Supporting Information
Intensified atmospheric plasma-based process for isolation of chitin biopolymer from waste crustacean biomass

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Materials and methods

1. Shrimp shell samples:

Shrimps (*Pandalus borealis*) were harvested by seagoing trawlers and frozen within two hours after the catch.On land, the frozen raw shrimps were thawed, steam-boiled and shelled using pilling machines. Samples of shrimp shells were taken out and stored at -18 °C. Prior to the experiment shrimp shells were defrosted and washed in distilled water and filteredthrough a vacuum filter (0.45 μm) to remove as much excess fluid as possible and dried at 65 °C for 24h. Dried shrimp shells were composed primarily of the shrimp abdomen and/or tail section and chopped (shredded) to small flakes (size distribution 2-5 mm and thickness 80±10μm) prior filling the reactor(Fig. SI 1A and 1B).

Water content of non-treated shrimp shells was 4.5% and for plasma treated samples ranged from 0.4% to 0.5%. Water content was evaluated by the mass change for the sample drying in a convective oven (SP- 55 C, Kambič, Slovenia), at 110 ^{o}C for the time needed to reach constant mass.

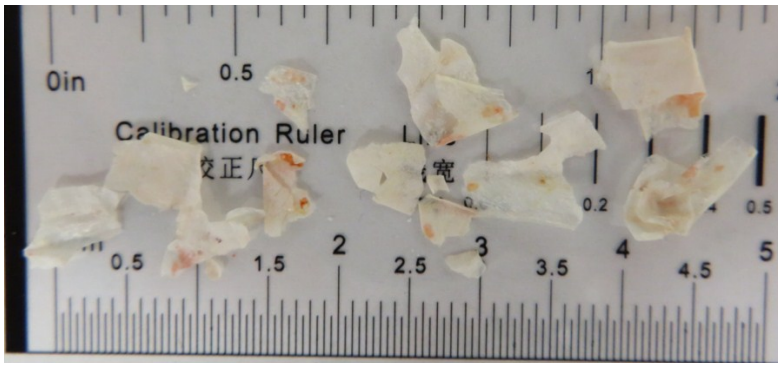
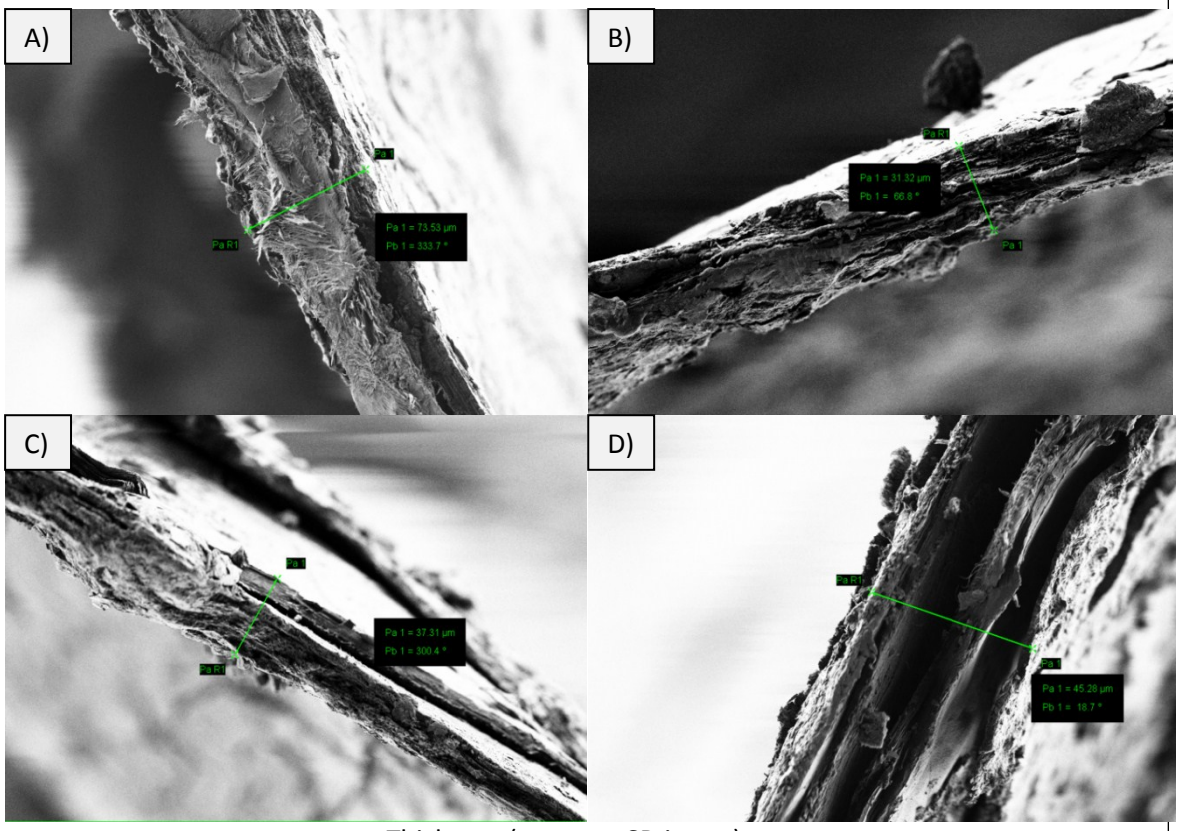


