

Translated English of Chinese Standard: GB/T22729-2008
www.ChineseStandard.net → Buy True-PDF → Auto-delivery.
Sales@ChineseStandard.net

GB

NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

ICS 67.040

X 80

GB/T 22729-2008

Oligopeptides powder of marine fish

海洋鱼低聚肽粉

Issued on: December 31, 2008

Implemented on: August 01, 2009

**Issued by: General Administration of Quality Supervision, Inspection and
Quarantine of PRC;
Standardization Administration of PRC.**

Table of Contents

Foreword.....	3
1 Scope.....	4
2 Normative references.....	4
3 Terms and definitions	5
4 Product classifications	5
5 Technical requirements	6
6 Test method	7
7 Inspection rules.....	11
8 Labeling, packaging, transportation, storage	13
Appendix A (Normative) Proportion of protein hydrolysates with relative molecular mass less than 1000 u (High performance gel filtration chromatography).....	14

Oligopeptides powder of marine fish

1 Scope

This standard specifies the product classification, technical requirements, test methods, inspection rules, labeling, packaging, transportation, storage of oligopeptide powder of marine fish.

This standard applies to the production, inspection, sales of oligopeptide powder products of marine fish.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Standard; however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB 2733 Hygienic standard for fresh and frozen marine products of animal origin

GB/T 4789.2 Microbiological examination of food hygiene - Aerobic plate count

GB/T 4789.3 Microbiological examination of food hygiene - Detection of Coliform bacteria

GB/T 4789.4 Microbiological examination of food hygiene - Examination of Salmonella

GB/T 4789.5 Microbiological examination of food hygiene - Examination of Shigella

GB/T 4789.10 Microbiological examination of food hygiene - Examination of staphylococcus aureus

GB/T 4789.15 Microbiological examination of food hygiene - Examination of molds and yeasts

GB/T 5009.3 Determination of moisture in food

results are not converted into protein coefficients. They are expressed in terms of total nitrogen mass fraction.

6.3 Oligopeptides

6.3.1 Method summary

Low-molecular-weight protein hydrolysates (including oligopeptides and free amino acids) are soluble in trichloroacetic acid solution. The high-molecular-weight proteins are easy to precipitate in trichloroacetic acid solution. After the sample is dissolved in the trichloroacetic acid solution, it is centrifuged, to separate the precipitated protein. Collect the centrifuged supernatant. According to the method specified in GB/T 5009.5, determine the acid-soluble protein hydrolysate content of the centrifuged supernatant. The acid-soluble protein hydrolysate content minus the free amino acid content, to obtain the content of oligopeptides.

6.3.2 Analytical procedures

6.3.2.1 Determination of acid-soluble protein hydrolysate content

Weigh 2 g (accurate to 0.001 g) of sample. Add 10 mL of 15% trichloroacetic acid solution. Mix well. Let it stand for 10 min. After centrifuging the sample solution for 10 min at 4000 r/min, take all the centrifuged supernatant. Determine the protein hydrolysate content of the supernatant, according to the method specified in GB/T 5009.5. Calculate the acid-soluble protein hydrolysate content in the sample. The protein conversion factor is 6.25. The test results are converted to a dry basis, based on the loss on drying of the sample.

6.3.2.2 Determination of free amino acid content

6.3.2.2.1 Determination method by amino acid automatic analyzer

Weigh 0.02 g ~ 0.03 g of sample, accurate to 0.0001 g. Use 3.5% sulfosalicylic acid solution, to dissolve it evenly. Transfer the sample solution into a 50 mL volumetric flask. Make the volume constant. Centrifuge the sample solution for 5 min, in a centrifuge, at a speed of 4000 r/min. Take the centrifuged supernatant. Then use a 0.45 μm microporous filter membrane, to filter the centrifuged supernatant. Transfer the filtrate into a 50 mL volumetric flask. Make its volume reach to the mark; shake it uniformly to prepare for use. Then determine its free amino acid content, by an automatic amino acid analyzer, in accordance with the method specified in GB/T 5009.124.

