Chitin Sponge, Extraction Procedure from Shrimp Wastes Using Green Chemistry

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ABSTRACT: Chitin sponges were obtained from shrimp residues using green chemistry procedures. Partial deproteinization was performed by grinding fresh residues with water during 30 s in a food blender obtaining 39.3% d.b. partial deproteinized cephalothorax (PDC). Protein reduction in this step was 80%, with 49% of mineral salts (measured as ashes), 97% fat, and 41% being chitin product. To separate chitin from PDC, a mixture of 8.75 mL MeOH, 16.25 mL water, and 25 g of $CaCl_2$ was used (solvent MAC) per gram of PDC. Chitin dissolved in MAC under mechanical agitation to form a sponge, which had an overall composition of 42% chitin, 46% ashes, and 11% proteins. Chitin sponge weathering and biodegradation tests showed that during 30 days, the sponge is stable at ambient conditions in southern Mexico City environment, and when placed in compost-soil mixtures is degraded in about two weeks. These results open new possibilities to the recycling of crustacean residues. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 3909–3916, 2007

Key words: chitin; foams; shrimp residues; protein; biopolymer

INTRODUCTION

Chitin, poly(*N*-acetyl-D-glucosamine), has become an interesting biopolymer for its abundance, biodegradability, and non toxicity.[1,2] Chitin is insoluble in water and in most organic solvents because of strong hydrogen bonding present between the polymer chains. One of the main sources to obtain chitin is the exoskeletons of crustaceans. These residues are mainly composed of chitin, proteins, and carbonates. Conventionally chitin separation procedures are based on the use of acids and alkalis to remove minerals and proteins, respectively.[3] However, acid treatment reduces the molecular weight of the resulting biopolymer product and the use of alkali produces deacetylations, which change the material properties.[3]

Porous biodegradable materials have a great demand for various applications such as drugs delivery systems, as support for enzymes immobilization, molecular fractioning, and transition metals absorption. These materials have been also widely explored in tissue engineering.[2,4]

Chitosan, the chitin deacetylated form, forms gels in dilute acetic acid. These gels, when lyophilized, produce chitosan sponges, which present applications in the biomedical field.[5] Chitin may be dissolved in mixtures of *N,N*-dimethylacetamide-5% LiCl (DMAc/5%LiCl) forming gels that coagulate with water.[6] Chitin sponges from these lyophilized gels are hazardous for human health because of solvent toxicity.

A less toxic solvent is the mixture of calcium chloride in methanol.[7] Chitin gels are formed when dissolved chitin coagulates in the presence of water. In situ preparation of calcium chloride can be done by adding HCl to the exoskeleton of crabs after extraction of proteins with alkali.[8]

Another proposed method to regenerate chitin is through chitosan lyophilization and further acetylation with acetic anhydride.[9] This indirect procedure can take long process time because it requires a previous step of chitin deacetylation to form chitosan, which might produce chain length reduction.

Chitin sponge can be obtained without lyophilization by submersion in an HCl solution of chitin gels, previously produced dissolving chitin in DMAc/5%LiCl, mixed with calcium carbonate.[4]

This research was focused on the extraction of chitin from crustacean residues using less aggressive chemical methods. The consideration of the experiments described in the previous paragraphs led to the use of a mixture of calcium chloride with less methanol and the addition of water to extract chitin. Also, the sponge formation can be done without lyophilization. Once the sponges were formed, their

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stability was assessed by weathering tests. Finally, biodegradability experiments on the chitin films were carried out and are described herein.

EXPERIMENTAL

Reagents

All reagents used were analytical grade (J. T. Baker, Mexico) with the exception of anhydrous calcium chloride, which was commercial grade, and it was used as dehumidifying agent (*Cero humedad*, Industrias Ralc, Mexico). Calcium chloride was dissolved in distilled water, filtered, recrystallized, and dried in an oven at 160°C during 5 h.

FTIR measurements

For the FTIR measurements, KBr pellets samples were prepared and predried at 100°C during 2 h. Spectra were obtained in a Nicolet equipment (Nicolet Instruments, Madison, WI, USA), model Impact 410, using a computer with the OMNIC-3.1a software, in frequency range of 4000–400 cm^{-1}. All spectra were recollected by accumulation of 64 scans with a resolution of 2 cm^{-1}.

Crustacean residues

Fresh shrimp cephalothorax was obtained from the central seafood market in Mexico City.

Characterization

The product characterization was performed following standard methodologies.[10] Chitin was quantified according to Black and Schwartz methodology.[11]

TABLE I
Factorial Design for Shrimp Powder Deproteinization with SDS

Variables in original units		Variables in codified units	
SDS (%) (w/w)[a]	Sodium carbonate (%) (w/w)[a]	X_1	X_2
0	0	−1	−1
0	0.1	−1	1
2	0	1	−1
2	0.1	1	1
1	0.05	0	0
1	0.05	0	0

[a] With respect to total dry weight: shrimp powder + SDS + sodium carbonate.

