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Lupin Seed for Human Consumption

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Abstract

The food quality of lupin seed, i.e. soaking, cooking, sprout growing and mold growing for fermentation, was investigated by using the seed of *Lupinus angustifolius* harvested in Western Australia. A method to produce lupin seed protein concentrate (LPC) was developed, and the usage of LPC in Korean food system was investigated. The water soaking rate of lupin seed was faster than that of soybean, but the cooking rate of lupin seed was much slower compared to soybean. The thermal softening time, D_{100}, was 345 min for lupin seed and 84 min for soybean. A two-phase solvent extraction system consisting of haxane-alcohol-water could effectively remove the residual bitter taste, lipid and yellow pigments of lupin seed flour, and the resulting LPC contained over 50% protein and had bland flavor and milky white color. Treatment of LPC with carbohydrate decomposing enzymes resulted in a product of more soluble and higher concentration of protein. Methods to produce lupin seed vegetable milk and lactic beverages from LPC products were discussed.

Introduction

Lupins have been grown as a pulse crop for human consumption in Mediterranean countries and the high lands of South America for several thousand years. The seeds are valued for their high protein contents, which varies from 30 to more than 40% on a dry weight basis. The primitive varieties grown are extremely bitter because of an alkaloid content of 1.0 to 2.5% in the dry seeds, but this is reduced to about 0.1% by boiling and prolonged steeping in brine before eating. During the period 1928-1935, German scientists selected true breeding mutant of several lupin species, having natural alkaloid contents of only 0.02%, which are called as "sweet" lupins.[1]

The studies on the utilization of Australian sweet white lupin seed, *Lupinus angustifolius*, in Korean food system have been initiated in 1982 at the Department of Food Technology, Korea University. The motivation of the study was that the seed of Australian sweet white lupin had an appearance and chemical composition similar to those of soybean, which is the most important legume for Asian people. If lupin seed can be used in place of soybean to prepare fermented bean products, vegetable milk and curd products and other high-protein food products, the economic advantage will be significant, especially in Asian countries.

The food processing characteristics of lupin seed, i.e. soaking, cooking, sprout growing and fermentation ability, were investigated in comparision with soybean. A method of processing lupin seed protein concentrate from dehulled lupin seed flour was developed, and the use and functional property of LPC were examined.

Structure of Lupin Seed

Table 1 compares the average seed weight and percentage of seed coat of lupin seed and soybean. The seed weight of lupin was lower than soybean. Lupin seed had very thick seed coat, 22.4% of whole seed, compared to soybean. The percentage of seed coat of lupins tended to increase as the seed weight decrease. Figure 1 shows the microstructure of the cotyledones of lupin seed and soybean. The cotyledonary cells of lupin seed was in egg-like shape and much (more than 4 times) larger than those of soybean. The microstructure of cotyledonary cells of lupin seed was characterized with thick cell wall having distinct pit-pairs. The protein

Table 1. Average seed weight and percent of seed coat of soybean and lupin seed

Beans	Ave wt of seed (g)	% seed coat
Lupin seed	0.17	22.4
Soybean	0.23	6.6

raw soybean cotyledon cells. (x 300)　　raw lupinseed cotyledon cells. (x 300)

Fig. 1. Microstructure of cotyledonary cells of lupin seed and soybean (Sudan Black B. Staining)

bodies in lupin seed cotyledone cell contained numerous crystaloids, which were absent in soybean.[2]

Table 2 shows the proximate chemical composition of lupin seed, seed coat and kernel. The seed coat contained very low level of crude protein and crude fat. The content of nitrogen free extract and fiber of the seed coat was 93.4%.[3] The seed coat of soybean was reported to contain 9.0% crude protein, 0.9% crude fat, 4.0% ash and 86.2% nitrogen free extract and fiber.[4] Compared to these, lupin seed coat contained larger amount of N-free extract and fiber, but less of protein and ash.

