (G1315B) diode array detector (Agilent Technologies, USA). Identification of the quercetin and isorhamnetin glucosides was based on spectra, standards, and literature data. External standards were used for identification and quantification, and results are presented as micrograms of quercetin 3,4′-glucoside per gram of dry weight, micrograms of quercetin 3′-glucoside per gram of dry weight, micrograms of quercetin 4′-glucoside per gram of dry weight, and micrograms of quercetin (aglycone) per gram of dry weight. The data were first calculated considering their actual molecular weight and then calculated to be expressed in μg/g DW. The quercetin or isorhamnetin glucosides tentatively identified—quercetin-3,7,4′-glucoside, quercetin-7,4′-glucoside, isorhamnetin-3,4′-glucoside, and isorhamnetin-4′-glucoside—were expressed as quercetin 4′-glucoside per gram of dry weight.

Validation of the Method. The within-laboratory reproducibility (day-to-day precision) was analyzed during a long-term investigation,

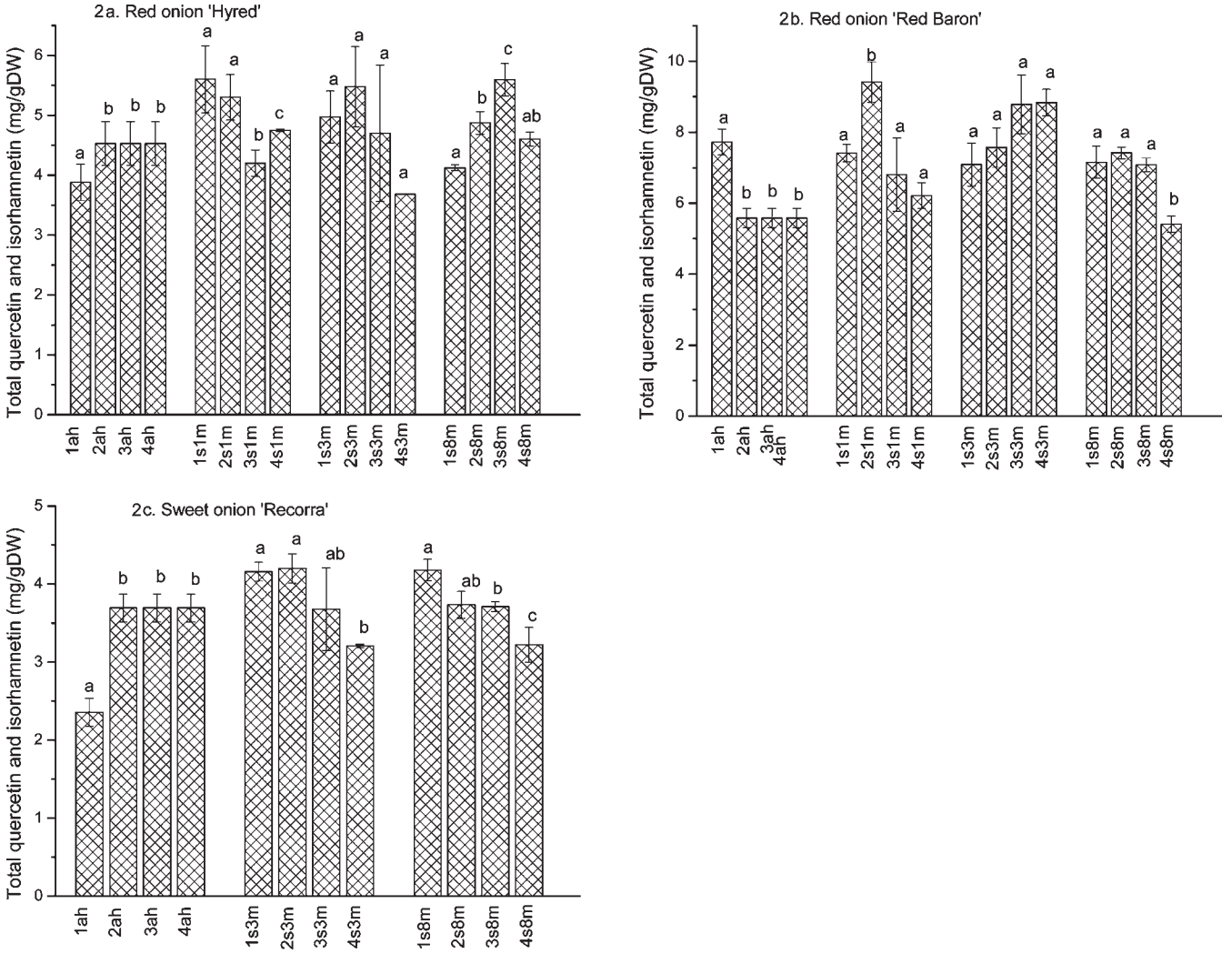

Figure 2. (**a**) Content of total quercetin and isorhamnetin in red onion 'Hyred', treatments 1−4 (T1−T4), at harvest and during storage: 1ah, treatment 1 after harvest; 1s1m, treatment 1 after storage for one month; 1s3m, treatment 1 after storage for three months; 1s8m, treatment 1 after storage for eight months; etc. Values are mean ± standard deviation (mg/gDW). Values denoted with the same letter within the same storage time (comparison between treatments, within a storage time) are not statistically significant at $p < 0.05$. (**b**) Content of total quercetin and isorhamnetin in red onion, 'Red Baron', treatments 1−4 (T1−T4), at harvest and during storage: 1ah, treatment 1 after harvest; 1s1m, treatment 1 after storage for one month; 1s3m, treatment 1 after storage for three months; 1s8m, treatment 1 after storage for eight months; etc. Values are mean ± standard deviation (mg/gDW). Values denoted with the same letter within the same storage time (comparison between treatments, within a storage time) are not statistically significant at $p < 0.05$. (**c**) Content of total quercetin and isorhamnetin in sweet onion, 'Recorra' treatments 1−4 (T1−T4), at harvest and during storage. 1ah: treatment 1 after harvest; 1s1m: treatment 1 after storage for one month; 1s3m: treatment 1 after storage for three months; 1s8m: treatment 1 after storage for eight months; etc. Values are mean ± standard deviation; (mg/gDW). Values denoted with the same letter within the same storage time (comparison between treatments, within a storage time) are not statistically significant at $p < 0.05$.

where analyses were performed 4 times within 8 months, and each time with 5−6 replicates. In addition, a short-term experiment was performed with two analysis times during 12 days. The within laboratory repeatability (within day precision) was analyzed during 3 different days within one day, and each day 5 times. Onion was used as plant material.

Extraction validation was performed by measuring the recovery over time of quercetin glucosides during the extraction time using acidified (150 mM HCl) 99.7% ethanol. The extraction efficiency was tested for methanol, ethanol, and mixtures of these, and also with HCl in different concentrations added.

