Grapeseed as a Possible Source of Food Protein

Communication of a work team directed by **Prof. Andrea Corrao** of the University of Palermo[1] in the ambit of a research program of the Italian C.N.R., Istituto Di Industrie Agrarie Dell'Universita' Viale Delle Scienze – Parco D'Orleans, Palermo, Italy

Materials of various provenience have been considered from the viewpoint of their protein content: 1. raw grapeseed, hand-separated from pomace and air-dried at room temperature; 2. toasted grapeseed from distillery; 3. defatted and defibered grapeseed meal of industrial provenience, for animal fee.

Apart from analytical results on the composition of these materials, which help to improve the knowledge of the grapeseed as a raw material of food interest, there will be reported here, as they seem particularly significant, only the results of some tests on grapeseed nitrogen extractability.

On a defatted and defibered meal (19.4% crude protein and 10.5% polyphenols) prepared from raw grapeseed, and comparatively on the same material partially derived of phenolics (21.9% crude protein and 3.8% polyphenols), the water extractability of nitrogen was examined at pH 2 through 11, at room temperature and 5% (w/v) meal conccentration.

Solubility curves showed that partial elimination of phenolics greatly improve the nitrogen solubility in the whole pH range from 2 to 9.

In particular the nitrogen extractable at pH 7, which represented only 5.7% of total nitrogen in the original meal, was increased to 38.5% in consequence of the partial elimination of phenolics.

At pH 8 the extractable nitrogen rose from 15.7 to 45.4% of total nitrogen.

Extraction of proteic constituents from raw grapeseed meal requires a preliminary minimization of phenolics to improve the nitrogen solubility at pH values favorable to protein integrity. From the partially phenolics-freed meal, a protein isolate containing 87.5% crude protein and 2.5% polyphenols was prepared by extraction at pH 8 and isoelectric precipitation. For the raw grapeseed meal, this polyphenol content seems to be the phenolic fraction which is more strongly bound to proteic constituents of grapeseed. The nitrogen solubility as a function of pH was examined as well, under the same conditions, in samples of grapeseed meal of industrial provenience. For this material containing partially oxidized polyphenols, a partial elimination of phenolics did not improve the nitrogen solubility, which was nearly 28% at pH from 7 to 9. Higher extractability values, but not above 50%, corresponded to pH levels between 10 and 11.

From an industrial meal containing 25.9% crude protein and 7.4% polyphenols, a protein isolate containing 87.6% crude protein and 0.5% polyphenols was prepared by extraction at pH 8 and isoelectric precipitation. The protein-to-polyphenols ratio, equal to 3.5 in the original meal, increased to 175 in the isolate.

Investigations are in progress on toasted grapeseed from a distillery. This represents the intermediate step of a technological chain from raw grapeseed to industrial meal.

The nitrogen extractability of a toasted grapeseed meal, without a preliminary elimination of polyphenols, was of 30-35% in the range of pH from 7 to 9 and reached 40% at pH 11.

These experimental results permit some preliminary considerations. 1. For raw grapeseed material rich in natural polyphenols, the partial elimination of these is necessary to extract protein with relatively good yield at convenient pH level. But raw grapeseed as a source of protein is only a reference material of scientific and experimental interest. 2. From industrial defibered meal containing partially oxidized polyphenols and showing a fairly good nitrogen solubility at pH from 7 to 9, it is possible to prepare protein isolates with a very low polyphenol content, without an elimination of phenolics, but with a low yield protein. 3. There are good reasons to believe that the toasted grapeseed from a distillery could become a convenient source of food protein through a process for simultaneous recovery of protein and oil.

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Development of Lupine Proteins

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ABSTRACT

Lupine is a potentially valuable seed protein producing crop for temperate climates. Alkaloid-free varieties have been developed. The true seed protein content varies from 30% to 45%. Protein quality and digestibility compare favorably with those of soya. Oil yields come upward of 10%, and the composition is similar to soy bean oil. Lupine is relatively free of antinutritional factors present in other legumes. Large scale nutritional trials have been carried out. Lupine isolates have been used in preparation of foods.