TABLE II
Factorial Design for Shrimp Powder Deproteinization with Vegetable Saponified Oil

Variables in original units		Variables in codified units	
Time (h)	SAP (%) (w/w)[a]	X_1	X_2
1	0	−1	−1
1	2	−1	1
2	0	1	−1
2	2	1	1
1.5	1	0	0
1.5	1	0	0

[a] With respect to total dry weight: shrimp powder + SAP (vegetable saponified oil).

Shrimp cephalothorax powder

Washed shrimp cephalothorax was dried at 60°C for 18 h and after it was ground in a cereal mill to about 149 μm and it was stored at 4°C.

Digestion solutions

Stock solutions were prepared with 1% (w/v) sodium dodecylsulfate (SDS), with 0.25% (w/v) sodium carbonate, and 1% (w/v) saponified vegetable oil (SAP). This saponified vegetable oil was prepared by reflux of 0.88 g commercial corn oil (human consumption quality), saponification index of 192, with 0.12 g NaOH, and 5 mL distilled water, and completed to 100 mL with distilled water. These digestion solutions were tested at different ratios (Tables I–III).

Deproteinization

Open reflux deproteinization

Table I shows the digestion conditions with SDS for 0.5 g of shrimp powder with 10 mL of digestion solution for a period of 1–2 h. The product obtained was filtered, washed, and dried, measuring deproteinization percentages as weight losses. For SAP digestion, the same procedure was followed, utilizing the concentrations and times listed in Table II.

Deproteinization using a microwave digestor

A Berghof Microwave Digestor MWS-1 was used, with six teflon digestion 30 mL vessels (Berghof Laborprodukte GmbH, Eningen, Germany). For the SDS digestion, 0.5 g of shrimp powder were weighed and introduced to the microwave digestor vessel, adding 10 mL of the digestion solution as indicated in Table I. Digestion conditions were 180°C during 10 and 30 min, in three stages: The

TABLE III
Factorial Design for Shrimp Powder Deproteinization with Vegetable Saponified Oil and Microwave Radiation

Variables in original units		Variables in codified units	
Time (min)	SAP (%) (w/w)[a]	X_1	X_2
10	0	−1	−1
10	2	−1	1
15	0	1	−1
15	2	1	1
12.5	1	0	0
12.5	1	0	0

[a] With respect to total dry weight.

first one the samples were kept 5 min with a heating power level of 80% to bring conditions to 180°C; the second one keeps the samples at 180°C during the programmed lapse of time with a heating power level of 80%; and the third stage is for cooling, during 10 min with a heating power level of 10% reducing the temperature to 100°C. Once the digestion is finished the product is recovered, filtered (using glass microfibre filters Whatman GF/A), washed (using 100 mL distilled water in five 20 mL portions), and dried (in oven at 100°C for 2 h), determining percentage of deproteinization as weight losses. For the SAP digestion, the procedure is similar, according to the conditions established in Table III at 180°C.

Deproteinization of fresh shrimp residues

The blending of 100 g of clean fresh shrimp wastes was carried out in a domestic *Osterizer* blender (Osterizer, USA), with an undetermined constant rotational speed using different shrimp : distilled water ratios (0, 200, 500 y, 1000 mL) for 30 s. Water was separated by filtration using a 1 mm mesh sieve, and the solid mass was blended a second time using the same shrimp : water ratio for other 30 s. Filtered solid material was dried in a lab oven at 60°C during 18 h.[1] Dried material was weighed to calculate the deproteinization as the weight loss (in dry basis).

Mixture methanol-water-calcium chloride

Tables IV and V present the methanol-water-calcium chloride (Solvent MAC) ratios tested. They were based in mixtures of 10 g anhydrous calcium chloride with distilled water and methanol using moderate heat at reflux to complete dissolution of the sample.

[1]Low temperature drying is recommended to avoid chitin molecules degradation.

TABLE IV
Methanol-Water-Calcium Chloride (Solvent MAC) Tested Mixtures

$CaCl_2$ (g)	MeOH (mL)	H_2O (mL)
10	5	5
10	3.5	6.5
10	2.5	7.5
10	1.5	8.5
10	0	10

Chitin extraction

Solvent selection

Partially deproteinized chitin (0.4 g) were mixed with 10 mL of a solution of calcium chloride in methanol-water at different ratios in a 125 mL Erlenmeyer flask, (Table IV). The Erlenmeyer flask outlet was hermetically closed with *Parafilm* (Pechiney Plastic Packaging, Menasha, WI, USA), to avoid both methanol losses and ambient moisture contamination. The mixture was maintained with magnetically stirred using during 24 h at ambient temperature to dissolve chitin. The following step was the filtration using a commercial polymeric tissue (*Magitel*, Colgate-Palmolive, Mexico) to remove solid particles. The solution is use for forming films and sponges.