Dry Matter Analyses and Visual Quality Assessment. Two portions of approximately 10 g of homogenized onion sample from each plot were placed in aluminum cups and dried at 70 °C for 24 h, followed by 105 °C for 1 h, and the dry matter content was calculated by dividing the weight of the dried samples with the initial weight of the fresh samples. The onions were visually inspected during storage, and onions showing rot or mold infection were noted and discarded.

Statistical Analyses. The results from the analyses were statistically analyzed in Minitab Release 14.1 (Minitab Inc., USA). Results were subjected to ANOVA. Significance was determined at $p < 0.05$, and results reported were significantly different at this level unless otherwise stated.

RESULTS

Validation of the Method. The within-laboratory reproducibility (day-to-day precision) was analyzed, and the coefficients of variation (CV) for the reproducibility were 4.07% for the quercetin-3,4′-diglucoside and 3.62% for the quercetin-4′-glucoside. The within laboratory repeatability (within day precision) was analyzed, and the CV for the repeatability was 1.80% for the quercetin-3,4′-diglucoside and 1.20% for the quercetin-4-glucoside.

Extraction validation was performed by measuring the recovery over time of quercetin glucosides during the extraction time, and after three days of extraction in acidified (150 mM HCl) 99.7% ethanol, 95% of the total amount was extracted, and after 12 days no increase in extracted amount was noted. Extraction efficiency

was tested for methanol, ethanol, and mixtures of these, and also with HCl in different concentrations added. The recovery was found to be highest in ethanol 99.7% with HCl added at a concentration of 150 mM.

Red Onion: Total Flavonols and Major Quercetin Glucosides. In general, there were no large differences between treatments (T1−T4) in the content of total flavonols (**Figure 2a** and **b**). In 'Hyred' and 'Red Baron', there were no significant differences between treatments in the content of total flavonols after storage for 3 months nor after harvest in 'Hyred', but in 'Red Baron', the content was higher in the nonfield dried onions (T1) as compared to the field dried onions (T2−T4). The 96 h heat treated onions (T4) had lower content of total flavonols than the 24 h heat treated onions (T3) after eight months of storage in both 'Hyred' and 'Red Baron'.

In general, there were no large changes in the content of total flavonols in 'Hyred' or 'Red Baron' during storage (**Figure 2a** and **b**, significance denotations not shown). In 'Hyred', the content in the nonfield dried treatment (T1) increased initially during storage, but it had decreased after eight months of storage (**Figure 2a**). In contrast, no changes in the content of total flavonols were found in the field dried onions (T2, T3, and T4) after the first month of storage in 'Hyred', and in T2 the content did not change during the rest of the storage time. In the field dried onions with 24 h of heat treatment (T3), the total flavonols increased at the end of the storage time, whereas, in the field dried onions with 96 h of heat treatment (T4), the content decreased after three months of storage and then increased again.