Lupine is being investigated in several countries as a potential seed protein producer. Its possibilities are particularly appealing for areas in the world where soy does not grow.

Lupine is tolerant towards a wide variation of soils and of climatic conditions. It is grown as an aestival crop in cold temperate areas and as a winter crop in temperate and warm temperate ones; it tolerates frost and drought. It requires sandy and silt-sandy soils from strongly acid to calcareous ones with preference for moderately acidic (1). The adaptability of lupine to poor soils on which other crops would not survive provided it with the sinister reputation that it conferred barrenness on land on which subsequently other crops were unsuccessfully cultivated. On the contrary, its nitrogen-fixing capacity causes a saving of fertilizers which is estimated at up to 80-100 kg N per hectare.

The presence of toxic quinolizidine alkaloids in the

TABLE I

Total Protein and Essential Amino Acid Content of Whole Lupine Seed[a]

	Lupinus species[b]					
	Albus	Angustifolius	Luteus	Mutabilis	Soya[b]	Whole hen's egg[c]
Total protein	35.4	31.2	44.0	42.9	35.0	---
Ile	5.12	3.84	4.55	4.68	4.54	6.64
Leu	8.53	6.63	8.96	7.42	7.68	8.85
Lys	5.56	5.17	6.10	5.95	6.38	6.45
Met+Cys	2.78	2.29	3.11	2.67	2.59	5.54
Phe+Tyr	8.87	7.11	6.91	7.91	8.08	10.03
Thr	4.14	3.54	3.99	4.01	3.86	5.07
Trp	0.94	0.91	0.86	0.84	1.28	1.60
Val	4.95	4.03	4.25	4.01	4.80	7.26
MEAA[d]	74.31	62.21	70.65	68.33	69.47	100

[a] Proteins are given as per cent of whole seed; amino acids as g per 16 g N.

[b] From ref. 2.

[c] Report of a Joint FAO/WHO Expert Group "Protein Requirements," n. 301, (1965).

[d] Modified Essential Amino Acid Index (see text).

original varieties has undoubtedly been a severe hindrance to the development of lupine as a major arable crop. It was grown for food and feed in the ancient Mediterranean civilizations and in pre-Colombian American in the Andean regions of Chile, Peru, and Bolivia. Its modern development can be traced to the interest of the Prussian king Frederick II in the seventeenth century, but significant improvement initiated during and after World War I in Germany. Gradually, "sweet" varieties with low alkaloids have replaced, in part, their original "bitter" counterparts. In recent years plant breeders in many different centers have given systematic attention to this problem.

In Europe lupine is cultivated in Poland where the estimated area was 400,000 hectares in 1964, including about 300,000 hectares of alkaloid-free varieties, but it later declined due to availability and use of fertilizers. In the USSR, mainly North Ukraine and Byelorussia, the total area sown to lupine was 1,150,000 hectares in 1960, of which all but 100,000 hectares were sweet lupine, and an expansion was forecasted. Experimental and limited commercial cultivation has been reported in the Netherlands, West Germany, Denmark, Sweden, and England. Outside Europe an important center for sweet lupine cultivation is the Cape Province in South Africa; in Swartaland it occupies some 25% of cultivated area in rotation with cereals. A limited area is cultivated in western Australia and some also in New Zealand. Cultivation of alkaloid-free lupines in the Gulf Coast States of the USA has been limited by frost and disease (1).

Protein content in lupine seeds shows significant interspecific variation, but in general it compares well with that of soya (Table I). Protein yield data can be confusing, as they may be quoted for whole seed or for dehusked material or for "flour," and in lupine the husks represent a high proportion of the whole dry seed, ranging from 15% in *L. albus* to 25% in *L. luteus,* whereas soybean has 9% hull on the average, and it has a very low protein content. Hove (3) quotes 2 to 3 per cent protein for the husk against 38 to 50 per cent for the kernels in his studies on *L. angustifolius,* Uniwhite, and *L. luteus,* Weiko III, respectively.

