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# AROMATIC COMPOUNDS IN THREE VARIETIES OF TURNIP GREENS HARVESTED AT THREE MATURITY LEVELS\*

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## ABSTRACT

*Turnip greens (Brassica rapa) are commonly consumed in the southern U.S.A. Typically, they have a bitter taste, which increases with maturity, probably because of increased levels of glucosinolates. While glucosinolate degradation products have been isolated from various members of the Brassica family, the effect of variety and maturity on these products has not been determined. This study focused on the glucosinolate degradation products of three varieties of turnip greens: Purple Top, Seven Top and Tokyo Cross, harvested 45, 60 and 75 days after planting. Four volatile components (benzene acetonitrile, benzene propane nitrile, 1H-indole-3-acetonitrile and benzene ethyl isothiocyanate) were isolated, identified and quantified. All increased significantly ( $P < 0.05$ ) as the greens matured; however, only*

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*benzene propane nitrile and 1H-indole-3-acetonitrile were significantly affected ( $P < 0.05$ ) by variety.*

## PRACTICAL APPLICATIONS

This project applies to those researchers examining levels of functional components in foods. It also applies to registered dieticians who counsel clients on functional foods. As the relationship between disease and diet evolves, research of this nature will become even more important.

## INTRODUCTION

Some epidemiological studies have indicated an inverse relationship between the consumption of cruciferous vegetables and the risk of cancer (Steinmetz and Potter 1996; Kurilich *et al.* 1999). This is partially due to glucosinolates, a class of phytochemicals found in cruciferous vegetables. It is thought that glucosinolates break down into bioactive components that are protective against cancer (Howard *et al.* 1997; Kurilich *et al.* 1999).

In addition to possibly being anticarcinogenic (Kushad *et al.* 1999), glucosinolates are also the major flavor precursors in the Brassica family (Heath and Reineccius 1986). Cruciferous vegetables are characterized by sulfurous aromas and flavors following tissue injury or cooking. Upon degradation, glucosinolates yield three types of products: glucose, bisulfate and mixtures of volatile compounds including organic nitriles and isothiocyanates (Heaney and Fenwick 1980).

Isothiocyanate, derived from glucosinolate precursors via the action of thioglucoside glucohydrolase (E.C.3.2.3.1, myrosinase), generally contributes to the desirable, pungent flavors of Brassicas (Heaney and Fenwick 1980; Chin and Lindsay 1994). Isothiocyanates are responsible for the hotness of watercress, mustard and radish, and contribute significantly to the overall flavor of other Brassica. The pungent flavor of mustard and its biting taste, as well as the characteristic flavors of radish, broccoli, cabbage and cauliflower, originate from the degradation of their respective glucosinolates (Shahidi 1994). Other possible glucosinolate aglycon products are less volatile; notably the various indole compounds formed from glucobrassicin and neoglucobrassicin as well as from glucosinalbin (Heaney and Fenwick 1980).

Purple Top and Seven Top are the two most popular varieties of turnip greens grown in Alabama (Garvin 1994). Tokyo Cross is an F<sub>1</sub> hybrid that has been cited as being mild in flavor (Carlson *et al.* 1987). Although several researchers have isolated glucosinolate degradation products from Brassica,

there is no information on the relationship between variety and maturity. Therefore, this study examined the glucosinolate degradation products of three varieties (Purple Top, Seven Top and Tokyo Cross) of turnip greens harvested at 45, 60 and 75 days after planting.

## MATERIALS AND METHODS

### Planting, Harvesting and Storage

Turnip greens were grown in 15 m × 15 m plots at Alabama A&M University's Winfred Thomas Agricultural Research Station, Hazel Green, AL. Samples were collected 45, 60 and 75 days after planting. The greens were washed, blanched in a Dixie steam blancher-cooler (Model M6; Dixie Canner Equipment Co., Athens, GA) for 3 min, bagged in ziplock freezer bags (500 g), labeled and frozen (-20C) until analyses could be performed.

### Extraction Procedure

Volatiles were extracted with methylene chloride and then subjected to the GC-MS for isolation, identification and quantification. This procedure was developed in collaboration with United States Department of Agriculture-Agricultural Research Service-Southern Regional Research Center, New Orleans, LA (Jones *et al.* 1995).

