AN EVALUATION OF THE PHYSICAL, CHEMICAL AND FUNCTIONAL CHARACTERISTICS OF THE WHITE AND NARROW-LEAFED LUPINES

(Lupinus albus and *Lupinus angustifolius)*

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Bassoodeo Oomah

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ABSTRACT

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AN EVALUATION OF THE PHYSICAL, CHEMICAL AND FUNCTIONAL CHARACTERISTICS OF THE WHITE AND NARROW-LEAFED LUPINES (*Lupinus albus* and *L. angustifolius*). Major Professor: W. Bushuk.

Seed of four lupine cultivars, harvested at different stages of maturity, was used to investigate compositional differences. Four other lupine varieties from two species *L. albus* and *L. angustifolius* were also included to study various physicochemical characteristics of lupines. Flours of three wheat cultivars (strong, medium and weak dough mixing strength) were used to examine the functional characteristics of lupine flours in the breadmaking quality of composite flours.

The total seed nitrogen of lupines increased slowly during the growth period; but the nitrogen contents of the *L. angustifolius* cultivars were higher than those of the *L. albus* at all times during seed development. Seeds in the pod of the lateral axes in the albus species had lower nitrogen content than those from the main axis.

Cultivars of *L. albus* had twice as much lipid as those of *L. angustifolius*. Oleic acid is the major fatty acid of all lupines. Total saturated fatty acid content decreased and the total unsaturated fatty acid content increased from 30 to 60 days after anthesis. Small amounts (1.5%) of erucic acid were present only in the *L. albus* cultivars.

Cellulose forms the major part of the total carbohydrate of lupines

(about 50%). The *L. albus* cultivars were richer in ethanol-soluble sugars than those of *L. angustifolius*. Cultivars of the latter species were richer in sucrose and verbascose but poorer in glucose and stachyose than those of *L. albus*. Starch was present in trace amounts. The other carbohydrate components were hemicelluloses and reducing sugars.

Results of the protein solubility fractionation experiments showed that varieties which were low in albumin had a high globulin content. Extraction of the lipids from lupine flour, reduced the solubility of the globulin fraction and increased that of the alkali-soluble fraction. It was found that distilled water alone can solubilize up to 75% of the total soluble lupine proteins. The molecular weight distribution of the proteins, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, revealed differences among cultivars and between the two species. Amino acid compositions showed that cultivars of the albus species had higher quantities of some essential amino acids than those of the angustifolius species.

A new resinoid disc dehuller was used on a batch basis to dehull lupines without affecting the seed. Pin-milling and air-classification studies revealed that lupine flour cannot be fractionated into flours of different protein content by air classification. However, dehulling and milling of lupine seeds into flour is possible on an industrial scale.

The breadmaking properties of composite flours comprising wheat flours (of strong, medium and weak dough mixing strength) and lupine flour were investigated. Lupine flour was used to replace 5, 10, 20 and 30% (flour basis) of the wheat flour. Lupine flour produced changes in the breadmaking properties suggesting a simple dilution of wheat protein.

Satisfactory bread can be produced from composite flours containing up to 10% lupine flour. Minor adjustments in the breadmaking procedures would be required if a weaker wheat is used as the base flour.

Differences in the cell structures of white lupines were apparent on the scanning electron microscope at approximately 20 days after anthesis. The cellular structure of the cotyledonary cells as well as that of the seed coat changed drastically from 20 to 40 days after anthesis. Protein forms the major storage product in mature white lupine cotyledonary cells. It is deposited in the form of protein bodies which occupy most of the cell volume at maturity.

Some microscope observations of the cellular structure of *L. albus* are described. The oil, probably membrane bound, is regularly oriented near the cell wall and around the protein bodies and is seen as small electron-transparent droplets. Protein bodies are of different sizes and shapes. Phytin occurs within protein bodies and appear as black specks. Starch granules, though not numerous, are present in the cotyledon cells. In mature seeds, the remaining organelles are compressed between the protein bodies.

