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Chitin Extraction from Black Tiger Shrimp (*Penaeus monodon*) Waste using Organic Acids

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Abstract: In chitin extraction from black tiger shrimp shell waste, HCl is the commonly used decalcifying agent. However, it is considered a harsh chemical. An alternate was found by using organic acids. Conditions for deproteinization were: 1 M NaOH at 95°C for 6 h and solid-to-solvent ratio of 1 : 15 (w/v). Demineralization involved treatment with 0.25 M HCl at ambient temperature for 30 min with agitation. The optimal solid-to-acid ratio was 1 : 30 (w/v) and this led to 86.5 ± 1.2% purification of chitin. With the same conditions, the optimal ratio of mixed acids (0.25 M HCOOH and 0.25 M $C_6H_8O_7$ at 1 : 2 (v/v)) was 1 : 28 (w/v) with chitin purification of 88.1 ± 1.8%.

Keywords: Chitin, depolymerization, demineralization, extraction, shrimp waste

INTRODUCTION

The black tiger shrimp industry in Thailand has risen rapidly in the last two decades. In 1994, Thailand was the world's top producer of black tiger

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shrimps, exporting 250,000 tons of the products worth US$ 1.7 billion. This equates to 25% of the world supply of pond-raised shrimp (1). However, the shrimp industry generates a significant amount of solid waste, about 70–80% of the landed catch. The waste has a potential for pollution and pose a disposal problem. The techniques available for its disposal include ocean dumping, incineration, and disposal to landfill sites (2). The waste has also been used in the production of animal feed (2).

Over the years, there have also been considerable efforts to develop techniques for the recovery and utilization of the biopolymers in the waste. Shrimp waste composes of a number of valuable chemicals such as chitin. Main sources of chitin are crustacean shell wastes from various species of crabs, shrimps, crawfish, krill, and prawns. Black tiger shrimp (*Penaeus monodon*) waste has the highest chitin content (about 40.4% dry basis (db)) (Table 1). Molecular structure of chitin is given elsewhere (7).

Recently, the commercial value of chitin has increased because of the beneficial properties of its soluble derivatives. The most useful one is chitosan. The derivatives from chitin are suitable in chemical, biotechnology, agriculture, food processing, cosmetics, veterinary, medicine, dentistry, environment protection, paper, and textile industries (7). However, the industrial production of chitin is limited due to environmental pollution caused by demineralization. Usually, demineralization is accomplished using HCl, which is not environmentally friendly. Besides, use of HCl in chitin production for pharmaceuticals and human-consuming products should be avoided.

Chitin is closely associated with proteins, inorganic material which is mainly $CaCO_3$, pigments and lipids (7). Therefore, the aim of chitin purification is to remove these impurities. Demineralization and deproteinization are most frequently carried out with HCl and NaOH treatments, respectively. However, other methods may be used, and the order of these two steps may be varied depending on the composition of the final product. In most instances, deproteinization has been carried out prior to demineralization (7).

Traditionally, shell wastes are deproteinized using aqueous solutions of NaOH or KOH. The other solutions being effective for deproteinization are

Table 1. Proximate composition on % dry basis of crustacean shell wastes (3–6)

	Chitin source	Protein	Ash	Lipids	Chitin
Crab	*Collinectes sapidus*	25.1	58.6	2.1	13.5
	Chinoecetes opilio	29.2	40.6	1.3	26.6
Shrimp	*Pandalus borealis*	41.9	34.2	5.2	17.0
	Crangon crangon	40.6	27.5	9.9	17.8
	Penaeus monodon	47.4	23.0	1.3	40.4
Crawfish	*Procamborus clarkia*	29.8	46.6	5.6	13.2
Krill	*Euphausia superba*	41.0	23.0	11.6	24.0
Prawn		61.6	29.4	1.4	33.0

Na_2CO_3, $NaHCO_3$, K_2CO_3, $Ca(OH)_2$, Na_2S, $CaHSO_3$, and Na_3PO_4 (2). The effectiveness of alkali deproteinization depends on process temperature, alkali concentration, and the ratio of its solution to the shells. Crustacean shell wastes are usually treated with dilute NaOH solution at concentrations ranging from 1% to 10% (w/v) and at an elevated temperature from 65 to 100°C. An increase in the sample-to-solution ratio above 1 : 4 (w/v) has only a minor effect on the efficiency of deproteinization (7). Reaction time usually ranges from 0.5 to 6 h. Different conditions used in deproteinization are listed in Table 2.

