Some Physicochemical and Nutritional Properties of a Sweet Lupin (*Lupinus albus* var. *multolupa*) Protein

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The lupin variety utilized in this study contained between 38–40% crude protein on a dry basis. The proteins were 55% soluble in water and 80–85% soluble in aqueous alkaline solution at pH 9.0. The pH of lowest solubility was 4.5. Fractionation of the alkaline extract by gel filtration on Sephadex G-100 gave three elution peaks. Sucrose gradient centrifugation furnished three bands with sedimentation coefficients of 8.1, 2.8, and 1.2 S, and the calculated molecular weights (mol wt) were 163 000, 53 000, and 9000, respectively. Electrophoresis on polyacrylamide gel revealed six protein bands. When the sample was treated with mercaptoethanol and sodium dodecyl sulfate (SDS) and the gel prepared in the presence of SDS, a maximum of 11 polypeptide monomers was detected ranging in molecular weight from 5500 to 66 000. Methionine was the first limiting amino acid and addition of 0.15% DL-methionine based on the protein raised the protein efficiency ratio from 0.9 to 2.2 for the rat.

Lupin as a crop plant has been known since ancient time. The two species *Lupinus albus* and *Lupinus mutabilis* have been cultivated for grains for 300 years or more in the Mediterranean basin and in the high lands of South America (Sengbusch, 1953). Several other species have come into cultivation only recently and are still in the process of adaptation to different ecological conditions.

A descriptive history and present status of lupins have been published by Gladstones (1970).

One of the main difficulties in the utilization of lupin as food or feed has been the widespread occurrence of bitter alkaloids in the seeds in concentrations ranging from 0.02 to 3% (Winther, 1938).

Modern breeding of lupins for grain and forage date back to Sengbusch (1937, 1938) who obtained the first alkaloid-free or "Sweet" seeds of *L. luteus* and *L. angustifolius*. Alkaloid-free cultivars of *L. albus* were also selected by Sengbusch and others in the 1930's.

The main species cultivated at present are *L. luteus*, *L. angustifolius*, and *L. albus* with a fairly large number of different varieties or cultivars. In South America, particularly in countries of the Andean region, predominates the species *L. mutabilis*, although the introduction of the three main varieties of sweet lupin is underway in many South American countries (Gross and von Baer, 1974; Junge, 1973).

The main interest in lupin for foods and feeds is related to its high content of protein and relatively high oil content in some varieties.

Tannous and Cowan (1967) tested samples of a bitter lupin from the Mediterranean region after submitting them to a debittering process of prolonged steeping and boiling in water. The average PER values for rats were elevated from 0.57 to 1.06 when the diet was supplemented with methionine, as compared with 3.05 for a casein standard. The low PER could have been the result of incomplete detoxification of the seeds or damage of the protein during the debittering process.

Hove (1974) studied the composition and the protein quality of some cultivars of *L. luteus* and *L. angustifolius*, obtaining PER values of 1.29 for the cultivar uniwhite of *L. angustifolius* and 1.39 for the *L. luteus* cultivar weiko III. The PER's were elevated to 2.40 and 2.56, respectively, by methionine supplementation and compared with 2.90 for the casein control diet. On the other hand, Ballester et al. (1976) reported protein efficiency ratio (PER) values of 0.48 for *L. albus* and 0.99 for *L. luteus*, values which were elevated to 2.48 and 2.30, respectively, by supplementation of the protein with 0.3% DL-methionine.

In this paper some physicochemical and nutritional properties of the proteins of a new variety of *Lupinus albus* are described.

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MATERIALS AND METHODS

The lupin seeds used in this study were of a sweet variety (*Lupinus albus* var. *Multolupa*) obtained from the Ministry of Agriculture Experimental Farm at "Sete Lagoas", state of Minas Gerais, Brazil.

Determination of crude protein (% N × 6.25) was done by a semi-microkjeldahl procedure as described by Villela et al. (1973).

Lipid determination was done by extracting the petroleum ether soluble material for 8 h in a Soxhlet apparatus and determining the extracted lipid gravimetrically after evaporation of the solvent.

