mers of M-3,5-G and M-3-G will be reported in a separate publication.

ACKNOWLEDGMENT

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Effect of Debittering Treatment on the Composition and Protein Components of Lupin Seed (*Lupinus termis*) Flour

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In the traditional Egyptian practice, lupin seed is debittered by steeping in water overnight followed by boiling for 20 min, soaking in water for 4 days, and addition of NaCl. About 15% of total solids are leached out in this method. The protein, available lysine, and ash content increased due to debittering, but the sugar and tannin content decreased. The lupin seed or debittered lupin seed flour did not show trypsin and α-amylase inhibitor activity and hemagglutinin activity. Lupin seed flour showed caseinolytic activity, but the debittered flour did not have this activity. The nitrogen solubility of the debittered flour was lower in water and 1 M NaCl solution (12.6%) but nearly the same in 0.02 M NaOH (92.6%). The in vitro digestibility of debittered lupin seed proteins with pepsin/pancreatin was slightly higher. Debittering treatment denatured lupin seed proteins and also dissociated the high molecular weight protein component.

In spite of its high (36%) protein content, lupin seed (*Lupinus termis*) is not fully utilized in the Egyptian diets unlike faba bean (*Vicia faba*) or other legumes. This limitation is mainly due to the presence of alkaloids and bitter substances (Mogghaddam et al., 1976). Lupin seed is debittered by a process of prolonged steeping, boiling, and salting, for edible use to a limited extent.

There is considerable information available on the chemical composition and protein quality (Hove, 1974; Ballester et al., 1980), nutritive value (Mogghaddam et al., 1976), factors affecting protein solubility (Ruiz and Hove,

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1976), and functional properties of several different species of lupinus (Sosulski et al., 1978; Malgarini and Hudson, 1980; Sathe et al., 1982). Recently, Morad et al. (1980) have reported that the natural amber color of lupin (*L. termis*) gives a desirable color to macaroni when blended with wheat flours at 2–6% levels. However, the changes in the chemical and physicochemical properties of the proteins that may occur due to the debittering treatment have not been studied, to the best of our knowledge. The method used in Egypt to remove the bitter substances and alkaloids from lupin seed was used in this study, and its effect on the chemical composition of the flour and the physicochemical properties of the proteins has been determined.

EXPERIMENTAL SECTION

Materials. Lupin seed, *L. termis*, of the variety Giza-I was obtained from the Plant Breeding Department, Min-

istry of Agriculture, Giza, Egypt. Pepsin, pancreatin, trypsin, and α-amylase were from Sigma Chemicals.

Preparation of Lupin Seed Flour. The dry lupin seeds were mechanically split into two halves in a high-speed blendor (Sumeet Super) for a few seconds. The hulls and seed coats were loosened from the kernels by hand and separated. Dehulled seeds were flaked in a Kavrnmaskiner (Type 5) flaking machine to a 0.2-mm thickness and defatted by repeated extraction with hexane. The defatted meal was air-dried at room temperature (~28 °C) and ground to a powder to pass through a 60-mesh (British Standard Screen) sieve. This flour was used for analysis and designated as sample A.

Preparation of Debittered Lupin Seed Flour. The traditional Egyptian method of debittering lupin seed was used. The dry seeds were steeped in tap water overnight followed by boiling for 20 min in another aliquot of tap water. The water was removed and the seed soaked in tap water at room temperature for 4 days. The water was changed every 3 h during the soaking. Enough water was used to cover the seeds. At the end of the soaking time NaCl was added to the seeds at approximately 2.6% on the wet weight of the seed. The debittered, salted, lupin seeds in the wet condition were dehulled and passed through meat mincer twice and then freeze-dried before fat extraction. The freeze-dried material was defatted as described before and the final flour designated as sample B. The fat content in both the flours was less than 0.5%.

Methods. Moisture, protein, and total ash were determined by the method of AOAC (1975).

Available Lysine. This was determined by the procedure of Carpenter (1960) using 1-fluoro-2-4-dinitrobenzene (FDNB) reagent. The results are expressed as grams of lysine per 16 g of nitrogen.