Demineralization can usually be achieved in 1 to 3 h extraction with dilute (1 to 8%) HCl at room temperature (8, 9). The process occurs when the acid reacts with $CaCO_3$ according to the following reaction (10):

$$CaCO_3 + 2HCl \longrightarrow CO_2^{\uparrow} + CaCl_2 + H_2O \quad (1)$$

The above process was also accomplished with 90% HCOOH, 22% HCl, 6N HCl or 37% HCl (11, 12). The HNO_3 is also used in demineralization (13). Full demineralization is possible when the amount of acid is stoichiometrically greater than the mineral content in the sample. To avoid chitin depolymerization, ethylenediamine-tetra-acetic acid (EDTA) can be used for removal of mineral salts (14, 15). The demineralization of the shells using CH_3COOH or H_2SO_4 has been also reported (11). Prolonged reaction time, up to 24 h, resulted only in a minimal drop in the ash content, but can cause chitin degradation (7). Alternative demineralization conditions in chitin extraction from various sources are listed in Table 3.

The objective of this study is to find the alternatives of chitin extraction from shell waste of black tiger shrimp and to select an optimum combination of organic acids. Additionally, amounts of acids are tried to be reduced for minimal environmental pollution.

MATERIALS AND METHODS

The experiment was divided into 2 modules. The purpose of module I was to differentiate and select the mixed acids with the highest yield of demineralization for module II. In Module I, demineralization was conducted using various organic acids. In module II, optimal amounts of HCl and mixed organic acids were obtained by varying solid-to-acid ratios from 1 : 20 to 1 : 40 (w/v). The purpose was to determine minimal amount of acids needed for demineralization.

Standard Chitin Amount

Total amount of nitrogen was measured using Automated Kjeldahl method, (#4.2.05 in (33)) by KJECTEC AUTO 1030 Analyser (Foss North America

Table 2. Conditions used for deproteinization in preparation of chitin extraction from various sea products

Source	Chemical type	Concentration	Temperature (°C)	Length of treatment (h)	Shell-alkali ratio (w/v)	Reference
Shrimp	NaOH	3.0% (w/w)	100	1	n/a	(13)
	NaOH	2.0 N	80	n/a	n/a	(16)
	NaOH	2.5 N	75	6	1 : 12.5	(17)
	NaOH	3.0%	100	1	n/a	(13)
	NaOH	1.0%	65	1	1 : 10	(18)
	NaOH	4.0%	100	1	n/a	(11)
	NaOH	1.0 N	100	1	1 : 6	(19)
	NaOH	5.0 N	100	1	n/a	(20)
	KOH	1.0%	90	2	1 : 20	(5)
Krill	$NaHCO_3$	0.02 M	90	2	n/a	(12)
	NaOH	3.5%	90–95	2	1 : 10	(21)
	NaOH	3.5%	25	2	n/a	(22)
	NaOH	4.0%	98	2.5	1 : 25	(11)
	KOH	3.0%	95	2	1 : 10	(12)

Lobster	NaOH	1.0 N	100	12 h, repeat 5 times	1 : 5.5	(23)
	NaOH	10.0%	Ambient	72	n/a	(24)
	NaOH	10.0%	100	2.5	1 : 50	(25)
	NaOH	15.0%	65	3	1 : 10	(1)
Prawn	NaOH	5.0%	Reflux	2	1 : 15-20	(26)
	NaOH	5.0%	100	0.5	1 : 1	(11)
	NaOH	0.5%	100	0.5	1 : 15	(11)
Crawfish	NaOH	3.5%	65	2	1 : 10	(9)
	NaOH	15.0%	65	3	1 : 10	(26)
Crab	NaOH	1.0 N	80	3 h, repeat 2 times	n/a	(27)
	KOH	2.0%	90	2	1 : 20	(5)
	NaOH	1.0 N	50	6	n/a	(28)
	NaOH	1.0 N	50	5	n/a	(29)
	NaOH	5.0%	65	1	1 : 15	(30)

Table 3. Conditions used for demineralization in preparation of chitin extraction from various sea products

