

Variation in the Quercetin Content in Different Colored Onions (*Allium cepa* L.)

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Abstract. The aglycone, or free quercetin, and total quercetin content of 75 cultivars and selections was analyzed by reverse-phase high-performance liquid chromatography. Quercetin glycosides were hydrolyzed into aglycones. Total quercetin content in yellow, pink, and red onions varied from 54 to 286 mg·kg⁻¹ fresh weight in different onion entries grown during 1992. White onions contained trace amounts of total quercetin. Free quercetin content in all the onions was low (< 0.4 mg·kg⁻¹) except in '20272-G' (12.5 mg·kg⁻¹ fresh weight). Bulbs stored at 5, 24, and 30C and controlled atmosphere (CA) for 0,1,2,3,4, and 5 months showed a most marked change in total quercetin content at 24C compared to other treatments, with a rise in mid-storage followed by a drop. Storage at 5 and 30C also demonstrated a similar change. However, total quercetin content did not vary significantly in bulbs stored at CA for 5 months. We conclude that genetic and storage factors affect quercetin content on onions.

Given the many positive effects of vegetables on human health, there is increasing interest in foods and beverages that contain quercetin. Onions provide a useful model system to investigate the genetics of plant flavonols (Brown, 1980; Kuhnau, 1976). Another major interest of the food industry in flavonols is their use as therapeutic agents, particularly in cancer. It is surprising that, even though data are available on the presence of quercetin in onion, there has been little research conducted to enhance the content of this compound.

It has been estimated that daily intake of flavonoids in the average American diet amounts to about 1 g, but in some cultures, dietary consumption may be as high as 2 to 3 g per day (Bier and Nigg, 1992; Kuhnau, 1976). Recently, Hertog et al (1993a) estimated quercetin intake of 16 mg·day⁻¹ in Netherland; however, the total flavonol and flavones is about 5-fold lower than previous findings of Kuhnau (1976). They also indicated that onions contribute about 13% flavonoid intake in Netherland, of which quercetin made up most of the flavonoid intake (63%).

Quercetin has been found to be mutagenic in either the absence or presence of S-9 metabolic activator (Ames, 1983; Bjeldanes and Chang., 1977). Although some evidence of quercetin carcinogenicity has been found (Pamukcu et al., 1980) in Japan, these results could not be confirmed in other studies on various animals (Morino et al., 1982; Saito et al., 1980). It was proposed that in vitro mutagenicity tests, like the Ames test, provide conditions and components required for generating hydroxyl radicals, but such conditions and components usually do not exist under normal physiological conditions (Leighton et al., 1992).

On the other hand, several scientists have undertaken systematic in vivo studies on the biological activity of flavonols. The

anticarcinogenic and antioxidant activity of quercetin has been reviewed (Patil et al., 1995). Quercetin intake is inversely associated with mortality from coronary heart disease (Hertog et al., 1993b).

Breeders and medical researchers are interested in developing onions with higher levels of quercetin. These onions may appeal to the health-conscious consumer. Location and genetic nature affect quercetin content; however, genetic characteristics appear to be predominant in determining the observed variation in quercetin content (Bajaj et al., 1990; Bilyk et al., 1984; Leighton et al., 1992). The need to check for population variation deserves emphasis, since it is often neglected despite of the fact that intraspecific chemical differences in flavonoids have been detected in well-known species (Harborne, 1986). This study was conducted to determine the variation of aglycone and total quercetin content in representative colored onions and the changes in total quercetin content due to storage conditions at different temperatures.

Materials and Methods

Sources. Quercetin (3,3',4',5,7 penta hydroxy flavone) was obtained from Sigma Chemical Co., St. Louis.

In 1992, 75 onion entries [37 from the Texas Agricultural Experiment Station, Weslaco; 37 entries grown in two locations—Griffin Brand (GB), McAllen, Texas, and Starr County (ST), Texas, provided by Asgrow Seed Co. (Kalamazoo, Mich.); and a 'Vidalia' onion (unknown cultivar) purchased from Blands Farms, Glenville, Ga.] were obtained.

The onion bulbs were harvested in late April and stored at 5C for 2 months. The onions were removed from the cooler and kept at room temperature (24C) overnight with air blowing through the boxes. Five bulbs per entry with three replications were evaluated in each experiment.