Sodium Chloride Content. This was determined in 1 g of flour by Mohr's method (Vogel, 1961).

Determination of Total Sugars. The flour was extracted with 70% ethanol for 2 h on a water bath and the ethanol extract used for the estimation of total sugars by the method of Dubois et al. (1956). Glucose was used as the standard.

Tannins. The tannins were extracted from the defatted flour (1 g) by refluxing with 80% ethanol for 30 min 3 times and estimated by using the Folin-Denis reagent (Swain and Hills, 1959).

Solubility of Protein. The solubility was determined by using 1 g of flour and 10 mL of the solvent. The suspension was shaken mechanically for 1 h at room temperature and centrifuged at 4000 rpm for 20 min at room temperature. The nitrogen content of the supernatant was determined by the Kjeldahl method; protein contents were calculated by using the factor 6.25. Distilled water, 1 M NaCl (pH 6.8), and 0.02 M NaOH (pH 8.5) were used as the solvents.

In Vitro Digestibility Index. This was estimated by the method of Akeson and Stahmann (1964), using pepsin and pancreatin. Flour (500 mg) was incubated with 12.5 mg of pepsin in 15 mL of 0.1 N HCl for 24 h at 37 °C in a water bath. After neutralization with 7.5 mL of 0.2 N NaOH and addition of 4 mg of pancreatin in 7.5 mL of pH 8.0, phosphate buffer, the digestion mixtures were incubated for an additional 24 h at 37 °C. The undigested protein was precipitated with 10% trichloroacetic acid (TCA) and the supernatant used for nitrogen estimation by Kjeldahl method. The results are expressed as percent of the flour nitrogen digested.

Trypsin Inhibitor Activity. It was determined as described by Kakade et al. (1969), using benzoyl-DL-argi-

nine-*p*-nitroanilide as the substrate for trypsin.

α-Amylase Inhibitor Activity. It was determined according to the method of Sathe et al. (1982). Soluble starch in 0.2 M phosphate buffer of pH 7.0 was incubated at 37 °C with α-amylase solution for 30 min, the reaction was stopped by heating in a water bath for 5 min, and the liberated sugars were estimated. The experiment was repeated in the presence of lupin flour extract. From the difference in the value of liberated sugars, α-amylase inhibitory activity was calculated.

Hemagglutinin Activity. The method of Liener and Hill (1953) used for soybean was adopted. One gram of the flour was extracted with 10 mL of 0.9% NaCl at 4 °C for 12 h and centrifuged for 20 min at 5000 rpm. The hemagglutinin activity of the supernatant was evaluated by using trypsinated rabbit blood erythrocyte suspension (4%).

Proteolytic Activity. Proteolytic activity was determined by using casein as the substrate by the method of Chrispeels and Boulter (1975). Casein (E. Merck, West Germany) was dissolved in 25 mM citrate–phosphate buffer of pH 5.7 at a concentration of 1%. Lupin flour extract as a source of protease was prepared by shaking 1 g of flour in 10 mL of 25 mM citrate–phosphate buffer of pH 5.7 for 1 h and taking the clear centrifuged extract. The assay mixture containing 1 mL of casein, 0.2 mL of flour extract, and 0.8 mL of water was incubated for 2 h at 37 °C in a thermostatic water bath. The reaction was stopped by the addition of 1 mL of 10% TCA, and the proteins were allowed to precipitate in the cold (4 °C) for 1 h. The absorbance of the supernatant obtained by centrifugation was read at 280 nm.

Absorption Spectrum. The ultraviolet (UV) absorption spectrum of protein in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl was recorded at room temperature (~28 °C) in a Perkin-Elmer double-beam recording spectrophotometer, 124, in the range of 240–300 nm. Approximately 0.1% solutions were used.

Fluorescence Spectrum. This was measured in a Perkin-Elmer fluorescence spectrophotometer, Model-203, at 25 °C. The emission spectrum of the protein solution in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl was measured in the range of 300–400 nm after excitation at 280 nm. The excitation spectrum was also measured in the range of 250–300 nm after fixing the emission maximum at 325 nm. Protein solution of 0.12 OD at 280 nm was used for the measurement.