Film formation

To precipitate dissolved chitin, 50 mL cold distilled water (7°C) were added to the solution. Using a *Millipore* membrane filter 0.45 μm (Millipore, Bedford, MA. USA), chitin was vacuum filtered and washed with an additional 50 mL of distilled water. It was then dried at 60°C for 2 h. The film was weighed to calculate chitin film yield from the 0.4 g partially deproteinized chitin, PDC.

TABLE V
Second Order Factorial Design to Obtain the Contour Graphs for the Methanol-Water-Calcium Chloride (Solvent MAC)

	Variables in original units		Variables in codified units	
$CaCl_2$ (g)	MeOH (mL)	H_2O (mL)	X_1	X_2
10	3.0	6.0	−1	−1
10	3.0	7.0	−1	+1
10	4.0	6.0	+1	−1
10	4.0	7.0	+1	+1
10	3.5	6.5	0	0
10	3.5	7.2	0	1.414
10	4.2	6.5	1.414	0
10	3.5	5.8	0	−1.414
10	2.8	6.5	−1.414	0

Solvent ratios effect on chitin extraction

In a 40 mL vial with hermetic lid, 0.5 g de PDC and 10 mL of the different MAC solutions tested (Table V) were covered, homogenized, and left resting during three days. After this period, contents were filtered using a lab filter press (Shop machined, Mexico DF) to remove solid particles. To precipitate chitin and prepare films, the same procedure described in the previous paragraph was followed.

Chitin sponge formation

Foams are formed by manual agitation of chitin solution using a glass stick. Foams (10 g) were placed in small Petri dishes (55 mm) and stored in a humidification chamber[2] (with a relative humidity contents higher than 90%) to promote the ambient moisture absorption by $CaCl_2$ because of its hygroscopicity, allowing the chitin precipitation as a sponge. The remaining excess liquor was extracted after 24 h using a micropipette leaving the chitin sponge in the Petri dish.

Chitin sponges weathering tests

Sponge disks were washed with distilled water to eliminate solvent residues and left to dry at room temperature to carry out the weathering tests and the biodegradation experiments. Method ASTM D 1435-85 modified by Flores[22] was employed to test weather effects on chitin sponge disks. Chitin sponge disks were placed on a flat plastic ''board'' and fixed with tacks. A polystyrene sponge disk of exactly the same size of the chitin sample was placed on a similar plastic ''board.'' Both plastic ''boards'' and their replicates were weighed, and then horizontally set face up on the roof of a building subject to day/night south Mexico City April–May environmental conditions for a period of 30 days. Twice a week the ''boards'' were weighed and weight losses were calculated.

Chitin sponges biodegradation experiments

The biodegradability of the chitin sponge disks were tested by introducing them along with polystyrene counterparts in a set of four compost containers at a 5 cm depth from top for a period of one month. Compost from domestic sources was aged for 3 months and their moisture contents were 45%. The containers were covered with aluminum foil to avoid compost moisture losses, and set in a temperature controlled room at 20°C. Once a week, the disk from one container was ''unearthed'' and photographed.

[2]Humidification chamber, kept at room temperature, is a covered glass desiccator with a thermometer coupled to the lid that instead of desiccant has distilled water.

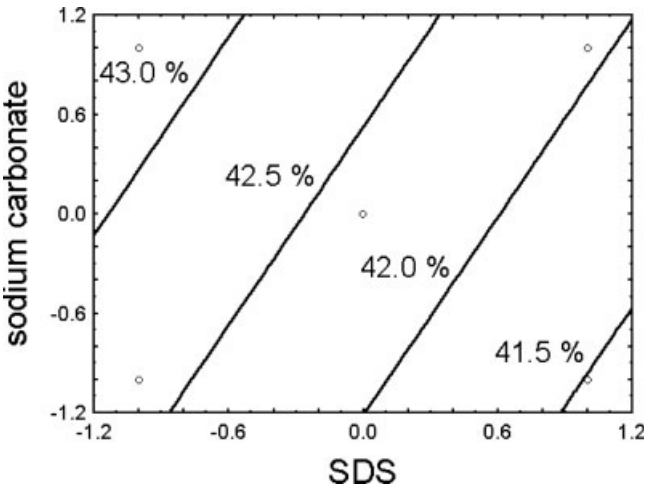

Figure 1 Contour plot for shrimp powder deproteinization with SDS and carbonate at open reflux conditions during two hours in codified units. Central point: 41.46 ± 0.02% (w/w). % Deprtn = 42.35 − 0.5725X + 0.2875Y.

