Figure 2. Suggested mechanism for the hydroxylation of carotenoids on Micro-Cel C. Possible Lewis acid sites on the surface of the adsorbent may function in a similar manner to the BF_3 reaction described by Petracek and Zechmeister (1956).

C was used, coupled with the positive reaction of 3,4-dehydro-β-apo-8′-carotenal, suggests a mechanism such as depicted in Figure 2. Lewis acid sites on Micro-Cel C are conceivable in light of the conversion of the 5,6-epoxide group of violaxanthin to the 5,8 isomer reported by Strain et al. (1967).

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Comparison of a High-Performance Liquid Chromatographic and Microbiological Method for the Determination of Niacin in Foods

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A high-performance liquid chromatographic (HPLC) method has been developed for the determination of niacin in foods. Samples are extracted in alkaline medium. The niacin is subjected to chromatography on both reverse-phase and anion-exchange columns to ensure adequate separation from interfering substances. Automatic column switching is used to transfer the niacin fraction from the reverse-phase to the anion-exchange column, and the niacin is detected by its absorption at 254 nm. This method was compared with a microbiological method. The results obtained by both methods agreed reasonably well.

A number of HPLC methods for the determination of niacin in foods have been published, and a review of the available methods is given by van Niekerk (1982). With the available single-column methods (using UV detection) we could not obtain chromatograms where the niacin peak was adequately separated from interfering peaks. A HPLC method, using the principle of column switching (Snyder and Kirkland, 1979) to improve separation, has, therefore, been developed by us.
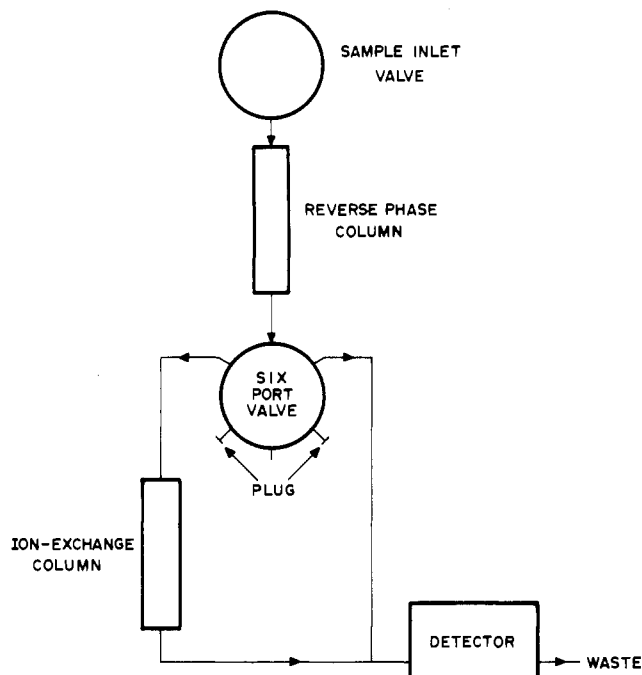
EXPERIMENTAL PROCEDURES

Apparatus. The HPLC equipment consisted of a Varian 5000 pump with three proportioning valves, a Valco inlet valve, a Model 1203 UV monitor from Laboratory Data Control with a 254-nm filter and a 3390 A reporting integrator from Hewlett-Packard. A motorized Valco

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six-port valve, controlled by the microprocessor of the pump, was used for column switching. A schematic diagram of the column configuration is shown in Figure 1. Stainless steel columns (15 × 0.46 cm) were slurry packed with Nucleosil 5 C_{18} for reverse-phase chromatography or Nucleosil 5 SB (Machery-Nagel) for anion-exchange chromatography.

A Metrohm potentiograph E 436 was used for titrations in the microbiological method.

Reagents. Mobile phase A was prepared by mixing 5.7 mL of glacial acetic acid with 800 mL of water, adjusting the pH to 3.0 with sodium hydroxide, and diluting to 1 L with water. Mobile phase B was prepared by mixing 5.7 mL of glacial acetic acid with 800 mL of water, adjusting to pH 3.0 with sodium hydroxide, diluting to 1 L with water and finally mixing this solution with methanol in a ratio of 5:95. Mobile phase C was prepared by mixing 22.8 mL of acetic acid with 800 mL of water, adjusting the pH to 3.0 with sodium hydroxide, and diluting to 1 L with water.

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Figure 1. Schematic diagram of the column configuration used for column switching.

Figure 2. Chromatogram of an alkaline extract of a millet sample on a reverse-phase column. Stainless steel column (15 × 0.46 cm i.d.) packed with Nucleosil 5 C_{18} with mobile phase A at 1 mL/min and UV detection at 254 nm.

