positively weighted by the second function while Centennial, which was rated better by the sensory panel, had more of the volatiles that were negatively weighted. These data suggest that volatiles 17, 22, 9, 3, and 7 contribute to good sweet potato flavor while excess amounts of 5, 18, 12, 19, 26, 14, and 2 may cause undesirable flavor. Volatiles 25, 4, 21, and 13, which are negatively weighted in the first function and positively weighted in the second function, may either increase or decrease flavor while increased amounts of 8, 20, and 24, which change from positive to negative, would probably detract from flavor because of the greater importance of the first function.

Lack of association with either a good- or a bad-flavored cultivar does not mean that a compound is not important to sweet potato flavor. If a compound that is very important to sweet potato flavor is present in adequate amounts in all sweet potatoes, there would be no correlation with sensory score and the amount of that compound. Some compounds that were correlated with sensory flavor scores may not contribute much but may be incidentally present with other compounds that do. If the unidentified components of sweet potato aroma can be identified, a few of the 27 constituents might be blended to produce good sweet potato flavor. Such a step would simplify both classification of cultivars by aroma profiles and selection of better flavored cultivars by chemical analysis. The quality of sweet potatoes is genetically controlled (Constantin et al., 1966). Many volatiles associated with the aroma of baked sweet potato are probably not present in raw sweet potatoes but are formed by baking. It is probable that the precursors of desirable aroma are genetically controlled and the amounts might be manipulated by breeding to improve the flavor of sweet potato. This study suggests the possibility of specifying baked sweet potato aroma on the basis of a few volatile compounds, thus enabling selection of cultivars on the basis of specific chemical content in an attempt to improve sweet potato flavor. It may also enable marketing and procurement of sweet potatoes with objectively stated flavor characteristics.

Registry No. Diacetyl, 431-03-8; hexane, 110-54-3; 2,3-pentanedione, 600-14-6; 2-methyltetrahydrofuran-3-one, 3188-00-9; furfuraldehyde, 98-01-1; xylene, 1330-20-7; isobutyronitrile 2-pyrone, 78-82-0; 2-furyl methyl ketone, 1192-62-7; benzaldehyde, 100-52-7; 5-methyl-2-furaldehyde, 620-02-0; mesitylene, 25551-13-7; octanal, 124-13-0; 2-pentylfuran, 3777-69-3; phenylacetaldehyde, 122-78-1; nonanal, 124-19-6; linalool, 78-70-6; decanal, 112-31-2; β-ionone, 79-77-6; 2-pyrone, 504-31-4.

LITERATURE CITED

Constantin, R. J.; Hernandez, T. P.; Miller, J. C. *Proc. Am. Soc. Hortic. Sci.* **1966**, *88*, 498.
Fitzgerald, F. K. *Ecol. Food Nutr.* **1976**, *5*, 107.
Galinat, W. C.; Everett, H. L. *Agron. J.* **1949**, *4*, 443.
Giri, N. C. "multivariate statistical inference"; Academic Press: New York, 1977.
Hamann, D. D.; Miller, N. C.; Purcell, A. E. *J. Food Sci.* **1980**, *45*, 992.
Hammett, H. L. "Processing Report"; LA State University: Baton Rouge, LA, 1980; National Sweet Potato Collaboratores Group Progress Report.
Hanson, H. L.; Kline, L.; Linweave, H. *Food Technol. (Chicago)* **1951**, *5*, 9.
Henry, D. R.; Block, J. H. *J. Med. Chem.* **1979**, *22*, 465.
Isphording, W. C.; Flowers, G. C. *J. Sediment. Petrol.* **1980**, *50*, 31.
Kays, S. J.; Horvat, R. J., 5th International Symposium of Tropical Root and Tuber Crops, Lima, Peru, 1973.
Klecka, W. R. "Statistical package for the social sciences"; McGraw-Hill: New York, 1975; p 435.
Law, J. M. *La. Agric. Exp. Stn., Bull.* **1977**, *No. 706.*
Marquardt, R. A.; Pearson, A. M.; Larzelere, G. H.; Grieg, W. S. *J. Food Sci.* **1963**, *28*, 421.
Purcell, A. E.; Later, D. W.; Lee, M. L. *J. Agric. Food Chem.* **1980**, *28*, 939.
Tiu, C. S. M.S. Thesis, Department of Food Science and Nutrition, Brigham Young University, Provo, UT, 1981.
USDA. "Agricultural statistics"; U.S. Printing Office: Washington, DC, 1979.
Wilson, L. G.; Averre, C. W.; Baird, J. W.; Sorenson, K. A.; Beasley, E. O.; Skroch, W. A. *N.C. Agric. Ext. Serv. Publ.* **1980**, *No. AG-09.*

