Effect of Gamma Irradiation on Quercetin on Onion (*Allium cepa* L.) Cultivars

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ABSTRACT

The role of quercetin in dietary anti carcinogenesis is well established. This study was undertaken to test the feasibility of radiation as a treatment for increasing the quercetin content in onion cultivars. Eleven cultivars (both diced and whole bulbs) were irradiated at 0, 0.8, and 1.2 kGy and quercetin content was analyzed by reverse-phase high performance liquid chromatography(RP-HPLC). Aglycone (free quercetin) content increased significantly (P =0.05) in diced onions treated both at 0.8 and 1.2 kGy in 'Cardinal', 'Dorado', and '20352G'. Total quercetin (both free and bound form) content in 'Dorado' (diced) and 'Cardinal' (whole bulb) increased significantly both at 0.8 and 1 .2 kGy. It was concluded that gamma irradiation can be used to increase quercetin content in specific onion cultivars.

RESUMEN

El papel de la quercetina en la dieta como anticarcinogénico está bien establecido. Este estudio se desarrolló para probar la factibilidad del uso de radiación como tratamiento para aumentar el contenido de quercetina en cultivares de cebolla. Se irradiaron once cultivares de cebolla (tanto bulbos enteros como cortados en cubitos) a 0, 0.8 y 1.2 kGy y se analizó el contenido de quercetina por cromatografía líquida de alto rendimiento de fase inversa (RP-HPLC). El contenido del aglicón (quercetina libre) aumentó significativamente (P = 0.05) en las cebollas cortadas en cubitos tratadas tanto con 0.8 y 1.2 kGy en los cultivares 'Cardinal', 'Dorado' y '20352G'. El contenido total de quercetina (tanto libre como en forma ligada) en 'Dorado' (cortado en cubitos) y en 'Cardinal' (bulbo entero) aumentó significativamente tanto a 0.8 como a 1.2 kGy. Se concluyó que la radiación gamma se puede utilizar para aumentar el contenido de quercetina en cultivares específicos de cebolla.

Additional index words: anticancer, phytochemicals, flavonoids, wounding

Onion consumption has increased due to the presence of pharmaceutically important compounds (Hanley and Fenwick, 1985). In the U.S., people consume about 1 g, of flavonoid daily and in some cultures this may be as high as 2-3 g, per day in the diet (Bier and Nigg, 1992). Recently, Hertog et al (1993) estimated quercetin intake of 16 mg kg^{-1} in Netherland; however, the total flavonol and flavones is approximately five fold lower than previous findings of Kuhnau (1976). Major dietary sources of quercetin show considerable geographical and cultural variation, in Italy the main source is red wine, in China the main source is tea and in the US and Northern Europe the main source is onion (Hertog et al., 1995). Although the majority of quercetin (Fig l) is present in onions as pharmacologically less active glycosides, glycosidases produced by the human intestinal flora "fecalases" are capable of hydrolysing a wide array of these quercetin glycosides to yield the aglycone quercetin (Tamura et al., 1980; McDonald et al., 1983; Parisis and Pritchard, 1983).

The formation of flavonoid glycosides is affected by light (Seigelman, 1969). A decrease in quercetin content was observed from the dry skin to inner rings in onion (Patil and Pike, 1995). In parsley, light exposure triggered expression of flavonoid biosynthesis (Bruns et al., 1986). Lees and Francis (1972) indicated that gamma irradiation exerts its effect on the flavonoid biosynthesis at an earlier metabolic point rather than the formation of these flavonol compounds. Neither boiling nor frying resulted in interconversion of the quercetin conjugates or production of free quercetin (Price et al., 1997). On the contrary, reports indicated that cooking lowered quercetin content with greater reductions being detected following microwaving and boiling compared to frying (Crozier et al., 1997). Many investigations have been undertaken to study the potential use of ionizing radiation for several other benefits like inhibition of sprouting (Urbain, 1986; Curzio and Croci, 1988; Wolters et al., 1990), disinfecting onions for quarantine purposes (Urbain, 1986) and improving the storage quality for international trade (Curzio and Urioste, 1993; Wolters et al., 1990). With the minimal