Figure 3. Chromatogram of an alkaline extract of a millet sample on an anion-exchange column. Stainless steel column (15 × 0.46 cm i.d.) packed with Nucleosil 5 SB with mobile phase C at 1 mL/min and UV detection at 254 nm.

Figure 4. Chromatogram of an alkaline extract of a millet sample on an anion-exchange column after cleanup on a reverse-phase column with column switching.

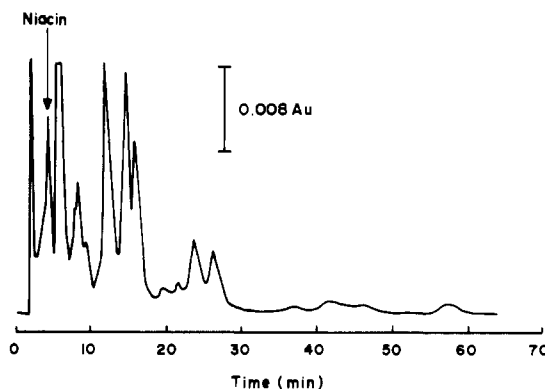The dried niacin assay medium was obtained from Difco Laboratories.

The diastase enzyme suspension was prepared by dissolving 68 g of sodium acetate (hydrated) in 130 mL of water, suspending 0.5 g of Clarase-1500 (Miles Laboratories) in this solution, and diluting the suspension to 200 mL with water.

Niacin standards for HPLC were prepared by dissolving 50 mg of niacin in 100 mL of water and diluting it to contain 1, 10, 25, 50, 100, and 250 μg/mL.

Methods. Acid extracts of the samples (1–5 g) were made by autoclaving with 60 mL of 0.1 M sulfuric acid for 1 h at 1 bar of pressure. After the mixture was cooled, 10 mL of the diastase enzyme suspension was added and the samples were kept in a water bath at 45 °C for 3 h. The suspension was cooled, diluted to 100 mL, and filtered before analyzing for niacin by HPLC or microbiologically.

Alkaline extracts of the samples (1–5 g) were made by adding 80 mL of water and 10 mL of a suspension containing 1 g of calcium hydroxide. After being heated for 30 min on a steam bath the samples were autoclaved at 1 bar of pressure for 30 min. The cooled suspension was diluted to 100 mL and left overnight in a refrigerator before centrifuging for 20 min at 1800 rpm. The supernatant was used for niacin analysis.
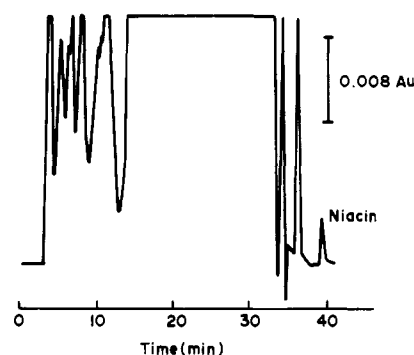
The extracts for HPLC analysis were filtered through Schleicher & Schüll regenerated cellulose membrane filters of 0.6-μm pore size, and 80 μL was injected onto the reverse-phase column. Mobile phase A was pumped through the reverse-phase column at a flow rate of 1 mL/min. The effluent from this column went directly to the detector and then to waste. During the period when the fraction, containing the niacin, was expected to elute, the effluent from the first column was diverted via the six-port valve to the ion-exchange column. The elution time of the fraction was determined prior to the analysis by spiking a sample with sufficient niacin to give a peak, readily distinguishable from the background. The valve was switched back, to complete elution of the reverse-phase column with mobile phase B at 2 mL/min, without disturbing the niacin fraction in the ion-exchange column. The niacin was subsequently eluted from the ion-exchange column by passing mobile phase C
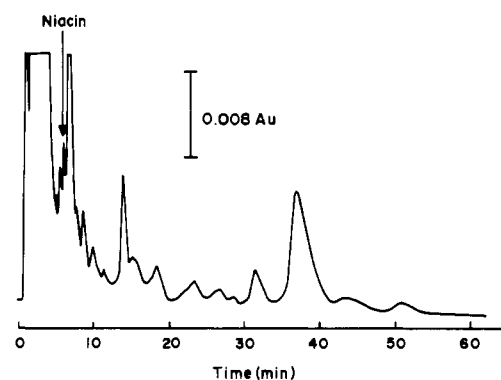
through both columns at 1 mL/min.

A calibration curve was obained by injecting 1–250 μg/mL niacin. Recovery determinations were conducted by adding niacin to a millet sample before extraction at a level of 1.2 mg/100 g.

The microbiological method is described by the Association of Vitamin Chemists, Inc. (1951). The standard curve was drawn by fitting a third degree polynomial by the method of least squares, and interpolation on the standard curve was done with the aid of the resultant equation.