RESULTS AND DISCUSSION

Reflux deproteinization

Figure 1 shows the contour lines for the 2 h digestion samples results. It can be seen that higher deproteinization is obtained when SDS concentration approaches zero and when sodium carbonate concentration increases. According to the first order equation, the negative effect (−0.57) that SDS has on the proteins extraction is twice higher than the positive effect (0.29) of the carbonates added. Deproteinization in the central point is 41.46% (w/w) with a standard deviation of 0.02. The small circles within the figure correspond to the experimental data. When digestion is carried out within 1 h, a similar behavior is appreciated but with a lower extraction percentage, being about 0.5% lower. Deproteinization with SAP (Fig. 2) had a different behavior. When open reflux is used, SAP improves deproteinization, 45% (w/w), and with SDS is 43% (w/w). According to the first order function (Fig. 2), the effect of digestion time on deproteinization is higher than with SAP concentration, 0.94 versus 0.77, respectively. Deproteinization in the central point is 44.08% (w/w) and the standard deviation is 0.18. Therefore, there is a significant difference ($P < 0.05$) between deproteinizing with SDS with respect to SAP at open reflux, favoring SAP over SDS.

Deproteinization using microwaves

Figure 3 presents the contour plot SDS results of the digestions during 30 min when microwave radia-

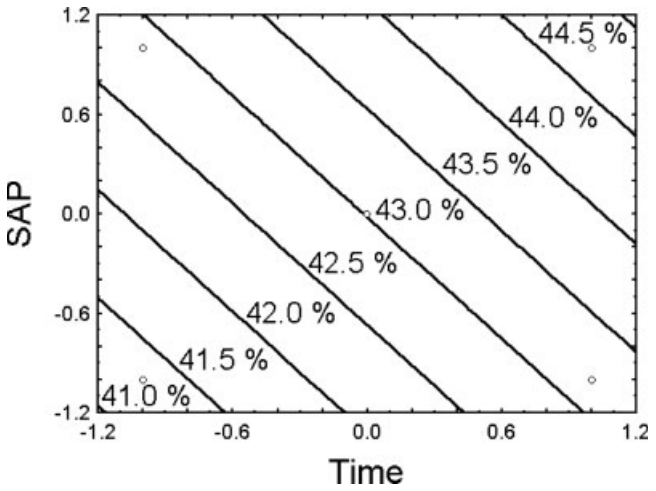

Figure 2 Contour plot for shrimp powder deproteinization with saponified vegetable oil, SAP, at open reflux conditions as a function time, in codified units. Central point: 44.08 ± 0.18% (w/w). % Deprtn = 43.0133 + 0.9375X + 0.7675Y.

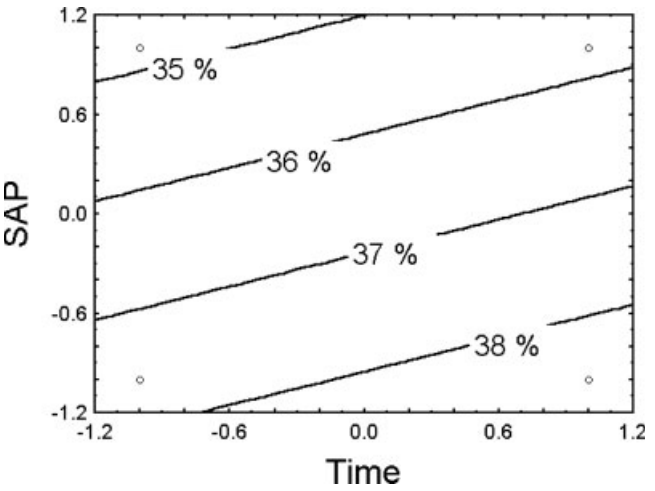

Figure 4 Contour plot for shrimp powder deproteinization with saponified vegetable oil, SAP, using microwave radiation, as a function of time in codified units. % Deprtn = 36.67 + 0.47X − 1.395Y.

tions were used. The behavior was similar to that for open reflux. According to the first order equation, the carbonates positive effect (0.30) on the deproteinization with microwave energy is as important as in the open reflux during 2 h (0.29). Deproteinization in the central point is 36.98% (w/w) with a standard deviation of 1.26. There is not a significant difference ($P < 0.05$) between digestion time of 30 min (36.98 ± 1.26) with digestion time of 10 min (35.20 ± 0.14). Considering the first order polynomial shown in Figure 4, SAP has a negative effect (−1.40) in deproteinization, that is, it does not favor the elimination of proteins and it is three times higher than the effect of the digestion time (0.47). There is a significant difference ($P < 0.05$) between deproteinizing with SAP at open reflux and with SDS in the microwave oven. Deproteinization with reflux is higher than with MW when SAP or SDS are employed.