A 50-g sample of turnip greens and 18.6 mL of distilled water were placed in a 250-mL beaker, covered with aluminum foil and cooked on a hot plate for 30 min. After cooking, the sample, a 50-mL methylene chloride and an internal standard, were blended for 15 s in a Sorvall Omni-mixer (model omni mixer 17105; DuPont Co., Newtown, CT). One hundred microliters of a 1,024-mg solution (in hexane) of 2-undecanone served as the internal standard. The sample was filtered under vacuum through Whatman's No. 1 filter paper (Whatman, Inc., Florham Park, NJ). The filtrate was placed in a separatory funnel, and after separation, the methylene chloride layer was removed and passed through anhydrous sodium sulfate. The volatiles were concentrated in a turbo-vap (model # ZW640-1; Zymark, Inc., Hopkinton, MA) under a stream of nitrogen. The samples were further concentrated under a stream of nitrogen to 100 µL, placed in a concentration vial, sealed and analyzed by GC-MS within 24 h (Jones *et al.* 1995).

### GC-MS Operational Procedures

The volatiles were isolated using gas chromatography, identified using mass spectrometry and quantified (Fig. 1). A Hewlett Packard (Palo Alto, CA)

$$\text{Concentration (ppm)} = \frac{(\text{RF}^1) \times (\text{area of volatile in sample})}{(\text{RF}^2) \times (\text{area of 2-undecanone in sample}) \times [\text{conc. of 2-undecanone in sample}]}$$

$$\text{Response factor (RF}^1) = \frac{\text{concentration of volatile in standard}}{\text{area of volatile in standard}}$$

$$\text{Response factor (RF}^2) = \frac{\text{concentration of 2-undecanone in standard}}{\text{area of 2-undecanone in standard}}$$

concentration of 2-undecanone in samples = 0.2048 ppm

Peak area from chromatograph.

FIG. 1. FORMULA TO CALCULATE CONCENTRATION OF VOLATILES

7673A autosampler injected 1  $\mu\text{L}$  of sample in a 5890A Hewlett Packard gas chromatograph equipped with a 50 m  $\times$  0.2 mm (5% phenylmethyl silicone) column. A Hewlett Packard 5970 mass selective detector, operating in the linear scanning mode for masses between 45 and 300 for compound identity and confirmation, was used to establish area counts. The temperature of the transfer line between the gas chromatograph and the mass spectrometer was 280C. The injector temperature was 250C. The oven temperature was held at 35C for 10 min, ramped at 4C/min to 250C, and held for 26.3 min. Total run time was 90 min.

### Statistical Analysis

A 3  $\times$  3 factorial arrangement (variety and maturity) was used as the experimental design. Data were collected in triplicate and were analyzed by analysis of variance (ANOVA) using general linear model (SAS Institute, Inc. 1990). When significant differences were found, Tukey's test was used to determine where the differences occurred.

## RESULTS AND DISCUSSION

Glucosinolate degradation products are thought to be responsible for the bitterness and anticarcinogenic properties of cruciferous vegetables. Four glucosinolate degradation products (benzene acetonitrile, benzene propane nitrile, 1H-indole-3-acetonitrile and benzene ethyl isothiocyanate) were isolated, identified and quantified in our samples. Other varieties or different harvest dates may have yielded different compounds.

### Variety

Benzene propane nitrile and 1H-indole-3-acetonitrile were significantly different ( $P < 0.05$ ) for variety (Table 1). Seven Top had significantly more

TABLE 1.  
MEAN CONCENTRATION OF GLUCOSINOLATE DEGRADATION PRODUCTS IN TURNIP  
GREENS AS AFFECTED BY VARIETY (AS IS BASIS) ( $n = 9$ )

Component	Variety			SEM
	Purple Top	Seven Top	Tokyo Cross	
Mean concentration (ppm)				
Benzene acetonitrile	0.018 <sup>a</sup>	0.026 <sup>a</sup>	0.025 <sup>a</sup>	±0.01
Benzene propane nitrile	1.128 <sup>a</sup>	2.504 <sup>b</sup>	1.732 <sup>ab</sup>	±0.65
1H-indole-3-acetonitrile	0.301 <sup>a</sup>	0.517 <sup>ab</sup>	0.595 <sup>b</sup>	±0.02
Benzene ethyl isothiocyanate	0.0004 <sup>a</sup>	0.0005 <sup>a</sup>	0.0007 <sup>a</sup>	±0.21

Mean concentrations in the same row with different superscripts are significantly different ( $P < 0.05$ ). SEM, standard error of the mean.

