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Chitin production by *Lactobacillus* fermentation of shrimp biowaste in a drum reactor and its chemical conversion to chitosan

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Abstract: Chitin was produced by fermenting shrimp heads and shells with *Lactobacillus plantarum* 541 in a drum reactor with an internal volume of 3 dm^3. The crude chitin yield from heads and shells was 4.5 and 13% respectively, comparable to the values obtained by the chemical method. For shrimp heads 83% deproteination and 88% demineralisation and for shrimp shells 66% deproteination and 63% demineralisation were achieved. The liquor obtained in both cases was of good sensory quality with a high content of essential amino acids and therefore with potential to produce protein powder for human consumption. The crude chitin was refined and converted to chitosan using 12.5 M NaOH. The chitosan obtained had a residual ash and protein content below 1%, a solubility of more than 98%, a viscosity in the range 50–400 cP and a degree of deacetylation of 81–84%. The molecular weight was in the range (0.8–1.4) × 10^6 Da. IR analysis indicated that the chitosan obtained through fermentation was similar to that obtained by the chemical method.

Keywords: chitin; shrimp shells; shrimp heads; chitosan; fermentation; drum reactor

INTRODUCTION

Crude shrimp biowaste contains chitin in a complex binding to 10–20% calcium and 30–45% protein.[1] If shrimp biowaste is fractionated effectively, valuable products such as chitin, chitosan, protein and asthaxanthin are obtained.[2] Chitosan, the deacetylated derivative of chitin, has numerous applications in the pharmaceutical, textile, food and cosmetic industries, in agriculture and in waste water treatment.[3]

Biowaste from the shrimp industry, a mixture of shrimp shells and heads, is used for the extraction of chitin. The protein in the waste is removed by treatment with 0.1 M NaOH and the minerals are removed by treatment with 0.13 M HCl. The main concern with the chemical method is the use of aggressive chemicals in large quantities at elevated temperatures. This affects the quality of the end product, the corrosion of the equipment[2] and the generation of considerable amounts of alkaline waste water high in biological oxygen demand (BOD) that cannot be released without further treatment.[4]

Fermentation of shrimp waste using lactic acid bacteria results in a solid fraction containing crude chitin[5,6] and the production of a liquor rich in natural shrimp protein, minerals and pigments. The action of the lactic acid bacteria is twofold. *Lactobacillus* produces a spectrum of proteases that detach the protein from the solid chitin–$CaCO_3$ complex by partial hydrolysis. In addition, the acid produced during fermentation dissolves a considerable part of the minerals, mainly $CaCO_3$, and increases the storability of the biowaste. The fermentation of shrimp biowaste has been optimised at pH 6.0 using 10% inoculum and 5% glucose, which results in simultaneous demineralisation and deproteination of the solid fraction.[7,8]

Lactobacillus species show aero-tolerant growth characteristics and prefer a relatively less agitated environment. In the case of shrimp fermentation a low degree of mixing is required owing to the generation of CO_2.[9,10] Therefore a laboratory-scale horizontal drum bioreactor was fabricated that could provide adequate mixing to ferment shrimp biowaste with *Lactobacillus plantarum* 541 at the desired pH and temperature conditions. The quality of chitin and chitosan prepared by the fermentation method and by the thermochemical process has been compared.

EXPERIMENTAL

Micro-organism and cultivation methods

L plantarum 541 was obtained from the Thailand Institute of Scientific and Technological Research (Bangkok, Thailand). The strain was stored in 40%

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glycerol at −80 °C or on MRS agar slants at 4 °C. The strain was routinely cultivated in MRS medium[11] containing glucose as substrate (20 g dm^{-3}). Overnight cultures in Erlenmeyer flasks with a cell density of (1–3) × 10^9 cells cm^{-3} were used as inoculum (5% v/v). Media were sterilised for 20 min at 115 °C.

Fermentation in drum reactor

Shrimp biowaste (heads or shells) obtained as fresh material and stored frozen at −10 °C was thawed and crushed in a mixer. In order to reduce uncontrolled decay, 1% (v/w) glacial acetic acid was added. The biowaste was weighed (1 kg of shells or 2 kg of heads) and loaded into the sieve drum, which is the interior compartment of the bioreactor. The reactor is described in detail in a later section. Inoculum (10% v/w) of *L plantarum* 541 and 5% (w/w) glucose dissolved in distilled water (10% w/v) were added to the biowaste. The pH of the fermentation mixture was measured continuously with a pH probe mounted in the interior of the reactor and maintained at a predetermined value using an α-digital pH controller (Eutech Instruments, Singapore). The acetic acid to be added was pumped directly from a reservoir into the reactor. The rotating shaft of the reactor was driven by a motor with a speed control. The motor was switched on at regular intervals of 1 h for a duration of 5 min. Water from a temperature-controlled water bath at 30 °C was circulated through the outer jacket of the reactor.

