
Method for treating a chitin containing material

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The invention relates to a method for treating a chitin containing material comprising (i) milling the chitin containing material, (ii) contacting the milled chitin containing material from step (i) with a mixture comprising an aqueous solution of an organic acid, and (iii) contacting the chitin
10 containing material with an alkaline solution comprising water:glycerol in a ratio of 50-100:1-50 (v/v) and potassium hydroxide at a concentration of from 0.2M to 5M; thereby treating the chitin containing material. The invention also relates to a chitin preparation, a calcium salt of an organic acid, a protein product, or an astaxanthin produced or producible from chitin containing material according to the inventive method and to the use of the produced or producible chitin preparation
15 in manufacture of a natural or synthetic polymer, in agriculture, in packaging, in construction, or in environmental protection, as an ingredient in the production of a medicament, a food, or a cosmetics, and/or as a solidified enzyme carrier.

20 Chitin is mainly found in the exoskeletons of crustaceans such as shrimps, crabs, crayfish and crawfish, but also in, for example, insects. It is abundant in nature, second only to cellulose. The macromolecular polysaccharide is a polymer composed of monomer N-acetylglucosamine linked by alpha-1,4 glycosidic bonds. The medical science community hailed it as the sixth vital element necessary for the human body after sugar, protein, fat, vitamins, and minerals (inorganic salts).
25 Due to its unique molecular structure and physical and chemical properties, as well as good biocompatibility and degradability, it has a wide range of uses in medicine, food, cosmetics, agriculture, environmental protection, and solidified enzyme carriers.

Crayfish (scientific name: *Procambarus virginalis*), also known as marbled crayfish, is popular
30 with people because of its delicious meat, being consumed mainly in Southeast Asia and African countries such as South Africa. However, the edible part of a crayfish is less than 20%. In the

traditional crustacean processing and production process, about 80% of the total mass of crayfish becomes waste, which wastes resources and pollutes the environment.

Shrimp and crab shells are today the main raw materials for chitin production. Deacetylation of chitin results in chitosan. Because chitin and its derivatives have excellent biological and chemical activities, they are widely used in many fields. For example, chitin fibers have long been regarded as natural polymer fibers with excellent comprehensive properties. Compared with general inorganic nano-reinforced materials, they have the characteristics of renewable, wide-ranging sources, low energy consumption, and low cost. Generally, chitin fibers are dispersed in natural or synthetic polymers directly or after light surface modification, and are widely used to improve the mechanical properties and thermal properties of polymer materials. In addition, chitin and its derivatives can also be used in food, cosmetics, packaging, building, tissue engineering and other fields with potential applications.

However, when considering to obtain chitin from crustaceans, the layered crystal structure formed by the close combination of the three components of protein, calcium carbonate and chitin in crustacean shells hinders the contact of general reagents and increases the difficulty of chitin extraction. Among them, the chitin polymer chain is bonded to a specific protein at one or more points in a non-covalent and covalent form to form a proteoglycan complex.

The classic chitin extraction method uses 1.5N sodium hydroxide and 1N hydrochloric acid to remove protein and ash from shrimp and crab shells, respectively. This treatment method requires not only the use of high-concentration, highly corrosive strong acid and alkali, but also a large amount of clean water. The whole production process is not only costly and wasteful, but also produces a large amount of wastewater with high salt and high ammonia nitrogen concentration and has a high chemical oxygen demand (COD). The treatment is difficult, the costs are high, and it is still difficult to meet the standard discharge conditions after treatment. Although the biological extraction method has mild reaction conditions and low pollution, it is time-consuming and costly, and cannot completely remove protein and calcium, and is not suitable for large-scale treatment.

Furthermore, the other two main components in shrimp and crayfish shells, calcium carbonate and

protein are rarely recycled and reused due to their high recycling cost and low product value. However, shrimp shell protein contains all kinds of essential amino acids, has high nutritional value, and has potential market value in agricultural farming and supplementation food. If the protein cannot be recycled in time, it will not only waste resources, but also decay quickly and easily cause environmental pollution. Therefore, strengthening the recycling of protein and calcium carbonate is also an urgent problem to be solved in the treatment of shrimp and crab shells. In fact, amino acids and calcium are essential nutrients for plant growth. Although the waste liquid extracted from chitin contains a large amount of protein and calcium, it cannot be directly used as a fertilizer. The main reasons are: 1. Traditional waste liquid contains a lot of sodium ions and chloride ions. At present, the level of NaCl in soil worldwide is seriously excessive and salinization is a very serious problem to consider. Excess salt can have a serious impact on plant growth and physiological activity. 2. After the neutralization of the protein-rich alkaline waste liquid in the traditional production process, the protein will precipitate in a large amount when it reaches the isoelectric point due to the large molecular weight, and the soluble protein or amino acid solution cannot be formed. The solid protein is difficult to be absorbed by the plant. Therefore, it is necessary to develop a sustainable method for utilization of crayfish and shrimp shell waste, which can improve the separation efficiency of chitin, reduce the cost, and effectively utilize protein and calcium, and reduce the discharge of waste and waste liquid.

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The technical problem underlying the present invention was thus the provision of an improved, preferably sustainable method, for extracting chitin from chitin containing materials, which overcomes the above-mentioned drawbacks. Furthermore, the method should not only make the chitin available, but also other components such as calcium carbonate and proteins, which are to be found in the chitin containing material.

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Thus, the present invention relates to a method for treating a chitin containing material comprising

- (i) milling the chitin containing material; and
- 30 (ii) contacting the milled chitin containing material from step (i) with a mixture comprising an aqueous solution of an organic acid; and/or

(iii) contacting the chitin containing material with an alkaline solution comprising water:glycerol in a ratio of 50-100:1-50 (v/v) and potassium hydroxide at a concentration of from 0.2M to 5M, preferably of from 0.5M to 2 M, more preferably of about 1M, most preferably of 1M;

5 thereby treating the chitin containing material.

The method proved to be a sustainable method and gave far better results than the classic method in view of less degradation of the final product and generated wastes. Furthermore, the sustainable method was the less aggressive method compared to the classic one and resulted in a slightly
10 higher yield. In addition, the sustainable method allowed the use of by-products, which have high market values.

In general, terms used herein are to be given their ordinary and customary meaning to a person of ordinary skill in the art and, unless indicated otherwise, are not to be limited to a special or
15 customized meaning. As used in the following, the terms "have", "comprise" or "include" or any arbitrary grammatical variations thereof are used in a non-exclusive way. Thus, these terms may both refer to a situation in which, besides the feature introduced by these terms, no further features are present in the entity described in this context and to a situation in which one or more further features are present. As an example, the expressions "A has B", "A comprises B" and "A includes
20 B" may refer to a situation in which, besides B, no other element is present in A (i.e. a situation in which A solely and exclusively consists of B) or to a situation in which, besides B, one or more further elements are present in entity A, such as element C, elements C and D or even further elements. Also, as is understood by the skilled person, the expressions "comprising a" and "comprising an" preferably refer to "comprising one or more", i.e. are equivalent to "comprising at
25 least one". In accordance, expressions relating to one item of a plurality, unless otherwise indicated, preferably relate to at least one such item, more preferably a plurality thereof. Further, as used in the following, the terms "preferably", "more preferably", "most preferably", "particularly", "more particularly", "specifically", "more specifically" or similar terms are used in conjunction with optional features, without restricting further possibilities. Thus, features introduced by these terms
30 are optional features and are not intended to restrict the scope of the claims in any way. The invention may, as the skilled person will recognize, be performed by using alternative features.

Similarly, features introduced by "in an embodiment" or similar expressions are intended to be optional features, without any restriction regarding further embodiments of the invention, without any restrictions regarding the scope of the invention and without any restriction regarding the possibility of combining the features introduced in such way with other optional or non-optional features of the invention. As used herein, if not otherwise indicated, the term "about" relates to the indicated value with the commonly accepted technical precision in the relevant field, preferably relates to the indicated value $\pm 20\%$, more preferably $\pm 10\%$, most preferably $\pm 5\%$ of deviation.

Preferably, the method comprises steps (i), (ii) and (iii), wherein steps (i), (ii) and (iii) are preferably carried out in consecutive order, so that step (i) is followed by step (ii), which is then followed by step (iii).

The milling in step (i) is preferably grinding in a knife mill.

In some preferred embodiments of the method, step (i) is followed by step (i-i) separation of the milled chitin containing material from step (i) into at least two fractions based on particle size, preferably by mechanical separation, more preferably by sieving, most preferably using a 18 mesh (1mm) sieve for the first fraction and a 45 mesh (354 μ m) sieve for the second fraction.

In some preferred embodiments of the method, step (i-i) is followed by step (i-ii) obtaining at least one fraction containing chitin containing material, wherein at least 70 weight-%, preferably at least 80 weight-%, more preferably at least 90 weight-% of said fraction have a particle size smaller than 18mesh (1 mm) and preferably larger than 45mesh (354 μ m), more preferably a particle size in the range of from smaller than 18mesh (1 mm) to larger than 45mesh (345 μ m) based on the total weight of said fraction being 100 weight-%. These particle size ranges preferably apply when steps (ii) and (iii) are intended not to be carried out in the same device, i.e. a change of device is intended, for example, for the filtering according to (ii-i) and the washing according to (ii-ii) as described in more detail herein below.

In another preferred embodiment of the method, in which at least steps (ii) and (iii) are carried out in the same device as outlined in more detail below, in step (i-ii) at least one fraction containing

chitin containing material is obtained, wherein at least 70 weight-%, preferably at least 80 weight-%, more preferably at least 90 weight-% of said fraction have a particle size smaller than 10 mesh (2mm) and preferably larger than 18 mesh (1mm), more preferably a particle size in the range of from smaller than 10 mesh (2mm) to larger than 14 mesh (1410 μ m).

