casein used in the assay was produced locally and was not of superior quality. The addition of 0.15% DL-methionine to the diet B doubled the PER value, indicating that methionine is the limiting amino acid in lupin protein. This result supports the amino acid composition data which also show methionine as the most limiting essential amino acid.

The data of Table V also show that heating the flour did not improve the biological value of the proteins, suggesting nonexistence of heat-labile toxic constituents. Assays for trypsin inhibitor and for phytohemagglutinin were negative, indicating absence of these antinutrients in the lupin seeds used in this study.

The very low biological value found for the isolated protein might be explained in terms of: (a) loss of sulfur amino acids during extraction and precipitation (Table III); (b) possible formation of cross-linkages and toxic material under the alkaline extraction condition (pH 9.0); (c) poor digestibility of the isolated protein.

These problems are being investigated further and will be the subject of a future communication.

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Analysis of Sorbic Acid in Dried Prunes by Gas Chromatography

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A gas chromatographic procedure for the quantitation of sorbic acid in processed dried prunes down to trace levels was developed. Elimination of interfering compounds in dried prunes and high recoveries of sorbic acid were obtained with a double-extraction procedure. Inclusion of a decanoic acid internal standard prior to the initial extraction eliminates many sources of error which can occur during the extraction procedure and subsequent *n*-butyl ester derivatization. Recoveries of added sorbic acid for this analytical procedure averaged 98.3%. Confirmation of the presence in processed prunes of 1 ppm added sorbic acid was obtained by comparing the chromatograms of the *n*-butyl esters from the final extract with and without permanganate oxidation which eliminates sorbic acid. Positive confirmation by gas chromatography–mass spectroscopy of down to 4 ppm of added sorbic acid was also accomplished.

Sorbic acid is used in many countries to preserve a wide variety of processed foods. It has been used for high-moisture dried prunes since the late 1950's. Analysis of sorbic acid is necessary to insure that a sufficient amount is present (150–450 ppm) in prunes, depending on their moisture level, to prevent spoilage (Nury and Bolin, 1962) and that the amount is within the tolerance (0–1000 ppm) set by the purchasing country (Dada, 1975). Recently, buyers have ordered prunes which are free of sorbic acid. Traces of sorbic acid can occasionally occur in prunes even when they have not been intentionally dipped or sprayed with sorbic acid, due to its presence on processing plant equipment.

Naturally occurring compounds, compounds which form during storage and impurities in reagents can cause erroneous results in trace level analysis and variable blank values in routine quantitative analysis. Currently used analytical methods are not able to accurately confirm the presence of trace levels of sorbic acid in prunes.

This paper presents a gas chromatographic analytical procedure for the quantitation and positive identification of sorbic acid down to trace levels in prunes. It utilizes a modification of a previously reported rapid double-ex-

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traction procedure (Stafford, 1976), using the less toxic dichloromethane for extraction instead of chloroform. Easily made *n*-butyl ester derivatives are used to avoid the problems associated with chromatographing free acids (Cochrane, 1975). The analytical procedure is also suitable for benzoic acid, which we have found to occur naturally at trace levels in dried prunes.

Gas chromatography–mass spectroscopy and a modification of the procedure described by Wilanowski (1974), in which sorbic acid is oxidized by an aqueous permanganate solution, are used for the positive confirmation of the presence of trace quantities of sorbic acid.

EXPERIMENTAL SECTION

Reagents. The following reagents were used in these experiments: (1) dichloromethane, reagent grade and glass distilled as needed; (2) Skellysolve B, glass distilled as needed; (3) boron trifluoride, 1-butanol, 14% by weight (Analabs, Inc.); (4) anhydrous sodium sulfate powder, reagent grade; (5) potassium permanganate, reagent grade, solution prepared by dissolving 15 g of $KMnO_4$ in distilled water, diluted to 100 mL, and filtered through glass wool; (6) sorbic acid (Eastman); (7) decanoic acid (Eastman), practical grade; (8) hydrochloric acid, reagent grade, diluted to 6 N.

Extraction. French variety prunes, harvested at mid and late season, were tunnel dried at this laboratory for use in these experiments. The prunes were prepared for extraction by blending 50 g of ground prunes with 450 mL of distilled water in a 1-qt blender jar for 5 min. A 50.0-g aliquot of the slurry was weighed into a 500-mL separatory funnel and a known amount of the internal standard, decanoic acid, diluted in dichloromethane was added by volumetric pipet. The mixture was extracted twice, first with 250 mL and then with 200 mL of dichloromethane, by shaking for 1 min each. To the combined extract, sufficient anhydrous sodium sulfate was added to remove residual water and the extract was reduced to approximately 25 mL in a vacuum rotary evaporator. The extract was transferred to a 250-mL separatory funnel and extracted twice with 25 mL of 0.5 N $NaHCO_3$ by shaking for 1 min. The dichloromethane layer was discarded and the combined aqueous extract was made acid to pH paper by carefully adding, with agitation, hydrochloric acid (6 N). The acidified aqueous extract was then extracted twice with 100 mL of dichloromethane and the combined extract was dried with anhydrous sodium sulfate.

