# Lipid Composition of Dry Beans and Its Correlation with Cooking Time

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The triglyceride and phosphatide contents of seven varieties or types of dry beans and the Alaska pea were determined. The triglyceride content ranged from 0.89% for Alaska peas to 1.54% for Michelite beans. The phosphatide content averaged near 1%, except for lima beans which averaged 0.88%. Gas-liquid chromatography was used to study the component fatty acids of the triglycerides and phosphatides. The main fatty acids of the triglycerides were palmitic, oleic, linoleic, and linolenic. Small amounts of myristic acid were found in all varieties except Great Northern beans. Lauric acid was found in Alaska peas. The list of component fatty acids of the phosphatides was similar to the triglycerides, except for the omission of linolenic acid in the phosphatides of the Great Northern, Michelite, Pinto, and lima beans. Palmitic acid constituted about 50% of the fatty acids in the phosphatides of the eight types of beans and peas studied. No significant simple correlation coefficients were obtained between triglyceride, phosphatide, or crude lipid content and the cooking time for the dry beans.

THE lipid content of dried beans is a relatively small percentage of the over-all composition and has not been studied extensively. Guerrant (*4*) determined the per cent fat and "lipoid" phosphorus in various *Leguminoseae* seeds, including navy and blackeye beans and green peas. Korytnyk and Metzler (*6*) determined the fatty acid composition of the glycerides from five types or varieties of dry beans. Labarre and Pfeffer (*7*) and Gambhir and Dutt (*3*) investigated the fatty acid composition of broad bean fixed oil. Lee and Mattick (*9*) investigated the changes which occur in the lipid fatty acids of frozen green peas after storage.

This report is concerned with the triglycerides and phosphatides and their constituent fatty acids in several types or varieties of dry beans and the Alaska pea. The purpose of this study was to determine whether there was any correlation between the various lipid fractions and the cooking time of dry beans.

## Procedure

**Preparation of Samples.** Table I lists the eight varieties or types of dry beans or peas studied and the location or year in which they were grown.

Beans from two locations grown during the same year were desired, but this was not always possible. Except for the broad and blackeye beans, all of the bean or pea varieties were obtained from the 1958 crop. Broadbeans from the 1957 and 1959 crops were utilized because of a crop failure in 1958. Because blackeye beans from two locations were not available from the 1958 crop, the 1958 and 1959 crops were used.

The beans were cooked for varying periods of time. The cooking time was defined as the time when 50% of the judges considered the beans and peas cooked (*13*). For chemical analyses only the cotyledons were analyzed because it was thought that after the beans had been hydrated the seed coat would not influence the cooking time. Taste panel tests indicated this may have been an erroneous assumption. The seed coat comprises 7 to 8% of the weight of lima, Michelite, and Great Northern beans; its lipid content ranges from 0.4 to 0.6% (*14*, *15*, *22*).

Various methods were used to remove the seed coats from the cotyledons. All the beans except the broad beans and peas required special soak treatments with water, 95% ethanol, or acetone to loosen the seed coat from the cotyledon. Because extended water treatment will initiate germination, water treatment was avoided when possible.

The blackeye beans grown in 1958 were soaked in water 15 minutes and the seed coats were removed by hand. The blackeye beans grown in 1959 were vacuum-infiltrated two times with water for 5 to 7 minutes and released 5 to 7 minutes. The lima beans from Oxnard, Calif., were vacuum-infiltrated with water as above, and the seed coats were removed immediately by hand. The lima beans from Los Angeles, Calif., and

Table I. Lipid Composition[a] of Cotyledons of Dry Beans and Peas and Correlation Coefficients with Cooking Time[b]