Source	Chemical type	Concentration	Temperature (°C)	Length of treatment (h)	Shell-acid ratio (w/v)	Reference
Shrimp	HCl	1.0 N	Ambient	0.5	n/a	(13)
	HCl	2.0 N	Ambient	2	n/a	(16)
	HCl	1.7 N	Ambient	6	1 : 9	(17)
	HCl	0.5 N	Ambient	n/a	1 : 11	(18)
	HCl	2.5%	20	1	1 : 10	(5)
	HCl	5.0%	Ambient	n/a	n/a	(11)
	HCl	8.0%	30	8	1 : 10	(31)
	HCl	0.75 N	Ambient	0.5	1 : 12	(19)
	HCl	1.25 N	Ambient	0.5	1 : 12	(19)
	CH_3COOH	1.75 N	25	12	1 : 15	(32)
	HCl	6.0 N	Ambient	1	n/a	(20)
Krill	HCl	22.0%	20	2	n/a	(12)
	HCl	0.6 N	Ambient	2	1 : 22	(21)
	HCl	3.5%	20	1.5	1 : 4	(22)
	HCl	2.0 N	15–25	0.5	1 : 25	(11)

Lobster	HCl	2.0 N	Ambient	5	1 : 9	(23)
	HCl	37.0%	−20	4	n/a	(24)
	CH_2O_2	90.0%	Ambient	18	1 : 10	(25)
	HCl	1.0 N	Ambient	2	1 : 15	(26)
Prawn	HCl	5.0%	Ambient	2	1 : 15–20	(11)
	HCl	5.0%	Ambient	1	1 : 2	(11)
	HCl	1.25 N	Ambient	1	n/a	(11)
	HCl	1.0 N	Ambient	2	1 : 15	(26)
Crawfish	HCl	1.0 N	Ambient	0.5	1 : 15	(9)
	HCl	1.0 N	Ambient	2	1 : 15	(26)
Crab	HCl	1.0 N	Ambient	12 h, repeat 2 times	n/a	(27)
	HCl	2.5%	20	1	1 : 10	(5)
	HCl	1.0 N	20	3	n/a	(28)
	HCl	0.1 N	Ambient	n/a	Excess	(29)
	HCl	1.0 N	Ambient	0.5	1 : 15	(30)

Inc., Brampton, Canada). Chitin nitrogen was determined according to the method of Black and Schwartz 1950 (34). Then the total amount of protein from shrimp shell was estimated. Calcium carbonate ($CaCO_3$) and the other mineral components were measured by slowly heating a sample to 600°C for 2 h and weighing the remaining product after cooling in a desiccator (method #4.1.10 in (33)). Lipid content was measured by Pet-Ether extraction using ANKOM Fat Analyzer (ANKOM Technology, Toronto, Canada), (method #4.5.01 in (33)).

Sample Collection

Black tiger shrimps were bought from the local supermarket, then peeled and dewatered. The wet shell materials (body, leg, and tail) at about 88% moisture content wet basis (wb) were then ground into finer pieces, packed in plastic containers, and kept frozen at −20°C. Ten replications were used for chemical analysis and data are given in Table 4.

Deproteinization

The sample was mixed in a beaker (1 liter size) with 1 M NaOH at 1 : 15 (w/v, db) and put in a waterbath (Ecoline 019, LAUDA, Germany) at 95°C for 6 h. The sample was removed from the waterbath and filtered using a filter paper (Whatman #1, Fisher Scientific). The sample was then washed with deionized water up to the neutral pH. The filter paper and collected material were dried in an oven (Isotemp 285A, Fisher Scientific) at 105°C for 24 h. Then a deproteinized sample was collected from the filter paper.

Module I: Demineralization

Demineralization was done by mixing deproteinized sample with 0.25 M acid at 1 : 40 (w/v) ratio and agitating using a magnetic stirrer (11-500-49S Fisher

Table 4. Proximate composition on % dry basis of black tiger shrimp shells[a]

Composition	Amount (%)
Protein	42.80 ± 0.99
Ash	31.20 ± 0.84
Lipids	0.27 ± 0.07
Chitin	25.73 ± 0.77

[a]Mean ± S.D. of ten determinations.

Scientific) for 30 min at ambient temperature. List of acids and mixed acids used in the experiments are shown in Table 5.