After 24 h, fermentation was stopped and the liquor was drained through the outlet port. To remove the last amount of liquor, the internal sieve drum was rotated at higher speed. The solid residue inside the reactor was washed with distilled water and then dried at 50 °C for further processing and analysis. The volume and weight of hydrolysate were measured. As reference, fermentation experiments were also conducted in 1 l beakers with 300 g of shrimp heads mixed manually at 1 h intervals. A solid residue was obtained after filtration of the slurry. All fermentation and chemical treatment experiments were conducted in duplicate.

Chemical treatment of shrimp heads and shells

To compare the fermentation and chemical production yields, heads and shells (1 kg) were subjected to 0.1 M NaOH and 0.13 M HCl. Two types of chitin were obtained by altering the sequence of NaOH and HCl treatments. This chitin was subjected to 12.5 M NaOH for deacetylation. The solids obtained were washed several times to obtain chitosan.

General analytical procedures

Samples for analysis were collected in triplicate from each fermentation vessel. Moisture content was measured by drying the samples in an oven at 105 °C for 24 h. Ash content was determined by burning the samples in a crucible at 600 °C in a muffle furnace (Sanyo, Gallenkamp, Loughborough, UK). Values of pH and pH drop (−dpH/dt, pH h^{-1}) were measured using a benchtop pH meter (Jenway 6051, Essex, UK). Protein content was measured using the standard biuret protein assay in samples before and after fermentation. The microbiuret assay was used to determine protein content in chitin and chitosan, where protein concentrations are very low. Total nitrogen content was determined by the Kjeldahl method (Kjeltech, Gallenkamp) in the initial biowaste as well as in the fermented solid residues, the difference being reported as protein nitrogen. Deproteination (%DP) was calculated using the equation $\%DP = [(P_O \times O) - (P_R \times R)] \times 100/(P_O \times O)$, where P_O and P_R are the protein concentrations (g g^{-1}) before and after fermentation respectively and O and R are the masses (g) of the original sample and the fermented residue respectively.

Demineralisation efficiency (%DM) was calculated using the above equation but replacing P_O and P_R in the equation by A_O and A_R, which represent the ash concentrations in the original sample and the fermented residue respectively. Chitin recovery (%CR) was computed as chitin derived (g) in reference to the original amount of chitin present in shrimp heads or shells. Chitin yield (%CY) was calculated as chitin derived (db, g) in reference to the original wet sample quantity of heads or shells. For lipid content the sample was subjected to solvent extraction with ethanol for 6 h and the extract was dried in a vacuum oven at 40 °C to remove traces of ethanol. Lipid content (%db) was calculated as the weight of extracted lipid divided by the original sample weight.

Quality of chitin and chitosan

Solubility

To determine the solubility of chitin, the sample was first dried for 24 h at 50 °C in a vacuum oven, then 1 g of dried chitin was dissolved in 100 cm^3 of dimethyl acetamide/lithium chloride (DMA/LiCl) solution for 12 h and subsequently centrifuged to determine the percentage of insoluble chitin. The DMA/LiCl solution was prepared by dissolving 8 g of anhydrous lithium chloride overnight in 100 cm^3 of DMA. The solubility of chitosan was determined by dissolving 1% (w/v) chitosan in a solution of 1% glacial acetic acid for 24 h under continuous stirring.

Degree of deacetylation (%DD)

The degree of deacetylation was determined by the HPLC method.[12] The %DD is obtained by analysing the acetic acid released upon hydrolysis of chitin by sulfuric acid in the presence of oxalic acid. Complete hydrolysis is obtained within 1 h. A known amount (10–50 mg) of vacuum oven-dried chitin or chitosan sample was placed in a 5 cm^3 ampoule into which 1.5 cm^3 of 12 M sulfuric acid and 1 cm^3 of 63 mg dm^{-3} oxalic acid were added. The ampoule was sealed gas-tight, incubated in an oven at 110 °C for 2 h and cooled in ice water for 2 h. The sample was then diluted 10-fold and filtered through a 0.45 μm membrane filter

before injection. The HPLC (Waters, Massachusetts, USA) used a 300 mm × 7.8 mm column packed with cation exchange resin (ORH-801). A flow rate of 0.8 cm^3 min^{-1} of 5 mM sulfuric acid under 1600 psi pressure was maintained. The oven and compartment temperatures were set to 45 and 25 °C respectively. The injection volume was kept at 30 mm^3 and a tunable absorbance detector (TAD, Waters™ 486) was used at 210 nm. Standards of acetic acid solutions were prepared with Merck GR-grade acetic acid (purity > 99.8%). Under the conditions stated above, the acetic acid peak eluted at 9.00 min and was normally completely separated from other peaks ($R_s > 1.84$).

