with much reduced antioxidant activity, resulting from the coupling of two PG free radicals. We (Dziedzic and Hudson, 1984b) found ellagic acid to be inactive in the Rancimat test. It may well be the same compound as we observed as an artifact in our PG degradation.

GENERAL CONCLUSIONS

There has been much controversy as to whether phospholipids are pro- or antioxidants and by what mechanism they exert their effects. The subject has been reviewed in some detail by Brandt et al. (1973). The work presented here sheds some light on this area of uncertainty. Phospholipids, as exemplified by DPE, present in an oil, will evidently degrade, whether or not primary antioxidants are also present, but much more rapidly in their absence than in their presence. It is possible that the mechanism of their degradation may differ in the two situations: we have already shown that the magnitude of their synergistic effects is dependent on the structures of the antioxidants used (Dziedzic and Hudson, 1984a). We therefore propose that the phospholipid or possibly its breakdown compounds interact with the free radicals from the primary antioxidant by regenerating the primary antioxidant and thus prolonging its useful life. The phospholipid must have this capability by virtue of its ability to generate hydrogen radicals or protons.

Phospholipids are partially removed during commercial oil refining by the "degumming" process. This is considered essential since their presence is often associated with undesirable flavors, odors, colors, and surface activity effects. On subsequent bleaching and deodorization the oil will be heated for a period of time at temperatures at or above of 100 °C. Such a system will, with the previous incomplete removal of phospholipid, promote the initial breakdown of the primary antioxidant. Thus, any subsequent storage, due to the lower level of antioxidant, will show a decreased stability and a prooxidant effect of the phospholipid. Such mechanisms may well be relevant to the controversy to date regarding pro- and antioxidant effects of phospholipids.

The growing concern over the safety of synthetic antioxidants has highlighted a need for new antioxidants and antioxidant systems. Phospholipid degradation products are presumably present in foods, e.g. in frying oils, and their incorporation may therefore greatly enhance the effect of an added or naturally present antioxidant.

Registry No. Propyl gallate, 121-79-9; DL-dipalmitoylphosphatidylethanolamine, 5681-36-7.

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Chirality Evaluation of 2-Methyl-4-propyl-1,3-oxathiane from the Yellow Passion Fruit

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In order to determine the chirality of 2-methyl-4-propyl-1,3-oxathiane (MPO), an essential trace component of the yellow passion fruit, a flavor concentrate of fresh yellow passion fruit was prepared by solvent extraction of the neutralized pulp with pentane–dichloromethane (2:1, v/v) and subsequent silica gel LC preseparation using a pentane–diethyl ether mixture (9:1, v/v). The concentrated eluate was further fractionated by repeated HPLC on Spherisorb CN (pentane–diethyl ether, 9:1 and 99:1, v/v), and the fractions were analyzed by coupled capillary gas chromatography–mass spectrometry using multi-ion detection. From the MPO-containing fraction, which still displayed a complex composition of volatiles, MPO was separated by Tenax-GC collection using capillary gas chromatography on Carbowax 20 M. After solvent desorption and concentration, finally, complexation gas chromatography on a chiral nickel(II) bis[3-(heptafluorobutyryl)-1(*R*)-camphorate] capillary column revealed the (2*R*,4*S*)-configurated *cis*-MPO by comparison of its retention time with that of the synthesized reference MPO.

Due to its delicate flavor the volatiles of yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) have been extensively studied (Hiu and Scheuer, 1961; Winter and Klöti, 1972; Huet 1973; Winter et al., 1976; Demole et al., 1979; Casimir et al., 1981; Chen et al; 1982; Engel and Tressl, 1983a, b). Sulfur-containing components and some unusual aliphatic esters have been reported to play important roles in the unique flavor of this tropical fruit. In particular, Winter et al. (1976) stressed the importance of *cis*- and trans-2-methyl-4-propyl-1,3-oxathiane (MPO), whose mixture showed, according to these authors, a strong and natural fruity odor with a green and slightly burnt note. Both MPO isomers have been found in yellow passion fruit in a 10:1 mixture with the cis-configurated compound as the main product.

