Williams, P. C.; Norris, K. H.; Gehrke, C. W.; Bernstein, K. Comparison of Near-infrared Methods for Measuring Protein and Moisture in Wheat. *Cereal Foods World* **1983**, *28*, 149-152.

Williams, P. C.; Preston, K. R.; Norris, K. H.; Starkey, P. M. Determination of Amino Acids in Wheat and Barley by Near-infrared Reflectance Spectroscopy. *J. Food Sci.* **1984**, *49*, 17-20.

Received for review March 8, 1988. Revised manuscript received September 27, 1989. Accepted December 21, 1989.

Inhibitory Effect of Phenolics on Carotene Bleaching in Vegetables

Jan Oszmianski† and Chang Y. Lee*

Department of Food Science and Technology, Cornell University, Geneva, New York 14456

Lipoxygenase is known to be involved in the indirect oxidation of carotene, and some phenolic compounds are known to prevent lipoxygenase activity. Inhibitory potencies of major phenolics in vegetables on carotene oxidation were studied by using both a model system and various vegetables. Catechin and epicatechin were the highest and *p*-coumaric and ferulic acids were the lowest in inhibitory efficacy. In general, in a model system flavans showed the highest inhibitory effect followed by flavonols and acidic phenolic compounds. The concentration of phenolics in vegetables appeared to have a high correlation with the inhibitory effect on carotene bleaching, in general, but spinach, which contains the highest concentration of phenolics among vegetables studied, did not show the same level of inhibitory potencies. This may be due to the fact that spinach phenolics consist mainly of methylated flavones and *p*-coumaric derivatives, compounds that have low inhibitory effects.

A recent report showed that the apparent increase in vitamin A value in canned peas as compared to fresh peas was not due to an actual increase but rather to the loss of carotenoids in fresh peas during analysis (Edwards and Lee, 1986). The endogenous enzyme system in raw vegetables was shown to have carotene-oxidizing activity (Booth, 1960). Lipoxygenase oxidizes fatty acids and produces peroxides that oxidize carotene by a secondary or coupled reaction (Walsh and Hauge, 1953). The addition of antioxidants, such as pyrogallol, during the analysis for carotene resulted in higher carotene values in fresh vegetables (Ueno et al., 1982). Certain phenolic compounds are known to prevent lipoxygenase activity (Rhee and Watts, 1966; King and Klein, 1987; Takahama, 1985). Lipoxygenase activity and phenolic content vary greatly among vegetables, with the result that carotene bleaching is affected (Lee and Smith, 1988). Little work, however, has been done to determine the effect of different phenolic groups on carotene bleaching by lipoxygenase in vegetables. In this study, the carotene bleaching effect of several major phenolics was investigated by using both a model system and several different vegetables.

MATERIALS AND METHODS

Materials. Spinach, broccoli, carrots, green beans, and peas were obtained from a local market. The β-carotene (1% CWS) was a gift from Hoffmann-LaRoche Chemical Co. The phenolic standards, (+)-catechin, (−)-epicatechin, phloretin glucoside, phloretin, quercetin, rutin, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid, linoleic acid, lipoxygenase, and Tween 80 were purchased from Sigma Chemical Co. Procyanidin B_2 was isolated from grapes (Lee and Jaworski, 1987).

Table I. Relative Inhibitory Effects (%) of Various Phenolics on Carotene Bleaching by Lipoxygenase

phenolics	concn 1.25 mM	2.5 mM	5 mM
(+)-catechin	45.0	75.2	96.4
(−)-epicatechin	33.4	61.6	97.0
procyannidin B_2	29.0	*a*	*a*
phloretin glucoside	7.8	10.9	17.0
phloretin	33.4[b]		
rutin	10.9[b]		
quercetin	11.2[b]		
chlorogenic acid	8.8	14.1	23.2
caffeic acid	9.2	15.9	26.6
p-coumaric acid	2.4	5.4	11.4
ferulic acid	2.0	5.0	8.8

[a] Precipitation took place. [b] Saturated solution, <0.02 mM.

Inhibitory Effect of Phenolics in Model Systems. All phenolic compounds, except for quercetin, rutin, and phloretin, were individually dissolved in pH 7 McIlvaine buffer at concentrations of 2.5, 5, and 10 mM. Quercetin, rutin, and phloretin were made to only one concentration (saturated, <0.2 mM) due to their poor solubilities. β-Carotene (840 μg/mL) was dissolved in McIlvaine's buffer at pH 7; linoleic acid (1.2 mg/mL) was dissolved in the same buffer except that it contained 800 μg/mL Tween 80. Lipoxygenase (1 mg/mL, 126 500 units/mg) was dissolved in distilled water.