Sample preparation. A 20-g sample of tissue from the middle section around the equator of the bulb without dry skin was blended with 80 ml ethanol (80%) for 1 min and filtered through MFS no. 5C filter paper. The filtrate was stored in screw-capped vials at -20C until analyzed. A 5-ml aliquot was evaporated to dryness under vacuum at 50C (Buchler evapomix) and resuspended in 1 ml ethanol (80%). The extracts were filtered through 0.45-µm nylon 66 filters (Alltech Associates, Deerfield, Ill.), and a 10-µl volume of this solution was injected into the high-performance liquid chroma-

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tography (HPLC) system.

Absorption spectra. The absorption spectra (190 to 820 nm) of quercetin dissolved in 80% ethanol was determined using a Hewlett Packard 8452A diode array spectrophotometer. Maximum absorbance of quercetin occurred at 374 nm.

Hydrolysis. Hydrolysis conditions have been optimized (Hertog et al 1992), but, in our study, complete hydrolysis with 99% recoveries of glycoside peaks were observed with 2 N HCl at 80°C for 30 min. The resulting aglycone was quantified by HPLC as described by Markham (1989). Individual fractions of quercetin glycosides were collected based on retention times of respective peaks. Complete hydrolysis of quercetin glycosides yielded aglycone; however, partial hydrolysis of quercetin diglycoside yielded both monoglycoside and aglycone.

Quercetin quantification. Aglycone quantification was performed by using an external standard (free quercetin). Peak areas

were normalized to the external standard, and a standard curve was fitted by linear regression (peak area vs. concentration in mg·ml⁻¹). Total quercetin content was calculated by combining total glycosides (from complete hydrolysis to aglycones) and aglycone (standard). Mean recovery for quercetin was 89% and long-term variability of quercetin analysis in the laboratory was low (coefficient of variation <6%). The coefficient of variation for five successive analysis of the same sample was 1% to 2% and for five samples from the same onion was 4% to 9%.

HPLC analysis. The HPLC system consisted of a Perkin-Elmer binary LC pump 250, a Perkin-Elmer LC-600 auto sampler, a Perkin-Elmer UV/Vis spectrometric detector LC-290, a PV Nelson 900 series interface, a Hewlett-Packard 3394 integrator, and a Bondapak C-18 column (250 × 4.6 mm, 10 μm) connected to a Bondapak C-18 guard column. The mobile phase consisted of solvent A (0.5% orthophosphoric acid in water) and solvent B (0.5% orthophosphoric acid in water) and solvent B (0.5% orthophosphoric acid in water)

Table 1. Variation of aglycone and total quercetin contents among different colored onion entries at different locations (mg·kg⁻¹ fresh wt).^z