Intrinsic viscosity for molecular weight determination

Chitosan was dissolved in sodium acetate buffer (0.1 M sodium acetate and 0.2 M acetic acid) to obtain concentrations of 0.010, 0.012, 0.014, ..., 0.030%. Intrinsic viscosity [η] was determined using a Cannon–Fenske (Cannon Instrument Co, State College, PA, USA) viscometer immersed in a unithermal bath (Yamato (Tokyo, Japan) model BR-61) at 30 ± 0.01 °C. The efflux time of the solution was between 200 and 300 s. Intrinsic viscosity was calculated using the equation $[\eta] = \lim \eta_{sp}/C$, with $C \rightarrow 0$, where [η] is the intrinsic viscosity, η_{sp} is the specific viscosity and C is the concentration of chitosan solution. The average molecular weight of chitosan samples was estimated using the Mark–Houwink relationship $[\eta] = K\ (MW)^a$, where K is a constant, MW is the average molecular weight and a is the Mark–Houwink constant. In this equation the K and a values depend on the degree of deacetylation of the chitosan sample.[13] For example, for a degree of deacetylation of 84% the values of a and K were 0.96 and 14.2×10^{-6} respectively, whereas for a degree of deacetylation of 91% the values of a and K were 0.88 and 65.9×10^{-6} respectively.

Apparent viscosity

Chitosan solution was prepared by dissolving 1% (w/v) chitosan in 1% (v/v) glacial acetic acid for 24 h. The solution was then filtered through a nylon cloth to remove any non-soluble particles. The viscosity of chitosan solution was measured using a Brookfield (Stoughton, USA) DV II+ viscometer, spindle no 63.

RESULTS AND DISCUSSION

Analysis of shrimp biowaste

The heads form the major fraction (50%) by weight of the material obtained after removal of the meat from the whole shrimp (Table 1). The moisture and ash contents in the shrimp biowaste (heads and shells) were in the ranges 70–83 and 18–20% respectively. The values for ash content were not significantly different in the three fractions of the shrimp waste material, indicating that minerals are distributed equally. In comparison with the shells, the heads were higher in protein and lower in chitin and contained some fat as well.

Chitin nitrogen was calculated from the nitrogen content in the residue after treatment with 0.1 M NaOH and 0.13 M HCl. After treating the residue with 0.1 M NaOH, protein is removed completely. The only nitrogenous compound in the residue is chitin. Chitin content (%) was calculated as chitin nitrogen × 14.25, as the chitin monomer contains approximately 7% nitrogen. The chitin content in shells (57%) was higher than that in heads (42.8%) and tails (45.6%). The shells were also cleaner and easier to handle for production of chitin owing to their lower protein (19.3%) and lipid (0.5%) contents. A mass balance was achieved based on data for minerals, protein, lipids and chitin. With an error <10%, this balance is reasonably accurate.

Design and fabrication of drum bioreactor

A prototype reactor with an internal volume of 3 dm^3 was fabricated using acrylic (perspex) material to be able to observe the changes in the biowaste texture and coloration during fermentation (Fig 1). The drum reactor consists of two concentric cylindrical tubes made of perspex, with a removable lid (k) on one side and a closed cover on the other side. The double layer (w) serves as a jacket in which water at the desired temperature is pumped through the inlet (x) and outlet (y) provided. The reactor jacket is supported by semicircular strips fixed to a four-legged frame (not shown in the figure). A perforated cylindrical sieve (d) of perspex fits inside the drum jacket. One side of the cylindrical sieve is closed with a fixed cover plate, while the other side is closed with a removable sieve plate (c) that is used for loading and unloading

Table 1. Composition (%db) of shrimp biowaste[a]

Shrimp biowaste fraction	Proportion of shrimp waste (g g^{-1})	Moisture content (%wb)	Ash	Protein[b]	Total lipid	Chitin nitrogen[c]	Chitin[d]	Mass balance error[e]
Head	0.50	74.4 ± 2.5	17.6 ± 1.3	24.6 ± 1.4	6.3 ± 1.3	3.0 ± 0.5	42.8	8.7
Shell	0.35	75.5 ± 3.2	19.3 ± 1.4	18.6 ± 2.4	0.5 ± 0.04	4.0 ± 0.3	57.0	4.6
Tail	0.15	74.2 ± 2.9	18.3 ± 1.0	26.7 ± 1.4	—	3.2 ± 0.4	45.6	9.4
Whole shrimp waste	1.00	83.8 ± 2.1	19.1 ± 1.2	28.6 ± 1.6	3.2 ± 1.5	3.6 ± 0.7	51.3	2.2

[a] Shrimp biowaste is shrimp minus meat.
[b] Assayed by biuret method.
[c] Nitrogen content measured in solid fraction after deproteination and demineralisation.
[d] Chitin (%) = chitin nitrogen × 14.25 (chitin contains ~7% N).
[e] Mass balance error = 100 − %(protein + ash + lipid).

