# AGRICULTURAL AND FOOD CHEMISTRY

### Quercetin Content in Field-Cured Onions (*Allium cepa* L.): Effects of Cultivar, Lifting Time, and Nitrogen Fertilizer Level

LARS M. MOGREN,\* MARIE E. OLSSON, AND ULLA E. GERTSSON

Department of Crop Science, Swedish University of Agricultural Sciences, P.O. Box 44, SE-230 53 Alnarp, Sweden

Variation in quercetin content was investigated in field-cured onions (*Allium cepa* L.) that had been supplied with different nitrogen fertilizer levels and lifted at different developmental stages. Quercetin content varied significantly between years and was well correlated to global radiation in August. Field curing generally resulted in significant increases in quercetin content compared to levels at lifting. Nitrogen fertilizer level did not affect quercetin content, suggesting that nitrogen leakage from soil may be minimized without effects on flavonol content. Lifting time had minor effects on quercetin content in field-cured onions. Cultivar differences in quercetin content were significant but not consistent in all years. Quercetin content increased significantly less in dark environments compared to field curing, but some quercetin synthesis occurred regardless of light. Field curing with or without foliage still attached did not affect quercetin content, suggesting that no transportation from the foliage to the scales occurred.

## KEYWORDS: *Allium cepa*; onion; quercetin glucosides; curing; drying; nitrogen fertilizer; lifting time; cultivar

#### INTRODUCTION

World onion production is currently  $\approx$ 44 million tonnes, making it the second most important horticultural crop after tomatoes (1). The common yellow onion (Allium cepa L.) belongs to the Lilliaceae family and is grown all over the world. Since antiquity, the onion has been traditionally used in folk medicine for its therapeutic action. Research directed at disease prevention rather than cure is currently attempting to produce onions with a high content of nutraceuticals (2). Reports of health benefits from onions include anticarcinogenic properties, antiplatelet activity, antithrombotic activity, and antiasthmatic and antibiotic effects (I). At present there is considerable debate about the specific components responsible for the beneficial health effects of onions. Two main groups of chemical compounds have been proposed: the flavor precursors alk(en)yl sulfoxides (ACSOs) and the flavonoids (1). Red-skinned varieties contain anthocyanins, and both red and yellow onions contain flavonols, mainly quercetin and its derivatives. The functions of flavonoids in plants include pigmentation (to attract pollinators), protection of the plant from UV light and microorganisms, defense against grazing animals, regulation of enzyme activity, and signal substances for nitrogen-fixing bacteria (3). In plants, flavonoids occur primarily as glycosides, with different sugar groups linked to one or more of the hydroxyl groups (4). Flavonoids are mainly found in the aboveground outer parts of plants, such as leaves, flowers, and fruit, whereas the content in stalks and roots is usually limited (5). The highest concentrations of flavonoids in onion are found in the outer dry scales, so the greatest loss of flavonoids takes place when the onions are peeled (6). The formation of flavonol glycosides normally depends on the action of light (7) and is induced by UV light (8). In onion, the main flavonol glycoside forms have been found to be quercetin 4'-monoglucoside and quercetin 3,4'diglucoside (9). Bioavailability of flavonols in the human body has been a controversial issue for a long time. The absorption from different foods seems to differ; for example, the absorption of quercetin forms from tea was found to be half that from onions (10), and the absorption rate from apples was one-third that from onions (11). Cooking, frying, or warm-holding of onions has only minor effects on the flavonoid content available (6), and the losses of onion flavonoids caused by gastric acid and intestinal juices seem to be low (12). It was long believed that flavonoid glycosides were not absorbed but hydrolyzed to their aglycones by bacterial enzymes in the intestine (13, 14), but later it was proposed that the flavonoid glycosides were in fact absorbed (15). However, in a recent review of the absorption and metabolism of flavonoids, it was concluded that a number of studies now support the view that quercetin glycosides are not absorbed intact in humans and are not able to reach the systemic circulation (16). Although the bioavailability of the aglycones formed after hydrolysis along the aerodigestive tract is considered to be low, conjugates formed during the metabolism of flavonoids, such as sulfate and glucuronic acid conjugates, have been found in relatively high concentrations. The biological importance of these metabolites and of degradation

<sup>\*</sup> Author to whom correspondence should be addressed (telephone +46 40 415363; fax +46 40462166; e-mail lars.mogren@vv.slu.se).

products formed during biological degradation of the flavonoid backbone remains to be elucidated (16).

Onions are a natural part of the daily diet for most of the world's population and, therefore, an important source of the potentially health-promoting compound quercetin and its derivatives. For example, in Denmark, quercetin was found to comprise 37% of the flavonoid intake, and 16% of the total flavonol and flavanone intake was from onions, which is equivalent to 10 g of onion per day (17). In Poland, onion has been ranked as the seventh most important fruit and vegetable source of polyphenolic compounds in the diet (18). In a Dutch study, tea and onions were found to be the most important sources of flavonoid consumption (19). Previous investigations have mainly analyzed quercetin content in onions bought at the local market (12, 20-27), and therefore information on how cultivation conditions and handling at harvest affect quercetin content is scarce. Reducing the leakage of nitrogen from the soil to the groundwater is an important goal of environmental care actions, but little is known about how reduced nitrogen fertilization affects the nutritional value and content of healthpromoting compounds of onions including the effect on quercetin. Late lifting generally results in higher yields, but the onions start to sprout earlier after long storage (28). After lifting, the common practice in Sweden is to leave the onions lying in the field for a couple of days to dry and cure in full sunlight, but sometimes forced air in dark environments is used instead. The aim of the present work was to investigate the role of nitrogen fertilizer level, lifting time, cultivar, and field curing in flavonol synthesis in the common yellow onion. Additional experiments were performed to investigate the role of curing with or without foliage and with or without light and variation in quercetin glucoside content between different onion scales.

