Comparison of Fermentation Profiles between Lupine and Soybean by *Aspergillus oryzae* and *Aspergillus sojae* in Solid-State Culture Systems

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To explore the possibilities of using lupine as a soybean replacement in fermented foods, fermentation profiles of lupine and soybean by *Aspergillus oryzae* and *A. sojae*, respectively, in a solid-state culture were compared. Biomass, spore concentration, oxygen consumption rate, carbon dioxide production rate, water activity, and production of three enzymes, namely amylase, protease, and cellulase, were measured during a 7-day fermentation. The similarity of fermentation profiles and the reported nutritive value of lupine and soybean lead to the conclusion that lupine can be used as a potential replacement of soybean in certain fermented foods.

Keywords: *Solid-state fermentation; fermented foods; lupine; soybean; Aspergillus oryzae; Aspergillus sojae*

INTRODUCTION

For worldwide sustainable development, there is an increasing need of protein foods of plant origin. In developing countries, the ever-growing population and the malnutrition problem caused by protein deficits necessitate an increased supply of food proteins. Food of animal origin has the disadvantage of being expensive to produce because of the biological inefficiency of converting plant protein into animal proteins. In developed countries, there is awareness of the increasing environmental burden caused by animal manure and consumers' concern about cholesterol-related diseases. Therefore, the development of protein foods of plant origin is becoming an inevitable trend.

Soybean has long been important raw materials for the production of food with meatlike texture such as soybean curd (tofu) and tempe (Steinkrauss, 1983). Soybean is also commonly used for the production of indigenous fermented amino acid/peptide sauce and pastes with meatlike flavor. *Aspergillus oryzae*, *Aspergillus sojae*, and *Rhizopus oligosporus* are strains commonly used for fermented food production in solid-substrate culture with soybean as raw material.

Lupine, a kind of bean, has an appearance similar to that of soybean. One of its particular features is that lupine can grow in places where the climate is not suitable for growing soybeans. In the unfermented state, lupine lacks the strong, unwanted beany flavor of soybean, and its price is lower than that for soybean. Lupine has been tested for tempe production (Kidby et al., 1977). However, it has not yet been tried in other food fermentation processes.

In food fermentation, soybean can be used not only to make fermented food products, such as soy sauce or fermented bean curd, but also as a material to produce enzymes necessary for further food fermentation processes. For example, soybean can be used for the production of protease in the process of soy sauce and soy paste production. If lupine can perform the same function as soybean in these food fermentations, then it can probably be used as a substitute for soybean in these fermented foods. The fermentation profile of lupine is very important to get information relating to the possibilities of using lupine for fermented foods.

The aim of the work presented here is to cultivate *A. oryzae* and *A. sojae* on soybean and lupine, respectively, and to compare their fermentation profiles to explore the possibility of using lupine for the development of protein foods.

MATERIALS AND METHODS

Microorganisms. *A. oryzae* SRRC 266 and *A. sojae* ATCC 42249 were used throughout the experiments. Spore suspension was obtained by harvesting 7-day-old fungi on potato dextrose agar (PDA) slant with 10 mL of sterile water. Wheat bran was used as a medium for the starter culture. Wheat bran (10 g) was mixed with 10 mL of water in a 250-mL Erlenmeyer flask and sterilized at 121 °C for 30 min. After cooling, 0.5 mL of spore suspension of *A. oryzae* or *A. sojae* was added for inoculation. The culture was done at 25 °C for 7 days.

Substrate, Inoculation, and Incubation. Sweet lupine and soybean were used as raw materials for fermentation. Sweet lupine was obtained from Cebeco Zaden BV Nederland (Deventer, Netherlands), and soybean was purchased from a local supermarket. The dry beans were cracked, and the hull was not removed from the endosperm. The cracked beans were soaked in water at a ratio of 1:1.2 (w/w) overnight. The water was totally absorbed by the beans, and drainage was not necessary. Consequently, no soluble components were lost during the soaking process. Sterilization was done at 121 °C for 30 min. In all experiments, each kind of bean was inoculated with 1% (w/w) starter culture.

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The inoculated beans were aseptically distributed over Petri dishes containing ~30 g of beans. The Petri dishes were placed in a climatic incubator controlled at 25 °C and 97% relative humidity. Sampling was done by taking one Petri dish from the incubator every 24 h throughout the 7-day fermentation period.