( $P < 0.05$ ) benzene propane nitrile than Purple Top. Tokyo Cross was not significantly different ( $P > 0.05$ ) from either Purple Top or Seven Top.

Tokyo Cross had significantly ( $P < 0.05$ ) more 1H-indole-3-acetonitrile than Purple Top (Table 1). There was no significant difference ( $P > 0.05$ ) between Seven Top and the other two varieties.

Overall, Seven Top and Tokyo Cross did not differ significantly in the level of degradation products. However, the consumer panelist preferred Purple Top and Tokyo Cross over Seven Top. The panelists commented that Seven Top was extremely bitter, had a strong aroma and left a bitter aftertaste. Tokyo Cross was generally regarded as having a mild flavor, a clean, fresh aroma and not too bitter. The flavor of Purple Top was rated between Seven Top and Tokyo Cross (Jones and Sanders 2002).

Numerous compounds in the Brassica family (Brussels sprouts, horseradish, turnip greens and others) produce a bitter taste. Although these compounds have a range of chemical structures, they are mainly nonvolatile and almost always water soluble. The bitterness of Brussels sprouts varies greatly between cultivars and may be related to the concentration of (–)5-vinyloxazolidine-2-thione, a compound derived from 2-hydroxy-3-butenyl glucosinolate. While cooking or blanching did not necessarily accentuate the bitterness, certain cultivars of Brussels sprouts were predisposed toward bitterness. Analysis of Brussels sprouts revealed that progoitrin, sinigrin, gluconapin and glucobrassicin are the predominant glucosinolates (Fenwick *et al.* 1983).

Horseradish, another member of the cruciferous family, has allyl and phenyl ethyl isothiocyanates as the principal bittering compounds. According to Gilbert and Nursten (1972), allyl and 2-phenylethyl (benzene ethyl) isothiocyanates are the principal pungent compounds of horseradish. The

2-phenylethyl isothiocyanate is the distinctive aroma of fresh watercress. It gives a tingy sensation, but does not appear to be pungent or lachrymatory. Even with its high proportion of 2-phenylethyl isothiocyanate, freshly grated horseradish has no aroma characteristic of this compound. This compound is either masked or blended with others, and loses its aroma qualities. An explanation of the differences in the aroma qualities among the varieties of horseradish could be attributable to changes in the proportions of the major aroma compounds. A small change in the proportions could be sufficient to significantly alter the overall aroma effect (Gilbert and Nursten 1972).

Because horseradish, Brussels sprouts and turnip greens are members of the same family, they have common aroma and flavor compounds. The aroma and flavor of turnip greens may be due not only to which compounds are present but also the proportion. For example, Tokyo Cross had more 1H-indole-3-acetonitrile (glucobrassicin) than Purple Top. According to Fenwick *et al.* (1983), only 21% of the panelists could detect bitterness in a 50-mg/100-ml solution of glucobrassicin. Even though Tokyo Cross may have significantly more 1H-indole-3-acetonitrile than Purple Top, this may not be the compound that most affects the bitterness. Additionally, there have been no reports on the possible synergistic effects nor the bitterness of the other compounds.

## Maturity

Although only two glucosinolate degradation products were significantly different ( $P < 0.05$ ) for variety, all were significant for maturity. The concentration increased from 45 to 75 days of maturity. Benzene acetonitrile (Table 2) and benzene propane nitrile significantly increased ( $P < 0.05$ ) from 60 to 75 days of maturity. However, the initial stages (45 and 60 days of maturity) did not have a significant effect ( $P > 0.05$ ) on these two compounds.