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Recently, the synthesis and analysis of all the MPO stereoisomers was achieved by our group (Heusinger and Mosandl, 1984). At the same time, it could be demonstrated that all the stereoisomers possess their own unique sensory properties (Mosandl and Heusinger, 1985). Therefore, we were interested to evaluate the chirality of the natural MPO occurring in traces in the yellow passion fruit. In this paper, the procedure to determine the chirality of this important chiral flavor compound is described.

EXPERIMENTAL SECTION

Materials. Yellow passion fruits (32 kg) (*P. edulis* f. *flavicarpa*) were obtained in full ripe state by air freight from Brazil.

Isolation of Volatiles. The fruits were cut into two pieces, and the pulp was separated by pressing through a sieve. The pH of the fruit pulp obtained (15 kg) was adjusted to pH 7.0 by adding 1 N aqueous solution of sodium hydroxide, and the neutral pulp was diluted with distillated water (1:1, v/v).

Liquid–Liquid Extraction. The diluted neutral fruit pulp was extracted with pentane–dichloromethane (2:1, v/v) in several batches over 24 h (Drawert and Rapp, 1968). The extracts were dried over anhydrous sodium sulfate and carefully concentrated to 10 mL by using a Vigreux column (45 °C).

Column Chromatography on Silica Gel. The concentrated extracts obtained from solvent extraction were fractionated on silica gel 60 (Merck), activity grade II, by using a pentane–diethyl ether gradient (Idstein et al., 1984). Cooled (11–13 °C) glass columns, 2.0 cm i.d. × 30 cm, were used. The elution rate was 60 mL/h, and three fractions were separated. Fraction I-S was eluted with 300 mL of pentane, fraction II-S was obtained by eluting with 1000 mL of pentane–diethyl ether (9:1, v/v), and fraction III-S was collected by eluting with 200 mL of pentane–diethyl ether (1:1, v/v). All eluates were dried over anhydrous sodium sulfate and concentrated to 20 mL.

High-Pressure Liquid Chromatography (HPLC). A Gilson HPLC system consisting of pump (Model 303), manometer modul (Model 8083), and UV detector as well as equipped with a 5-μm Spherisorb CN column (250 × 8 mm; Bischoff) was used. Repeated HPLC separations were carried out under different conditions. First series of runs: As mobile phase, a pentane–diethyl ether mixture (9:1, v/v) was used. The flow rate was 4 mL/min, and the pressure was 50 bar. The eluted compounds were detected at 220 nm. In total, 40 runs with injections each of 450–500 μL of II-S were performed and the eluates collected by cutting into two fractions. Fraction I-H was cut from 2.5 to 4.4 min, and fraction II-H was obtained by cutting between 4.4 and 18 min. Fraction I-H was concentrated to approximately 8 mL by distilling off the solvent on Vigreux column (45 °C). Second series of runs: For HPLC rechromatography, a pentane–diethyl ether mixture (99:1, v/v) was now used as mobile phase. The other conditions of fractionation were the same as outlined for the series of first runs. In total, 14 runs with injections each of 450 μL of I-H were performed. The eluates were cut into three fractions by collecting from 2.2 to 3.8 min (I-HH), 3.8 to 5.8 min (II-HH), and 5.8 to 8.2 min (III-HH). Fraction II-HH was concentrated to 2 mL on a Vigreux column (45 °C) for further analysis.

Capillary Gas Chromatography. A Carlo Erba Fractovap 4160 gas chromatograph with fid equipped with a J & W fused silica DB-wax capillary column (30 m, 0.31 mm i.d., film thickness 0.25 μm) and a 2-m uncoated fused silica capillary precolumn as the "retention gap" (Grob and Müller, 1982) was used. On-column injection with an air-cooled injection system was employed. The temperature program was 3 min isothermal at 50 °C and then 50-250 °C at 4 °C/min. The flow rates for the carrier gas were 2.5 mL/min He, for the make-up gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. The detector temperature was kept at 220 °C. Volumes of 0.3 μL were injected.