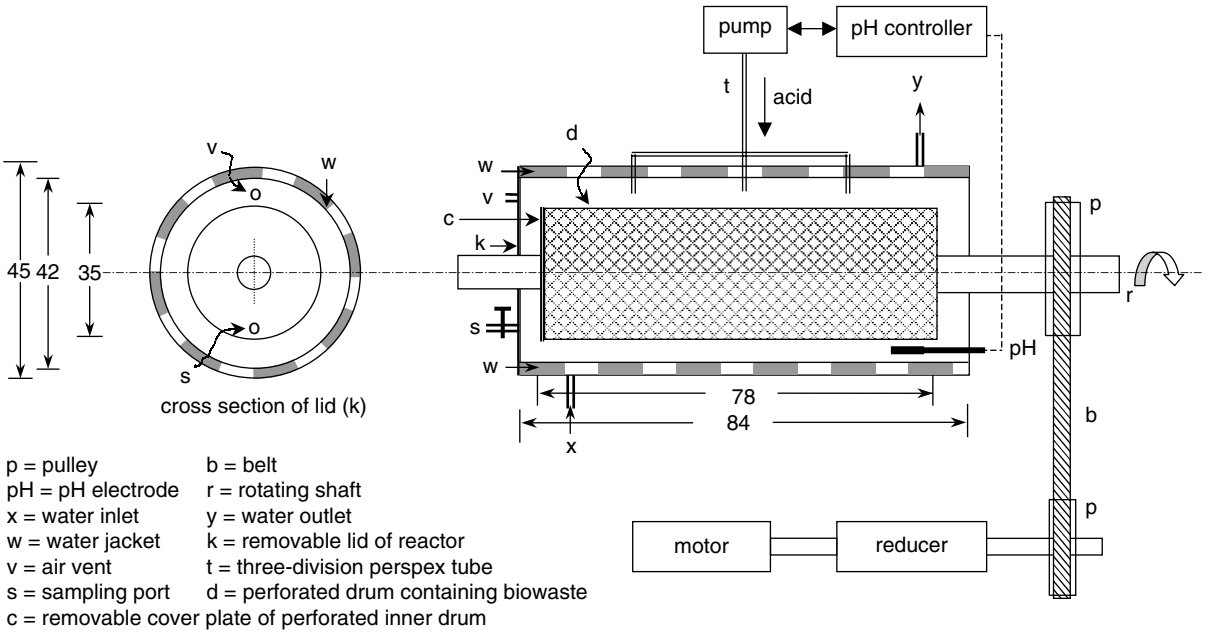

Figure 1. Schematic diagram of 3 dm^3 perspex drum reactor (all dimensions in mm).

the sample. The rotation is provided by a rotating shaft (r) fixed to a belt (b) and pulley (p) arrangement attached to a reducer and a motor.

The removable lid (k) has two vents, one for air (v) to keep the reactor at atmospheric pressure and the other for sampling (s) and draining of liquor at the end of fermentation. The cylindrical sieve with its shaft hangs on ball-bearings for free rotation fixed at both sides on the covers and the lid respectively. The pH controller (pH) is set with lower and upper limits, transferring the signal to the pump to operate. The acid is pumped into the reactor through a three-division perspex tube (t). In comparison with the design of drum reactor proposed earlier,[10] this bioreactor is based on conducting the fermentation at controlled pH in just 24 h.

Effect of *Lactobacillus plantarum* 541 and acetic acid on pH of biowaste

The pH of shrimp biowaste is in the range 8.2–8.5; if, after thawing, the pH is not adjusted to lower values, the waste becomes spoiled within a few hours. Acetic acid and *Lactobacillus* inoculum can lower the pH to a level where spoilage is delayed or does not occur at all. An optimum value of pH 6.0 for demineralisation and deproteination of shrimp heads was determined by conducting several tests at different pH values.[7] Figure 2 shows the pH profile of shrimp biowaste