In contrast to 'Hyred', in 'Red Baron', the content of total flavonols did not change in the nonfield dried onions (T1), but in all field dried onions (T2, T3, and T4), the content increased during storage (**Figure 2b**). In T2 the content increased after one month of storage, whereas in T3 and T4 the content increased after three months of storage and then decreased after eight months of storage.

Quercetin-4′-glucoside was present in the highest amounts, followed by quercetin-3,4′-diglucoside, in 'Red Baron', though the opposite was the case for 'Hyred'. The levels were higher in 'Red Baron' (range 2370−4690 μg/g DW; 2349−4431 μg/g DW) than in 'Hyred' (range 1484−2406 μg/g DW; 1940−3079 μg/g DW) (**Figure 3a** and **b**). The content of quercetin-4′-glucoside in the different treatments (T1−T4) did not show any consistent changes during storage in the two cultivars. In both 'Hyred' and 'Red Baron', the content in the nonfield dried onions, T1, had decreased after eight months of storage, but in 'Hyred' it first increased after one month of storage. In T2, the same pattern of an increase at the beginning of storage and a decrease after eight months of storage could be seen in both cultivars, though it was only significant in 'Red Baron'. In the heat treated 'Red Baron' onions (T3 and T4), quercetin-4′-glucoside first increased during storage and then decreased, while no such changes could be found in 'Hyred', though the content decreased during storage in T4. Quercetin-3,4′-diglucoside increased during storage in both 'Hyred' and 'Red Baron' in T3 and T4, though it had decreased again after eight months of storage in 'Red Baron'. No consistent pattern could be found in T1 and T2 (**Figure 3a** and **b**).

Red Onion: Minor Quercetin and Isorhamnetin Glucosides. For some of the minor components of flavonol glucosides in both 'Hyred' and 'Red Baron', an increase of the content could be found during storage, sometimes followed by a decrease (**Tables 1** and **2**). Quercetin-3,7,4′-triglucoside increased during storage in all treatments in both 'Hyred' and 'Red Baron'. Also, quercetin-7,4′-diglucoside increased during storage in all treatments in both 'Hyred' and 'Red Baron', but in 'Red Baron' the content decreased at the end of the storage time in the field dried onions