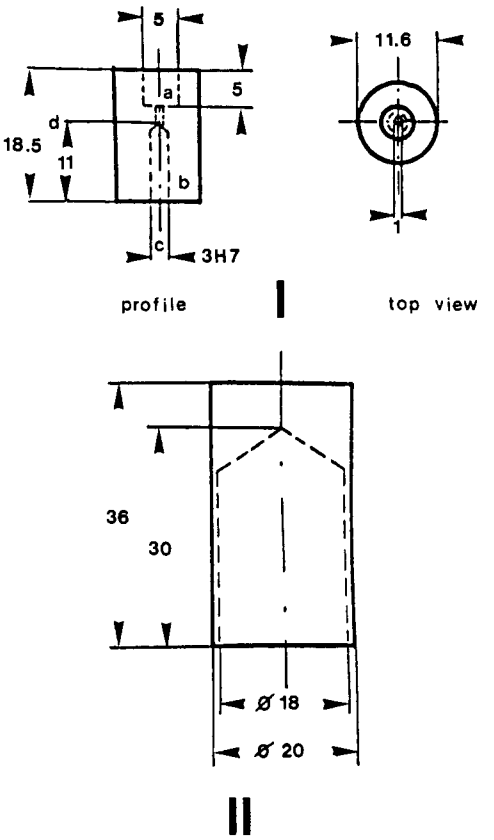


Figure 1. I: Teflon connection (b) to the detector base (c) with Tenax-GC collection tube (a) and end of capillary column (d). II: Hood (material brass) to avoid temperature loss.

Tenax-GC Collection by Capillary Gas Chromatography. A Dani 6500 gas chromatograph with fid equipped with a Hewlett-Packard fused silica CW 20 M capillary column (30 m, 0.31 mm i.d., film thickness 0.25 μm) column was used. PTV injection with solvent split was employed. The temperature program was 4 min isothermal at 50 °C and then 50–210 °C at 4 °C/min. The flow rates for the carrier gas were 2.0 mL/min N_2, for the make-up gas 30 mL/min N_2, and for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. The detector temperature was kept at 250 °C.

Under these conditions the linear retention time of MPO was determined as R_t 1514. Then, instead of using the fid (detector, 250 °C; detector gases switched off), a special Teflon tube containing 10 mg of purified Tenax-GC (Enka) was applied by a suitable Teflon connection (Figure 1) to the detector base, allowing the collection of gas chromatographic eluate at the desired retention time (1514). Ten injections (approximately 4 μL) of II-HH were carried out to collect enough MPO for subsequent chiral capillary gas chromatography.

Chiral Capillary Gas Chromatography. Apparatus and general conditions were the same as described above for capillary gas chromatography on CW 20 M. A 25-m fused silica chiral phase Ni(HFC)$_2$ capillary column (0.125 M in OV 101) was used at isothermal 90 °C (Mosandl et al., 1984).

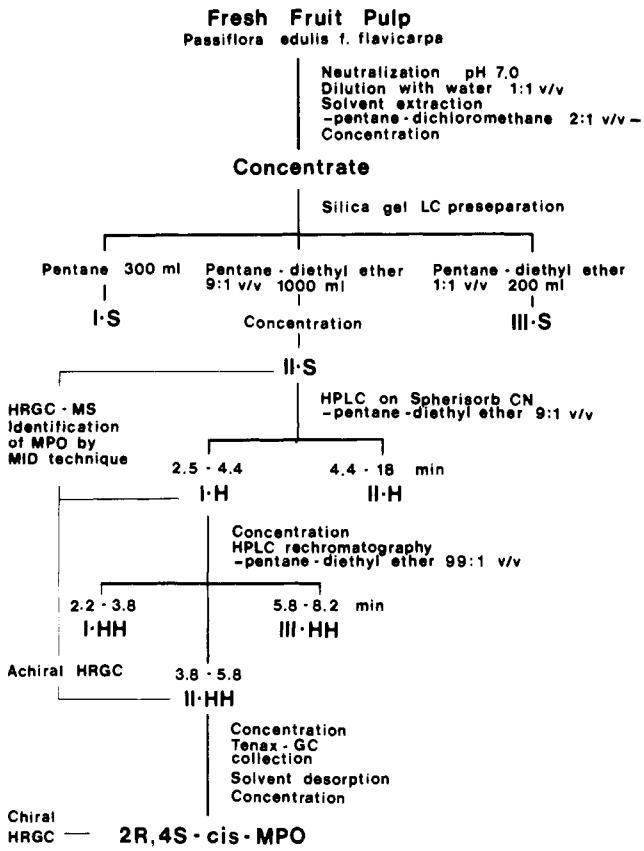


Figure 2. Analytical steps to evaluate the configuration of *cis*-2-methyl-4-propyl-1,3-oxathiane (MPO) from yellow passion fruit.