temperature rise associated with radiation, adverse changes in the food, such as altered flavor, odor, color, texture and loss of nutritional quality are minimized (IFT expert panel, 1983; Lu et al., 1987). Sensory quality of irradiated bulbs was better than unirradiated ones (Urioste et al., 1990). Furthermore, under the permitted irradiation, fresh onions did not show any changes in the aroma constituents which are the most unstable flavor component in onion (Kobayashi et al., 1994). Although doubts have been raised about the prospect for gamma irradiation exposures as a postharvest treatment for fresh produce (Maxie et al., 1971), the Food and Drug Administration has approved the treatment of fruits and vegetables with gamma irradiation up to 1 kGy (USDHHS, 1986). Presently, at least 21 countries irradiate different foods on a commercial scale.

Quercetin has a powerful growth inhibiting activity on human breast (Markaverich et al., 1988), leukemic (Larocca et al., 1990), ovarian (Scambia et al., 1990), and gastrointestinal (Nosokawa et al., 1990; Yoshida et al., 1990) tumor cells. The anticarcinogenic and antioxidant effects of quercetin have been reviewed (Patil et al., 1995b). Epidemiological studies have indicated that a relationship between a diet rich in flavonols and a reduced incidence of heart disease (Hertog, 1995).

On the other hand, Ames et al (1975) reported that mutation due to quercetin have occurred. The interesting part is mutagenicity of quercetin could be due to the nature of Ames test and the type of in vitro systems. With in vitro studies such as Ames test in the presence of trace metals copper and iron, quercetin produces superoxide, hydrogen peroxide and other molecular oxygen that can cause DNA strand breakage (Fazal et al., 1990; Rahman et al., 1992). In order to accumulate enough hydrogen peroxide in the inoculation medium these assays require prolonged incubation. In contrast, mammalian cells in vivo have sufficient catalase, superoxide dismutase (SOD), and glutathione peroxidasc to defend against hydrogen peroxide and superoxide anion radical formation (Stocker and Frei, 1991). It was also proposed that in vitro mutagenicity tests, like Ames-test, provide conditions and components required for generation of hydroxyl radicals, but such conditions and components usually do not exist under normal physiological conditions (Leighton et al., 1992).

This study was undertaken to determine the feasibility of gamma irradiation as a treatment for increasing the level of aglycone and total quercetin content and also to see if radiation treatment could convert quercetin glycosides to the aglycone.

MATERIALS AND METHODS

Onion Sources. The 'Texas Grano lOl5Y' and two breeding lines were obtained from the TAES breeding program (Pike et al., 1988). Eight commercial cultivars of onions grown in different regions of the United States were provided by Asgrow Seed Co., Kalmazoo, MI. USA.

All cultivars were stored at 4C until they were analyzed. Onions were removed from the cooler and kept at 24C overnight with air blowing through the boxes. Five to ten bulbs per cultivar were evaluated with three replications.

Flavonoid Source. Quercetin (3,3,4,5,7 Penta hydroxy flavone) was obtained from Sigma Chemical Co., St. Louis, MO, USA.

Sample Preparation. Cultivars of four colors (four yellow, three red, two each of pink and white) were used. The edible part of the bulb was cut into equal parts (20 g, each). Samples were packed in polyethylene bags. The same number of whole onion bulb samples were used for gamma radiation treatment. After irradiation treatment, samples were chopped, mixed with 80 ml ethanol (80%), blended for 1 min and filtered through MFS # 5C filter paper. The filtrate was stored in screw capped vials at -20C. Aliquots (5 ml) were evaporated to dryness under vacuum at 5OC (Buchler evapomix). Dried material was re-suspended in 1 ml ethanol (80%). The extracts were filtered through 0.45 µm nylon 66 filters (Altech Associate Inc., Deerfield, IL), and 10 µl volume of this solution was injected into the HPLC system.