RESULTS AND DISCUSSION

The chromatogram of an alkaline extract of a millet sample, chromatographed on the reverse-phase column with mobile phase A, is shown in Figure 2. A chromatogram of the same extract on the ion-exchange column,

Table I. Timing for Mobile Phase Changes and Column Switching

time, min	mobile phase	flow rate, mL/min	columns[a] through which the mobile phase is passed	comment
0	A	1	1	sample on column 1
3	A	1	1 and 2	place niacin fraction on column 2
5.5	A	1	1	stop flow through column 2, elute column 1
7	B	1.5	1	clean column 1 with mobile phase B
25	C	1.5	1	equilibrate column 1 with mobile phase C
30	C	1	1	change flow rate
33	C	1	1 and 2	start eluting column 2 with mobile phase C; reset base line
45	A	1	1 and 2	equilibrate columns 1 and 2 with mobile phase A
55	A	1	1	flow through column 1 only; ready for next sample

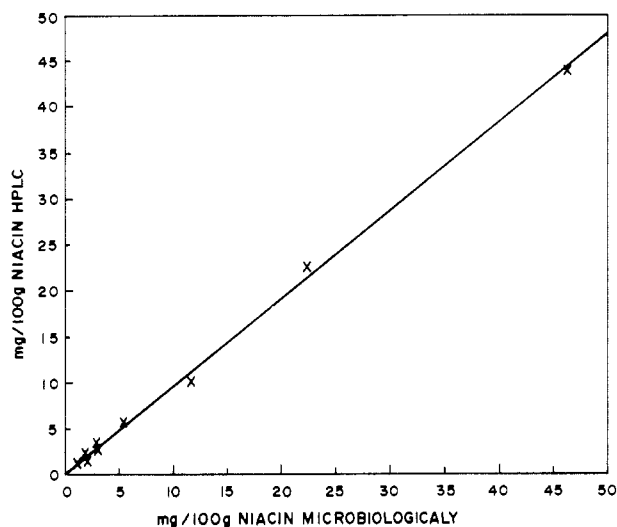[a] Column 1 refers to the reverse-phase column and column 2 to the anion-exchange column.

Figure 5. Comparison of the results obtained by the microbiological and HPLC methods on alkaline extracts of various samples.

using mobile phase C, is shown in Figure 3. The niacin is inadequately separated from the other peaks in both chromatograms and can, therefore, not be quantitated with accuracy. Separation on both columns, with column switching, ensures adequate resolution from interfering substances (Figure 4). Using mobile phase A, the niacin elutes from the first (reverse-phase) column with a capacity factor $k' = 1.6$. The niacin is transferred to the second (ion-exchange) column with the same mobile phase. With this mobile phase the niacin is strongly retained on the top of the column. It is, therefore, concentrated on the second column, ensuring a narrow inlet band that was finally eluted with mobile phase C. The high methanol content of mobile phase B ensures that all the strongly retained compounds are eluted from the first column before elution of the second column is commenced. The timing for mobile phase changes and column switching is given in Table I.

A linear calibration curve ($y = 22.84x + 26.14$, correlation coefficient $r = 0.99989$) for peak area against concentration in the range of 1–250 μg/mL niacin was obtained.

Nine different samples were extracted in alkaline and acid media, and the niacin was determined by HPLC and microbiologically. The samples consisted of maize meal, a high-fiber biscuit, rice, spaghetti, mushrooms, breakfast cereal, atchar powder, soybean meal, and mopani worms (an African delicacy). A niacin standard (0.15 mg) was also

Table II. Niacin Content of Samples Analyzed by the HPLC and Microbiological Methods

	niacin, mg/100 g			
	acid extract		alkaline extract	
sample	micro-biological	HPLC	micro-biological	HPLC
high-fiber biscuit	22.0	19.8	21.6	22.7
	22.6	20.0	22.7	22.2
maize meal	1.2	0.4	1.3	1.1
	1.2	0.5	1.3	1.3
rice	5.1	2.4	5.5	5.6
	5.0	2.4	5.5	5.6
spaghetti	1.9	1.0	2.2	2.4
	1.9	0.9	2.2	2.5
mopani worms	11.8	4.5	11.6	10.6
	11.7	4.4	11.5	9.9
mushrooms	46.8	29.6	45.8	44.2
	46.5	30.7	46.3	43.7
breakfast cereal	2.7	1.0	3.1	3.0
	2.6	1.0	3.1	3.3
atchar powder	2.3	1.3	2.2	1.6
	2.3	1.2	2.3	1.4
soybean meal	3.3	2.3	3.2	2.8
	3.2	2.0	3.2	2.8
pooled CV for 9 samples, %	1.4	7.0	1.6	5.8
standard (0.15 mg), mg	0.146	0.152	0.157	0.149
	0.146	0.148	0.148	0.153
	0.150	0.148	0.148	0.151
	0.150	0.149	0.155	0.149
	0.149	0.150	0.151	0.147
CV for standard, %	1.8	1.1	2.7	1.5

