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(54) Title: PROCESS FOR PRODUCING A HYDROLYSATE

(57) Abstract: The present invention relates to a process for producing a hydrolysate by using enzymes.



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PROCESS FOR PRODUCING A HYDROLYSATE

Field of the invention

5 The present invention generally relates to the fields of enzymology and industrial hydrolysate production. Particularly, the present invention relates to processes for producing hydrolysates. More particularly, the present invention relates to enzymes and their use in processes for producing hydrolysates.

Background of the invention

10 The fish industry has shown significant global growth in recent years. During fish processing 50-70% of the original fish material ends up as waste. The high volumes of waste represent a challenge from an environmental point of view as most of the fish waste is discarded. Optimizing the use of fish waste is not only beneficial for the environment, but also a promising
15 method to reduce the cost of disposal and increase the revenue of producers.

 Fish scales constitute about 5% of the fish waste. They are known to be a rich source of valuable components including proteins such as gelatin, collagen and chitin which are important components in food and biomedical industries.

 Thus far, research efforts have been directed at developing various means of extracting
20 valuable components from fish scales as well as exploring various possible applications. Despite the progress in extraction technology, the quality of the obtained components and the extraction yield can still be further optimized. Therefore, still a need remains in the art to provide improved processes for fish scale hydrolysate production.

Summary of the invention

25 An object of the invention is a process for producing fish scale hydrolysates by using enzymes. Optimization and improvement lie *inter alia* in the pretreatment as well as the enzymes used in the process.

Detailed description of the invention

30 Throughout the present specification and the accompanying claims, the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows. The articles "a" and
35 "an" are used herein to refer to one or to more than one (*i.e.* to one or at least one) of the grammatical object of the article. By way of example, "an element" may mean one element or more than one element. As will be apparent to those of skill in the art upon reading this application, each of the individual embodiments described and illustrated herein has discrete components and

features which may be readily separated from or combined with the features of any of the other embodiments without departing from the scope or spirit of the present invention described herein. Any recited process can be carried out in the order of events recited or in any other order which is logically possible.

5 The invention relates to a process for producing a fish scale hydrolysate comprising the steps of (a) pretreating fish scale for 1 to 60 minutes at a temperature ranging from 80°C to 120°C and a pH ranging from 5 to 12 and (b) adding at least a first enzyme composition to the pretreated fish scale and incubating the obtained mixture for 1 to 5 hours at a temperature ranging from 40°C to 70°C to obtain the fish scale hydrolysate.

10 Before pretreatment, the fish scale is removed from the fish. The removal of fish scale is called descaling. Descaling can be performed manually. However, large fish processing industries make use of descaling processes wherein automated fish descaling machinery is used.

 The fish scale can be derived from marine as well as freshwater fish. Examples include, but are not limited to, bonito, mackerel, drum, sea bass, tuna, swordfish, anchoveta, herring, shad,
15 skipjack, yellowfin and pompano.

 Several types of fish scale exist such as ganoid scale, cycloid scale, ctenoid scale and placoid scale. In an embodiment the process as described herein can be applied to any of these types of fish scale.

 After the fish scale is removed from the fish, the fish scale may be washed first with either
20 processing, tap, distilled or de-ionised water to remove dusts, dirt and other unwanted particles.

 In an embodiment before pretreatment the fish scale is washed first with an alkaline solution and then with water to rinse.

 In an embodiment the fish scale is decalcified. Decalcification is done before pretreatment by washing the fish scale with acid and then with an alkaline solution.

25 In an embodiment the pretreatment of the fish scale is done in a reactor. In an embodiment the pretreatment may also be done in two, three, four, five, six, seven, eight, nine, ten or even more reactors. So, the term "reactor" is not limited to a single reactor but may mean multiple reactors. In an embodiment the pretreatment is done in a reactor having a volume of 0.5 - 200 m³, preferably of 1 - 100 m³. In case multiple reactors are used in the pretreatment of the processes as described
30 herein, they may have the same volume, but also may have a different volume.