TABLE 2.  
MEAN CONCENTRATION OF GLUCOSINOLATE DEGRADATION PRODUCTS IN TURNIP GREENS AS AFFECTED BY MATURITY (AS IS BASIS) ( $n = 9$ )

Component	Maturity (days)			SEM
	45	60	75	
	Mean concentration (ppm)			
Benzene acetonitrile	0.013 <sup>a</sup>	0.019 <sup>a</sup>	0.037 <sup>b</sup>	±0.01
Benzene propane nitrile	1.004 <sup>a</sup>	1.684 <sup>a</sup>	2.675 <sup>b</sup>	±0.65
1H-indole-3-acetonitrile	0.303 <sup>a</sup>	0.497 <sup>ab</sup>	0.613 <sup>b</sup>	±0.02
Benzene ethyl isothiocyanate	0.0003 <sup>a</sup>	0.0007 <sup>b</sup>	0.0007 <sup>b</sup>	±0.21

Mean concentrations in the same row with different superscripts are significantly different ( $P < 0.05$ ). SEM, standard error of the mean.

Benzene ethyl isothiocyanate (Table 2) significantly increased ( $P < 0.05$ ) between 45 and the remaining harvest dates. However, there was no significant increase ( $P > 0.05$ ) between 60 and 75 days of maturity.

The 1H-indole-3-acetonitrile (Table 2) significantly increased as the greens matured. A significant difference ( $P < 0.05$ ) occurred between 45 and 75 days of maturity. No significant difference ( $P > 0.05$ ) occurred between 45 and 60 or between 60 and 75 days of maturity.

These results did agree with those of the consumer panel. As the greens matured, the preference decreased. Those harvested at 75 days after planting were significantly less preferred over those harvested at 45 and 60 days after planting (Jones and Sanders 2002).

The research of Gil and MacLeod (1980) confirmed that glucosinolate degradation products varied with the age of watercress. According to their study, benzyl thiocyanate was only produced by the younger plants and could not be detected in extracts of any seedlings or plants over 16 days of age. Thiocyanate production ceased immediately after the true leaves appeared. Although benzyl thiocyanate was not found in our samples, compounds did vary with age. As the greens matured, the amount of compounds increased. Different compounds and concentrations may have been found had the samples been tested at other times.

According to a study by Cole (1980), almost all volatiles detected in the seeds and 3-day-old seedlings disappeared by the fourth day and appeared again after 4 weeks. However, 2-phenyl ethyl isothiocyanate was detected only after hydrolysis of mature plants, not seeds or seedlings. This agrees somewhat with our study. The sample collected at 45 days of maturity had the least amount of benzene ethyl (2-phenyl ethyl) isothiocyanate, while those collected at 60 and 75 days of maturity had increased levels.

According to Tollsten and Bergström (1988), macerated buds of various Brassica contained nitrogen and sulfur compounds in greater number and quantity than in mature leaves. Results of their study indicated that compounds change with age. It seems that some compounds appear, disappear and sometimes reappear in the growth cycle of a particular plant (Tollsten and Bergström 1988) The exact relationship between maturity and rate of production of specific glucosinolates has not been thoroughly examined. Therefore, it is not possible to determine where specific glucosinolates appear in the growth cycle.

### **Maturity $\times$ Variety Interaction**

There was no significant interaction ( $P > 0.05$ ) between samples for benzene acetonitrile at 45 or 60 days of maturity (Table 3). However, between 60 and 75 days of maturity, there was a significant interaction ( $P < 0.05$ ). As the amount of benzene acetonitrile in Purple Top and Seven Top increased, Tokyo Cross decreased significantly ( $P < 0.05$ ).