#### MATERIALS AND METHODS

**Location of Field Study.** The field study was performed in 2002, 2004, and 2005 at Torslunda Research Station, Öland, in southeastern Sweden ( $56^{\circ}$  38' N,  $16^{\circ}$  30' E). Öland is one of Sweden's most important onion-growing districts. The soil at the site is a sand with a medium organic matter content (3%) and a low clay content.

**Growing Technique.** In 2002, insecticide-coated (carbamate type) seeds of two onion (*Allium cepa* L.) cultivars, cv. Barito F1 and cv. Summit F1, were sown. In 2004 and 2005, seeds of cv. Hyskin F1 without insecticide coating were also used. The seeds were sown directly in the field in the beginning of April each year. The rows were placed 0.5 m apart, and seed rate was  $\approx$ 39 seeds per row-meter. Pest and weed management was performed according to conventional commercial practices in Sweden. Water was supplied by irrigation as required on the basis of rain and evaporation data until the middle of July. In August the onions were lifted from the ground and left for field drying and curing in windrows for  $\approx$ 10 days before extraction of flavonoids.

**Experimental Design.** The experiment was carried out in five completely randomized blocks in 2002 and in four blocks in 2004 and 2005. In 2002 and 2004, each cultivar was grown with two different nitrogen fertilizer levels in each block as described below and lifted at two different times. In 2005, only one nitrogen fertilizer level was used in the whole experiment.

**Fertilizer Treatments.** Soil core samples (0-30 cm) were taken 2 days before sowing, and the N<sub>min</sub> content of each soil sample was analyzed as follows. Fresh soil samples were shaken with 2 M KCl for 2 h and then filtered. The content of mineralized nitrogen (ammonium and nitrate) was analyzed in these extracts by flow injection analysis (FIA). On the basis of these soil analyses and using the target value of 40 kg of N ha<sup>-1</sup> suggested by Gertsson and Bjorklund (29), nitrogen was supplied as NPK-micro. Additional phosphorus was supplied as PK, and additional potassium was supplied as KMg and KS, to ensure that there was sufficient sulfur in the soil. All fertilizers

were spread and harrowed down into the soil before sowing. In the beginning of June each year, new soil samples were taken in the same way as described above and analyzed. Low nitrogen fertilizer treatments were supplied with calcium nitrate on the basis of analysis of the content of mineralized nitrogen in the soil ( $N_{min}$ ) to a target value for June of 72 kg of N ha<sup>-1</sup>. The high nitrogen fertilizer treatments received 80 kg of N ha<sup>-1</sup> more than the low levels each year and are comparable to the nitrogen level used by many commercial growers.

**Lifting Times.** In August the onions were lifted from the ground at different stages determined by the approximate number of onions with fallen leaves. In 2002, 2004, and 2005 onions were lifted when 50% (early lifting) or 80% (late lifting) of the plants in the stands had fallen leaves.

**Curing Times.** After lifting, the onions were left on the field in windrows to dry and cure for 10-14 days, which is still common practice in many countries. After this time, the onion bulbs had developed papery outer scales and the necks were dry and tight.

Extraction Method. Directly after completion of field curing, onions with a diameter of 55–70 mm and a weight of  $\approx 100$  g were chosen for extraction. Each onion sample consisted of 10 onions from each experimental plot. The roots, the leaves, and the outer dry skins were removed to mimic domestic peeling. Each onion was divided longitudinally from the top to the base into eight (2002) or four (2004 and 2005) wedge-shaped pieces. Two opposite pieces from each onion were chopped and homogenized in a Waring blender. Four portions (2002 and 2004) or three portions (2005) 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) 98% ethanol. This long extraction method was found to be convenient and resulted in good extraction efficiency and repeatability (unpublished data). The extracts were centrifuged at 16500g for 10 min at 4 °C, transferred into Eppendorf tubes, and stored at -20 °C until analysis. Before HPLC analysis, the Eppendorf tubes were thawed and centrifuged at 16500g for 5 min at 20 °C.

HPLC Analysis Method. The analyses of the onion extracts were performed on an Agilent 1100 HPLC system. The column used was a Phenomenex Luna 5u C18(2) ( $150 \times 4.6 \text{ mm}, 5 \mu \text{m}$ ). The mobile phase consisted of (A) 50 mM acetic acid (HAc) in Millipore ultrapure water with 5% acetonitrile (v/v) and (B) acetonitrile with 5% methanol (v/ v). The flow rate was 1.0 mL min<sup>-1</sup> and the injection volume 20  $\mu$ L (2002) or 10  $\mu$ L (2004 and 2005, respectively). 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. External standards used for identification and quantification were quercetin (Sigma-Aldrich Chemie Gmbh) and quercetin 4'-glucoside (Extrasynthese). The absorbance was measured at 370 nm using an Agilent 1100 (G1315B) diode array detector (Agilent Technologies). Results are presented as milligrams of quercetin 4'-glucoside equivalent per kilogram of fresh weight of onion (mg kg-1 of fw) for all forms of quercetin.

