

Quercetin and Isorhamnetin Glycosides in Onion (*Allium cepa* L.): Varietal Comparison, Physical Distribution, Coproduct Evaluation, and Long-Term Storage Stability

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During onion processing, the outer dried protective layer (outer paper layer) and first two fleshy leaf layers are removed. This coproduct material is a potential commercial source of flavonoids especially quercetin. In the following study, the flavonoid composition was determined in coproduct materials and the press cake (material generated after juice extraction) in several commercially important onion varieties grown in California. Flavonoids were characterized and quantified using LC-(ESI)MS/MS and HPLC. The long-term stability of quercetin glycosides was assessed in dried coproduct materials stored at 4 and 22 °C over a 12 month period. In all varieties, the predominant forms of quercetin were the quercetin 3,4'-*O*-glucoside and 4'-*O*-glucoside. The first layer had significantly higher levels of flavonoids than the outer paper, second, and inner flesh layers on a DW basis ($p < 0.05$). *Allium cepa* "Milestone" contained the highest levels ($p < 0.05$) of flavonoids (1703 mg/100 g on a dry weight basis (DW)). Onion press cake had significantly higher levels of total quercetin as compared with fresh onions ($p < 0.05$). The levels of 4'-*O*-glucoside significantly decreased during the first month of storage and remained stable for 12 months of storage at either 4 or 22 °C ($p < 0.05$).

KEYWORDS: Onion; flavonol; quercetin; antioxidant; HPLC; LC-(ESI)MS/MS; glucoside; stability; storage; variety; coproduct

INTRODUCTION

Epidemiological studies associate the consumption of flavonoid-rich foods (fruits and vegetables) with protection against cardiovascular disease (1) and, to a lesser extent, against cancer and other age-related diseases (2). Among flavonoids, quercetin chelates certain transition metals, acts as a potent electron donor, and scavenges free radicals. The radical scavenging ability of quercetin inhibits lipid oxidation in vitro (3, 4), and it is theoretically possible for dietary flavonoids to help prevent atherosclerosis via the same mechanism in vivo. Major dietary sources of quercetin include onions (21.4 mg/100 g), apples (3.13 mg/100 g), and tea (1.99 mg/100 mL) (5). Flavonoids such as quercetin are nonpolar and are therefore linked with one or more water-soluble sugar molecules in plants (glycosides). Flavonoid glycoside profiles are species and variety specific. The flavonoid profile affects GI absorption and bioavailability (6).

Onions (*Allium cepa* L.) are a primary source of dietary quercetin in the Western diet. California is the major onion growing region in the United States with an estimated yearly production of 460,000 tons (7). Onion processors remove 40% of the onion as coproduct material. It is estimated that between 5

and 50 tons are generated daily. The processing waste includes the dry outer protective layer (outer paper layer), the two outer flesh layers, and onion press cake (8, 9). There are limited uses for onion waste (e.g., animal feeds and fertilizer), and the refuse results in high costs for transportation and landfill fees for the industry.

Currently, there is little information on the variety composition and distribution of quercetin glycosides in onions, and there are no studies on the varietal differences and distribution of quercetin glycosides in onions from California. More importantly, there is no information on the disposition of quercetin in onion coproduct materials; however, these materials are increasingly of commercial interest as a source of quercetin for use as an antioxidant in foods and cosmetics. Additionally, quercetin in the form of a glycoside may have potential use as a dietary supplement.

The present investigation characterizes the quercetin glycoside composition of several commercially important onion varieties grown in California (i.e., Cougar, Don Victor, Gobi, Milestone, Natasha, and Warrior). In addition, the levels and disposition of quercetin glycosides in the coproduct materials of four intermediate-day type varieties (i.e., Chief, Denali, Sequoia, and Cowboy) were evaluated along with the quercetin glycosides in onion press cake. As these coproduct materials have potential use in the food industry, the long-term stability of quercetin glycosides in dried onion was also investigated.

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Table 1. Onion Varieties, Day Types, and Growth Regions

variety	day type	growth region
Cougar	short-day	Imperial Valley, CA
Don Victor	short-day	Imperial Valley, CA
Gobi	short-day	Imperial Valley, CA
Milestone	storage	Salinas Valley, CA
Natasha	short-day	San Joaquin Valley, CA
Warrior	intermediate-day	San Joaquin Valley, CA
Chief	intermediate-day	San Joaquin Valley, CA
Cowboy	intermediate-day	San Joaquin Valley, CA
Denali	intermediate-day	San Joaquin Valley, CA
Sequoia	intermediate-day	San Joaquin Valley, CA