Absorption Spectra. The absorption spectra (190-820 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 2N HCI at 80°C for 30 min (Patil et al., 1995a).

Quantification of Quercetin. Quantification of aglycone (free quercetin) was performed by using the established method (Patil et al., 1995b). Mean recoveries for quercetin was 89% and long term variatibility of quercetin analysis in the laboratory was low (C_{VR} series $\leq 6\%$). The coefficient of variation for five successive analysis of the same sample was in the range of 1-2%, and also for five samples from the same onion in the range of 4-9%.

HPLC Analysis. The HPLC system consisted of a Perkin-Elmer Model 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*4.6mm, 10µ) connected to a Bondapak C-18 guard column (Waters Corp., Milford, Massachusetts, USA). The mobile phase consisted of solvent A (0.5% orthophosphoric acid in water) and solvent B (0.5% orthophosphoric acid in methanol) with the following gradient :(% B): 10 mm, 40-90%; 3.5 mm, 90%. The flow rate was 1 ml/min. Aglycone content was quantified

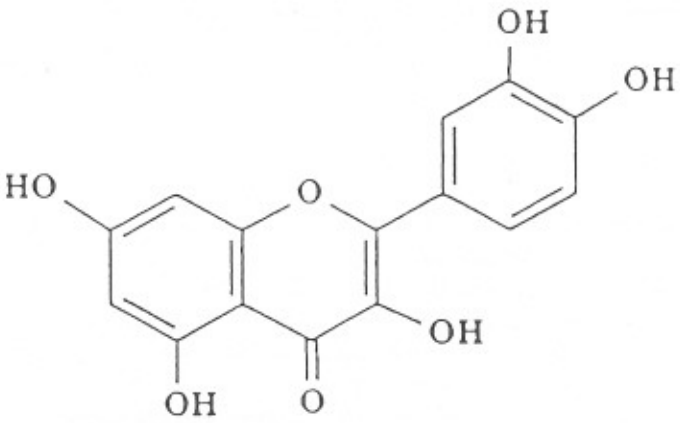

Fig. 1. Structure of quercetin (3,3', 4', 5,7' Penta hydroxy flavone

Table 1. Effect of gamma irradiation on the aglycone and total quercetin content in diced onions (mg/kg fresh wt)[z].

Cultivar	Dose (kGy)	Quercetin	
		Free	Total (Free and glycosides)
Red onions			
Cardinal	0.0	0.73b[y]±0.57	164.76a±26.97
	0.8	13.60a±2.06	229.06a±36.18
	1.2	12.77a±2.15	179.09a±31.92
Kadavan	0.0	0.15a±0.01	106.04a±12.38
	0.8	4.49a±2.92	137.68a±28.78
	1.2	3.45a±1.51	116.20a±15.19
Pink onions			
20352G	0.0	0.17a±0.01	130.40a±13.06
	0.8	15.15a±5.60	167.89a±18.83
	1.2	5.75a±2.98	146.47a±15.86
20366G	0.0	0.15a±0.01	109.32a±7.46
	0.8	0.46a±0.06	132.18a±8.18
	1.2	0.39a±0.02	119.21a±17.92
Yellow onions			
Dorado	0.0	0.15b±0.01	132.86b±10.4
	0.8	15.40a±3.62	187.13a±28.43
	1.2	12.28a±3.35	160.04ab±16.8
Sweet	0.0	0.26b±0.01	144.80a±13.70
Savannah	0.8	0.42a±0.23	145.61a±11.95
	1.2	0.33b±0.04	143.27a±12.56
TG502ST	0.0	0.20a±0.02	124.21a±16.94
	0.8	0.37a±0.03	146.60a±24.04
	1.2	0.42a±0.05	141.65a±25.44
TG502GB	0.0	0.38a±0.01	105.56ab±14.5
	0.8	0.53a±0.18	124.60a±13.62
	1.2	0.49a±0.06	122.08b±11.27
Henry	0.0	0.14a±0.01	60.71a±8.58
Special	0.8	0.28a±0.08	73.07a±12.34
	1.2	0.27a±0.06	61.69a±2.70
TG1015Y	0.0	0.14a±0.10	55.95a±28.50
	0.8	0.35a±0.11	70.15a±30.34
	1.2	0.28a±0.06	57.60a±22.73
White Onions			
Contessa	0.0	ND[x]	ND
	0.8	ND	0.18a
	1.2	ND	0.14a
Perla	0.0	ND	0.17a
	0.8	ND	0.18a
	1.2	ND	0.14a

