



## Chitin-Chitosan Yield of Freshwater Crab (*Potamon potamios*, Olivier 1804) Shell

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### ABSTRACT

In this study, freshwater crab (*Potamon potamios*, Olivier 1804), economically unevaluated, was used to obtain chitin-chitosan. Chitin-chitosan was extracted with standardized modified chemical method. Chitosan was extracted with demineralization, deproteinization, decoloration (chitin) and deacetylation (chitosan). Grinded shell was obtained as 60% after boiling, drying and grinding processes. Chitosan yield of crab shell was determined as 4.65% from grinded crab shell after demineralization (yield is 34.32%), deproteinization (yield is 7.25%), decoloration (yield is 6.83%) and deacetylation processes. Moreover, freshwater crab stock was estimated with catch per unit effort (CPUE) data in Eğirdir Lake for calculation of quantity of chitosan. Population size was estimated between 12.85±11.88 and 23.86±25.39 tones and freshwater crab was determined as an appropriate crustacean to obtain chitin-chitosan.

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### INTRODUCTION

Fresh-water crab, *Potamon potamios* Olivier is found in both aquatic and terrestrial habitats (Warburg *et al.*, 1982). The genus *Potamon* has been described in circummediterranean regions with several crab species. Three species viz., *Potamon fluviatilis*, *P. ibericum* and *P. potamios* live in Mediterranean regions while *P. ibericum* live in north and northwestern of Turkey, around the Black sea. *P. potamios* also lives in south and southwestern inland water of Turkey (Charmantier, 1992; Özbek and Ustaoglu, 2006). Although the stock in inland water has not yet been estimated within Turkey, it is believed that plenty of freshwater crabs exist. However, these populations have not been commercially evaluated. Furthermore, while catching the crayfish, trapped crabs are killed and discarded by the fishermen. In fact, freshwater crabs are the important source of chitin like the other crustaceans.

Chitin is a polysaccharide formed by N-acetyl-D-glucosamine units. Over the world, after cellulose, it is the second most abundant biopolymer, and it is present in invertebrates, insects, marine diatoms, algae, fungi and in crustacea like crab, shrimps and lobsters (Synowiecki and Al-Khateeb, 2003). Chitosan is a polyaminosaccharide obtained by the alkaline deacetylation of chitin, and its closed formula is C<sub>4</sub>H<sub>11</sub>NO<sub>4</sub>. Chitosan is hardly ever dissolved in water, but it weakly soluble in organic acids, like formic and acetic acids. Shell constitutes the 65% of

mussel weight; the 85% of oyster, and its chitin constituent is about 3 - 6% (Mol, 2004). The shell weight of blue crab (*Callinectes sapidus* Rathbun, 1896) is 42.81% (57.19% meat yield) in females and 33.33% (66.67%) in males. It is 71.77% (28.23% meat yield) in female sand crabs (*Portunus pelagicus* Linne, 1758), and 58.01% (41.99%) in males (Türel *et al.*, 2000). The shell waste of crustaceae is composed of 30–40% protein, 30–50% calcium carbonate and 20–30% chitin. However, these compositions are variable, depending on the species and seasons (Cho *et al.*, 1998). It was determined that crab (*Chionoecetes opilio*) and shrimp (*Pandalus borealis*) contain about 17-32.2% chitin (Shahidi and Synowiecki, 1991) while the chitin isolated from crab (*Chionoecetes opilio*) contains 26.6% chitin (Hong and Mun, 1995). Synowiecki and Al-Khateeb (2003) reported that shrimp shell waste, *Crangon crangon* contains 17.8% chitin. Hertrampf and Piedal-Pascual (2000) isolated 10.6% chitin from crab (*Chionoecetes opilio*) while Chakrabarti (2002) estimated that, chitin content of raw brown shrimp's (*M. monaceros*) shell waste is 4.5-7%. The chitin content of blue crab was reported to be 14% (Tharanathan and Kittur, 2003). Due to its ability to form semi-permeable film, chitosan has been successfully used as food wraps, thus having the property of extending shelf life (Muzzarelli, 1986). Chitosan films are tough, long lasting, flexible, and very difficult to tear. They have moderate water permeability and are extremely good barriers for the permeability to oxygen. The chitin and

chitosan have the potential to be used as food additive due to their low toxicity and digestibility and having the cholesterol lowering ability in human blood (Knorr, 1983). Furthermore, chitin and chitosan, obtained from crustacean, can be used in pharmacy, agriculture and the industrial applications other than food (Tharanathan and Kittur, 2003). In food industry, it has been used as covering material, in applications of packing, gelling agent additive, antimicrobial protector, and functional material of food (Ohshima, 1998; Shahidi *et al.*, 1999).