**Dry Matter Analyses.** Two portions of  $\approx 10$  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.

Extended Curing Experiment, 2005. In 2005, the effects of light, temperature, and relative humidity during curing were investigated more thoroughly. Besides ordinary field curing, three replicates, of 10 onions each, of the three cultivars were placed directly after lifting in one of five different environments: a tent covered with transparent plastic, a tent covered with dark nontransparent plastic, a stone wall shed, a climate chamber, or a sealed plastic carrier bag within the climate chamber. The tent was composed of a wooden structure  $\approx 1$  m high built on a concrete base. Half of it was covered with a transparent plastic film and the other half with two layers of black nontransparent plastic film. The transparent film reduced the UV light transmittance by 20% (measured by Philips TL 12, 4W, UV light lamp). The stone wall shed was completely dark, with ambient temperature (average = 15.7 °C) and high relative humidity (99%). The climate chamber was completely dark and had a higher average temperature (18.7 °C) and a lower relative humidity (75%). Within the climate chamber, onions were also placed in large plastic bags, which were sealed and not opened before extraction. Temperature and relative humidity were recorded with

**Table 1.** Temperature (T, °C) and Relative Humidity (RH, Percent) in the Different Curing Treatments, 2005

treatment	T <sub>mean</sub>	T <sub>max</sub>	T <sub>min</sub>	RH <sub>mean</sub>	RH <sub>max</sub>	$RH_{min}$
plastic bag in climate chamber	19.1	20.8	18.0	100		
climate chamber	18.7	19.9	17.7	75	86	62
stone wall shed	15.7	16.2	14.8	99	100	98
transparent plastic covering	23.2	42.0	13.1	66	98	25
dark plastic covering field curing	18.7 16.7	28.7 25.1	12.8 10.6	78 79	95 97	48 48

Tinytag Plus dataloggers (Gemini Data Loggers Ltd.). The mean temperature and mean relative humidity for each environment during the extended curing experiment in 2005 are summarized in **Table 1**. The tent covered with transparent plastic resulted in high daytime temperatures. The tent covered with dark plastic had almost the same conditions as normal field curing except for the absence of light. The stone wall shed had high relative humidity, resulting in slow drying and curing. The climate chamber had higher temperature but lower relative humidity compared to the stone wall shed, and this resulted in attractive looking, partly cured onions with brownish foliage. The onions put in sealed large plastic bags within the climate chamber had a fresh appearance, but the green foliage was greasy and had a strong unattractive smell. Due to condensation, the datalogger could not measure relative humidity in the sealed plastic bags.

**Extended Curing Experiment, 2003.** In 2003, Barito F1 onions were obtained from a commercial grower's field in the same geographical region as where the other experiments were performed. Onion samples were taken from different parts of the field at early and late stages (see Lifting Times) and analyzed as in 2002. In addition, onions with fallen and erect leaves from the same rows were collected once the leaves had just started to fall down. Wedge-shaped pieces were extracted after field curing as in 2002. In addition, the remaining scale parts of the same onions were separated into three portions, the two outer fleshy scales ("outer"), the next two scales ("middle"), and the rest of the scales ("inner"), and the portions were extracted and analyzed separately. To investigate the role of the foliage (the green leaves) for flavonol synthesis during curing, additional early- and late-lifted onions with the foliage cut off were left for field curing beside the other onions, which had their foliage still attached.

Weather Data. Weather data during all experimental years were recorded using an Adcon Telemetry (Adcon Telemetry Gmbh) weather station placed in the onion field.

**Statistical Analyses.** The results from the analyses were statistically analyzed in Minitab release 14.1 (Minitab Inc.). Results were subjected to ANOVA. If not stated, no interaction between factors was significant. Significance was determined at  $p \le 0.05$ , and results reported were significantly different at this level unless otherwise stated.

#### **RESULTS AND DISCUSSION**

No interactions were found between quercetin content in the onions after field curing and the factors nitrogen fertilizer level, lifting time, and cultivar within any of the years. Therefore, each factor was analyzed separately with the whole data material as a basis.

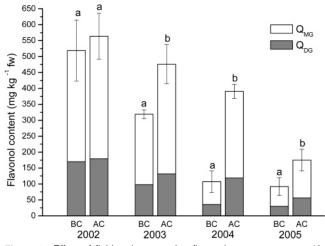
Weather Data and Quercetin Content. The mean levels of total quercetin after field curing differed significantly between the years (**Table 2**) with a total mean content for all years of 280 mg kg<sup>-1</sup> of fw. This level is comparable to Danish results showing yellow onion quercetin content to be 348 mg kg<sup>-1</sup> of fw (*30*). In Texas, total quercetin concentration (sum of quercetin glucosides) was found to range from 256 to 513 mg kg<sup>-1</sup> of fw and represented 88% of the total flavonol content (*31*).

The global radiation, measured as W m<sup>-2</sup>, during the bulbgrowing months of June–August was highest in the season of 2002. In 2003 it was somewhat lower, in 2004 it was even lower,

**Table 2.** Mean of Total Quercetin Glucosides,  $Q_{\text{mean}}$  (mg kg<sup>-1</sup> of fw) for Each Year, Global Radiation, R (W m<sup>-2</sup>), and Mean Temperature, T (°C), for Each of the Months When Onion Bulb Growth Occurs, and Total Global Radiation and Mean Temperature for the Whole Period from June through August 2002–2005 at Torslunda Research Station, Sweden

			Ju	June		July		August		mean
year	Q <sub>mean</sub> <sup>a</sup>	n	R	Т	R	Т	R	Т	R	Т
2003 2004	$\begin{array}{c} 564 \pm 72 \text{ a} \\ 475 \pm 61 \text{ b} \\ 391 \pm 70 \text{ c} \\ 175 \pm 34 \text{ d} \end{array}$	24 48	6788 6341	15.5 14.2	5842 5666	18.5 15.6	5550 5143	17.3 17.8	18180 17150	17.9 17.1 15.9 16.3

<sup>a</sup> Values are mean  $\pm$  standard deviation; values with the same letter are not significantly different at p < 0.05.