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ABBREVIATIONS

aa	Amino acids
AACC	American Association of Cereal Chemists
ADF	Acid detergent fiber
AOAC	Association of Official Analytical Chemists
BU	Brabender Unit
CSL	Calcium stearoyl-2-lactylate
CW	Cell Walls
D	dextro (used before carbohydrates and amino acids to show that the groups at the asymmetric carbon atom are placed at the right)
D	Dictyosomes
DAA	Days after anthesis
db	dry basis
DEAE	Diethylaminoethyl
DM	Dry matter
DMCS	Dimethyldichlorosilane
ER	Endoplasmic reticulum
FAO	Food and Agriculture Organization
G	Globoids
GG	Groove Gauge
GRL	Grain Research Laboratory
HMDS	Hexamethyldisilizane
IS	Intercellular space
L	Levo (in configurational sense only)

L	Lipids
LF	Lupine flour
LWFB	Lupine wheat flour blend
M	Mitochondria
M	Molar
MA	Milliampere
MEAA	Modified essential amino acid(s)
N	Nucleus
NFDM	Non fat dried milk
Nu	Nucleolus
OD	Optical density
PB	Protein bodies
α-PDG	Phenyl α-D-Glucopyranoside
PER	Protein efficiency ratio
Ph	Phytin
Pl	Plasmodesmata
Pp	Proplastids
S	Starch
S	Subunits
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscope
SSL	Sodium stearoyl 2-lactylate (Emplex)
TCA	Trichloracetic Acid
TEM	Transmission Electron Microscope
TFA	Trifluoroacetic Acid
V	Vacuoles

v/v	per cent "volume in volume"
W	Wire (for sieves)
w/v	per cent "weight in volume"

I. INTRODUCTION

Lupine, a legume crop, is being investigated in several countries as a potential seed protein producer. Its possibilities are particularly appealing for areas in the world where soybean are not adapted. The adaptability of lupines to poor soils on which other crops do not survive has further increased the potential of this new crop. Its nitrogen-fixing ability produces a saving of fertilizers which is estimated at up to 80-100 kg N per hectare (Cerletti and Duranti, 1979).

Lupine is not entirely new to Canada, records show that over 53 species of lupines existed in North America as far back as 1873. Dunn and Gillett (1966), in their monograph "THE LUPINES OF CANADA AND ALASKA", reported that five annual and 14 perennial species of lupine have been found in Canada. However, the North American lupines have not been exploited in commercial agriculture.

Lupines obtained from West Germany and Australia were introduced for field-testing in 1972 at Saskatoon and Morden (Stauffer) and at the University of Manitoba (Furgal, 1974). In the Miscellaneous Nursery, several entries produced seeds containing 42-45% protein, but the yields were extremely low. It was found that generally, the species of *L. albus* performed substantially better than either *L. angustifolius* or *L. luteus*.

The objective of the present study was to characterize lupine seed by determining relevant biochemical, physical and functional properties as a basis for its potential use as a feed and food crop. The project also includes examination of intra and interspecific variations of the two promising species, *L. albus* and *L. angustifolius*. On the premise that factors responsible for biochemical properties are more likely to be evident during early kernel development, the changes in protein content, fat content and morphological characteristics were examined for the two species at various stages of maturity. The study also emphasizes some technological characteristics relevant to the potential use of lupines in composite flours for bread and other baked products.

II. LITERATURE REVIEW

This review of the publications on the chemistry and technology of lupines will be presented in two sections. The first section will review the literature on the biology of lupines in the context of its potential as a new agricultural crop. The subsequent sections will review relevant literature on the protein and carbohydrate components and functional characteristics of lupines.

A. Biological Characteristics of Lupines

The genus Lupinus is one of the legumes in which mankind has been interested since ancient time. Recently Cristofolini and Chiapella (1977), in a serological study of the tribe GENISTEAE (Fabaceae) found only a partial concurrence with Hutchinson's classification in four tribes of the Genisteae species (Cystiseae, genisteae, laburneae and lupineae). They suggested that the lupinus species is very dissimilar and therefore should not be included with other genera of the tribe Genisteae.