Received for review February 21, 1984. Revised manuscript received June 4, 1984. Accepted October 15, 1984.

Distribution of Quercetin and Kaempferol in Lettuce, Kale, Chive, Garlic Chive, Leek, Horseradish, Red Radish, and Red Cabbage Tissues

Alexander Bilyk* and Gerald M. Sapers

The quercetin and kaempferol contents of 13 varieties of lettuce were determined. Leaf lettuce varieties contained 2–54 mg of quercetin/kg, while head lettuce varieties contained 1–28 mg/kg, more in the outer leaves than in the inner leaves. These samples also contained 0–2 mg of kaempferol/kg. Chives contained 55 mg of kaempferol and 9 mg of quercetin per kg in green portions and lesser amounts in white portions, while leek contained 20 mg of kaempferol/kg in green portions and no detectable quercetin in either portion. Two varieties of kale contained 7–20 mg of quercetin and 13–30 mg of kaempferol per kg. Other vegetables examined contained lesser amounts of these flavonols. No myricetin was detected in these samples.

Certain flavonols that are widely distributed in fruits and vegetables (Herrmann, 1976) have been shown to be mutagenic by the Ames test (Bjeldanes and Chang, 1977; Hardigree and Epler, 1978; MacGregor and Jurd, 1978) as well as by other assays for mutagenicity (Meltz and MacGregor, 1981; Watson, 1982). Evidence for the carcinogenicity of the mutagenic flavonols has been obtained by Pamukcu et al. (1980) and Hatcher et al. (1983) but not by Fukuoka et al. (1980), Morino et al. (1982), or Taka-

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

nashi et al. (1983). The mutagenicity of flavonoids has been reviewed by Brown (1980) and Horowitz (1981).

Because of the possibility that mutagenic flavonols might have adverse effects on human health and that as a consequence, information on important dietary sources of these compounds would be useful, we have investigated varietal differences in the distribution of quercetin, kaempferol, and myricetin in various fruits and vegetables consumed in the United States and reported to contain high concentrations of these compounds. Previously, we reported that several varieties of sweet Spanish onions contained greater concentrations of quercetin than did other varieties of onions examined (Bilyk et al., 1984). In the present study, we report varietal differences in the distribution of quercetin and kaempferol in the edible portions of various flavonol-rich vegetables.

EXPERIMENTAL SECTION

Lettuce, kale, chive, and garlic chive samples were obtained from the experiment station of the W. Atlee Burpee Co. in Doylestown, PA. Four varieties of leaf lettuce were examined whole: Crispy Sweet, Green Ice, Ruby, and Salad Bowl. Samples of five head lettuce varieties (Augusta, Buttercrunch, Minneto, Summer Bibb, and Tom Thumb) were separated into outer leaf, inner leaf, and apical leaf portions, each of which was examined individually. Samples of Barcarolle, Burpee Bibb, Fordhook, and Paris White head lettuce were examined without separation. Two varieties of kale, Dwarf Siberian and Vates Blue Curled Dwarf, were examined whole. Chives and garlic chives were separated into green and white portions before analysis. In addition, samples of horseradish, leek, red cabbage, and red radish (varieties not known) were obtained from local food stores. The leeks were subdivided into green and white portions while the other vegetables were examined whole.