**Figure 1.** Effect of field curing on onion flavonol content:  $n_{2002} = 40$ ;  $n_{2003} = 24$ ;  $n_{2004} = 48$ ;  $n_{2005} = 24$ ;  $Q_{MG}$  = quercetin 4'-monoglucoside;  $Q_{DG}$  = quercetin 3,4'-diglucoside; BC = before curing; AC = after curing. Within each year bars with the same letter are not significantly different at p < 0.05.

and in 2005 the global radiation was lower than in 2002 but higher than 2003 (Table 2). The mean temperature was lowest in 2004 and highest in 2002 (Table 2). The total mean quercetin content for all treatments after field curing (Table 2) was well correlated to global radiation in August ( $R^2 = 0.83$ ), but, in an earlier study, the correlation was found to be even higher before field curing  $(R^2 = 0.98)$  (32). The correlation was much lower for July ( $R^2 = 0.34$ ) and for the whole onion growth period of June-August ( $R^2 = 0.07$ ). The correlation between mean temperature in August and content of quercetin glucosides was not as high as for global radiation  $(R^2 = 0.74)$ , and the correlation for the whole onion growth period of June-August was low ( $R^2 = 0.53$ ). In the year 2002, with high flavonol content at lifting, almost no increase in flavonol content occurred during field curing. In all other years, however, the content of both quercetin monoglucoside and quercetin diglucoside increased significantly during field curing (Figure 1), but never reached levels higher than in 2002.

Growing season temperature can explain some annual and regional variability in onion flavor (33). We did not analyze flavor, and no clear effect of growing temperature on flavonol content could be found in our experiment. It has been proposed that UV-B light is important for quercetin biosynthesis in onions. In experiments on harvested onions, it has been demonstrated that the content of quercetin almost doubles in onion slices irradiated with UV light. Three different UV lamps were used, and the effect was the same for all, leading to the conclusion

**Table 3.** Effect of N Fertilizer Level (Low, High), Lifting Time (Early, Late), and Cultivar (Barito, Summit, Hyskin) on Content (mg kg<sup>-1</sup> of fw) of Quercetin 3,4'-Diglucoside ( $Q_{DG}$ ), Quercetin 4'-Monoglucoside ( $Q_{MG}$ ), and Quercetin Aglycone (Q) in Onions with a Diameter of 55–70 mm after Field Curing<sup>a</sup>

year treatment			Q <sub>MG</sub>	Q	total	increase during curing (%)			
	п	n Q <sub>DG</sub>				Q <sub>DG</sub>	Q <sub>MG</sub>	total	
2002	low N high N	20 20	177 ± 22 a 180 ± 20 a	384 ± 54 a 387 ± 53 a	tr <sup>b</sup> tr	561 ± 74 a 567 ± 72 a	2 9	9 12	7 11
2004	low N high N	24 24	119 ± 21 a 119 ± 23 a	$278 \pm 44$ a $265 \pm 56$ a	tr tr	397 ± 63 a 384 ± 78 a	213 261	248 327	236 304
2002	early lifting late lifting	20 20	180 ± 22 a 177 ± 20 a	394 ± 57 a 377 ± 48 a	tr tr	$574 \pm 78$ a $554 \pm 67$ a	5 5	15 6	12 6
2003	early lifting late lifting	12 12	$147 \pm 18 \text{ a}$ 116 $\pm$ 13 b	$344 \pm 35$ a $344 \pm 58$ a	tr tr	491 ± 51 a 460 ± 69 a	50 18	76 40	67 34
2004	early lifting late lifting	24 24	$114 \pm 20$ a 124 $\pm$ 22 a	$270 \pm 50 \text{ a}$ $273 \pm 51 \text{ a}$	tr tr	384 ± 69 a 397 ± 72 a	235 235	291 274	273 261
2005	early lifting late lifting	12 12	$\begin{array}{c} 50\pm5 \text{ a} \\ 62\pm10 \text{ b} \end{array}$	106 ± 13 a 131 ± 27 b	tr tr	156 ± 18 a 194 ± 36 b	100 77	112 77	108 78
2002	Barito Summit	20 20	183 ± 23 a 174 ± 17 a	$404\pm53$ a $367\pm46$ b	tr tr	$587 \pm 75 \text{ a} \\ 540 \pm 62 \text{ b}$	12 0	26 0	22 0
2003	Barito	24	$132\pm23$	$344\pm47$	tr	$475\pm62$	35	56	49
2004	Barito Hyskin Summit	16 16 16	114 ± 13 a 142 ± 17 b 101 ± 10 c	$278 \pm 29 \text{ a}$ $318 \pm 30 \text{ b}$ $219 \pm 29 \text{ c}$	tr tr tr	392 ± 39 a 460 ± 45 b 320 ± 38 c	208 294 197	239 382 232	229 351 220
2005	Barito Hyskin Summit	8 8 8	53 ± 7 a 63 ± 11 a 52 ± 7 a	$116 \pm 19 \text{ ab}$ $135 \pm 29 \text{ b}$ $106 \pm 17 \text{ a}$	tr tr tr	169 ± 26 ab 198 ± 40 b 158 ± 23 a	89 91 79	97 99 80	94 96 80

<sup>a</sup> Values are mean  $\pm$  standard deviation. Values with the same letter in each column, in each treatment group and within each year, are not significantly different at *p* < 0.05. <sup>b</sup> Trace (<10 mg kg<sup>-1</sup> of fw).

that quercetin synthesis may respond to a wide range in the UV spectrum (34). In another study, exposure of leaves of *Brassica napus* to supplementary UV-B radiation resulted in an overall increase in the amount of soluble flavonoids of  $\approx 150\%$  compared to control plants, with a specific increase in the amount of quercetin glycosides of up to 36-fold (35). These findings support our conclusion that global radiation rather than mean temperature is the determining factor for quercetin glucoside biosynthesis in onions.