6.3.2.2.2 Determination by high performance liquid chromatograph

6.3.2.2.2.1 Method summary

After precipitating the protein with trichloroacetic acid, use o-phthalaldehyde (OPA) and fluorene methyl chloroformate (FMOC-Cl) as the derivatization reagents, for primary and secondary amino acids, respectively. Use the pre-column automatic derivatization reversed-phase high-efficiency liquid chromatography, to determine the total free amino acids in oligopeptide powder of marine fish.

6.3.2.2.2 Instruments

- a) High performance liquid chromatograph: It is equipped with quaternary gradient pump, UV detector, autosampler, ChemStation software, online degasser.
- b) Analytical balance: Sensitivity 0.0001 g.
- c) pH meter.

6.3.2.2.3 Reagents

Triethylamine, tetrahydrofuran, acetic acid, boric acid are all analytically pure; methanol, acetonitrile, amino acid standards, o-phthalaldehyde (OPA), fluorene methyl chloroformate (FMOC-Cl) are all chromatographically pure; ultrapure water.

6.3.2.2.4 Determination

Weigh the 0.5 g of sample, accurate to 0.0001 g. Add 10 mL of 5% trichloroacetic acid to a 25 mL volumetric flask, to precipitate for 2 hours. Make its volume reach to the mark. Shake it uniformly. Filter it. Centrifuge at 10000 r/min for 15 min. Take the supernatant. Load it in the machine. Use an external standard method for determination.

Chromatographic conditions: Analytical column: Hypersil AA-ODS, 5 μ m, 200 mm $\times$ 2.1 mm (inner diameter). Mobile phase: Solution A: The 20 mmol/L sodium acetate buffer, which contains 0.018% triethylamine (TEA); use dilute acetic acid to adjust to pH7.2; add 0.3% tetrahydrofuran (THF). Solution B: The 100 mmol/L sodium acetate buffer solution of 20%; use dilute acetic acid to adjust to pH7.2; add 40% acetonitrile and 40% methanol. Gradient elution program: The solution A is 100% at 0 min; the solution B is from 0% to 60% within 17 min. The solution B is 100% at 18 min, which is kept to 24 min. At 25 min, the solution B returns to 0%. Flow rate: 0.45 mL/min before 18.1 min; it rises to 0.8 mL/min at 18.5 min, which is kept to 23.9 min; at 24 min, it drops back to 0.45 mL/min. UV detection: Use 338 nm for the determination of primary amino acids AND 262 nm for the determination of secondary amino acids (after 15 min). Fluorescence detection: Determine the excitation wavelength of the primary amino acid at 340 nm AND the emission wavelength at 450 nm; after 14.5 min, the wavelength is switched to 266 nm (excitation) and 305 nm

Measure in accordance with the method specified in GB/T 5009.15.

6.12 Methyl-mercury

Measure in accordance with the method specified in GB/T 5009.17.

6.13 Total number of colonies

Measure in accordance with the method specified in GB/T 4789.2.

6.14 Coliform flora

Measure in accordance with the method specified in GB/T 4789.3.

6.15 Molds and yeasts

Measure in accordance with the method specified in GB/T 4789.15.

6.16 Pathogenic bacteria

Salmonella, Shigella and Staphylococcus aureus are tested according to the methods, which are specified in GB/T 4789.4, GB/T 4789.5, GB/T 4789.10, respectively.

7 Inspection rules

7.1 Inspection classification

7.1.1 Exit-factory inspection

The items of the exit-factory inspection include sensory characteristics, total nitrogen, oligopeptides, proportion of protein hydrolysates with a relative molecular weight of less than 1000 u, loss on drying, ash content (except collagen oligopeptide powder of marine fish bone), the total number of colonies, coliform flora.

7.1.2 Type inspection

Type inspection items include all the items, which are specified in 5.2, 5.3, 5.4, 5.5.

For the products, which are produced throughout the year, they shall be type-inspected once a year. However, they shall also be type-inspected in any of the following situations:

- When the new product is put into production;
- When there are major changes in raw materials and processes;

7.3.2.2 In the type inspection items, if the microbial indicators do not meet this standard OR if malignant impurities (such as glass, metal, insects, etc.) are found, they shall not be re-inspected BUT judged as unqualified products.

7.3.2.3 In the type inspection items, except for the indicator as mentioned in 7.3.2, if other indicators do not meet this standard, it may double sampling from the same batch of products, to re-inspect the unqualified items. If the result of the re-inspection still does not meet the requirements of this standard, it will be judged as unqualified product.