Flavonol isolation, purification, identification, and quantitation were performed according to procedures described previously (Bilyk et al., 1984). The chopped vegetable samples were extracted with absolute methanol at a 1:5 ratio (fresh weight/volume). Extracts were treated with activated carbon to remove chlorophyll and waxy materials. After solvent removal, the extracted flavonol glycosides were hydrolyzed to aglycons with 2 N HCl. Aglycons were separated by thin-layer chromatography (TLC) on silica gel plates with benzene–pyridine–formic acid (65:25:10) as the developing solvent and by reverse-phase high-performance liquid chromatography (HPLC) on an octadecyl bonded silica packing with methanol–water–acetic acid (50:42:8) as the mobile phase. Quantitative analyses for quercetin and kaempferol in vegetable extracts were performed in triplicate by HPLC, using purified flavonols as external standards. The standards were prepared from quercetin and kaempferol (Sigma Chemical Co., St Louis, MO) by preparative TLC as described above, extraction of the separated bands with methanol, filtration, and evaporation of the solvent at room temperature in vacuo. The standards were more than 99% pure by HPLC analysis. Calibration was carried out weekly or whenever a column was replaced by analysis in duplicate or triplicate of 10 μL of standard solution containing 0.05% each of freshly purified quercetin and kaempferol in methanol. Flavonol recovery was determined by "spiking" methanolic extracts of chopped lettuce with quercetin and rutin and measuring losses during carbon treatment, solvent removal, acid hydrolysis, and hydrolysate extraction by HPLC analysis. The completeness of the extraction procedures was confirmed independently by HPLC analysis of successive extracts of vegetable samples and hydrolysates. UV absorption spectra were obtained with methanol solutions of separated TLC bands and standards.

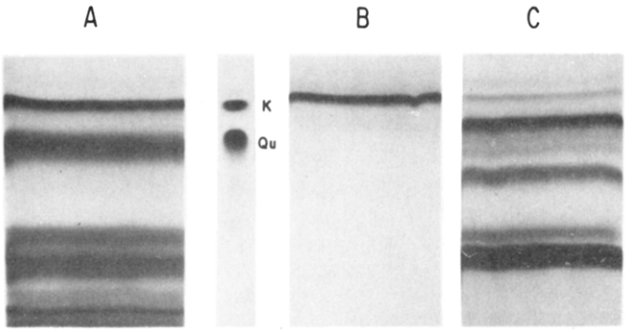

Figure 1. TLC separation of quercetin (Qu) and kaempferol (K) in extracts of Vates Blue Curled Dwarf kale (A), chives (B), and Ruby lettuce (C).

Table I. Quercetin and Kaempferol Contents of Leaf Lettuce Varieties

variety	flavonol content, mg/kg of fresh wt[a]	
	quercetin	kaempferol
Crispy Sweet	2 ± 0.13	ND
Green Ice	54 ± 0.32	ND
Ruby	31 ± 0.39	2 ● 0.41
Salad Bowl	10 ± 0.35	ND

[a] Mean for triplicate determinations ± standard deviation; ND = not detectable.

RESULTS AND DISCUSSION

Quercetin and kaempferol but not myricetin were detected in extracts of the vegetables examined in this study. Identifications were based on comparisons of TLC R_f values (Figure 1), HPLC retention times, and UV absorption spectra for unknowns and standards, as described previously (Bilyk et al., 1984). Variability in HPLC retention times, due to column aging and variation in chromatographic conditions, was small, typical retention time values for quercetin and kaempferol (means of 14 successive calibration runs) being 7.52 ± 0.13 and 11.04 ± 0.28 min, respectively. Coefficients of variation for the calibration factors used in flavonol quantitation (determined for 14 successive calibration runs) were 20.4% and 16.5% for quercetin and kaempferol, respectively. Spiking experiments demonstrated that the recovery of quercetin, added to the lettuce extract as the aglycon or as rutin and carried through the analytical procedure was quantitative, losses being less than 2%.