Fifteen microliters of lipoxygenase solution was added to a solution containing 0.5 mL of β-carotene, 0.5 mL of linoleic acid,

† On leave from the Agricultural University, 50-375 Wroclaw, Norwida 25, Poland.

0021-8561/90/1438-0688$02.50/0

Table II. Phenolic Content of Various Vegetables and Their Inhibitory Effects on Carotene Bleaching by Lipoxygenase

	phenolics,[a] mg/100 g				inhibition, %			
vegetable	neutral fraction A	neutral fraction B	acidic fraction	total	neutral fraction A	neutral fraction B	acidic fraction	total
beans	4.3	18.8	13.2	36.3	2.1	11.6	13.2	26.9
broccoli	14.8	78.4	46.6	139.8	10.1	21.2	33.3	64.6
carrots	1.6	7.0	49.3	57.9	5.1	4.4	20.9	30.4
peas	8.7	69.6	17.8	96.1	4.1	10.0	34.4	48.5
spinach	10.8	147.7	51.9	210.4	5.5	1.5	16.3	23.3

[a] Calculation is based on chlorogenic acid as a standard.

and 1 mL of phenol solution. The rate of bleaching at 20 °C was measured at 460 nm with a Hewlett-Packard 8452A spectrophotometer equipped with a diode array detector and a series 300 computer.

Extraction of Phenolics from Vegetables. Vegetable phenolics were fractionated into three groups: (1) neutral fraction A, consisting mainly of flavans and other polar phenolics; (2) neutral fraction B, mainly flavonols that are less polar; and (3) acidic phenolics. Vegetable sample (50 g) was homogenized with 100 mL of 80% methanol with a Polytron for 1 min. To prevent oxidation, 0.5 g of ascorbic acid was added. The homogenate was filtered through Whatman filter paper no. 2. This extraction was repeated twice. Methanol and a portion of water were removed with a vacuum evaporator, and the extract was washed with 50 mL of hexane three times to remove carotenoids and other nonpolar compounds. The phenolic extract was brought to a final volume of 50 mL with distilled water. Ten milliliters of the extract was adjusted to pH 7.0 with 1 and 0.2 N NaOH and passed through two C18 Sep-Pak columns (Waters Associates) connected together and preconditioned with 5 mL of methanol and 5 mL of distilled water. After the Sep-Pak columns were washed with 5 mL of distilled water and 5 mL of 0.01 N HCl (acidic fraction), the adsorbed neutral phenolic fraction A was eluted with 5 mL of 10% CH_3CN solution acidified to pH 2. The neutral fraction B was eluted with 5 mL of 40% CH_3CN. The acidic fraction was adjusted to pH 2 with 0.2 N HCl and passed through a C18 Sep-Pak preconditioned with 5 mL of methanol and 5 mL of 0.01 N HCl. The adsorbed acidic phenolics were eluted with 5 mL of 40% CH_3CN. The solvent (CH_3CN) was removed from each fraction with a vacuum evaporator, and the phenolic compounds were dissolved in 5 mL of McIlvaine's buffer at pH 7. They served as the source of phenolics.

Inhibitory Effect of Vegetable Phenolics on Carotene Bleaching. One milliliter of phenolic extract was mixed with 0.5 mL of β-carotene and 0.5 mL of linoleic acid solution. Lipoxygenase solution (15 μL) was added, and the rate of carotene bleaching was measured as described above.

HPLC Analysis. Each phenolic fraction was subjected to HPLC analysis of phenolics according to previously described methods (Jaworski and Lee, 1987; Oleszek et al., 1988). All tests and analyses were carried out in duplicate on duplicate samplings.