Soaking Property

The soaking rate of lupin seed was faster than soybean, in spite of the thicker seed coat and larger fiber content in the seed coat of lupin. Figure 2 compares the rate of water absorption of lupin seed and soybean in water. The rate of water absorption was accelerated when the beans were dehulled. The dehulled cotyledones absorbed water to their maximum amount (120-140% absorption) within 4 hrs of soaking, whereas whole beans reached to their maximum absorption after 12 hrs of soaking. The rate of water absorption and the maximun amount of water absorbed by lupin seed were always greater than soybean. Similar results were found

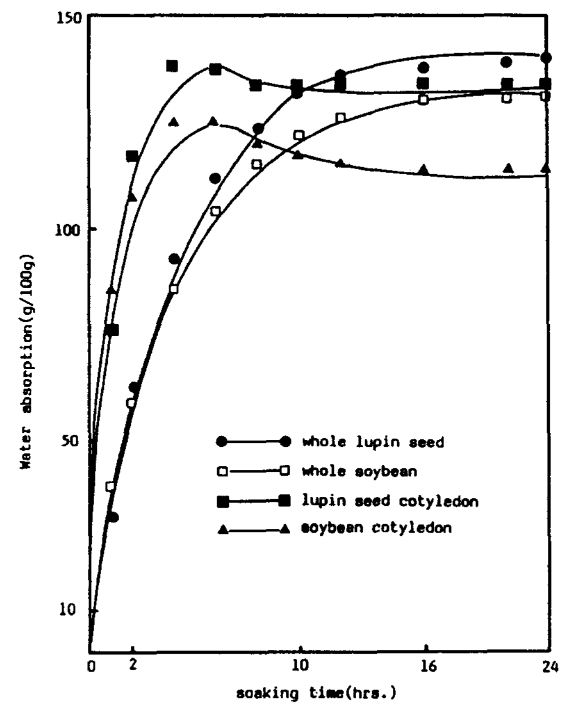

Fig. 2. Water absorption of beans during soaking in water at 25°C

Table 2. Chemical compositions of lupin seed, seed coats and kernels (%) (dry basis)

	Moisture	Crude protein	Crude fat	Ash	Total sugars	Crude fiber
Seed	10.88	31.32	7.87	2.71	36.66	21.44
Seed coat	9.86	2.37	1.38	2.89	25.0	58.36
Kernel	11.53	39.72	8.07	2.31	29.16	20.74

with the soaking media containing NaCl, $NaHCO_3$, Na_2CO_3 or sodium tripolyphosphate.[3]

Cooking Property

The cooking rate of the beans sufficed the first order reaction kinetics, as shown in Fig. 3. A straight line relationship was obtained by plotting ln (a_0/a_t) versus cooking time, which indicated that ln (a_0/a_t) = Kt is sufficed, where a_0 is puncture force at zero time (soaked beans), a_t is puncture force at time t. From this curve, the first order reaction constant, K, was calculated.[3]

Table 3 summarizes the cooking rate constant of lupin seed and soybean at different cooking temperature. The cooking rate constant of lupin seed soaked in water was 6.7 × 10^{-4}/min at 100°C and increased by the increase of cooking temperature. The cooking rate of lupin seed was enhanced by soaking treatment in NaCl-$NaHCO_3$ solution. The cooking rate of soybean was 4-6 times higher than that of lupin seed, depending on the cooking temperature. The temperature dependence of the cooking rate constants could be fitted to the Arrhenius equation. The cooking rates of lupin seed and soybean were also evaluated in terms of decimal reduction time (D value) and Z value, same as used in canning process.

The semi-log plots for the softening of lupin seed and soybean by cooking time at various temperatures could be approximated as a straight line as shown in Figure 4.[3]

Table 3 shows that D_{100} of lupin seed was 345 min compared to 84 min of soybean. Soaking in NaCl-$NaHCO_3$ solution instead of water could reduce the D value of lupin seed by 2/3, whereas soybean was not significantly affected by the soaking medium. As shown in Table 4, slight differences were noticed in Z value and activation energy for the texture softening between lupin seed and soybean.[3]