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In some preferred embodiments of the method, the organic acid used in step (ii) in the mixture comprising an aqueous solution of an organic acid is citric acid, maleic acid, or a mixture thereof. Preferably, said aqueous organic acid has a concentration of from 0.2M to 5M, more preferably of from 0.5M to 2M, more preferably about 1M, most preferably 1M.

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Herein, several ratios are defined as mass to volume (m/v) ratios meaning the ratio of the respective solid material mass, expressed, preferably, in gram, to the volume of the respective liquid, expressed, preferably, in milliliters. In addition, ratios are defined as mass to mass (m/m) ratios. Said ratios are the ratio of solid material mass, expressed, preferably, in gram, to another solid material mass, expressed, preferably, in gram.

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In some preferred embodiments of the method, the chitin containing material to aqueous organic acid ratio during step (ii) is of from 1:2 (m/v) to 1:50 (m/v), more preferably is of from 1:5 (m/v) to 1:20 (m/v), more preferably of from about 1:10 (m/v), most preferably of 1:10 (m/v).

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In some preferred embodiments of the method, step (ii) is performed at a temperature of from 20°C to 100°C, more preferably of from 50°C to 90°C, more preferably about 70°C, most preferably of 70°C.

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In some preferred embodiments of the method, said step (ii) is performed for of from 0.1h to 30h, more preferably for of from 0.5h to 30h, more preferably for of from 1h to 30h, preferably of from 1.5h to 12h, more preferably of from 2h to 5h, more preferably about 2.5h, most preferably 2.5h; or more preferably of from 1h to 2h, more preferably about 2h, most preferably 2h.

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In some preferred embodiments of the method, step (ii) is followed by step (ii-i) filtering the chitin containing material of step (ii) from the aqueous organic acid.

In some preferred embodiments of the method, step (ii) and optionally step (ii-i) is followed by step (ii-ii) washing to neutral pH, preferably in the range of from pH 6 to 9, more preferably of from pH 6.5 to 8, still more preferably about pH 7; wherein washing to neutral pH is preferably
5 done with an aqueous medium, wherein preferably at least 95 weight-% of said aqueous medium are water. In some preferred embodiments of the method, in the range of from 0.1 to 15 liter, preferably in the range of from 1 to 10 liter, more preferably in the range of from 1 to 5 liter, of aqueous medium are used for (ii-ii) per kg of chitin containing material obtained from (ii) or (ii-i). In some preferred embodiments, the aqueous medium comprises at least one basic and/or at least
10 one acidic compound. Preferably, the at least one basic compound comprised in the aqueous medium is potassium hydroxide (KOH). In some preferred embodiments, when at least steps (ii) and (iii) are carried out in the same device as outlined in more detail below, an aqueous medium comprising KOH obtained from step (iii-i) is partially or completely used as aqueous medium in step (ii-ii). Using the aqueous medium comprising KOH obtained from step (iii-i) for washing to
15 neutral pH in step (ii-ii) offers the advantage of a re-usability of said aqueous medium comprising KOH obtained from step (iii-i), thus resulting in a reduction of amount of aqueous medium required for the overall process. Moreover, due to the basic KOH, this measure allows reaching neutral pH in washing step (ii-ii) faster and with less amount of aqueous medium required.

20 In some preferred embodiments of the method, the ratio of chitin containing material to water/glycerol mixture in step (iii) is from 1:2 (m/v) to 1:50 (m/v), more preferably is of from 1:5 (m/v) to 1:20 (m/v), more preferably of from about 1:10 (m/v), most preferably of 1:10 (m/v).

In some preferred embodiments of the method, step (iii) is performed at a temperature of from
25 40°C to 100°C, more preferably of from 50°C to 90°C, more preferably about 70°C, most preferably of 70°C.

In some preferred embodiments of the method, said step (iii) is performed for of from 0.1h to 30h, more preferably for of from 0.5h to 30h, more preferably for of from 1h to 30h, more preferably of
30 from 3h to 12h, more preferably about 6h, most preferably 6h, or more preferably of from 1h to 10h, more preferably about 3h, most preferably 3h.

In some preferred embodiments of the method, step (iii) is followed by step (iii-i) filtering the chitin containing material of step (iii) from the alkaline solution.

5 In some preferred embodiments of the method, step (iii) and optionally step (iii-i) is followed by step (iii-ii) washing to neutral pH, preferably in the range of from pH 6 to 9, more preferably of from pH 6.5 to 8, still more preferably about pH 7; wherein washing to neutral pH is preferably done with an aqueous medium, wherein preferably at least 95 weight-% of said aqueous medium are water. In some preferred embodiments of the method, in the range of from 0.1 to 15 liter,
10 preferably in the range of from 1 to 10 liter, more preferably in the range of from 1 to 5 liter, of aqueous medium are used for (iii-ii) per kg of chitin containing material obtained from (iii) or (iii-i). From washing step (iii-ii), an aqueous medium comprising KOH is obtained. In some preferred embodiments, said aqueous medium comprising KOH obtained from step (iii-ii) is recycled to washing step (ii-ii) and reused there.

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In some preferred embodiments of the method, said method further comprises step (iv) contacting the chitin containing material with a binary solvent being a mixture of a first organic solvent and a second organic solvent or with a first organic solvent. Preferably, said first organic solvent is a C1 to C3 alcohol, or mixture thereof, more preferably is ethanol. Said second organic solvent is
20 preferably a C3 to C5 ketone, more preferably is propanone. In some preferred embodiments of the method, the first organic solvent to second organic solvent ratio in said binary solvent is 50-100:1-50 (v/v).

In some preferred embodiments of the method, the chitin containing material to binary solvent ratio
25 or the chitin containing material to first organic solvent ratio in step (iv) is of from 1:2 (m/v) to 1:50 (m/v), preferably is of from 1:5 (m/v) to 1:20 (m/v), more preferably of from about 1:10 (m/v), most preferably of 1:10 (m/v).

Preferably, step (iv) is performed at a temperature of from 10°C to 50°C, more preferably of from
30 15°C to 40°C, more preferably of from 20°C to 35°C, most preferably of from 25°C to 30°C. Preferably, step (iv) is performed for of from 0.1h to 10h, preferably for of from 0.5h to 10h, more

preferably of from 1h to 4h, more preferably about 2h, most preferably 2h, or more preferably of from 0.5h to 2h, more preferably about 1h, most preferably 1h.

In some preferred embodiments of the method, step (iv) is followed by step (iv-i) filtering the chitin
5 containing material of step (iv) from the binary solvent or from the first organic solvent.

In some preferred embodiments of the method, at least steps (ii) and (iii), optionally (iv) and optionally (iv-i) are carried out in the same device, preferably at least steps (ii), (ii-i), (ii-ii), (iii), (iii-i), and (iii-ii), optionally (iv) and optionally (iv-i) are carried out in the same device. Preferably,
10 the device is a closed reactor, wherein matter exchange with the surroundings is prevented or at least reduced compared to matter exchange in an open system. For maintaining the pressure below a predetermined maximum pressure value, the closed reactor has one or more pressure relief valve(s). These valves act as safety mechanisms that automatically open when the pressure in the closed reactor exceeds the predetermined maximum pressure value, wherein said maximum
15 pressure value is in some preferred embodiments 30 bar, more preferably 10 bar, more preferably 3 bar, more preferably 2 bar, more preferably 1.5 bar. In some alternative preferred embodiments, said maximum pressure value is preferably 0.75 bar, more preferably 0.5 bar. In some preferred embodiments of the method, the closed reactor comprises a filtering unit, preferably in the lower part of the reactor, more preferably at the bottom of said reactor. Said filtering unit at the bottom of
20 the closed reactor is also called filtering floor unit. The filtering unit is preferably a filter plate. Materials and dimensions of filter plates are known to the skilled person. Preferably, the filtering unit comprises or consists of a polyamide, preferably nylon (PA 6.6). Preferably, the filtering unit is a woven fabric. The filtering unit has preferably a mesh size of $>500\text{ }\mu\text{m}$, more preferably of $\geq 600\text{ }\mu\text{m}$, more preferably of $\geq 700\text{ }\mu\text{m}$, more preferably of $\geq 800\text{ }\mu\text{m}$, more preferably of $\geq 900\text{ }\mu\text{m}$
25 and/or preferably a mesh size of $<2500\text{ }\mu\text{m}$, more preferably of $\leq 2000\text{ }\mu\text{m}$, more preferably of $\leq 1800\text{ }\mu\text{m}$, more preferably of $\leq 1500\text{ }\mu\text{m}$, more preferably of $\leq 1200\text{ }\mu\text{m}$. Preferably, the filtering unit has a mesh size in the range of from >500 to $<2500\text{ }\mu\text{m}$, more preferably in the range of from 600 to 2000 μm , more preferably in the range of from 700 to 1800 μm , more preferably in the range of from 800 to 1500 μm , more preferably in the range of from 900 to 1200 μm .

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The reactor is preferably in controllable fluid connection with a suction unit. "Controllable"

regarding fluid connection means that, for example, a valve is used, which allows either a fluid connection when opened or prevents a fluid connection when closed.