 In an embodiment the incubation of the obtained mixture of the first enzyme composition and pretreated fish scale is done in a reactor. In an embodiment the incubation may also be done in two, three, four, five, six, seven, eight, nine, ten or even more reactors. So, the term "reactor" is not limited to a single reactor but may mean multiple reactors. In an embodiment the enzymatic
35 incubation is done in a reactor having a volume of 0.5 - 200 m³, preferably of 1 - 100 m³. In case multiple reactors are used in the enzymatic incubation of the processes as described herein, they may have the same volume, but also may have a different volume.

In an embodiment the pretreatment and the enzymatic incubation are performed in the same reactor(s). In another embodiment they are performed in separate reactors.

In the processes as described herein, pretreated fish scale is added to the reactor in which the enzymatic incubation takes place. This can be done batch-wise, fed-batch wise or continuously.

5 In an embodiment the first enzyme composition is added to the reactor in which the enzymatic incubation takes place. This can be done batch-wise, fed-batch wise or continuously.

In an embodiment the first enzyme composition comprises a protease. In other words, the first enzyme composition comprises at least one protease. The first enzyme composition may also comprise more than one protease, for example two, three, four, five, six or even more different
10 proteases. The first enzyme composition may be an aqueous composition.

The term "protease" as used herein is defined as an enzyme that hydrolyses peptide bonds. It includes any enzyme belonging to the EC 3.4 enzyme group (including each of the subclasses thereof). The EC number refers to Enzyme Nomenclature from NC-IUBMB which is regularly supplemented and updated. Proteases are also called peptidases, proteinases, peptide
15 hydrolases or proteolytic enzymes. The term protease includes not only natural derived or wild-type proteases but also mutants, variants, fragments etc. thereof exhibiting protease activity, as well as synthetic proteases, such as shuffled proteases, and consensus proteases. Such genetically engineered proteases can be prepared by methods generally known in the art.

In an embodiment the first enzyme composition comprises an alkaline protease and/or a
20 neutral protease. In other words, the first enzyme composition may comprise an alkaline protease or the first enzyme composition may comprise a neutral protease or the first enzyme composition may comprise an alkaline protease and a neutral protease. In an embodiment the first enzyme composition may comprise more than one alkaline protease and/or more than one neutral protease. In case the first enzyme composition comprises more than one alkaline protease, the alkaline
25 proteases may differ from one another. In case the first enzyme composition comprises more than one neutral protease, the neutral proteases may differ from one another.

The term "neutral protease" is intended to be an enzyme classified according to the Enzyme Commission number EC 3.4.24.28. Neutral proteases are active at neutral pH. The term "alkaline protease" is intended to be an enzyme classified according to the Enzyme Commission
30 number EC 3.4.21.62. Alkaline proteases are active at neutral to alkaline pH.

In an embodiment at least a second enzyme composition is added to the pretreated fish scale. In an embodiment the second enzyme composition is added together with the first enzyme composition to the pretreated fish scale. In a preferred embodiment the second enzyme composition is added to the pretreated fish scale after the first enzyme composition has been added
35 to the pretreated fish scale. The second enzyme composition may be an aqueous composition.

In a preferred embodiment the second enzyme composition is added during incubation. Preferably, the second enzyme composition is added at least 1 minute, at least 2 minutes, at least 3 minutes, at least 4 minutes, at least 5 minutes, at least 6 minutes, at least 7 minutes, at least 8

minutes, at least 9 minutes, at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, at least 60 minutes, at least 70 minutes, at least 80 minutes, at least 90 minutes, at least 100 minutes, at least 120 minutes, at least 150 minutes, at least 180 minutes, at least 210 minutes, at least 240 minutes, at least 270 minutes, after the start of the incubation.

In an embodiment the second enzyme composition comprises a protease. In other words, the second enzyme composition comprises at least one protease. The second enzyme composition may also comprise more than one protease, for example two, three, four, five, six or even more different proteases.

In an embodiment the second enzyme composition comprises an alkaline protease and/or a neutral protease. In other words, the second enzyme composition may comprise an alkaline protease or the second enzyme composition may comprise a neutral protease or the second enzyme composition may comprise an alkaline protease and a neutral protease. In an embodiment the second enzyme composition may comprise more than one alkaline protease and/or more than one neutral protease. In case the second enzyme composition comprises more than one alkaline protease, the alkaline proteases may differ from one another. In case the second enzyme composition comprises more than one neutral protease, the neutral proteases may differ from one another. In an embodiment the protease(s) in the first enzyme composition differ from the protease(s) in the second enzyme composition. In an embodiment the protease(s) in the first enzyme composition are the same as the protease(s) in the second enzyme composition.

