HYDROTHERMAL TREATMENT OF PERSIAN CRAB SHELL TO PRODUCE CHITIN AND CHITOSAN

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ABSTRACT
The production of useful materials from persian golf crab shell using sub-critical water treatment were assessed. Protein and calcium carbonate decompoition were successfully attained by addition of aqueous acetic acid solution and highly pure chitin (> 95%) was produced. This process was conducted in a relatively low reaction temperature of 458 – 498 K and short reaction time of 1 – 10 min in one step procedure that is cosiderably acceptable compared with conventional method. Moreover, it was possible to produce chitosan in the temperature range of 458–498 K and very short reaction time of 1–4 min by the deacetylation of chitin using 40 wt% sub-critical aqueous NaOH solution. Deacetylation rate was found relatively low in 5–20 wt% NaOH aqueous solution and at reaction time of 60 min. The deacetylation degree of 93.4% was obtained under the condition of 40 wt% aqueous NaOH solution, reaction time of 4 min and reaction temperature of 498 K.

Keywords: Chitin; Chitosan; Sub-ceritical water; Crab shell; Deacetylation; Depolymerization

INTRODUCTION
Chitin is a natural bio-polymer of N-acetyl-D-glucosamine (Glc-NAc) units through a β (1-4) linkage and it is the second most abundant biopolymer with various interesting functions in the fields of biotechnology, food and pharmaceutical industry, cosmetics and environmental engineering [1]. Chitin and its derivatives have high economic value owing to their versatile biological activities and agrochemical applications [2-3]. However, biotechnologically produced chitin is at present not commercially available, but offers new perspectives for production of high viscosity chitosan that is a cationic polymer derived from chitin comprising copolymers of β (1→4)-glucosamine and N-acetyl-d-glucosamine. The physicochemical and biological properties of chitosan make it an excellent material for the preparation of drug delivery systems and for the development of new biomedical applications in many fields from skin to bone or cartilage [4-5]. The chemical structure of chitin and chitosan are illustrated in **Figure 1**.
The popularity of using subcritical water as a solvent to extract a variety of organic compounds has grown over the last 10 years compared with conventional extraction solvents [6].

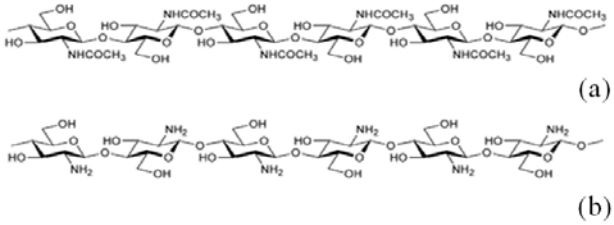

Figure 1: Chemical structure of chitin (a) and chitosan (b)

Conventional extraction processes are multi-stage and require non- or semi-polar solvents to achieve high extraction yields. These solvents are often toxic and then removal of such organic solvent is often required where as the extract is to be used as a food or pharmaceutical compounds. Solvent removal is expensive, time-consuming and large waste water treatment for the neutralization is required. Sub-critical water treatment (SBCW), in general, had higher extraction yields than conventional extraction alternatives [7-9]. In this regard, SBCW is an ideal candidate for use as a solvent [6].

In this study, the quite new valuable chitin producing method from crab shell using sub-critical aqueous acetic acid solution was developed not only to produce chitin with the high purity, but also to decrease the initial and running cost in comparison with the conventional method. In addition, the efficient method for production of valuable substances and chitosan by depolymerization of chitin were investigated using sub-critical NaOH aqueous solution.

MATERIALS AND METHODS

Crab shells of *Portunus pelagicus* (*portunidae* crab family) were collected form Persian gulf coast, Iran. The samples were washed with weighted method in saltwater and freshwater and finally dried in a hot air oven for 24 h at 65℃. A stainless tube (SUS 316, id 14.9 cm × 1.8 cm) with Swagelok caps was used as a reactor (reactor volume 34 cm^3). Aqueous acetic acid solution (Merck, Germany) for chitin production and aqueous NaOH solution (Merck, Germany) for chitosan production were used. The reactions were conducted in temperature range of 458–498 K with sub-critical aqueous acetic acid solution (stoichiometric molar ratio of acetic acid was 3:1 for calcium carbonate). Chitin flakes (0.2 g) and aqueous acetic acid solution (2 gr) were loaded in the reactor, and the reactor was put into a contact furnace. The starting point was set at the moment when the reactor was put into the contact furnace consequently the reaction time included the initial heating period. After a given reaction timeand to terminate the reaction, the reactor was removed and rapidly quenched in a water bath to cool down to room temperature. After cooling, the products were collected from the reactor and divided into the water-soluble and solid fractions (chitin) using a membrane filter (pore size: 0.20μm, Millipore). After SBCW treatment, the chitin was dried at 90 ˚C for 24 h.