8 Labeling, packaging, transportation, storage

8.1 Label

8.1.1 The product label shall indicate the product name, net content, name and address of the manufacturer and distributor, production date, production batch number, shelf life, product standard number, etc.

8.1.2 The product name shall meet the requirements in Chapter 4.

8.2 Packaging

The packaging container shall meet the hygienic standards of food containers and packaging materials.

8.3 Transportation

The tools and vehicles, which are used to transport products, shall be clean, hygienic, dry and free of pollutants. During the transportation of the product, it shall be covered, protected from rain and sun exposure. It cannot be mix-transported with toxic, harmful, or odorous items.

8.4 Storage

8.4.1 Products shall not be stacked in the open. The finished product warehouse shall be clean, dry, ventilated, free of rodents and insects.

8.4.2 The product shall be stacked with backing plates. It shall be more than 10 cm above the ground and 20 cm away from the wall.

8.4.3 Products shall not be stored in the same warehouse, with toxic, harmful, odorous, perishable, or damp items.

detector AND chromatographic workstation or integrator, with GPC data processing software.

A.3.2 Mobile phase vacuum filtration and degassing device.

A.3.3 Ultrasonic oscillator.

A.3.4 Analytical balance: Sensitivity 0.0001 g.

A.4 Chromatographic conditions and system adaptability experiment

Chromatographic column: TSKgel G2000 SWXL 300 mm × 7.8 mm or other gel columns of similar performance, suitable for the determination of proteins and peptides.

Mobile phase: Acetonitrile: water: trifluoroacetic acid is 45:55:0.1 (volume ratio).

Detection wavelength: UV220 nm.

Flow rate: 0.5 mL/min.

Column temperature: 30 °C.

Injection volume: 10 µL.

In order to make the chromatographic system meet the detection requirements, it is stipulated that, under the above chromatographic conditions, the column efficiency of the gel chromatography column, that is, the number of theoretical plates (N), is calculated based on the peak of the tripeptide standard (Gly-Gly-Gly), which is not less than 5000; the distribution coefficient (K_d) of oligopeptides shall be within 0 ~ 1.

A.5 Preparation of relative molecular mass calibration curve

Respectively, use the mobile phase to prepare 0.1% (mass concentration) above-mentioned peptide standard solutions of different relative molecular mass. After filtering with polytetrafluoroethylene or nylon filter membrane, which has a pore diameter of 0.2 µm ~ 0.5 µm, make sample injection, respectively, to obtain the chromatograms of the series of standard products. Use the logarithm of the relative molecular mass (lgM_r) to plot the retention time, OR make a linear regression, to obtain the relative molecular mass calibration curve and its equation.

A.6 Sample preparation

Weigh 20.0 mg of sample, in a 10 mL volumetric flask. Use mobile phase to dilute the volume to the mark. Oscillate ultrasonically for 10 min, to dissolve and mix the sample thoroughly. After filtering by polytetrafluoroethylene or nylon

This is an excerpt of the PDF (Some pages are marked off intentionally)

Full-copy PDF can be purchased from 1 of 2 websites:

1. <https://www.ChineseStandard.us>

- SEARCH the standard ID, such as GB 4943.1-2022.
- Select your country (currency), for example: USA (USD); Germany (Euro).
- Full-copy of PDF (text-editable, true-PDF) can be downloaded in 9 seconds.
- Tax invoice can be downloaded in 9 seconds.
- Receiving emails in 9 seconds (with download links).

2. <https://www.ChineseStandard.net>

- SEARCH the standard ID, such as GB 4943.1-2022.
- Add to cart. Only accept USD (other currencies - <https://www.ChineseStandard.us>).
- Full-copy of PDF (text-editable, true-PDF) can be downloaded in 9 seconds.
- Receiving emails in 9 seconds (with PDFs attached, invoice and download links).

Translated by: Field Test Asia Pte. Ltd. (Incorporated & taxed in Singapore. Tax ID: 201302277C)

About Us (Goodwill, Policies, Fair Trading...): <https://www.chinesestandard.net/AboutUs.aspx>

Contact: Wayne Zheng, Sales@ChineseStandard.net

Linkin: <https://www.linkedin.com/in/waynezhengwenrui/>

----- The End -----