In an embodiment the first enzyme composition and/or the second enzyme composition comprises additional enzymes. These additional enzymes can be selected from the group consisting of acetyl esterases, alpha-galactosidases, alpha-glucanases, alpha-glucosidases, aminopeptidases, amylases, arabinases, arabinofuranosidases, asparaginases, beta-galactosidases, beta-glucosidases, carbohydrases, carbonic anhydrases, carboxypeptidases, catalases, cellulases, chitinases, chymosins, cutinases, cyclodextrin glycosyltransferases, deoxyribonucleases, endoglucanases, epimerases, esterases, expansins, glucan lysases, glucoamylases, glucose oxidases, glucuronidases, glycosyl hydrolases, hemicellulases, hexose oxidases, hexosyltransferases, hydrolases, hyaluronic acid synthase, invertases, isomerases, laccases, ligninases, lipases, lyases, mannosidases, mutanases, oxidases, oxidoreductases, pectinases, pectate lyases, pectin acetyl esterases, pectin depolymerases, pectin methyl esterases, pectinolytic enzymes, peptidases, perhydrolases, peroxidases, phenoloxidases, phytases, polygalacturonases, polyol oxidases, rhamno-galacturonases, ribonucleases, transferases, transport proteins, transglutaminases, xylanases and combinations thereof.

The pH during pretreatment is ranging from 5 to 12. In other words, the pH during pretreatment is in the range of 5 to 12. In an embodiment the pH during pretreatment is in the range of 6 to 11. In an embodiment the pH during pretreatment is in the range of 7 to 11.

The temperature during pretreatment is ranging from 80°C to 120°C. In other words, the temperature during pretreatment is in the range of 80°C to 120°C. In an embodiment the temperature during pretreatment is in the range of 85°C to 115°C. In an embodiment the temperature during pretreatment is in the range of 90°C to 110°C. In an embodiment the temperature during pretreatment is in the range of 95°C to 105°C.

The pretreatment is done for 1 to 60 minutes. In an embodiment pretreatment is done for 5 to 55 minutes. In an embodiment pretreatment is done for 10 to 50 minutes. In an embodiment pretreatment is done for 15 to 45 minutes. In an embodiment pretreatment is done for 20 to 40 minutes.

The temperature during incubation is ranging from 40°C to 70°C. In other words, the temperature during incubation is in the range of 40°C to 70°C. In an embodiment the temperature during incubation is in the range of 45°C to 65°C. In an embodiment the temperature during incubation is in the range of 50°C to 60°C. In an embodiment the temperature during incubation is in the range of 52°C to 58°C. In an embodiment the temperature during incubation is in the range of 54°C to 56°C.

The incubation is done for 1 to 5 hours. In an embodiment incubation is done for 1.5 to 4.5 hours. In an embodiment incubation is done for 2 to 4 hours.

In an embodiment the first enzyme composition is added at a dosage ranging from 0.1% to 2.0% (w/w pretreated fish scale (dry matter)). In an embodiment the first enzyme composition is added at a dosage ranging from 0.2% to 1.8% (w/w pretreated fish scale (dry matter)). In an embodiment the first enzyme composition is added at a dosage ranging from 0.3% to 1.7% (w/w pretreated fish scale (dry matter)). In an embodiment the first enzyme composition is added at a dosage ranging from 0.4% to 1.6% (w/w pretreated fish scale (dry matter)).

In an embodiment the second enzyme composition is added at a dosage ranging from 0.1% to 2.0% (w/w pretreated fish scale dry matter). In an embodiment the second enzyme composition is added at a dosage ranging from 0.2% to 1.8% (w/w pretreated fish scale dry matter). In an embodiment the second enzyme composition is added at a dosage ranging from 0.3% to 1.7% (w/w pretreated fish scale dry matter). In an embodiment the second enzyme composition is added at a dosage ranging from 0.4% to 1.6% (w/w pretreated fish scale dry matter).