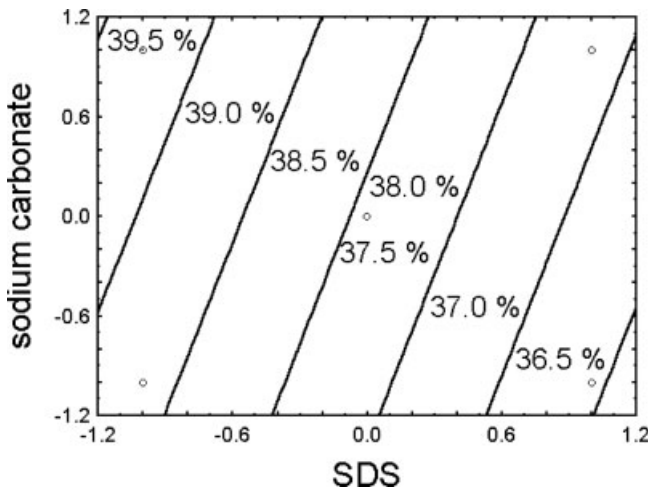

Figure 3 Contour plot for shrimp powder deproteinization with SDS and carbonate using microwave radiation during 30 min in codified units. Central point: 36.98 ± 1.26% (w/w). % Deprtn = 37.9217 − 1.045X + 0.305Y.

These results indicate that the use of surfactants (SDS and SAP) is not suitable for shrimp residues powder deproteinization, whereas it is suitable indeed with the use of open reflux or MW, with the exception of open reflux with SAP. This might be due to the fact that secondary reactions might be taking place during digestion reducing its efficiency to dissolve proteins. When MW is used, as temperature increases proportionally to pressure within the hermetically closed vessels, exists the possibility of decomposition of the reagents, as well as undesirable products formation.[12] Surfactants may also form less soluble calcium salts thus reducing its efficiency.

SAP helps shrimp residues powder deproteinization only when open reflux is used, possibly because no temperature alteration occurs. Also, it might contribute to maintain alkaline conditions. Sodium carbonate, which maintains alkaline conditions, seems to have a positive effect on deproteinization as well. Although MW energy shortens digestion time, it seems to alter surfactants characteristics reducing its efficiency.

Deproteinization of fresh shrimp residues

Deproteinization results for 100 g fresh shrimp residues with 200 mL of a SDS solution at 0.1% and its water control were 59.4 ± 1.3% and 60.7 ± 4.9%, respectively. No significant differences between both were found ($P < 0.05$). It was expected that the sur-

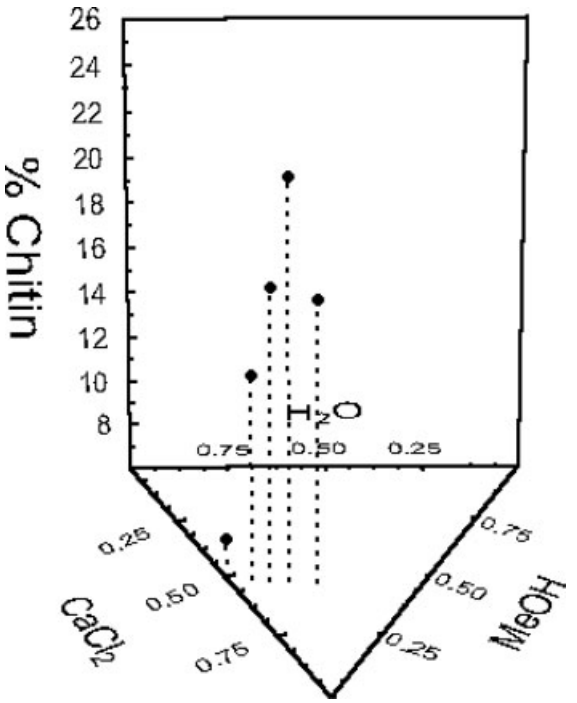

Figure 5 Chitin extraction as a function of the mixture $CaCl_2$, MeOH, and H_2O (MAC), with magnetic stirring.

factant would help to extract fats and proteins but this assumption was erroneous and no increase of fat extraction was detected. It has been reported that proteins are more easily removed from fresh cephalothorax,[13] particularly those weakly associated, with ca. two-thirds of the proteins present in the fresh shrimp residues removed in this way. Remaining proteins are those more strongly bonded to chitin, even after intensive alkaline treatment.[14,15] However, all the deproteinization options tested partially remove proteins from samples, the preferred method is its extraction from fresh shrimp residues, that is not only the easiest one but the most effective.[16] Besides, due to the mild extraction conditions applied, it is not aggressive with the chitin molecule.