Entry	Growing location	Quercetin	
		Aglycone	Total
Red onions			
20356G	TAES	0.20 ± 0.01	202.2 ± 12.7
20355G	TAES	0.16 ± 0.0	157.16 ± 10.3
Kadavan	LC	0.32 ± 0.11	153.32 ± 17.4
20354G	TAES	0.23 ± 0.01	150.23 ± 13.5
4172	BG	0.17 ± 0.01	141.17 ± 16.2
Red Bone	GB	0.30 ± 0.06	117.38 ± 9.0
Pink onions			
20352G	TAES	0.19 ± 0.0	158.19 ± 8.8
20366G	TAES	0.21 ± 0.01	128.21 ± 17.5
20357G	TAES	0.20 ± 0.01	118.2 ± 14.3
Yellow onions			
Sweet Savannah	ST	0.28 ± 0.02	286.40 ± 16.9
4128	GB	0.40 ± 0.03	286.28 ± 14.6
XPH 6068	GB	0.22 ± 0.02	214.22 ± 17.1
XPH 6023	GB	0.28 ± 0.02	207.28 ± 23.6
4101	GB	0.23 ± 0.01	179.23 ± 25.7
4157	GB	0.23 ± 0.02	175.23 ± 13.2
4122	ST	0.18 ± 0.02	159.18 ± 11.7
XPH 6022	ST	0.31 ± 0.03	157.31 ± 17.8
4144	ST	0.21 ± 0.01	153.21 ± 8.2
TG1015Y	TAES	0.38 ± 0.12	150.38 ± 10.2
XPH 6028	GB	0.24 ± 0.02	150.24 ± 16.7
2027.5G	TAES	0.15 ± 0.0	150.15 ± 10.3
20277G	TAES	0.19 ± 0.01	146.19 ± 10.1
415.5	ST	0.27 ± 0.04	145.27 ± 9.8
4018	GB	0.24 ± 0.09	141.24 ± 9.0
TG502	ST	0.28 ± 0.03	140.28 ± 12.3
Granex 33	ST	0.24 ± 0.01	139.24 ± 15.6
Granex 429	ST	0.21 ± 0.01	138.21 ± 10.5
20270G	TAES	0.25 ± 0.09	137.25 ± 17.2
4143	GB	0.16 ± 0.0	137.16 ± 7.1
4130	GB	0.23 ± 0.01	136.23 ± 12.3
4141	ST	0.32 ± 0.13	136.32 ± 7.4
4120	ST	0.12 ± 0.03	135.12 ± 6.3
20272G	TAES	12.5 ± 0.98	127.50 ± 6.4
4143	ST	0.19 ± 0.0	126.19 ± 11.7
4149	GB	0.19 ± 0.01	124.19 ± 13.1
Henry special	GB	0.20 ± 0.01	123.2 ± 11.8
4146	GB	0.40 ± 0.08	119.4 ± 10.9

acid in methanol) with the following gradient: (% B): 10 min, 40% to 90%; 3.5 min, 90%. The flow rate was 1 ml·min⁻¹. Aglycone content was quantified using external standards.

Storage studies. 'Texas Grano 1015Y' bulbs were stored at 5, 24, and 30°C and controlled atmosphere (CA) (99% N₂ and 1% O₂ at 4.4°C) for 5 months. Relative humidity of 50% to 60% was maintained in all storage temperatures. Five bulbs for each temperature with three replications were taken out for extraction every month and total quercetin content was analyzed by reverse-phase HPLC.

Statistical analysis. For variation of quercetin content among different entries, a completely randomized block design was used. Analysis of variance with one-way test was performed with SAS (Cary, N.C.). Storage studies data were analyzed by 4 × 6 factorial design with three replications. Mean separations with Duncan's multiple range test were performed at *P* = 0.05.

Results and Discussion

Onions contain quercetin and its glycosides (Bilyk et al, 1984; Herrmann, 1976). In a preliminary experiment, extraction efficiency of several solutions of ethanol and methanol at concentrations of 20%, 40%, 60%, 80%, and 100% were tested. Quercetin levels were up to 20% higher at 80% with ethanol and methanol compared to other solution concentrations (results not shown). Ethanol phase partitioning efficiently extracted flavonols from onions, since the extraction method using methanol yielded the same levels of recovery as determined by absorbance at 374 nm. This specific wavelength corresponds to the maximal absorbance value in extracts from onion. Thus, measuring absorbance at 374 nm provided a useful quantitative estimation of total quercetin content.

Table 1. Continued.