Amino acid determination in the defatted flour and in the isolated proteins was performed on an acid hydrolysate by ion-exchange chromatography in a Beckman 120C Amino Acid Analyzer following the procedure recommended by the instrument's manufacturer.

Crude fiber and ash contents were determined according to the procedures described in the AOAC (1970).

Protein solubility, under various pH conditions, was determined as follows: a series of 5-g samples of defatted flour was suspended in 100 mL of distilled water and the pH of each sample adjusted to a different value with 2 N HCl or NaOH solution and then agitated for 30 min with a magnetic stirrer. At the end of the agitation period the suspensions were centrifuged (12 000*g*) for 10 min, the supernatant collected, and the volume adjusted to 100 mL. The extracted nitrogenous matter was determined by the semi-microkjeldahl procedure using 2–10 mL of the supernatant. Percent protein solubility (% N × 6.25) in relation to the crude protein content in the flour was plotted as a function of pH.

Isolation of the lupin proteins was done by first extracting the flour in an aqueous medium in which the pH was adjusted to 9.0 with a 2 N NaOH solution. The soluble material was separated from the insoluble residue by centrifugation (12 000*g*) for 10 min. Most of the extracted

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Table I. Proximate Composition of a Seed Variety of Sweet Lupin (*Lupinus albus* var. *multolupa*)

seed component	percent of whole seed (dry basis)
hull (seed coat)	17.0
total lipid (PE extract)	10.6
crude protein (% N × 6.25)	38.0
crude fiber	13.0
ash	3.4

proteins was precipitated by lowering the pH of the extract to 4.5, which coincided with the pH of lowest solubility of the lupin proteins. The precipitate was recovered by centrifugation (8000*g*) for 10 min.

Gel filtration of the aqueous (pH 9.0) extract was performed in a Sephadex G-100 column (55 × 2.5 cm) equilibrated with a 0.05 M, pH 8.5 sodium phosphate buffer. Three-milliliter fractions were collected at an elution rate of 2 mL/h, and the fractions were analyzed for absorbance at 280 nm and by the method of Lowry et al. (1951) at 660 nm. Absorbance readings were plotted against tube number.

Polyacrylamide disc gel electrophoresis was performed in tubes (7.5 × 0.5 cm) according to the procedure of Davis (1964).

The electrophoresis in SDS–polyacrylamide gel for determining the molecular weight of the monomers was performed by the method of Weber and Osborn (1969). For the calculation of molecular weight, a standard curve was constructed using the following proteins from Sigma Chemical Co; lactic dehydrogenase (mol wt 36 000); bovine serum albumin (mol wt 65 000); pepsin (mol wt 35 000); and lysozyme (mol wt 14 100). The protein bands were stained by a 1% Amido Schwartz solution in 7.5% glacial acetic acid. After immersion of the gels in this solution for 30 min, the background color was removed by repeated washings with a 7.5% acetic acid solution. The mobilities of the proteins in the gel were plotted against molecular weights on a semilog paper.

The molecular weight of the proteins in a pH 9.0 extract was determined by sucrose gradient centrifugation (5 to 20% sucrose) using lactic dehydrogenase (LDH) with sedimentation coefficient of 7.4 S as a marker. The preparation was centrifuged for 15 h at 175 000*g* in a Beckman SW-65 type rotor at 4 °C. The molecular weights were calculated using the procedure of Martin and Ames (1961).

The biological value of the proteins was determined by the protein efficiency ratio (PER) method, first described by Osborne and Mendel (1917).

The percent composition of the diets was: protein, 10; vegetable oil, 8; vitamin diet fortification mixture, 2 (NBC); salt mixture, 4 (Rogers and Harper, 1965); starch, 50; and sucrose, to 100. The carbohydrate and lipid contents of the lupin flour were taken into consideration in the preparation of the diets. Weaning rats of the Wistar strain were caged individually and received water and food ad libitum for 28 days.

Trypsin inhibitor activity was tested in the unheated flour extract by the procedure of Kakade et al. (1969).

Phytohemagglutinin activity was performed in the unheated flour extract by the method of Kabat (1964).