According to Zhukovsky (1929), the genera Lupinus consist of 250-300 species, but more recent data of Cristofolini and Chiapella (1977) suggest a total of about 100 species with only about ten species in Europe. The differences in the number of species reported might be due to the reconstruction and reclassification of the taxa. Furthermore, Harborne (1971) reports 100-200 species of lupines according to his taxa.

Five lupine species grow wild in the Prairie Provinces (Boivin, 1967). They are *L. polyphyllus*, Lindley., *L. nootkanensis*, Donn., *L. argenteus*, Pursh., *L. sericeus* and *L. pusillus*. Dunn and Gillet (1966) have reviewed the introgression among these Canadian species. They found that all the five annuals have a haploid number of twenty-four (2N=48).

Although the lupine genus comprises over a hundred species, only fifteen of them have been cultivated as an agricultural crop. The most commonly grown species are *L. albus*, *L. angustifolius*, *L. luteus*, *L. consentinii*, *L. mutabilis* and *L. polyphyllus*. All these are large-seeded lupine species. They are listed in Table 1, along with their chromosome numbers.

The genus is unique in being distributed over both the Old and the New Worlds. Species of the genus grow in the warm tropical countries Brazil, Sudan, Somalia as well as in the cold Arctic region of Alaska. In Portugal, Spain, Italy, Greece and many other countries, species like *L. albus*, *L. angustifolius* and *L. luteus* are widespread both in cultivated and wild forms. However, significant differences of these species in both forms of lupines do not exist. Zhukovsky (1929) reports that lupines belong to a small group of plants, which despite the process of evolution, shows no great difference between the domesticated forms and their progenitors. In other words, lupines, unlike other crops, lack genetic variability. Such crops are adapted to the rather crude land preparation, seeding, weeding and harvesting procedures of traditional agriculture (Harlan 1975). They are also adapted to low soil fertility.

L. albus is the oldest-established cultivated species among the Mediterranean and African lupines (Gladstones 1974). The Ancients used *L. albus* for green manure and the seeds for cattle feed and human food, after steeping to remove the water-soluble alkaloids, as well as for

Table 1. AGRICULTURALLY IMPORTANT LUPINUS SPECIES, THEIR COMMON NAMES AND CHROMOSOME NUMBERS

Species	Common Name	Chromosome Number	
		2n[1]	n[2]
L. albus	White lupine	50	25
L. angustifolius	Narrow-leafed lupine	40	20
L. luteus	Yellow lupine	52	26
L. consentenii	Sandplain lupine	32	--
L. mutabilis	Sweet lupine	48	--

[1] Gladstones (1970).
[2] Confirmed by Pazy *et al.* (1977)

medicinal and cosmetic purposes. Present-day use of *L. albus* in the Mediterranean region hardly differs from that described by the early Greek and Roman writers. It is now little used for green manuring except under special conditions such as in irrigated rice fields. The recent history of cultivated *L. albus* and its modern development as a crop plant have been described elsewhere (Gladstones 1970).

L. angustifolius has not been cultivated in classical Greece or Rome. Botanists of the 16th and 17th centuries called it *L. sylvestris* (wild lupines), reported to be still prevalent in Canada, in contrast to *L. albus* which was known as *L. sativus*. Nevertheless, there is evidence that seeds of wild or volunteer stands have probably been used casually for a long time. Bauhin et al (1651) and Savi (1798) record its common name as "lupino salvatico" and Maratti (1822) as "Fusgalia salvatico" presumably implying a role in times of need. The French botanists Boreau (1849) and Tourlet (1908) speak of wild lupines as being cultivated in Anjou under the names "pois a cafe", "cafe turc" and "cafe". Foury (1950) also gives "lupin a cafe" as a common name for *L. angustifolius* in Morocco. Spread of *L. angustifolius* in cultivation and its recent development as a crop are described by Gladstones (1970).

B. Physical Characteristics

1. Seed Size

The potential for growing and using lupine species depends on a number of factors such as seed size and protein content and proportion of seed coat. Of four lupine species studied by Withers *et al.* (1975)

L. albus had the highest seed size (362 mg) as well as the highest seed and protein yield. *L. luteus* ranked second in seed and protein yield although it had the lowest seed size (133 mg).