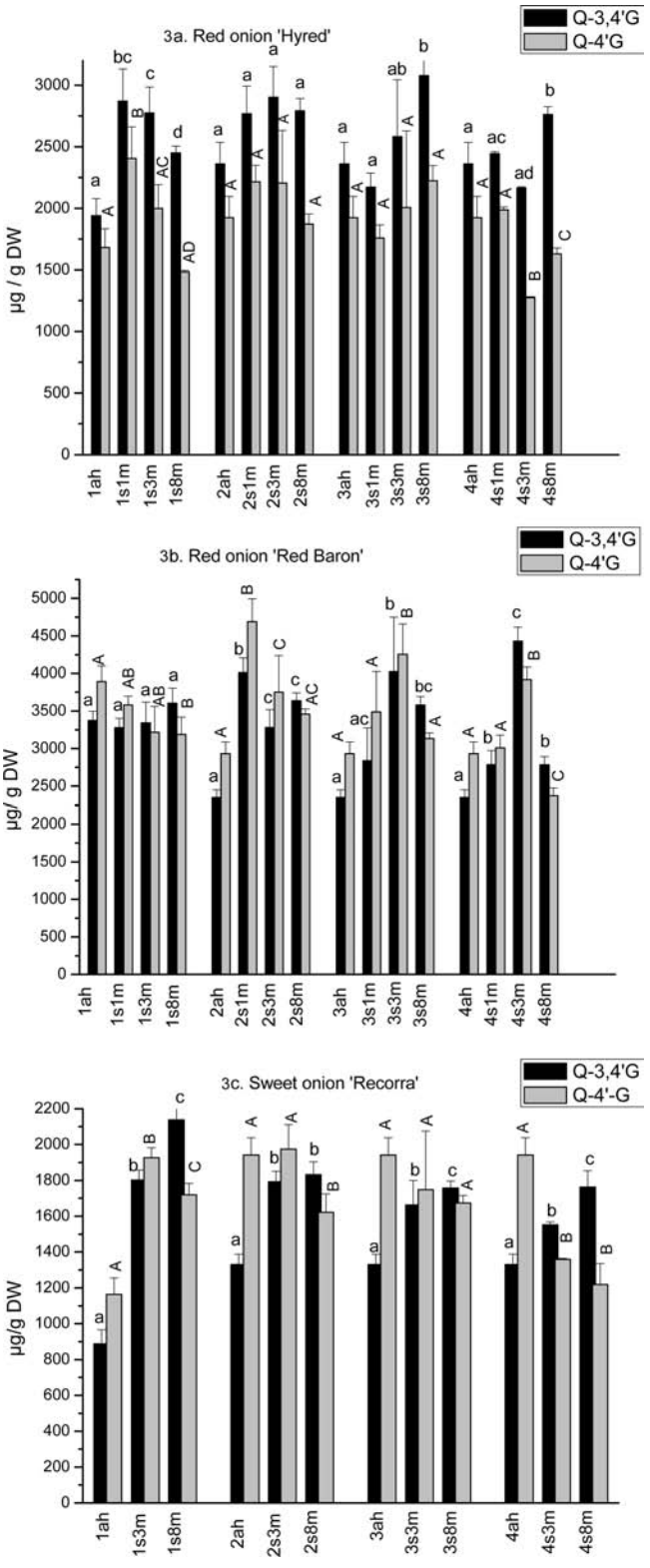

Figure 3. (**a**) Red onion 'Hyred', (**b**) red onion 'Red Baron', and (**c**) sweet onion 'Recorra' contents of the two major quercetin glucosides, quercetin-3,4′-diglucoside and quercetin-4′-glucoside, after harvest and during storage: 1ah, treatment 1 after harvest; 1s1m, treatment 1 after storage for one month; 1s3m, treatment 1 after storage for three months; 1s8m, treatment 1 after storage for eight months; etc. Values are mean ± standard deviation (mg/gDW). Values denoted with the same letter within the same treatment (1, 2, 3, or 4) (comparisons between storage times, within a treatment) are not statistically significant at $p < 0.05$.

Table 1. Red Onion 'Hyred' Content (μg/g DW) of Individual Quercetin or Isorhamnetin Glucosides, Minor Components, after Harvest and after Storage[a]

	quercetin and isorhamnetin glucosides, minor components					
treatment	Q-3,7,4′G[b]	Q-7,4′G[b]	IR-3,4′G[b]	Q-3G[c]	IR-4′G[b]	Q(agl)[c]
1ah	17.5 ± 0.6a	28.8 ± 2.3 a	29.4 ± 1.3 a	53.4 ± 4.4a	103.0 ± 5.3 a	24.2 ± 3.3a
1s1m	30.7 ± 2.6 bc	47.4 ± 5.3 bc	39.0 ± 3.6 b	58.7 ± 9.8 a	110.7 ± 16.6 a	39.7 ± 10.3 b
1s3m	33.5 ± 1.1 bd	43.5 ± 3.1 cd	40.8 ± 1.9 b	42.8 ± 4.0 a	95.6 ± 9.8 a	12.8 ± 3.7 a
1s8m	35.1 ± 1.4 cd	38.0 ± 1.1 d	nd	tr	109.3 ± 1.8 a	nd
2ah	19.5 ± 1.2 a	33.6 ± 2.4 a	31.2 ± 2.4 a	46.4 ± 4.9 a	100.8 ± 10.4 a	11.1 ± 1.7 a
2s1m	29.6 ± 2.5 b	47.8 ± 4.1 b	39.8 ± 3.2 ab	56.3 ± 4.5 a	89.6 ± 8.1 a	56.5 ± 2.6 b
2s3m	33.8 ± 2.2 b	46.0 ± 6.9 b	48.0 ± 9.2 b	45.4 ± 5.5 a	126.0 ± 35.9 a	21.3 ± 10.2 a
2s8m	45.1 ± 1.8 c	47.8 ± 1.2 b	nd	tr	109.0 ± 7.2 a	nd
3ah	19.5 ± 1.2 a	33.6 ± 2.4 a	31.2 ± 2.4 a	46.4 ± 4.9 b	100.8 ± 10.3 a	11.1 ± 1.7 a
3s1m	25.1 ± 0.8 b	31.8 ± 1.6 ab	37.9 ± 1.8 b	54.4 ± 2.4 b	97.7 ± 5.8 a	25.5 ± 13.6 a
3s3m	34.0 ± 2.4 c	42.5 ± 8.2 ac	49.6 ± 3.2 c	41.1 ± 5.2 bc	128.7 ± 26.2 a	13.1 ± 5.8 a
3s8m	43.9 ± 2.0 d	51.3 ± 2.7 d	nd	36.4 ± 2.5 a	152.2 ± 7.5 b	tr
4ah	19.5 ± 1.2 a	33.6 ± 2.4 a	31.2 ± 2.4 a	46.4 ± 4.9 b	100.8 ± 10.4 a	11.1 ± 1.7 a
4s1m	29.5 ± 0.3 b	42.7 ± 1.4 b	56.6 ± 1.8 c	37.9 ± 2.5 a	130.8 ± 1.8 c	21.1 ± 3.7 b
4s3m	30.9 ± 0.4[d] b	32.4 ± 0.5[d] a	44.8 ± 0.2[d] b	36.6 ± 0.2[d] a	98.3 ± 0.8[d] a	nd[d]
4s8m	42.9 ± 1.4c	48.0 ± 1.1 c	nd	nd	121.8 ± 2.8 b	nd