In an embodiment the process as described herein further comprises adjusting the pH of the fish scale before pretreatment to a pH ranging from 7 to 11. If needed, additional pH adjustment is done during pretreatment. In an embodiment pH adjustment is done by adding a hydroxide of an alkali metal and/or a hydroxide of an alkaline earth metal to the fish scale.

In an embodiment the hydroxide of an alkali metal and/or the hydroxide of an alkaline earth metal are selected from the group consisting of aluminium hydroxide, barium hydroxide, calcium hydroxide, caesium hydroxide, potassium hydroxide, lithium hydroxide, magnesium hydroxide, sodium hydroxide, rubidium hydroxide, strontium hydroxide and any combination thereof. In a preferred embodiment the hydroxide is sodium hydroxide.

After enzymatic incubation of the pretreated fish scale, the enzymes may be inactivated. Inactivation may, for example, be done at elevated temperatures of around 75 to 100°C applied for 5 to 30 minutes. Inactivation may be done in the reactor used for enzymatic incubation. Alternatively, inactivation may also be done in a different reactor. In an embodiment inactivation is done by means of the pasteurization step and/or the drying step.

To extract specific compounds and/or fractions from the fish scale hydrolysate, the fish scale hydrolysate may be subjected to one or more purification steps using methods known in the art such as centrifugation, decantation, plate filtration, frame filtration, microfiltration, ultrafiltration and nanofiltration.

The fish scale hydrolysate and/or the specific compounds and/or fractions extracted from the fish scale hydrolysate may be dried. Drying can be done by means of known technologies such as spray drying, freeze drying and roller drum drying. Before drying, the fish scale hydrolysate and/or the specific compounds and/or fractions extracted from the fish scale hydrolysate may be concentrated.

The present invention also relates to a fish scale hydrolysate obtainable by a process as described herein. All embodiments and features described above for the process for producing a fish scale hydrolysate also apply to the fish scale hydrolysate obtainable by a process as described herein.

In an embodiment the fish scale hydrolysate as described herein comprises peptides. The term "peptides" as used herein means short chains of amino acids, typically comprising 2–50 amino acids. The amino acids in a peptide are connected to one another in a sequence by bonds called peptide bonds. In an embodiment the fish scale hydrolysate as described herein comprises peptides comprising a molecular weight of 5 kD or lower. In an embodiment at least 80% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 85% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 90% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 91% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 92% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 93% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 94% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 95% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 96% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 97% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 98% of the peptides in the fish scale hydrolysate as

described herein comprise a molecular weight of 5 kD or lower. In an embodiment at least 99% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower. In an embodiment 100% of the peptides in the fish scale hydrolysate as described herein comprise a molecular weight of 5 kD or lower.

5 To measure the molecular weight of the peptides molecular weight distribution analysis methods can be applied. In an embodiment the following molecular weight distribution analysis method is used. In case the sample is gellated, it is first heated to liquidize the gel. Liquid samples are kept at room temperature. After homogenization on a vortex stirrer (Scientific Industries G-560E), approximately 50 mg of each sample is mixed with approximately 4950 μ l 100 mM sodium
10 phosphate buffer + 400 mM sodium chloride pH 6.4. The added buffer volume is adjusted to the sample weight to obtain a 100 times (w/v) dilution of each sample. The diluted samples are centrifuged for 15 minutes at 20000xg at room temperature (Eppendorf 5417C). 5 μ l of the supernatants are injected in a biocompatible Waters ACQUITY HClass-Bio UPLC system, equipped with a pump (Waters 186015041), autosampler (Waters 186015040), column heater
15 (Waters 186015011) and photodiode array detector (Waters 186015032). The running buffer is 100 mM sodium phosphate buffer + 400 mM sodium chloride pH 6.4. Separation is performed on a Waters ACQUITY UPLC Protein BEH SEC Column, 125Å, 1.7 μ m, 4.6 mm X 150 mm (Waters 186006505) at 40°C and 0.300 ml/min. Size exclusion chromatography (SEC) profiles are recorded using ultraviolet (UV) detection at 214 nm. Standards bovine serum albumin (Sigma P0914),
20 ovalbumin (Bio-Rad 1511901), equine myoglobin (Bio-Rad 1511901), [Glu]-fibrinopeptide B (Sigma F3261), glutathione oxidized (Sigma G4376), and glutathione reduced (G4251) are injected to calibrate the column and to assign molecular weight fractions of >50 kDa, 15-50 kDa, 5-15 kDa, 1-5 kDa and <1 kDa. The peak areas of each molecular weight fraction of the hydrolyzate samples are exported and evaluated.