| Variety or Type | Location or Year | Crude Lipid | % Triglyceride[c] | % Phosphatide[d] |
|---|---|---|---|---|
| Pinto, *Phaseolus vulgaris* | Michigan | 3.08 | 1.15 | 1.06 |
| | Idaho | 2.92 | 1.32 | 0.82 |
| Great Northern, *Phaseolus vulgaris* | Nebraska | 3.00 | 0.96 | 1.02 |
| | Idaho | 3.31 | 1.26 | 0.94 |
| Michelite, *Phaseolus vulgaris* | Michigan | 3.46 | 1.46 | 1.00 |
| | Idaho | 3.63 | 1.56 | 1.07 |
| Small Red, *Phaseolus vulgaris* | Nampa, Idaho | 3.02 | 1.22 | 1.03 |
| | Twin Falls, Idaho | 3.00 | 1.32 | 0.99 |
| Lima, *Phaseolus lunatus* | Oxnard, Calif. | 2.98 | 1.21 | 0.74 |
| | Los Angeles, Calif. | 3.04 | 1.15 | 0.82 |
| Broad, *Vicia faba* | 1957 (Calif.) | 3.56 | 1.27 | 1.05 |
| | 1959 (Calif.) | 3.78 | 1.34 | 0.99 |
| Blackeye, *Vigna sinensis* | 1958 (Calif.) | 3.26 | 1.36 | 0.95 |
| | 1959 (Calif.) | 3.03 | 1.30 | 1.02 |
| Alaska peas, *Pisum sativum* | Moscow, Idaho | 2.57 | 0.90 | 0.97 |
| | Grangeville, Idaho | 2.68 | 0.89 | 1.02 |
| Correlation coefficient, $r$[e] | | 0.032 | 0.040 | 0.192 |

[a] Dry weight basis. Average of duplicate determinations on two replicates from each location or year.
[b] Cooking time when 50% of judges considered beans cooked (*13*).
[c] Triglyceride calculated as tristearin from glycerol analysis.
[d] Phosphatides calculated as phosphatidylcholine from phosphorus analysis.
[e] All $r$ values nonsignificant at the 5% level.

all locations of the Great Northern and Michelite beans were vacuum-infiltrated with 95% ethanol instead of water. The Small Red beans were dried overnight at 100° C. before vacuum infiltration with ethanol. The beans were allowed to stand in ethanol 20 minutes after the final release of the vacuum. The seed coats of the Pinto beans from both locations were scarified to allow solvent penetration. The Pinto beans from Twin Falls, Idaho, were vacuum infiltrated with water for 10-minute intervals and the vacuum was released for 20 minutes. The Pinto beans from Nampa, Idaho, were soaked in acetone for 2 minutes.

After the dry beans were treated as above, the samples were dried in an oven at 70° C., then coarsely ground in a large hammer mill. The seed coats were separated from the cotyledons with a seed hull blower. This was easily done for all varieties except the Pinto and Small Red beans. With the latter, some seed coat material remained attached to the cotyledons and was removed by hand. The cotyledon material was ground in a Wiley mill equipped with a 60-mesh screen and dried overnight in a vacuum oven at 60° C. This ground material was used for the lipid determinations.

**Lipid Determinations.** A crude lipid determination was obtained by extracting the ground beans with an ethanol-benzene azeotrope (32 to 68, v./v.) for 36 hours in a Soxhlet extractor. The residue from the filtrate after evaporation of the solvent was designated as crude lipid.

Chloroform-methanol (2 to 1, v./v.) was used to extract the lipid from the beans for the quantitative triglyceride and phosphatide analyses. This solvent system is used extensively to extract total lipid from plant and animal tissues.

Preliminary extraction experiments on the bean material with chloroform-methanol, water-saturated 1-butanol, 95% ethanol, and ethyl ether showed that the chloroform-methanol gave good extraction of phosphorus (for phospholipids) and glycerol (for triglycerides). The water-saturated butanol extracted slightly more phosphorus and approximately 25% less glycerol than the chloroform-methanol. Hot 95% ethanol resulted in low phosphorus and glycerol extractions. Ether extracted practically no phosphorus.

For the extraction of the lipid for the triglyceride and phosphatide determinations 5 grams of dried bean powder were mixed with 18 ml. of chloroform-methanol (2 to 1, v./v.) in a 50-ml. centrifuge tube. The mixture was first heated at 60° C. for 10 minutes with occasional stirring and then shaken for 15 minutes. Marinetti *et al.* (*11*) found that heating in the 60° C. water bath had no adverse effect on the extraction of phosphatides from animal tissue. To prevent splattering of the sample, the water bath temperature was not allowed to rise above 60° C. The mixture was centrifuged and the supernatant liquid decanted. The sample was extracted four more times with 12 ml. of chloroform-methanol as above, except that the 15-minute shaking period was omitted. Preliminary experiments showed that 96% of the phosphatides and 99% of the triglycerides were removed after five extractions, compared to the total of 10 extractions. The crude lipid extracts from the five extractions were placed in a 60° C. water bath to evaporate the solvent. A gentle stream of nitrogen was directed on the surface of the solvent to prevent oxidation and to facilitate evaporation. The crude lipid residue was immediately dissolved in 5 ml. of chloroform and applied to a silicic acid column to separate the triglycerides and phosphatides.