The sample was filtered using a filter paper (Whatman #1) and washed with deionized water up to the neutral pH. The filter paper and collected material were then dried in the oven at 105°C for 24 h. The residue, containing mainly chitin, was collected. The mixed acids with the highest extraction yield were selected for module II.

Module II: Demineralization

Demineralization was done using 0.25 M HCl and mixed organic acids were selected from module I. The cycle was completed in 30 min at ambient temperature. Agitation was also applied by using a magnetic stirrer (Fisher Scientific). However, ratio between sample and acid was no longer fixed but varied from 1 : 20 to 1 : 40 (w/v) in step of 4. Additional experiments were conducted to get optimal acid-to-solid ratio, by increasing the ratio by an increment of 2. After demineralization, the residue was filtered and dried in the oven at 105°C for 24 h.

Design of Experiment and Data Analysis

To obtain chitin amount, protein, ash, and lipid contents of samples were determined. Ten replicates of each treatment were conducted for this analysis. In module I, the fixed variables during demineralization were extraction time, temperature, acid concentration and solid-to-acid ratio. The

Table 5. List of acids and mixed acids used in experiments

#	Acid name and composition
1	0.25 M HCl
2	0.25 M CH_3COOH
3	0.25 M HCOOH
4	0.25 M $C_6H_8O_7$
5	0.25 M CH_3COOH and 0.25 M HCOOH at 1 : 1 (v/v)
6	0.25 M CH_3COOH and 0.25 M HCOOH at 1 : 2 (v/v)
7	0.25 M CH_3COOH and 0.25 M HCOOH at 2 : 1 (v/v)
8	0.25 M CH_3COOH and 0.25 M $C_6H_8O_7$ at 1 : 1 (v/v)
9	0.25 M CH_3COOH and 0.25 M $C_6H_8O_7$ at 1 : 2 (v/v)
10	0.25 M CH_3COOH and 0.25 M $C_6H_8O_7$ at 2 : 1 (v/v)
11	0.25 M HCOOH and 0.25 M $C_6H_8O_7$ at 1 : 1 (v/v)
12	0.25 M HCOOH and 0.25 M $C_6H_8O_7$ at 1 : 2 (v/v)
13	0.25 M HCOOH and 0.25 M $C_6H_8O_7$ at 2 : 1 (v/v)

variables varied were types of acids and their mix ratios. To compare extraction efficiency of the organic acids with HCl (standard acid), two-way analysis of variance (ANOVA) concerning types and mix ratios could not be applied because HCl was not mixed with the organic acids. Consequently, results from 13 different types of acids and mixed acids were analyzed by one-way ANOVA. Three replicates were conducted for each treatment.

Demineralization using HCl was conducted first. Then other treatments were conducted after complete randomization. Data were analyzed by ANOVA. For module I, one-way CRD (Completely Randomized Design) and Duncan's multiple-range test were used to choose the acid or mixed acid providing the highest extraction yield for module II. Differences were considered significant at $P < 0.05$ ($\alpha = 0.05$).

In module II, the fixed variables were temperature and acid concentration. Data from HCl and mixed organic acids were analyzed following the same procedure as that for module I. Then two-way ANOVA factorial design and least significant difference (LSD) test were applied to observe the relationship between type of acid and solid-to-acid ratio. The results of demineralization by HCl and by mixed organic acids were compared and considered significantly different at $P < 0.05$ using statistical analysis system (SAS) (35).

RESULTS AND DISCUSSION

Black tiger shrimp shells contain about $42.80 \pm 0.99\%$ protein and $31.20 \pm 0.84\%$ ash (Table 4). Deproteinization and demineralization are therefore the critical steps of chitin extraction.

HCl is considered a harsh chemical and may be the cause of detrimental effects on the intrinsic properties of the purified chitin. Generally, concentration of HCl used in demineralization was 1 M or higher (Table 3). To minimize HCl amount, this study used it at 0.25 M, which is below all used concentrations except one. The concentration of organic acids and their mixed acids used in demineralization were all equal to 0.25 M, which is the same level as that of HCl. The demineralization yields are given in Table 6.