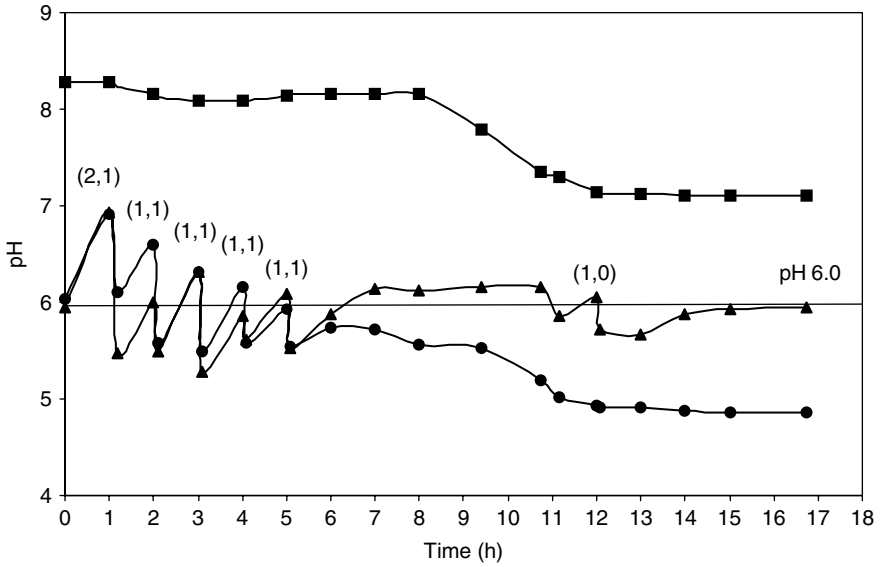

Figure 2. Changes in pH during (a) autofermentation of shrimp biowaste without addition of acetic acid and *Lactobacillus* inoculum (■), (b) autofermentation with pH maintained at 6.0 with acetic acid and no *Lactobacillus* inoculum (▲) and (c) *Lactobacillus* (10% v/w)-assisted fermentation with pH maintained below 6.0 with acetic acid (●). The values in parentheses at different time intervals indicate the amounts of glacial acetic acid added (cm^3) under conditions (b) and (c) respectively.

with addition of glacial acetic acid and/or *Lactobacillus* inoculum during fermentation.

Fermentation of heads and shells in perspex drum reactor

Four successful fermentation experiments were carried out in the 3 dm^3 drum reactor, two with shrimp heads and two with shrimp shells. The reactor basket could hold 2 kg of heads but only 1 kg of shells, as the bulk density of shells is very low. As reference, experiments were conducted with 300 g of heads or shells in 2 dm^3 beakers. Heads and shells were crushed before fermentation. Inoculum (10% v/w) and glucose (5% w/w) were added and the pH was maintained at or below a value of 6.0 by addition of glacial acetic acid. For comparison of results, all data in Table 2 are presented for 1000 g of sample.

The treatment of heads (2 kg) was very efficient in the drum reactor and resulted in 128–142 g of fermented residue (64–71 g from 1000 g of heads; Table 2). The performance of the 3 dm^3 drum reactor was satisfactory to achieve good fermentation of the biowaste. The pH was maintained within limits and good separation of residue and liquor was observed at the end. The average deproteination and demineralisation efficiencies were 83 and 88% respectively. In the beakers, lower efficiencies of deproteination (76%) and demineralisation (77%) were obtained. In the case of both drum reactor and beaker experiments the chitin yield (%CY), expressed as the percentage of crude chitin derived from the original biowaste quantity, was about 4.4% (Table 2). The higher efficiency of deproteination and demineralisation in the drum reactor as compared with the beaker experiments could be attributed to better mixing conditions in the reactor.

The deproteination and demineralisation of shells were less efficient. The average deproteination and demineralisation efficiencies were 66 and 63% respectively (Table 2). The lower performance of *Lactobacillus* fermentation in deproteinating and demineralising shell waste might be due to the compact structure of the shells. As a result, the *Lactobacillus* proteases might not reach the internal structure of the shells. Also, the protein layer protects $CaCO_3$ against exposure to lactic acid. Since the fermentation of shells was not efficient, the solid residue after fermentation still amounted to 13% of the original dry weight. This crude residue may not be termed chitin as it has high amounts of protein (34%) and minerals (37%) still attached to it. Fermentation of shells in the beakers resulted in only 53% deproteination and 50% demineralisation and therefore the chitin recovery and chitin yield have not been calculated and reported here. In real terms there is little change in shell structure with the high amounts of protein and minerals still attached and this product needs complete chemical treatment to convert it to chitin. From these results one can infer that fermentation of shells is less efficient.

Comparison of fermentation and chemical chitin extraction

The chitin yield through the chemical route was 4.4% for heads and 7.2% for shells (Table 3). For heads the yield was similar to that obtained by the fermentation route (Table 2). The chitin recovery through the chemical route for both heads (38%) and shells (45%) is low. This indicates that, in both fermentation and chemical extraction routes, 50–60% of solid material, assumed to be chitin, is lost. Two hypotheses explaining the loss of chitin can

Table 2. Deproteination and demineralisation of biowaste after fermentation in drum reactor