Fertilizer Level, Lifting Time, and Cultivar. It has previously been shown that nitrogen levels and temperature can influence flavor intensity and onion quality (36, 37). In the present study, no flavor studies were performed. A decrease in soil nitrogen concentration may be associated with an increase in total quercetin concentration (9), and a limited nitrogen supply has been associated with higher levels of phenolics in plant (8). However, in the present study, high or low levels of nitrogen fertilizers during growth did not result in differences in quercetin content after field curing in any of the years (Table 3). Furthermore, there were no significant differences in percentage of nonmarketable onions, onion size, or yield between the high and low nitrogen levels in any of the years. The nitrogen levels used in this study were normal or slightly low compared to conventional production, and therefore no conclusions can be drawn about extreme nitrogen levels. From the results we conclude that it may be possible to grow and field cure onions with commercial yields and equally high quercetin content with minimized nitrogen fertilization based on soil analysis. The risk of nitrogen leakage from the soil can thereby be reduced, which may have a positive impact on the environment.

Early lifting of onions (50% fallen leaves or less) postpones the onset of sprouting after long-term storage, but the yield is reduced (28). Early-lifted onions have been observed to exhibit lighter color (38), which could indicate a lower quercetin content. The levels of phenolics in plant tissues can be influenced by the maturity of the plant (8), but it has been proposed that growth stage is not important for determining quercetin concentration (39). In our field study, late lifting resulted in significantly higher contents of both quercetin diglucoside and quercitin monoglucoside after field curing in 2005, but not in the other years (**Table 3**). Our conclusion is that the time of lifting seems to be a minor determinant of quercetin content in field-cured onions.

It is generally stated that differences in flavonol content within a commodity need to be understood in terms of maturation and environmental effects before cultivar differences can be exploited (7). There are differences between resistant and susceptible cultivars in their polyphenol content, suggesting that these compounds play an important role in defense mechanisms (40). However, in our study no correlation was found between quercetin content and percentage of nonmarketable onions.

It has been found that onion cultivar differences in quercetin content can be high, up to 11-fold (41) The majority ( $\approx$ 90%) of the total flavonols have been shown to be confined to the epidermal tissue of each onion scale. This means that scale thickness or any other factor that alters the ratio of epidermal to storage tissue could indirectly affect gross flavonol concentration. Such factors may account for some inter- and intracultivar variability (42). In the present study, no visible differences in color or scale thickness were observed when the onion samples were being prepared for analysis. Quercetin monoglucoside and quercetin diglucoside have been reported to account for 93% of the total flavonoid content in the brown onion cv. Rijnsburger, with the diglucoside as the main component (9) and with only traces of free quercetin aglycone (43). We found that quercetin monoglucoside was the main onion flavonol component, in contrast to Price and Rhodes (9), who found higher contents of quercetin diglucoside. However, Lombard et al. (31) found that

**Table 4.** Content (mg kg<sup>-1</sup> of fw) of Quercetin 3,4'-Diglucoside (Q<sub>DG</sub>), Quercetin 4'-Monoglucoside (Q<sub>MG</sub>), and Quercetin Aglycone (Q) in Onions with a Diameter of 55–70 mm before and after Drying and Curing in Different Curing Treatments,  $2005^a$ 

treatment	Q <sub>DG</sub>	Q <sub>MG</sub>	Q	total
before treatment	31 ± 7 a	$63\pm19~\mathrm{ab}$	tr <sup>b</sup>	95 ± 26 a
plastic bag in climate chamber	27 ± 4 a	54 ± 11 ab	tr	81 ± 14 a
climate chamber	$43\pm8$ b	$81\pm17$ bc	tr	$124\pm25$ b
stonewall shed	$40\pm7$ b	$84\pm17~{ m bc}$	tr	$123\pm23$ b
transparent plastic covering	$45\pm8~{ m bc}$	$78\pm16~{ m bc}$	tr	$123\pm23$ b
dark plastic covering	$47\pm 6~bc$	88 ± 15 c	tr	$135\pm20$ b
field curing	$53\pm7$ c	117 ± 18 d	tr	$170\pm25~{ m c}$
-				

<sup>*a*</sup> Values are mean  $\pm$  standard deviation (n = 12). Values with the same letter in each column are not significantly different at p < 0.05. <sup>*b*</sup> Trace (<10 mg kg<sup>-1</sup> of fw).

the monoglucoside content in all cultivars analyzed was  $\geq 20\%$ higher than the quercetin diglucoside content. The mean content for golden onion cultivars in northern Italy was found to be 572 mg kg<sup>-1</sup> of fw quercetin monoglucoside compared to 49 mg kg $^{-1}$  of fw quercetin diglucoside (2). The different relative proportions of these quercetin glucoside forms stated in different reports and countries could be an effect of different cultivars, as well as result of differences in analysis technique. It has been found that maceration can lead to the loss of guercetin diglucoside and the appearance of quercetin monoglucoside and free quercetin aglycone (9). However, there are also reports that both quercetin diglucoside and quercetin monoglucoside in onions are virtually unaffected by chopping (44). Quercetin has been found to be formed by deglucosidation of quercetin glucosides on the border between drying and dried brown onion scale areas (23). In our study, all partly dried scales were removed to mimic domestic peeling, thereby reducing the risk for differences in deglucosidation.

In our study, differences in flavonol content between cultivars after field curing were significant in all years (**Table 3**). In 2002 and 2004, cv. Barito had higher flavonol levels than cv. Summit, but in 2005 no statistical difference could be found. In 2004, cv. Barito had significantly lower levels of flavonols than cv. Hyskin, but in 2005 the same tendency was not significant. In 2002 and 2005, the levels of quercetin diglucoside were the same in all cultivars, but in 2004 all cultivars showed statistically different levels. Our conclusion is that there were some differences in quercetin levels after field curing between the cultivars in our study, but the differences were not consistent throughout the years, suggesting that the cultivars react differently to annual variations in climatic factors.

