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Chemical Composition of a New Variety of the Andean Lupin (*Lupinus mutabilis* cv. Inti) with Low-Alkaloid Content

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The chemical composition of the seeds of the low-alkaloid variety "Inti" of the Andean lupin (*Lupinus mutabilis*) grown in the south of Chile was examined. The contents of (% of dry weight) total alkaloids, protein, lipids, and oligosaccharides were found to be 0.0075, 51.0, 16.0, and 14.7%, respectively. The low levels of the sulfur amino acids (2.38% of total protein) made them the first amino acids to limit the protein quality of these lupin seeds. The fatty acid pattern was C16:0, 13.9%; C18:0, 3.0%; C18:1, 41.8%; C18:2, 38.9%; and C18:3, 2.6% of total fatty acids. The α-galactoside amounted to 13.5% of dry weight (raffinose, 2.49%; stachyose, 10.1%, verbascose, 0.85%). It can be stated that "Inti" is highly interesting as a potential protein energy crop for a temperate climate.

INTRODUCTION

For several millennia, the Andean lupin (*Lupinus mutabilis*) has been used for soil enrichment and as a food crop in the Andean highland. The European invasion of the Incaic culture centers was responsible for the destruction of both the traditional forms of cultivation and the nutritional habits developed by the precolombian population which were perfectly adapted to their ecology (Gross and von Baer, 1976). As a result, the cultivation of lupins was constantly being reduced and replaced by other legumes.

Only recently have efforts been made to stimulate the growth of autochthonous cultivated crops that are based on the ecological and sociocultural conditions of the different regions (Vietmeyer, 1980). In this context German technical cooperation with different South American countries resulted in the promotion of lupin production and utilization (Gross and von Baer, 1975).

The main obstacle to a broader utilization of the Andean lupin in nutrition is its high content of bitter and poisonous quinolizidine alkaloids (Hatzold *et al.*, 1983). Only through a laborious leaching process over a period of several days could the highland Indians traditionally extract the bitter alkaloids from the grain and render them palatable.

Von Sengbusch (1942) had succeeded in reducing the alkaloid content by approx 99% in the Mediterranean lupin species *L. luteus, L. angustifolius,* and *L. albus.*

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Several breeders also tried to reduce the content of bitter components in *L. mutabilis*. Von Sengbusch (1942) was the first to report findings of seeds from Andean lupins with a low-alkaloid content. According to Hackbarth (1961) these plants were lost due to their late ripening period. Brücher (1971) and Pakendorf (1974) produced mutants with a low-alkaloid content through irradiation treatment. However, their work was not continued. Gross and von Baer (1975) initiated a breeding program for the selection of low-alkaloid forms of *L. mutabilis*. Two years later, descendants with a low-alkaloid content of 0.3% had been selected (von Baer and Gross, 1977). Six years later, a first so-called "sweet" form—that is, grains with an alkaloid content of less than 0.05%—was noted (von Baer and Gross, 1983).

The aim of this work is to describe some substances of nutritional impact found in the grain of the variety Inti and discuss their potential role in nutrition.

EXPERIMENTAL

Experimental Design

Two low-alkaloid seed samples were sent with their descriptions from the breeder to the coordinator of the experiment. There the samples were milled and homogenized and then distributed. The analyses were made in the form of a blind test at different laboratories. Crude protein and amino acids were determined by Degussa AG, and the analyses of the lipid fraction by the Institute of Plant Breeding and Plant Production of the University of Giessen. The oligosaccharide analyses were carried out in the Institute of Nutrition of the Federal University of Rio de Janeiro and finally the total quinolizidine alkaloids were analyzed in the Institute of Pharmaceutical Biology of the University of Munich. The results of the analyses were sent back to the coordinator for interpretation and then discussed with all the authors together.

All samples were analyzed twice, and the average of the two analyses was taken as result. When the data of the two analyses differed by more than 5%, a third analysis was carried out to check variation within acceptable limits.

Sample Description

The seed samples analyzed in the present work correspond to whole seeds of *L. mutabilis* harvested in isolated plots at Campex (Temuco, Chile) in February 1986 and were not submitted to any previous treatment, such as cooking or dehulling, before milling in a hammer mill (100 mesh = 0.149 mm) and homogenizing. *L. mutabilis* cv. Inti corresponded to a selected low-alkaloid, so-called "sweet" strain of 215 plants. All of them gave a negative result for the presence of alkaloids (von Baer and von Baer, 1986). A representative sample of this strain was obtained by forming a pool by taking one seed from each harvested plant.

As a control the sister-line 2150 of Inti was included in the study. This line corresponded to a bulk sample of a low-alkaloid strain derived from the same original material as Inti.