Another point in favor of lupine is the high yields per hectare. For *L. angustifolius* and *L. mutabilis* field yields above 5 metric tons of beans per hectare have been reported (4,5); this means for the latter variety about 2 tons per hectare overall protein produced. Yields of beans on a dry matter basis are nevertheless lower than in other legumes, and this suggests that substantial genetic improvement can still be achieved.

Determinations of the essential amino acids for the seed proteins of four main lupine species are compared in Table I with those of soy and with egg protein. *L. luteus* is seen to be limiting in valine, but the other species are all limiting, like soy protein, in the sulfure-containing amino acids. Judging protein quality by the *Modified Essential Amino Acid* index (MEAA), which includes the contribution of all essential amino acids and represents protein quality better than the chemical score, only one species, *L. angustifolius,* is inferior to soy bean, one is marginally lower, and two, *L. albus* and *L. luteus,* are much better. Supplementation with 0.6% methionine (or with Quinoa flour) has been found to raise the protein efficiency ratio of lupine flour above that of casein added with 5% methionine (6).

Data in the literature, though based in incomplete resolution, indicate that seed globulins consist of several fractions with similar properties in different lupine species. The most detailed information has been obtained on *L. albus* (7,8), and we will refer to it. Globulins are 84% of total proteins of the dehusked seed. They divide into six major fractions with M.W. 430,000, 330,000, 300,000, 260,000, 225,000, 187,000, which all contain a carbohydrate. One has isoelectric point 7.9; all the others have values in the acidic range between 5.7 and 6.2. All dissociate into several different subunits; most associations depend on rather loose ionic interactions, but some are due to disulfide bridges. Some subunits of different fractions appear highly similar (7). Three of the fractions do not contain any cysteine or methionine or have only traces of them; three have both these amino acids at a higher level than soy protein. Fraction 1 represents only 5.7% of total globulins. The two other fractions with sulfur-containing amino acids are very much like in subunit composition and together make up 45% of total globulins; in Table II they are given jointly as fraction 7. Cysteine is present in apparently identical subunits (7,8).

The albumin component of seed has a better amino acid composition than globulins but is scarce and contains a large number of different molecular species.

Results obtained on *L. angustifolius* suggest that biosynthetic regulation affects the globulin fractions as separate entities, though their changes are interrelated. Indeed deficiency of sulfur in the nutrient medium decreases two sulfur-rich protein components, which move in electrophoresis like our fractions 1 and 7 respectively, and increases a component poor in sulfur with same electrophoretic behavior as fractions 4,5 and 6 (9). The selective amino acid distribution in separate molecules and the possibility of shifting their ratios give hope that a genetic approach may be effective in improving the quality of lupine globulins.

The digestibility of lupine proteins is good both in vitro and in vivo and compares favorably with that of soy proteins (2). Indeed only a very modest antitryptic and urease activity have been reported for *L. albus* and a saponin content similar to that in soybeans, but lupine is free of

TABLE II

Essential Amino Acids of Globulin Fractions of Lupine Seeds (g/16 g N)

	Total globulin extract	Fraction 1	Fraction 4	Fraction 5	Fraction 6	Fraction 7
Ile	4.50	4.91	4.89	3.93	4.17	4.92
Leu	7.36	9.35	7.31	6.49	7.12	8.11
Lys	3.90	6.34	4.10	3.78	3.56	3.67
Met+ Cys	1.75	3.37	0.00	0.03	0.00	3.87
Phe+Tyr	8.43	8.40	9.40	9.68	11.23	8.02
Thr	3.08	7.43	3.04	2.85	2.68	5.01
Trp	0.49	1.11	0.04	0.00	0.00	0.53
Val	3.16	6.30	3.09	2.39	3.00	4.10
MEAA[a]	54.80	81.68	26.33	19.89	22.30	65.80

[a] Modified Essential Amino Acid index (see text).