Wet and Dry Matter Weight. Change of weight was determined by measuring the weight of a Petri dish before and after incubation. Dry matter content was determined by measuring the change of weight of ~1.0 g of fermented beans before and after drying at 106 °C for 16 h.

Water Activity Measurement. An electric hydrometer (type EK 84/3H/63T, sensor type enBSK-4, Novasina, Pfafficon, Switzerland) was used to measure the water activity of samples.

Oxygen Consumption Rate (OCR) and Carbon Dioxide Production Rate (CPR). OCR and CPR were calculated from the change in volume fraction of oxygen and carbon dioxide over time, using the setup described elsewhere (Smits et al., 1996a). This setup contained a cell in which three Petri dishes were placed and O_2 and CO_2 levels were measured by pumping the air from the cell through a paramagnetic O_2 analyzer (Servomex Series 1100, Servomex, Zoetermeer, Netherlands) and an infrared CO_2 analyzer (Servomex Series 1400). Decreases in O_2 and increases in CO_2 level were measured and recorded simultaneously for at least 20 min. OCR and CPR were calculated with the formula of Smits et al. (1996a).

Glucosamine. A modified version of the method described by Lin and Cousin (1985) was used for hydrolysis of the samples for glucosamine analysis. About 0.25 g of fermented bean was hydrolyzed with 2.5 mL of 4 M HCl for 3 h at 100 °C in a sealed tube. The hydrolysates were diluted to 10 mL with distilled water after cooling. Concentrations of sugars were then measured by HPLC ion exchange chromatography (CarboPac PA-1 column with quard column; Dionex, Sunnyvale, CA) with pulse amperometric detection at 25 °C using (+)-D-glucosamine hydrochloride (Sigma, St. Louis, MO) as the reference solution. As eluent, 18 mM NaOH was used.

Spore Counting. Spores were harvested from 1 g of fermented beans with 9 mL of 0.1% (w/v) Tween 80.

A direct visual method was used for spore counting by using a counting chamber.

Enzyme Activities. *Amylase.* About 1 g of fermented bean was extracted with 15 mL of 0.1% (w/v) Tween 80 for 30 min. After centrifugation at 3000 rpm for 5 min, the supernatant was used for measuring amylase activity, which was done by incubating 0.5 mL of supernatant with 0.5 mL of 2.0% (w/v) soluble starch in 0.05 M citric acid buffer (pH 4.8) at 50 °C for 20 min. The reaction was stopped by adding dinitrosalicylic acid solution (DNS) and further treated as described by Wood and Bhat (1988). Activities were calculated against a glucose standard and corrected for activities found in blanks without substrate and blanks without enzyme. Activity is expressed in units (U), with 1 U being defined as 1 μmol of glucose released per minute at 50 °C.

Cellulase. Extracts used for the amylase assay were also used for cellulase measurement. A 1-mL portion of 0.05 M citric buffer (pH 4.8) was added to a test tube followed by the addition of 0.5 mL of sample properly diluted in the same buffer and a 1 cm × 6 cm strip of Whatman No. 1 filter paper curled around a glass rod. The reaction mixture was incubated at 50 °C for 60 min, and then 3 mL of DNS reagent was added to stop the reaction. The suspension was well mixed, and the tubes were transferred to a boiling water bath for exactly 5 min and then cooled in ice. The tube contents were well mixed after 20 mL of distilled water had been added. Finally, the tubes were allowed to stand for at least 20 min to allow the pulp to settle, and the color formed was read in a spectrophotometer at 540 nm. Enzyme blank, reagent blank, and glucose standard solutions were treated in the same way. Using this standard, the absorbance of the samples, after subtraction of enzyme and reagent blanks, was translated into milligrams of glucose produced per gram of fermented bean.

Protease. Extraction for protease activity measurement was done by adding 10 mL of 100 mM phosphate buffer (pH 7.0) to 1 g of fermented bean. After centrifugation, the supernatant was used for analysis. Protease activity was measured as described elsewhere (Smits et al., 1996b). Dimethylcasein (10 mg/mL in 20 mM phosphate buffer, pH 7.0) was used as substrate. Alanine release was determined as a measure of expression of protease activity. One unit is defined as 1 μmol alanine released per minute per gram of fermented bean.