ANALYTICAL METHODOLOGY

Protein and Amino Acids

Crude protein and amino acids were determined by Degussa AG, Federal Republic of Germany, according to the method of AOAC (1984) and Spindler *et al.* (1984),

respectively. Samples were defatted in a Soxhlet unit with petroleum ether. The nitrogen content was determined by the μKjeldahl technique. Crude protein was calculated by multiplying the *N* content by 6.25.

Methionine and cystine were converted to the stable forms cysteic acid and methionine sulfone by oxidation with performic acid. For oxidation, samples were mixed with performic acid reagent for 16 h. After the addition of hydrobromic acid (HBr), the samples were hydrolized with 6 *N* HCl over 24 h (Spindler *et al.*, 1984).

The amino acids were determined by ion-exchange chromatography with norleucine as the internal standard. As a dilution buffer sodium citrate (pH 2.20) was used.

Equipment used for analysis was an automatic amino acid analyzer (Biotronic Amino Acid Analyzer LC 2000) with an 0.6-cm-diameter cation-exchange column and a two-channel strip recorder. The column temperature started at 48.5°C and increased to 60°C after 48 min. Wavelength for detection was at 440 and 570 nm.

All amino acids were computed by a Spectra Physics 4270 integrator except proline, which was determined by manually measuring the 440-nm absorption peak area.

Tryptophan is extremely acid labile and sensitive toward oxidation. This amino acid was therefore determined after alkaline hydrolysis with lithium hydroxide (4.3 mol/liter) for 16 h at 120°C under exclusion of oxygen, with high-performance liquid chromatography (HPLC) of the hydrolyzate.

Separation of tryptophan is achieved by HPLC using a reversed-phase column (Hypersil ODS 5 μm, 10 cm × 21 mm), followed by uv detection at 217 nm. Tryptophan appears as a single peak with a retention time of about 6 min. Calibration was carried out using tryptophan AR (Serva) in standard solutions from 0.15 to 1.2 mg/100 g[3] tryptophan.

Histidine, tyrosine, and phenylalanine show reduced recovery rates after oxidation pretreatment. Therefore these amino acids were determined after direct hydrolysis with 6 *N* hydrochloric acid.

Fat and Fatty Acids

The total oil content was measured in whole dried seeds (3 h at 150°C) by NMR using a Newport Analyzer MK II (Oxford Instruments, UK.) with 6.5-ml sample assembly. Four grams of pure soybean oil was used as a reference for all measurements (Madsen, 1976). For fatty acid analysis, samples were milled and 1 g was extracted with 10 ml petroleum ether (bp below 40°C). The solvent was evaporated under vacuum and the fatty acids were directly transmethylated at room temperature according to the method of Thies (1971). As methylating agent a sodium methylate solution (0.5 g sodium methylate, 20 ml isooctane, 75 ml methanol) was used in the ratio 2 ml solution:100 mg fat. The fatty acid methylesters were extracted with 0.5 ml isooctane and injected into the gas chromatograph, Carlo Erba 4100, equipped with a flame ionization detector.

The separation of fatty acid methylesters was carried out under the following conditions: 2-m glass column packed with 10% DEGS on Chromosorb W, AW, DMCS; carrier gas, N_2 35 ml/min; injector and detector temperature, 300°C; oven, 195°C.

The peaks were integrated and the concentrations calculated by Spectra Physics System I electronic integrator using the normalization method without correction factors.

TABLE 1

CHEMICAL COMPOSITION OF THE GRAIN OF TWO SWEET STRAINS OF *Lupinus mutabilis* (% OF DRY WEIGHT)

	Inti	Line 2150
Protein ($N \times 6.25$)	51.0	52.6
Lipids	16.0	16.2
Oligosaccharides	14.8	15.4
Alkaloids	0.0075	0.015

Oligosaccharides

The milled lupin flour was defatted with petroleum ether (40–60°C). The defatted meal (2 g) was extracted according to the method described by Macrae and Zand-Moghaddam (1978). The sample was treated with 40 ml of a methanol:water solution (4:6, v/v) under reflux in a water bath at 90°C for 2 h. The suspension was centrifuged and the residue was extracted twice more with a boiling mixture of aqueous methanol. The combined extracts were evaporated to dryness using a rotary evaporator below 50°C. The residue was dissolved in 20 ml distilled water, cleared with zinc ferrocyanide, and diluted to 25 ml. The precipitated material was centrifuged off and the supernant filtered through a Millipore filter (0.45 μm). This filtrate was used for chromatography.