**Separation of Triglycerides and Phosphatides.** Borgstrom (*2*) demonstrated that a quantitative separation can be obtained with a silicic acid column using chloroform and methanol as eluting agents.

Three grams of silicic acid were mixed with 1 gram of Hyflo Super-Cel. This mixture was placed in a 1.7-cm. column and washed according to the method of Marinetti *et al.* (*11*). The crude lipid was dissolved in 5 ml. of chloroform and applied to the silicic acid column. Three rinses using 10-ml. portions of chloroform were added to the column. Each portion was allowed to settle into the adsorbent before application of the next portion. Preliminary elution studies showed that at least 35 ml. of chloroform were required to elute the triglycerides. The last phosphatide component (lecithin) to be removed from the column by methanol exhibits a considerable amount of tailing. Therefore 50 ml. of methanol were required to elute the phosphatides. The triglyceride eluate was diluted with chloroform to 50 ml. in a volumetric flask and the phosphatide eluate was diluted to 100 ml. with chloroform.

The triglyceride content was determined from the glycerol content of the triglyceride eluate. The triglycerides were saponified by a modified procedure of Van Handel and Zilversmit (*18*). A 5-ml. aliquot of the triglyceride extract was evaporated to dryness in a hot water bath. The triglycerides were saponified by adding 3 ml. of 0.2*M* methanolic potassium hydroxide and heating the sample at 60° to 70° C. for 15 minutes. Two milliliters of 5*N* sulfuric acid were added and the test tube was placed in boiling water to remove the methanol. The water level of the bath was kept slightly above the level of the reaction mixture in order to avoid excess evaporation of water from the tube. The Lambert and Neish (*8*) procedure was followed for estimation of glycerol in the sample, except that the addition of sulfuric acid was omitted.

The presence of sugars, polysaccharides, or serine interferes with the determination of glycerol. However, after column chromatography none of these interfering substances was detected in the triglyceride fraction.

The phosphatide content of the dry beans was calculated from the phosphorus content of the phosphatide eluate. A 3-ml. aliquot of the phosphatide solution was evaporated to dryness in a hot water bath. The phosphorus content was determined by the method of Harris and Popat (*5*). Gas chromatography studies showed that the dried bean phosphatide contained about 50% palmitic acid and 50% of 18 carbon fatty acids. The phosphatide values were therefore calculated on the basis of one palmitic acid and one stearic acid group in the molecule. Phosphatidylcholine was chosen to represent the bean phosphatides because it was the predominant phosphatide in Michelite beans (*17*).

**Gas-Liquid Chromatography of Fatty Acids in Triglycerides and Phosphatides.** Gas-liquid chromatographic analyses of the methyl esters of the fatty acids in the triglyceride and phosphatide fractions of all eight varieties of bean and pea seeds were performed on a Beckman GC-2 gas chromatograph equipped with a 9-foot diethylene glycol–succinate column (*10*). The operating conditions were: filament current, 320 ma.; chart speed, 0.5 inch per minute; carrier gas, helium; flow rate, 81 ml. per minute; temperature, 199° C. The methyl esters of the fatty acids were prepared by transmethylation with sodium methoxide (*21*). The final pentane solution was reduced to a small volume (approximately 1 ml.) and between 10 and 20 μl. of the solution were injected into the gas chromatograph. The methyl esters were determined quantitatively by measuring the area under the peaks. Conversion factors for each fatty acid were obtained to convert peak area to mole per cent. Known reference compounds used to verify the quantitative analyses were the methyl esters of lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic acids. Fatty acids of 20 or more carbons were not determined in this investigation.