[a] Values are the means of triplicate samples unless otherwise stated ± standard deviation. Q-3.7,4′G, Q-7,4′G, IR-3,4′G, and IR-4′G are expressed as Q-4′G. [b] Tentatively identified by spectral data and the literature. [c] Identified by reference standards. [d] Values are the means of duplicate samples. For each compound, values denoted with the same letter within the same treatment (1, 2, 3, or 4) are not statistically significant. Abbreviations: tr, traces; 1ah, treatment 1 after harvest; 1s1m, treatment 1 after storage for one month; 1s3m, treatment 1 after storage for three months; 1s8m, treatment 1 after storage for eight months; etc. Q-3,7,4′G, quercetin-3,7,4′-triglucoside (1); Q-7,4′G, quercetin-7,4′-diglucoside (2); IR-3,4′G, isorhamnetin-3,4′-diglucoside (4); Q-3G, quercetin-3-glucoside (5); IR-4′G, isorhamnetin-4′-glucoside (7); Q(agl), quecetin (aglycone); numbers in parentheses refer to structures shown in **Figure 1**.

Table 2. Red Onion 'Red Baron' Content (μg/g DW) of Individual Quercetin or Isorhamnetin Glucosides, Minor Components, after Harvest and after Storage[a]

	quercetin and isorhamnetin glucosides, minor components					
treatment	Q-3,7,4′G[b]	Q-7,4′G[b]	IR-3,4′G[b]	Q-3G[c]	IR-4′G[b]	*Q*(agl)[b]
1ah	24.0 ± 1.4 a	50.1 ± 3.2 a	23.9 ± 0.7[d] a	195.9 ± 8.8 d	111.6 ± 4.6 a	65.8 ± 43.5 a
1s1m	30.2 ± 1.8 b	56.0 ± 2.5 ac	42.5 ± 1.2 b	130.3 ± 4.2 c	166.4 ± 7.2 c	130.9 ± 16.9 b
1s3m	42.2 ± 3.5 c	68.4 ± 6.5 b	65.3 ± 7.2 c	108.5 ± 10.2 ab	143.3 ± 12.5 b	59.3 ± 7.8 a
1s8m	53.2 ± 3.0 d	63.2 ± 2.9 bc	nd	92.3 ± 7.0 a	123.9 ± 8.8 a	31.2 ± 2.9 a
2ah	16.6 ± 0.4 a	35.3 ± 1.5 a	nd	100.5 ± 6.0 ab	122.9 ± 5.0 a	23.3 ± 9.1 a
2s1m	33.6 ± 3.0 bc	71.8 ± 5.6 c	90.2 ± 23.5 a	163.2 ± 16.8 c	202.8 ± 19.8 b	151.6 ± 24.9 c
2s3m	35.2 ± 7.4 c	64.5 ± 6.8 c	70.0 ± 8.6 a	120.5 ± 9.3 b	135.7 ± 24.6 a	77.5 ± 16.7 b
2s8m	43.9 ± 1.3 d	55.1 ± 1.0 b	nd	94.2 ± 3.3 a	121.7 ± 6.8 a	tr
3ah	16.6 ± 0.4 a	35.3 ± 1.5 a	nd	100.5 ± 6.0 a	122.9 ± 5.0 a	23.3 ± 9.1 a
3s1m	24.1 ± 5.0 ab	50.2 ± 7.6 ab	75.1 ± 16.7 a	109.5 ± 12.2 a	160.6 ± 20.8 b	55.9 ± 9.0 a
3s3m	39.4 ± 12.8 c	74.3 ± 13.5 c	83.4 ± 10.5 a	109.3 ± 34.1 a	138.3 ± 12.0 ab	105.5 ± 42.7 b
3s8m	44.0 ± 1.2[d] c	59.5 ± 1.0 b	nd	98.1 ± 2.4 a	145.3 ± 4.4 ab	39.7 ± 3.9 a
4ah	16.6 ± 0.4 a	35.3 ± 1.5 a	nd	100.5 ± 6.0 c	122.9 ± 5.0 b	23.1 ± 9.1 a
4s1m	32.1 ± 3.4 ab	49.0 ± 4.4 b	57.3 ± 5.0 a	108.8 ± 3.9 c	135.2 ± 10.0 b	32.7 ± 1.5 a
4s3m	53.1 ± 2.0 b	83.0 ± 2.3 c	81.2 ± 3.7 b	76.8 ± 4.3 a	135.7 ± 8.2 b	55.3 ± 2.4 b
4s8m	tr	50.2 ± 2.0 b	nd	88.0 ± 3.9 b	102.8 ± 4.8 a	nd

[a] See footnote in **Table 1**. [b] Tentatively identified by spectral data and the literature. [c] Identified by reference standards. [d] See footnote in **Table 1**.