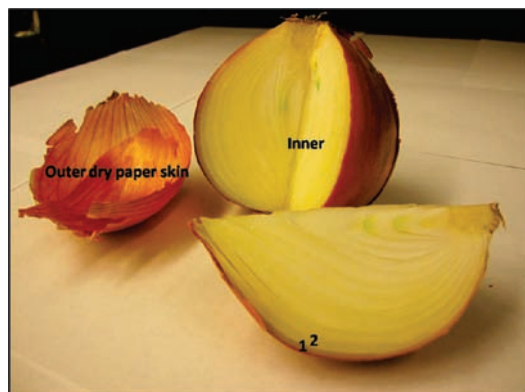
MATERIALS AND METHODS

Chemicals. Quercetin dihydrate (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one dehydrate) was purchased from Sigma-Aldrich (St. Louis, MO). Quercetin 3-*O*-glucoside, quercetin 4'-*O*-glucoside, quercetin 3,4'-*O*-diglucoside, and isorhamnetin 3-*O*-glucoside were obtained from Extrasynthese (Genay, France). The HPLC-grade methanol used for extraction was purchased from Fisher Scientific (Fair Lawn, NJ).

Samples. Onions and onion press cake were obtained from Gills Onions, LLC (Oxnard, CA) in the summer of 2008. Onion varieties, type, and growth region are shown in **Table 1**. All varieties were yellow onions. Onions (i.e., Gobi and Vaquero), used for producing press cake, were grown in the Imperial Valley of CA and Salinas Valley of CA, respectively. Plants were grown on mineral soil and fertilized with Calcium Ammonium Nitrate (17%). The watering process followed normal practices; less than weekly at prebulbing stage and twice weekly at the peak demand period for about 3 weeks. The quercetin composition was characterized in the outer paper layer and first two flesh layers of the following varieties: Cougar, Don Victor, Gobi, Milestone, Natasha, and Warrior. Four intermediate-day type varieties of yellow onions were used to investigate the distribution of quercetin glycosides in onions (i.e., Chief, Cowboy, Denali, and Sequoia). The onions (11.34 kg of Cowboy, Chief, and Denali varieties and 22.70 kg of Sequoia variety) were harvested, bagged, and shipped directly to UC Davis. Around 20 onion bulbs per variety were used for the composite samples. The onions were divided into four parts, the outer paper, first, second, and inner flesh layers and randomly assigned to one of three composite samples for each variety. Onion press cake is the coproduct material produced during onion juice extraction under high pressure. The onion peels were shredded, and high pressure was applied to extract onion juice. The remaining cake was dried and pulverized. The dried press cake samples were shipped immediately to UC Davis. *Allium cepa* "Sequoia" was used to assess the long-term (12 month) stability of quercetin glucosides in dried onion. The Sequoia onions and press cake samples were lyophilized in a VirTis freeze-drier (SP Industries Inc., Warminster, PA, USA) until the weight of the sample reached a constant value or air-dried at room temperature to a constant value and stored at -80 °C until analysis.

Flavonoid Extraction. The flavonoid extraction was performed following the method of Caridi et al. (10) with slight modifications. Quercetin glucosides and quercetin aglycone were extracted from 1 g of the dried onion sample or press cake using 50 mL of 80% aqueous methanol. The mixture was homogenized for 1 min, allowed to stand for 20 min, and the supernatant was filtered through a 0.45- μ m PTFE membrane filter. The extracts were stored at -4 °C until analysis. The samples were stored up to 0–1 days before analysis. The total extraction process was done in triplicate on each composite sample. Recovery was assessed by observing the amounts of added authentic standards recovered from a sample matrix. Quercetin 4'-*O*-glucoside and quercetin aglycone standards, in the amount of 2.00 mg and 0.6 mg, respectively, were added to the 1.00 g onion sample. The experiment, which was repeated five times, indicated that the recoveries of quercetin 4'-*O*-glucoside and quercetin aglycone from onions are 104.5 \pm 0.7% and 86.7 \pm 6.1%, respectively.

HPLC Analysis. Flavonoid levels were quantified using a Hewlett-Packard series 1200 liquid chromatograph (Agilent, Palo Alto, CA) equipped with a multiple wavelength detector (G1365B) monitoring 370 nm. Chromatographic separation was carried out according to the method of Caridi et al. (10), with slight modification. Separations of flavonol glucosides and quercetin aglycone were performed on an Agilent

**Figure 1.** Different layers of onions as defined in this study.

Zorbax XDB C₁₈ column (4.6 \times 250 mm, 5 μ m) with a C₁₈ guard column (4.6 \times 12.5 mm, 5 μ m). The flow rate was 1.0 mL/min, and the injection volume was 20 μ L. The mobile phase consisted of a linear gradient of water (A) and methanol (B) as follows: time 0–10 min, 20–80% B; and time 10–20 min, 80% B. The column was re-equilibrated between injections using 10 mL of the initial mobile phase. Quantification of quercetin 3,4'-*O*-diglucoside, quercetin 3-*O*-glucoside, quercetin 4'-*O*-glucoside, and quercetin aglycone was achieved using the authentic standards. Isorhamnetin 4'-*O*-glucoside was quantitated using isorhamnetin 3-*O*-glucoside. The detection limits of flavonol glucosides and quercetin aglycone were all 0.5 μ g/mL. The linear ranges of quantification of flavonol glucosides and quercetin aglycone were 1–100 μ g/mL. Extracts that exceeded the concentration range of the standard curve were diluted before injection.