## *Results and Discussion*

**Quantitative Lipid Determination.** The results for the crude lipid, triglyceride, and phosphatide determinations are given in Table I. The per cent phosphatide is constant at 1%. Guerrant (*4*) using an ether-alcohol solution found a "lipoid" phosphorus content of 0.0452% in navy beans, 0.0784% in blackeye beans, and 0.0784% in green peas (New Era variety). The phosphatide phosphorus obtained in this investigation was: Michelite (navy bean), 0.0425 and 0.0400%; Great Northern, 0.0374 and 0.0409%; blackeye beans, 0.0381 and 0.0407%; Alaska peas, 0.0386 and 0.0407%. Large differences between the two studies on the blackeye beans and peas are apparent but the cause is not known. Vidal (*19*) analyzed the following seeds and obtained lecithin plus cephalin values as follows: blackeye, 0.372%; green peas, 0.554%; lima beans, 0.711%; broad beans, 0.288%.

The crude lipid content of the dry beans was approximately 3%, except for 3.5 to 3.8% for the Michelite and broad beans. The Alaska peas contained less than any of the dry beans.

**Fatty Acids of Bean Triglycerides and Phosphatides.** The main component fatty acids in the triglycerides of the eight varieties were found to be palmitic, stearic, oleic, linoleic, and linolenic. With a few exceptions, the fatty acid contents of the triglycerides were similar (Table II). In the broad bean and Alaska pea, the oleic acid was much higher and the linolenic acid content was much lower than in the other varieties. The blackeye and lima beans and Alaska pea contained more palmitic acid than the other varieties or types of beans. Small amounts of myristic and stearic acids could be detected in all varieties except the Great Northern beans. Lauric acid was detected in Alaska peas. The linoleic acid content ranged from 25 to 40%. The triglyceride fatty acid content of all varieties of *Phaseolus vulgaris* was very similar in per cent composition.

Korytnyk and Metzler (*6*) determined the fatty acid composition of the glycerides of a number of types of dry beans, including lima, blackeye, and pinto. The only fatty acid above $C_{18}$ was 2.5% behenic acid in the blackeye beans. Small or trace amounts of fatty acids which appeared to be *n*-pentadecanoic and *n*-heptadecanoic acids were also found. Otherwise the per cent composition of the present investigation and those of Korytnyk and Metzler were similar when compared on an equivalent basis. Takahashi (*16*) found what may have been cerotic acid in addition to the fatty acids found in this study.

Gambhir and Dutt (*3*) found 1.8% palmitic, 7.46% stearic, 1.0% arachidic, 0.11% behenic, 27.29% linoleic, 11.61% linolenic, and 41.70% oleic acids in broad beans. Labarre and Pfeffer (*7*) did not report any linolenic or arachidic acids. In the present study palmitic acid made up 21 mole % of the triglyceride and 48 mole % of the phosphatide fatty acids of the broad beans.

Some of the large differences in composition between the results of this study and those of Gambhir and Dutt may arise from different source material. Gambhir and Dutt obtained an 8.1% yield of oil by extracting the broad beans with petroleum ether, while the present study showed only 3.7%.

Baker *et al.* (*1*) studied the petroleum ether extract of *Vigna sinensis* and found fatty acids of $C_{15}$, $C_{17}$, $C_{20}$, $C_{22}$, $C_{24}$, $C_{26}$, and $C_{18}$ with one double bond in addition to the ones determined in this study. The total weight per cent of the former fatty acids was 7.2%. The remaining per cent fatty acid composition was similar except for a higher linolenic acid content in the present investigation.

In the phosphatides (Table III) the Great Northern, Pinto, Michelite, and lima beans had only negligible amounts of linolenic acid. The oleic acid of the pinto and lima bean phosphatides were considerably higher than the other varieties or types. The palmitic acid was uniformly high in all cases. Definite varietal differences of the per cent composition of the fatty acids of the phosphatides of *Phaseolus vulgaris* were apparent. The major saturated fatty acid found in the triglycerides and phosphatides of all the varieties or types of beans was palmitic acid.

Comparison of the fatty acids found in the phosphatides and triglycerides shows an increase in saturated fatty acids in the phosphatides with the increase being palmitic acid. Wagenknecht (*20*) studied various lipid fractions of raw green beans and found fatty acids from $C_6$ to $C_{20}$ but only a very small amount of $C_{20}$ fatty acid.