Entry	Growing location	Quercetin	
		Aglycone	Total
4122	GB	0.24 ± 0.02	116.24 ± 11.5
20303G	TAES	0.16 ± 0.0	111.16 ± 14.3
20291G	TAES	0.21 ± 0.06	110.21 ± 22.1
20274G	TAES	0.21 ± 0.01	108.21 ± 16.2
4127	ST	0.16 ± 0.01	107.16 ± 13.8
4124	GB	0.22 ± 0.01	105.22 ± 8.5
0001	GB	0.16 ± 0.0	100.16 ± 8.9
20288G	TAES	0.19 ± 0.01	98.19 ± 23.4
20276G	TAES	0.19 ± 0.0	96.19 ± 7.9
20280G	TAES	0.30 ± 0.09	95.3 ± 15.4
TG502	GB	0.22 ± 0.02	95.22 ± 11.4
20290G	TAES	0.16 ± 0.01	94.16 ± 11.3
4135	GB	0.21 ± 0.05	93.21 ± 6.9
20301G	TAES	0.15 ± 11.3	87.15 ± 13.9
Rio Bravo	ST	0.18 ± 0.01	86.72 ± 8.7
20253G	TAES	0.19 ± 0.0	85.19 ± 8.3
20330G	TAES	0.27 ± 0.1	81.27 ± 8.8
Vidalia	GA	0.17 ± 0.0	80.18 ± 5.2
20263G	TAES	0.18 ± 0.01	77.18 ± 11.6
20316G	TAES	0.20 ± 0.03	76.2 ± 10.2
20338G	TAES	0.33 ± 0.05	74.33 ± 9.1
20251G	TAES	0.15 ± 0.0	74.15 ± 4.6
20305G	TAES	0.14 ± 0.0	71.14 ± 17.3
202596	TAES	0.19 ± 0.0	70.19 ± 9.8
20249G	TAES	0.16 ± 0.0	68.16 ± 7.4
20257G	TAES	0.16 ± 0.0	56.16 ± 15.8
4145	GB	0.34 ± 0.03	54.34 ± 0.02
White onions			
20223G	TAES	0.56 ± 0.0	1.41 ± 0.17
20319G	TAES	0.26 ± 0.12	0.91 ± 0.19
20240G	TAES	0.14 ± 0.0	0.56 ± 0.12
Contessa	GB	ND	0.54 ± 0.05
20234G	TAES	ND	0.37 ± 0.07
4168	GB	ND	0.37 ± 0.02
20216G	TAES	ND	0.36 ± 0.05
20304G	TAES	0.13 ± 0.0	0.41 ± 0.03
20235G	TAES	ND	0.27 ± 0.05
20221G	TAES	ND	0.26 ± 0.05
20221G	TAES	ND	0.21 ± 0.01

²Data are given as means of 15 samples ±SE. Growing locations are ST = Starr County, Texas; GB = Griffin and Brand, McAllen, Texas; TAES = Texas Agricultural Experiment Station, Weslaco; GA = Blands Farms, Glenville, GA; LC = Las Cruces, NM. ND = not detectable.

Table 2. Changes in the total quercetin content of 'TG1015Y' onions stored at different temperatures for 5 months (mg·kg⁻¹ fresh wt).

Month	Storage temp			
	5C	24	30	CA ^y
0	77.65 a ^x ± 7.9	78.83 c ± 11.7	64.62 ab ± 6.1	72.09 a ± 9.3
1	54.48 b ± 9.6	121.54 ab ± 14.3	75.99 ab ± 8.9	66.78 a ± 7.3
2	92.87 a ± 8.7	96.35 bc ± 9.8	86.57 a ± 9.7	67.50 a ± 6.8
3	52.83 b ± 4.1	140.24 a ± 10.2	89.97 a ± 17.5	61.45 a ± 5.1
4	72.18 ab ± 8.3	75.80 c ± 9.7	51.05 b ± 5.5	65.24 a ± 5.7
5	53.73 b ± 5.4	81.50 c ± 6.0	68.59 ab ± 6.7	63.53 a ± 11.3

^xData are given as means of 15 samples + SE.

^yCA = controlled atmosphere: CO₂ = 99% and O₂ = 1% at 4.4C.

^zMean separation within columns at *P* = 0.05 by Duncan's multiple range test.

Variation of aglycone and total quercetin content among different colored entries. Onions are unusual in the complexity of their flavonol components. The major flavonols in onions are all derivatives of quercetin and monoglycoside, diglycoside, or free aglycone. In all onions studied, spiraeoside was the main quercetin-containing compound present (data not shown). This is consistent with the previous reports (Koeppen and Vander-Spuy, 1961; Leighton et al., 1992).