Polyacrylamide Gel Electrophoresis (PAGE). This was carried out by the procedure described by Davis (1964) using 7.5% gel and 0.01 M sodium phosphate buffer of pH 7.8. Lupin seed flour protein was extracted in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl to extract the maximum amount of protein (flour to solvent ratio 1:10; extraction time 1 h at room temperature), and the extract was dialyzed against 0.01 M buffer without NaCl for 24 h. About 200 μg of protein was loaded. Electrophoresis was carried out for 3 h at a constant current of 3 mA/tube. The gels were stained with a staining solution of 0.5% Amido black for 30 min and destained with 7.5% acetic acid.

Ultracentrifugation. The protein was extracted in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl by shaking for 1 h at room temperature. The flour to solvent ratio was 1:10 (w/v). After centrifugation at 5000 rpm for 20 min, the clear supernatant was dialyzed against the same buffer overnight and 0.6% protein solution was used. The experiment was done with a Spinco Model E analytical ultracentrifuge fitted with a rotor

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Table I. Proximate Composition of Defatted Lupin Seed Flours, Raw (A) and Debittered (B)[a]

	sample	
constituent	A	B
protein, $N \times 6.25$	51.5 ± 2.1	57.7 ± 2.3
total sugars, % glucose	16.6 ± 0.21	2.6 ± 0.19
total ash, %	3.8 ± 0.10	8.2 ± 0.12
available lysine, g/16 g of N	2.52 ± 0.51	3.18 ± 0.48
sodium chloride, %	–	7.5
tannins, %	0.86 ± 0.05	0.11 ± 0.03

[a] Average of three measurements and expressed on a dry weight basis.

Table II. Nitrogen Solubility and in Vitro Digestibility of Lupin Seed Flours[a]

	nitrogen solubility, %			pepsin–pancreatin digested protein, %
sample	H_2O	1.0 M NaCl	0.02 M NaOH	
A (raw)	24.3 ± 2.2	86.3 ± 5.2	98.3 ± 5.1	81.8 ± 3.1
B (debittered)	8.9 ± 2.4	12.6 ± 4.8	92.6 ± 5.3	86.6 ± 2.9

[a] Average of three measurements.

temperature indicator and control (RTIC) unit and phase plate schlieren optics. It was carried out at 50 740 rpm and at room temperature. Photographs were taken at different intervals of centrifugation. From the photographs, s_{app} was calculated by the standard procedure (Schachman, 1959). Enlarged tracings were used to calculate the proportion of the proteins in the extract.

RESULTS AND DISCUSSION

Proximate Composition. The proximate chemical composition of lupin seed flour before and after debittering was determined in triplicate, and the mean value and standard error are given in Table I. The debittered flour showed higher contents of total protein, total ash, and available lysine, whereas the original flour showed higher contents of total sugar and polyphenols. Soaking and steeping of lupin seed in water for a long time removed most of the soluble material, particularly simple sugars, minerals, low molecular weight polypeptides, and tannins. This possibly lead to an increase in the protein content of the debittered flour. The reductions were 84 and 87% for sugars and tannins, respectively. Sample B showed a higher value for total ash, and this was expected because sodium chloride was added to it according to the traditional practice. Mogghaddam et al. (1976) have reported that *L. mutibilis* (bitter variety) contains up to 2% alkaloids, especially lupanine and hydroxylupanine. The sweet varieties of lupin evolved through plant breeding contain less than 0.1% alkaloids. Sosulski and Garratt (1976) and Sosulski and Youngs (1979) have reported a wide variation in chemical composition of lupin, and this may be due to either climatic conditions or differences in genetic characteristics among the varieties. Also, Mogghaddam et al. (1976) have reported very significant interspecies variation and even variation between the cultivars.

Sample B had 0.1% tannins, value that is considered characteristic of sweet lupin.