RESULTS AND DISCUSSION

Proximate composition for the variety *multolupa* of sweet lupin seed is shown in Table I. The high protein content (38%) is comparable to that found in soybean seeds. The seed coat (hull) accounts for 17% of the dry seed and is responsible for the high crude fiber (13%) of the whole seed. This high fiber content indicates that for most uses (feed or human foods) the seed coat should be removed.

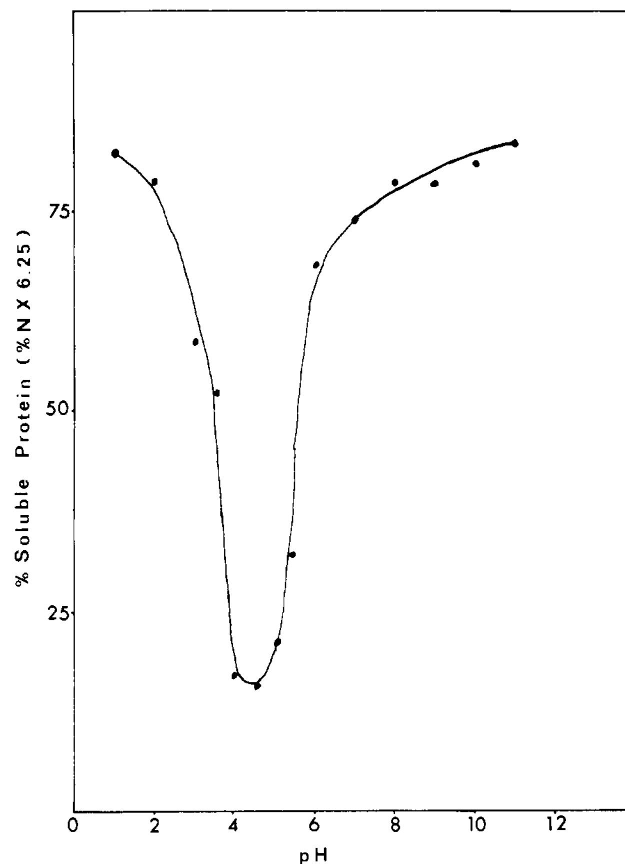

Figure 1. Solubility curve of lupin (*Lupinus albus* var. *multolupa*) protein as a function of pH.

The lipid content (10.6%) of this variety is within the range of values reported in the literature for other species and varieties (Hove, 1974; Junge, 1973).

The remaining 18% unaccounted for in Table I could be attributed to carbohydrates and other undetermined materials.

The solubility curve as a function of pH is shown in Figure 1. The solubility is greater than 80% at pH 1.0, dropping to 50% at 3.5 and to 15% at pH 4.5. The slope of the solubility curve is very steep from pH 4.5 to near neutrality, reaching 70% at pH 6.0. As the medium becomes alkaline, the solubility continues to increase to levels of 80–85% at pH 9.0, showing in this range a lower pH dependence. Higher pH, although more efficient in extracting the proteins, should be avoided due to possibility of disruption of the protein structure and degradation of certain amino acids.

Table II illustrates the solubility property of the lupin proteins which is of practical application in the laboratory for obtaining protein isolates. After extracting the proteins in alkaline solution, approximately 80% of them can be precipitated by lowering the pH to 4.5. The precipitate is recovered by centrifugation to give a material with 85–90% protein on a dry basis.

The nitrogenous compounds which are soluble at pH 4.5 comprise some of the proteins and the nonprotein nitrogenous substances. The proteins were precipitated from the remaining supernatant by adding trichloracetic acid (TCA) to a final concentration of 5% (w/v). The TCA soluble material represents 5–6% of the extracted nitrogenous matter and constitutes the nonprotein fraction.