Sample type	Sample wt (g) *a*	MC (%) *b*	DW (g) *c*	Protein (%) *d*	Protein (g) *e*	Ash (%) *f*	Ash (g) *h*	Chitin (g) *j*	DP (%)	DM (%)	CR (%)	CY (%)
Heads (drum reactor)												
Original	1000	74.7	253.0	28.0	70.8	20.2	51.1	115.2	—	—	—	—
R1	64.5	5.9	58.8	21.0	10.1	10.7	5.5	43.2	85.7	89.2	37.5	4.3
R2	71.0	6.9	65.8	22.0	14.4	9.6	6.3	45.1	79.6	87.6	39.1	4.5
Heads (beaker)												
Original	1000	78.4	216.0	27.7	60.0	18.2	39.3	103.1	—	—	—	—
R1	76.7	8.5	68.7	20.1	14.4	12.9	9.0	45.3	76.0	77.4	43.9	4.5
R2	74.3	8.2	68.2	21.2	14.5	13.1	8.9	44.8	75.8	77.3	43.5	4.4
Shells (drum reactor)												
Original	1000	72.6	273.5	23.1	62.9	18.5	50.5	158.7	—	—	—	—
R1	184	10.0	165.2	12.5	20.7	10.9	18.0	126.5	67.4	64.3	79.7	12.6
R2	194	10.5	173.2	12.8	22.2	11.9	19.4	131.6	64.7	61.6	82.9	13.2
Shells (beaker)												
Original	1000	70.4	296.4	23.4	69.4	20.2	59.9	165.6	—	—	—	—
R1	258.7	9.5	234.1	14.1	33.0	13.8	32.3	163.1	52.4	46.1	—	—
R2	246.3	10.2	221.2	13.9	30.7	11.9	26.4	162.9	55.7	55.9	—	—

R1, R2 = wet weight of residues after fermentation; MC (*b*) = moisture content in *a*; DW (*c*) = dry weight of *a* = [(100 − *b*)/100]*a*; *d*, *e*, *f*, *h* = protein and ash contents by % and weight respectively; DP (deproteination) = [(*e* in original − *e* in R1 or *e* in R2)/(*e* in original)] ×100; DM (demineralisation) = [(*h* in original − *h* in R1 or *h* in R2)/(*h* in original)] ×100; chitin computed in grams (*j*) = *c* − *e* − *h*− (lipid content from Table 1); CR (chitin recovery) = [(*j* in R1 or *j* in R2)/(*j* in original)] ×100; CY (chitin yield) = [(*j* in R1 or *j* in R2)/(*a* in original)] ×100.

Table 3. Deproteination and demineralisation of heads and shells by chemical method

Sample type	Sample wt (g) *a*	MC (%) *b*	DW (g) *c*	Protein (%) *d*	Protein (g) *e*	Ash (%) *f*	Ash (g) *h*	Chitin (g) *k*	CR (%)	CY (%)
Heads (original)	1000	74.7	253.0	28.0	70.8	20.2	51.1	115.2	—	—
Residue (R1)[a]	50	12.0	44.0	1.0	0.44	0.8	0.35	43.2	37.5	4.4
Shells (original)	1000	72.6	273.5	23.1	62.9	18.5	50.5	158.7	—	—
Residue (R1)[a]	80	10.0	72.0	0.5	0.36	0.8	0.56	71.1	44.8	7.2

[a] Average from two beakers subjected to chemical treatment.
See footnote to Table 2 for explanation of *a*, *b*, *c*, *d*, *e*, *f*, *h* and *k*.

be formulated. (i) The waste might contain a large amount of nitrogenous compounds that are not chitin and do not contain peptide bonds, and this material is lost during the extraction steps. This would infer that the initial chitin content in actual terms is much lower. (ii) Chitin occurs in the shrimp heads or shells partially in aggregates of small particle size, which are lost during filtration or sequential washing. In the standard procedures, chitin particles are supposed to be retained by cloth filtration, but small chitin particles might be lost during cloth filtration.

The loss of either nitrogenous non-chitin material or chitin occurs in both fermentative and chemical processing. In spite of the fact that the fermentation and chemical procedures are widely different, it is remarkable that the value of 4.4% yield of chitin from heads is found for both processes. This similarity contributes to the confidence that the value of 4.4% as chitin yield is reliable.

Advantages of fermentation process over chemical extraction of chitin

In addition to obtaining a high-value by-product in the form of a liquor rich in protein, minerals and asthaxanthin, a major advantage of the fermentation process is the reduction in the use of chemicals. As a result, the quantity of chemicals and protein in the process waste water is considerably reduced. The waste streams of the fermentation process contain much less protein, because most of it has been removed during fermentation, contributing to a reduction in costs for treatment before disposal. The additional treatment with alkali and acid after fermentation cannot be avoided, but only a fraction of the chemicals are needed as compared with the chemical procedure. This is due to the fact that the quantity of the solid fraction after fermentation is 5–8 times lower as compared with the original biowaste, as the major portion of protein and minerals is already removed.

Chitin production routes

The qualities of chitin and chitosan differ substantially depending on the production route chosen.[2] The chitin fraction after fermentation was subjected to two production routes, A and B (Table 4), to remove the protein and minerals still remaining after fermentation. In route A the residue after fermentation was first deproteinated and subsequently demineralised, whereas in route B the residue was demineralised first and deproteinated later. For comparison, non-fermented shrimp shells were used and treated as per routes A and B.