Figure SI 1A: The characteristic sizes of the shrimp shell particles.

All the samples were positioned vertically on sample holder so that the sample thickness was exposed to the objective lens. Thickness was recorded by the entire length of the sample. Also, we used different pieces of the same material. The SEM conditions used are described in section 8.



Thickness (average±SD in µm)			
A) Raw shrimp shell	B) Deproteinized	C) 10% O_2 in N_2 plasma (6min)	D) N_2 plasma (6min)
80±10 µm	30±10 µm	35±5 µm	44±8 µm

Figure SI 1B: SEM images with the measured thickness and the average values for multiple particles and positions along a particle for the A) raw ; B) 10% O_2 in N_2 plasma treated; C) pure N_2 plasma treated and; D) deproteinized shrimp shell.

2. Plasma treatment and GC analysis

For the treatment of shrimp shell samples, atmospheric pressure dielectric barrier discharge plasma was used (Figure SI-2). The plasma reactor is made of a quartz tube with inner diameter 7 mm and outer diameter 10 mm and mounted vertically with metallic clamps. A stainless steel rod was used as the inner electrode, which is fixed in the center of this reactor tube and connected to a high voltage source. An outer electrode derived from aluminum tape was wrapped around the glass reactor at a length of 5 cm was grounded. The upper end of the reactors is connected to the gas inlet through mass flow controller units and the other end to the sample collecting unit (liquid condenser) that can be cooled up to –15 °C. A Teflon tube was used to connect the liquid condenser with the micro GC for the analysis of the gaseous products released after plasma-shrimp shell chemical interactions.

The plasma was generated in the reactor tube in a volume of about 3.1 cm^3. The mass of the sample (cca. 0.1 g) inside the quartz tube was measured on a precise scale (with an accuracy of 0.01%) before

and after the treatment. At least three parallel experiments were performed for each treatment time and average values for the mass loss were calculated.

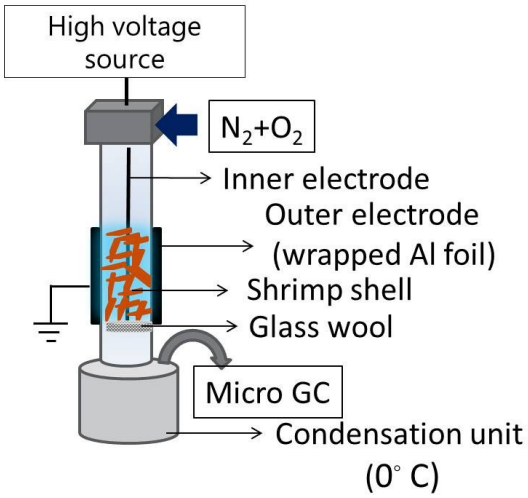


Figure SI 2: Schematic of the dielectric barrier discharge reactor system.

The composition of outlet gas was determined by gas chromatography (Micro GC Fusion® Gas Analyzer, INFICON, Switzerland) using Rt-Molsieve 5A and RT-U-Bond columns.