Lee and Mattick (*9*) studying frozen green peas after one-year storage at −17.8° C. found very large differences in the fatty acid content of the various lipid fractions between peas which had been heat-treated (blanched) to inactivate enzymes and those with no treatment. The free palmitic acid from the untreated peas was 2563.4 mg. and that from the enzyme-inactivated peas was 10.5 mg. per 1500 grams of frozen peas. Other large differences in the free fatty acids were also found. With untreated peas the largest proportion of the fatty acids was in the free fatty acid form; with heat-treated peas only very small amounts of free fatty acids were found. Enzymes may have similar effects in dried beans, since Morris and Wood (*12*) have reported that beans with high moisture content stored at high temperature for a long period develop rancid off-flavors. Taste panel tests in the present study indicated that rancidity may have developed in the Michelite beans with high moisture content (*13*).

Lee and Mattick (*9*) reported no fatty acids longer than $C_{18}$ in any lipid fraction. Very small amounts of capric and a trace amount of caprylic acid were found. In the current study, the dried peas contained no capric or caprylic acid.

Small unidentified peaks at retention volumes (relative to methyl palmitate) of 1.29 and 1.50 were observed in the methyl esters of fatty acids from the phosphatides. One of these peaks may represent methyl palmitoleate, because theoretically its retention volume should be within these ranges. There were also unidentified peaks at retention volumes of 0.73 and 0.84 of the lima bean triglycerides.

No significant (5% level) correlation coefficients were obtained between cooking times for the dry beans and the triglyceride, phosphatide, and crude lipid content (Table I). The low values indicate that the lipids analyzed are not important in determining cooking time of dry beans.

Table II. Fatty Acids in Eight Varieties of Bean and Pea Triglycerides

| | Mole % of Fatty Acid | | | | | | |
|---|---|---|---|---|---|---|---|
| Variety | Lauric | Myristic | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| Great Northern bean, *Phaseolus vulgaris* | | | 19.7 | | 2.6 | 32.2 | 45.4 |
| Michelite bean, *Phaseolus vulgaris* | | 0.1 | 15.8 | 1.0 | 13.3 | 26.9 | 42.6 |
| Pinto bean, *Phaseolus vulgaris* | | 0.1 | 16.0 | 0.5 | 8.7 | 26.7 | 47.9 |
| Red bean, *Phaseolus vulgaris* | | 0.2 | 19.3 | 1.4 | 8.9 | 25.6 | 44.5 |
| Alaska pea, *Pisum sativum* | 0.7 | 0.7 | 33.6 | 3.4 | 33.0 | 24.4 | 4.3 |
| Blackeye bean, *Vigna sinensis* | | 0.1 | 36.7 | 2.1 | 6.2 | 25.7 | 28.8 |
| Broad bean, *Vicia faba* | | 0.2 | 20.7 | 2.4 | 30.6 | 41.0 | 5.0 |
| Lima bean, *Phaseolus lunatus* | | 0.3 | 30.0 | 2.8 | 9.3 | 37.7 | 19.9 |

Table III. Fatty Acids in Eight Varieties of Bean and Pea Phosphatides

| | Mole % of Fatty Acid | | | | |
|---|---|---|---|---|---|
| Variety | Palmitic | Stearic | Oleic | Linoleic | Linolenic |
| Great Northern bean, *Phaseolus vulgaris* | 58.3 | 5.1 | 9.8 | 26.8 | |
| Michelite bean, *Phaseolus vulgaris* | 53.6 | 3.4 | 8.1 | 35.0 | |
| Pinto bean, *Phaseolus vulgaris* | 40.0 | 0.6 | 49.5 | 10.0 | |
| Red bean, *Phaseolus vulgaris* | 49.7 | 3.1 | 9.3 | 18.7 | 19.3 |
| Blackeye bean, *Vigna sinensis* | 61.4 | | 10.2 | 12.3 | 16.1 |
| Broad bean, *Vicia faba* | 48.4 | 2.2 | 14.3 | 16.7 | 18.4 |
| Lima bean, *Phaseolus lunatus* | 34.0 | 3.2 | 36.5 | 26.3 | |
| Alaska pea, *Pisum sativum* | 49.5 | 1.0 | 10.4 | 21.7 | 17.3 |

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**NUTRIENT DISTRIBUTION IN GRAIN**