The solubility in different solvents showed that sample A had solubility higher than sample B in all the solvents used (Table II). Solubility in 1 M NaCl and 0.02 M NaOH was higher than that in water, and sodium hydroxide showed the highest solubility. The low solubility of sample B in water and 1 M NaCl solution is perhaps due to the heat treatment used in the debittering treatment. Ruiz and Hove (1976) have reported a value of 85% for the solubility of lupin seed protein in 5% NaCl solution and
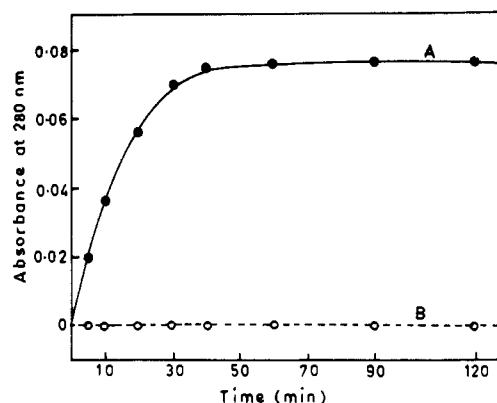

Figure 1. Proteolytic activity (caseinolytic) of untreated and treated lupin seed flours. (A) Raw flour extract. (B) Debittered flour extract.

94% in dilute alkali at pH 8.5: these values agree with our data.

Digestibility of the pepsin–pancreatin system showed a trend different to N-solubility behavior in aqueous solvents. Sample A gave a value (80%) comparable to that of casein, but sample B showed a slightly higher value (86.6%). Apparently boiling the lupin seeds for 20 min slightly improved the in vitro digestibility of the seed proteins. This could be due to denaturation of proteins and increased susceptibility toward enzyme attack. The improvement of in vitro digestibility of sample B on heating observed in this work is in agreement with the reported improvement in protein digestibility of proteins of *Phaseolus vulgaris* (Romero and Ryan, 1978; Chang and Satterlee, 1980; Iyer et al., 1980), winged beans [*Psophocarapus tetragonolobus* (L) DC] (Ekpenyong and Borchers, 1980), and lupin seed (*L. mutabilis*) (Sathe et al., 1982).

Antienzyme Activities. Samples A and B did not show trypsin and α-amylase inhibitor activity and hemagglutinin activity. Sathe et al. (1982) have reported the absence of trypsin, chymotrypsin, and α-amylase inhibitor activity in lupin seed flour and concentrate. Our results are in agreement with this. Thus, lupin flour (especially the sweet variety) may have good nutritional quality. Also, it was observed in this study that lupin seed flour extract did not show any hemagglutinin activity.

Proteolytic activity of lupin seed flours is given in Figure 1. Sample A showed some activity, but sample B did not. This could be due to the heat treatment applied to sample B. The activity reached a maximum value after 40 min of incubation at 37 °C and remained constant up to 2 h. The proteolytic (caseinolytic) activity of lupin seed flour is very low compared to that of soybean or guar (Nath et al., 1978).