A Knauer liquid chromatograph (Dr. H. Knaur KG, FRG) consisting of a pump (Model 64) and a refractive index detector (differential refractometer) at 4× was used. Sample injection was by means of a Rheodyne injection valve (Model 7120, Rheodyne Ltd., U.S.A.). A Spherisorb-5-amino analytical column (250 × 5 mm i.d., Phase separation Ltd., UK) was used attached to a guard column (30 × 5 mm i.d.), packed with the same phase material. The mobile phase was acetonitrile:water (72:28, v/v) at a flow rate of 1.0 ml/min. Quantification was achieved by area comparison with external standards of sucrose, raffinose, stachyose (Sigma Chemical Co., U.S.A.), and verbascose (fraction donated by Nestect Ltd., Switzerland), using an electronic integrator (Carlo Erba Mega Series, FRG).

Alkaloids

One gram of seed material was suspended in 15 ml of 0.5 *M* HCl and extracted for 1 h at 20°C. The extract was made alkaline with 4 *M* NaOH, applied to an Extrelute column (Merck) and the alkaloids were eluted with CH_2Cl_2. After evaporation the crude alkaloid extract was analyzed by capillary GLC, using a Perkin–Elmer GC 8500 instrument and a 15 m × 0.23 mm DB 1 column. Identification and quantification of the alkaloids was peformed as in Wink *et al.* (1983).

RESULTS

Table 1 presents the chemical composition of the two sweet strains of the Andean lupin. The protein content of both samples was over 50% of dry weight, whereas the oil content was over 16%.

TABLE 2

AMINO ACID PATTERN OF THE SEED OF TWO SWEET STRAINS OF *Lupinus mutabilis* (g AMINO ACID/16 g N)

	Inti	Line 2150	FAO/WHO (1973) reference protein
Aspartic acid	9.8	10.3	
Threonine	3.5	3.6	4.0
Serine	5.1	5.3	
Glutamic acid	22.8	23.8	
Proline	3.8	3.7	5.0
Glycine	3.9	3.7	
Alanine	3.3	3.4	
Valine	3.7	3.4	
Cystine[a]	1.6	1.7	
Methionine[b]	0.8	0.8	
Total sulphur-containing amino acids	2.4	2.5	3.5[c]
Isoleucine	3.9	3.6	4.0
Leucine	6.8	6.9	7.0
Tyrosine	4.6	[e]	
Phenylalanine	3.8	[e]	
Total aromatic amino acids	8.4	[e]	6.0[d]
Lysine	5.2	5.3	5.5
Tryptophane	0.8	[e]	1.0
Histidine	3.2	[e]	
Arginine	9.1	9.3	
NH_3	2.5	2.5	
Total	98.3	87.3	

[a] As cysteic acid.
[b] As methionine acid.
[c] Total sulfuric amino acids.
[d] Total aromatic amino acids.
[e] Present but not analyzed.

As a part of the carbohydrate fraction the samples contained approximately 15% of dry matter in the form of oligosaccharides. More accentuated differences between both strains were found in the alkaloid content. Whereas Inti displayed an alkaloid content of only 0.0075%, the sister strain contained 0.0156%, i.e., twice as much. Lupanine was the main alkaloid and accounted for more than 80% of total alkaloids. It was accompanied by tetrahydrorhombifoline, 4-hydroxylupanine, and 13-hydroxylupanine as minor components.

The amino acid pattern of both samples is shown in Table 2, in comparison with the FAO (1973) reference profile, which recommends the amino acid composition of a foodstuff suitable for human nutrition. Due to the small quantity available of the sample, not all amino acids could be quantified. All the amino acids essential for human beings were found in the range of the reference protein. However, the first limiting amino acid group is the sulphur amino acids which barely constitute 60% of the recommended quantity. The second rank seems to be occupied by valine.

TABLE 3

FATTY ACID COMPOSITION OF GRAIN SAMPLES OF TWO SWEET STRAINS OF THE ANDEAN LUPIN (% OF TOTAL FATTY ACIDS)

	Inti	Line 2150
Palmitic acid	13.9	13.4
Stearic acid	3.0	2.8
Oleic acid	41.7	41.2
Linoleic acid	38.8	39.6
Linolenic acid	2.6	3.0

The fatty acid composition of the two samples of the Andean lupin is reported in Table 3. The patterns differ only slightly. The main fatty acid, with over 41%, is oleic acid, closely followed by linoleic acid with 39%. Palmitic, stearic, and linoleic acids are together responsible for approx 20%.

Table 4 gives a summary of the individual oligosaccharide composition and the total α-galactoside content of the two strains. The main oligosaccharide is stachyose which is nearly 70% of the total. The second important sugar found is raffinose with 17%, followed by sucrose (approx 10%) and verbascose (approx 6%). The α-galactoside content is approx 13.5% of dry weight. Again, as observed in all other chemical characteristics except the alkaloid content, both samples show a similar oligosaccharide pattern.