(T2−T4). Isorhamnetin-3,4′-diglucoside increased during storage in all treatments in both 'Hyred' and 'Red Baron', but it could not be detected at the end of the storage time in both cultivars, nor was it detected at harvest of the field tried onions (T2−T4) in 'Red Baron'. The content of quercetin-3-glucoside was 2- to 3-fold higher in 'Red Baron' than in 'Hyred'. In 'Hyred' the content of quercetin-3-glucoside decreased at the end of the storage time, whereas no consistent changes could be found in 'Red Baron'. Also for isorhamnetin-4′-glucoside and quercetin aglycone the content was higher in 'Red Baron' than in 'Hyred'. In 'Red Baron' an initial increase of the content of isorhamnetin-4′-glucoside during storage was found in T1−T3, though in T4 the content decreased after eight months of storage. Isorhamnetin-3,4′-diglucoside initially increased and then decreased during storage in 'Red Baron' in T1−T3, while no changes in 'Hyred' in T1−T3 could be found during storage. For quercetin aglycone, in both 'Hyred' and 'Red Baron', an increase of the content was found initially during storage, followed by a decrease, and it was not detected in any of the treatments after eight months of storage in 'Hyred'.

Sweet Onion: Total Flavonols and Major Quercetin Glucosides. In general, there were no large differences between treatments (T1−T4) in the content of total flavonols, with the exception of the lower content of the nonfield dried onions, T1, directly after harvest (**Figure 2**). The content of the heat treated onions, T3 and T4, was lower after eight months of storage than that of the onions without heat treatments, T1 and T2.

Table 3. Sweet Onion 'Recorra' Content (μg/g DW) of Individual Quercetin or Isorhamnetin Glucosides, Minor Components, after Harvest and after Storage[a]

	quercetin and isorhamnetin glucosides, minor components					
treatment	Q-3,7,4′G[b]	Q-7,4′G[b]	IR-3,4′G[b]	Q-3G[c]	IR-4′G[b]	Q(agl)[c]
1ah	nd	13.0 ± 1.0 a	25.8 ± 1.2 a	41.0 ± 2.6 a	206.5 ± 9.9 a	19.1 ± 5.3 a
1s3m	nd	27.4 ± 1.6 b	52.6 ± 2.2 b	67.9 ± 0.5 b	260.1 ± 4.4 c	41.3 ± 9.6 a[d]
1s8m	nd	nd	nd	75.9 ± 3.3 c	238.2 ± 9.5 b	tr
2ah	nd	19.4 ± 0.6 a	32.0 ± 1.4 a	65.6 ± 3.5 a	287.6 ± 18.4 b	18.1 ± 5.1 a
2s3m	16.6 ± 0.4	29.4 ± 0.8 b	48.1 ± 0.5 b	63.2 ± 4.6 a	258.4 ± 10.1 b	41.9 ± 11.9 b
2s8m	nd	nd	nd	64.8 ± 2.2 a	215.6 ± 13.5 a	nd
3ah	nd	19.4 ± 0.6 a	32.0 ± 1.4 a	65.6 ± 3.5 b	287.6 ± 18.4 b	18.1 ± 5.1 a
3s3m	tr	27.6 ± 2.1 b	46.4 ± 0.6 b	52.4 ± 7.6 a	219.4 ± 54.2 a	32.8 ± 1.8 b
3s8m	nd	nd	nd	55.4 ± 2.4 a	223.0 ± 4.3 a	nd
4ah	nd	19.4 ± 0.6 a	32.0 ± 1.4 a	65.6 ± 3.5 b	287.6 ± 18.4 b	18.1 ± 5.1
4s3m	18.7 ± 0.5	25.6 ± 0.2 b	45.8 ± 0.5 b	45.8 ± 0.3 a	157.9 ± 1.1 a	nd
4s8m	nd	nd	nd	58.6 ± 5.1 b	181.1 ± 11.8 a	nd

[a] See footnote in **Table 1**. [b] Tentatively identified by spectral data and the literature. [c] Identified by reference standards. [d] $p = 0.06$.

In general, there were no large changes of total flavonol content in sweet onion 'Recorra' during storage. As in 'Hyred', the content of total flavonols increased in the nonfield dried onions 'Recorra' (T1) initially during storage but did not change thereafter (**Figure 2c**, significance denotations not shown). As in 'Red Baron', the content of total flavonols in T2 increased in 'Recorra' after three months of storage and then decreased after eight months of storage. The content of total flavonols in 'Recorra' did not change during storage in T3 but decreased during storage in T4.