The mixed acids (HCOOH and $C_6H_8O_7$) at 1 : 2, 1 : 1, and 2 : 1 (v/v) are the top three alternatives providing higher extraction yields. In addition, such yields are not significantly different comparing to that of HCl treatment (control). The mixed acids of HCOOH and $C_6H_8O_7$ (1 : 2 v/v), providing the highest yield, was then chosen for module II work. The aim of module II was to find the optimal volume of acids required to demineralize ash, mainly $CaCO_3$. Comparison based on extraction yield between HCl and mixed acids (0.25 M HCOOH and 0.25 M $C_6H_8O_7$ at 1 : 2 (v/v)) was investigated. Deproteinization was based on the same procedure as that in module I, i.e. the samples were mixed with 1 M NaOH at 1 : 15 (w/v) and heated in the waterbath at 95°C for 6 h. As shown in Fig. 1 for demineralization, the amounts of acids used were not fixed at 1 : 40. The ratio between sample

Table 6. Percentage of deproteinization by 1 M NaOH, demineralization by 0.25 M HCl, 0.25 M CH_3COOH, 0.25 M HCOOH, 0.25 M $C_6H_8O_7$, and their mixed acids at different ratios and total purification of chitin*

Acid	Ratio (v/v)	Deproteinization		Demineralization		Purification of chitin (%)**	% Demineralization/ % total extraction
		Extraction (%)	Yield (%)**	Extraction (%)	Yield (%)**		
HCl	—	38.6 ± 0.9	90.2 ± 2.2	28.8 ± 1.7	93.8 ± 2.3	90.8 ± 1.2	42.7 ± 2.0^{ab}
CH_3COOH	—	38.8 ± 0.8	90.6 ± 1.9	19.2 ± 0.9	61.4 ± 3.0	78.0 ± 0.8	33.1 ± 1.4^{e}
HCOOH	—	38.3 ± 2.3	89.5 ± 5.3	25.6 ± 0.3	82.2 ± 0.8	86.1 ± 2.7	40.1 ± 1.6^{c}
$C_6H_8O_7$	—	39.0 ± 0.8	91.2 ± 1.8	26.8 ± 2.3	85.8 ± 7.3	88.6 ± 4.1	40.6 ± 1.6^{bc}
$CH_3COOH + HCOOH$	1 : 1	37.9 ± 1.9	88.7 ± 4.4	22.6 ± 0.3	72.5 ± 1.0	81.5 ± 2.2	37.4 ± 1.5^{d}
$CH_3COOH + HCOOH$	1 : 2	36.5 ± 0.7	85.4 ± 1.7	23.9 ± 0.4	76.7 ± 1.2	81.4 ± 1.3	39.6 ± 0.5^{c}
$CH_3COOH + HCOOH$	2 : 1	36.7 ± 0.4	85.8 ± 1.0	21.1 ± 0.5	67.8 ± 1.6	77.9 ± 0.5	36.5 ± 0.8^{d}
$CH_3COOH + C_6H_8O_7$	1 : 1	39.8 ± 0.6	93.0 ± 1.5	26.9 ± 0.4	86.3 ± 1.4	89.9 ± 1.3	40.4 ± 0.3^{c}
$CH_3COOH + C_6H_8O_7$	1 : 2	37.5 ± 1.5	87.5 ± 3.5	28.1 ± 1.4	90.0 ± 4.6	88.3 ± 1.4	42.9 ± 2.1^{ab}
$CH_3COOH + C_6H_8O_7$	2 : 1	38.6 ± 0.5	90.2 ± 1.1	26.9 ± 0.3	86.1 ± 0.8	88.1 ± 0.8	41.0 ± 0.3^{abc}
$HCOOH + C_6H_8O_7$	1 : 1	37.4 ± 1.6	87.3 ± 3.7	28.3 ± 0.2	90.6 ± 0.8	88.4 ± 2.2	43.1 ± 1.0^{a}
$HCOOH + C_6H_8O_7$	1 : 2	37.7 ± 1.3	88.1 ± 3.0	28.9 ± 0.4	92.5 ± 1.2	89.7 ± 1.8	43.4 ± 0.9^{a}
$HCOOH + C_6H_8O_7$	2 : 1	37.3 ± 1.3	87.0 ± 3.0	28.2 ± 0.2	90.3 ± 0.7	88.1 ± 1.6	43.1 ± 0.9^{a}

*Mean ± S.D. of three replications.

**Data based on the results from Table 4.

The means with the same letters in a column are not statistically different ($P > 0.05$).