In some preferred embodiments, the closed reactor comprises at least a reactor with a filtering unit, preferably a filtering floor unit, a valve, preferably arranged below the filtering floor unit, wherein said valve is connected to a suction unit, and at least a receiver vessel. Preferably, the suction unit is a Nutsche filtering unit. Preferably, the receiver vessel is a three-neck round-bottom flask with bottom drain and PTFE stopcock for solvent exchange, preferably automated solvent exchange. Overall, for example, once step (ii) is completed, the valve below the filtering floor unit can be opened and step (ii-i), i.e. the filtering of the chitin containing material of step (ii) from the aqueous organic acid, can be conducted in the same vessel by suction via the suction unit of the aqueous organic acid while the solid chitin containing material remains on the filtering floor unit (in the reactor) and the aqueous organic acid is transferred into the receiver vessel, followed by washing of the chitin containing material in step (ii-ii) on the filtering floor unit by addition of an aqueous medium onto the filtering floor unit, again followed by suction via the suction unit, now of the aqueous medium. Preferably, the closed reactor is further equipped with one or more devices for solvent addition such as funnel(s). Preferably, the closed reactor further comprises means for stirring and means for pH measurement such as a stirrer, preferably a lining stirrer, and a pH sensor, as well as means for temperature control such as heating jackets and coolers/condensers. Suitable devices are known in the art and are sold, for example, by company Ablaze Glass Works Pvt. Ltd. One suitable device is the ablaze® Glass Nutsche Filter reactor schematically shown in Fig. 12. Using such a device enables to reduce the amount of washing solvents and enables to avoid solvent losses due to solvent evaporation.

In some preferred embodiments of the method, at least steps (ii) and (iii), optionally (iv) and optionally (iv-i), preferably at least steps (ii), (ii-ii), (iii), and (iii-ii), optionally (iv) and optionally (iv-i) are carried out at a pressure in the range of from 0.1 to 30 bar, preferably in the range of from 0.1 to 10 bar, more preferably in the range of from 0.1 to 3 bar, more preferably in the range of from 0.1 to 2 bar, more preferably in the range of from 0.1 to 1.5 bar.

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In some preferred embodiments of the method, at least steps (ii) and (iii), optionally (iv) and

optionally (iv-i), preferably at least steps (ii), (ii-ii), (iii), and (iii-ii), optionally (iv) and optionally (iv-i) are carried out at a pressure of at most 0.75 bar, preferably at most 0.5 bar, preferably at a pressure in the range of from 0.2 to 0.75 bar, more preferably at a pressure in the range of from 0.2 to 0.5 bar.

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In some preferred embodiments of the method, said method further comprises step (iv-ii) evaporating the binary solvent or the first organic solvent after of step (iv-i), thereby obtaining astaxanthin.

10 In some preferred embodiments of the method, said method is a method of producing a calcium salt of an organic acid and preferably comprises an additional step of drying said calcium salt of an organic acid obtained as a filtrate in step (ii-i).

15 In some alternatively preferred embodiments of the method, said method is a method of producing a protein product and preferably comprises drying the protein product obtained as a filtrate in step (iii-i).

In some alternatively preferred embodiments of the method, said method is a method of producing an astaxanthin, and preferably comprises drying the astaxanthin obtained in the filtrate of step (iv-
20 i).

In some alternatively preferred embodiments of the method, said method is a method of producing chitin and comprises step (v) obtaining chitin as the product of step (ii), (iii), or (iv), or as the retentate of step (iv-i).

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Preferably, the chitin obtained in step (v) has a purity of at least 90%, more preferably of at least 95%, more preferably of at least 98%, more preferably of at least 99%.

As indicated also below, the chitin containing material is preferably crustacean shells. Crustacean
30 shells comprise a plurality of compounds, one compound being chitin, wherein the crustacean shells comprise up to 50 weight-% of chitin based on the total weight of the crustacean shells (being

100 weight-%), i.e. the chitin containing material milled in step (i) comprises up to 50 weight-% of chitin based on the total weight of the chitin containing material (the crustacean shells). In accordance with the present invention, the yield (weight of chitin obtained in step (v) in relation to the total weight of crustacean shells) is preferably in the range of from 20 to 90 %, more preferably in the range of from 20 to 50 %, more preferably in the range of from 20 to 40 %, or more preferably in the range of from 20 to 30 %. Preferably, the crustacean shells in this case are from Shrimp. Also in accordance with the present invention, the yield (weight of chitin obtained in step (v) in relation to the total weight of crustacean shells) is preferably in the range of from 20 to 50 %, more preferably in the range of from 20 to 40 %. Preferably, the crustacean shells in this case are from Crayfish.

In some alternatively preferred embodiments of the method, said method is a method for producing chitin and at least one of a calcium salt of an organic acid, a protein product, and astaxanthin from chitin containing material.

In some preferred embodiments of the method, the chitin containing material are crustacean shells, preferably crustacean shells from crayfish, more preferably crustacean shells from marbled crayfish (*Procambarus virginalis*).

In some preferred embodiments of the method, said method comprises steps (i) to (iv) in consecutive order.

The present invention further relates to a method for producing a fertilizer, comprising the steps of the method as described above and optionally the steps of formulating the product obtained as fertilizer.

The present invention further relates to a method of producing a food or feed, comprising the steps of the method as described above and optionally the step of formulating the product as food or feed.

The present invention further relates to a method of producing a feed or food additive, comprising

the steps of the method as described above and optionally the step of formulating the product as food or feed additive.

5 The present invention further relates to a method of producing a chitin and at least one of a calcium salt of an organic acid, a protein product, and astaxanthin from chitin containing material, the comprising the steps as described above.

10 The present invention further relates to a chitin preparation, a calcium salt of an organic acid, a protein product, or an astaxanthin produced or producible from chitin containing material according to a method as described above.

The chitin preparation, calcium salt of an organic acid, protein product, or astaxanthin preferably comprises chitin at a concentration of from 15% to 50%, preferably from 25% to 40%, most preferably of 35% (m/m).

15 The chitin preparation, calcium salt of an organic acid, protein product, or astaxanthin preferably comprises acetyl-glucosamine and/or glucosamine at a concentration of from 15% to 50%, preferably from 25% to 40%, most preferably of 35% (m/m).

20 Preferably, the temperature of the first thermal event of the chitin preparation in thermal gravimetric analysis is at most 55°C, more preferably at most 52°C, more preferably at most 51°C, most preferably at most 49°C.

25 The present invention further relates to a use of a calcium salt of an organic acid, a protein product, or an astaxanthin as described above as a fertilizer, a food or feed, a feed or food additive, a cosmetic or an antioxidant.

30 The present invention further relates to a use of a chitin preparation as described above in manufacture of a natural or synthetic polymer, in agriculture, in packaging, in construction, or in environmental protection, as an ingredient in the production of a medicament, a food, or a cosmetics, and/or as a solidified enzyme carrier.

In view of the above, the following embodiments are particularly envisaged:

- 5 Embodiment 1: A method for treating a chitin containing material comprising
- (i) milling the chitin containing material;
 - (ii) contacting the milled chitin containing material from step (i) with a mixture comprising an aqueous solution of an organic acid; and
 - (iii) contacting the chitin containing material with an alkaline solution comprising
- 10 water:glycerol in a ratio of 50-100:1-50 (v/v) and potassium hydroxide at a concentration of from 0.2M to 5M, preferably of from 0.5M to 2 M, more preferably of about 1M, most preferably of 1M;
- thereby treating the chitin containing material.
- 15 Embodiment 2: The method of embodiment 1 comprising (i), (ii) and (iii), wherein (i), (ii) and (iii) are preferably carried out in consecutive order, so that (i) is followed by (ii), which is then followed by (iii).
- Embodiment 3: The method of embodiment 1 or 2, wherein said milling is grinding in a knife mill.
- 20 Embodiment 4: The method of any one of embodiments 1 to 3, wherein step (i) is followed by step (i-i) separation of the milled chitin containing material from step (i) into at least two fractions based on particle size, preferably by mechanical separation, more preferably by sieving.
- 25 Embodiment 5: The method of embodiment 4, wherein step (i-i) is followed by step (i-ii) obtaining at least one fraction containing chitin containing material, wherein at least 70 weight-%, preferably at least 80 weight-%, more preferably at least 90 weight-% of said fraction have a particle size smaller than 18mesh (1mm) and preferably larger than 45mesh (354 μ m), based on the total weight of said fraction being 100 weight-%.
- 30 Embodiment 6: The method of any one of embodiments 1 to 5, wherein said organic acid is citric

acid, maleic acid, or a mixture thereof.

Embodiment 7: The method of any one of embodiments 1 to 6, wherein said aqueous organic acid has a concentration of from 0.2M to 5M, preferably of from 0.5M to 2M, more preferably about 1M, most preferably 1M.

Embodiment 8: The method of any one of embodiments 1 to 7, wherein the chitin containing material to aqueous organic acid ratio during step (ii) is of from 1:2 (m/v) to 1:50 (m/v), preferably is of from 1:5 (m/v) to 1:20 (m/v), more preferably of from about 1:10 (m/v), most preferably of 1:10 (m/v).

Embodiment 9: The method of any one of embodiments 1 to 8, wherein step (ii) is performed at a temperature of from 20°C to 100°C, more preferably of from 50°C to 90°C, more preferably about 70°C, most preferably of 70°C.

Embodiment 10: The method of any one of embodiments 1 to 9, wherein said step (ii) is performed for of from 0.1h to 30h, more preferably for of from 0.5h to 30h, more preferably for of from 1h to 30h, more preferably of from 1.5h to 12h, more preferably of from 2h to 5h more preferably about 2.5h, most preferably 2.5h, or more preferably of from 1h to 2h, most preferably about 2h, more preferably about 2h, most preferably 2h.