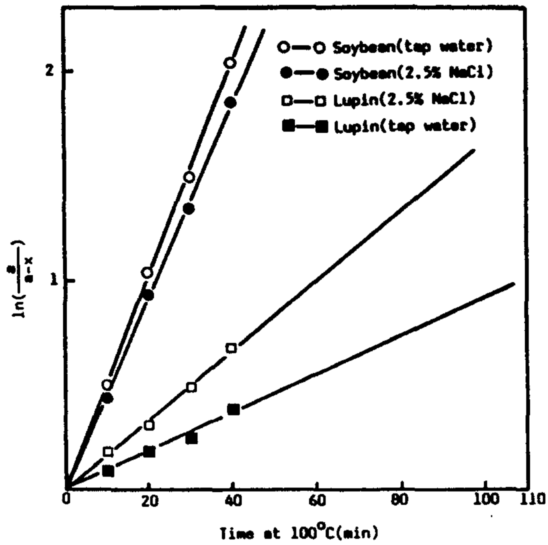

Fig. 3. A first order reaction curve for the thermal softening of soybean and lupinseed: plotted according to the equation:

$\ln \frac{a_0}{a_t} = Kt$ where t = time of heating at 100°C (min): a = puncture force at zero time, a_t = puncture force at time t: slope (K) = first order reaction rate constant

Table 3. First order rate constants for cooking beans at various temperatures

Soaked for 24 hrs in	Cooking temp (°C)	Lupin seed K	Lupin seed D	Soybean K	Soybean D
Tap water	100	0.0067	344.9	0.0269	84.0
	105	0.0094	277.8	0.0269	59.5
	110	0.0108	250.0	0.0468	61.1
	121	0.0162	163.9	0.0867	32.2
2.% NaCl + 0.5% $NaHCO_3$	100	0.0082	277.8	0.0314	73.0
	105	0.0124	200.0	0.0541	50.7
	110	0.0156	161.3	0.0571	50.0
	121	0.0219	116.3	0.0851	32.8

Table 4. Z value and activation energy (E) for the softening of beans by cooking

Sample names	Soaked for 24 hrs in	Z (°C)	E cal/g mole
Lupin seed	tap water	55.4	11620
	2.5% NaCl+ 0.5% $NaHCO_3$	47.5	13224
Soy-bean	tap water	39.1	14960
	2.5% NaCl+ 5.0% $NaHCO_3$	43.6	12540

Sprout Making

The root of lupin seed grew faster than soybean, when the seeds were treated same as the soybean sprout cultivation. The dry weight of lupin seed sprout increased by 30% for 3 days of germination, whereas that of soybean increased only 10% for the same period.(5)

Both the diameter and length of lupin seed root were larger than those of soybean root grown for 4 days. The color, freshness and crispiness of lupin seed sprout salad were excellent. However, the residual bitter taste of lupin seed sprout salad was significant and it was the major limiting factor for the acceptance.

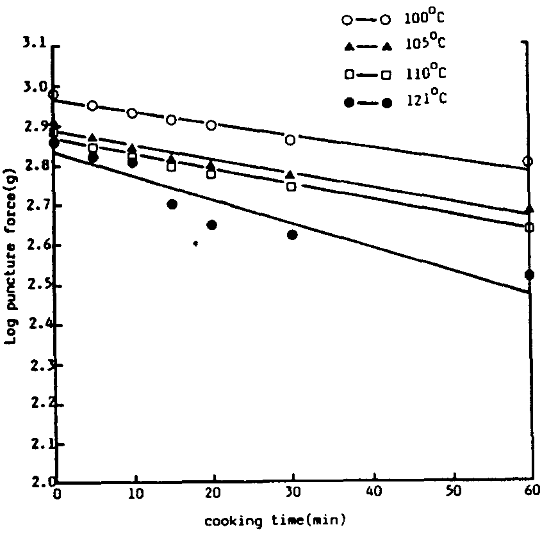

Fig. 4. Semi-log plots for the softening of lupin seed by cooking time at various temperature (soaking medium: tap water)

Lupin Seed Sauce Fermentation

The methods of determining the growth rate of mold on cooked beans were investigated in order to compare the growth of *Aspergillus oryzae* on lupin seed to that on soybean. The measurement of hypae length on cooked bean could be used as a simple and rapid method of estimating the growth of mold on different substrates. The inoculation of mold on intact surface of seed coat retards the growth compared to the cutted surface inoculation.(6) The growth of *A. oryzae* was particulary hindered on the surface of lupin seed which had thick seed coat.