Physicochemical Properties. The UV absorption spectrum of samples A and B in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl is given in Figure 2. Sample A gave a spectrum with a maximum at 275 nm and a minimum at 250 nm; sample B had a maximum at 263 nm and a minimum at 245 nm. Sample B gave a broad maximum, whereas sample A gave a sharp maximum. The ratio of the absorbance at the maximum to that at the minimum was 1.39 for sample A and 1.15 for sample B; proteins not conjugated with nucleic acid and other impurities give a ratio of 1.5 (Layne, 1957). From this criterion both samples A and B were contaminated with UV-absorbing impurities, possibly polyphenols. In the case of sample B, the minimum had shifted to lower wavelength. A similar effect of heat has been reported in the case of groundnut protein (Srikanta and Narasinga Rao, 1974) and guar protein (Nath, 1980).
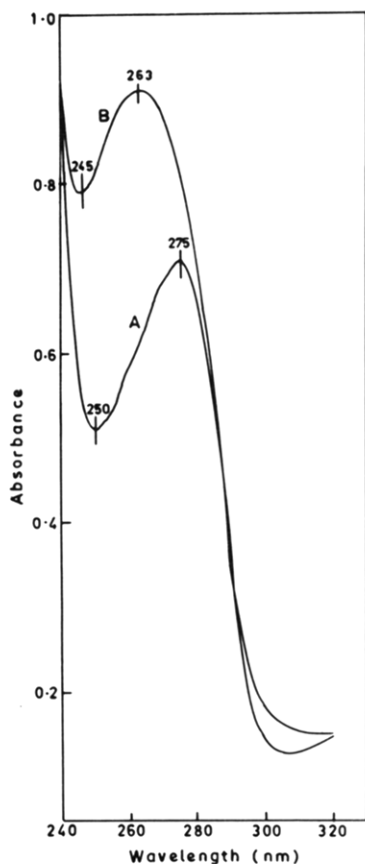
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Figure 2. Ultraviolet (UV) absorption spectrum of lupin seed proteins in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl. (A) Raw flour proteins. (B) Debittered flour proteins.
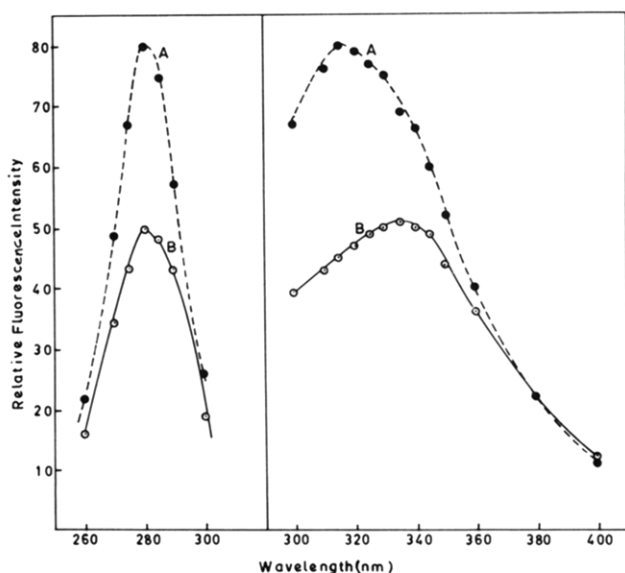

Figure 4. Polyacrylamide gel electrophoresis pattern of lupin seed proteins in 0.01 M sodium phosphate buffer of pH 7.8 and 7.5% gel. (A) Raw flour extract. (B) Debittered flour extract.

Figure 5. Sedimentation velocity pattern of lupin seed proteins in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl; photograph was taken after 57 min after the attainment of the maximum speed of 50 740 rpm at 25 °C. Sedimentation proceeds from left to right. (Top) Debittered flour extract. (Bottom) Raw flour extract. Numbers refer to the s_{ap+} of the peaks.

Figure 3. Fluorescence spectrum (excitation and emission spectrum) of lupin seed proteins in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl. (A) Raw flour extract. (B) Debittered flour extract.

The fluorescence spectrum (excitation and emission spectrum) is shown in Figure 3. Both the samples had fluorescence excitation maximum at 280 nm; however, the emission maximum was different in the two samples. The emission maximum was at 315 nm for sample A and at 335 nm for sample B. The treated sample (B) showed not only a shift in the emission maximum toward higher wavelength
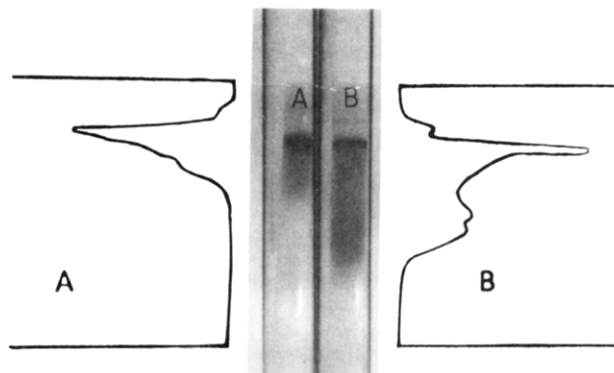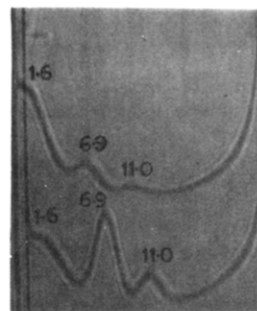
but also quenching of fluorescence. A red shift in the emission maximum and fluorescence quenching are caused by the denaturation of the proteins (Chen et al., 1969). From this criterion the proteins appeared to have denatured by the debittering treatment.