Table II. Extraction and Fractionation of Lupin (*Lupinus albus* var. *multolupa*) Nitrogenous Substances

fraction description	total (% N × 6.25) (g/100 g of flour)	% of total in the flour	% of total extracted
defatted flour (8.35% water)	39.7	100.0	
alkaline extract (pH 9.0)	33.8	85.2	100.0
precipitated (pH 4.5)	26.8	67.5	79.2
soluble (pH 4.5)	7.0	17.7	20.8
soluble pH 4.5 precipitated by 5% TCA	5.0	12.5	14.6
Nonprotein nitrogen	2.0	5.0	5.8

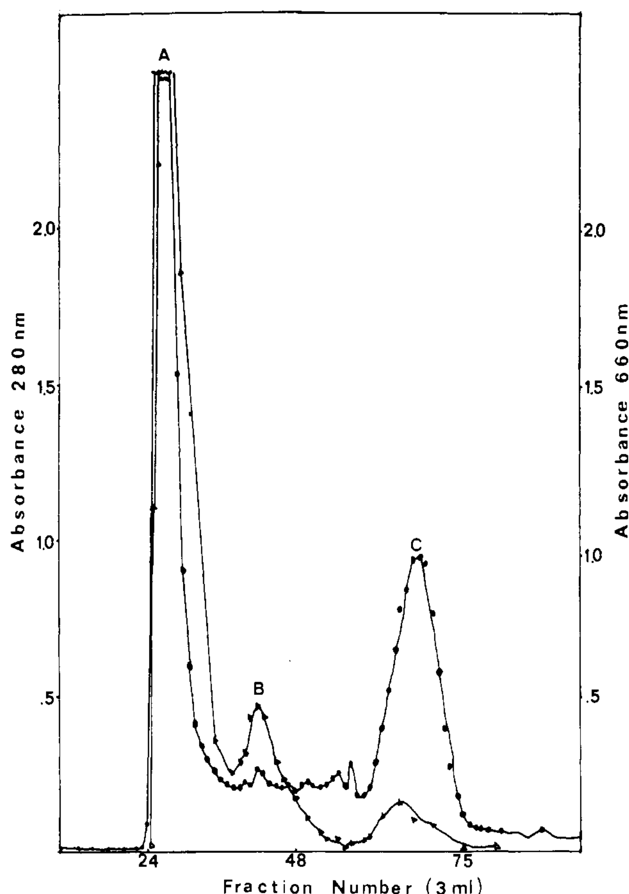

Figure 2. Gel filtration of a pH 9.0 extract of sweet lupin seed (*Lupinus albus* var. *multolupa*) proteins: absorbance at 280 nm (○—○); absorbance at 660 nm (△—△).

The total amino acid profiles of the defatted flour and those of the proteins in the isolate are quite similar and are shown in Table III. The most significant differences in the amino acid composition of defatted flour and of the isolate are the contents of cysteine (half-cystine) and of methionine which appear in lower concentration in the isolated proteins. This is of nutritional significance since the sulfur-containing amino acids, particularly methionine, is the first limiting amino acid in the lupin protein. It is worth mentioning that acid hydrolysis of proteins and ion-exchange chromatography of the amino acids generally introduce a certain loss of the sulfur amino acids, particularly of cysteine, which may have contributed to the low values found for this amino acid, mainly in the isolate.

Table III. Amino Acid Composition of a Sweet Lupin (*Lupinus albus* var. *multolupa*) Protein

amino acid[a]	defatted flour	protein isolate
lysine	4.4	4.9
histidine	1.9	1.9
arginine	9.2	9.6
aspartic acid	11.8	14.2
threonine	3.8	4.2
serine	6.7	6.4
glutamic acid	32.9	32.9
proline	4.3	4.7
glycine	4.6	4.3
alanine	3.6	3.7
half-cystine	1.7	trace
methionine	0.8	0.6
valine	3.5	4.5
isoleucine	4.1	5.0
leucine	8.8	9.2
tyrosine	4.7	3.8
phenylalanine	3.8	3.3

[a] Amino acid concentrations are expressed in grams of amino acid/16 g of nitrogen.