The aim of this study was to obtain chitin and chitosan from the shells of freshwater crab which is known as Mediterranean crab, has not been economically evaluated in Turkey that has the utility in many industrial areas, and to determine the potential quantity of chitin–chitosan depending on the size of crab population.

### MATERIALS AND METHODS

Freshwater crab (*Potamon potamios*, Olivier 1804), known as Mediterranean crab and a natural species of Lake of Eğirdir, were used. The population size was estimated by using the CPUE data (Ricker, 1975). Crabs were caught during April to July, 2008, from 5 stations chosen randomly as 150 specimens per trap at each station. Length (1/50 inch measuring calipers) and weight (to the nearest 0.1 g) of 500 crabs randomly chosen were recorded, and the average length and weight of population was calculated. To isolate chitin – chitosan, following boiling for 10 min, the crabs were taken out of their shells. The shells were dried in the dry air sterilizers at 60°C for 6 hours. The shells were ground with a TETRA mill (Model HD-702 Istanbul-Turkey) with a 2 mm mesh screen and sieved with 40 mesh (0.425mm) sieve (No *et al.* 2003). The isolation of chitin–chitosan from the crab shells was carried out with 4 repetitive analyze. Chitosan was derived from shells with mineralization, deproteinization, decoloration, and deacetylation. The shell was demineralized with 1N HCl for 30 min at ambient temperature with a solid/solvent ratio of 1:15 (w/v) (No and Lee, 1995). Following demineralization, the decalcified shell was collected on a filter paper (Whatman No. 3) in Buchner funnel, washed to neutrality in running tap water, and rinsed with deionized water. Deproteinization was accomplished under standard autoclaving conditions (15psi/121°C). The demineralized shell was treated with 3% NaOH for 10 min at 15 psi/121°C and solid/solvent of ratio of 1:10 (w/v) (Cho and No, 1999). The residue was then washed and filtered as mentioned above. The chitin residue was bleached with 0.32% sodium hypochlorite solution for 3 min with a solid/solvent ratio of 1:10 (w/v) (No and Lee, 1995). The decolored chitin was collected, washed and dried at 60°C for 4 h in a forced-air oven. Deacetylation was achieved by treatment of chitin under conditions of 15 psi/121°C with 50% NaOH for 30 min and a solid/solvent ratio of 1:15 (w/v) (No *et al.* 2000). The resulting chitosan was collected, washed and dried at 60°C for 4 h in a forced-air oven. The potential amount of chitosan was calculated according to population size and yield of chitosan.

### Statistical analysis

Statistical analyses were performed using SPSS 16.0 for Windows software. Differences in the means between

groups were analyzed by one-way analysis of variance (ANOVA) and post hoc multiple contrast (DUNCAN), and at  $P < 0.05$  was considered statistically significant.

### RESULTS

The average lengths and weights of the 500 crabs randomly collected from the catching during the period of study are presented in Table 1. Stock amounts calculated using the data of the cumulative catching and catching efforts during various months varied between 12848.74 and 23857.64 Kg. However, the stock amounts decreased gradually from April to June but increased in July (Table 2). The amount of chitosan obtained from freshwater crabs was calculated with 4 repetitive analyze. The crab shell wet weights were calculated for dried and grind states with the ratio of 60.050% and results were presented in Table 3 and Fig. 1.

As the result of the chemical analysis of the crab samples obtained after drying and grinding, amount of chitin was determined with the ratio of 6.83% and that of chitosan with the ratio of 4.651% (Table 3, Fig. 1). Depending on the population size, the potential amount of chitosan was calculated between 358.56 and 665.77Kg for only Eğirdir Lake (Table 4).