Selection of the best MAC ratio

Figure 5 indicates that 25 g $CaCl_2$, 8.75 mL MeOH, and 16.25 mL H_2O/gram of partially deproteinized cephalothorax was the best solvent ratio to obtain the highest chitin extraction. It should be emphasized that when the terms chitin films and sponges are mentioned they mean compounds that have chitin and carbonate in a high proportion with some proteins and fats, and not pure chitin products (Fig. 7). An interesting feature of the proposed process is that the methanol-water-calcium chloride mixture is recyclable and can be employed to prepare more new solvent.

The hydrogen bond $O—H\ldots O$ between two glucosamine bonded units and the bond $N—H\ldots O$ of the acetylamine group and the carbonyl of the two adjacent chains confer to the α-chitin molecule a very high water insolubility.[17] Depending upon the number of hydrogen bonds, chitin will present hydrophilic or hydrophobic regions. Thus, to dissolve chitin, MAC solvent breaks these hydrogen bonds forming a soluble complex chitin-calcium salt that modifies the hydrophilic and hydrophobic regions arrangement changing the chitin crystalline structure.[18] The process is reversible because upon addition of water, calcium ions leave the chitin molecule regenerating the hydrogen bonds and precipitating it. Water replaces the MAC solvent and chitin forms a water gel. It is hypothesized that methanol helps to transport calcium chloride within the chitin structure to break the hydrogen bonds, since as chitin is highly hydrophobic, calcium chloride dissolved in water has difficulties to access these sites. As calcium chloride is not too soluble in methanol, added water helps to increase its solubility. Because of the mild dissolving conditions it is expected that molecular weight and acetylation degree of the chitin molecule are not affected.

Contour plot shown in Figure 6 present the positive effect of water, that is, the elimination of proteins. According to the polynomial function of second order the positive effect of water (0.94) is approximately twice the effect of methanol (0.43). Besides, there is a slightly positive effect (0.06) between the water and methanol interaction. The region for highest chitin dissolving characteristics is between 6.5–7.0 mL water and 3.5–4.2 mL methanol

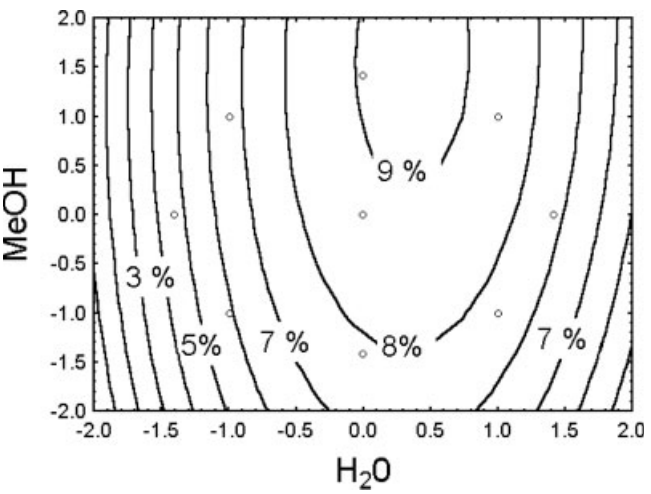

Figure 6 Contour plot for the chitin extraction from partially deproteinized shrimp cephalothorax with different MAC solvent ratios (mixture $CaCl_2$, MeOH, and H_2O), during three days. Variables are in codified units. % Chitin = $8.7389 + 0.937X + 0.4347Y–1.4066X^2 + 0.0575XY - 0.1412Y^2$.

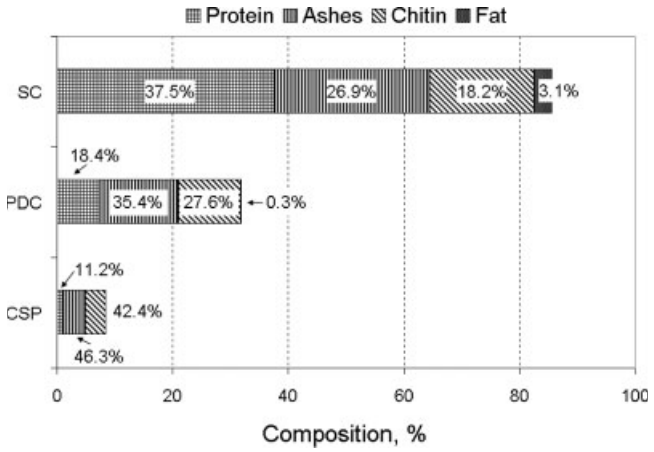

Figure 7 Shrimp cephalothorax (SC), partially deproteinized cephalothorax (PDC), and chitin sponge (CSP) composition. For chitin sponge, ''ashes'' contents was calculated by difference after determining protein and chitin contents.