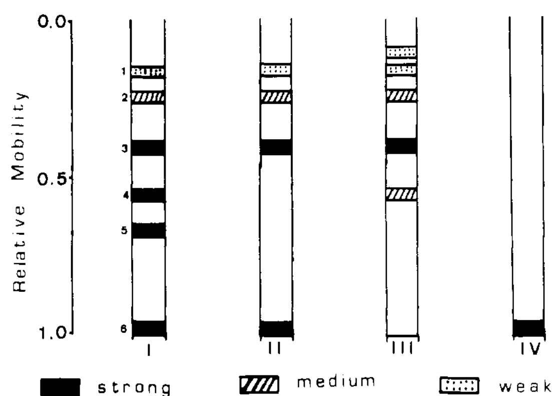

Figure 3. Electrophoretic patterns of lupin proteins on polyacrylamide gels: (I) pH 8.5 sodium phosphate buffer extract; (II) water-soluble proteins (2% NaCl extract) after dialysis against distilled water; (III) peak A, eluted from Sephadex G-100 (Figure 2); (IV) peak B, eluted from Sephadex G-100.

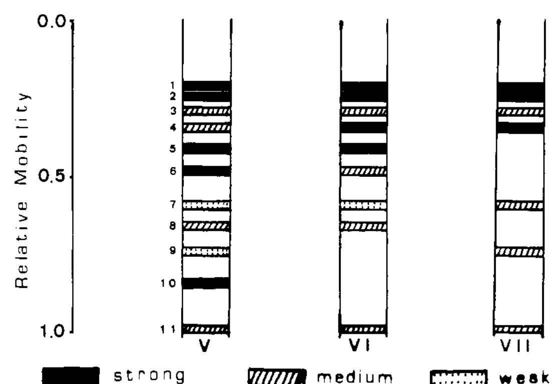

Figure 4. Electrophoretic patterns of lupin proteins subjected to SDS–polyacrylamide gel electrophoresis: (V) pH 8.5 sodium phosphate extract; (VI) aqueous extract; (VII) water-soluble proteins (2% NaCl extract) after dialysis against distilled water.

Some amino acids (lysine, aspartic acid, arginine, threonine, proline, valine, isoleucine, and leucine) appear in increased concentration in the isolate.

The elution pattern for the gel filtration of a pH 9.0 extract on Sephadex G-100 column appears in Figure 2 and shows three elution peaks (A, B, and C). Sucrose gradient ultracentrifugation of the same extract gave three bands with sedimentation coefficients 8.1, 2.8, and 1.2 S. The

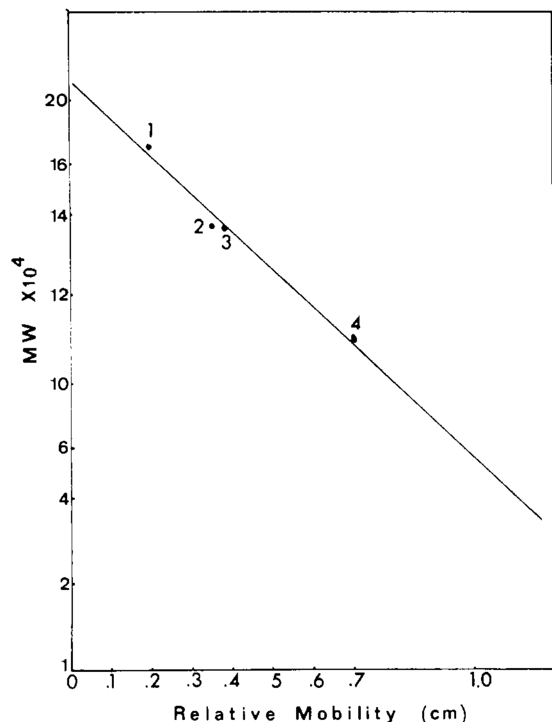

Figure 5. Monologue plot of molecular weight as a function of mobility of SDS-polyacrylamide gel electrophoresis.

molecular weights estimated by using lactic dehydrogenase (7.4 S) as a marker were 163000, 53000, and 9000, respectively.

