Table VIII. Absorbance of Rat Urine at 266 nm from in Vivo Trial 3

Treatment	Absorbance[a]
Control	0.176
HSD	1.362
HSD extract	0.973
HSD residue	0.153
Standard error	±0.055

[a] Each mean is the average of six male rats.

Hatfield, 1972). The second effect is on post absorptive nitrogen metabolism and may be related to the nitrogen needed to detoxify or excrete the problem compound(s). Glycine is used to detoxify benzene and the product hippuric acid is excreted (White et al., 1968).

Urine from the HSD and HSD extract fed rats was noticeably darker in color than for the control or residue treatments. All urines were scanned in the UV region. The dark urines showed an absorption peak at 266 nm. Urine from rats fed control and HSD residue diets showed little absorption at 266 nm (Table VIII). The 95% ETOH extract was also scanned and showed a typical UV spectra for lignin (Sarkanen and Ludwig, 1971) with an absorption peak near 280 nm. Syringyl alcohol and a guaiacyl derivative which are components of wood lignin show an absorption peak of 266–8 nm (Sarkanen and Ludwig, 1971). These are similar to the absorption peak in the urine of HSD and HSD extract fed rats. When the pH of these compounds is raised to 14, the absorption peak of the syringyl alcohol shifts to 245–6 nm and the guaiacyl derivative to 290 and 315 nm. The UV absorption spectrum of rat urine raised to pH 14 showed an absorption peak shift to 253 nm. It is not possible from these data to determine what compound(s) are being excreted by the rats, but we can assume these UV absorbing compound(s) are from lignin or other polyphenol degradation products present in the sawdust and released during pressure and acid hydrolysis.

LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis", 9th ed, Washington, D.C., 1960.
Butterbaugh, J. W., Johnson, R. R., *J. Anim. Sci.* **38**, 394 (1974).
Dehority, B. A., Johnson, R. R., *J. Dairy Sci.* **44**, 2242 (1961).
Driedger, A., Hatfield, E., *J. Anim. Sci.* **34**, 465 (1972).
Erlinger, L. L., Ph.D. Dissertation, University of Nebraska, Lincoln, Nebraska, 1974.
Erlinger, L. L., Klopfenstein, T., *J. Anim. Sci.* **41**, 1189 (1975).
Hudson, J. M., Ph.D. Dissertation, University of Nebraska, Lincoln, Nebraska, 1971.
Lawton, E. J., Bellamy, W. D., Hungate, R. E., Bryant, M. P., Hall, E., *Science* **113**, 380 (1951).
McDougall, A. I., *Biochem. J.* **43**, 99 (1948).
Mellenberger, R. W., Satter, L. D., Millet, M. A., Baker, A. J., *J. Anim. Sci.* **32**, 756 (1971).
Sarkanen, K. V., Ludwig, C. H., "Lignins—Occurence, Formation, Structure and Reactions", Wiley, New York, N.Y., 1971, pp 244, 245.
Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics", McGraw-Hill, New York, N.Y., 1960.
Stranks, N. W., *Forest Prod. J.* **9**(7), 228 (1959).
Tilley, J. M. A., Terry, R. A., *J. Brit. Grassland Soc.* **18**, 104 (1963).
White, A., Handler, P., Smith, E. L., "Principles of Biochemistry", 3rd ed, McGraw-Hill, New York, N.Y., 1964, pp 25, 528.
Wilson, R. K., Pigden, W. J., *Can. J. Anim. Sci.* **44**, 122 (1964).

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COMMUNICATIONS

Protein Classification and Nitrogen Extractability of Grape Seed Meal

Grape seed meal is investigated for the first time in order to evaluate its potential for food use. The amino acid composition shows that grape seed meal is deficient in sulfur-containing amino acids and in lysine, but contains the other essential amino acids in adequate levels. Protein classification based on solubility indicates that most of the protein N was residual, while glutelin, albumin, and prolamin fractions include the largest amount of simple proteins. The N extractability profiles of grape seed meal determined over the pH range 2.0–12.0 are reported.