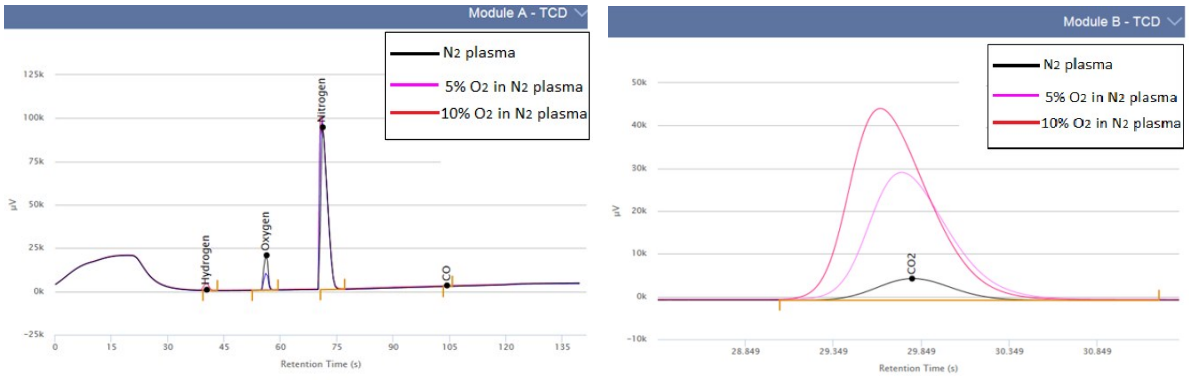


Figure SI 3: Chromatograms of the detected gaseous species at different plasma gas mixtures at the end of 2 minutes.

3. Chitin determination

0.2-0.4 g of the dried raw or plasma treated sample was placed in a beaker with 50 ml of 1M HCl and heated for 1 hat 100 ˚C. Sample was filtered through sintered glass crucible and washed with distilled water. Residue was placed back into a beaker with 100 ml of 5% NaOH solution and heated for 1 h at 100 ˚C. Shrimp shells were filtered through sintered glass crucible and washed with distilled water two times and twice with 15 ml of acetone. Samples were dried in a crucible at 110 ˚C to constant weight

and incinerated the content in a furnace at 600 ˚C for 6 hours. Loss in weight represented chitin content in a sample.

4. Total nitrogen determination

Kjeldahl method is used for determining nitrogen content of organic and inorganic substances, and it contains three major steps: digestion, distillation and titration. 0.1g of sample was placed in Kjeldahl flask with 7g of K_2SO_4, 0.3g HgO and 5ml of concentrated H_2SO_4. Mixture was heated for around 40 minutes. Digested sample was transferred to round flask, and distilling apparatus was set. Around 4 g of Devard alloy and 100ml of 33% NaOH was added to solution to convert NH_4^+ to NH_3, followed by boiling and condensation of the NH_3 gas in a receiving solution (0.1M HCl). After, receiving solution was titrated by 0.1M NaOH to quantify the amount of ammonia, and used volume of NaOH was recorded. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution. The excess acid in the receiving solution is neutralized by standardized 0.1M NaOH and end point of the titration is recorded by color change.

5. Ash content

Ash content in samples was determined by incinerating samples at 600 ˚C for 6 hours, and weighing samples before and after incineration.

6. Shrimp shell deproteinization protocol

Dried shrimp shells were mixed with 1M NaOH in 1:20 ratio and incubated at 80°C for 1 hour, with stirring. After deproteinization, shrimp shells were washed three times with distilled water and dried at 65°C for 24h.

7. XPS

XPS primary survey and high-resolution spectra were recorded by using PHI-TFA X-ray photoelectron spectrometer. The analyzed depth of each measurements were about 3-5 nm with an accuracy of binding energy of about ±0.3 eV. The recorded spectra were analyzed by using the software Multipak v8.1, provided by the instrument supplier.