Breeding onions for improved yield and physiological characters has been well documented. Selection may also affect the quercetin content of plants. It was therefore of interest to investigate a food plant, which had undergone considerable genetic selection, to determine if there are environmental differences that affect quercetin concentration (Herrmann and Woldecke, 1977). Knowledge of genetic differences in quercetin content in onions may be of potential value to breeders if it is confirmed that quercetin confers healthful properties to consumers. Among 75 entries (55 yellow, three pink, six red, and 11 white) analyzed for total quercetin content, the concentration of this flavonol differed significantly, ranging from 0.21 to 286 mg·kg⁻¹ fresh weight (Table 1). The marked variation in total quercetin content was about 1360-fold over entries of all colors including white onions. This variation in total quercetin content among different-colored onions may be genetic in origin (Leighton et al., 1992). The concentration of flavonols is usually $\mu\text{g}\cdot\text{kg}^{-1}$ fresh weight in root vegetables, even though it might be nearly 1000-fold higher in leaves. In contrast to root vegetables, onion bulbs (leaf tissue) contain more flavonols (Pierpoint, 1986). It was evident from our data that yellow, red, and pink onions contain higher concentrations of total quercetin, while white onions contain only trace amounts (Table 1). The yellow onion 'Sweet Savannah' had the highest total quercetin content, the red onion '203566' had a medium amount of total quercetin (202 mg·kg⁻¹), while white onions contained only trace amounts. The low amount of total quercetin in white onions was also found by other investigators (Herrmann, 1976; Leighton et al., 1992).

The high quercetin content reported by Kuhnau (1976) could be due to analyses of whole onions as opposed to edible parts, as in this study. Further, supporting evidences for significant differences between edible vs. nonedible onion parts have been reported elsewhere (Patil and Pike, 1995). The variation in total quercetin content among yellow, pink, and red onions, was 5-fold. It was clear that yellow onions contained more total quercetin than red onions. This conflicts with the results of Kiviranta et al (1988). Although previous studies have indicated that color might be due to the presence of quercetin (Bajaj et al., 1990; Laul et al., 1984), our results demonstrated that other factors are involved in determining high quercetin. In contrast, the reports of Bajaj et al. (1990) suggest that there could be higher quercetin content in red onions than

white onions if we consider only white and red onions. Although pink onions are uncommon in the market, all three pink genotypes from the Texas Agricultural Experiment Station breeding program contained moderate amounts of total quercetin. Since various glycosides of the same aglycone do not alter flower color in geranium (Asen, 1982) the variation in total quercetin content of yellow and red onions might be due to genetic factors other than pigments.

Evidence suggests that aglycone has greater pharmacological activity than its glycosides. Therefore, onions bred for high aglycone content may have more health benefits. Interestingly enough, the yellow onion '20272-G' had high aglycone content (12.50 mg·kg⁻¹), while no other entry had >0.6 mg·kg⁻¹, irrespective of the color. High aglycone content in '20272-G' may be under genetic control rather than environmental influence since all entries were grown in the same field and analyzed similarly.

Variation of total quercetin content due to storage conditions. Little data on storage effect on changes in quercetin content has been published. Changes in total quercetin content in 'TG1015Y' stored at three temperatures (5, 24, and 30C) and CA for 5 months is presented in Table 2. Total quercetin content in bulbs stored at 24C showed significant (*P* = 0.05) changes during 1, 2, and 3 months, while quercetin content was similar at 0, 4, and 5 months. We observed bulb sprouting in all storage conditions except CA storage. The nonconsistent pattern of quercetin content in storage conditions (5, 24, and 30C) may be due to bulb sprouting. CA storage did not produce significant changes in quercetin content during storage. The nonsignificant differences in quercetin content for 5 months in CA storage could be due to the fact that internal decay of bulbs was not observed at 4.4C (Adamicki and Kepka, 1974). Since our samples presented in Table 1 were analyzed within 2 months of storage at 5C, our data could have been only slightly influenced by storage effects on total quercetin content. However, diglycoside content did not show any increasing trend at the expense of the monoglycosides during any of our storage conditions, which conflicts with previous results (Starke and Herrman, 1976).

Knowledge of differences in quercetin content among different onion entries is a basic requirement for breeders who are dealing with onions for health benefits. In summary, our results indicated that red, pink, and yellow onions had more quercetin than white onions. However, color may not be the only criterion affecting total quercetin content, suggesting that there is the potential to breed for onions with high quercetin content independent of color.

Previous researchers assumed that quercetin might be mutagenic and toxic and reported that varietal differences in quercetin could be a basis for breeding for reduced quercetin content (Bilyk et al., 1984). Considering the biological effects of quercetin, it is generally unclear whether quercetin is beneficial or harmful to humans.

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