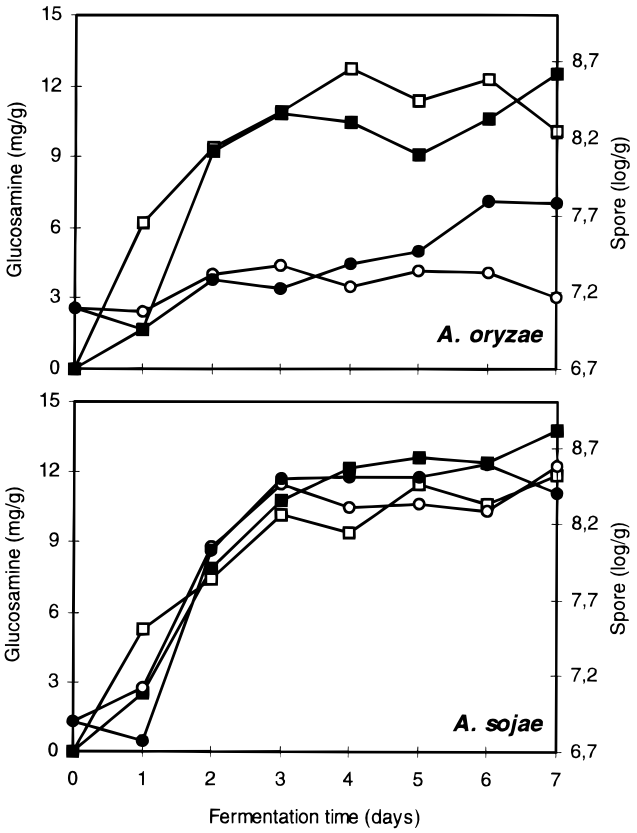

Figure 1. Course of glucosamine and spore counting during fermentation: (□) glucosamine in lupine; (■) glucosamine in soybean; (○) spore concentration in lupine; (●) spore concentration in soybean.

RESULTS AND DISCUSSION

Glucosamine and Spore Concentration. Glucosamine content and spore concentration are shown in Figure 1. To describe the growth of fungi, the background level of the bean was subtracted from the glucosamine level. A maximum glucosamine level was obtained after 4−5 days of fermentation, and almost no differences in glucosamine content were seen between fungi growing on lupine and fungi growing on soybean. Also, no differences in glucosamine content between the culture with *A. oryzae* and that with *A. sojae* were observed. The profile of spore formation differed between the strains, with *A. sojae* having a higher spore concentration than *A. oryzae*.

OCR and CPR. The courses of OCR and CPR are shown in Figure 2. *A. oryzae* and *A. sojae* had the same OCR and CPR patterns. The highest activities were found in the first day of fermentation. This result differs from previously reported results (Smits et al., 1996a) with *Trichoderma reesei* in wheat bran, for which maximum activities were seen at 53 h of fermentation. The OCR and CPR profiles were the same for lupine and soybean.

Enzyme Production. Activities of three enzymes, namely amylase, protease, and cellulase, were deter-

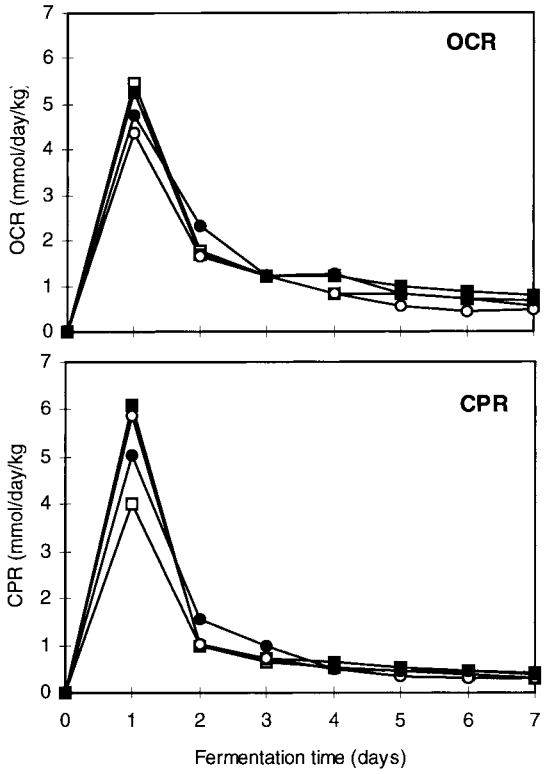

Figure 2. Course of OCR and CPR during fermentation: for *A. oryzae*, (□) in lupine, (■) in soybean; for *A. sojae*, (○) in lupine, (●) in soybean.