Quercetin-4′-glucoside was present in the highest amounts in 'Recorra' (range 1219.3−1974.7 μg/g DW), and quercetin-3,4′-diglucoside was present in the second largest amounts in all treatments (range 889−2139 μg/g DW) (**Figure 3c**). Quercetin-4′-glucoside decreased during storage in T2 and T4, but the amount increased in T1 during storage as compared to directly after harvest. The lowest values after storage were found in the 96 h heat-treated onions (T4). Quercetin-3,4′-diglucoside increased after harvest during storage in all treatments. In T1, T3, and T4 it increased until after storage for eight months, and in T2, it increased until storage for three months and then no change occurred. The highest amount, 2139 μg/g DW, was found in T1 after eight months of storage.

Sweet Onion: Minor Quercetin and Isorhamnetin Glucosides. In general, of the minor components of flavonol glucosides, there were higher amounts of isorhamnetin-4′-glucoside and lower of quercetin-3,7,4′-triglucoside and quercetin-7,4′-diglucoside in sweet onion 'Recorra' than in the investigated red cultivars (**Table 3**). Quercetin-3,7,4′-triglucoside was only detected in 'Recorra' in low amounts in T2, T3, and T4 after storage for three months. Quercetin-7,4′-diglucoside, isorhamnetin-3,4′-diglucoside, and quercetin aglycone ($p = 0.06$ in T1) increased after three months of storage, and they were not detected after eight months of storage in any of the treatments (T1−T4, traces of quercetin aglycone in T4). In all treatments with field dried onions, T2−T4, isorhamnetin-4′-glucoside decreased during storage.

Red and Sweet Onion: Dry Matter and Visual Quality. There were no large differences in dry matter content between the different treatments of the red onions (all values not shown). The nonfield dried, nonheated onions (T1) of 'Hyred' and 'Red Baron' had somewhat lower dry matter content (average content after harvest and all storage times 13.64 and 12.99%, respectively), as compared with the field dried onions (T2−T4; 14.63 and 13.32%, respectively). The heat treatments resulted in minor changes in the dry matter content. The field dried onions of 'Hyred' and 'Red Baron', T2, had somewhat lower dry matter content (average content after harvest and all storage times 14.32 and 13.21%, respectively) as compared with the heat treated T3 (14.54 and 13.47, respectively) or T4 (14.99 and 13.32, respectively).

The sweet onion 'Recorra' had on average lower dry matter content than the red onion cultivars 'Hyred' and 'Red Baron' (average of all treatments, after harvest and all storage times 10.44, 13.82, and 13.23%, respectively). There were no large differences in dry matter content between the different treatments (all values not shown). The nonfield dried onions (T1) of 'Recorra' had somewhat lower dry matter content (average content after harvest and all storage times 9.95%), as compared with the field dried onions (T2−T4; 10.63%). The heat treatments resulted in minor changes in the dry matter content. The field dried onions, T2, had somewhat lower dry matter content (average content after harvest and all storage times; 10.47%) as compared with the heat treated T4 (10.89%), but the dry matter content of T3 was not significantly different.

The heat treatments did not result in any changes in visual quality during storage, nor were any differences found in this investigation between the heat treated onions (T3 and T4) and the nonheat-treated onions (T1 and T2) in amount of discarded onions due to rot or mold during storage (values not shown). There were very few onions discarded during the whole storage period, independent of treatment or cultivar.

DISCUSSION

In other crops, heating has been used to reduce the incidence of pathogen infection and to modify the response to different types of stress, and to maintain quality during storage (*22*), and curing of sweet onion for three days at 40 °C has previously been found to increase the storability of the onions (*23*). In this investigation, no differences were found between the heat treated onions (T3 and T4) and the non-heat-treated onions (T1 and T2) in amount of discarded onions due to rot or mold during storage. The conditions at harvest, and the growing conditions during the season when the experiment was conducted, might have been unfavorable to pathogen infection, resulting in good keeping quality during the whole storage period, as these factors have been suggested to affect the storability of the onions (*24*, *25*). It

cannot be excluded that another season, resulting in more quality problems during storage, may have given more differences between treatments. The sweet onion 'Recorra' had somewhat lower dry matter content, in accordance with previous investigations, and this has been suggested as a cause of a lower storability (*26*). However, the sweet onions in this investigation had good storability. In another investigation, using the same cultivar at the same growing site, the sugar content was found to be in the lower range (*13*) in comparison with sweet onions grown at sites with a warmer climate (*27*). This could be due to the growing conditions at the experimental site in Norway, and the lower sugar content may be a cause of better storability.