25 In an embodiment a fish scale hydrolysate as described herein comprises peptides in range of 1 to 20% (w/w). In an embodiment a fish scale hydrolysate as described herein comprises peptides in range of 2 to 18% (w/w). In an embodiment a fish scale hydrolysate as described herein comprises peptides in range of 3 to 16% (w/w). In an embodiment a fish scale hydrolysate as described herein comprises peptides in range of 4 to 14% (w/w). In an embodiment a fish scale
30 hydrolysate as described herein comprises peptides in range of 5 to 12% (w/w). In an embodiment a fish scale hydrolysate as described herein comprises peptides in range of 6 to 10% (w/w).

In an embodiment a fish scale hydrolysate as described herein comprises a protease. In other words, a fish scale hydrolysate as described herein comprises at least one protease. The fish scale hydrolysate may also comprise more than one protease, for example two, three, four, five,
35 six or even more different proteases. The one or more proteases in the fish scale hydrolysate are inactivated.

In an embodiment the fish scale hydrolysate comprises an alkaline protease and/or a neutral protease. In other words, the fish scale hydrolysate may comprise an alkaline protease or

the fish scale hydrolysate may comprise a neutral protease or the fish scale hydrolysate may comprise an alkaline protease and a neutral protease. In an embodiment the fish scale hydrolysate may comprise more than one alkaline protease and/or more than one neutral protease. In case the fish scale hydrolysate comprises more than one alkaline protease, the alkaline proteases may differ from one another. In case the fish scale hydrolysate comprises more than one neutral protease, the neutral proteases may differ from one another.

Next to the protease, the fish scale hydrolysate as described herein may also comprise at least one compounds selected from the group consisting of collagen, fat, lecithin, sclerotin, vitamin, gelatin, hydroxyapatite, guanine, chitin, chitosan, mucopolysaccharides, calcium phosphate, magnesium, iron, zinc and calcium.

In an embodiment the fish scale hydrolysate is a powder.

The present invention also relates to a food, feed, pharmaceutical, cosmetic, biomedical or biosorbent product comprising a fish scale hydrolysate as described herein. All embodiments and features described above for the process for producing a fish scale hydrolysate and/or all embodiments and features described above for the fish scale hydrolysate of the invention also apply to the food, feed, pharmaceutical, cosmetic, biomedical or biosorbent product as described herein.

EXAMPLES

Example 1

Fish scale hydrolysate production using different pretreatment and enzymatic hydrolysis conditions

40 grams of decalcified fish scales and 400 grams of water were added to 500 ml vials. The vials were placed in a water bath and pretreated under the conditions as shown in Table 1.

After pretreatment, the samples were subjected to enzymatic hydrolysis. The vials were placed in ice or boiling water to quickly reach the appropriate temperatures for incubation. Next, enzymes were added to the vials and incubated in a water bath during 3 hours under the conditions as shown in Table 2. The enzymes used were Sqzyme PS-KL, an alkaline protease (pH optimum 7-12 from Suntaq) and Maxipro NPU, a neutral protease (pH optimum 5-8 from DSM).

After the incubation, supernatant of each sample was obtained by centrifugation at 2000xg for 5 minutes and the supernatant was analyzed using a refractometer (ATAGO PAL-1). The refractometer was used to measure total solids (expressed as Brix value). The results are shown in Table 3.

The data in Table 3 reveal that pretreatment at a temperature of 80°C or higher resulted in a high amount of total solids in the samples. Moreover, the data demonstrate that increasing temperature and/or time of incubation further increases the amount of total solids in the samples.

Example 2

Fish scale hydrolysate production using pretreatment and one or more enzymes during enzymatic hydrolysis

40 grams of decalcified fish scales and 400 grams of water were added to 500 ml vials. The vials were placed in a water bath and pretreated at 100°C for 30 minutes.