Capillary Gas Chromatography–Mass Spectrometry. A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injector was coupled by an open-split interface to a Finnigan MAT 44 mass spectrometer. A J & W DB-wax fused silica column (30 m, 0.31 mm i.d., film thickness 0.25 μm) connected to a 2-m uncoated piece of fused silica capillary column as the "retention gap" (Grob and Müller, 1982) was used. The conditions were as follows: temperature, isothermal for 5 min at 60 °C and then from 60 to 250 °C at 5 °C/min; carrier gas flow rate, 2.5 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mV; multiple-ion detection (*m/e* 160, 145); injection volumes, 0.3 μL.

RESULTS AND DISCUSSION

In Figure 2 the steps of our procedure to evaluate the chirality of the trace component 2-methyl-4-propyl-1,3-oxathiane (MPO) from fresh yellow passion fruit (*P. edulis* f. *flavicarpa*) are schematically outlined. Isolation of MPO was achieved by solvent extraction of the neutralized and diluted fruit pulp using pentane–dichloromethane (2:1, v/v). The concentrated extract was preseparated by liquid chromatography on silica gel into three fractions (I-S to III-S), and MPO was detected in fraction II-S by using coupled capillary gas chromatography–mass spectrometry (HRGC–MS). Multi-ion detection (MID) technique was applied monitoring the $M^+ - (m/z\ 160)$ and the $(M - 15)^+$ peaks. In the following, fraction II-S was subjected to repeated HPLC fractionation on Spherisorb CN. In a first series of runs, two fractions were obtained, collecting the eluates from 40 injections each of 450–500 μL of II-S in two cuts: fraction I-H from 2.5 to 4.4 min and fraction II-H from 4.4 to 18 min. As shown by MID technique during HRGC–MS, fraction I-H (Figure 3a) contained MPO. The corresponding analysis by capillary gas chromatography

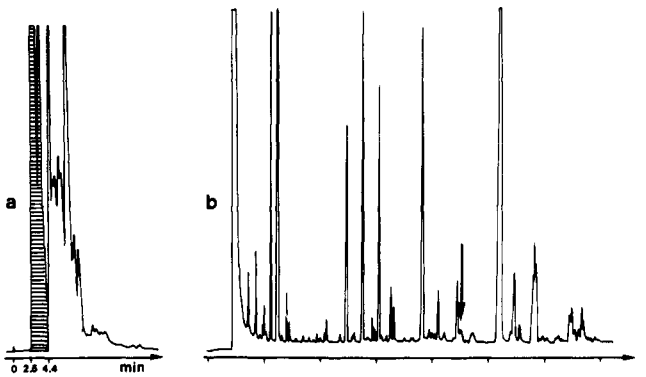


Figure 3. (a) Cut from the first series of HPLC runs (fraction I-H), eluted with pentane–diethyl ether (9:1, v/v). (b) HRGC separation of I-H on a J & W 30 m × 0.31 mm fused silica WCOT capillary DB-Wax, df = 0.25 μm. The arrow indicates the partially resolved MPO.

(HRGC) on CW 20 M still displayed a complex mixture of volatiles with a partially overlapped MPO peak at R_t 1514 (Figure 3b). Therefore, in a second series of HPLC runs fraction I-H was further separated into three fractions (I-HH to III-HH) as outlined in Figure 1. MPO was eluted in fraction II-HH (Figure 4a) and HRGC on CW 20 M showed that it could be now analyzed without any interaction by matrix compounds (Figure 4b). The 70-eV mass spectrum of MPO recorded by HRGC–MS from this fraction was in full accordance with that previously published by Winter et al. (1976).