Previous investigations have shown that concentrations of flavonols in onion may to some extent be affected by processing (*28*), and this may at least partly be due to degradation in connection to heating. At the more moderate temperatures of the heating treatments used in this investigation, 36 °C, in general, no negative effects of heating in the content of total flavonols in 'Hyred', 'Red Baron', and 'Recorra' could be found, with the exception of a lower content in the onions heated for the longest time, T4, after eight months in all the investigated cultivars (in 'Recorra' also after three months). At this time of storage, this reduction in the content could not be due to degradation of quercetin glucosides directly at heating, but possibly rather could occur as a consequence of the heating treatments affecting metabolism in the onions unfavorably. Heating is known to induce 'heat shock proteins', and the heating may result in different stress reactions involving reactive oxygen species in the heat stress signaling (*29*), which in this case may have affected the content of total flavonols negatively after storage. To the best of our knowledge, no studies have been published previously of how heat treatment/curing at these moderate temperatures affects flavonol glucoside content during storage.

This investigation confirms that quercetin-4′-glucoside and quercetin-3,4′-diglucoside were the two major flavonols in onion, as previously has been found in several investigations (e.g. refs *18*−*20*). The concentrations of these quercetin glucosides are in accordance with previous investigations (*13*, *30*). Also the content of some of the quercetin glucosides present in lower amounts (quercetin-3,7,4′-triglucoside, quercetin-7,4′-diglucoside, and quercetin-3-glucoside) confirms earlier studies (*31*). The content of isorhamnetin-3,4′-diglucoside and isorhamnetin-4′glucoside was in accordance with the results of ref *28*, though ref *31* reported up to 10-fold, and 2- to 3-fold higher content, respectively.

In general the flavonol glucosides have been found to possess good stability during storage (*18*−*20*), and decreased content of quercetin-4′-glucoside and quercetin-3,4′-diglucoside in two yellow onion cultivars was found during storage at variable storage temperatures in a commercial store (*19*). In this investigation the analyzed flavonols were in general stable during storage under all treatments, though sometimes a reduction could be found at the end of storage, especially for some of the minor components, which at this time were below the detection level.

Onion flavonols are comparably well absorbed in humans (*32*), and bioavailability has been found to be the same for both of the major quercetin glucosides in onion, quercetin-4′-glucoside, and quercetin-3,4′-diglucoside (*33*). Furthermore, in animal models, the distribution of the different metabolites was found to be unique for the different target tissues (*34*). As knowledge increases of the differential effects of the individual flavonols in human tissues and organs, and of the specific bioactivity of individual flavonol glucoside species, it also becomes increasingly important to understand how the content in the onions of these glucosides is affected by cultivation practice and postharvest handling. Isorhamnetin has been proposed to have specific human bioactivity and several physiological functions. In one investigation, isorhamnetin showed an antitumor effect, both *in vitro* and *in vivo* in mice (*35*), and in another study, isorhamnetin permeated the cell membrane into the cell and showed cytotoxicity against hepatocellular human carcinoma cells (*36*). Individual isorhamnetin glucoside species were shown to have different preventive activity against liver injury in mice. Isorhamnetin 3-glucoside showed preventive activity whereas isorhamnetin 3,7-diglucoside did not, indicating a structure−activity relationship (*37*). Further, coadministration of quercetin and isorhamnetin was found to increase permeability and absorption, and they were found to reduce the secretion permeation of each other in Caco-2 cells. The permeability ratio was increased by 4.3 and 2.2 times, respectively. Also, the absorption of a single dose of quercetin and isorhamnetin was increased in rats, if they were administered together (*38*). Methylation is a common pathway of flavonoid metabolism in animals, and the primary metabolite of quercetin was found in one study to be isorhamnetin (3-*O*-methyl-quercetin), with tamarixetin (4-*O*-methyl-quercetin) as a secondary metabolite (*34*). However, in a study in rats, no transformation of isorhamnetin into quercetin or kaempherol occurred, indicating that demethylation of the 3′-oxymethyl group of isorhamnetin did not occur at absorption (*38*), and isorhamnetin 3-glucoside has also been suggested to be directly absorbed in mice without modification (*37*). Therefore, the content of isorhamnetin found in onion may be interesting from a human health point of view. In this investigation, the content of isorhamnetin glucosides was fairly low, comprising about 5−10% of total flavonols, but the content was in general relatively stable during storage, with the sweet onion cultivar 'Recorra' having a higher content. However, the longest heating treatment in this investigation, T4, caused decreasing content of isorhamnetin-4′-glucoside in red onion 'Red Baron' and sweet onion 'Recorra' after eight months of storage as compared with directly after harvest. Also, isorhamnetin-3,4′-diglucoside showed a lower stability during storage and was not detected in any of the treatments after eight months of storage.

In conclusion, in the investigated sweet onion as well as both red onion cultivars, neither storage nor heat treatment caused any major differences in total flavonol content. However, for some of the minor quercetin and isorhamnetin glucosides, both storage and heat treatment resulted in significant changes during storage. Isorhamnetin glucosides, which possibly might be of special interest from a health point of view, were present in a higher amount in the sweet onion cultivar than in the two red cultivars.

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