Confirmatory Test. For qualitative analysis half of the combined dichloromethane extract was transferred to a 250-mL separatory funnel. Two milliliters of the potassium permanganate solution was added and the mixture was shaken for 1 min. The dichloromethane layer was drained off and dried with anhydrous sodium sulfate before proceding with the *n*-butyl ester formation described below.

Esterification. The dichloromethane extract was taken to dryness in a vacuum rotary evaporator. Two milliliters of BF_3–butanol was added to the dry residue and the mixture was refluxed on a steam table using a cold water condenser for 20 min. The reaction mixture was allowed to cool and 2 mL of Skellysolve B was added before transferring to a 60-mL separatory funnel. The mixture was then extracted twice with 25 mL of distilled water by shaking for 1 min to remove excess butanol and inactivate the boron trifluoride. The Skellysolve B layer was transferred to a 5-mL volumetric flask containing sufficient anhydrous sodium sulfate to remove residual water.

Gas Chromatography. A Tracor Model 222 gas chromatograph equipped with a flame ionization detector was operated isothermally at 135 °C. The 6 ft × $^1/_4$ in o.d. stainless steel column was packed with 80/100 mesh Chromosorb W, HP coated with 10% AT-1000 (Alltech Associates). Helium flow at the detector was 50 mL/min and the detector and inlet were operated at 250 °C. A 1 to 10 μL injection was used depending on the concentration of *n*-butyl esters present.

Table I. Sorbic Acid Recovery

sorbic acid added, ppm	% recovery[a]	standard error of mean
4.3	94.8	0.78
42.6	99.2	0.94
211.0	101.0	0.25

[a] Average of four runs.

The presence of sorbic acid was confirmed by the loss of the *n*-butyl sorbate peak from the permanganate oxidized extract. Quantitation was accomplished by measuring the areas of sorbic acid and decanoic acid peaks with a planimeter and applying the formula:

$$\text{ppm sorbic acid} = \frac{\text{area sorbic acid}}{\text{area decanoic acid}} \times \text{wt (mg) decanoic acid} \times 200$$

GC–Mass Spectroscopy. A H.P. 5721A was interfaced with an all-glass jet separator by VG Micromass to a VG Micromass 70/70 F mass spectrometer. The 8 ft × $^1/_8$ in o.d. stainless steel column was packed with 100/120 mesh Chromosorb W, HP coated with 5% AT–1000. Linear flow was 50 mL/min. The gas chromatograph was operated isothermally at 115 °C. A 1-μL injection was used for all analyses. The jet separator was maintained at 180 °C and the yield was 25%. The chromatographic records were reconstructed gas chromatograms based on the total ion current of each scan. The scan rate was 3 s/decade.

RESULTS AND DISCUSSION

The extraction efficiencies for the analytical procedure at three levels of added sorbic acid are shown in Table I. Addition of the chemically similar decanoic acid internal standard at the start of extraction eliminates many possible sources of error which can occur during extraction and subsequent esterification. The use of 1-butanol–boron trifluoride produces, in a short time, *n*-butyl esters which are nonvolatile and relatively water insoluble.

In the unprocessed dried prune controls no *n*-butyl sorbate was found at the detection limit <1 ppm with the gas chromatograph and <4 ppm with the GC–mass spectrometer. In a sample with 4 ppm sorbic acid added, *n*-butyl sorbate was positively identified by the GC–mass spectrometer and the molecular ion at m/e 168 was observed. The presence of 0.7 and 1.6 ppm of added sorbic acid in two processed prune samples was confirmed using the qualitative permanganate procedure. Gas chromatography of the *n*-butyl esters from the unoxidized extracts showed the presence of a peak with retention time of *n*-butyl sorbate. Enrichment with a *n*-butyl sorbate standard confirmed this. The peak was not present in the extracts which had been subjected to permanganate oxidation before the esterification procedure was accomplished, but three new small peaks <1 ppm each were formed. These peaks can be differentiated by their retention times from *n*-butyl sorbate, but their presence does indicate a need for caution when interpreting chromatograms from permanganate oxidized extract.