Qualitative Analysis by LC(ESI)-MS/MS. LC(ESI)-MS/MS analyses of extracts were carried out with a HPLC system (Shimadzu Scientific, Columbia, MD) interfaced to a Quattro LC triple-quadrupole tandem mass spectrometer (Micromass, Altrincham, U.K.) equipped with an SIL-10A auto sampler, and binary LC 10AD pumps and Z spray. MassLynx software (v. 3.5) was used for data acquisition and processing. The HPLC eluent was split in a ratio of 1:1 (using the same gradient as described above). The capillary, cone, and extractor voltages were set to 3.0 kV, 30 V, and 2 V, respectively. The source temperature and the desolvation gas temperatures were 145 and 400 °C, respectively. Nitrogen was used with a flow of 550 L/h. Total ion scanning over a mass range of *m/z* 250–800 was applied to identify all possible flavonol glucosides and quercetin aglycone, both in negative and positive modes. The peaks of analytes corresponding to possible flavonol glucosides and quercetin aglycone were further investigated with negative and positive LC(ESI)-MS/MS by applying optimum collision energy for different analytes.

Statistical Analysis. Statistical analysis was performed using SPSS software (v. 16.0, SPSS, Inc., Chicago, IL). Multivariate analysis of variance (MANOVA) was applied to evaluate the effect of varietal differences of onions, the effect of layers on flavonoid levels, and variety \times layer interaction. Significant differences of flavonoid levels among onion varieties and among layers in the same variety were determined using one-way ANOVA followed by the Duncan's multiple range test at $p < 0.05$. The flavonoid level changes for long-term storage were also determined by ANOVA followed by the Duncan's multiple range test at $p < 0.05$. Variety differences of press cake onions was evaluated with an independent *t*-test at $p < 0.05$. The differences between flavonoids in outer layers of fresh Gobi onion and Gobi press cake were analyzed using an independent *t*-test at $p < 0.05$.

RESULTS AND DISCUSSION

Identification of Quercetin and Isorhamnetin Glycosides by LC(ESI)-MS/MS. In typical onion processing, the outer paper, first, and second layers are removed and discarded (**Figure 1**). However, early studies on hydrolyzed materials indicate that these materials are rich in the bioflavonoid quercetin (11). Unfortunately, little information is available on the quercetin glycoside composition of this material; yet it is recognized that the quercetin glycosides are more bioavailable than the aglycone form of

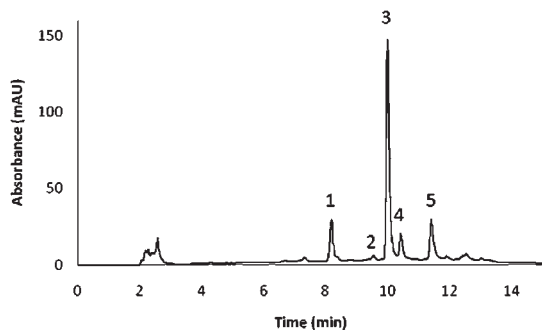


Figure 2. HPLC chromatogram of onion extracts monitored at 370 nm. Peak 1, quercetin 3,4'-*O*-diglucoside; peak 2, quercetin 3-*O*-glucoside; peak 3, quercetin 4'-*O*-glucoside; peak 4, isorhamnetin monoglucoside; and peak 5, quercetin aglycone.