The second part of experiments was related to chitosan production. Chitin deacetylation was performed with aqueous solution of NaOH (5 to 40 wt. %) in the temperature range of 458-498 K and residence time of 1 to 60 min. The molecular weight of obtained chitosan was estimated using size exclusion chromatography. The degree of deacetylation was determined using Fourier Transform Infrared Spectroscopy (IR 550, Hayward, California, USA) in transmittance mode.

RESULTS

In crustacean cuticles, chitin is intimately associated with protein, inorganic salts such as calcium carbonates and lipids, so its isolation needs purification. Effect of reaction time at different temperatures on the ratio of residual calcium carbonate and chitin for sub-critical acetic acid treatment is shown in **Figure 2a** and **2b**. Graph 2a clearly demonstrate that calcium carbonate was removed to about 95% of initial amount and about the 90% of initial chitin remained in the solid phase during the experiment time.

There is a comparison between commercial and pure chitin in term of ratio of residual solid at different reaction temperatures and time of experiment. According to the previously published articles in conventional chitin producing methods [10], crab shell contained protein (30%), calcium carbonate (35%) and chitin (35%). Figure 2b Shows that after 1 minute chitin become similar to the commercial grade produced from the crab shell with the conventional method. Furthermore, almost 100% purity chitin was obtained in the additional sub-critical treatment of 2–10 minutes. It was proven that the very high purity chitin could be produced in one stage process, short time treatment, and remarkably low cost in process without strong base and large waste water treatment for the neutralization.

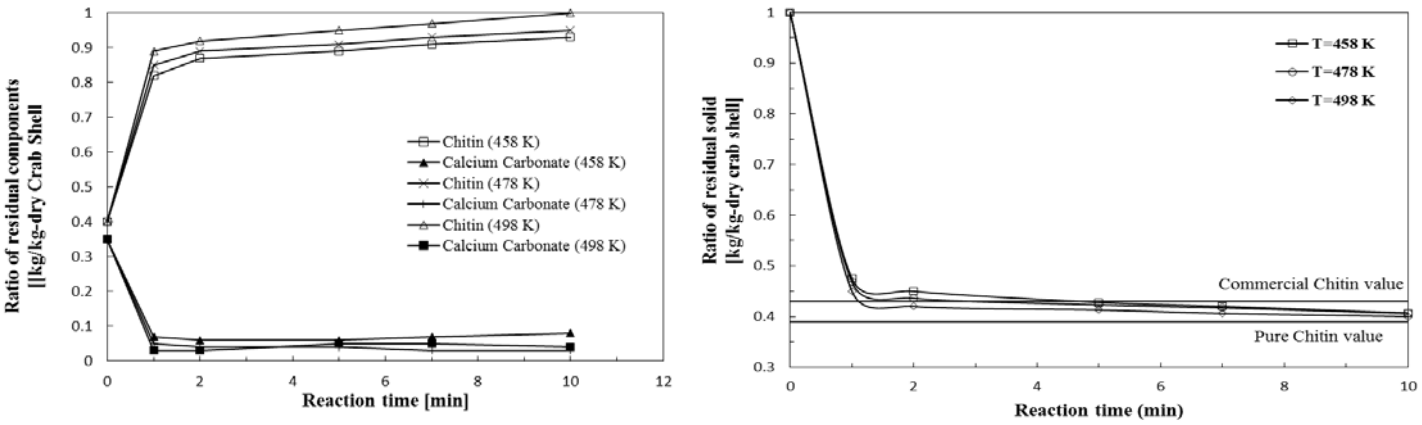

Figure 2: Effect of reaction time and temperature on ratio of residual components, a) residual chitin and calcium carbonate, b) commercial and pure chitin value

To analyze the effect of aqueous NaOH solution on chitin deacetylation rate, different NaOH wt% at 498 K were experimented. For 5-20 wt%, deacetylation degree was relatively low and need a long reaction time (up to 60 min), while the highest achievable rate (93.4%) was attained at 40 wt% in a very short reaction time. The deacetylation ration of chitin is illustrated in **Figure 3a** and **3b**. Due to the higher deacetylation rate at 40 wt% aqueous NaOH solution, degree of deacetylation was obtained at different reaction temperatures and 40 wt%. As illustrated in Figure 3b and the maximum rate was detected at 498 K.