TABLE III

Yield and Component Fatty Acids of Whole Lupine Seed Oil and of Soy Bean Oil[a]

	Fatty acids: Carbon atoms/double bonds						
	16:0	18:0	18:1	18:2	18:3	22:1	Oil yield, %
L. albus	8.3	1.7	54.4	18.2	8.5	3.0	5.1 to 11.9
L. angustifolius	12.7	6.3	34.6	38.7	4.6	3.5	5.5 to 6.1
L. luteus	8.5	2.7	28.8	46.7	5.5	3.6	3.3 to 5.6
L. nutabilis	13.3	8.2	44.3	30.2	2.4	0	7.2 to 14.3
L. termis	8.1	2.1	46.2	19.2	9.2	n.d.	9.5
L. nootkatensis	14.3	1.7	32.1	41.1	8.8	n.d.	10.7
Soy	11.5	3.9	24.6	52.0	8.0	0	13 to 20

[a] Data from ref. 2 and 6.

haemagglutinins, isoflavones, and other components typical in legumes. Also the content of flatus-inducing oligosaccharides is relatively low.

A further reason to consider lupine as a substitute for soy is its potential as an oilseed, though, as shown in Table III, it yields less oil than soybeans. The component fatty acids in lupine seed bear a general resemblance to those of soybeans except in the ratio between 18:1 and 18:2. Linolenic acid (18:3), an undesirable component from the point of view of oil quality and keeping properties, is present in all species at a level only lightly higher than in soy. Small amounts are found of another undesirable compound, erucic acid (22:1), which is absent in soy. However, *L. mutabilis* is free of this acid and also contains much less linolenic acid than soy. Linoleic acid (18:2), the principal essential fatty acid, is always an important component. The total content of saturated acids is usually quite low. On balance, therefore, lupine seed oil can be considered nutritionally good. Processing of lupins to oil and meal is similar to the processing of soybeans and can be performed in existing oil processing plants; dehulling is facilitated by the weak adhesion between hull and kernel.

A prime objective of breeding programs is to raise the oil yield to levels which have already been achieved by a similar procedure in the case of soybean, and which are necessary economically in order to justify a modern oil seed extraction plant. Indeed the technological utilization of proteins depends on the availability at economical costs of a defatted and debittered intermediate.

Breeding programs have been successful in virtually removing alkaloids from commercial varieties of *L. angustifolius* and *L. luteus*. Of the principal components, lupanine is the most dangerous: 20 mg/kg administered intraperitoneally is toxic to guinea pigs (10). Hydroxylupanine has only one-tenth of the toxicity of lupanine. In humans, reports on fatal cases involving infants and children give estimated alkaloid doses ranging from 11 to 25 mg per kg body weight (11). Nonfatal cases of poisoning of adults have been reported involving estimated doses of 25 to 46 mg of alkaloids per kg body weight. It can be concluded that in varieties in which total alkaloid is below 0.1 per cent, an amount of lupine flour containing 100 g protein can be safely consumed daily by an adult. Moreover, toxic components are separated during technological treatments of the flour. At present, "sweet" lupin flour is approved for use in infant food at levels up to 15% in Chile.

Gross and associates in Peru have carried out large scale nutritional trials with food containing up to 40% flour from locally grown white lupine low in alkaloids. No disturbances were observed and several items were better accepted than the corresponding traditional food. A program for producing an alkaloid-free protein isolate is in progress (5,12,13).

Debittered lupine flours were used successfully in breadmaking and in cereals on an experimental basis as early as 1919. The relatively high lysine and deficient sulfur-containing amino acids point out a complementary effect with grain flours: indeed, 3% lupine flour added to rye improved by 27% the biological value of bread (6). In pasta and bakery items, lupine flour may substitute for egg also because of the yellow color it confers to the finished product. Pompei and Lucisano (14) found that proteins of a lupine isolate added to wheat flour (40% as compared to the proteins of wheat) gave properties of hard wheat flour to a typical bread-making flour. Lupine proteins extracted by wet milling then precipitated at the isoelectric point and dried promoted water-binding in a meat-protein system, whereas dry milling and alkaline extraction enhanced the emulsifying capacity (14). Experimental lupin products were made in Chile into items such as gruels, gravies, and baked goods (15).

A German patent of 1942 (16) covers spinning fibers for food use from lupine seeds; preliminary texturization runs at INTEC (Chile) gave promising results. The major expected technological advance now is to produce from lupine, as has been done for soy, structured, semimanufactured products that may substitute for meat in human nutrition.