[z]Results are given as means of 5 bulbs ± S. E.
[y]Means in a column followed by the same letter are not significantly different at P=0.05.
[x]ND = Not detectable.

using external standards.

Irradiation Treatment. Both diced and whole bulb samples were treated with gamma irradiation (Cobalt-60) at SteriGenic International in Fort Worth, Texas, USA. Onions were irradiated with doses of 0.8 kGy and 1.2 kGy. Control (non-irradiated) samples were transported along with the other samples to avoid environmental effect. After irradiation, onions were stored at ambient temperature (20-24C) for 24 h. Samples were analyzed by HPLC as described previously.

Wounding Treatment. Edible parts of onion bulbs were cut into small pieces (0.5 cm), and mixed thoroughly. Samples (20 g) were packed in polyethylene bags. For fresh weight analysis, samples were stored at -20C. The wounded or cut samples were analyzed for quercetin content after 1, 3, 5 and 7 days storage at 23C. For dry weight analysis samples were lyophilized for 72 h. Dried samples (0.5 g) were mixed with 80% ethanol using a 1: 60 ratio and filtered using MFS # 5C filter paper. Total quercetin and aglycone content was measured on both a dry weight and fresh weight basis as previously described.

Statistical Analysis. A split of a completely randomized design and randomized block design with sampling for the

Table 2. Effect of gamma irradiation on the aglycone and total quercetin content in whole onion bulbs (mg/kg fresh wt)[z].

Cultivars	Dose (kGy)	Quercetin	
		Free	Total (free and glycosides)
Red onions			
Cardinal	0.0	0.17b[y]±0.0	122.54b±19.4
	0.8	0.53a±0.24	274.79a±36.18
	1.2	0.25b±0.32	163.02a±31.92
Kadavan	0.0	0.16a±0.01	134.57a±12.38
	0.8	0.20a±0.04	158.70a±28.78
	1.2	0.18a±0.15	142.05a±15.19
Pink onions			
20352G	0.0	0.15a±0.0	119.86a±13.06
	0.8	0.82a±0.64	189.82a±18.83
	1.2	0.40a±0.57	147.05a±15.86
20366G	0.0	0.14a±0.0	108.27a±7.46
	0.8	0.97a±0.36	152.63a±8.18
	1.2	0.25a±0.60	112.61a±17.92
Yellow onions			
Sweet	0.0	0.14a±0.0	151.05b±10.4
Savannah	0.8	0.58a±0.24	203.61a±28.43
	1.2	0.44a±0.36	144.46a±16.8
Dorado	0.0	0.15a±0.0	99.94a±13.70
	0.8	0.25a±0.02	159.86a±11.95
	1.2	0.20a±0.06	135.79a±12.56
TG502GB	0.0	0.18a±0.01	92.40a±16.94
	0.8	4.70a±3.86	132.5a±24.04
	1.2	0.80a±0.07	110.26a±25.44
TG502ST	0.0	0.25a±0.06	69.12ab±14.5
	0.8	0.69a±0.23	133.76a±13.62
	1.2	0.66a±0.47	98.66ab±11.27
TG1015Y	0.0	0.14a±0.0	72.83a±8.58
	0.8	0.71a±0.34	80.54a±12.34
	1.2	0.38a±0.17	44.25a±2.70
Henry	0.0	0.15a±0.02	64.96a±28.50
Special	0.8	0.41a±0.27	87.92a±30.34
	1.2	1.07a±0.32	81.95a±22.73
White Onions			
Contessa	0.0	ND[x]	0.41a±0.12
	0.8	ND	0.76a±0.07
	1.2	ND	0.71a±0.12
Perla	0.0	ND	0.19a±0.02
	0.8	ND	1.71a±0.54
	1.2	0.16a±0.1	0.96a±0.27

[z]Results are given as means of 5 bulbs ± S. E.
[y]Means in a column followed by the same letter are not significantly different at P=0.05.
[x]ND = Not detectable.

diced onion and whole bulb experiments, respectively were used. Data were analyzed by analysis of variance, and Duncan's multiple range test was employed to determine significant differences among treatment effects (SAS,l988).