The electrophoretic characterization of the lupin proteins on polyacrylamide gel is seen in Figures 3 and 4. Figure 3 (gels I to IV) illustrates the number of protein components in the pH 8.5 sodium phosphate buffer extract (I) in the albumin or water-soluble fraction (II) and in the materials from peaks A and B eluted from Sephadex column (III and IV), respectively. In the pH 8.5 buffered extract (I), six protein bands were detected as compared to only four bands (1, 2, 3, and 6) in the water-soluble fraction (II). This fraction did not show two major proteins of R_M 0.56 and 0.67 (bands 4 and 5) which appear in the crude extract (I). The material of peak A (III) originated five protein bands and peak B (IV) only one. On the other hand, peak A revealed a weak band which was not seen in the electrophoretic pattern of the crude extract (I) or in the water-soluble (albumin) fraction (II). Since this band was appeared in several different runs, it does not seem to be an analytical artifact and may suggest a different state of aggregation of the protein molecules in this isolated material. Peak C could not be characterized by gel electrophoresis due to interference of a strong pigmented material and possibly to complexation with phenolic compounds existent in the seeds.

Figure 4 shows the results of electrophoresis done on samples pretreated with mercaptoethanol and using gels containing SDS, according to the method of Weber and Osborn (1969). Under these conditions the pH 8.5 phosphate buffer extract (V) gave 11 bands, the aqueous extract (VI) revealed nine bands, and the albumin fraction (VII) gave seven protein bands.

The standard curve for calculating the molecular weight of the polypeptides is shown in Figure 5, and the values for all the bands detected in the SDS gels are shown in Table IV. Numbers for the bands on Figure 4 and Table IV are correspondent to facilitate comparisons. The calculated molecular weight for the polypeptide monomers ranged from 5500 to 66000.

Table IV. Subunit Molecular Weight of Sweet Lupin (*L. albus* var. *multolupa*) Proteins by SDS-Polyacrylamide Gel Electrophoresis

	mol wt		
band no.	alkaline extract (pH 9.0)	water extract	albumins[a]
1	66 000	66 000	66 000
2	54 000	58 000	58 000
3	48 500	48 500	49 000
4	38 500	42 500	41 000
5	32 000	34 500	
6	24 500	29 000	
7	18 000	21 000	20 000
8	15 000	15 500	
9	12 000		13 000
10	9 200		
11	5 450	5 600	5 450

[a] Water-soluble proteins after dialysis of a 2% NaCl extract.

Table V. Protein Efficiency Ratio (PER) of Sweet Lupin (*L. albus* var. *multolupa*) as the Only Source of Protein, Compared to a Casein Diet

protein source in the diet	PER[a] ± 1 SD
A. casein (standard	2.10 ± 0.079
B. dehulled, full-fat lupin flour heated (10 min at 97 °C) ± 0.15% DL-methionine	2.02 ± 0.125
C. dehulled, full-fat lupin flour heated (10 min at 97 °C)	0.81 ± 0.101
D. dehulled, full-fat unheated lupin flour	0.81 ± 0.070
E. dehulled, defatted unheated lupin flour	1.03 ± 0.130
F. integral unheated lupin flour	1.03 ± 0.049
G. protein isolated by precipitation at pH 4.5	0.22 ± 0.061

[a] Calculated PER are mean value of six rats.

The large number of polypeptide structural units encountered in the SDS gel, compared with the maximum of six protein bands revealed by electrophoresis on simple gel, suggests a high degree of polymerization for the structures of some of these proteins. The fact that gel filtration on column of Sephadex G-100 produced only three peaks indicated a poor fractionation of the proteins by this technique.

The fractionation of the proteins in the crude extract by sucrose gradient ultracentrifugation does not offer a good resolution either, and the molecular weight calculated for these protein bands should be interpreted as average molecular weights of heterogeneous material merely indicating the approximate range of molecular weights of the proteins in the crude extract.

The results of the biological evaluation of the proteins in the lupin flour and of the lupin protein isolated by precipitation at pH 4.5 are shown in Table V.

The differences between mean values of PER were tested applying nonparametric statistics (Wilcoxon test) as described by Jaroslav (1969). The standard casein diet (A) did not differ significantly ($P \geq 0.05$) from the heated full-fat lupin flour supplemented with 0.15% DL-methionine, on the protein basis. On the other hand, diets A and B differed significantly ($P \geq 0.05$), being superior to diets C to G. Diets C, D, E, and F were not different statistically from each other, but they were all superior from diet G ($P \geq 0.05$).

Additional interpretation of the experimental data calls for the following comments: the PER value for casein (2.10) was low as compared with reported values. The

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.

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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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