Cooked lupin seed could be made into excellent Meju, the fermentation starter for Korean soybean sauce and paste. The amylase activity of the paste of Meju-brine mixture made from lupin seed was considerably higher than those of soybean. Meju made from intact seed coat of lupin seed had higher amylase activity than cutted seed coat samples. On the other hand, cutted seed coat samples showed higher protease activity. The rate of free amino acid formation during lupin seed Meju-brine mixture was similar to that of soybean Meju, but the rate of dark-brown color formation was much greater with lupin seed Meju than with soybean Meju.(7)

In sensory evaluation, the flavor scores of lupin seed sauce and paste were slightly lower than that of soybean products, but the overall quality of fermented lupin seed sauce was acceptable.(7)

Preparation of lupin seed protein concentrate

An excellent quality protein ingredient was produced from dehulled lupin seed flour by two-phase solvent extraction system. Fig. 5 shows a schematic diagram for the preparation of lupin seed protein concentrate (LPC) by using two-phase solvent extraction method. A mixture of haxane, ethanol and water (10:7:3) was used to remove oil, yellow color and bitter taste from lupin seed flour. The resulting protein ingredient had milky white color and bland taste.(8) The protein concentration was further elevated by removing the carbohydrate fraction which was sölubilized with carbohydrate decomposing enzymes. As shown in Table 5, the protein concentration could be increased up to 78% level by treating with a complex carbohydrate decomposing enzyme preparation, SP 249 (Novo, Denmark).(9)

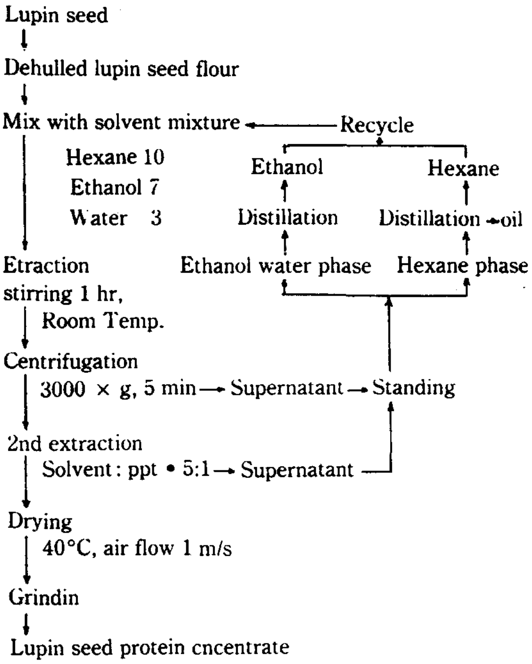

Fig. 5. Schematic diagram for the preparation of lupin seed protein concentrate (LPC) by using two-phase solvent extraction method

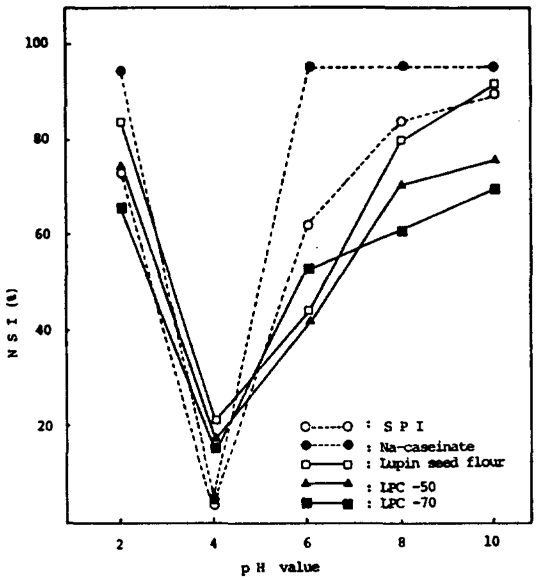

Fig. 6. Effect of pH on nitrogen solubility index of protein products in distilled water

Functional properties of lupin seed protein concentrate

The functional properties of lupin seed protein concentrate (LPC-50) and its further concentrated form (LPC-70) by removing the fraction solubilized with carbohydrate decomposing enzymes were compared with those of soybean protein isolate (SPI) and Na-caseinate. The LPC products exhibited a protein solubility pattern similar to SPI, and a flow behavior similar to Na-caseinate, as shown in Figures 6 and 7.