RESULTS AND DISCUSSION

Irradiation Effect on Total Quercetin Content in Diced Onions. Significant increase in total quercetin content was observed in 'Dorado' and 'TG5O2GB'. However, in general, gamma irradiation treatment of diced onions increased total quercetin content in yellow and red colored cultivars, but had no effect on quercetin content in white onion cultivars. Onions exposed to 0.8 kGy had the higher total quercetin content. The 1.2 kGy treatment also resulted in a slight increase in total quercetin content (Table 1). The increase in total quercetin content after irradiation treatment may be due to stimulation of PAL (Phenylalanine ammonia lyase) and flavonoid biosynthesis (Riov and Monselise, 1969). Hahlbrock and Grisebach (1979) reported that PAL is the rate limiting enzyme in flavonoid glycoside biosynthesis in response to irradiation.

On a fresh weight basis, 'Cardinal' (red) showed a marked

increase in total quercetin (229.06 mg kg^{-1}) content at 0.8 kGy, compared with non-irradiated samples (164.76mg kg^{-1}), and 'Cardinal' samples irradiated with 1.2 kGy showed a slight increase (179.09 mg kg^{-1}) in total quercetin content (Fig. 2). Increased total quercetin content in red onions in response to irradiation may be attributed to greater initial content and/or greater phenylpropanoid metabolism. White onions contained negligible amounts of initial quercetin compared to colored onions (Patil et al., 1995a). Although, total quercetin content at 1.2 kGy increased relative to control samples, the slight increase in total quercetin at 1.2 kGy compared to 0.8 kGy may be attributed to removal of quercetin by oxidation and/or decrease in PAL activity (Riov et al., 1968).

Irradiation Effect on Aglycone Content in Diced Onions. Of eleven (different colored) cultivars of onions analyzed, aglycone content increased significantly (P=0.05) in diced onions treated with both 0.8 and 1.2 kGy in 'Cardinal', 'Dorado', and '203-52-G' (Table 1). The significant increase in aglycone content by irradiation could be due to partial hydrolysis and/or autolysis of quercetin diglycoside yielding aglycone and/or stimulation of specific enzymes in the flavonoid biosynthesis pathway (Price et al., 1997).

Irradiation treatment significantly (P=0.05) increased aglycone content in 'Cardinal' onion bulbs (13.60mg kg^{-1}) at 0.8 kGy compared to non irradiated samples (0.73 mg kg^{1}); and the aglycone content (12.77mg kg^{-1}) increased less at 1.2 kGy compared to 0.8 kGy (Table 1 and Fig. 2). Several reports indicated that aglycone has greater pharmacological activity than quercetin glycosides (Tamura et al., 1980; McDonald et al., 1983). Different quercetin glycosides present in onions include: quercetin 3,4' diglycoside, quercetin 7,4' diglycoside, and spiraeoside (quercetin 4' monoglycoside) (Brandwein, 1965; Kiviranta et al., 1988; Leighton et al., 1992; Tsushida and Suzuki, 1995). In view of the anti tumor or tumor inhibiting properties, partial or complete hydrolysis of glycosides can occur either by irradiation or various glucosidases present in the human gut, mouth, and feces (Bokkenheuser and Winter 1988; Leighton et al., 1992).