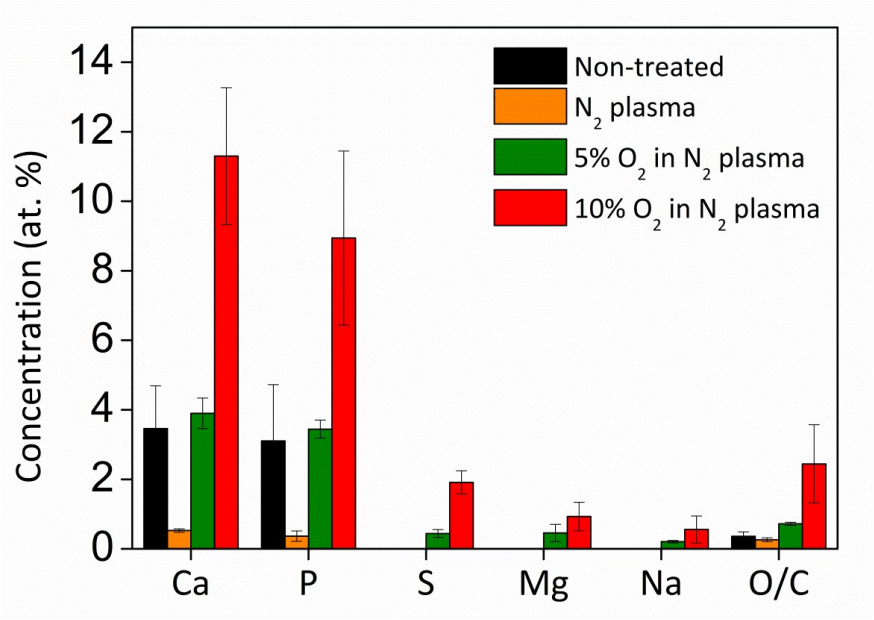


Figure SI 4:Mineral content non-treated and plasma treated shrimp shells for 3 minutes as analyzed by XPS

In non-treated samples and samples treated with N_2 plasma had no minerals other than calcium and phosphorus detected, while in samples treated with 5% and 10% O_2 in N_2 plasma, sulphur, magnesium and sodium were detected on the surface of the sample. Samples treated with 10% O_2 in N_2 plasma showed significant increase higher concentration of minerals in general on the sample surface.

In shrimp shell minerals are located on the surface and in the inner layers of the material. Using plasma treatment, proteins were removed and minerals from inner layers were exposed on the surface. Calcium concentration in non-treated sample was 3.46% and in sample treated with 10% O_2 in N_2 plasma 11.3%. The same trend was observed for P, S, Mg and Na concentration. All plasma treated samples showed a significant increase of minerals.

Oxygen to carbon ratio (O/C) increased in samples treated with O_2 in N_2 plasma, meaning that on the surface of the samples are located functional groups containing oxygen.

8. SEM analysis

The SEM analysis of different areas of the raw shrimp shells and plasma treated shrimp shells were performed on scanning electron microscope(SUPRA 35 VP, Carl Zeiss, Jena, Germany) operating at 1 kV. The elemental composition of the shrimp shell surface was determined by using energy dispersive X-ray spectroscopy (EDX) (Inca 400, Oxford Instruments, Tubney Woods, UK) operated at 20 kV.

9. ATR FT-IR

The Fourier Transform Infra-Red (FT-IR) spectra of shrimp shells before and after plasma treatment were recorded using FTIR spectrophotometer (Perkin Elmer, FT-IR spectrophotometer, Spectrum Two, Manchester, UK), in the range between 400 and 4000 cm^{-1}, using diamond ATR mode of operation with 4 accumulated scans, at a resolution of 4 cm^{-1}.

Background spectra, which were collected under identical conditions, were subtracted from the sample spectra automatically. Water and carbon dioxide were excluded from detection during spectra collecting.

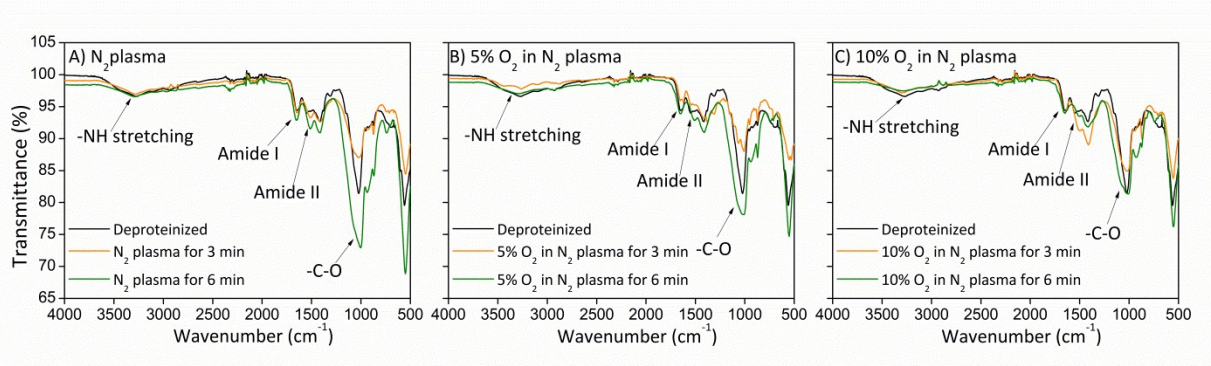


Figure SI 5: FTIR spectra of deproteinized shrimp shell (with 1M NaOH, at 80˚C for 1 hour) and shrimp shells treated in A) N_2 plasma, B) 5% O_2in N_2 plasma and C) 10% O_2in N_2 plasma for 3 and 6 minutes are shown.

(Deproteinization was performed as described in section 6.)