**Table 1: The average length (mm) and weight (g) values of the populations of freshwater crab of Eğirdir Lake *Potamon potamios* (Olivier, 1804).**

Parameter	Mean ± SE	Min-Max
Length	49.66 ± 5.56	32-66
Weight	51.78 ± 16.32	12-118.72

**Table 2: The population size of cumulative catch and catching effort obtained from the stations during different months**

Months	Population size ± 95 % Confidence limits	
	Individuals	Kg
April	276799 ± 30342 <sup>b</sup>	14332.65 ± 1571.11
May	268454 ± 27965 <sup>b</sup>	13900.55 ± 1448.03
June	248142 ± 22952 <sup>b</sup>	12848.74 ± 1188.46
July	460750 ± 49030 <sup>a</sup>	23857.64 ± 2538.77

Values in the same column with different superscripts are significantly different ( $P \leq 0.05$ ).

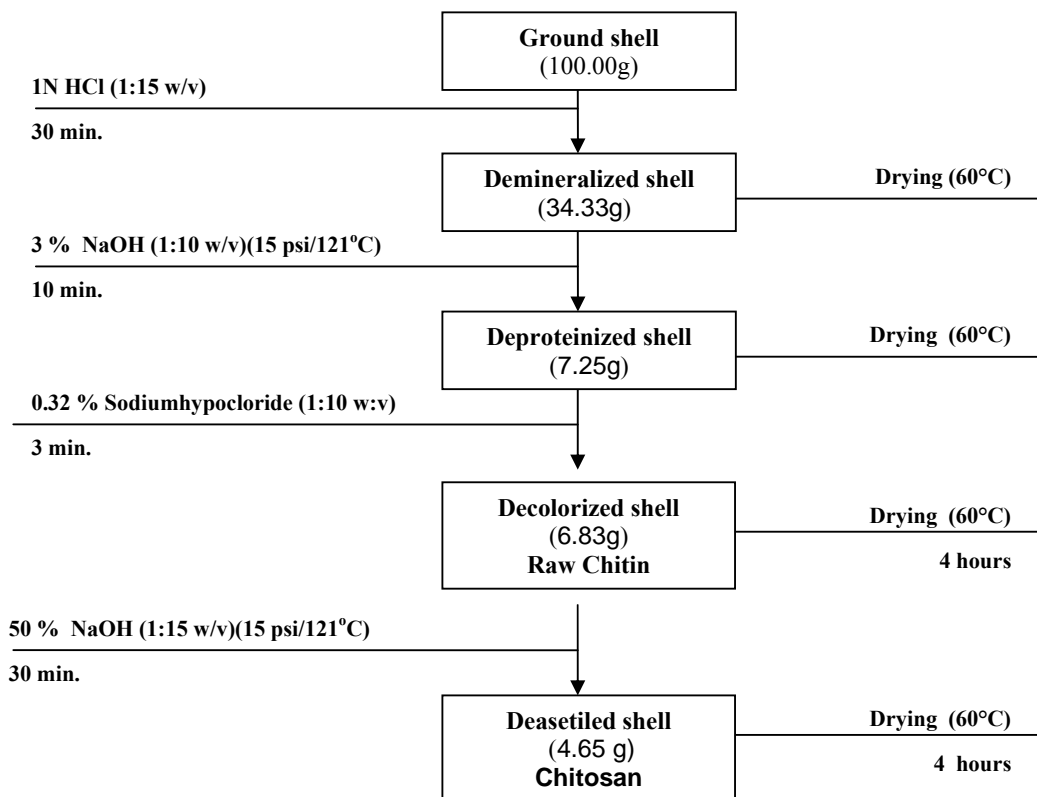
**Table 3: The yield (%) at wet and dried crab shells**

Stages of Production	% yield	S.E.
<b>Wet weight</b>	100	
Weight of shell boiled	62.625 <sup>a</sup>	2.529
Weight of shell dried	60.75 <sup>b</sup>	3.228
Weight of shell ground	60.05 <sup>b</sup>	2.102
<b>Dry weight</b>		
Ground sample	100.00	0.000
Demineralized sample	34.32	0.27
Deproteinized sample	7.25	0.08
Decolorized sample (Raw Chitin)	6.83	0.08
Deasetiled sample (Chitosan)	4.65	0.04

Values in the same column with different superscripts are significantly different ( $P \leq 0.05$ ).