The increasing interest for the serious food protein shortage in many parts of the world has led to intensive efforts toward exploring novel and indigenous plant protein sources at present not adequately utilized. Among the different vegetables proposed, oilseeds are the most economic, acceptable, and safe types of crops for protein production. In fact, oilseed flours as by-product of oil industry require a minimum of processing, have good biological value, and are relatively free from antinutritive factors and fermentable sugars.

Grape seed ranks as second in importance among Italian sources of vegetable oils and its production in 1976 has been of 160 000 tons (FAO, 1976). The defatted meal (10% protein) is a by-product of the oil industry, not currently marketed as animal feed because of its high crude fiber content. Grape seed meal has not yet been studied in order to obtain proteins to supplement foods. The dehulling and the presence of polyphenolic chromogens are the major problems in using grape seed meal for human diets; nevertheless, the high seed production in Italy and the very low cost of the meal lead to consider it as an available and economic protein source.

Values for proximate composition of grape seed meal have been reported (Defrancesco et al., 1976), but there

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has not been research on the protein classification and N extractability.

The object of the present work was to study the amino acid composition and the protein solubility of grape seed meal in order to evaluate its potential use for human consumption.

EXPERIMENTAL SECTION

Sample Preparation. Whole grape seeds were obtained from the wine-making process of the grape type Marsala. After the fermentation of the must, skins and seeds were submitted to the pressing. The residue separated from the skins and dried constituted the grape seeds used in the present investigation. Seeds were successively ground at 4 °C in a Sorvall omnimixer, and oil extraction was carried out with *n*-hexane at a solvent to meal ratio of 3:1 (v/w) for 16 h at 25 °C. The slurry was filtered under vacuum and the residue was dried by air aspiration and reground in a Buhler mill Model MLI-204 to pass a 100-mesh screen. The resulting product is listed grape seed meal and has been used for the present work.

Analytical Methods. Moisture, protein, lipid, crude fiber, ash, and tannin contents were determined according to AOAC procedures (1975). Total sugars were measured following the method of Dubois et al. (1956).

Amino acid analyses were performed on a LKB 4101 autoanalyzer according to Spackman et al. (1958). Methionine and cystine were determined by the procedure of Moore (1963) by oxidation with performic acid. The N to protein conversion factor was measured by the amino acid composition according to the method of Tkachuck (1969).

Protein Classification. Protein fractionation based on solubility following the classical Osborne method (1907) was performed essentially as described by Lund and Sandstrom (1943). Duplicate 1-g samples of the meal were three times successively extracted, 1 h for the first extraction, 20 min for the second, and the third, respectively, with 20 mL of double distilled water (pH 6.6), 5% NaCl (pH 7.0), 70% aqueous ethanol at 70 °C, and 0.2% NaOH (pH 12.2). The extracts with each solvent corresponding to the albumin, globulin, prolamin, and glutelin fractions, respectively, were pooled and centrifuged at 27 000*g* for 15 min. The N content of each protein fraction and of the insoluble residue was calculated by the microKjeldahl method and expresed as percent of the total N in the meal. Nonprotein N content was determined by adding to the meal 10% trichloroacetic acid.

Extraction of N. The N extractability values of grape seed meal were obtained according to Gheyassudin et al. (1970). Four-gram samples were extracted with 100 mL of water or salts over a pH range of 2.0–12.0; the pH was kept constant during the extraction using 0.5 N HCl or 0.5 N NaOH. Extracts were centrifuged at 27 000*g* for 15 min, filtered through Whatman No. 3 paper and the filtrate analyzed for Kjeldahl N. All values reported are the average of triplicate experiments.

RESULTS AND DISCUSSION

Proximate composition of defatted grape seed meal was 9.0% moisture, 10.1% protein (expressed as N × 5.7), 1.8% lipid, 3.6% ash, 57.4% crude fiber, 9.7% total sugar, and 1.4% tannin. The composition of grape seed meal was similar to that of the high fiber fraction of a typical oilseed flour, while the low level in tannins was probably due to the diffusion of these compounds in alcohol during the previous wine-making process.