Table 4. Production of chitin and chitosan by different treatments (A and B) of fermented residue and shrimp shells

Path A	Path B
4% NaOH, 50 °C, 16 h, 1:10	4% HCl, 60 °C, 4 h, 1:10
↓	↓
Wash and dry	Wash and dry
↓	↓
4% HCl, 60 °C, 4 h, 1:10	4% NaOH, 50 °C, 16 h, 1:10
↓	↓
Chitin	Chitin
↓	↓
50% NaOH, 50 °C, 48 h, 1:20	50% NaOH, 50 °C, 48 h, 1:20
↓	↓
Chitosan	Chitosan

Chitin quality analysis

Chitin is characterised on the basis of its ash content, protein content, solubility and degree of deacetylation. The ash content was lowest in path A, the reason being that, when the protein has already been removed, the acid has better penetration into the solid material, and minerals are dissolved easily. On the other hand, in path B a layer of protein is still present during the acid treatment and, as a result, the acid is unable to attack the chitin backbone easily. The residual protein content did not differ in residues obtained via routes A and B. The solubility of chitin in DMA/LiCl was high (>98%) for fermented samples with low ash content, whereas chitin prepared according to route B did not dissolve more than 90% (Table 5). The colour of chitin from fermented residues of the heads was darker than that of chitin obtained by chemical treatment. Path B resulted in more whiteness. In the case of the fermented product the ash content was comparatively low for both shells and heads. The differences in residual ash values can be observed at the chitin stage itself, proving that lactic acid produced during fermentation contributes significantly to demineralisation.

Chitosan quality analysis

Chitin was converted to chitosan by treatment with 12.5 M NaOH (Table 4). The use of 50% NaOH nullified possible effects that might be caused by

Table 5. Quality of chitin and chitosan obtained from fermentation and chemical methods

	Fermentation				Chemical	
	Heads		Shells		Shells	
Property	Path A	Path B	Path A	Path B	Path A	Path B
Chitin						
Protein (%)	<1.35	<1.51	<0.94	<1.05	<0.98	<1
Ash (%)	0.11	0.25	0.02	0.08	0.27	0.69
Solubility (%)	>98	85–98	>98	85–98	>98	>98
Chitosan						
Protein (%)	<0.8	<1.0	<0.54	<0.95	<0.5	<0.6
Ash (%)	0.05	0.15	0.01	0.07	0.21	0.19
Degree of deacetylation (%)	81.0 ± 0.2	82.3 ± 0.2	83.4 ± 0.1	86.1 ± 0.3	92.3 ± 0.2	92.1 ± 0.2
Turbidity (NTU)	166.0	89.8	74.5	50.6	14.0	12.2
Solubility (%)	>98	>98	>98	>98	>99	>99
MW × 10^4 (Da)	83.0	105.3	99.0	139.7	86.2	124.8
Apparent viscosity (cP)	49	82	55	395	206	1107

See Table 4 for details of paths A and B.

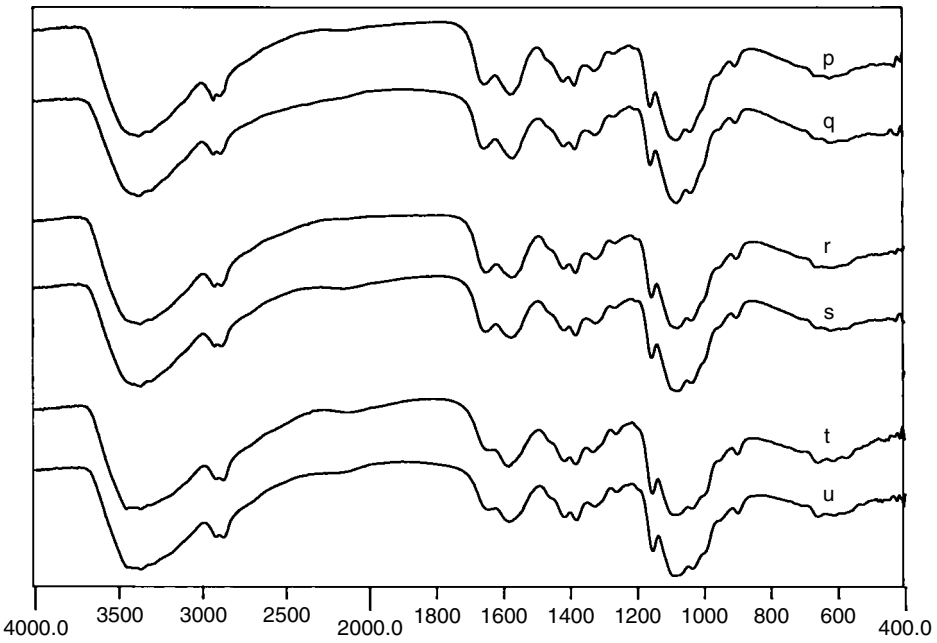

Figure 3. FT-IR spectra (4000–400 cm^{-1} region) of chitosan obtained from different processing routes (Table 4): p, shell fermentation/path A; q, shell fermentation/path B; r, head fermentation/path A; s, head fermentation/path B; t, shell chemical/path A; u, shell chemical/path B.