After pretreatment, the samples were subjected to enzymatic hydrolysis. The vials were placed in ice water to quickly reach the incubation temperature of 55°C. Next, enzymes were added to the vials and incubated in a water bath during 3 hours under the conditions as shown in Table 4. The enzymes used were Sqzyme PS-KL, an alkaline protease (pH optimum 7-12 from Suntaq) and Sqzyme PS-NL, a neutral protease (pH optimum 5.5-9 from Suntaq).

After the incubation, supernatant of each sample was obtained by centrifugation at 2000xg for 5 minutes and the supernatant was analyzed using a refractometer (ATAGO PAL-1). The refractometer was used to measure total solids (expressed as Brix value). The results are shown in Table 5.

The data in Table 5 show that the combined use of alkaline protease and neutral protease achieved a higher extraction yield on total solids than the use of alkaline protease or neutral protease alone at the same dosage.

Example 3

Fish scale hydrolysate production using different pH conditions during pretreatment

40 grams of decalcified fish scales and 400 grams of water were added to 500 ml vials. The pH of samples 2 and 3 were adjusted using sodium hydroxide. Then, vials were placed in a water bath and pretreated at 100°C for 30 minutes.

After pretreatment, the samples were subjected to enzymatic hydrolysis. The vials were placed in ice water to quickly reach the incubation temperature of 50°C. Next, enzymes were added to the vials and incubated in a water bath during 3 hours under the conditions as shown in Table 6. The enzymes used were Alcalase 2.4L, an alkaline protease (pH optimum 6-12 from Novozymes) and Maxipro NPU, a neutral protease (pH optimum 5-8 from DSM).

After the incubation, supernatant of each sample was obtained by centrifugation at 2000xg for 5 minutes and the supernatant was analyzed using a refractometer (ATAGO PAL-1). The refractometer was used to measure total solids (expressed as Brix value). The results are shown in Table 7.

The data in Table 7 show that increasing the pH during pretreatment leads to higher extraction yields on total solids.

Table 1: Pretreatment conditions during fish scale hydrolysate production process.

Sample number	Temperature(°C)	Time (minutes)	pH
1	28	30	6.45
2	100	30	6.45
3	60	30	6.45
4	80	30	6.45
5	100	5	6.45
6	100	15	6.45
7	100	30	6.45

Table 2: Temperatures and enzymes applied during enzymatic hydrolysis.

Sample number	Temperature (°C)	Enzymes added at the beginning of incubation and their dosage (w/w fish scale)	Enzymes added after 1-hour incubation and their dosage (w/w fish scale)
1	55	Sqzyme PS-KL; 1%	Sqzyme PS-NL; 1%
2	55	Sqzyme PS-KL; 1%	Sqzyme PS-NL; 1%
3	50	Maxipro NPU; 1%	-
4	50	Maxipro NPU; 1%	-
5	50	Maxipro NPU; 1%	-
6	50	Maxipro NPU; 1%	-
7	50	Maxipro NPU; 1%	-

5 Table 3: Brix value of samples.

Sample number	Brix (%)
1	7.1
2	9.2
3	3.8
4	7.3
5	7.8
6	8.1
7	8.4

Table 4: Temperatures, pH and enzymes applied during enzymatic hydrolysis.

Sample number	pH	Temperature (°C)	Enzymes added at the beginning of incubation and their dosage (w/w fish scale)	
1	6.45	55	-	Sqzyme PS-NL; 1%
2	6.45	55	Sqzyme PS-KL; 1%	-
3	6.45	55	Sqzyme PS-KL; 1%	Sqzyme PS-NL; 1%
4	6.45	55	Sqzyme PS-KL; 0.5%	Sqzyme PS-NL; 0.5%

Table 5: Brix value of samples.

Sample number	Brix (%)
1	9.1
2	9.3
3	9.6
4	9.5

5 Table 6: Temperatures, pH and enzymes applied during fish scale hydrolysate production process.

Sample number	pH	Temperature (°C)	Enzymes added at the beginning of incubation and their dosage (w/w fish scale)	
1	6.5	50	Alcalase 2.4L; 0.4%	Maxipro NPU; 0.4%
2	8.0	50	Alcalase 2.4L; 0.4%	Maxipro NPU; 0.4%
3	10.0	50	Alcalase 2.4L; 0.4%	Maxipro NPU; 0.4%

Table 7: Brix value of samples.