Wounding Effect on Aglycone And Total Quercetin Content. Exposure of plants to various forms of abiotic stress is known to induce the synthesis of phenolics (Ecker and Davis, 1987). Results indicated that wounding alone was not responsible for increased total quercetin content in irradiated diced onions. No increase in total quercetin or aglycone content was observed when samples were analyzed on either a fresh or dry weight basis (results not presented). Similar results were obtained with tomatoes (Lee et al., 1970) subjected to a radiation dose of 200 krad. Wounding of plant tissue is known to induce a number of physiological changes, including ethylene biosynthesis (Ap Rees, 1966; McGlasson, 1969). Stimulation of ethylene biosynthesis by gamma irradiation may be responsible for increased phenylpropanoid metabolism and production of quercetin and quercetin glycosides.

Irradiation Effect on Total Quercetin Content in Whole Onion Bulbs. The results of radiation effect on total quercetin content in whole onion bulbs are presented in Table 2. In all cultivars tested, radiation increased total quercetin content both at 0.8 and 1.2 kGy compared with non-irradiated samples.

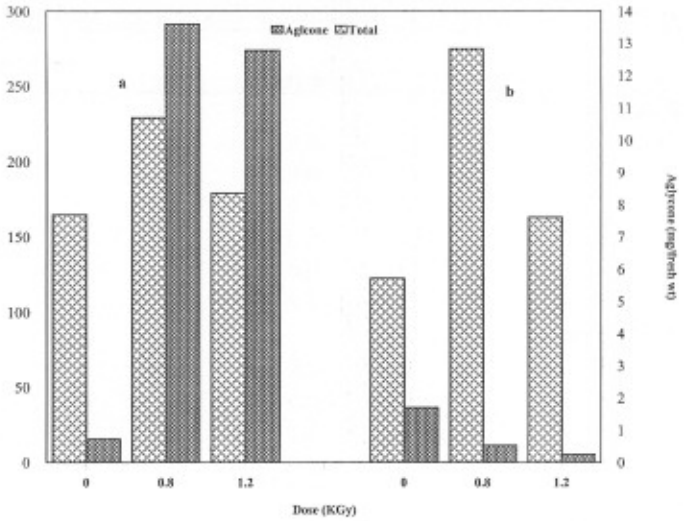

Fig. 2. Gamma irradiation effect and aglycone and total quercetin content in 'Cardinal' a) Diced onion and b) Whole onion bulbs.

However, bulbs treated at 1.2 kGy had lower total quercetin content than bulbs treated at 0.8 kGy. In 'Cardinal' a significant (P=0.05) increase in total quercetin content (274.79 mg kg^{-1}) occurred in samples treated with 0.8 kGy and 1.2 kGy compared to untreated (122.54mg kg^{-1}) onion bulbs (Fig. 2). Results indicate that the radiation effect was similar in whole onion bulbs and in diced onions.

Irradiation Effect on Aglycone Content in Whole Onion Bulbs. Aglycone level in whole onion bulbs changed little when exposed to gamma irradiation (Table 2). In contrast to diced onions, aglycone content in whole bulbs of 'Cardinal' increased slightly after irradiation treatment (Fig. 2). This lack of marked irradiation effect on aglycone content in whole onion bulbs may be attributed to the larger variation in aglycone content between bulbs of the same cultivar unlike diced onions where the same bulb was used for all the treatments.

CONCLUSIONS

The biological effects of quercetin have tremendous potential for the prevention of chronic diseases such as cancer and heart disease. Furthermore, glycosylated forms of quercetin are not a hindrance as glycosidic bonds are broken down readily by gastrointestinal bacteria. The deglycosylation in the gut and the degree of absorption in to the body need to be studied further. Our results indicated that gamma irradiation may be used to increase aglycone and total quercetin content in certain onion cultivars. Although some cultivars (white) exhibited no increase in total quercetin content, several cultivars (red, pink and yellow) showed a marked increase in aglycone content in both diced and whole onion bulbs. Increasing levels of quercetin aglycone in the diet is important since it has been shown to have potential anticarcinogenic properties. The data may also provide a base for epidemiological studies investigating the relationship between the intake of quercetin and risk of chronic diseases such as cancer and coronary diseases.

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