RESULTS AND DISCUSSION

Various phenolics commonly found in vegetables were examined in model systems for their inhibitory effects on carotene bleaching by lipoxygenase. As shown in Table I, increased concentrations of individual phenolics increased the inhibitory effects on all 11 phenolics studied. Catechin and epicatechin were especially potent inhibitors of lipoxygenase: at a concentration of 5 mM, both compounds produced over 95% inhibition. Procyanidin B_2, a dimer of epicatechin, also showed a strong inhibitory effect at 1.25 mM, an activity that was comparable to that of epicatechin at that concentration. It was not possible to measure inhibition at higher concentrations of procyanidin B_2 due to the precipitation of the reactants. Phloretin and quercetin derivatives were lower in their inhibitory effects compared to those of the flavans. Since these compounds have very low solubilities, it was not possible to compare them closely with the other phenolics. All acidic phenolic compounds were weak inhibitors of lipoxygenase: chlorogenic acid, the most commonly known acidic phenolic in vegetables, showed less than one-third the inhibitory effect of catechin or epicatechin. Ferulic and *p*-coumaric acids were the weakest inhibitors among all of the phenolics that were studied. Overall, flavans possessed the strongest inhibitory capacity, followed by flavonols and then the acidic phenolic compounds.

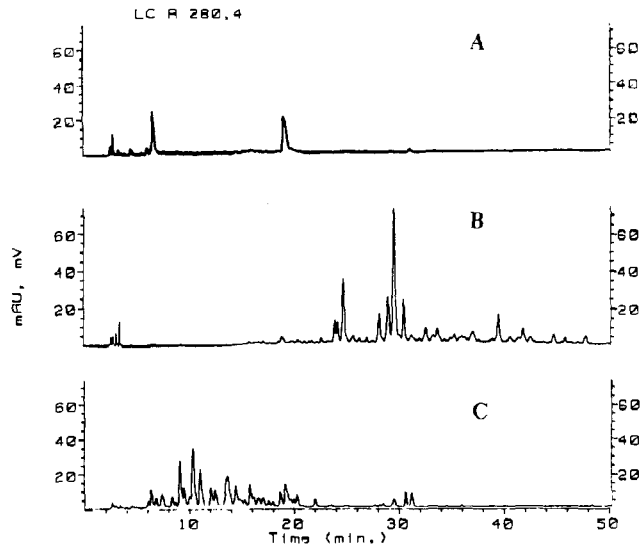

Figure 1. High-performance liquid chromatograms of three phenolic groups in green beans: (A) flavans and polar phenolics; (B) flavonols; (C) acidic phenolics.

The fractionation procedures separated the complex phenolic constituents of vegetables into three major groups that could be readily analyzed by HPLC. Studies on the phenolic compounds in the five vegetables showed that the major phenolics consisted of flavonols (neutral fraction B) and acidic phenolic compounds. No attempt was made to identify individual phenolics in this study. Figure 1 shows a typical representative chromatogram of phenolics in green beans. In general, the number of peaks and the peak area of neutral fraction B (flavonols) were larger than those of neutral fraction A (flavans and other polar phenolics) and the acidic phenolic compounds (C) in all of the vegetables studied. Table II shows the relative amount of phenolics in the five vegetables. Spinach contained the highest concentration of flavonols and acidic phenolics. It was followed by broccoli. Carrots were very low in both flavans and flavonols, while acidic phenolic compounds, especially chlorogenic acids, appeared to be the major phenolics. Overall, beans and carrots were low in phenolic compounds. A moderate concentration of phenolics was found in peas.

Table II also shows the inhibitory effects of the fractions from each vegetable on carotene bleaching by lipoxygenase. Total inhibitory effects for each vegetable were

proportional to the total concentration of phenolics, except for spinach. Broccoli phenolics showed nearly 65% inhibition followed by peas, 48.5%; carrots, 30.4%; beans, 26.9%;, and spinach, 23.3%. In spite of the highest concentration of flavonols and acidic phenolic compounds among the vegetables studied, the phenolics of spinach inhibited carotene bleaching the least. This can be explained by the fact that flavones in spinach were mostly methylated compared to other vegetables (Hermann, 1988) and methylation led to a considerable reduction of antioxidant activity (Letan, 1966). In addition, the acidic phenolic compounds in spinach consisted mainly of *p*-coumaroyl derivatives (Tadera and Mitsuda, 1971; Hermann, 1978), compounds that showed very low inhibitory effects on carotene bleaching by lipoxygenase (Table I).

A careful examination of Table II reveals that neutral fraction A, which consisted mainly of flavans such as catechins and procyanidins and other polar phenolics, had a closer correlation ($r = 0.74$) to the inhibitory effect than neutral fraction B ($r = 0.18$) and the acidic phenolic compounds ($r = 0.01$). This agrees with the observations that were made with the standard phenolics in the model system. Therefore, it is concluded from this study that among the various phenolic groups, flavans and other polar phenolics are the major inhibitors of the lipoxygenase that bleaches carotene in vegetables.