TABLE 3.  
MEAN CONCENTRATIONS OF GLUCOSINOLATE DEGRADATION PRODUCTS FOR  
THREE VARIETIES OF TURNIP GREENS AT THREE MATURITY LEVELS ( $n = 9$ )

Component	Variety	Maturity (days)			SEM
		Mean concentration (ppm)			
		45	60	75	
Benzene acetonitrile	Purple Top	0.008 <sup>c</sup>	0.011 <sup>c</sup>	0.035 <sup>ab</sup>	±0.002
	Seven Top	0.009 <sup>c</sup>	0.018 <sup>bc</sup>	0.052 <sup>a</sup>	
	Tokyo Cross	0.021 <sup>bc</sup>	0.03 <sup>abc</sup>	0.025 <sup>bc</sup>	
Benzene propane nitrile	Purple Top	1.074 <sup>a</sup>	0.857 <sup>a</sup>	1.453 <sup>a</sup>	±0.176
	Seven Top	1.104 <sup>a</sup>	1.922 <sup>a</sup>	4.485 <sup>b</sup>	
	Tokyo Cross	0.834 <sup>a</sup>	2.273 <sup>a</sup>	2.089 <sup>a</sup>	
1H-indole-3-acetonitrile	Purple Top	0.282 <sup>bc</sup>	0.180 <sup>bc</sup>	0.44 <sup>abc</sup>	±0.054
	Seven Top	0.363 <sup>bc</sup>	0.341 <sup>bc</sup>	0.848 <sup>bc</sup>	
	Tokyo Cross	0.266 <sup>bc</sup>	0.969 <sup>a</sup>	0.55 <sup>abc</sup>	
Benzene ethyl isothiocyanate	Purple Top	0.001 <sup>a</sup>	0.001 <sup>a</sup>	0.001 <sup>a</sup>	±0*
	Seven Top	0.001 <sup>a</sup>	0.001 <sup>a</sup>	0.001 <sup>a</sup>	
	Tokyo Cross	0.001 <sup>a</sup>	0.002 <sup>b</sup>	0.001 <sup>a</sup>	

Mean volatile concentrations within the same component with different superscripts are significantly different ( $P < 0.05$ ).

\* Standard error of the mean (SEM) was negligible; therefore, the number was reduced to 0.

Samples showed no significant interaction for benzene propane nitrile from 45 to 60 days of maturity. At 75 days of maturity, the amount of benzene propane nitrile in Seven Top increased significantly ( $P < 0.05$ ). The concentration of benzene propane nitrile in Seven Top and Purple Top increased through all maturity dates. Tokyo Cross increased at 60 days and decreased by 75 days.

A significant interaction ( $P < 0.05$ ) for 1H-indole-3-acetonitrile occurred between 45 and 60 days of maturity. At 60 days of maturity, Tokyo Cross had significantly higher concentration of 1H-indole-3-acetonitrile than the other two varieties. The concentration of 1H-indole-3-acetonitrile for Purple Top and Seven Top increased throughout this study, while that of Tokyo Cross increased and then decreased at 75 days.

A significant interaction ( $P < 0.05$ ) for benzene ethyl isothiocyanate occurred between 45 and 60 days of maturity. For Purple Top and Seven Top, the level remained constant throughout all maturity dates, while at 60 days of maturity, Tokyo Cross was twice as high as the other two varieties.

Gil and MacLeod (1980) examined the effect of age and variety on the volatile compounds of watercress. With curled watercress, the production of nitrile decreased considerably as the plant aged. Isothiocyanate production also increased but to a lesser extent. With plain watercress, the opposite was true.

Ju *et al.* (1980) reported that the metabolic pathways for glucosinolate biosynthesis function differently in some cultivars. Differences in metabolism may occur in various stages during the biosynthesis of glucosinolates, or in the uptake, transport or synthesis of substrates for this biosynthesis. Their work demonstrated that the accumulation of different glucosinolates in roots of rutabaga and turnip occurs at different times during the growing season. Evidence indicates that certain amino acids, especially those containing sulfur, are the progenitors of the aglycon moiety of glucosinolates. The decreasing trend of glucosinolates after periods of major synthesis or accumulation may be associated in some way with the utilization of progenitor amino acids for protein synthesis (Ju *et al.* 1980).

## CONCLUSIONS

The results of this study indicate that glucosinolate degradation products vary with variety and maturity. Seven Top, rated by a sensory panel as the most pungent at 75 days of maturity, also had the highest concentration of three of the four components. The concentration of compounds also increased as the samples matured. Future studies that include more varieties would aid in examining varietal differences. A longer maturity time with more harvest dates would allow the researcher to determine a pattern for the production of volatile compounds.

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