Varieties of *L. angustifolius* are generally early maturing, have a much higher coefficient of reproduction and in addition have many other agronomic advantages over *L. albus*. Pouhalskaya (1976) studied five varieties of the *L. angustifolius* species with seed size ranging from 80 to 180 mg. She observed heterosis in seed size in intervarietal crosses. Seed size was the dominant factor in the first (F_1) generation whereas in the second (F_2) generation only maternal effect on seed size was observed. Linear correlations between seed size of any two generations were obtained. The correlation coefficient between seed size of F_2/F_3, F_3/F_4 and F_4/F_5 were 0.424, 0.809 and 0.755 respectively. Therefore, selection for seed size can be made with great probability, using F_3 and F_4 plants.

Within varieties, larger lupine seeds have lesser proportions of seed coat. Hove (1974) found highly significant linear correlation (-0.62 to -0.88) between seed size and the proportion of seed coat for different varieties.

2. Seed Coat

The seed coat contains negligible protein and more than 50% fibre. At 20 to 25% of the seed, it substantially dilutes the protein nutritional value. The seed coat, added to a normal diet, depresses apparent digestibility of the protein and other organic matter. The PER (protein efficiency ratio) of the whole seed has been found to be consistently slightly less than that of dehulled seeds (Hove 1974). On the other hand, the hull contains no growth depressants. Substantial proportions of its fibre are digested by rats and pigs. This has been attributed to the absence of lignin in the seed coat. A peculiarity of the seed coat is that it contains no phosphorus, and most of the calcium of the seed. In addition, the seed coat contains nearly twice as much lysine and half of the arginine present in whole seed.

3. Inflorescence

In lupines, the fruits (pods) develop from flowers borne in terminal racemose inflorescences on the erect main axis and on a hierarchy of lateral axes formed on branches that arise in the leaf axils on the main axis (Gladstones 1958, Farrington and Greenwood 1975). Farrington (1976) noted several differences in phenological development of two lupine species, *L. angustifolius* and *L. consentinii*. A striking feature of the pattern of seed development on each axial order is the rapid increase in weight per seed which occurs almost concurrently on the main axis, the first order apical axes, and the second order apical axes. Seeds on the main axis generally attain a weight similar to those on the first and second order apical axes. The smallest seed, as reported by Farrington (1976), were found on the highest apical axes in *L. angustifolius*. However, later developing seeds (i.e., on the higher order apical axes) had a lower nitrogen content (Golovchenko 1975).

4. Dry Matter

Dry weight of the lupine plant decreases during maturation, while that of the maturing seed increases. Seed weight in angustifolius and consentinii species studied by Farrington (1976), remained low until 23 weeks after seeding, subsequently it increased rapidly. Initially, most of the weight increase was in the pod valves but, in the last five weeks prior to maturity, seed weight increased sharply. Pate and Hocking (1978) found that sequential harvesting of fruits produced a pattern of dry weight which increased as the fruit swelled during the first seven weeks after anthesis and then levelled off on account of seed dehydration and filling of the storage reserves.

5. Fruit Development

Fruit development of legumes can be characterized on the basis of embryogenetic processes by three main stages. During the first stage, seeds remain relatively small and growth is mainly dependent on cell division. The pods grow rapidly during this stage to their full size. During the second stage, cell expansion is the prevailing growth mechanism in the cotyledons. The seeds increase rapidly in size and weight, while the dry weight of the pod decreases slightly. During the third stage, the pods decay, the seeds ripen and enter the resting state (Dure 1975, Manteuffel *et al.* 1976).

The three stages of fruit development according to Vavilov *et al.* (1976) correspond to seed formation, pod filling and ripening phases of the lupines. The period from flowering to full maturity is twice as long for *L. albus* as for cereal grains. The stage of pod formation requires more than 40-45 days. At the end of this period, the seed contains only 30% of the dry matter but by the end of the pod filling stage, it reaches