differences in the protein content of chitin. Solubility was high in both cases. FT-IR analysis confirmed the identity of chitosan obtained from heads and shells by fermentation (Fig 3). The fermentation samples were higher in turbidity as compared with those obtained by chemical treatment (Table 5).

The chitosan produced by path B showed a higher viscosity than that from path A. In path A the protein layer is removed by the first NaOH treatment and the protein protection is lost, exposing the mineral backbone to strong acid. The molecular weight was high for all fermented samples, but corresponding values of apparent viscosity were low. This infers that, although the glycosidic chain was long, interaction between the chitosan molecules was low in acetic acid solution. This is probably related to the lower degree of deacetylation, which allows the dissolved chitosan molecules in a hydrodynamic environment to have a more compact structure.[14] The chitosan from heads generally had a lower molecular weight and a lower apparent viscosity than that from shells.

Applications for shrimp hydrolysate

The fermentation process, in comparison with the chemical method, has the advantage of yielding a liquor rich in protein and asthaxanthin that has not been exposed to acid and alkali. It should be noted that the additional chemical treatment after fermentation mentioned above only concerns the chitin production and not the liquor fraction. The liquor from shrimp head fermentation was analysed and found to contain all the essential amino acids, qualifying it as a rich

Table 6. Characterisation of shrimp hydrolysate

Amino acid	Content (g per 100 g)			
	Shrimp protein	Beef[c]	Milk powder (low fat)[c]	Egg (whole)[c]
Threonine[a]	3.40 ± 0.01	0.95	1.52	0.36
Valine[a]	3.38 ± 0.05	1.18	1.61	0.48
Methionine[a]	1.86 ± 0.01	0.48	0.67	0.24
Lysine[a]	5.34 ± 0.00	1.79	2.51	0.63
Isoleucine[a]	3.42 ± 0.01	1.02	1.62	0.46
Leucine[a]	5.38 ± 0.03	1.35	2.25	0.71
Phenylalanine[a]	3.62 ± 0.01	3.69	1.23	0.40
Histidine[b]	2.29 ± 0.01	0.73	0.82	0.19
Arginine[b]	5.07 ± 0.03	1.31	1.01	0.63
Aspartic acid	6.14 ± 0.01	1.63	2.14	1.04
Serine	2.35 ± 0.01	0.70	1.54	0.60
Glutamic acid	9.97 ± 0.05	2.72	6.56	1.09
Glycine	5.23 ± 0.03	1.05	0.49	0.24
Alanine	5.14 ± 0.02	1.08	0.86	0.41
Proline	3.76 ± 0.02	0.85	3.22	0.31
Cystine	0.40 ± 0.01	0.14	0.35	0.25
Tyrosine	2.51 ± 0.02	0.61	1.18	0.29

[a] Essential amino acid.
[b] Essential amino acid for children.
[c] Ministry of Health, Nutrition Department, Thailand, 1995.

source of protein (Table 6). The hydrolysate had about 14% protein, measured by the biuret method, and a density of 1.04 g dm^{-3}. It had a dark pink colour, indicating a high amount of the shrimp pigment asthaxanthin. The shrimp hydrolysate had a nice shrimp flavour and a uniform viscosity. When dried under vacuum, the powder could become a source of protein, minerals, flavour and colour as a food supplement. It is, like other commercially available protein powders, rich in glutamate, aspartate, lysine and leucine. In addition, it resembles beef protein, which is rich in phenylalanine (Table 6). Tests are under way with the objective of standardising and applying this powder for human consumption.

In the chemical route there is no recovery of protein and minerals for human use. The fermentation technique is sustainable and outweighs the chemical route in revenues owing to the recovery of a protein-rich hydrolysate. Since 1 kg of biowaste (wb) produces about 700 ml of extract, it results, after evaporation and vacuum drying, in approximately 100 g of powder rich in proteins and minerals. If properly processed, this powder will have at least the same net economic value as the chitosan obtained from shrimp biowaste.[15] Therefore the by-product obtained in the form of a protein/mineral liquor gives the fermentation process added economic value.

CONCLUSIONS

The results show that chitin for the production of chitosan can be obtained by fermentation. The advantages over chemical extraction are no exposure of chitin to strong acids, a large reduction in chemicals needed and generation of a protein-rich liquor with high nutritional value that has potential after further treatment for the production of high-value protein powder for human consumption.

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