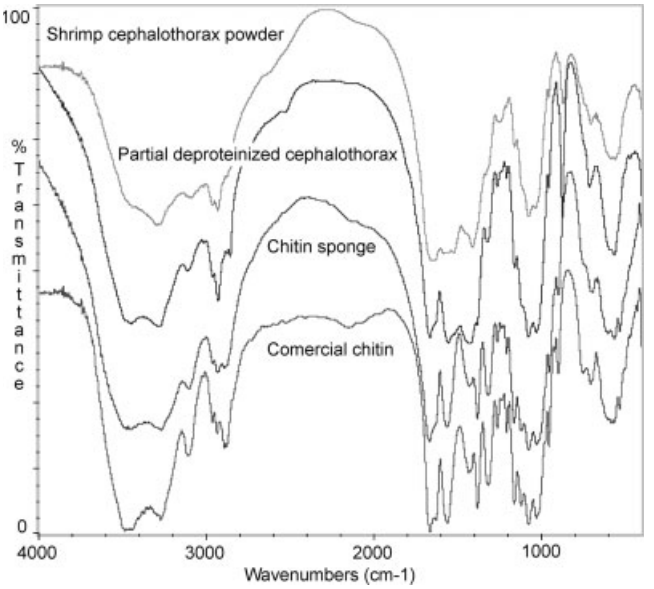

Figure 8 Infrared spectrographs for samples of shrimp cephalothorax (SC), partially deproteinized cephalothorax (PDC), chitin sponge (CSP), and commercial (Sigma) pure chitin.

with 10 g calcium chloride for 0.5 g partially deproteinized shrimp cephalothorax.

More chitin is dissolved when extraction is performed with stirring compared to resting. One inconvenience associated with stirring is that viscosity of the chitin mixture increases energy consumption.

Chitin sponge formation

Sponge formation from chitin solutions prepared using the MAC solvent is an alternative and less expensive procedure for lyophilization as well as environmentally friendlier. Figure 7 presents the reductions obtained after blending of fresh shrimp cephalothorax in proteins (80%), minerals as ''ashes'' (49%), fat (97%), and chitin (41%). Final partially deproteinized cephalothorax contains 28% chitin. Although the presence of protein is somewhat detrimental to chitin dissolution, it is necessary for chitin foam formation.

To separate chitin from PDC, a mixture of 8.75 mL MeOH, 16.25 mL H_2O, and 25g $CaCl_2$, (solvent MAC) was used per gram of PDC. MAC dissolved chitin under mechanical stirring to form a sponge which had an overall composition of 42% chitin, 46% ashes, and 11% proteins.

FTIR measurements

Figure 8 presents the infrared spectrographs for samples of shrimp cephalothorax (SC), partially deproteinized cephalothorax (PDC), chitin sponge (CSP), and commercial crab shells pure chitin (Sigma Chemical, St. Louis, MO, USA). It may be observed that as purification steps take place, the samples peaks approach to the pure chitin characteristic peaks. These results indicate that samples contain chitin.

Shrimp cephalothorax is a mixture of chitin, proteins, and carbonates. Thus, characteristic chitin peaks are not readily appreciated,[19–21] in the original samples. The same behavior occurs with the samples of partially deproteinized cephalothorax. However, as chitin extraction processes take place and the proteins and carbonates are eliminated, the chitin characteristic peaks become more defined.

Chitin sponge and commercial chitin characteristic peaks can be easily observed in the region of 3200–3500 cm^{-1}, corresponding to the —NH and —OH stretching vibrations, and the 3099 cm^{-1} to the secondary amide. In the region of 3000–2800 cm^{-1} the peaks related to the bonds C—H can be easily observed for commercial chitin and for chitin sponge.

Chitin sponge and the commercial chitin have amide bands at 1660, 1562, and 1311 cm^{-1}, and C—H bonds at 1429 and 1378 cm^{-1}, whereas for the

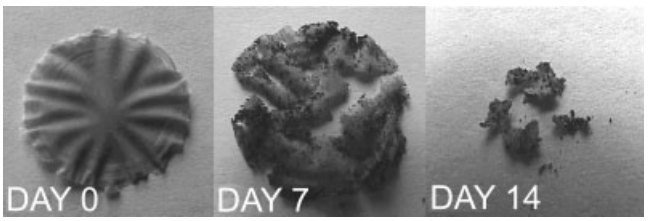

Figure 9 Biodegradation of chitin films in compost-soil mixtures (chitin film was completely degraded after 17 days).

shrimp and partially deproteinized cephalothorax they are not readily observed.