Samples of representative leaf and head lettuce varieties contained as much as 54 mg of quercetin/kg of fresh weight but only traces (≤2 mg/kg) of kaempferol (Tables I and II). Varietal differences were great, the quercetin content of leaf lettuce varying between 2 and 54 mg/kg for four varieties of leaf lettuce and between 0 and 28 mg/kg for nine varieties of head lettuce. With the latter, more quercetin was found in the outer and apical leaves than in the inner leaves. Our values for the quercetin and kaempferol contents of lettuce varieties available in the United States were lower than the quantities reported previously by Wöldecke and Herrmann (1974) for three European varieties, i.e., 31, 98, and 276 mg of quercetin/kg for Blanco, Apollo, and Valentine varieties, respectively. These varieties also contained more quercetin in the outer leaves than in the inner leaves and much more quercetin than kaempferol. It should be pointed out that our method

Table II. Quercetin and Kaempferol Contents of Head Lettuce Varieties

variety	portion[a]	flavonol content, mg/kg of fresh wt[b] quercetin	kaempferol
Augusta	A (37.9)	ND	ND
	B (25.2)	ND	ND
	C (36.9)	ND	<1 ± 0.00
Buttercrunch	A (30.6)	2 ± 0.08	1 ± 0.03
	B (35.5)	ND	< ± 0.13
	C (33.9)	<1 ± 0.05	ND
Minneto	A (28.9)	1 ± 0.26	2 ± 0.31
	B (35.4)	ND	<1 ± 0.02
	C (35.6)	ND	ND
Summer Bibb	A (36.0)	10 ± 0.02	1 ± 0.04
	B (31.1)	2 ± 0.02	ND
	C (32.9)	8 ± 0.09	ND
Tom Thumb	A (40.7)	38 ± 0.46	ND
	B (39.8)	9 ± 0.65	ND
	C (19.4)	12 ± 0.75	ND
Barcarolle	whole	9 ± 0.48	ND
Burpee Bibb	whole	28 ± 0.33	ND
Fordhook	whole	17 ± 0.18	ND
Paris White	whole	1 ± 0.43	ND

[a] A = outer leaves; B = inner leaves; C = apical leaves. Values in parentheses are percentages by weight. [b] Mean for triplicate determinations ± standard deviation; ND = not detectable.

of flavonol determination was based on separation by HPLC, while Wöldecke and Herrmann carried out photometric analyses of bands separated by TLC (Wildanger and Herrmann, 1973a). This procedural difference as well as the characteristics of the varieties examined may explain the difference between our results and those reported by Herrmann and co-workers.

Quantities of quercetin and kaempferol found in samples of chives, garlic chives, leek, red cabbage, horseradish, and red radish and in two varieties of kale are given in Table III. These vegetables generally contained more kaempferol than quercetin. With both chives and leek, we found higher flavonol levels in the green leaves than in the white leaf portion. Starke and Herrmann (1976) also reported more kaempferol than quercetin in leek, flavonol contents being greater in green leaves than in white leaves and in outer leaves than in inner leaves. However, in contrast to our results they reported more quercetin than kaempferol in chives. Both quercetin and kaempferol were present in kale leaves, Dwarf Siberian containing more of the latter flavonol and Vates Blue Curled Dwarf containing more of the former. Herrmann (1976) reported more kaempferol than quercetin in a German variety of kale, "Halbhoher grüner extra-krauser". Quercetin and kaempferol levels reported in this study generally were less than those found by Herrmann and co-workers (Wildanger and Herrmann, 1973b; Eloesser and Herrmann, 1975; Starke and Herrmann, 1976).

Van der Hoeven et al. (1983) detected mutagenic properties in extracts of lettuce and other vegetables by means of the Ames test. They reported a 7-fold difference in mutagenic response among the five varieties compared. Whether such mutagenic responses are relevant to human health remains to be seen. Horowitz (1981) considers the risks associated with flavonol ingestion to be minimal.

Our results suggest that a large reduction in the quercetin content of leaf and head lettuce could be achieved by breeding, should such a goal become desirable. Any effort in this direction should include an assessment of maturation and environmental effects on the flavonol composition of the commodity in question.