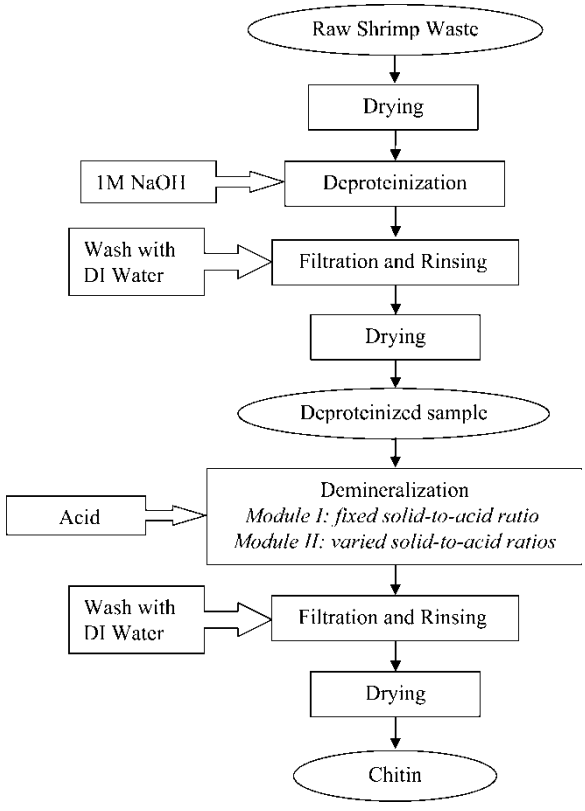

Figure 1. Procedure schematic of chitin extraction from black tiger shrimp shell waste (modules I and II). DI = deionized.

and acid was varied from 1 : 20 to 1 : 40 in step of 4. Then after closer observation the ratio was varied in step of 2 to obtain optimal ratio.

On HCl extraction (Fig. 2), further analysis was conducted at 1 : 30 (w/v) ratio. The yield at 1 : 30 represents the optimal ratio where 0.25 M HCl demineralized 88.1% $CaCO_3$ and the purification, with proper deproteinization, was achieved at 86.5% (Table 7). At this point, it is assumed that the acid was almost used and, therefore, there was very little excess acid left in the solution. Above 1 : 30 ratio, the yields are not statistically different (Table 7).

The mixed organic acids ratios lead to better demineralization yield than that by HCl at ratios below 1 : 32 (Figure 2). However, HCl gave slightly higher yields above this ratio. The volume of mixed organic acids is optimal at ratio 1 : 20 (w/v), which is lower than the optimal ratio by HCl treatment. Further studies on demineralization by mixed organic acids were repeated at ratios 1 : 16, 1 : 22 and 1 : 26 (w/v). Increasing ratio above 1 : 20 (w/v) provided fluctuating yields which are insignificantly different

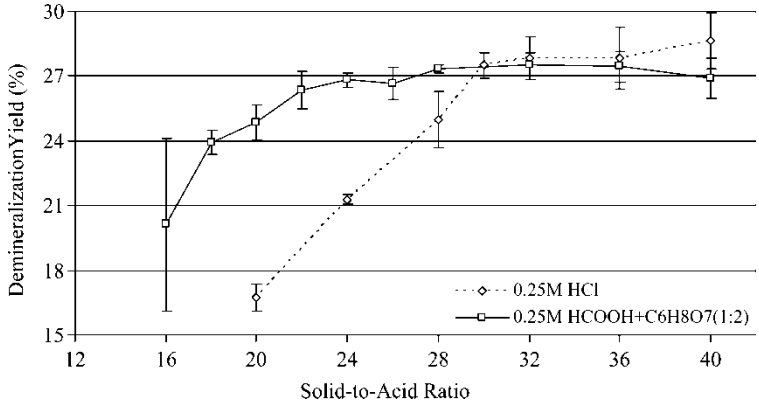

Figure 2. Comparison between demineralization (extraction %) with 0.25 M HCl and 0.25 M HCOOH + 0.25 M $C_6H_8O_7$ (1 : 2 v/v) at various solid-to-acid ratios.