Extended Curing Experiment, 2005. There were no significant interactions between the factors cultivar and curing treatment. The increase in total flavonol content during normal field curing was 79% (Table 4). Onions cured in plastic bags showed no increase in flavonol content at all, and all other treatments resulted in an  $\approx$ 33% increase in both quercetin diglucoside and quercetin monoglucoside (Table 4). These results suggest that humidity and temperature during curing have small effects compared to light. In the absence of light, there was still an increase in quercetin glucosides during curing, but field curing in full sunlight resulted in the highest increase. The 20% UV light transmittance reduction could not fully explain the surprisingly low content of quercetin in the transparent plastic covering treatment. The high temperatures (Table 1) may have reduced quercetin synthesis in the transparent plastic treatment.

Price et al. (43) found that following the curing and drying process at 28 °C for 10 days, a significant 54% reduction in quercetin monoglucoside content occurred. No new peaks appeared in the chromatograms, so the likely explanation was that a thicker outer dry skin was removed after curing to make the sample representative of an edible part of the onion (43). In our study, we did not find this kind of reduction in quercetin glucoside content following field curing (**Table 4**).

**Extended Curing Experiment, 2003.** In 2003, onions with the leaves still erect and onions with fallen, but still green, leaves in the same row were lifted on the same date, when the leaves had just started to fall down, and then left for field curing for 10 days. After field curing, the onions with erect leaves had small but significantly higher levels of quercetin glucosides in the outer and middle scales (**Table 5**), but when the whole onions were analyzed, no statistical difference could be found between onions that had erect or fallen leaves at lifting.

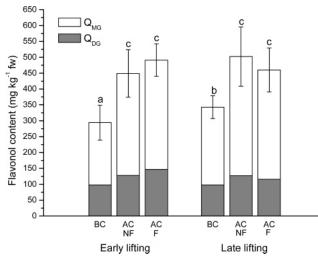
There is a gradient in quercetin content from the outer to the inner scales of onion bulbs (45). The content of quercetin glucosides also varies within each scale from base to top, with highest content in the top (25). Both of these observations were explained by older cells in the outer scales and the upper part of each scale. During aging, the molar ratio of quercetin diglucoside/monoglucoside decreases, which means that the onion scales develop a higher monoglucoside content with age (25). In our study, no significant difference in quercetin glucoside ratio was found in any onion part or between onions that had erect or fallen leaves at lifting in 2003 (**Table 5**).

As a general rule, onion foliage should be allowed to desiccate as much as possible after lifting and before topping (46). The greatest number of rotted bulbs in storage has been shown to

**Table 5.** Content (mg kg<sup>-1</sup> of fw) of Quercetin 3,4'-Diglucoside ( $Q_{DG}$ ), Quercetin 4'-Monoglucoside ( $Q_{MG}$ ), and Quercetin Aglycone (Q) after Curing in Different Parts of Cv. Barito Onions with a Diameter of 55–70 mm in the Same Row with Fallen or Erect Leaves, Lifted at the Stage When the Leaves Had Just Started To Fall Down, 2003<sup>a</sup>

onion part le		Q <sub>DG</sub>	Q <sub>MG</sub>	Q	total	increase during curing (%)		
	leaf status					Q <sub>DG</sub>	Q <sub>MG</sub>	total
outer scales	erect fallen	$365 \pm 37$ a 295 $\pm$ 39 b	1030 ± 83 a 865 ± 91 b	22 ± 3 a 21 ± 7 a	1417 ± 103 a 1181 ± 130 b	152 31	237 67	214 59
middle scales	erect fallen	77 ± 10 a 71 ± 3 a	244 ± 32 a 196 ± 10 b	tr <sup>b</sup> tr	321 ± 40 a 266 ± 12 b	492 97	804 145	703 129
inner scales	erect fallen	25 ± 4 a 27 ± 4 a	$77\pm10$ a $63\pm14$ a	tr tr	102 ± 14 a 91 ± 19 a	1150 440	1825 600	1600 550
whole onion	erect fallen	117 ± 7 a 126 ± 24 a	$341 \pm 36 \text{ a} \\ 332 \pm 19 \text{ a}$	tr tr	466 ± 57 a 449 ± 26 a	139 40	199 74	186 60

<sup>a</sup> Values are mean  $\pm$  standard deviation (n = 8). Values with the same letter in each column and pair are not significantly different at p < 0.05. <sup>b</sup> Trace (<10 mg kg<sup>-1</sup> of fw).



**Figure 2.** Effects of field curing in 2003, with or without foliage attached, on onion flavonol content: n = 12;  $Q_{MG}$  = quercetin 4'-monoglucoside;  $Q_{DG}$  = quercetin 3,4'-diglucoside; BC = before curing; AC = after curing; NF = no foliage attached during curing; F = foliage still attached during curing. Bars with the same letter are not significantly different at p < 0.05.

occur when onions are topped before field curing, probably as a result of rainfall within 12 h after topping (38). In our study, the early-lifted onions had significantly lower contents of quercetin monoglucoside at lifting (**Figure 2**) compared to latelifted onions. After field curing, no difference was found in quercetin content between early- and late-lifted onions. Whether or not the foliage was still attached to the onions during the field-curing period had no significant effect on the content of quercetin glucosides, which is in line with the findings of a New Zealand study that timing of foliage removal had no effect on mean skin color score (38). This result suggests that the quercetin synthesis is situated within the onion scales and that no transportation from the foliage to the onion scales occurs.