**Table 4: Potential amounts of chitosan according to population size and rational values of chitosan**

Months	Stock	Ground shell (Kg)	Chitosan (Kg)
April	14332.65 ± 1571.11	8599.59 ± 942.67	399.97 ± 43.84
May	13900.55 ± 1448.03	8340.33 ± 868.82	387.91 ± 40.41
June	12848.74 ± 1188.46	7709.24 ± 713.08	358.56 ± 33.16
July	23857.64 ± 2538.77	14314.58 ± 1523.26	665.77 ± 70.85

**Fig. 1: The stages of process for recovery of chitin-chitosan from crab shells and the values of yield**

## DISCUSSION

Among the 4500 species of crab over the world, only 22 crab species have been economically evaluated (Siddiquie *et al.*, 1987; Cumberlidge *et al.*, 2009). Crabs are defined as a biological indicator in the ecological studies (Philips, 1977). Many researches have been carried out on biological, systematic, anatomic and morphological characteristics and food chemistry of crabs (Ünlüsayın, 2003; Gülle *et al.*, 2007) but population size has not been studied yet in Turkey. Using the data of CPUE, the population size of crab was determined as 12848.74 to 23857.64Kg. It has been observed that the crab stock decreases from April to June and significantly increases during July ( $P < 0.05$ ). This rise could be due to more active movement of crabs before the spawning season that increased their catchability by fishing gears at various statuses in the study area.

The exoskeleton of crustacean is the most important industrial source for production of chitin now-a-days. All over the seas of the world, the amount of this biopolymer is estimated to be 1.560 million tons (Cauchie, 1997). In every year, about 100 billion tones of chitin are produced

by crustaceans, mollusks, insects, and fungi. Annual synthesis of this polysaccharide in freshwater ecosystem is estimated to be about 600 million tons (Cauchie, 1997). Chitin is the most underexploited biomass resource available on the Earth (Knoor, 1984). The crabs whose 1280 species exist in the fresh water can be evaluated as an important source of chitin indicated that the production of chitin and chitosan is currently based on crab and shrimp shells discarded by the canning industries in Oregon, Washington, Virginia and Japan and by various finishing fleets in the Antarctic. Several countries possess large unexploited crustacean resources, e.g. Norway, Mexico and Chile. The production of chitosan from crustacean shells obtained as a food industry waste is economically feasible, especially if it includes the recovery of carotenoids. The shells contain considerable quantities of astaxanthin that is marketed as a fish food additive in aquaculture, especially for salmon to give red and pink colour to the flesh (Majeti and Kumar, 2000).

In the present study, the yield after boiling was found as 62.625% which was statistically significant according to yield of dried and ground freshwater crab shell. Similar results were obtained with the sand crab (Türeli *et al.*,

2000). The chitin content of the ground shells was determined as 6.83%, which is consistent with the results reported by Mol (2004) as mussel and oyster having chitin around 3-6%. Hertrampf and Piedal-Pascual (2000) reported that chitin content of crab (*Chionoecetes opilio*) is around 10.6% that of brown shrimp (*M. Monoceros*) is 4.5-7% in the shell wastes (Chakrabarti, 2002). Content of chitin was determined as 14% in blue crab (Tharanathan and Kittur, 2003), in *Chionoecetes opilio* and *Pandalus borealis* between 17 and 32.2% (Shahidi and Synowiecki, 1991), in *Chionoecetes opilio* 26.6% (Hong and Mun, 1995), and in shrimp (*Crangon crangon*) 17.8% (Synowiecki and Al-Khateeb, 2000). It has been reported that the differences in the quantities of chitin between different crabs varied according to the species and season (Cho *et al.*, 1998).

Chitosan is a polyaminosaccharide provided by the alkaline deacetylation of chitin, and it is quite easy to derive methodologically. In this study, the chitosan content of freshwater crab has been determined to be 4.65%. Depending on the population size, the chitosan may be extracted from crabs of Eğirdir Lake between 358.56 and 665.77Kg. The economic value of chitosan has been calculated as 484.000-898.000 Euro (1350 Euro/Kg chitosan). Freshwater crab may be considered as alternative fishing material in the freshwaters that have already been over-fished. On the other hand, freshwater crab can also be cultured as a source of chitin because it is easier and cheaper way to collect and catch than that of marine crustaceans. Moreover, every country having freshwater crustaceans can commercially produce chitin and chitosan because of simplicity of its processing for chitin recovery.

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