Table I shows the amino acid composition of grape seed meal. The limiting amino acids appear to be sulfur-containing amino acids (1.40%) and lysine (3.14%). The other essential amino acids were found present in adequate amounts, particularly leucine (8.16%), phenylalanine (4.59%), and threonine (4.09%). The amino acid composition of grape seed meal has been compared with that of the meal of Italian sunflower varieties (Canella et al., 1976); grape seed meal contained more leucine, isoleucine, and threonine and less sulfur-containing amino acids and lysine than sunflower meal. The N to protein conversion factor for grape seed meal calculated from the amino acid composition corresponds to 5.11.

Table I. Amino Acid Composition of Grape Seed Meal (g/100 g of Protein)

Amino acid	Grape seed meal	Sunflower meal[a]	FAO ref protein[b]
Lys	3.14	3.36	4.2
Met + Cys	1.40	3.57	4.2
Phe	4.59	4.23	2.8
Tyr	2.72	2.28	2.8
Trp	*c*	1.09	1.4
Ile	4.98	3.76	4.2
Leu	8.16	5.78	4.8
Thr	4.09	3.25	2.8
Val	5.54	4.51	
His	2.72	2.19	
Arg	6.89	8.57	
Gly	7.90	5.39	
Ser	4.99	3.80	
Ala	5.34	3.87	
Asp	9.14	8.61	
Glu	23.74	22.30	
Pro	5.67	3.80	

[a] Canella et al. (1976). [b] FAO (1970). [c] Tryptophan was not determined because of destruction during acid hydrolysis.

Protein classification of grape seed meal based on solubility differences provided the following results. Most of the protein N (79.5%) was residual (insoluble in the solvents used to extract proteins). Only 17.6% of the total N made up simple proteins. Glutelin (7.9%), albumin (5.7%), and prolamin (3.1%) fractions included the largest amount of simple proteins, while globulin was negligible (0.9%) and nonprotein N was 2.6% of the total. The residual protein may include proteins that are denatured or conjugated with different nonprotein groups, constituting glucoproteins, lipoproteins, chromoproteins, and others. The high amount of residual proteins in grape seed meal could be ascribed, not only to the presence of these derived proteins, but also to the high content of fiber bodies which, by occluding proteins, prevent their extraction.

The N extractability profiles for grape seed meal are reported in Figure 1. The curve in water shows a broad pH range (2.0–8.0) of minimum solubility (about 10%). The increase of N extracted at alkaline pH values is due to an increased ionization of proteins resulting in solubilization. The pH dependence of solubility in either 1 M NaCl or 1 M $CaCl_2$ is not much different from that in the absence of salts, but the percent of N extracted is found lower.

The low content of soluble proteins remarkably reduces the potential of grape seed meal for applications in foods. An effective dehulling of the seeds, removing a part of the fiber should probably increase the protein solubility. Unfortunately, the dehulling is difficult because of the size of the grape seed. Another problem to be solved consists in the removing of the polyphenolic constituents which impart a dark-brown color to the alkaline protein extracts. The extraction of these pigments might be achieved by means of an appropriate hydroalcoholic mixture, as that used to remove chlorogenic acid from sunflower meal

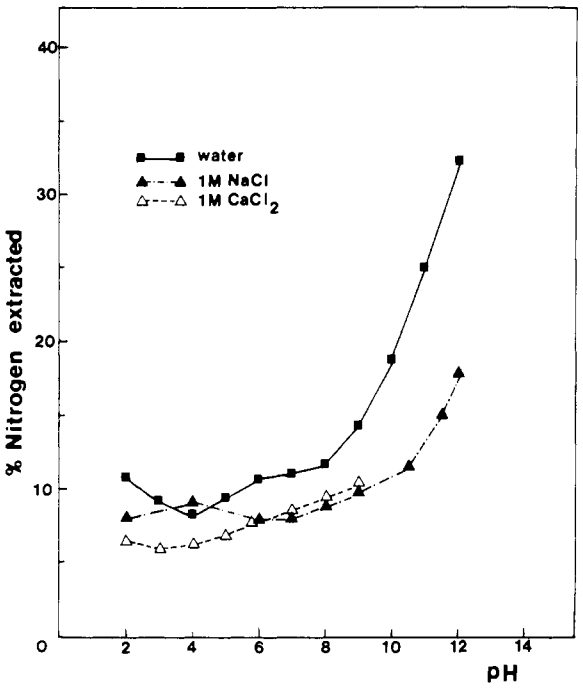

Figure 1. Solubility of the N of grape seed meal in water and salt solutions as a function of pH.