**Dry Matter Differences.** Onions of cv. Barito had significantly lower dry weight after field curing than the other cultivars in all years, but the levels were somewhat different in the different years. In 2002, cv. Barito had a lower dry weight (12.1%) compared to cv. Summit (12.9%). In 2004, cv. Barito had a lower dry weight (11.6%) than both cv. Hyskin (12.7%) and cv. Summit (12.9%). In 2005, cv. Barito had a lower dry weight (11.9%) than cv. Hyskin (12.2%), which in turn had a lower dry weight than cv. Summit (12.9%). No other significant differences in dry weight were found between curing treatments, onions that were lifted early or late, or onions that received high or low nitrogen levels in any of the years.

Moisture loss in early-lifted sweet onions (with the foliage still erect) can be up to 10% during curing (47). In our study, the dry weight after field curing compared to the dry weight at lifting was only between 0 and 0.9% higher. These findings agree with those of Grevsen and Sorensen (28), who found that onions lifted at the stage when 50% had fallen leaves had approximately the same dry matter content as onions lifted at 80% fallen leaves. The annual variation they found in dry weight was between 11.6 and 12.3% for cv. Hyton in 1997–1998 (28), which is similar to our results.

In conclusion, the amount of global radiation in August seemed to be the major determinant of quercetin glucoside content in field-cured yellow onions. No transport of quercetin glucosides from the green leaves to the onion scales was detected during field curing. In most experimental years, a significant increase in level of quercetin glucosides was found in fieldcured onions compared to the content at lifting, and the levels were higher in all onion scales. The increase was highest when the onions were cured in the field in full sunlight, but there was also a significant increase in completely dark environments, irrespective of temperature and relative humidity. Cultivar differences in quercetin glucoside levels varied and were not consistent between years. Nitrogen fertilizer level and lifting time were of minor importance for final quercetin glucoside content in onions after field curing.

#### ACKNOWLEDGMENT

We thank the staff at Torslunda Research Station for field assistance. We acknowledge Karl-Erik Gustavsson and Elisabet Modig for technical and laboratory assistance.

#### LITERATURE CITED

- Griffiths, G.; Trueman, L.; Crowther, T.; Thomas, B.; Smith, B. Onions—a global benefit to health. *Phytother. Res.* 2002, *16*, 603–615.
- (2) Marotti, M.; Piccaglia, R. Characterization of flavonoids in different cultivars of onion (*Allium cepa* L.). *J. Food Sci.* 2002, 67, 1229–1232.
- (3) Meltzer, H. M.; Malterud, K. E. Can dietary flavonoids influence the development of coronary heart disease? *Scand. J. Nutr.* 1997, 2, 50–57.
- (4) Aherne, S. A.; O'Brien, N. M. Dietary flavonols: chemistry, food content, and metabolism. *Nutrition* 2002, 18, 75–81.
- (5) Rasmussen, S. E.; Breinholt, V. M. Non-nutritive bioactive food constituents of plants: bioavailability of flavonoids. *Int. J. Vitam. Nutr. Res.* 2003, 73, 101–111.
- (6) Ewald, C.; Fjelkner-Modig, S.; Johansson, K.; Sjoholm, I.; Akesson, B. Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chem.* **1999**, *64*, 231–235.
- (7) Bilyk, A.; Cooper, P. L.; Sapers, G. M. Varietal differences in distribution of quercetin and kaempferol in onion (*Allium cepa* L.) tissue. J. Agric. Food Chem. **1984**, 32, 274–276.
- (8) Parr, A. J.; Bolwell, G. P. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.* 2000, *80*, 985–1012.
- (9) Price, K. R.; Rhodes, M. J. C. Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. J. Sci. Food Agric. **1997**, 74, 331–339.
- (10) de Vries, J. H. M.; Hollman, P. C. H.; Meyboom, S.; Buysman, M. N. C. P.; Zock, P. L.; van Staveren, W. A.; Katan, M. B. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. *Am. J. Clin. Nutr.* **1998**, *68*, 60–65.
- (11) Hollman, P. C. H.; van Trijp, J. M. P.; Buysman, M. N. C. P.; v. d. Gaag, M. S.; Mengelers, M. J. B.; de Vries, J. H. M.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418*, 152–156.
- (12) Shon, M.-Y.; Choi, S.-D.; Kahng, G.-G.; Nam, S.-H.; Sung, N.-J. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem. Toxicol.* **2004**, *42*, 659–666.
- (13) Griffiths, L. A.; Barrow, A. Metabolism of flavonoid compounds in germ-free rats. *Biochem. J.* **1972**, *130*, 1161–1162.
- (14) Bokkenheuser, V. D.; Shackleton, C. H. L.; Winter, J. Hydrolysis of dietary flavonoid glycosides by strains of intestinal Bacteroides from humans. *Biochem. J.* **1987**, *248*, 953–956.
- (15) Hollman, P. C. H.; Katan, M. B. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol.* **1999**, 37, 937–942.