Weathering and biodegradation tests

Weathering

Sunlight and rain were present during these tests. Polystyrene samples did not suffer appreciable weight losses. Chitin samples suffered at early stages a significant weight change but later on they kept their weight constant. Total chitin sponges weight loss was 60%. At the end of the experiments, chitin samples showed a slightly darker color than its unweathered counterparts.

Biodegradation

One of the containers was opened every week and the polystyrene and chitin disks were ''unearthed.'' After the second week, chitin disks were only some fragments. No chitin fragments were found in the third and fourth week containers. They were completely degraded (Fig. 9). The polystyrene sample did not suffer any changes. It was concluded that this synthetic polymer is very stable, both in weathering and in biodegradation tests. Chitin samples were stable during weathering tests but they were easily degraded when in contact with microbial communities present in compost mixtures. This behavior exhibited by chitin sponge disks indicate that packaging materials may have a relatively long shelf life but when deposited in a municipal refuse disposal site they are easily degraded.

CONCLUSIONS

Deproteinization experiments indicate that open reflux using no surfactant agents is the best option to remove proteins from the shrimp cephalothorax powder, when compared with microwave energy.

Chitin may be obtained from partially deproteinized cephalothorax, PDC, using a mixture of 8.75 mL MeOH, 16.25 mL H_2O, and 25g $CaCl_2$, per gram of PDC, instead of corrosive substances such as hydrochloric acid and sodium hydroxide. The mixture used, can be reused to further dissolve chitin.

When chitin is dissolved in the MAC mixture, the formation of sponges is facilitated, representing an alternative and less expensive procedure than lyophilization as well as an environmentally friendlier one.

Chitin sponges are biodegradable and may be used as substitutes of polystyrene foams in the fast food disposable items, and have a long enough shelf life for this purpose. These results open new possibilities to the recycling of crustacean residues, using simple separation processes and environmentally friendly systems.[16]

Although this research is focused towards the use of chitin foams to substitute synthetic plastics, the use of porous biodegradable materials is much more diversified, going from biomedical applications as wound dressings up to drug delivery systems or as chirurgical implants, as well as many other uses.

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References

1. Khor, E. Curr Opin Solid State Mater Sci 2002, 6, 313.
2. Ravi Kumar, M. N. V. React Funct Polym 2000, 46, 1.
3. Percot, A.; Viton, Ch.; Domard, A. Biomacromolecules 2003, 4, 12.
4. Chow, K.; Khor, E. Biomacromolecules 2000, 1, 61.
5. Seol, Y.; Lee, J.; Park, Y.; Lee, Y.; Ku, Y.; Rhyu, I.; Lee, S.; Han, S.; Chung, Ch. Biotechnol Lett 2004, 26, 1037.
6. Yusof, N.; Lim, L.; Khor, E. Carbohydr Res 2004, 339, 2701.
7. Mitsunori, L.; Toshihisa, K. Jpn. Pat. 7,316,202 (1995).
8. Seiichi, T.; Yutaka, T. Jpn. Pat. 036,109 (2005).
9. Ryogo, H.; Masa, I. Jpn. Pat. 7,179,649 (1995).
10. AOAC. In Official Methods of Analysis of the Association of Official Analytical Chemists, 13a ed.; Horwitz, W., Ed.; AOAC International: Washington, DC, 1980; pp 14, 289, 291, 376.
11. Black, M.; Schwartz, H. Analyst 1950, 75, 185.
12. Hall, N., Ed. The New Chemistry; Cambridge University Press: Cambridge, Great Britain, 2000.
13. Singer, N.; Wooten, J. U.S. Pat. 0,060,610 (2003).
14. No, H.; Meyers, S.; Lee, K. J Agric Food Chem 1989, 37, 575.
15. Chang, K.; Tsai, G. J Agric Food Chem 1997, 45, 1900.
16. Flores, R.; Barrera-Rodríguez, S.; Durán-Domínguez, M. C. PA. Pat. 009,517 (2004). IMPI, Distrito Federal, México.
17. Muzzarelli, R. Chitin; Pergamon Press: London, Great Britain, 1977.
18. Reeves, W.; Chen, L. U.S. Pat. 0,143,388 (2003).
19. Burkhanova, N.; Yugai, S.; Pulatova, Kh.; Nikononvich, G.; Milusheva, R.; Voropaeva, N.; Rashidova, S. Chem Nat Comp 2000, 36, 352.
20. Van de Velde, K.; Kiekens, P. Carbohydr Polym 2004, 58, 409.
21. Cardenas, G.; Cabrera, G.; Taboada, E.; Miranda, P. J Appl Polym Sci 2004, 93, 1876.
22. Flores, R. Tesis de Maestría en Ciencias, UNAM, Mexico, Distrito Federal, 2004.