Table III. Quercetin and Kaempferol Contents of Various Vegetables

vegetable	portion[a]	flavonol content,[b] mg/kg of fresh wt quercetin	kaempferol
chive	green (50.0)	9 ± 0.42	55 ± 0.52
	white (50.0)	ND	16 ± 0.10
garlic chive	green (31.0)	4 ± 0.02	6 ± 0.02
	white (69.0)	ND	28 ± 0.21
leek	green (32.1)	ND	20 ± 0.01
	white (67.9)	ND	ND
kale, Dwarf Siberian	whole	7 ± 0.05	30 ± 0.08
kale, Vates Blue Curled Dwarf	whole	20 ± 0.44	13 ± 0.16
red cabbage	whole	2 ± 0.09	ND
horseradish	whole	ND	6 ± 0.01
red radish	whole	ND	4 ± 0.62

[a] Values in parentheses are percentages by weight. [b] Mean for triplicate determinations ± standard deviation; ND = not detectable.

ACKNOWLEDGMENT

We thank Lois A. Stringer of the W. Atlee Burpee Co. for providing us with vegetable samples. We also acknowledge the technical assistance of Phyllis Lewis and Melissa Weaver, employees of the Eastern Regional Research Center.

Registry No. Quercetin, 117-39-5; kaempferol, 520-18-3.

LITERATURE CITED

Bilyk, A.; Cooper, P. L.; Sapers, G. M. *J. Agric. Food Chem.* **1984**, *32*, 274.
Bjeldanes, L. F.; Chang, G. W. *Science (Washington, D.C.)* **1977**, *197*, 577.
Brown, J. P. *Mutat. Res.* **1980**, *75*, 243.
Eloesser, W.; Herrmann, K. *Z. Lebensm.-Unters. -Forsch.* **1975**, *159*, 265.
Fukuoka, M.; Yoshihira, K.; Natori, S.; Sakamoto, K.; Iwahara, S.; Hosaka, S.; Hirono, I. *J. Pharmacobio-Dyn.* **1980**, *3*, 236.
Hardigree, A. A.; Epler, J. L. *Mutat. Res.* **1978**, *58*, 231.
Hatcher, J. F.; Pamukcu, A. M.; Ertürk, E., Bryan, G. T. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1983**, *42*, 786.
Herrmann, K. *J. Food Technol.* **1976**, *11*, 433.
Horowitz, R. M. *ACS Symp. Ser.* **1981**, *170*, 43.
MacGregor, J. T.; Jurd, L. *Mutat. Res.* **1978**, *54*, 297.
Meltz, M. L.; MacGregor, J. T. *Mutat. Res.* **1981**, *88*, 317.
Morino, K.; Matsukura, N.; Kawachi, T.; Ohgaki, H.; Sugimura, T.; Hirono, I. *Carcinogenesis (London)* **1982**, *3*, 93.
Pamukcu, A. M.; Yalciner, S.; Hatcher, J. F.; Bryan, G. T. *Cancer Res.* **1980**, *40*, 3468.
Starke, H.; Hermann, K. *Z. Lebensm.-Unters. -Forsch.* **1976**, *161*, 25.
Takanashi, H.; Aiso, S.; Hirono, I.; Matsushima, T.; Sugimura, T. *J. Food Saf.* **1983**, *5*, 55.
Van der Hoeven, J. C.; Lagerweij, W. J.; Bruggeman, I. M.; Voragen, F. G.; Koeman, J. H. *J. Agric. Food Chem.* **1983**, *31*, 1020.
Watson, W. A. F. *Mutat. Res.* **1982**, *103*, 145.
Wildanger, W.; Herrmann, K. *J. Chromatogr.* **1973a**, *76*, 433.
Wildanger, W.; Herrmann, K. *Z. Lebensm.-Unters. -Forsch.* **1973b**, *152*, 134.
Wöldecke, M.; Herrmann, K. *Z. Lebensm.-Unters. -Forsch.* **1974**, *156*, 153.

Received for review June 5, 1984. Revised manuscript received November 5, 1984. Accepted November 13, 1984. Presented at the 186th National Meeting of the American Chemical Society, Washington, DC, Aug 1983. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.