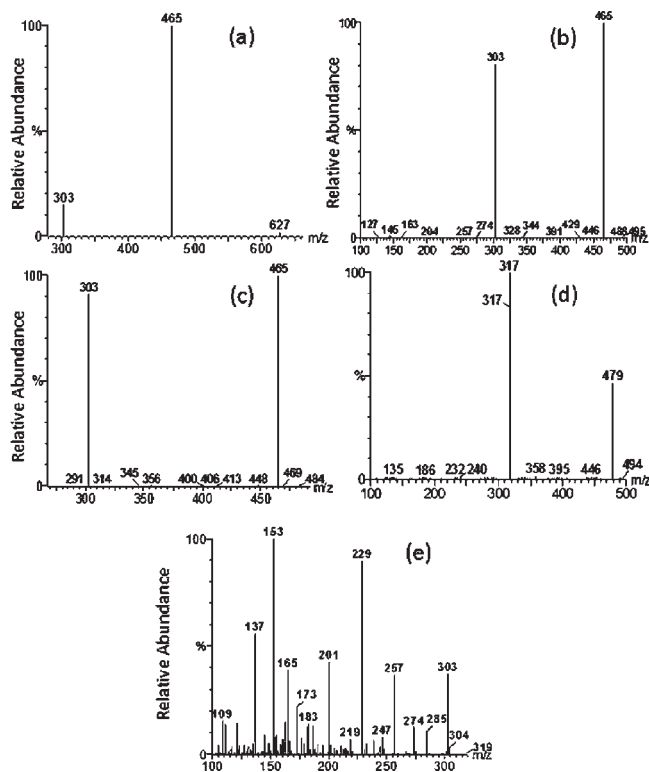


Figure 3. LC(ESI)-MS/MS spectra of chromatographic peaks obtained from onions. (a) Peak 1 (quercetin 3,4'-*O*-diglucoside); (b) peak 2 (quercetin 3-*O*-glucoside); (c) peak 3 (quercetin 4'-*O*-glucoside); (d) peak 4 (isorhamnetin mono glucoside, probably isorhamnetin 4'-*O*-glucoside); and (e) peak 5 (quercetin aglycone) in positive ion mode.

quercetin (12, 13). Therefore, we examined the quercetin glycoside content of extracts from numerous varieties of onion. Our results indicate that the primary flavonoids in onion include quercetin 3,4'-*O*-diglucoside, quercetin 3-*O*-glucoside, quercetin 4'-*O*-glucoside, isorhamnetin 4'-*O*-glucoside, and quercetin aglycone. A typical chromatogram of an onion extract is given in **Figure 2**. Peaks were identified by comparing the HPLC retention times and MS/MS spectra with authentic flavonoid standards. Typical LC(ESI)-MS/MS positive mode spectra of flavonoids identified in onion extracts are given in **Figure 3a–e**.

The first eluting peak (peak 1) produced a strong pseudomolecular ion at m/z 627 $[M + H]^+$ and fragment ions at m/z 465 $[M + H - 162]^+$ and m/z 303 $[M + H - 162 - 162]^+$ corresponding to the loss of two molecules of glucose (**Figure 3a**). This peak was identified as the diglucoside. MS spectra for peaks 2 and 3

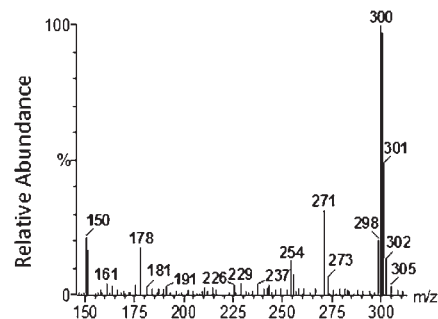


Figure 4. LC(ESI)-MS/MS negative mode spectra of peak 1 as identified in the HPLC chromatogram described in **Figure 2**.

(**Figure 3b** and **c**) indicate that these peaks correspond to monoglucosides. MS/MS spectra demonstrated an ion at m/z 303 $[M + H - 162]^+$ which corresponds to the loss of a glycosyl group. At increased collision energies, this ion produced fragments corresponding to the quercetin aglycone (m/z of 229, 153, and 137).

Isorhamnetin (methyl quercetin) 4'-*O*-glucoside is the main form of isorhamnetin found in onions (14). Peak 4 matched the retention time of an authentic standard of isorhamnetin 3-*O*-glucoside and resulted in the formation of a pseudomolecular ion at m/z 479 $[M + H]^+$ corresponding to methylated quercetin. MS/MS spectra indicate that this ion gives a fragment ion at m/z 317 $[M + H - 162]^+$ corresponding to the loss of a glycosyl group from methylated quercetin (**Figure 3d**). Upon the basis of these results, peak 4 was tentatively identified as isorhamnetin 4'-*O*-glucoside.

The last eluting peak (peak 5) produced a pseudomolecular ion at m/z 303 $[M + H]^+$, and MS/MS spectra demonstrated the characteristic fragmentation pattern of quercetin aglycone (m/z of 229, 153, and 137) (**Figure 3e**).

In ESI negative mode, quercetin mono- and diglucosides are predicted to produce quercetin aglycone (m/z 301) after the loss of glycosyl units. Interestingly, peaks 1, 2, and 3 produced fragments at both m/z 301 and m/z 300 (**Figure 4**). The ion at m/z of 300 likely arises through the loss of one proton, and the formation of the quinone or occurs through the formation of the deprotonated radical aglycone during heterolytic cleavage of glucoside (14, 15).