Benzoic acid was not detected at the 1 ppm level in the two lots of fresh prunes used in these experiments. However, benzoic acid was detected at the 4 ppm level by gas chromatography on the above two lots of fresh prunes

after they were dried and this presence was confirmed by GC–mass spectroscopy. The benzoic acid was evidently formed from the oxidation of naturally occurring benzaldehyde during drying.

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Analyses of Vitamin B_6 in Extractives of Food Materials by High-Performance Liquid Chromatography

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The B_6 vitamin components, pyridoxal, pyridoxine, and pyridoxamine, from extractives of fruits and vegetables were analyzed by use of high-performance liquid chromatography (LC). The B_6 components were obtained free from interfering materials by ion exchange. The 5′-phosphate esters of the B_6 components were hydrolyzed enzymatically with no adverse effects, thus permitting the determination of total B_6 content in the food samples. The LC columns (SCX) were developed with buffered phosphate solutions. Recovery of pyridoxine was quantitative; recovery of pyridoxal and of pyridoxamine was 60 and 85%, respectively. The consistency of the results and a precision of ±2% indicate this method is applicable to quantitative as well as qualitative analyses of these essential nutrients.

With the increasing interest in nutrition and emphasis on nutritive labeling we undertook to develop an accurate and rapid method suitable to fill these needs. Although the analysis of the B_6 vitamers (namely pyridoxal, pyridoxine, and pyridoxamine) using high-performance liquid chromatography (LC) is no longer new, the application has been only to pharmaceutical products or pure vitamin preparations (Cole et al., 1973). There is no information in the literature reporting on the successful use of LC for the determination of B_6 vitamins in foodstuffs.

B_6 vitamins occur naturally both in free and phosphate-bound forms (5′-phosphate, as coenzyme). In order to determine the total B_6 activity these bound forms must be cleaved. Therefore, it was necessary to ascertain the stability of the B_6 compounds when they are exposed to the rigorous hydrolysis treatment, which usually meant incubating the extracts at elevated temperatures and low pH for an extended period of time.

A second factor was the desirability of being able to use the extract prepared for the analyses of other vitamins such as riboflavin, thiamin, and ascorbic acid for the B_6 analysis without further handling. This would represent a substantial savings in time and effort of the analyst. If this was not feasible, then a third factor, the quantitative isolation of the desired B_6 components from the food extracts, would have to be developed.

The selection of correct column packing and developing solvent systems to achieve a rapid, high-resolution, and quantitative operation with the LC apparatus was of primary importance. Williams and Cole (1975) described the use of Aminex A-5 resin for the analysis of the B_6 isomers but did not apply this procedure to foodstuffs. Other investigators have used various ion-exchange resins such as Dowex AG50W to separate the pyridoxyl components, but have used either the usual bioassay (Toepfer and Lehmann, 1961) or fluorometric methods for the final quantitations (Chin, 1975; Gregory and Kirk, 1977). We report here a procedure which uses ion exchange to clean up food extracts, followed by vitamin B_6 quantitation with LC.

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EXPERIMENTAL SECTION

Apparatus and Materials. A Perkin-Elmer Model 601 LC unit equipped with an LC-55 variable wavelength detector was used for liquid chromatography.

Columns were stainless steel, 0.023 mm i.d. × 50 cm or 1 m, packed with Zipax SCX (Dupont).

The authentic B_6 vitamers (pyridoxal·HCl, pyridoxine·HCl, and pyridoxamine·H_2O·2HCl) were obtained from Calbiochem Laboratory.

Taka-diastase (Parke-Davis) was used as the phosphatase, papain, NF VIII (Difco Lab) as the protease enzymes. Dowex AG50W resins were obtained from Bio-Rad Laboratory.

Phosphate buffered solutions were prepared from reagent grade KH_2PO_4, and the pH was adjusted by using KOH or H_3PO_4 solutions.

Methods and Procedures. Standard stock solutions of authentic B_6 vitamers were made in glass distilled water. These solutions contained 0.1 mg (100 μg)/mL of each component and were diluted to lower concentrations with water. Samples of solutions containing individual B_6 vitamers as well as 1:1:1 mixtures of all three were injected onto the LC columns. The volume of samples injected onto the LC column was 20 μL. The lower limit of sensitivity of the detector was determined to be in the magnitude of 0.002 μg/20 μL (0.0002 aufs) equivalent to 100 μg or 0.1 mg/L.

Extracts of fruits and vegetables were prepared according to the procedure described by Pippen et al. (1975). The procedure was blended with equal weight of 95% ethanol. Twenty-five milliliter aliquots of each slurry were transferred to 100-mL actinic red volumetric flasks. Control B_6 mixtures of various concentrations were dissolved in 25 mL of water. All samples and controls were treated in the following manner. Sixty milliliters of 0.1

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