mined (Figure 3). There was almost no variation in amylase activity during the whole fermentation period. The measured activity of amylase comes probably from the starter. The course of cellulase activity is shown in Figure 3B. The highest activity was found after 3 days of fermentation and was higher in lupine than in soybean for both *A. oryzae* and *A. sojae*. *A. sojae* produced relatively more cellulase than *A. oryzae*. *A. sojae* produced more protease than *A. oryzae* (Figure 3C), whereas protease activity was higher in soybean than in lupine for both strains. Protease activity increased dramatically during 2 days of fermentation and did not increase significantly afterward.

Relationship between Glucosamine and Spore Concentration. The glucosamine method is a popular method for the determination of fungal biomass in solid-state culture. The accuracy of this method depends on establishing a reliable conversion factor relating glucosamine to mycelial dry weight. Because the glucosamine content of fungal biomass in liquid culture is not applicable to fungal biomass in solid-state culture (Smits et al., 1996b), we used the amount of glucosamine for representing fungal biomass, without converting it into mycelial dry weight. Figure 1 shows that each strain has its own particular pattern in the relationship of glucosamine content with amount of spores. Spore formation of *A. oryzae* is very slow, and *A. oryzae* produced fewer spores than *A. sojae*. Since the optimum water activity (a_w) value is lower for sporulation than for growth, the higher spore concentration of *A. sojae* is caused by a faster decrease in a_w, as shown in Figure 4. With respect to soy sauce and other amino acid fermentations, a low spore concentration is favorable for the final product, albeit the quality of the final product is also affected by the second fermentation (Röling et al., 1994; Yokotsuka, 1977).

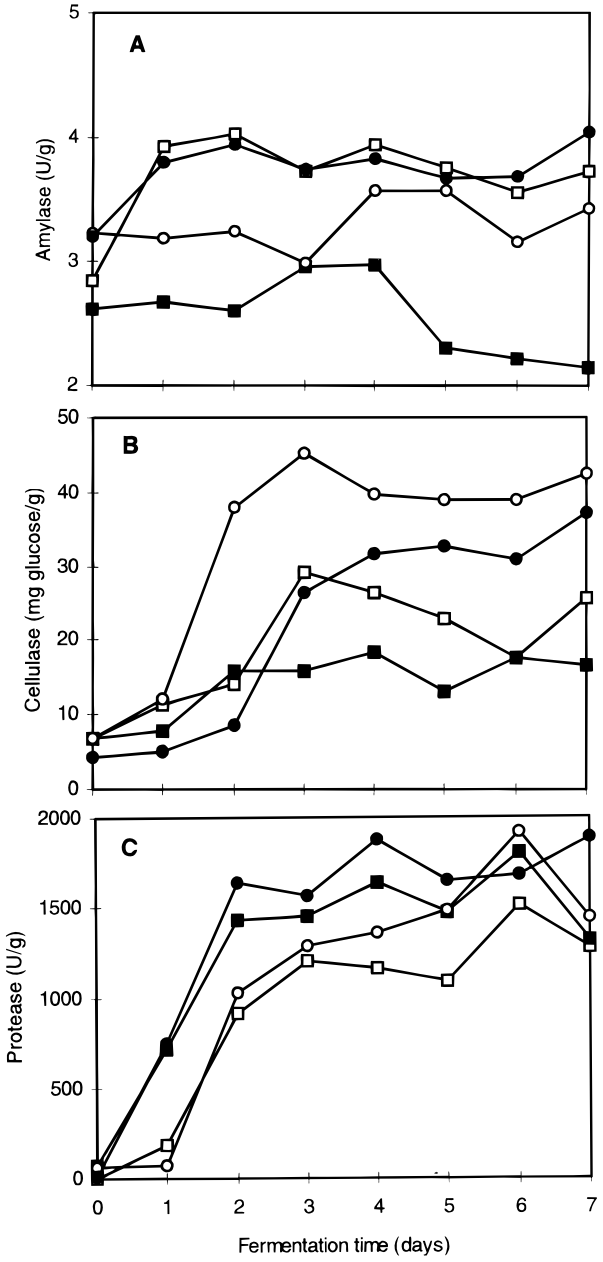

Figure 3. Course of enzyme production during fermentation as shown by activities of amylase (A), cellulase (B), and protease (C): for *A. oryzae*, (□) in lupine, (■) in soybean; for *A. sojae*, (○) in lupine, (●) in soybean.