DISCUSSION

After a 12-year breeding program it finally became possible to obtain a variety of *L. mutabilis* with a negligible percentage of bitter substances that met the requirements, regarding the alkaloid content, for human food and animal feed.

The variety Inti has an alkaloid content of 0.0075%, which is far below the recommended 0.02% (Culvenor and Petterson, 1986). Although the alkaloid content in the grains of the sister-line 2150 was twice as high, due to some bitter plants remaining in the strain, the recommended limit of alkaloid still was not surpassed. The average content of bitter substances in the grain of the Andean lupin lies around 3% (Hatzold

TABLE 4

OLIGOSACCHARIDE COMPOSITION (% OF TOTAL OLIGOSACCHARIDES) AND TOTAL α-GALACTOSIDE CONTENT (% OF DRY WEIGHT) OF SEEDS OF TWO STRAINS OF *Lupinus mutabilis*

	Inti	Line 2150
Sucrose	9.0	9.9
Raffinose	16.9	16.6
Stachyose	68.3	67.7
Verbascose	5.8	5.8
α-galactosides	13.5	13.9

et al., 1983), the content of these antinutritive substances was reduced by more than 99% through breeding.

Several studies have confirmed the high protein content of approximately 42% in the seeds of *L. mutabilis* (Hudson *et al.*, 1976; Torres *et al.*, 1976; Arai *et al.*, 1978; Gross *et al.*, 1983a). Both strains, Inti and Line 2150, reveal an exceptionally high protein content of over 50% of dry weight (Table 1). On the other hand, the oil content of 16% is slightly lower than the mean observed oil content of varieties of Andean lupins (Gross *et al.*, 1983a). The lower oil content explains the somewhat scarce oleic–linoleic acid ratio of both samples, Inti and Line 2150, of 1.07 and 1.04, respectively (Table 3). Common oleic–linoleic acid ratios are around 2 (Gross and von Baer, 1981). The reduced oil content however effects mainly the quantity of the higher saturated storage lipids whereas the higher unsaturated functional lipids, mainly membrane lipids, decrease only slightly (Gross and von Baer, 1981). Thus, the low content of storage lipids requires an explanation. Is it due to a genetically determined phenomenon or to insufficient ripeness of the grain?

It is known from different oil seeds, such as sunflower (Marquard *et al.*, 1977), that their fatty acid composition can be influenced drastically by environment, principally by the temperature during ripening. Cold temperatures, which do not allow a satisfactory ripening of the grain, result in a higher percentage of linolenic acid, which has been noted in maize (Jahn-Deesbach *et al.*, 1975), as well as in lupins (Gross *et al.*, 1983b; Römer *et al.*, 1986), since the content of linoleic acid is high in the membrane lipids. A higher percentage of linolenic acid could not be observed. Therefore it seems that both the lower oil content and the oleic–linoleic acid ratio are genetically determined. This observation agrees with earlier chemical analyses carried out with material which had the same genetical basis (Schoeneberger *et al.*, 1982).

Another indicator of environmental influence on the quality of the grain is the composition of the oligosaccharides, since it is known that the sucrose–verbascose ratio in well-ripened grains is higher than in insufficiently ripened grains (Trugo *et al.*, 1988). From the end of the vegetation cycle of the plant until the ripening phase of the seed, the lower molecular sugars such as sucrose start to be converted into higher molecular sugars, such as raffinose and verbascose (Saini and Lymberg, 1983). In both of the Andean lupin samples examined, the sucrose and verbascose content is relatively low (Table 4). Again this may be due mainly to a genetic characteristic of the grain. However, the genetic and environmental influence of the chemical composition of Inti can only be ascertained by a comparative cultivation trial at different locations.

Since the economic feasibility of oil extraction in seeds generally starts at an oil content in the seeds of about 18%, it would be important to increase the oil yield of this strain. If there is a genetic determination for low-oil content, as suggested by the high-protein content, then it should be considered whether Inti could be crossed with a lipid-rich cultivar.

The high content of α-galactosides which are minimally digestible for monogastrics become preeminent where the stachyose predominates. There exists substantial evidence that this high stachyose content certainly reduces the digestibility of carbohydrates and thus of total energy. If in future a reduction of the α-galactoside content could be achieved through breeding, the economy of the grain could be further improved.

In conclusion, we can state that the low-alkaloid-containing variety Inti of the Andean lupin (*L. mutabilis*) represents a new, interesting protein-energy crop for temperate climates.

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