Variety Comparisons of Quercetin Glycosides. The outer paper layer and the first two flesh layers of six different commercially important varieties of onions were analyzed. The levels of quercetin 3,4'-*O*-diglucoside, quercetin 3-*O*-glucoside, quercetin 4'-*O*-glucoside, isorhamnetin 4'-*O*-glucoside, and quercetin aglycone in the outer layers of onions ranged between 10–541, 4–54, 56–536, 7–79, and 20–1047 mg/100 g dry weight (DW), respectively (**Table 2**). Total quercetin and isorhamnetin content ranged between 93 and 1703 mg/100 g DW. The quercetin content varied significantly between cultivars, ranging up to an 18-fold difference. Genetic and external factors are crucial for flavonoid biosynthesis. The length of day required for developing onion bulbs varies. Onion types are classified by the length of day: short-day type requiring 10–11 h of day length, intermediate-day type (12–13 h of day length), and long-day type (≥ 14 h of day length) (16). The longer-day type varieties have been shown to contain higher amounts of quercetin aglycone than the shorter-day type varieties (17). The Cougar variety was grown in the same field with Don Victor and Gobi varieties. Interestingly, Cougar had higher quercetin levels (505 mg quercetin aglycone equivalent/100 g DW) than Don Victor and Gobi varieties (86–237 mg quercetin aglycone equivalent/100 g DW). This result suggests that Cougar onions are a high-quercetin variety.

Milestone, a storage onion, had the highest levels of quercetin flavonoids (1703 mg/100 g DW) as compared with the other

Table 2. Variety Comparisons of Flavonoids in the Outer Layers of Onions (mg/100 g DW)^a

variety	quercetin 3,4'- <i>O</i> -diglucoside	quercetin 3- <i>O</i> -glucoside	quercetin 4'- <i>O</i> -glucoside	isorhamnetin 4'- <i>O</i> -glucoside	quercetin aglycone	sum
Cougar	541 ± 4 f	42 ± 1 c	480 ± 4 e	79 ± 0 f	256 ± 2 b	1398 ± 3 e
Don Victor	10 ± 0 a	below LOQ ^b	56 ± 1 a	7 ± 0 a	20 ± 0 a	93 ± 2 a
Gobi	95 ± 3 d	10 ± 0 b	103 ± 2 c	21 ± 1 d	30 ± 0 a	258 ± 4 c
Milestone	49 ± 0 c	54 ± 1 d	536 ± 3 f	17 ± 0 c	1047 ± 29 c	1703 ± 30 f
Natasha	36 ± 3 b	4 ± 0 a	89 ± 2 b	12 ± 0 b	26 ± 0 a	167 ± 5 b
Warrior	242 ± 1 e	10 ± 0 b	230 ± 2 d	34 ± 0 e	23 ± 0 a	539 ± 3 d

^a Values are mean ± SD. Mean values followed by the different letters (a–f) within each column are significantly different at $p < 0.05$. ^b Detected but below LOQ (limit of quantitation).

Table 3. Variety Comparisons of Flavonoid Distribution (mg/100 g DW)^a

variety	quercetin 3,4'- <i>O</i> -diglucoside	quercetin 3- <i>O</i> -glucoside	quercetin 4'- <i>O</i> -glucoside	isorhamnetin 4'- <i>O</i> -glucoside	quercetin aglycone	sum
Chief						
inner	128 ± 1 Ba	5 ± 0 Ca	90 ± 0 Ba	13 ± 0 Ba	below LOQ	237 ± 1 Ba
second	1027 ± 21 Cd	44 ± 2 Db	964 ± 24 Db	110 ± 5 Cb	29 ± 1 Ba	2175 ± 39 Db
first	943 ± 20 Dc	55 ± 1 Dc	2392 ± 79 Cd	217 ± 6 Cc	160 ± 6 Cb	3766 ± 62 Dd
paper	305 ± 8 Db	below LOQ ^b	1612 ± 18.5 Dc	166 ± 2 Dd	423 ± 7 Dc	2505 ± 16 Dc
Cowboy						
inner	159 ± 1 Ca	4 ± 0 Ba	96 ± 1 Ca	18 ± 0 Ca	below LOQ	277 ± 1 Ca
second	347 ± 6 Ab	10 ± 0 Ab	237 ± 3 Bb	41 ± 1 Ab	below LOQ	634 ± 10 Bb
first	690 ± 21 Bc	30 ± 1 Ac	688 ± 11 Ac	102 ± 1 Ad	39 ± 1 Ba	1548 ± 32 Ad
paper	157 ± 3 Ca	below LOQ	687 ± 4 Bc	73 ± 1 Bc	326 ± 2 Cb	1243 ± 4 Cc
Denali						
inner	186 ± 7 Db	7 ± 0 Da	101 ± 1 Da	22 ± 0 Da	below LOQ	316 ± 9 Da
second	334 ± 7 Ac	12 ± 0 Bb	189 ± 2 Ab	45 ± 2 Ab	below LOQ	581 ± 10 Ab
first	800 ± 2 Cd	44 ± 2 Cc	646 ± 2 Ac	135 ± 7 Bd	26 ± 1 Aa	1651 ± 9 Bd
paper	140 ± 7 Ba	below LOQ	706 ± 3 Cd	103 ± 6 Cc	157 ± 7 Ab	1106 ± 18 Bc
Sequoia						
inner	106 ± 2 Ab	4 ± 0 Aa	62 ± 1 Aa	8 ± 0 Aa	below LOQ	179 ± 2 Aa
second	548 ± 0 Bd	25 ± 0 Cb	444 ± 2 Cb	64 ± 0 Bc	29 ± 0	1109 ± 1 Cc
first	507 ± 8 Ac	42 ± 0 Bc	1666 ± 27 Bd	108 ± 0 Ad	186 ± 5	2507 ± 38 Cd
paper	87 ± 2 Aa	below LOQ	637 ± 3 Ac	43 ± 1 Ab	289 ± 6	1055 ± 11 Ab