(Table 8). The higher yields by the mixed organic acids at low ratios (Fig. 2) can be explained based on the chemical reaction between acids and $CaCO_3$. The reaction is mainly driven by the amount of H^+ ion. According to the chemical structures, one molecule of $C_6H_8O_7$ gives $3H^+$ while HCl, HCOOH and CH_3COOH give only one ion of H^+ (36). In addition, concentration of acids in this study is based on "Molarity", which accounts for the amount of acid molecules in 1 litre of solution. Based on this unit, $C_6H_8O_7$ is advantageous upon any other acids in this study. However, the extraction trends would be different if "Normality" was used where acid concentration is no longer based on the amount of molecules but the amount of H^+ ions.

Tables 7 and 8 show that 0.25 M HCl is to be optimal at 1 : 30 (w/v) ratio while the optimal ratio of mixed organic acids is 1 : 20 (w/v). The data were further analyzed to study the relationship between the type of acid and its solid-to-acid ratio required (Table 9). When the yields of extraction from two demineralizing agents are compared, the amount of mixed organic acids needed to equalize the highest demineralization level of HCl is 1 : 28 (w/v). Purification of chitin using mixed organic acids at 1 : 28 (w/v) ratio is 88.1%. The yield is comparable to the previous work (37), where chitin yield was 88.0%. Chitin was twice deproteinized using NaOH at 0.81 M and 1.41 M at 80–85°C, and each cycle took 30 min. The sample was demineralized using 1.63 M HCl at 70°C for 2 h. The process was continuous in a stirring tank at 1 : 5.6 solid-to-solution ratio (w/v). When compared to the previous work, demineralization in this study required lower acid concentration (0.25 M acids), energy (ambient temperature) and process time (30 min duration).

The yield obtained in this study is low compared to the extraction elsewhere (38) where chitin purification was 97.2%. Deproteinization was accomplished using 1 M NaOH with 1 : 10 (w/v) solid-to-solution ratio after

Table 7. Percentage of deproteinization by 1 M NaOH, demineralization by 0.25 M HCl at different solid-to-acid ratios and total purification of chitin*.

Solid-to-acid ratio	Deproteinization		Demineralization		Purification of chitin (%)**	% Demineralization/ % total extraction
	Extraction (%)	Yield (%)**	Extraction (%)	Yield (%)**		
1 : 20	37.8 ± 1.0	88.4 ± 2.4	16.7 ± 0.6	53.6 ± 2.0	73.5 ± 0.8	30.7 ± 1.3^{d}
1 : 24	37.1 ± 0.7	86.7 ± 1.6	21.3 ± 0.2	68.2 ± 0.7	78.6 ± 0.9	36.4 ± 0.5^{c}
1 : 28	37.3 ± 1.2	87.2 ± 2.7	25.0 ± 1.3	80.0 ± 4.1	83.9 ± 0.2	40.1 ± 2.0^{b}
1 : 30	36.8 ± 0.3	85.9 ± 0.7	27.5 ± 0.6	88.1 ± 2.0	86.5 ± 1.2	42.8 ± 0.3^{a}
1 : 32	37.3 ± 0.8	87.2 ± 1.8	27.8 ± 1.0	89.2 ± 3.2	87.7 ± 0.5	42.7 ± 1.4^{a}
1 : 36	37.5 ± 0.7	87.5 ± 1.6	27.8 ± 1.4	89.2 ± 4.6	87.9 ± 1.2	42.6 ± 1.7^{a}
1 : 40	37.1 ± 1.0	86.7 ± 2.3	28.6 ± 1.3	91.8 ± 4.2	88.5 ± 1.7	43.5 ± 1.5^{a}

*Mean ± S.D. of three replications.

**Data based on the results from Table 4.

The means with the same letters in a column are not statistically different ($P > 0.05$).

Table 8. Percentage of deproteinization by 1 M NaOH, demineralization by 0.25 M HCOOH + 0.25 M $C_6H_8O_7$ (1 : 2 v/v) at different solid-to-acid ratios and total purification of chitin*