Embodiment 11: The method of any one of embodiments 1 to 10, wherein step (ii) is followed by step (ii-i) filtering the chitin containing material of step (ii) from the aqueous organic acid.

Embodiment 12: The method of any one of embodiments 1 to 11, wherein step (ii) and optionally step (ii-i) is followed by step (ii-ii) washing to neutral pH, preferably in the range of from pH 6 to 9, more preferably of from pH 6.5 to 8, still more preferably about pH 7; wherein washing to neutral pH is preferably done with an aqueous medium, wherein preferably at least 95 weight-% of said aqueous medium are water.

Embodiment 13: The method of embodiment 12, wherein in the range of from 0.1 to 15 liter,

preferably in the range of from 1 to 10 liter, more preferably in the range of from 1 to 5 liter, of aqueous medium are used for (ii-ii) per kg of chitin containing material obtained from (ii) or (ii-i).

Embodiment 14: The method of any one of embodiments 1 to 13, wherein the ratio of chitin containing material to water/glycerol mixture in step (iii) is from 1:2 (m/v) to 1:50 (m/v), preferably is of from 1:5 (m/v) to 1:20 (m/v), more preferably of from about 1:10 (m/v), most preferably of 1:10 (m/v).

Embodiment 15: The method of any one of embodiments 1 to 14, wherein step (iii) is performed at a temperature of from 40°C to 100°C, preferably of from 50°C to 90°C, more preferably about 70°C, most preferably of 70°C.

Embodiment 16: The method of any one of embodiments 1 to 15, wherein said step (iii) is performed for of from 0.1h to 30h, more preferably for of from 0.5h to 30h, more preferably for of from 1h to 30h, preferably of from 3h to 12h, more preferably about 6h, most preferably 6h, or for preferably of from 1h to 10h, more preferably about 3h, most preferably 3h.

Embodiment 17: The method of any one of embodiments 1 to 16, wherein step (iii) is followed by step (iii-i) filtering the chitin containing material of step (iii) from the alkaline solution.

Embodiment 18: The method of any one of embodiments 1 to 15, wherein step (iii) and optionally step (iii-i) is followed by step (iii-ii) washing to neutral pH, preferably in the range of from pH 6 to 9, more preferably of from pH 6.5 to 8, still more preferably about pH 7; wherein washing to neutral pH is preferably done with an aqueous medium, wherein preferably at least 95 weight-% of said aqueous medium are water.

Embodiment 19: The method of embodiment 18, wherein in the range of from 0.1 to 15 liter, preferably in the range of from 1 to 10 liter, more preferably in the range of from 1 to 5 liter, of aqueous medium are used for (ii-ii) per kg of chitin containing material obtained from (ii) or (ii-i).

Embodiment 20: The method of any one of claims 1 to 19, wherein at least steps (ii) and (iii) are

carried out in the same device, preferably at least steps (ii), (ii-i), (ii-ii), (iii), (iii-i), and (iii-ii), are carried out in the same device.

Embodiment 21: The method of any one of embodiments 1 to 20, wherein said method further
5 comprises step (iv) contacting the chitin containing material with a binary solvent being a mixture of a first organic solvent and a second organic solvent or contacting the chitin containing material with a first organic solvent.

Embodiment 22: The method of any one of embodiments 1 to 21, wherein said first organic solvent
10 is a C1 to C3 alcohol, or mixture thereof, preferably is ethanol.

Embodiment 23: The method of any one of embodiments 1 to 22, wherein said second organic solvent is a C3 to C5 ketone, preferably is propanone.

15 Embodiment 24: The method of any one of embodiments 1 to 23, wherein the first organic solvent:second organic solvent ratio in said binary solvent is 50-100:1-50 (v/v).

Embodiment 25: The method of any one of embodiments 1 to 24, wherein the chitin containing material to binary solvent ratio or the chitin containing material to first organic solvent ratio in step
20 (iv) is of from 1:2 (m/v) to 1:50 (m/v), preferably is of from 1:5 (m/v) to 1:20 (m/v), more preferably of from about 1:10 (m/v), most preferably of 1:10 (m/v).

Embodiment 26: The method of any one of embodiments 1 to 25, wherein step (iv) is performed at a temperature of from 10°C to 50°C, preferably of from 15°C to 40°C, more preferably of from
25 20°C to 35°C, most preferably of from 25°C to 30°C.

Embodiment 27: The method of any one of embodiments 1 to 26, wherein step (iv) is performed for of from 0.1h to 10h, preferably for of from 0.5h to 10h, more preferably of from 1h to 4h, more preferably about 2h, most preferably 2h, or more preferably of from 0.5h to 2h, more preferably
30 about 1h, most preferably 1h.

Embodiment 28: The method of any one of embodiments 1 to 27, wherein step (iv) is followed by

step (iv-i) filtering the chitin containing material of step (iv) from the binary solvent or from the first organic solvent.

Embodiment 29: The method of any one of embodiments 21 to 28, wherein at least steps (ii) and (iii), optionally (iv) and optionally (iv-i) are carried out in the same device, preferably at least steps (ii), (ii-i), (ii-ii), (iii), (iii-i), and (iii-ii), optionally (iv) and optionally (iv-i) are carried out in the same device.

Embodiment 30: The method of embodiment 20 or 29, wherein the device comprises a closed reactor, said closed reactor preferably comprising a filtering unit, preferably at the bottom of said reactor (filtering floor unit), the filter (floor) unit preferably being a filter plate, wherein the reactor is optionally in controllable fluid connection with a suction unit.

Embodiment 31: The method of embodiment 30, wherein the closed reactor comprises at least a reactor with a filtering unit, a valve connected to a suction unit and at least a receiver vessel.

Embodiment 32: The method of any one of embodiments 29 to 31, wherein the filtering unit comprises or consists of a polyamide, preferably nylon (PA 6.6).

Embodiment 33: The method of any one of embodiments 29 to 32, wherein the filtering unit is a woven fabric.

Embodiment 34: The method of any one of embodiments 29 to 33, wherein the filtering unit has a mesh size of $>500\text{ }\mu\text{m}$, preferably of $\geq 600\text{ }\mu\text{m}$, more preferably of $\geq 700\text{ }\mu\text{m}$, more preferably of $\geq 800\text{ }\mu\text{m}$, more preferably of $\geq 900\text{ }\mu\text{m}$ and/or wherein the filtering unit has a mesh size of $<2500\text{ }\mu\text{m}$, preferably of $\leq 2000\text{ }\mu\text{m}$, more preferably of $\leq 1800\text{ }\mu\text{m}$, more preferably of $\leq 1500\text{ }\mu\text{m}$, more preferably of $\leq 1200\text{ }\mu\text{m}$.

Embodiment 35: The method of any one of embodiments 29 to 34, wherein the filtering unit has a mesh size in the range of from >500 to $<2500\text{ }\mu\text{m}$, preferably in the range of from 600 to 2000 μm , more preferably in the range of from 700 to 1800 μm , more preferably in the range of from 800 to

1500 μm , more preferably in the range of from 900 to 1200 μm .

Embodiment 36: The method of any one of embodiments 29 to 35, wherein in step (i-ii) at least one fraction containing chitin containing material is obtained, wherein at least 70 weight-%, preferably at least 80 weight-%, more preferably at least 90 weight-% of said fraction have a particle size smaller than 10 mesh (2mm) and preferably larger than 18 mesh (1mm), more preferably a particle size in the range of from smaller than 10 mesh (2mm) to larger than 14 mesh (1410 μm).

Embodiment 37: The method of any one of embodiments 29 to 36, wherein at least steps (ii) and (iii), optionally (iv) and optionally (iv-i), preferably at least steps (ii), (ii-ii), (iii), and (iii-ii), optionally (iv) and optionally (iv-i) are carried out at a pressure in the range of from 0.1 to 30 bar, preferably in the range of from 0.1 to 10 bar, more preferably in the range of from 0.1 to 3 bar, more preferably in the range of from 0.1 to 2 bar, more preferably in the range of from 0.1 to 1.5 bar, or at a pressure of at most 0.75 bar, preferably at a pressure in the range of from 0.2 to 0.75 bar.

Embodiment 38: The method of any one of embodiments 29 to 37, wherein from washing step (iii-ii), an aqueous medium comprising potassium hydroxide is obtained, which is recycled as to washing step (ii-ii) and used as aqueous medium in said washing step (ii-ii).

Embodiment 39: The method of any one of embodiments 1 to 38, wherein said method further comprises step (iv-ii) evaporating the binary solvent or the first organic solvent after step (iv-i), thereby obtaining astaxanthin.

Embodiment 40: The method of any one of embodiments 11 to 41, wherein said method is a method of producing a calcium salt of an organic acid and preferably comprises an additional step of drying said calcium salt of an organic acid obtained as a filtrate in step (ii-i).

Embodiment 41: The method of any one of embodiments 17 to 42, wherein said method is a method of producing a protein product and preferably comprises drying the protein product obtained as a filtrate in step (iii-i).

30

Embodiment 42: The method of any one of embodiments 28 to 41, wherein said method is a method

of producing an astaxanthin, and preferably comprises drying the astaxanthin obtained in the filtrate of step (iv-i).

Embodiment 43: The method of any one of embodiments 1 to 42, wherein said method is a method
5 of producing chitin and comprises step (v) obtaining chitin as the product of step (ii), (iii), or (iv), or as the retentate of step (iv-i).

Embodiment 44: The method of embodiment 43, wherein the chitin obtained in (v) has a purity of
10 at least 90%, preferably of at least 95%, more preferably of at least 98%, more preferably of at least 99%.