^a Values are mean ± SD. Mean values followed by different capital letters in different varieties for the same layer are significantly different at $p < 0.05$. Mean values followed by different lowercase letters within each column in the same variety for different layers are significantly different at $p < 0.05$. ^b Detected but below the limit of quantitation (LOQ).

varieties evaluated (93–1398 mg/100 g DW) ($p < 0.05$). This variety is typically stored up to 4 months at a temperature of 1–4 °C before it is processed. In the outer layers of all varieties examined, the dominant flavonoids were quercetin 3,4'-*O*-diglucoside and quercetin 4'-*O*-glucoside (70–87%), with the exception of Milestone in which the quercetin aglycone dominated. The ratio of quercetin aglycone to total quercetin glucosides was also higher in the Milestone variety. Quercetin aglycone has more potent antioxidant activity than quercetin glycosides (18). The sugar moiety in quercetin glycosides may not contribute the antioxidant activity. The superoxide anion radical-scavenging activity of quercetin aglycone (209 SOD units/mg) is superior to vitamins C and E (23 and 9 SOD units/mg, respectively). Generally, flavonoids are known to have the ability to protect vitamins C and E from oxidation during food storage (19). This result suggests that high quercetin aglycone levels of Milestone onions may be a factor contributing to the enhanced storage-ability of this variety.

Variety Comparisons of Flavonoid Distribution. The flavonoid composition was determined in the inner combined, second, first, and outer paper layers of the following varieties of onions: Chief, Cowboy, Denali, and Sequoia. The MANOVA (Wilks' Lambda = 0.000, $p < 0.001$) confirmed the effects of variety and location (layer) on flavonoid levels as well as two-way interactions

between variety and flavonoid distribution (Table 3). Of the varieties tested, Chief contained the highest levels of total flavonoids (2175 mg/100 g DW in the second, 3766 mg/100 g DW in the first, and 2505 mg/100 g DW in the outer paper layers) with the exception of the inner combined layers, which were highest in the Denali onion (237 mg/100 g vs 316 mg/100 g DW, respectively). This is an important observation as it is the inner layers that are considered the edible portion of the onion in commercial processing.

In general, the first layer contained the highest level of total flavonoids by dry weight and was noted before (20). The coproduct materials (i.e., the outer paper, first, and second layers) had significantly higher levels of total flavonoid than the inner edible parts ($p < 0.05$). This result can be explained by the high activity of phenylalanine ammonia-lyase, which synthesizes flavonol glucosides, in the outer layers (21). The average total flavonoid levels of all investigated cultivars ranged from 252 mg/100 g DW in the inner, 1125 mg/100 g DW in the second, 2368 mg/100 g DW in the first, and 1478 mg/100 g DW in the outer paper layer. The content of quercetin 3,4'-*O*-diglucoside and quercetin 4'-*O*-glucoside ranged from 106 to 186 and 62–101 mg/100 g DW, respectively. These levels were less than those reported in German yellow onion varieties (quercetin 3,4'-*O*-diglucoside, 20–400 mg/100 g DW, and quercetin 4'-*O*-glucoside, 0–240 mg/100 g DW)

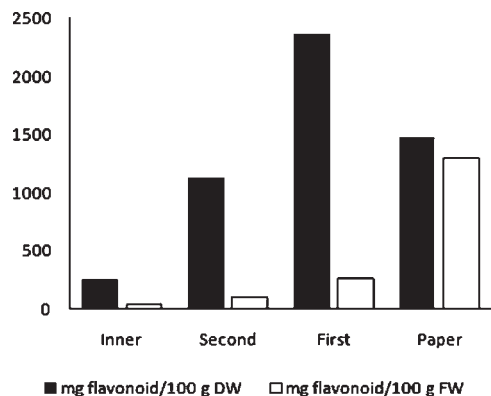


Figure 5. Average flavonoid levels in different layers of onions (cv. Chief, Cowboy, Denali, and Sequoia) in mg/100 g onion DW and fresh weight (FW).