Sample number	Brix (%)
1	9.0
2	9.2
3	9.5

CLAIMS

1. A process for producing a fish scale hydrolysate comprising the steps of:
 - a) pretreating fish scale for 1 to 60 minutes at a temperature ranging from 80°C to 120°C and
5 a pH ranging from 5 to 12,
 - b) adding at least a first enzyme composition to the pretreated fish scale and incubating the
obtained mixture for 1 to 5 hours at a temperature ranging from 40°C to 70°C to obtain the
fish scale hydrolysate.
- 10 2. The process according to claim 1, wherein the first enzyme composition comprises a protease.
3. The process according to claim 1 or 2, wherein the first enzyme composition comprises an
alkaline protease and/or a neutral protease.
- 15 4. The process according to any one of the claims 1 to 3, wherein during incubation at least a
second enzyme composition is added to the pretreated fish scale.
5. The process according to claim 4, wherein the second enzyme composition is added at least
10 minutes after the start of the incubation.
- 20 6. The process according to claim 4 or 5, wherein the second enzyme composition comprises a
protease.
7. The process according to any one of the claims 1 to 6, wherein the pH during the pretreatment
25 is ranging from 7 to 11.
8. The process according to any one of the claims 1 to 7, wherein the temperature during
incubation is ranging from 50°C to 60°C.
- 30 9. The process according to any one of the claims 1 to 8, wherein the first enzyme composition is
added at a dosage ranging from 0.1% to 2.0% (w/w pretreated fish scale (dry matter)).
10. The process according to any one of the claims 1 to 9, wherein the second enzyme composition
is added at a dosage ranging from 0.1% to 2.0% (w/w pretreated fish scale dry matter).
- 35 11. The process according to any one of the claims 1 to 10, further comprising adjusting the pH of
the fish scale before pretreatment to a pH ranging from 7 to 11.

12. The process according to claim 11, wherein the pH adjustment is done by adding a hydroxide of an alkali metal and/or a hydroxide of an alkaline earth metal to the fish scale.

13. The process according to any one of the claims 1 to 12, wherein the fish scale is decalcified.

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14. A fish scale hydrolysate obtainable by a process according to any one of the claims 1 to 13.

15. The fish scale hydrolysate according to claim 14, wherein the fish scale hydrolysate comprises a protease.

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16. A food, feed, pharmaceutical, cosmetic, biomedical or biosorbent product comprising the fish scale hydrolysate according to claim 14 or 15.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/078191

A. CLASSIFICATION OF SUBJECT MATTER INV. A23J3/04 A23J3/34 C12P21/06 ADD. 		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A23J C12P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, EMBASE, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHANG YIQI ET AL: "Fish Scale Valorization by Hydrothermal Pretreatment Followed by Enzymatic Hydrolysis for Gelatin Hydrolysate Production", MOLECULES, vol. 24, no. 16, 19 August 2019 (2019-08-19), page 2998, XP055912121, DOI: 10.3390/molecules24162998	1-16
Y	the whole document, in particular p. 9-10, paragraphs 3.2. and 3.3. ----- -/--	1-13
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 9 January 2023		Date of mailing of the international search report 25/01/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Bassias, Ioannis

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2022/078191

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Izzati H ET AL: "Low Molecular Weight Collagen from Tilapia Fish Scales for Potential Cosmetic Application", Der Chemica Chemica, 1 January 2017 (2017-01-01), pages 108-114, XP055912263, Retrieved from the Internet: URL:https://www.derpharmachemica.com/pharmaceuticals/low-molecular-weight-collagen-from-tilapia-fish-scales-for-potential-cosmetic-application.pdf [retrieved on 2022-04-13]	14-16
Y	the whole document, in particular p.109, "Experimental"	1-13

X	CN 111 345 339 A (UNIV JIANGXI NORMAL) 30 June 2020 (2020-06-30)	14-16
Y	the whole document	1-13

X	CN 109 371 082 B (UNIV ZHEJIANG OCEAN) 18 June 2021 (2021-06-18)	14-16
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2022/078191

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CN 109371082	B	18-06-2021	NONE	

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