Embodiment 45: The method of any one of embodiments 1 to 44, wherein said method is a method
for producing chitin and at least one of a calcium salt of an organic acid, a protein product, and
astaxanthin from chitin containing material.

15

Embodiment 46: The method of any one of embodiments 1 to 45, wherein the chitin containing
material are crustacean shells, preferably crustacean shells from crayfish, more preferably
crustacean shells from marbled crayfish (*Procambarus virginalis*).

20 Embodiment 47: The method of embodiment 46, wherein the crustacean shells from crayfish,
wherein the yield (weight of chitin obtained in step (v) in relation to the total weight of crustacean
shells) is in the range of from 20 to 50 %, preferably in the range of from 20 to 40 %.

Embodiment 48: The method of any one of embodiments 1 to 45, wherein the chitin containing
25 material are crustacean shells, preferably crustacean shells from shrimp.

Embodiment 49: The method of embodiment 48, wherein the crustacean shells are from shrimp,
wherein the yield (weight of chitin obtained in step (v) in relation to the total weight of crustacean
shells) is in the range of from 20 to 90 %, preferably in the range of from 20 to 50 %, more
30 preferably in the range of from 20 to 40 %, or more preferably in the range of from 20 to 30 %.

Embodiment 50: The method of any one of embodiments 1 to 49, wherein said method comprises steps (i) to (iv) in consecutive order.

Embodiment 51: A method for producing a fertilizer, comprising the steps of the method of any one of embodiments 11 to 0 and optionally the steps of formulating the product obtained as fertilizer.

Embodiment 52: A method of producing a food or feed, comprising the steps of the method of any one of embodiments 17 to 51 and optionally the step of formulating the product as food or feed.

Embodiment 53: A method of producing a feed or food additive, comprising the steps of the method of any one of embodiments 17 to 52 and optionally the step of formulating the product as food or feed additive.

Embodiment 54: A method of producing a chitin and at least one of a calcium salt of an organic acid, a protein product, and astaxanthin from chitin containing material, comprising the steps of any one of embodiments 1 to 53.

Embodiment 55: A chitin preparation, a calcium salt of an organic acid, a protein product, or an astaxanthin produced or producible from chitin containing material according to a method of any one of embodiments 1 to 54.

Embodiment 56: The chitin preparation, calcium salt of an organic acid, protein product, or astaxanthin of embodiment 55, comprising chitin at a concentration of from 15% to 50%, preferably from 25% to 40%, most preferably of 35% (m/m).

Embodiment 57: The chitin preparation, calcium salt of an organic acid, protein product, or astaxanthin of embodiment 55 or 56, comprising acetyl-glucosamine and/or glucosamine at a concentration of from 15% to 50%, preferably from 25% to 40%, most preferably of 35% (m/m).

Embodiment 58: The chitin preparation of any one of embodiments 55 to 56, wherein the

temperature of the first thermal event in thermal gravimetric analysis is at most 55°C, preferably at most 52°C, more preferably at most 51°C, most preferably at most 49°C.

Embodiment 59: Use of a calcium salt of an organic acid, a protein product, or an astaxanthin
5 according to any one of embodiments 55 to 58 as a fertilizer, a food or feed, a feed or food additive, a cosmetic or an antioxidant.

Embodiment 60: Use of a chitin preparation according to any one of embodiments 55 to 59 in
10 manufacture of a natural or synthetic polymer, in agriculture, in packaging, in construction, or in environmental protection, as an ingredient in the production of a medicament, a food, or a cosmetics, and/or as a solidified enzyme carrier.

All references cited in this specification are herewith incorporated by reference with respect to their
15 entire disclosure content and the disclosure content specifically mentioned in this specification.

FIGURES

- 20 **Fig. 1:** shows the IR Spectrum of chitin extracted from Shrimp shells via sustainable method.
- Fig. 2:** shows the IR Spectrum of chitin extracted from crayfish shells via sustainable method.
- Fig. 3:** shows the IR Spectrum of chitin extracted from crayfish shells via classic method.
- 25 **Fig. 4:** shows the IR Spectrum of dry shrimp shells.
- Fig. 5:** shows the IR Spectrum of dry crayfish shells.
- Fig. 6:** shows the XRD Spectrum of chitin extracted from shrimp shells via sustainable method.
- Fig. 7:** shows the XRD Spectrum of chitin extracted from crayfish shells via sustainable
30 method.
- Fig. 8:** shows the XRD Spectrum of chitin extracted from crayfish shells via classic method.

Fig. 9: shows the thermal decomposition graphic of chitin extracted from shrimp shells via sustainable method; wherein “Cel” represents “°Celsius”.

Fig. 10: shows the thermal decomposition graphic of chitin extracted from crayfish shells via sustainable method; wherein “Cel” represents “°Celsius”.

5 **Fig. 11:** shows the thermal decomposition graphic of chitin extracted from crayfish shells via classic method; wherein “Cel” represents “°Celsius”.

Fig. 12: shows a schematic representation of a closed, semi-automated reactor (Glass Nutsche Filter reactor of company Ablaze Glass Works Pvt. Ltd.) comprising a 5 Liter jacketed reactor (1), vessel cover (lid) (2), Lab type condenser (3), S bend (4),
10 additional funnel (5), PTFE propeller type stirrer (6), GFT filter plate (7), zero hold drain valve (8), receiver vessel (9), quick release clamp (10), clamp (11), structure support (12), utility manifold (13), Bola connectors (14), hose connector (15), cowl seal (16), VELP motor (17), heating system (18). The semi-automated reactor further has (not shown in Fig. 12 as being located behind the propeller type stirrer
15 (6)) at least one pressure relief valve.

Fig. 13 shows the chitin recovery efficiency achieved for Experiment IX compared to the chitin recovery efficiency achieved for Experiments IV to VIII.

20 EXAMPLES

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

25 1 Extraction Protocols

1.1 Pre-treatment

1.1.1 Shrimp shells

30

Shrimp, *Litopenaeus vannamei*, were obtained from the company Villa Pescados

(<https://villapescados.com.br/>), located in the city of Parnamirim - RN- Brazil and company Boutique Oceânica (<https://www.boutiqueoceanica.com/>), located in the city of Vargem Pequena - RJ – Brazil. The shrimp collected (type 80/100) has an average weight of 11g and length of 12 cm. After a careful separation of the protein part of the shrimp, the sample of crustacean exoskeletons (shrimp shell residues) was washed in hot water at a temperature of 80°C for 2h with stirring. After washing, protein residues still attached to the shells were carefully removed with the help of tweezers. Afterwards, the samples were dried in an oven with air circulation for 24h at 90°C. Once dried, the exoskeleton samples were grounded in a willey knife mill and sieved to obtain particles smaller than 18 mesh (1000µm).

1.1.2 Crayfish shells

Crayfish, *Procambarus virginalis*, were collected from lake Reilingen in the state of Baden-Württemberg in Germany with the approval of local fishery authorities in Germany and under research permits No. 58/19/MEDD/SG/DGF/DSAP/SCB.Re. After peeling, shells were washed and protein residues still attached to the shells were carefully removed with the help of tweezers. Afterwards, the samples were dried in an oven with air circulation for 24h at 90°C and grounded in a knife mill and sieved to obtain particles sizes between 18 and 45 mesh (1000 - 354 µm).

1.2 Classic Method for chitin extraction (State of the art)

1.2.1 Demineralization

The demineralization of the material was carried out at room temperature (25 °C) with a magnetic stirrer for 6h, with a ratio of 1:10 (m/v) using an aqueous solution of HCl at a concentration of 1M. The demineralized product was washed and filtered until a neutral pH was reached. After washing, the material was dried in an oven for 12 hours at 70°C.

1.2.2 Deproteinization

After the previous step (1.2.1), the dried and demineralized material obtained was suspended in

a solution containing water and 1M NaOH, with a ratio of 1:10 (m/v), at room temperature for 6 hours, then the samples were dried in an oven for 12h at 70°C.

1.2.3 Deodorization/Clarification

5

In the clarification step, the material from the previous step (1.2.2) was added to an aqueous solution of 0.5M sodium hypochlorite. The purpose of this step was to reduce the odor from the material and the removal of pigments. It was then washed with water to remove the remaining sodium hypochlorite, until pH is neutral. After washing, it was necessary to dry the product
10 obtained (wet chitin). The drying occurred at a temperature of 60 °C for 24 hours before the obtainance of the final product for laboratory analysis.

1.3 Sustainable Method for chitin extraction (inventive method)

1.3.1 Demineralization

15

The demineralization of the material was carried out at a temperature of 70 °C with a magnetic stirrer for 6h, with a ratio of 1:10 (m/v) using an aqueous solution of citric acid at a concentration of 1M. After 2.5h the material precipitated. The demineralized product was
20 washed with distilled water and filtered until it reached neutral pH, after washing the material was dried in an oven for 12 hours at 70°C or until the material was completely dry. After drying, the product's dry mass and yield of this step was determined.

1.3.2 Deproteinization

25

After the previous step (1.3.1), the dry and demineralized material obtained, with a ratio of 1:10 (m/v) in relation to the total volume, was suspended in a solution containing water/glycerol in a ratio 1:1 (v/v) and KOH at 1M concentration. The mixture was heated at 70°C for 6h, then the sample was filtered and washed with distilled water, until the sample was clear. Afterwards,
30 the sample was dried in an oven for 12 hours at 70°C or until it was completely dry. After this process, the dry mass of the product obtained was measured using a precision balance. These

data on the weight obtained was registered to calculate the final yield (see summary below, Table 4).