Recently, Mosandl et al. (1984) demonstrated that nickel(II) bis[3-(heptafluorobutyryl)-1(*R*)-camphorate] [Ni(HFC)$_2$; Schurig and Bürkle, 1982] is an efficient optically active stationary phase for the analytical enantiomer resolution of racemic 1,3-oxathianes without any derivatization procedure. Enantiomer analysis by complexation gas chromatography on a chiral metal chelate such as Ni(HFC)$_2$ is based on the possibility of formation of reversible diastereoisomeric chelate complexes via electron-donor functions of the volatile chiral molecule to be analyzed. However, in our study of natural MPO from yellow passion fruit, a direct analysis by Ni(HFC)$_2$/MS could not be realized because of the occurrence of other complexing volatiles present in the extract of fruit pulp in much higher concentrations than MPO (Figure 4b). Thus, overloading and decreased resolution of the high selective chiral Ni(HFC)$_2$ stationary phase was observed.

To overcome this problem, MPO was separated from fraction II-HH by Tenax-GC collection during HRGC on CW 20 M. Ten injections of II-HH were carried out to collect pure MPO at the desired retention time (R_t 1514). After solvent desorption with diethyl ether and careful concentration, MPO was reinjected onto a chiral Ni(HFC)$_2$ capillary column with fid detection. By comparison of its retention time with those of the four synthesized stereoisomeric MPO (Mosandl and Heusinger, 1985) (Figure 5), it could be demonstrated that *cis*-MPO occurring in traces in yellow passion fruit displayed the (2*R*,4*S*) configuration. As outlined in Figure 6 this stereoisomeric MPO is characterized by a sulfurous, herbaceous green odor quite different from that of the other MPO stereoisomers.

By this laborious way, the stereochemical analysis of an essential sulfur-containing chiral trace component from yellow passion fruit was carried out for the first time. In future studies, separation of trace flavor compounds may be facilitated by, e.g., two-dimensional HRGC using a combination of achiral/chiral capillary columns (Schom-

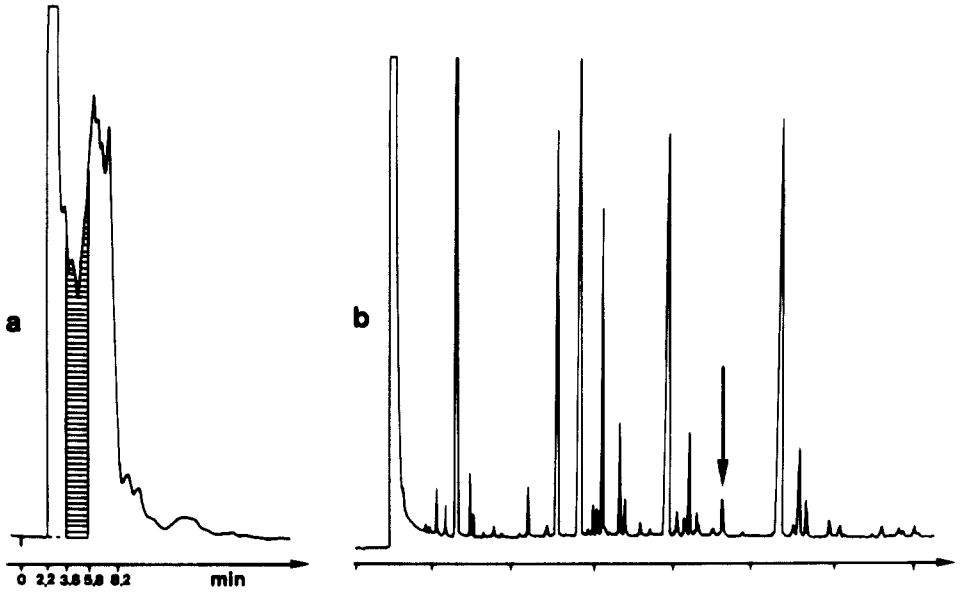


Figure 4. (a) Cut from the second series of HPLC runs (fraction II-HH), eluted with pentane–diethyl ether (99:1, v/v). (b) HRGC separation of II-HH on a J & W 30 m × 0.31 mm fused silica WCOT capillary DB-Wax, df = 0.25 μm. The arrow indicates the now totally resolved MPO.