Relationship between Glucosamine and Enzyme Activities. The relationship between glucosamine and enzyme activities is shown in Figure 5. Because protease activities are associated with glucosamine content (Figure 5A), protease activities are related with fungal growth. Specific activities of protease (units per milligram of glucosamine) remain relatively constant except for the first day of fermentation (data not shown). In soy sauce production, protease is very important because it contributes to flavor and biochemical changes in the second fermentation. *A. sojae* has higher cellulase activity than *A. oryzae* (Figure 5B). Because of the higher cellulase activity, more sugars are released into the water phase of the medium. The higher solute concentration was held to make a greater contribution to a_w reduction, so a_w decreased more rapidly, as shown in Figure 4. The higher activity of cellulase also contributes to a stronger dry matter weight loss. After

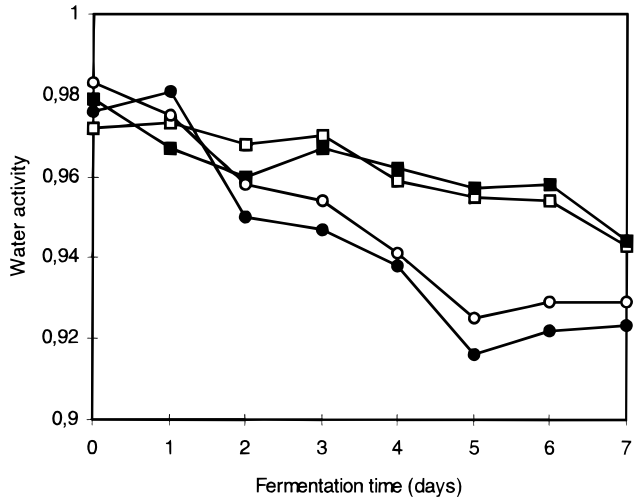

Figure 4. Water activity (a_w) measurement during fermentation: for *A. oryzae*, (□) in lupine, (■) in soybean; for *A. sojae*, (○) in lupine, (●) in soybean.

4 days of fermentation, dry matter weight losses in lupine and soybean were 0.2048 and 0.1283 g/g of initial dry matter, respectively, for *A. oryzae*, and 0.1524 and 0.1295 g/g, respectively, for *A. sojae*.

For soy sauce fermentation, it is advisable to stop fermentation after 3 days because cellulase activity has reached its highest level by then and no changes in protease activity can be expected after 2 days of fermentation.

Table 1. Qualitative Overview of Fermentation Profiles of Lupine and Soybean for *A. oryzae* and *A. sojae*

		lupine	soybean
A. oryzae	amylase	±[a]	±
	protease	+	++
	cellulase	++	+
	glucosamine	+	+
A. sojae	amylase	±	±
	protease	++	+++
	cellulase	+++	++
	glucosamine	+	+

[a] ±, no changes in activity during fermentation; value higher with increasing number of + signs.

A qualitative overview of fermentation profiles is given in Table 1. It is clearly shown that fermentation profiles for lupine are similar to those for soybean.

A mixture of fungal hydrolysis enzymes is very important for solid-state food fermentation. Higher cellulase activity in lupine improves the substrate utilization of lupine in fermentation. Higher dry matter weight loss in lupine indicates that macromolecules are degraded at a higher rate in lupine than in soybean. Comparison of the composition of lupine and that of soybean, as shown in Table 2 (Sijtsma et al., 1995), and of the fermentation profiles discussed above implies that lupine can likely be used as a replacement for soybean in fungal fermentation by *A. oryzae* and *A. sojae* in solid-state culture. This conclusion is based in particular on protease and cellulase profiles and the growth of fungi.