- (16) Walle, T. Absorption and metabolism of flavonoids. *Free Radical Biol. Med.* 2004, *36*, 829–837.
- (17) Justesen, U.; Knuthsen, P.; Lyhne Andersen, N.; Leth, T. Estimation of daily intake distribution of flavonols and flavanones in Denmark. *Scand. J. Nutr.* **2000**, *44*, 158–160.
- (18) Cieslik, E.; Greda, A.; Adamus, W. Contents of polyphenols in fruit and vegetables. *Food Chem.* **2006**, *94*, 135–142.
- (19) de Vries, J. H. M.; Janssen, P. L. T. M. K.; Hollman, P. C. H.; van Staveren, W. A.; Katan, M. B. Consumption of quercetin and kaempferol in free-living subjects eating a variety of diets. *Cancer Lett.* **1997**, *114*, 141–144.
- (20) Bonaccorsi, P.; Caristi, C.; Gargiulli, C.; Leuzzi, U. Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). *J. Agric. Food Chem.* **2005**, *53*, 2733–2740.
- (21) Chu, Y. H.; Chang, C. L.; Hsu, H. F. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. 2000, 80, 561–566.
- (22) Escarpa, A.; Perez-Cabrera, C.; Gonzalez, M. C. Optimization and validation of a fast liquid gradient for determination of prominent flavan-3-ols and flavonols in fresh vegetables. J. High Resolut. Chromatogr. 2000, 23, 637–643.
- (23) Takahama, U.; Hirota, S. Deglucosidation of quercetin glucosides to the aglycone and formation of antifungal agents by peroxidasedependent oxidation of quercetin on browning of onion scales. *Plant Cell Physiol.* **2000**, *41*, 1021–1029.
- (24) Fossen, T.; Pedersen, A. T.; Andersen, O. M. Flavonoids from red onion (*Allium cepa*). *Phytochemistry* **1998**, 47, 281–285.
- (25) Hirota, S.; Shimoda, T.; Takahama, U. Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. J. Agric. Food Chem. **1998**, 46, 3497–3502.
- (26) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590–595.
- (27) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 1996, 44, 3426– 3431.
- (28) Grevsen, K.; Sorensen, J. N. Sprouting and yield in bulb onions (*Allium cepa* L.) as influenced by cultivar, plant establishment methods, maturity at harvest and storage conditions. J. Hortic. Sci. Biotechnol. 2004, 79, 877–884.
- (29) Gertsson, U.; Bjorklund, I. Strategies for determining optimum nitrogen supply to onions. *Acta Hortic.* 2002, 571, 181–185.
- (30) Justesen, U.; Knuthsen, P.; Leth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromtography with photo-diode array and mass spectrometric detection. *J. Chromatogr. A* **1998**, *799*, 101–110.
- (31) Lombard, K. A.; Geoffriau, E.; Peffley, E. Flavonoid quantification in onion by spectrophotometric and high performance liquid chromatography analysis. *HortScience* 2002, *37*, 682–685.
- (32) Mogren, L. M.; Olsson, M. E.; Gertsson, U. E. Effects of cultivar, lifting time and nitrogen fertiliser level on quercetin content in onion (*Allium cepa* L.) at lifting. *J. Sci. Food Agric.* 2006, in press.

- (33) Coolong, T. W.; Randle, W. M. Temperature influences flavor intensity and quality in 'Granex 33' onion. J. Am. Soc. Hortic. Sci. 2003, 128, 176–181.
- (34) Higashio, H.; Hirokane, H.; Sato, F.; Tokuda, S.; Uragami, A. Effect of UV irradiation after the harvest on the content of flavonoid in vegetables. *Acta Hortic.* 2005, 682, 1007–1012.
- (35) Olsson, L. C.; Veit, M.; Weissenbock, G.; Bornman, J. F. Differential flavonoid response to enhanced UV-B radiation in *Brassica napus. Phytochemistry* **1998**, *49*, 1021–1028.
- (36) Coolong, T. W.; Randle, W. M. Ammonium nitrate fertility levels influence flavour development in hydroponically grown 'Granex 33' onion. J. Sci. Food Agric. 2003, 83, 477–482.
- (37) Randle, W. M. Increasing nitrogen concentration in hydroponic solutions affects onion flavor and bulb quality. J. Am. Soc. Hortic. Sci. 2000, 125, 254–259.
- (38) Wright, P. J.; Grant, D. G.; Triggs, C. M. Effects of onion (*Allium cepa*) plant maturity at harvest and method of topping on bulb quality and incidence of rots in storage. *N. Z. J. Crop Hortic. Sci.* 2001, 29, 85–91.
- (39) Patil, B. S.; Pike, L. M.; Hamilton, B. K. Changes in quercetin concentration in onion (*Allium cepa L.*) owing to location, growth stage and soil type. *New Phytol.* **1995**, *130*, 349–355.
- (40) Lachman, J.; Orsak, M.; Pivec, V. Flavonoid antioxidants and ascorbic acid in onion (*Allium cepa*). Zahradnictvi-UZPI 1999, 26, 125–134.
- (41) Yang, J.; Meyers, K. J.; van der Heide, J.; Liu, R. H. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. J. Agric. Food Chem. 2004, 52, 6787–6793.
- (42) Trammell, K. W.; Peterson, C. E. Quantitative differences in the flavonol content of yellow onion, *Allium cepa* L. J. Am. Soc. *Hortic. Sci.* **1976**, *101*, 205–207.
- (43) Price, K. R.; Bacon, J. R.; Rhodes, M. J. C. Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). J. Agric. Food Chem. **1997**, 45, 938–942.
- (44) Makris, D. P.; Rossiter, J. T. Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): effect on flavonol content and antioxidant status. J. Agric. Food Chem. 2001, 49, 3216–3222.
- (45) Patil, B. S.; Pike, L. M. Distribution of quercetin content in different rings of various coloured onion (*Allium cepa* L.) cultivars. J. Hortic. Sci. 1995, 70, 643–650.
- (46) Wright, P. J.; Grant, D. G. Effects of cultural practices at harvest on onion bulb quality and incidence of rots in storage. *N. Z. J. Crop Hortic. Sci.* **1997**, *25*, 353–358.
- (47) Maw, B. W.; Mullinix, B. G. Moisture loss of sweet onions during curing. *Postharvest Biol. Technol.* 2005, 35, 223–227.

Received for review April 7, 2006. Revised manuscript received June 21, 2006. Accepted June 22, 2006. This work was supported by a strategic research grant from the Swedish University of Agricultural Sciences.

JF060980S