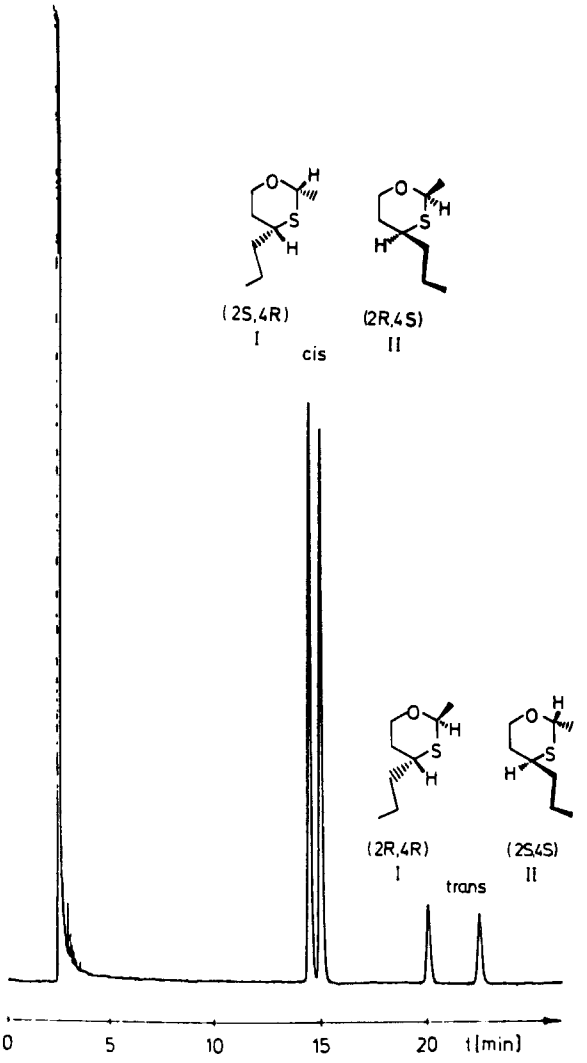
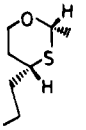
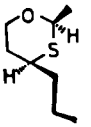
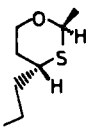
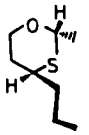


Figure 5. Enantiomer resolution of the *cis*/*trans*-MPO racemates (80:20) on the chiral nickel(II) bis[3-(heptafluorobutyryl)-1-(*R*)-camphorate] capillary (Mosandl et al., 1984).

burg et al., 1984). However, all work on chirality evaluation implies the knowledge of the absolute configuration of reference compounds, i.e. synthesis of the desired stereoisomers.

config	$[\alpha]^{20}_{D}$[a] (*c* 5–15, CCl_4)		sensory characteristics
	2*S*,4*R*	+56.12	odor fatty, fruity green, tropical fruits, grapefruit taste fatty fruity, tropical fruits, passion fruit, guava
	2*R*,4*S*	−56.07	odor sulfurous, herbaceous green, roasty, linseed oil like, onion taste caramel nut, with a slight fruity roasty note
	2*R*,4*R*	+119.4	odor green grass root, earthy red radish note taste herbaceous green
	2*S*,4*S*	−117.6	odor sulfurous, slight bloomy sweet odor taste dry, sticky floury

[a] Optical purity in all cases higher than 99.7%.

Figure 6. Structures as well as odor and taste qualities of stereoisomeric MPO.