# Location of Nonprotein Nitrogenous Substances in Corn Grain

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Nonprotein nitrogenous substances in corn contribute to the flavor and nutritional quality of the processed grain in food products and feeds. Because their presence in dry-milled products is determined by their location in the kernel, the amounts of amino acids, quaternary nitrogen compounds, nucleosides, purines, and pyrimidines in germ, endosperm, and bran fractions were measured. These compounds were extracted with aqueous ethanol from the corn fractions, separated by ion-exchange chromatography, and quantitatively determined by specific spectrophotometric procedures. Amino acids, which contribute over 50% of the nonprotein nitrogen in whole corn or its fractions, are distributed almost equally between the endosperm and the smaller germ fraction, with minor amounts in the bran. The concentration of free amino acids in the germ is several times that in the endosperm. Only small differences exist between the types of amino acids found in germ and endosperm. Quaternary nitrogen and heterocyclic nitrogen compounds were primarily in the germ.

A SMALL but significant fraction of the nitrogen in whole corn grain has been shown to be present in low-molecular-weight substances such as free amino acids, amines, amides, quaternary nitrogen compounds, purines, and pyrimidines (*7*). These nonprotein nitrogenous substances contribute to the nutritional value, flavor, and other factors important to products derived from corn. For the preparation of certain foods and feed products from corn, the grain is dry-milled to yield endosperm, bran, and germ fractions which may be processed separately. Therefore, the disposition of the nonprotein nitrogen and its effect upon product quality will depend upon the initial concentration of these substances in the grain parts. This report describes a quantitative study of the distribution of the major nonprotein nitrogenous compounds in the parts of corn grain. The information obtained is also of interest relative to the roles of these substances in seed metabolism.

Determinations of these substances were facilitated by improved methods for the ion exchange chromatography of amino acids (*15, 18*), quaternary nitrogen compounds (*6*), and purines and pyrimidines (*8*). These procedures employed columns of finely pulverized classified cation exchange resins and sodium citrate buffer eluent. Improved resolution, decreased degradation of labile compounds, and increased rate of analysis were advantages of these methods.

## *Materials and Methods*

**Hand-Dissected Corn Samples.** Whole grain yellow dent hybrid seed corn (Doubet Variety 25) was picked at maturity, air-dried, and immediately transferred to cold storage. This corn was grown under average farm conditions in central Illinois in 1960 on soil balanced in phosphorus, potash, and nitrogen but without added minor elements. Fertility level of the soil was medium high with a yield of 100 bushels per acre. The kernels were manually dissected into germ, endosperm, and combined tip cap and pericarp fraction according to the procedure of Hopkins and coworkers (*13*) as modified by Earle, Curtis, and Hubbard (*12*). Completeness of separation was determined by microscopic examination. Samples of whole corn, endosperm, and bran (combined tip cap and pericarp) were air-dried and ground in a hammer mill to pass a 0.027-inch screen. The germ was first flaked in a roller mill and its oil removed with petroleum ether before grinding. The germ contained 37.5% oil. The germ sample was analyzed as lipid-free material, but values in tables are corrected to whole germ. Moisture content of the ground samples was determined by drying under vacuum at 100° C. over phosphorus pentoxide (Table I). Data obtained with respect to yields of fractions, nitrogen content, and amounts of nonprotein nitrogenous constituents were converted to a moisture-free basis in all of the tables.

**Isolation of Nonprotein Nitrogen Constituents.** The previously described method for extracting nonprotein nitrogen from whole corn (*7*) was modified considerably to facilitate analysis of corn fractions. One hundred grams of the finely ground sample were stirred in 1 liter of 80% aqueous ethyl alcohol at room temperature for 10 minutes. The suspension was cooled to 4° C. and centrifuged to obtain a solution of material soluble in 80% ethanol. The residue was re-extracted with 1 liter of 80% ethanol, centrifuged at 4° C., and then washed with an additional volume of 200 ml. of cold 80% ethanol. Alcohol was removed from the combined washes and extracts by vacuum distillation. Zein and other water-insolubles were removed at various stages in the reduction of volume to prevent occlusion of minor constituents with the precipitate. Precipitates were washed before being discarded, and all washings were combined with the extract. The combined extract containing water-soluble nonprotein nitrogenous substances was concentrated to dryness under vacuum at a bath temperature of 40° C., then made up to volume with water. The amount of nitrogen extracted was determined by semimicro-Kjeldahl and compared with total nitrogen (Table I). All extracts