and Australian yellow onion varieties (quercetin 3,4'-*O*-diglucoside, 153–404 mg/100 g DW, and quercetin 4'-*O*-glucoside, 58–286 mg/100 g DW) (10, 20). The quercetin 3,4'-*O*-glucoside content of Californian onion varieties were higher than those of Italian varieties (41–73 mg/100 g DW); whereas quercetin 4'-*O*-glucoside levels were lower in Californian varieties than Italian varieties (374–725 mg/100 g DW) after correcting the FW values to DW on the basis of the 90% estimated moisture content (22). Japanese yellow onion varieties contained higher levels of quercetin 3,4'-*O*-glucoside (208–412 mg/100 g DW) and quercetin 4'-*O*-glucoside (172–392 mg/100 g DW) after correcting the FW values to DW values (23). Genetic and environmental factors can account for these differences as well as differing extraction and measurement methods, storage conditions, and UV light exposure (24–28).

The average content of isorhamnetin 4'-*O*-glucoside in this study ranged from 8 mg/100 g DW to 22 mg/100 g DW and is in good agreement with reported values (5–51 mg/100 g DW) after correcting FW values to DW on the basis of a 90% estimated moisture content (29–31).

The levels of quercetin 3,4'-*O*-diglucoside, quercetin 4'-*O*-glucoside, and aglycone in the first layer of onions range between 507 and 943 mg/100 g, 646–2392 mg/100 g, and 26–186 mg/100 g DW, respectively. These values are similar to those reported by Beesk et al. (20).

In the outer paper layer, the levels of quercetin 3,4'-*O*-diglucoside, quercetin 4'-*O*-glucoside and aglycone ranged from 87 to 305 mg/100 g, 637 to 1612 mg/100 g and 157 to 423 mg/100 g DW respectively and were again similar to those reported by Beesk et al. (20).

The levels of total flavonoids per fresh weight were calculated using the moisture content of each layer (Figure 5). When expressed on a fresh weight basis, the flavonoid levels were ~11% of DW in the first, second, and inner layers. However, as the outer paper layer was already dry, the levels of flavonoids based upon fresh weight were ~89% of DW. Our data suggest that the outer paper and first layers of the onion can be considered as commercially important targets for the isolation of quercetin glycosides for commercial use.

In all varieties, quercetin 3,4'-*O*-diglucoside (47% of flavonoids) and quercetin 4'-*O*-glucoside (39% of flavonoids) predominated. This result was previously reported (11, 20). Relative ratios of quercetin diglucoside, monoglucoside, and aglycone are presented in Figure 6. Quercetin monoglucoside and quercetin aglycone levels increased from the inner layers to the outer paper layer. The levels of quercetin diglucoside followed the opposite trend, with levels decreasing from the outer layer to the inner layers. In the outer paper layer, quercetin aglycone was the second

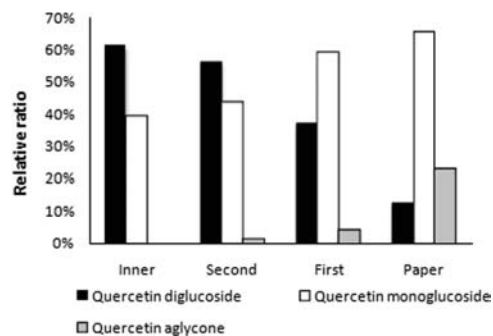


Figure 6. Average relative ratio of quercetin 3,4'-*O*-diglucoside, quercetin monoglucoside (3-*O*-glucoside and 4'-*O*-glucoside), and quercetin aglycone in different layers of the following varieties of onions: Chief, Cowboy, Denali, and Sequoia.

Table 4. Flavonoid Compositions in Press Cake Derived from Gobi and Vaquero Onions (mg/100 g DW)^a

flavonoid	Gobi (mg/100 g DW)	Vaquero (mg/100 g DW)
quercetin 3,4'- <i>O</i> -diglucoside	57 ± 1	37 ± 0 ^b
quercetin 3- <i>O</i> -glucoside	13 ± 0	13 ± 0
quercetin 4'- <i>O</i> -glucoside	159 ± 5	672 ± 4 ^b
isorhamnetin 4'- <i>O</i> -glucoside	20 ± 1	67 ± 0 ^b
quercetin aglycone	73 ± 0	175 ± 2 ^b
sum	293 ± 6	898 ± 5 ^b

^a Values are mean ± SD. ^b Significantly different from Gobi at $p > 0.001$.

predominant flavonoid in all varieties. The percentage of quercetin aglycone compared with total quercetin increased from the inner layers (0%) to the outer paper layer (6%). A possible explanation for the high levels of aglycone in the outer paper layers is that the cells of outer paper layers are more aged than the inner layers (21). Aging causes enzymatic hydrolysis of quercetin glycosides, resulting in the accumulation of quercetin aglycone in the outer paper layers. This observation may have important commercial implications as quercetin aglycone has lower bioavailability than the monoglucoside (12, 13). These distribution patterns are in accordance with previous published results of the distribution pattern of quercetin diglucoside, monoglucoside, and aglycone in different layers of yellow onion cultivars (20, 26).