subjected to the two extractions. The results are given in Table II. The niacin results obtained microbiologically, on both alkaline and acid extracts, showed no difference (as determined by a pairwise T test on the means, $P \le 0.5$ for the samples and $P \le 0.05$ for the means of the standard). The HPLC method, however, gave lower values ($P \ge 0.05$) for the acid than for the alkaline extracts, except for the standard, which showed no difference at $P \le 0.5$. This might indicate that part of the niacin in the acid extracts was not present as free niacin but existed in a form available to the microorganisms. The results for the HPLC method on the alkaline extracts were plotted against the results of the microbiological method (Figure 5) and a straight line ($y = 0.9625x - 0.038$, $r = 0.9992$) was fitted to the data by the method of least squares. On testing whether the line differed from the theoretical line $y = x$ (Bowker and Lieberman, 1959), it was found that it dif-

fered marginally (F = 5.4) at $P \le 0.05$. Independent tests on the slope and y intercept indicated that the slope did not differ from 1 (t = 2.35, $P \le 0.05$) and the intercept did not differ from 0 (t = 0.123, $P \le 0.05$). A pairwise T test on the data did not indicate a difference (t = 1.65 at $P \le 0.1$). It is therefore apparent that the two methods gave results which were in fair agreement (on the alkaline extract). The microbiological method has better precision than the HPLC method. This is to be expected since the values for the microbiological method is estimated from the mean of five different dilutions of the sample.

The recovery by the HPLC method on the millet sample was 100.1% (coefficient of variation CV = 4.5%, n = 10) and for the microbiological method 101.1% (CV = 6.6%, n = 5). The HPLC method has a detection limit of 0.5 mg of niacin/100 g of sample when a 5-g sample is extracted and diluted to 100 mL.

A chromatogram can be completed within ca. 1 h. The HPLC method would, therefore, have an advantage when a small number of samples must be analyzed. For a large number of samples, the HPLC method would only be able to compete with the microbiological method, in the number of samples analyzed, if an automatic sampler is used to enable the chromatograph to be run overnight.

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Progel and Gel Formation and Reversibility of Gelation of Whey, Soybean, and Albumen Protein Gels

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Dynamic shear measurements were used to determine the kinetics of development of structural rigidity in thermally produced egg white (EW), whey protein concentrate (WPC), and Promine D (PD) gels by measuring the change in the storage modulus (G') during heating and cooling. EW showed rapid and extensive G' value development with heating time whereas G' development in WPC was much slower and less extensive. When cooled, both protein systems showed a reversible cooling "set" with the WPC "set" approximately 10× greater than that of EW. Thermal development of G' values could be described by a first-order kinetic equation of the form $G' = G'_\infty(1 - e^{-kt})$. G' development in PD gels was characterized by the presence of considerable elasticity in the aqueous suspensions prior to heat application and rapid development of G' values on heat application. This development could not be described as first order and no cooling set was observed in PD gel samples. Scanning electron microscopy showed EW and WPC gels to consist of a network of spherical particles apparently adhering together. PD gels consisted of very large particles embedded in a gel matrix.

The basic texture and structure of many foods depends upon the formation of relatively rigid structures by protein through the process of thermal gelation. Despite this obvious importance of protein thermal gelation to food formation, little is known of gel formation following initial denaturation (Shimada and Matsushita, 1980, 1981) and few methods exist to accurately measure gel formation (Hermansson, 1979). Potentially, valuable information on the degree and mechanism of gelation would be available if it were possible to follow the time course of gelation (Catsimpoolas and Meyer, 1970), but following formation of structure in a heated protein solution or suspension is difficult. Commonly, rheological instruments are designed to measure the "gel strength" of the preformed gel (Beveridge et al., 1980; Schmidt et al., 1979) or the apparent viscosity of heated solutions or suspensions (Hermansson, 1975, 1978; Catsimpoolas and Meyer, 1970). None of these methods are very suitable for following the time course of gelation since either the gel must be performed or the shearing forces generated during measurement destroy the forming structure.

Dynamic shear measurements are made at small total strains and the rheological properties measured are essentially those of the undisturbed material (Elliott and Ganz, 1975), avoiding the problems associated with structural breakdown during measurement. Evaluation of dynamic shear stress response to small amplitude oscillation requires evaluation of two moduli, the storage modulus (G'), a measure of energy stored due to elastic deformation of the sample, and the loss modulus (G''), a measure of energy dissipated as heat due to viscous flow within the sample. The sinusoidally varying shear stress and strain signals are separated by a phase difference, the tangent of which, the loss tangent, is numerically equal to the ratio G''/G'. In principle, as gel networks form, the sample becomes more elastic in nature and G' values will rise while the loss tangent values fall.

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