LPC-70 showed excellent emulsifying capacity and the performance was similar to that of SPI when 0.1-0.5M salt was added. The foaming capacities of LPC-50 and LPC-70 were similar to that of SPI, but the stability was very low at pH 7.0. Lupin seed protein concentrates showed high oil and water absorption capacities, and LPC-70 had showed exceptionally high oil absorption capacity.[10]

Table 5. Changes in yield during the enzymatic (SP 249 enzymes, Novo Co. Product) hydrolysis of lupin seed protein concentrate (6%) Reaction condition: pH = 4,5, Temperature = 40°C, Enzyme/Substrate = 1 : 500)

Treatment times hr	Total[a] yield %	Protein[a] concentration %	Protein yield[a] % of total protein
1	68.00	60.81	79.93
2	60.16	68.30	79.43
3	56.83	72.70	79.32
4	54.83	77.90	82.57

a: Mean triplicate determinations on dry weight basis

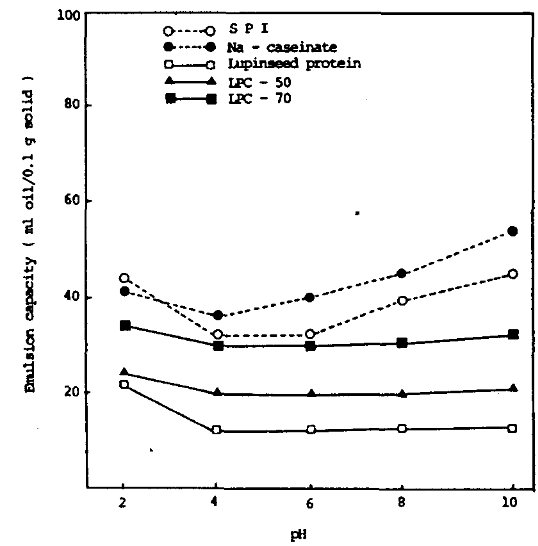

Fig. 7. Changes in the apparent viscosity of protein products having various concentrations (r= 36.71 sec⁻)

These functional properties indicated the use of lupin seed protein concentrates for saussage making, vegetable milk processing and doughnut and other deep frying snack manufacturing. The improvement of the functional properties of LPC-50 and LPC-70 could justify the labour and cost for the processing of lupin seed protein concentrates.

Preparation of lupin seed milk from LPC

Fig. 8 shows the formula and flow chart for the preparation of lupin seed milk from LPC. Carbohydrate decomposing enzymes were applied for the improvement of physical property and sensory quality of lupin seed milk. The viscosity of lupin seed milk decreased and the dispersion stability increased by the treatment with SP 249 enzyme preparation. The optimum condition of enzyme treatment for the improvement of lupin seed milk was the hydrolysis of LPC water dispersion with a mixture of cellulase and hemicellulase of the enzyme/substrate ratio 0.2-1.0%, at 40-50°C for 5 hrs. The lupin seed milk prepared by this method was physically stable. Fig. 9 compares the picture of lupin seed milk bottle stored at 5°C for 7 days. The grittiness of lupin seed milk disappeared by this method, and the sensory quality was comparable to that of commercial soybean milk.[9]

Mixing LPC and water (Waring blender, 6000 rpm, 2 min and 12000 rpm 1 min)
↓ ← Enzyme treatments
Heating treat ment (95°C, 5 min)
↓ ← Oil, sugar, flavoring agent, monoglyceride, salt
Mixing (Waring blender, 6000 rpm, 2 min and 12000 rpm, i min)
↓
Homogenizing (3500 / 500 psi)
↓
Heating treatment (110°C, 10 min)
↓
Homogenizing (as above)
↓
Bottling
↓
Lupin seed milk