(Sodini and Canella, 1977). Further work is required to establish if a dehulled grape seed meal with a subsequently more soluble protein content and free of color-forming precursors would represent an interesting source of economic proteins to utilize in food preparation.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, D.C., 1975.
Canella, M., Castriotta, G., Mignini, V., Sodini, G., *Riv. Ital. Sostanze Grasse* **53**, 156 (1976).
Defrancesco. F., Margheri, G., Avancini, D., Casagrande, S., *Riv. Soc. Ital. Sci. Alim.* 1, 15 (1976).
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F., *Anal. Chem.* **28**, 350 (1956).
FAO, "Amino Acid Content of Foods and Biological Data on Proteins", Vol. 24, Rome, 1970, p 100.
FAO Yearbook, Rome, 1976.
Gheyassudin, S., Cater, C. M., Mattil, K. F., *Food Technol.* **24**, 242 (1970).
Lund, A. P., Sandstrom, W. M., *J. Agric. Res.*, **66**, 349 (1943).
Moore, S., *J. Biol. Chem.* **238**, 235 (1963).
Osborne, T. B., Publication No. 84, Washington, D.C., 1907.
Sodini, G., Canella, M., *J. Agric. Food Chem.* **25**, 822 (1977).
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30**, 190 (1958).
Tkachuck, R., *Cereal Chem.* **46**, 419 (1969).

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Biosynthesis of Flavor Compounds by Microorganisms. 3. Production of Monoterpenes by the Yeast *Kluyveromyces lactis*

The monoterpenes citronellol, linalool, and geraniol were found in the odorous constituents produced by the yeast *Kluyveromyces lactis* in aerobic submersed culture. Their biosynthesis did not require special precursors. Citronellol and linalool were produced at about 50 μg/L, while geraniol could be detected only in traces; added geraniol was reduced nearly quantitatively to citronellol. By changing the culture conditions it is possible to influence the yield of monoterpenes as demonstrated for citronellol. With increasing temperature and with increasing concentrations of asparagine, as a nitrogen source, citronellol was formed at a higher rate.

Monoterpenes are of interest because of their structural characteristics and their physiological and sensory activities. These properties result in broad applications in flavor industry, food industry, and pharmacy. In 1972 Devon and Scott listed about 400 monoterpenes, which are primarily products of higher plants. Little is known about the occurrence and biosynthesis of monoterpenes in microorganisms. In 1964 Katayama found geraniol, *d*-limonen, and α-pinen in algae. Collins and Halim (1970, 1972) and Halim and Collins (1971) reported the presence of monoterpenes in cultures of the fungi *Ceratocystis variospora*, *Trametes odorata*, and in various species of the fungus *Phellinus*. The mechanism of biosynthesis of monoterpenes in fungi was studied by Lanza and Palmer (1977) by feeding radioactive precursors to *Ceratocystis moniliformis*. As part of our studies on the biosynthesis of flavor compounds by microorganisms (Schreier et al., 1975, 1976) we found that the yeast *Kluyveromyces lactis* also produces monoterpenes.

EXPERIMENTAL SECTION

Kluyveromyces lactis No. 2359 was obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The organism, maintained on YM-agar (Difco), was cultivated in 1-L Pasteur flasks, plugged with cotton wool, containing 300 mL of modified (Difco) Yeast-Carbon-Base (C, N sources and ratios, vitamin supply). The inoculated media were shaken at 180 rpm, usually at 25 °C. After a cultivation period of 2 to 4 days the cells were removed by centrifugation. The clear culture broth was extracted with pentane–methylene chloride (2:1) in an apparatus for liquid–liquid extraction, free acids were removed with 5% $NaHCO_3$ solution, and the pentane–methylene chloride phase was then dried over anhydrous Na_2SO_4 and concentrated on a Vigreux column at 45 °C (Drawert et al., 1969). Gas chromatography was carried

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