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Sesame Protein: A Review and Prospectus

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ABSTRACT

Sesame is one of the earliest condiments and crops grown for edible oil. Sesame is consumed directly as sweetmeat, a "peanut butter-like" product, a candy ingredient, bread condiments, and snack foods. The world production of sesame is about 2,000,000 metric tons. China and India are the largest producers but internally consume their production. Sudan is the largest exporter of seed. Sesame contains 50% oil, which is highly resistant to oxidation, and 25% protein, which has a unique balance of amino acids. Dehulling of sesame for human consumption is important since the hull contains 2-3% oxalic acid, which chelates calcium and has a bitter flavor. Dehulled, defatted meal contains 60% protein, is bland, and contains limited qualities of flatulence-causing sugars and high quantities of phytic acid. Aqueous processing yields isolated protein containing 72% protein and recovers 56% of the seed protein. Sesame protein is very stable to heat and contains large quantities of methionine. Sesame meal has a PER of about 1.35. Sesame is low in lysine and requires supplementation or can be blended with soy protein to give PERs nearly equivalent to casein. Sesame protein is composed of nearly 80% α-globulin and 20% β-globulin. Limited attempts have been made to characterize these 2 fractions. Sesame protein has low solubility that limits food applications in its native form. Sesame protein performs better than other oilseeds in baking applications. Production of sesame is limited to countries where labor is plentiful and inexpensive until indehiscent varieties and/or improved mechanical harvesting techniques are developed. However, intense breeding and engineering research programs are in progress.

INTRODUCTION

Sesame, *Sesamum indicum L,* may be the earliest condiment used and the oldest crop grown for edible oil. The seed has been called the "queen of the oilseed crops" because of the high yield of oil and quality of the seed, oil, and meal (1). Accounts of ancient history and mythology document early recognition of sesame seed as a source of high quality food. According to the Assyrians, the world was not created until the gods first refreshed themselves with sesame wine. Sesame was the symbol of immortality in early Hindu legend (2). Archeological evidence indicates that sesame was cultivated in Palestine and Syria around 3000 BC and in the civilizations of Babylonia, 1750 BC, and Indus Valley, 2500 BS (3). An Egyptian tomb bears a 4,000-year-old drawing of a baker adding sesame to bread dough. Archeologists have found sesame seed mash in the ruins of the Old Testament kingdom of Araret. In 1298 Marco Polo observed the Persians using sesame oil for cooking, body massage, medicinal purposes, illumination, cosmetics and lubricating primitive machinery.

WORLD PRODUCTION

Sesame is grown primarily in less developed tropical and subtropical areas of Asia, Mediterranean, and South America. Current world production is estimated at about 2,000,000 metric tons annually, placing sesame behind soybean, peanut, cottonseed, sunflower, linseed and rapeseed, in the quantity of world oilseed production (4,5). Acreage, yield and production on a world basis has largely remained the same over the past five years. In 1976, 1,900,000 metric tons were grown on 6,400,000 hectares with an average yield of 307 kg/hectare. The relative standing of the world's 10 leading sesame-producing countries in 1976 is summarized in Table I. India devotes the greatest acreage to sesame, but has one of the lower records for yield per hectare. India produces nearly 21.4% of the world sesame crop, followed by China at 19.6% and Sudan at 13.5%. Sudan is the major world exporter (6). Asia and Africa produce nearly 90% of the world supply of sesame. Most of the seed is consumed in the countries where it is produced; less than 5% of world production enters export trade (1).

Harvesting characteristics of sesame have precluded the development of a successful crop in the U.S. and other developed countries. The seed capsules of normal dehiscent (shattering) sesame varieties open at maturity. Considerable care is required to prevent excessive seed loss. The shattering of the seed pod is desirable in China and India where an adequate supply of cheap labor enables hand harvesting and threshing. The lack of uniform ripening of pods has further complicated mechanical harvesting techniques and breeding efforts. Seed pods at the base of the plant may be opening while the upper part of the plant is still flowering. Where mechanization is the basis for successful crop pro-