1.3.3 Deodorization/Depigmentation

After the previous step (1.3.2), the product obtained was added to a binary mixture of ethanol 85%/propanone 90% (1:1) or to ethanol only and in the proportion of 1:10 (m/v), at room temperature and under stirring for 2h. Then the precipitate was filtered and washed until excess ethanol was removed and dried at 70 °C for 12h before submitting it for laboratory analysis.

Herein below, any “yield” indicated in % is based on weight of chitin obtained (mP = product mass after all extraction protocol in grams) in relation to the total weight of crustacean shells used (mT = total mass of biomass used in grams), i.e. $\text{Yield} = \text{mP}/\text{mT}$.

2 Examples related to method validation

2.1 Experiment I (method validation)

To carry out the first experiment, 31.8879 g of dry shrimp shells (<18 mesh (<1000µm)) were used. The extraction was done according to the inventive sustainable protocol for chitin extraction described in 1.3. The product mass was measured in a precision analytical balance and 22.37g was obtained. After this process, the resulting sample had to be sealed and stored to prevent the entry of moisture and placed in refrigeration (-15°C). The extracted sample was quite clarified and there was no need for the clarification step. The result found was a yield of 26.69%.

2.2 Experiment II (method validation)

For the realization of the Experiment II, the same procedure as in “Experiment I” was used, wherein the extraction was done according to the inventive sustainable protocol for chitin

extraction described in 1.3., with 74.8950g of dried shrimp shell. The main difference was in the particle size, 18 to 45 mesh (1000µm to 354µm) were used. The result found was a yield of 81.52%

5 **2.3 Experiment III (method validation)**

In Experiment III, the extraction was done according to the inventive sustainable protocol for chitin extraction described in 1.3., using the same parameters as in experiment Experiment I. Total of 79.9385g of dried crayfish shells 18 to 45 mesh (1000µm to 354µm) were used. The result found was a yield of 20.10%

2.4 Experiment IV and V (method validation)

In Experiment IV and Experiment V, 30.0182g of dry shrimp shell and 30.1800g of dry crayfish shell were used. Both experiments were carried out according to the inventive sustainable protocol for chitin extraction described in 1.3., wherein for each shell type 18 to 45 mesh (1000µm to 354µm) were used. The result was a yield of 42.85% (IV) and 33.40% (V).

20 **3 Examples related to standardization**

3.1 Experiments VI and VII (standardization)

For Experiment VI and Experiment VII, 30.0125g of shrimp shell and 29.9367g of crayfish shell were used. The extraction was done according to the inventive sustainable protocol for chitin extraction described in 1.3., without any change in the method described in topic 1.3.2. The final yield of this extraction was 24.62% and 26.04% respectively. The extraction of chitin from the crayfish shell showed a slightly higher yield. 18 to 45 mesh (1000µm to 354µm) were used. The result found was a yield of 24.62% (VI) and 26,04% (VII).

30

3.2 Comparative Experiment VIII (standardization)

In Comparative Experiment VIII, the classic chitin extraction protocol described above 1.2. was used, with 30.0849 g of dried crayfish shell 18 to 45 mesh (1000 μ m to 354 μ m). It only had a modification in the deproteinization step with 1M NaOH, which was performed by heating at 70 °C for 6 hours. This change caused an increase in the clarification of the sample and also resulted in a decrease of total yield extraction. The result found was a yield of 14.29%.

4 Analysis

4.1 Infrared spectroscopy (FTIR)

FTIR spectra of chitins were acquired using a FTIR spectrometer (Thermo Fisher Scientific) over the wavenumber range of 4000–500 cm^{-1} . FTIR spectra were recorded by accumulation of at least 64 scans, with a resolution of 4 cm^{-1} using the OMNIC software (Thermo Fisher Scientific).

4.1.1 Chitin from shrimp and crayfish shells (sustainable method)

Chitin from shrimp and crayfish shells extracted by a sustainable method proved to be very similar to each other, presenting the same characteristic peaks: the peak at 3400 cm^{-1} was attributed to the -NH₂ and -OH groups stretching vibration and intermolecular hydrogen bonding. In both spectrums, the two bands at 1659 and 1624 cm^{-1} corresponded to the stretching of amide. The band at 1659 cm^{-1} was assigned to stretching of the C=O group hydrogen bonded to N-H of the neighboring intra-sheet chain, and the 1624 cm^{-1} band could indicate a specific hydrogen bond of C=O with the hydroxyl-methyl group of the next chitin residue of the same chain.

In both FTIR spectrums 3 peaks at about 1650, 1620 and 1550 cm^{-1} indicated that chitin from shrimp shells and crayfish extracted by sustainable method was in alpha form (Fig. 1 and 2). Alpha form of chitin is quite common and it was found in numerous studies.

4.1.2 Chitin from crayfish shells (classic method)

Unlike the spectrums from chitin by sustainable method (Fig. 2), a single peak was observed
5 in chitin by classic method at about 1412 cm⁻¹ (Fig. 3), not showing characteristics of chitin in
alpha form, this indicates a high degree of degradation with the classic method which is more
aggressive than the sustainable method used and doesn't allow the recovery of by-products
generated in each step, furthermore negatively affecting the properties of the extracted chitin,
the further processing by injection/extrusion and application of the chitin in molded bioplastic
10 products.

4.1.3 Dry shrimp and crayfish shells

As observed in the spectrums of dry shrimp and crayfish shells (Fig. 5 and Fig. 6) there was no
15 characteristic in both spectrums of the dried shells similar to those of the extracted chitins.

4.1.4 X-ray diffractometry (XRD)

XRD analyses were performed from 5° to 50° using a diffractometer without Ni-filtered, Cu-
20 K α radiation ($\lambda=1.542\text{\AA}$). The diffractometer was operated at 40 kV and 40 mA with 1°
diverging and receiving slits and the scans were carried out with a step size of 0.020° and a step
time of 0.2s.

The XRD charts of the chitins extracted showed a high degree of crystallization in their
25 structures and in general were very similar, reinforced with a 2° angle in the approximate
positions, indicating similarity in how chains were organized. The similarity was greater
between chitin extracted from shrimp and crayfish via sustainable method (Fig. 6 and Fig. 7),
while the classic method crayfish chitin presented a graphic that characterized a slightly more
accentuated crystallinity (Fig. 8). It was possible to see that the X-ray patterns showed refraction
30 peaks located at 8.9, 12.0, 18.9, 22.8 and 26.0°, which were characteristic of the crystalline form
of α -chitin and consistent with the reports of other works. Alpha-chitin was the structure with

the highest degree of crystallinity, when compared to β or γ -chitin, due to the antiparallel structure of the polymer chains. The peaks located at 8.8° and 18.8° had great intensity and distinction, which could indicate the crystallinity of the samples.

5 *4.1.5 Thermal Analysis - Thermogravimetry (TGA) and Differential Scanning Calorimetry (DSC)*

TGA analysis was performed with a thermogravimetric analyzer (DuPont Instrument). Samples (about 10 mg) were heated in open alumina pans, in a helium atmosphere from
10 30°C up to about 800°C at a heating rate of $10^\circ\text{C}/\text{min}$. Ash content (%) and moisture content (%) were determined from the TGA thermogram using Universal Analysis software (TA instruments). The quantification of carbon content was a powerful technique for the measurement of thermal stability of materials including Biopolymers.

15 Fig. 9 shows the thermal decomposition graphic of chitin extracted from shrimp shells via sustainable method, Fig. 10 shows the thermal decomposition graphic of chitin extracted from crayfish shells via sustainable method, and Fig. 11 shows the thermal decomposition graphic of chitin extracted from crayfish shells via classic method.

20 The 1st main thermal event was a very broad endothermic peak beginning at around 40°C and centered at around 40 to 50°C , ascribed to the release of weakly (sorbed) and then strongly hydrogen-bonded water. Other peak (endo) ranged very narrowly from around 147 to 152°C , with no significant variations among samples.

25 The 2nd main thermal event was an exothermic peak (chitin did not melt but decomposed) centered at around 340 to 350°C , associated with the main step, the amine residue elimination of the chemically complex thermal decomposition of chitins.

30 Comparing the thermal decomposition graphics of the three samples, it could be seen that chitin extracted from crayfish via classic method had the lowest decomposition temperature (328.2°C), in addition to at least 3 clear decomposition regions in the degradation profile, as

shown by differential thermal gravimetry (DTG, at approximately 328°C, 500°C and 666°C), the first and third being the most notable. From the DSC curve, it could be seen that the first two decompositions were safely related to exothermic mass loss events. Mass loss between room temperature and 200°C was water loss. The chitin extracted from crayfish via sustainable method presented a generally similar profile, with water loss between room temperature and 200°C, but it also presented particularities. The first mass loss after 200°C that was observed and the exothermic decomposition temperature at 345.2°C, a little higher than in the case of the chitin from shrimp. Then there were two more decompositions around 472°C and 617°C, the first certainly exothermic. This sample differed from the chitin classic method one at the decomposition point close to 617°C. This sample had this region of decomposition more defined and prominent, in addition to having a higher temperature, indicating a structural difference between the materials. In the chitin extracted from shrimp, the degradation profile was significantly different. This material presented the usual water loss of these samples between room temperature and 200°C, but the rest of the profile differed significantly. In comparison, it had only the first peak similar and at a higher temperature (355°C) of an exothermic nature. In addition, there was an exothermic mass loss event between about 400°C and 680°C where there was no set peak and the mass loss occurs more or less gradually. The material of all samples was almost completely decomposed around 700°C. An overview of the main thermal events is shown in Table 1 below:

Table 1: Main thermal event in DSC experiments performed

Sample/method	1st Thermal event T(°C)	2nd Thermal event T(°C)	3rd Thermal event T(°C)	4th Thermal event T(°C)	5th Thermal event T(°C)
Chitin extracted from crayfish shells / classic method	56.7	343.7	491.4	528.1	666.7
Chitin extracted from crayfish shells / Sustainable method	48.6	345.2	346.8	472.1	617.9

Chitin extracted from shrimp shells / Sustainable method	50.7	355.2	364.6	378.8	679.1
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4.1.6 Potentiometric titration

Chitin (0.25 g) was dissolved in 12.5 ml of 0.1014M standard HCl solution. The solution was then topped up to 50 ml with distilled water and calculated amounts of KCl (0.28 g) were added to adjust the ionic strength to 0.1. The titrate was a standard solution of 0.04943M NaOH. The titrate was added until the pH value reaches 2.0. The standard NaOH was then added stepwise and the pH values of the solution were recorded and a curve with two inflection points was obtained. The difference of NaOH solution volumes between these points corresponds to the acid consumed for salinification of the amine groups of chitin and allowed the determination of DD% (degree of deacetylation) of the chitin.