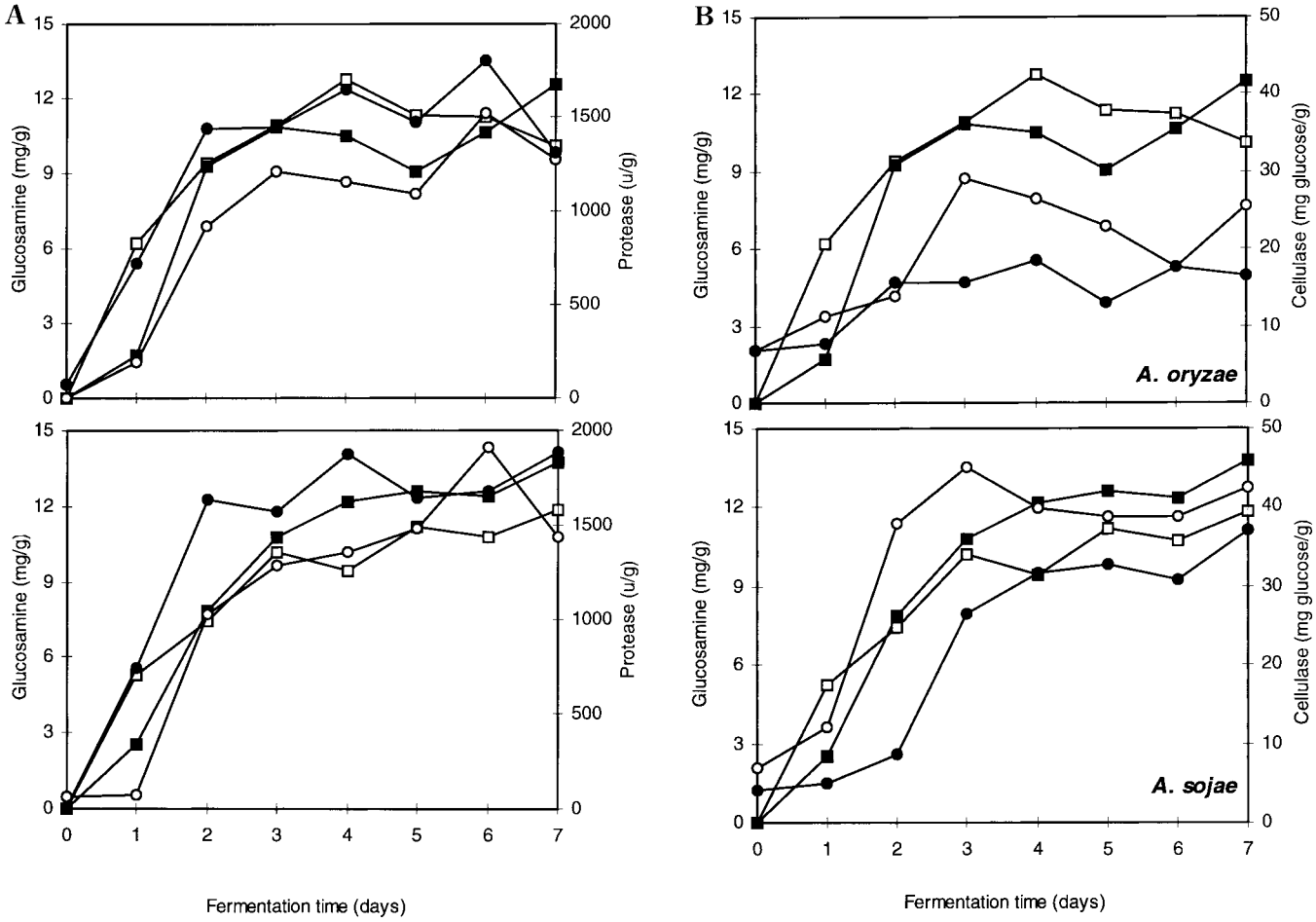

Figure 5. (A) Relationship between glucosamine and protease activity during fermentation: for glucosamine, (□) in lupine, (■) in soybean; for protease activity, (○) in lupine, (●) in soybean. (B) Relationship between glucosamine and cellulase activity during fermentation: for glucosamine, (□) in lupine, (■) in soybean; for cellulase activity, (○) in lupine, (●) in soybean.

Table 2. Comparison of Lupine and Soybean

	lupine	soybean
protein (% of dry wt)	42.9	35−40
limiting amino acids	methionine, cystine	methionine, cystine
coeff of digestion (%)	85−92	85−87
antinutritional factors	alkaloids, protease inhibitors	protease inhibitors, lectins, antivitamins A, B_{12}, D, E

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