The polyacrylamide gel electrophoresis pattern and the densitometric tracing of the two samples in 0.01 M phosphate buffer of pH 7.8 are shown in Figure 4. Sample A showed a minor band near the origin, a major band, and a shoulder. Sample B showed, in addition to these bands, two bands with higher mobility; these bands were not well resolved. The proportion of these two fast-moving bands was high (~40% of the total). The fast-moving bands may be due to dissociation of the protein by heat.

The sedimentation velocity pattern of the proteins in 0.1 M sodium phosphate buffer of pH 7.8 containing 0.5 M NaCl is given in Figure 5. In general, both the patterns consisted of three peaks, having s_{app} values of 1.6S, 6.9S, and 11.0S. Sample A had the 6.9S protein as the major component (~50%) whereas 1.6S was the major protein component of sample B (~60%). The increase in the proportion of the low molecular weight protein fraction in sample B indicated that high molecular weight fractions of sample A had undergone dissociation. Chakraborty et al. (1979) have reported a similar ultracentrifugation pattern for raw lupin seed proteins.

Thus, the debittering treatment for lupin seed not only changed the chemical composition of the meal but also caused dissociation of the high molecular weight protein and denaturation. The debittered meal had improved in vitro protein digestibility.

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Dissociation of Yeast Nucleoprotein Complexes by Chemical Phosphorylation

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Chemical phosphorylation of yeast nucleoproteins using $POCl_3$ caused dissociation of nucleoprotein complexes. Subsequent precipitation of the phosphorylated proteins at pH 4.2 resulted in a protein preparation with low levels of nucleic acid contamination. However, there was an appreciable decrease in the recovery of proteins after phosphorylation. The advantages of the phosphorylation method over that of other chemical modification methods for decreasing the nucleic acid content of yeast proteins are also discussed.

Proteins from microbial sources, especially yeast, could significantly supplement the ever increasing demand for the world supply of food protein (Tannenbaum and Wang, 1975). However, the exploitation of these proteins for direct human nutrition is limited because of the high nucleic acid content and poor functionality of these proteins (Kihlberg, 1972; Sinskey and Tannenbaum, 1975; Vananuvat and Kinsella, 1975; Lipinsky and Litchfield, 1974). There are several methods currently available to reduce the nucleic acid content of single cell proteins (Newell et al., 1975a,b; Robbins et al., 1975; Shetty and Kinsella, 1979). These methods involve chemical and enzymatic treatment of homogenized yeast cells. Although these treatments effectively reduce the nucleic acid content, they have several detrimental effects on the nutritional and functional qualities of the isolated protein. For example, alkali treatment causes formation of potentially toxic compounds such as lysinoalanine (Shetty and Kinsella,

1980a,b) and enzymatic hydrolysis of nucleic acids results in concomitant degradation of proteins by endogenous proteases (Lindbloom, 1977). Chemical modification of yeast proteins with acid anhydrides such as succinic and citraconic anhydrides effectively reduced the nucleic acid content of yeast proteins (Shetty and Kinsella, 1979, 1980a,b). However, the nutritional safety and acceptability of acylated proteins are yet to be determined. In our continuing effort to develop a simple and safe alternative method for the isolation of yeast proteins with low levels of nucleic acids, we studied chemical phosphorylation of yeast nucleoproteins using phosphorus oxychloride (PO-Cl_3).

EXPERIMENTAL SECTION

Brewer's yeast (Saccharomyces carlbergensis) was obtained from Genesee Brewing Co. (New York). The yeast cells were washed 3 times with cold distilled water and disrupted by using a Dyno-Mill (Type KDL, Willy A. Bachofen, Manufacturing Engineers, Basel, Switzerland) at 5 °C. The pH of the homogenate was adjusted to 9.0, and the mixture was stirred for 30 min at 5 °C. The solution was then centrifuged at 15000g for 30 min at 5 °C to remove the cell wall and other insoluble materials.

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