Figure 4 illustrates the depolymerization of chitosan in 40 wt% aqueous NaOH solution at various temperature (458 to 498 K) and reaction time (0 to 4 min). It can be seen that produced chitosan from chitin was depolymerized a little after reaction was started.

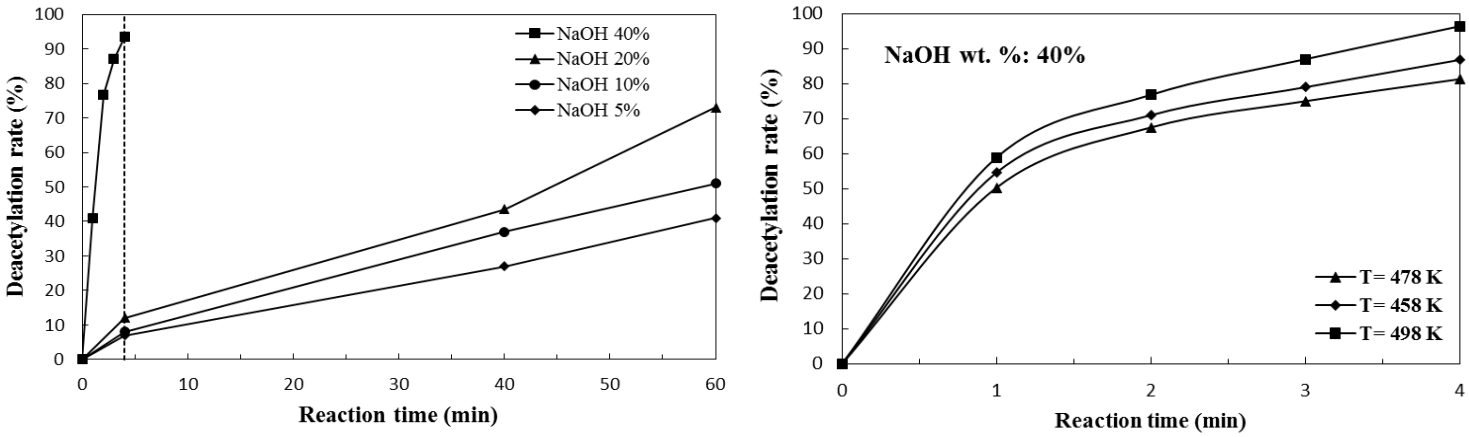

Figure 3: Deacetylation rate of chitin to Chitosan, a) effect of different percentage of NaOH at T= 498 K and b) effect of different reaction temperature at NaOH 40%

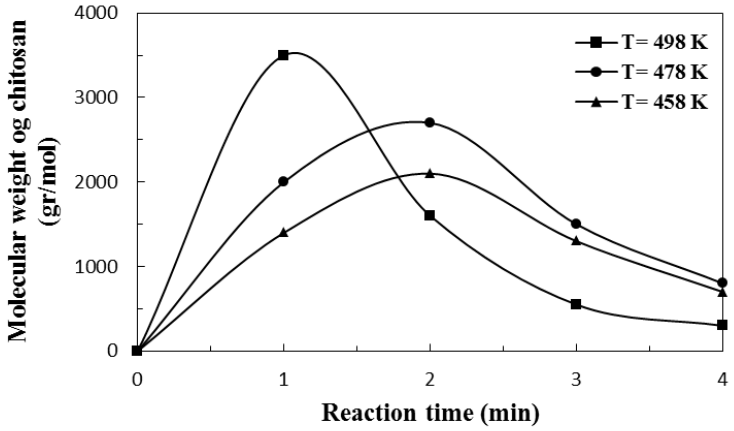

Figure 4: Depolymerization of chitosan in sub-critical aqueous NaOH solution (NaOH 40%)

CONCLUSION

The production of valuable substances was investigated using supercritical water treatment as a relatively new method. Various decomposition parameters including temperature, reaction time and NaOH wt% was carried out for producing of chitin and chitosan. Using sub-critical aqueous acetic acid solution provide the chitin with highest purity. In addition, maximum chitosan was producet at 498 K and 40 wt% NaOH aqueous solution. Short reaction time and single stage process as well as high purity products confirmed that this method is an economical and feasible technology.

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