The DD% was calculated from the relation:

$$DD\% = \frac{(1 - 161Q)}{(1 + 42Q)}$$

$$\text{Where } Q = \frac{N\Delta V}{m}$$

ΔV was the volume of NaOH consumed between the two inflection points (in liter), N was the concentration of NaOH (0.05 mol/L) and m was the dry weight of chitosan (in g)

To perform the titration, the following pH standards were used:

20 Buffer Solution pH 4.00 + 0.01 to 25.0°C + 0.2°C.

Buffer Solution pH 7.00 + 0.02 to 25.0°C + 0.2°C.

Buffer Solution pH 9.00 + 0.02 to 25.0°C + 0.2°C.

Table 2 below shows the results.

25

Table 2: Potentiometric titration and deacetylation degree (DD%) results

Sample / method	V1 (ml)	pH 1	V2 (ml)	pH 2	Δ Volume	DD%
Chitin extracted from crayfish shell / sustainable	15.6	6.418	17.59	8.521	1.99	38.04%
chitin extracted from crayfish shell / classic method	12.9	4.15	18.52	8.37	5.62	38.23%
Chitin extracted from shrimp shell / sustainable	23.80	4.633	25.1255	8.29	1.323	37.90%

4.1.7 Standard Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement – ASTM D792

5 Method: B (alcohol)

Laboratory Temperature: 21.9°C Laboratory Humidity: 46% Immersion

Fluid Temperature: 22.5°C

Immersion Fluid: Ethyl Alcohol Immersion

Fluid Density: 0.7911 g/cm³

10 Equipment: Shimadzu Analytical Scale, model AUW220D

Table 3 shows the results.

Table 3: Density results (g/cm³)

Sample / method	1	2	3	4	5	Average density	Standard deviation
Chitin extracted from crayfish shells / classic method	1.4584	1.453	1.4498	1.459	1.453	1.4546	0.0039
Chitin extracted from crayfish shells / sustainable method	1.6071	1.5076	1.6042	1.5597	1.6011	1.5759	0.0428

Chitin extracted from shrimp shells / sustainable method	1.3244	1.3188	1.3165	1.357	1.358	1.3349	0.0208
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4.2 Summary

Yields in general were within the range between 25% and 40%, with an advantage of crayfish, which has a higher yield and has a greater potential for the use of by-products from the extraction process, compared to shrimp. A summary of all experiments performed is shown below in Table 4:

Table 4: Summary of all experiments performed.

Experiments	Biomass/ Method	mT (g)	mP (g)	Yield (mP/mT)
I	Shrimp/ Sustainable	31.8879	8.5152	26.69%
II	Shrimp/ Sustainable	74.8926	61.0514	81.52%
III	Crayfish/ Sustainable	79.9385	20.8617	20.10%
IV	Shrimp/ Sustainable	30.0182	12.8628	42.85%
V	Crayfish/ Sustainable	30.1800	10.0803	33.40%
VI	Shrimp/ Sustainable	30.0125	7.3904	24.62%
VII	Crayfish/ Sustainable	29.9367	7.7961	26.04%
VIII	Crayfish/ Classic	30.0849	4.2989	14.29%

mT = total mass of biomass used in grams
mP = product mass after all extraction protocol in grams

The sustainable method recommended as standard operating procedure demonstrated better results in relation to less degradation of the final product and generated wastes, it was the less aggressive method compared to the classic one and with slightly higher yield. In addition, the sustainable method allowed the use of by-products which have a high market values.

FTIR analysis indicated a certain level of degradation with the classic method which was more aggressive than the sustainable method used, and indicated the alpha form in all chitins structures analysed.

The XRD charts of the chitins extracted showed a high degree of crystallization in their structures and in general were very similar, reinforced with a 2° angle in the approximate positions, indicating similarity in how chains were organized. The similarity was greater between chitin extracted from shrimp and crayfish via sustainable method. The chitin extracted by the classic method was more brittle than that extracted by the sustainable method.

In thermal analysis (TGA and DSC) chitin extracted from shrimp showed a higher decomposition rate and thermal events at lower temperatures compared to that extracted from crayfish, which indicated greater susceptibility to degradation and lower molecular weight and stability of chitin from shrimp. Chitins extracted from crayfish showed a slower rate of thermal degradation and higher molecular weight and stability compared to those extracted from shrimp. Chitin extracted from crayfish showed better processability and superior thermal properties when compared to chitin extracted from shrimp.

The deacetylation degree (DD%) results showed results around 40%, which was expected, compared to other studies, this indicated that the production of chitosan from chitin required another step in the extraction process (deacetylation) to reach values from 80% to 90% of DD%.

Density analyzes showed that chitin extracted from crayfish by the sustainable method had a higher density compared to that extracted from shrimp, this could indicate a higher molecular weight and better thermomechanical properties of chitin extracted from crayfish by the

sustainable method.

Comparing chitins from different origins (marbled crayfish and White shrimp), a great similarity between the materials was observed, both in basic composition, as well as in morphology and molecular arrangement (alpha chitin - free form). Marbled crayfish showed a good potential as a chitin source for bioplastic products and with even greater possibility of using the by-products of the purification process, which have great commercial potential, such as astaxanthin, proteins and calcium salts of organic origin. The purification process directly impacted the possibility or not of reusing these residues from the production of chitin.

5 Examples related to optimization

5.1 Experiment IX (open system)

In Experiment IX, 200 g crayfish shells with 18 to 45 mesh (1000 μm to 354 μm) were used and the extraction was done according to the inventive sustainable protocol for chitin extraction described in 1.3., using the same parameters as in Experiment I. The Experiment was done under atmospheric pressure (in the range of from 1000 to 1200 mbar). Demineralisation according to 1.3.1, deproteinization according to 1.3.2 and deodorization/depigmentation according to 1.3.3, where each done in an open vessel, wherein for filtration and washing, the demineralized product, the demineralized and deproteinated product as well as the precipitate obtained after deodorization/depigmentation had to be transferred from the open vessel to a filtration device and returned to the open vessel afterwards. The result was a yield of 32.5 %.

5.2 Experiment X (reactor set-up of closed, semi-automated reactor)

A closed, semi-automated, reactor (Glass Nutsche Filter reactor of company Ablaze Glass Works Pvt. Ltd.) is schematically shown in Fig. 12. In general, the closed reactor comprised an integrated jacketed glass reactor for homogeneous temperature control. The reactor was equipped with a lining stirrer, a pH sensor, a filtering floor unit, and a flush bottom valve connected to a Nutsche filtering

unit and a three-neck round-bottom flask with bottom drain and PTFE stopcock (receiver vessel), for automated solvent exchange, enabling a closed system operation. For maintaining the pressure below a predetermined maximum pressure value, the closed reactor had one or more pressure relief valve(s). These valves acted as safety mechanisms that automatically open when the pressure in the closed reactor exceeded the predetermined maximum pressure value, wherein said maximum pressure value was preferably 0.75 bar, more preferably 0.5 bar. The filtering floor unit was a woven filter fabric made of nylon having a mesh size of 1000 μm .

In Experiment X, 200 g of Crayfish shell with 10 to 18 mesh (2000 to 1000 μm) were used and the experiment was carried out according to the inventive sustainable protocol for chitin extraction described in 1.3 but in a closed, semi-automated, reactor as described above at a pressure in the range of from 0.2 to 0.5 bar without any change of device for filtration and washing. The result was a yield of 39.17 %.

5.3 Comparison of the Experiments

The Experiments comprised washing steps, in that the demineralized product obtained in 1.3.1 and the deproteinized product obtained in 1.3.2 had to be washed with distilled water and filtered until it reached neutral pH.

In Experiment IV to VIII and Experiment IX above, which were performed in an open system, washing steps were thus required after demineralization and after deproteinization, wherein the shells had to be rotated for 10 minutes in 10 liter of water per 1 kg of shells for each washing step. After the washing, the shells had to be transferred to a set-up of drainers equipped with different mesh pore sizes (10, 20, and 50 microns). This draining system allowed for the collection of the supernatant while minimizing shell losses through the mesh pores, ensuring efficient separation and recovery of the rinsed shells.