Flavonoids in Onion Press Cake. Onion press cake is the solid residue produced during onion juice extraction under high pressure. The range of flavonoids in press cake derived from the industrial processing of Gobi and Vaquero onions is presented in Table 4. Total flavonoid levels in press cake were 293 mg/100 g DW for Gobi and 898 mg/100 g DW for Vaquero onions. Quercetin 4'-*O*-glucoside predominated in press cakes. With the exception of the quercetin 3,4'-*O*-diglucoside, flavonoid levels were significantly higher in the Vaquero press cake ($p < 0.001$).

The onion press cake obtained from processing Gobi onions contained significantly higher levels ($p < 0.001$) of quercetin than fresh onions of the Gobi variety (Figure 7). The level of aglycone in onion press cake was significantly higher ($p < 0.001$) than the levels found in the fresh onion. This is an important observation as the glycosidic form of quercetin is more bioavailable than the aglycone (12, 13). Differences in the quercetin glycoside profiles in fresh and processed foods or in extracts from different sources may help explain the discrepancies often found in results obtained from feeding trials which employed quercetin derived from a whole food (i.e., glycosides) with quercetin derived from supplements (i.e., aglycone). Understanding the flavonoid profile in coproduct materials is critical for tailoring their use in supplements, cosmetics, and as adjuncts in food processing.

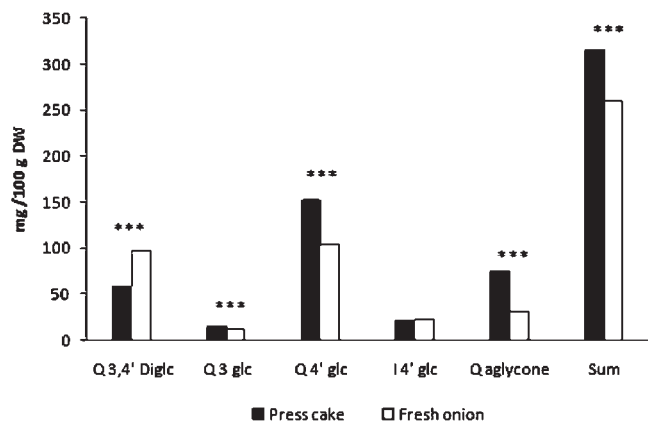


Figure 7. Flavonoid levels in onion press cake and in fresh onion of the Gobi variety. ***Significant difference in the levels of each flavonoid at $p < 0.001$.

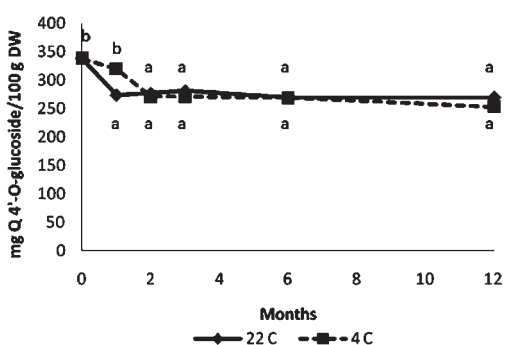


Figure 8. Long-term stability of quercetin 4'-O-glucoside in dried onion (cv. Sequoia) stored at 22 and 4 °C. The same letters are not significantly different at $p < 0.05$.

Long-Term Stability of Quercetin Glucoside (4'-O-Glucoside). Freeze-dried and powdered onion samples were archived and stored at 22 and 4 °C to determine the long-term stability of quercetin 4'-O-glucoside in what is considered the coproduct material from onion processing (the outer three layers). The results of a 12-month storage study indicated that quercetin 4'-O-glucoside was stable in dried onion stored at ambient and refrigerated temperatures and that little degradation occurred even with long-term storage of these materials (Figure 8). A previous study indicated a 50% initial loss of quercetin 4'-O-glycoside in the edible parts of onions and that quercetin composition was hardly changed during 6 months of storage (32). The quercetin 4'-O-glucoside may be more protected in the coproduct material from onion processing initially due to thicker dried outer layers than in the edible parts of onions.

In conclusion, this study provides initial information describing the quercetin profiles in onion processing coproduct materials. Considerable varietal differences in total quercetin and quercetin glycoside levels were observed. Additional varietal differences in the levels and distribution of quercetin glycosides were observed in the different layers of onions. The highest level of quercetin aglycone was present in the outer paper layers of all varieties investigated. Stability studies indicate that quercetin glucosides are stable in dried materials stored for extended periods of time (12 months) at either ambient or at refrigerated temperatures.

ABBREVIATIONS USED

DW, dry weight.

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