LITERATURE CITED

Booth, V. H. Inactivation of the Carotene-oxidizing System in Iris Leaf. *J. Sci. Food Agric.* **1960**, *11*, 8–13.

Edwards, C. G.; Lee, C. Y. Measurement of Provitamin A Carotenoids in Fresh and Canned Carrots and Green Vegetables. *J. Food Sci.* **1986**, *51*, 534–535.

Hermann, K. Nonessential Constituents of Vegetables. III. Carrots, Celery, Parsnips, Beets, Spinach, Lettuce, Endives, Chicory, Rhubarb and Artichokes. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 262–273.

Hermann, K. On the Occurrence of Flavonol and Flavone Glycosides in Vegetables. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 1–5.

Jaworski, A. W.; Lee, C. Y. Fractionation and HPLC Determination of Grape Phenolics. *J. Agric. Food Chem.* **1987**, *35*, 257–259.

King, L. K.; Klein, B. P. Effect of Flavonoids and Related Compounds on Soybean Lipoxygenase-1 Activity. *J. Food Sci.* **1987**, *52*, 220–221.

Lee, C. Y.; Jaworski, A. W. Phenolic Compounds in White Grapes Grown in New York. *Am. J. Enol. Vitic.* **1987**, *38*, 277–281.

Lee, C. Y.; Smith, N. Effect of Lipoxygenase and Phenolics on the Carotene Analysis of Fresh Vegetables. IFT Annual Meeting, 1988, Abstract 86.

Letan, A. The Relation of Structure to Antioxidant Activity of Quercetin and Some of its Derivatives. 1. Primary Activity. *J. Food. Sci.* **1966**, *31*, 518–523.

Oleszek, W.; Lee, C. Y.; Jaworski, A. W.; Price, K. R. Identification of Some Phenolic Compounds in Apples. *J. Agric. Food Chem.* **1988**, *36*, 430–432.

Rhee, K. S.; Watts, B. M. Effect of Antioxidants on Lipoxygenase Activity in Model Systems and Pea Slurries. *J. Food. Sci.* **1966**, *31*, 669–674.

Tadera, K.; Mitsuda, H. Isolation and Chemical Structure of a New Fluorescent Compound in Spinach Leaves. *Jp. Agric. Biol. Chem.* **1971**, *35*, 1431–1435.

Takahama, U. Inhibition of Lipoxygenase-Dependent Lipid Peroxidation by Quercetin: Mechanism of Antioxidative Function. *Phytochemistry* **1985**, *24*, 1443–1446.

Ueno, T.; Maekawa, A.; Suzuki, T. Effect of Lipoxygenase on the Determination of Carotene in Vegetables. *J. Vit. Soc. Jpn.* **1982**, *56*, 83–89.

Walsh, K. A.; Hauge, S. M. Carotene: Factors Affecting Destruction in Alfalfa. *J. Agric. Food Chem.* **1953**, *1*, 1001–1004.

Received for review September 14, 1989. Accepted December 20, 1989.

Proteins from Double-Zero Rapeseed

Bin Zhou, Zai-qing He, Hua-min Yu, and Kumar D. Mukherjee*

Federal Center for Lipid Research, Institute for Biochemistry and Technology, H. P. Kaufmann-Institute, Piusallee 68, D-4400 Münster, Federal Republic of Germany

Solubility profiles of protein, phytic acid, and glucosinolates in aqueous electrolytes of varying pH were found to be similar for defatted meals from two varieties of double-zero (low glucosinolate/low erucic acid) rapeseed (*Brassica napus*) and conventional Chinese rapeseed (*Brassica juncea*). All three meals yield upon extraction with aqueous electrolytes at pH 4–5 products that are enriched in protein to the extent of 20–35%. Simultaneously, the level of phytic acid in the extracted meals is reduced by about one-third while the glucosinolates are virtually eliminated. Extraction of the defatted meals at pH 12–13 results in dissolution of >90% of the protein, one-third of the phytic acid, and <10% of the glucosinolates. The resulting extract provides upon precipitation of the protein at pH 4–5 protein isolates (>90% protein) in yields of about 60% having phytic acid content of 2–2.5% without any detectable levels of glucosinolates. The amino acid compositions of the meals and protein isolates indicate favorable nutritive value of such products.

Rapeseed and related cruciferous oilseed crops are a rich source of protein (Finlayson, 1976). Technological processes have been suggested in the past for the removal of certain undesirable constituents of rapeseed, such as

0021-8561/90/1438-0690$02.50/0