In Experiment X, however, due to the use of the closed, semi-automated reactor, it was possible to reduce the amount of water required for washing per washing step from 10 l per kg of material (10l water per kg shells) to 5 l per kg material (5l water per kg shells). This reduction was achieved

due to the intensive homogenization and closed system design of the reactor, which minimized water waste. Furthermore, due to the use of the closed, semi-automated reactor, losses caused by solvent evaporation could be avoided, improving both the safety and efficiency of the process by eliminating the risk of solvent exposure and minimizing solvent consumption. As shown in Table 5, especially the last step according to 1.3.3 (deodorization/depigmentation) was majorly improved in that evaporation of the solvent could be prevented.

Table 5: Comparison of open system of Experiment IX and closed system of Experiment X

	Citric Acid(aq)			Glycerol/KOH(aq)			Ethanol		
	Input (ml)	Output (ml)	Loss (% (w/w))	Input (ml)	Output (ml)	Loss (% (w/w))	Input (ml)	Output (ml)	Loss (% (w/w))
Experiment IX (Open system)	2000	1940	3.00	2000	1980	1.00	2000	1650	17.50
Experiment X (Closed reactor)	2000	1970	1.50	2000	1980	1.00	2000	1960	2.00

The use of the closed, semi-automated reactor, in Experiment X also allowed to reduce the incubation time for the demineralization, deproteinization, and deodorization/depigmentation steps by up to 50%. Using the closed, semi-automated reactor allowed for incubation times of 2 hours, 3 hours, and 1 hour for the demineralization, deproteinization, and deodorization/depigmentation steps, respectively. Furthermore, the chitin recovery efficiency was significantly improved to 39.17% (w/w), for Experiment X compared to 32.50% (w/w) for an open system as used in Experiment IX as shown in Fig. 13.

The purity of the extracted chitin was analysed in that the protein contamination was determined by Bradford assay quantification and is listed below in Table 6.

Table 6: Chitin purity - Protein quantification

	Concentration (mg/ml)	% (w/w) of the total Chitin content
Experiment IX (Open system)	0.7865	0.7865
Experiment X (Closed reactor)	0.6265	0.6265

The purity of extracted chitin is crucial for various applications, and minimizing protein contamination was a key objective during the isolation process. While using an open system already achieved a great purity with less than 1% (w/w) (benchmark level accepted by the scientific community - Trung T.S., *et.al.* Improved method for production of chitin and chitosan from shrimp shells. Carbohydr. Res. 2020;489:107913. doi: 10.1016/j.carres.2020.107913.) of protein contamination in the final chitin product, the use of the closed, semi-automated reactor, in Experiment X resulted in a considerable reduction in protein contamination. Bradford assay quantification revealed that the purified chitin obtained through this method contained only 0.63% (w/w) residual protein, a significant improvement over the open extraction method.

The reduced protein contamination achieved with the closed extraction method could be attributed to the improved control over reaction conditions, minimizing exposure to air, and potentially enhancing the efficiency of the deproteinization step.

Furthermore, the ash content of extracted chitin was determined by weighting and burning as indicated in more detail below and the results are listed below in Table 7.

Table 7: Ash content measurement comparing open and closed method

	Initial weight (g)	Final weight (g)	SD	Ash content (% (w/w))
Experiment IX (Open system)	1	0.0092	0.00081	0.92
Experiment X (Closed reactor)	1	0.00492	0.0002	0.49

The ash content of extracted chitin represents the residual inorganic material or mineral content remaining after the extraction process. It is an important indicator of the effectiveness of the

demineralization step in removing calcium carbonate and other minerals from the crustacean shells. The ash content was determined by weighing a sample of the extracted chitin, burning it in a muffle furnace at high temperatures (around 600°C) to remove all organic matter, and then calculating the percentage of the non-combustible inorganic ash residue relative to the initial sample weight.

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The results showed that using the closed, semi-automated reactor, in experiment IX resulted yielded chitin with an ash content below 0.5% (w/w), significantly lower than the 0.93% (w/w) ash content obtained from the open extraction method. This lower ash content of less than 0.49% (w/w) demonstrated the superior demineralization efficiency of the closed system, resulting in a purer chitin product with minimal residual mineral contamination (Tissera WMJCM, et.al. An improved extraction and purification method for obtaining high-quality chitin and chitosan from blue swimmer (*Portunus pelagicus*) crab shell waste. Food Sci Biotechnol. 2021 Nov 26;30(13):1645-1655. doi: 10.1007/s10068-021-01002-x.).

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6 Summary

The implementation of the closed, semi-automated reactor significantly improved the crustacean shell recycling process yielding a better quality chitin. The process was now safer, faster, more efficient, and environmentally friendly, with reduced water consumption and solvent loss, while maintaining the same chitin recovery efficiency compared to the open system.

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Importantly, an ash content below 1% (w/w), preferably around 0.3-0.8% (w/w), was generally considered acceptable for high-quality chitin extracted from crustacean sources like shrimp, crab, or other shellfish. Minimizing the ash/mineral content through efficient demineralization was crucial to obtain pure chitin. The newly developed closed extraction system achieved remarkable improvements in demineralization efficiency, yielding chitin with an ash content below 0.5% (w/w). This was significantly lower than the 0.9% (w/w) ash content obtained from the open extraction method.

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The closed system's controlled environment and optimized conditions facilitated superior removal

of minerals, resulting in a purer chitin product with minimal residual ash or mineral contamination. This advancement in chitin extraction technology represented a significant step towards obtaining high-purity chitin that met the stringent quality standards required for various applications.

List of reference numbers

	1	Closed, semi-automated reactor comprising a 5 Liter jacketed reactor;
	2	Vessel cover (lid);
5	3	Lab type condenser;
	4	S bend;
	5	Additional funnel;
	6	PTFE propeller type stirrer;
	7	GFT filter plate;
10	8	Zero hold drain valve;
	9	Receiver vessel;
	10	Quick release clamp;
	11	Clamp;
	12	Structure support;
15	13	Utility manifold;
	14	Bola connectors;
	15	Hose connector;
	16	Cowl seal;
	17	VELP motor;
20	18	Heating system.

Claims

1. A method for treating a chitin containing material comprising
 - (i) milling the chitin containing material;
 - (ii) contacting the milled chitin containing material from step (i) with a mixture comprising an aqueous solution of an organic acid; and
 - (iii) contacting the chitin containing material with an alkaline solution comprising water:glycerol in a ratio of 50-100:1-50 (v/v) and potassium hydroxide at a concentration of from 0.2M to 5M;thereby treating the chitin containing material.
2. The method of claim 1, wherein step (i) is followed by step (i-i) separation of the milled chitin containing material from step (i) into at least two fractions based on particle size, preferably by mechanical separation, more preferably by sieving; wherein optionally step (i-i) is followed by step (i-ii) obtaining at least one fraction containing chitin containing material, wherein at least 70 weight-%, preferably at least 80 weight-%, more preferably at least 90 weight-% of said fraction have a particle size smaller than 250 μm , based on the total weight of said fraction being 100 weight-%.
3. The method of any one of claims 1 or 2, wherein said organic acid is citric acid, maleic acid, or a mixture thereof; and/or wherein said aqueous organic acid has a concentration of from 0.2M to 5M.
4. The method of any one of claims 1 to 3, wherein the chitin containing material to aqueous organic acid ratio during step (ii) is of from 1:2 (m/v) to 1:50 (m/v).
5. The method of any one of claims 1 to 4, wherein the ratio of chitin containing material to water/glycerol mixture in step (iii) is from 1:2 (m/v) to 1:50 (m/v).
6. The method of any one of claims 1 to 5, wherein at least steps (ii) and (iii) are carried out

in the same device, wherein the device preferably comprises a closed reactor, more preferably a closed reactor comprising a filtering unit.

7. The method of any one of claims 1 to 6, wherein said method further comprises step (iv) contacting the chitin containing material with a binary solvent being a mixture of a first organic solvent and a second organic solvent or contacting the chitin containing material with a first organic solvent; wherein said first organic solvent is preferably a C1 to C3 alcohol, or mixture thereof, more preferably is ethanol; and/or wherein said second organic solvent is preferably a C3 to C5 ketone, more preferably is propanone.
8. The method of claim 7, wherein at least steps (ii), (iii) and (iv) are carried out in the same device, wherein the device preferably comprises a closed reactor, more preferably a closed reactor comprising a filtering unit.
9. The method of claim 7 or 8, wherein step (iv) is followed by step (iv-i) filtering the chitin containing material of step (iv) from the binary solvent or from the first organic solvent.
10. The method of any one of claims 1 to 9, wherein said method is a method of producing chitin and comprises step (v) obtaining chitin as the product of step (ii), (iii), or (iv), or as the retentate of step (iv-i).
11. The method of any one of claims 1 to 10, wherein said method is a method for producing chitin and at least one of a calcium salt of an organic acid, a protein product, and astaxanthin from chitin containing material.
12. The method of any one of claims 1 to 11, wherein the chitin containing material are crustacean shells.
13. A method for producing a fertilizer or a food or feed or a feed or food additive, comprising the steps of the method of any one of claims 1 to 12 and optionally the steps of formulating the product obtained as fertilizer or optionally the step of formulating the product as food

or feed or optionally the step of formulating the product as food or feed additive.

14. A method of producing a chitin and at least one of a calcium salt of an organic acid, a protein product, and astaxanthin from chitin containing material, comprising the steps of
5 any one of claims 1 to 12.
15. A chitin preparation, a calcium salt of an organic acid, a protein product, or an astaxanthin produced or producible from chitin containing material according to a method of any one
10 of claims 1 to 12.
16. Use of a chitin preparation according to claims 15 in manufacture of a natural or synthetic polymer, in agriculture, in packaging, in construction, or in environmental protection, as an ingredient in the production of a medicament, a food, or a cosmetics, and/or as a solidified
15 enzyme carrier.