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Determination of Total Nonstructural Carbohydrates in Forage Tissue by *p*-Hydroxybenzoic Acid Hydrazide Flow-Injection Analysis

James D. Trent[1] and Scott Christiansen*

An extraction procedure for total nonstructural carbohydrates (TNC) in grasses was developed for *p*-hydroxybenzoic acid hydrazide (PAHBAH) flow-injection analysis. The extraction requires a minimum of 40 min of hydrolysis in 10 mL of boiling 2.5 mM H_2SO_4 to hydrolyze sucrose and fructans. Recoveries of added glucose and fructose ranged from 100 to 107%. The boiling treatment also served to gelatinize starch, allowing rapid starch hydrolysis using amyloglucosidase. Gelatinized preparations of corn, potato, and wheat starch were hydrolyzed to about 80–89% reducing sugars in less than 1 h after enzyme addition. Shoots and stem bases of wheat (*Triticum aestivum*) and Caucasian bluestem (*Bothriochloa caucasica*) and rhizomes of johnsongrass (*Sorghum halepense*) were analyzed for completion of hydrolysis by 0-, 24-, 48-, or 72-h incubations treated with a 0.5% solution of amyloglucosidase at 50 °C. Nearly complete TNC extraction of wheat and Caucasian bluestem occurred at 0 h while a 24-h incubation was necessary for johnsongrass rhizomes to allow effective tissue penetration by amyloglucosidase. Samples stored in the refrigerator for up to 50 days gave similar values when reanalyzed for TNC.

Plants allocate carbon into structural and nonstructural forms (White, 1973). Structural forms include cellulose and hemicellulose and nonstructural carbohydrates include starch (amylose, amylopectin), fructans (inulins, levans), disaccharides (sucrose, maltose), and monosaccharides (glucose, fructose) (Smith, 1981; White, 1973). Glucose and fructose are reducing sugars that react with a variety of compounds to produce colors that are measured spectrophotometrically. The major poly- or disaccharides present in most forage plants can be hydrolyzed to glucose or fructose chemically or enzymatically (Weir et al., 1977; Smith, 1981); hence, the total nonstructural carbohydrates (TNC) of a tissue sample may be estimated spectrophotometrically.

Many methods have been used for measuring TNC as reducing sugars in plant tissues. Common procedures include potassium ferricyanide (FCN) adapted for use with an autoanalyzer (Gaines, 1973; Wolf, 1975; Weir et al., 1977) and the Shaffer–Somogyi titrimetric method using copper reduction (Smith, 1981). Several authors (Powell and Lever, 1972; Lever, 1972, 1973; Lever et al., 1973; Fingerhut, 1973; Davis, 1976) have suggested a colorimetric method based on the reaction of *p*-hydroxybenzoic acid hydrazide (PAHBAH) with reducing sugars. Fingerhut (1973) suggested that PAHBAH was more specific for glucose than is the FCN procedure. In addition, Davis (1976) found that PAHBAH was satisfactory for measuring reducing sugars in tobacco. He suggested that the PAHBAH method was superior to the FCN method because substances other than glucose and fructose contributed to the reducing power of tobacco samples analyzed with FCN.

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Table I. Integrator Input Parameters (s) Required for the *p*-Hydroxybenzoic Acid Hydrazide (PAHBAH) and Ferricyanide (FCN) Flow-Injection Schemes

	PAHBAH	FCN		PAHBAH	FCN
cycle time	92	83	load time	45	45
sample time	8	19	window delay	182	214
load delay	17	29	peak window	90	81

Little information is currently available regarding TNC measurement with flow-injection analysis. Because the CUI and FCN methods are used routinely for measuring TNC in plants, our intention was to develop an automated PAHBAH and FCN procedure using flow-injection that is comparable to the Shaffer–Somogyi CUI method and to document the hydrolysis, incubation, and storage time for TNC extraction from forage tissue.

MATERIALS AND METHODS

Apparatus. The flow-injector was a Lachat Quickchem System IV, Model 170 analyzer [Lachat Chemicals Inc., Mequon, WI; mention of a trade name does not constitute endorsement by the USDA]. Schematic drawings of the flow-injection systems for PAHBAH and FCN are shown in Figures 1 and 2, respectively. Flow-injection and sample loop tubing were made of Teflon and had internal diameters of 0.813 and 0.559 mm, respectively (Lachat Chemicals, Inc.). Other tubing within the system had an internal diameter of 0.813 mm. The heating coil was made of Teflon tubing (i.d. = 1.6 mm). The proportioning pump speed was set on 80% maximum. Injected sample volumes were calculated from internal tubing dimensions. Approximate sample volume was 44.9 μL with a sample loop

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