Fig. 8 . Schematic diagram for the preparation of lupin seed milk from LPC

Fig. 9 . Lupin seed milk treated with enzyme SP 249 stored at 5°C for 7 days

Preparation of Lactic Fermented Lupin Seed Milk Beverage From LPC

Seven different strains of lactic bacteria and 13 combinations of those microorganisms were tested for their acid forming capacity on a vegetable milk made from lupin seed protein concentrate. When the milk was not treated with carbohydrate decomposing enzyme (SP 249), *L, mesenteroides* was appeared to be the most suitable microorganism having the maximum cell concentration of 1.0×10^9 / ml and the final pH 4.40 with the acidity 0.46%. On the other hand, for the enzyme treated lupin seed milk, mixed culture *L. acidophilus* and *S. thermophilus* showed the best performance having 1.9 $\times$ 10^9 / ml of maxmum cell mumber and the final pH and acidity were 3.69 and 1.13%, respectively, as shown in Table 6.[11] The results of sensory evaluation indicated that *S. lactis, L. casei,* mixed culture of *S. lactis* and *L. mesenteroides* and mixed culture of *L. acidophilus* and *S. thermophilus,* grown on enzyme hydrolized lupin seed milk, could produce acceptable lactic acid beverages.[11]

Future Research Needs

The large portion of seed coat to the whole lupin seed and the thick wall of the cotyledonary cells appeared to cause the long cooking time and difficulties in microbial gorwth during fermentation. Application of extrusion cooking process may be beneficial to reduce the cooking energy and accelerate microbial growth for the fermentation.

Table 6. Growth and acid production by lactic acid organisms in lupin seed milk

Organism	Control			Enzyme treatment		
	pH*	Total acid* %	Viable counts/ml**	pH*	Total acid* %	Viable counts/ml**
Intitial range	5.51	0.19	–	5.42	0.29	–
L. acidophilus	4.50	0.38	5.70×10^8	3.70	1.00	1.70×10^9
L. casei	4.60	0.37	4.10×10^8	3.70	1.01	7.00×10^8
S. lactis	4.45	0.36	1.50×10^8	4.15	0.57	4.00×10^8
L. mesenteroides	4.40	0.46	1.00×10^9	4.30	0.48	2.30×10^8
L. acidophilus × S. thermophilus	4.43	0.41	8.00×10^8	3.69	1.13	1.90×10^9
S. lactis × *L. mesenteroides*	4.40	0.37	5.40×10^8	4.25	0.65	8.30×10^9

* pH and titratable acidity were determined after 24hr.

** The viable counts represent the maximum attained during the 24-hr incubation period.

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루우핀 콩의 식품이용

이 철 호

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루우핀 콩의 식품가공 적성을 평가하기 위하여 서호주에서 생산된 Lupinus angustifolius의 종실을 삶음속도, 콩나물 성장속도 및 균체성장속도 등을 검토하고 또한 루우핀 종실로부터 만들어진 단백질 농축물(LPC)을 우

리나라 식품제조에 활용하는 방안등을 조사하였다.

루우핀 콩의 수분 침지속도는 대두보다 컸으나 삶음 속도는 대두보다 크게 떨어졌다. 가열에 의한 연화속도 D_{100}은 대두의 경우 84분이었으나 루우핀 콩의 경우 345분이었다. 루우핀 콩에 포함되어 있는 잔유 쓴맛성분(주로 알카로이드)와 황색색소 및 지방은 헥산-알콜-물을 혼합한 이상용매로 제거할 수 있으며 여기에서 얻어진 단백질 농축물은 단백질 함량 50% 이상을 함유하며 유백색의 순한 풍미를 가지는 물질로 우수한 식품재료로 판단되었다. 루우핀콩 단백질 농축물에 탄수화물(주로 셀룰로오스) 분해효소 처리를 하면 가용성이 높고 단백질 함량이 증가된 제품을 얻을 수 있다. 이들 재료들을 이용하여 식물성 대용유와 유산균 발효음료의 제조 가능하였으며 이들 제조방법의 제문제를 검토하였다.