Solid-to-acid ratio	Deproteinization		Demineralization		Purification of chitin (%)**	% Demineralization/ % total extraction
	Extraction (%)	Yield (%)**	Extraction (%)	Yield (%)**		
1 : 16	38.6 ± 0.6	90.1 ± 1.4	20.1 ± 4.0	64.5 ± 12.9	79.0 ± 5.4	34.1 ± 4.7^{c}
1 : 18	38.3 ± 0.4	89.5 ± 0.9	23.9 ± 0.6	76.7 ± 1.8	83.8 ± 0.3	38.5 ± 0.8^{b}
1 : 20	38.4 ± 0.4	89.7 ± 1.0	24.8 ± 0.8	79.6 ± 2.6	85.2 ± 1.2	39.3 ± 0.9^{ab}
1 : 22	38.6 ± 0.8	90.4 ± 1.8	26.3 ± 2.9	84.4 ± 2.8	87.0 ± 1.3	41.0 ± 1.3^{ab}
1 : 24	39.1 ± 2.1	91.4 ± 5.0	26.8 ± 0.3	86.0 ± 1.0	88.8 ± 2.6	40.7 ± 1.6^{ab}
1 : 26	37.4 ± 1.2	87.4 ± 2.9	26.6 ± 0.8	85.4 ± 2.4	86.8 ± 2.3	41.3 ± 1.0^{a}
1 : 28	38.1 ± 1.2	89.1 ± 2.8	27.3 ± 0.2	87.5 ± 0.6	88.1 ± 1.8	41.7 ± 0.6^{a}
1 : 32	37.6 ± 1.0	87.8 ± 2.3	27.5 ± 0.5	88.3 ± 1.7	87.7 ± 1.4	42.3 ± 0.9^{a}
1 : 36	38.0 ± 0.5	88.8 ± 1.1	27.4 ± 0.7	87.9 ± 2.4	88.8 ± 1.6	41.9 ± 0.4^{a}
1 : 40	37.6 ± 0.5	87.9 ± 1.1	26.9 ± 0.9	86.3 ± 3.0	86.9 ± 1.8	41.7 ± 0.6^{a}

*Mean ± S.D. of three replications.

**Data based on the results from Table 4.

The means with the same letters in a column are not statistically different ($P > 0.05$).

Table 9. Comparison of demineralization percentage divided by total extraction percentage for 0.25 M HCl and 0.25 M HCOOH + 0.25 M $C_6H_8O_7$ (1 : 2 v/v) at different ratios*

	% Demineralization/% total extraction	
Solid-to-acid ratio	0.25 M HCl	0.25 M HCOOH + 0.25 M $C_6H_8O_7$ (1 : 2 v/v)
1 : 20	30.7 ± 1.3^{a}	39.3 ± 0.9^{ce}
1 : 24	36.4 ± 0.5^{b}	40.7 ± 1.6^{cef}
1 : 28	40.1 ± 2.0^{c}	41.7 ± 0.6^{cdf}
1 : 30	42.8 ± 0.3^{d}	—
1 : 32	42.7 ± 1.4^{d}	42.3 ± 0.9^{df}
1 : 36	42.6 ± 1.7^{df}	41.9 ± 0.4^{cdf}
1 : 40	43.5 ± 1.5^{d}	41.7 ± 0.6^{cdf}

*Mean ± S.D. of three replications.

The means with the same letters in a column are not statistically different ($P > 0.05$).

24 h at ambient temperature. The sample was demineralized using 1.1 M HCl with 1 : 17.5 (w/v) solid-to-acid ratio at ambient temperature for 12 h. However, demineralization using high acid concentration in long period of time could cause depolymerization where physical property of chitin is degraded (39).

The proposed procedure can be applied on a larger scale and will lower amounts of acid needed in the process. The organic acids used in this research are widely used in various food industries and are food grade compared to HCl. Thus the alternatives presented could be highly beneficial when chitin is required for pharmaceutical or human-consuming products, i.e. glucosamine supplement, cosmetics, dentistry, and eye dressing.

CONCLUSIONS

In chitin extraction from shell waste of black tiger shrimp, HCl was replaced by CH_3COOH, HCOOH, $C_6H_8O_7$ and their combinations. Among the organic acids used, the mixture of 0.25 M HCOOH and 0.25 M $C_6H_8O_7$ at 1 : 2 (v/v) provided the highest yield of extraction. The optimal solid-to-acid ratio is 1 : 28 (w/v). The replacement using the mixed organic acids indicated satisfactory extraction yield. The optimal selection provided $87.5 \pm 0.6\%$ during demineralization and $88.1 \pm 1.8\%$ purification of crude chitin after deproteinization. Additionally, $C_6H_8O_7$ is the key alternative in demineralization. When $C_6H_8O_7$ mixed with CH_3COOH or HCOOH

at different ratios, the demineralization yields reached acceptable level comparing to that by HCl.

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