

VOLUNTARY MONOGRAPH FOR SALMON PROTEIN HYDROLYSATE TYPE 2

DEFINITION

Purified protein hydrolysate obtained from fresh farmed fish of the family *Salmonidae*, with minimum 90 % of the hydrolysed proteins having a molecular weight below 10 000, and with a maximum degree of hydrolysis that complies with the test for α -amino nitrogen. The salmon protein hydrolysate is water soluble and may be manufactured in liquid or solid form.

PRODUCTION

The fish shall only be given feed with a composition that is in accordance with the relevant EU or other applicable regulations. The fresh raw materials may be from salmon viscera. Hydrolysis is carried out by addition of exogenous proteases, and at temperature not exceeding 60°C. After hydrolysis the proteases are inactivated by heating to 90°C for a time period (typically 15 minutes) as recommended by the supplier of proteases. After removal of fat and solid substances, water is removed at product temperatures not exceeding 80°C. The production shall comply with relevant EU regulations¹.

CHARACTERS

The liquid form is a brownish, moderately viscous aqueous solution, while the solid form is a yellow-brownish powder.

IDENTIFICATION

The product to be examined complies with the limit in the assay.

TESTS

Biological digestibility (to be determined in mink as described by Skrede (1979)): minimum 95%.

Dry matter (ISO 6496): minimum 60 % for the liquid² and 94-96 % for the solid form.

Protein content (ISO 5983): minimum 80 % of dry matter.

¹ REGULATION (EC) No 853/2004 laying down specific hygiene rules for food of animal origin (http://eur-lex.europa.eu/LexUriServ/site/en/oj/2004/l_139/l_13920040430en00550205.pdf)

² A minimum of 60 % dry matter is required for stability reasons.

Lipid content (ethyl acetate extraction, NS 9402): maximum 2.5 % for the liquid and 4.0 % for the solid form.

Ash (ISO 5984): maximum 4.5 % for the liquid and maximum 8.0 % for the solid form.

pH (10 % aqueous solution of dry matter at room temperature): 6-7.

α -amino nitrogen (analyse as described by Nielsen *et al.* (2006)):

Calculate the percentage α -amino nitrogen of total nitrogen from the following expression:

$$\frac{AN}{TN} \times 100$$

AN = α -amino nitrogen

TN = total nitrogen; the value is obtained from the determination of protein content.

Limit: maximum 22 %.

Total aerobic microbial count (NordVal 012): less than 10^4 per gram.

Salmonella (NordVal 023 or NMKL 71): absent in a 25 gram sample.

Yeasts and moulds (NordVal 016): less than 20 per gram.

Enterobacteriaceae (ISO 21528-1): less than 10 per gram.

ASSAY

Percentage of hydrolysed proteins with molecular weight below 10 000.

Size-exclusion chromatography (as described by RUBIN Report no. 4617/115

(http://www.rubin.no/files/documents/4617-115_peptidstorrelse_hydrolysat2.pdf), or by

Aksnes *et al.* (2006) using 0.1 M Na-phosphate buffer, pH=6.8 (including 0.1 M NaCl, 0.3 % SDS and 0.001 N NaN₃) as eluting buffer).

Calculate the percentage content of hydrolysed proteins with molecular weight (MW) below 10 000 using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram,

B = Sum of the areas of the peaks with a retention time equal to or less than the calculated retention time of a component with molecular weight of 10 000.

The retention time of a component with molecular weight of 10 000 is calculated from a calibration curve using the following standards³:

³ These standards may be purchased from Sigma-Aldrich.

	<i>MW</i>
Albumin, bovine serum	66 000
Ovalbumin	45 000
Pepsin	34 700
Carbonic anhydrase	29 000
Lysozyme	14 600
Cytochrome C	12 400
Aprotinin	6 500
Polymyxin	1 470
Leu-gly-gly	245.3
Glycyl-glycin (Diglycin)	132.12

Limit: minimum 90 %.

STORAGE

Avoid direct sunlight. Store in closed containers (liquid and solid form), alternatively in paper bags in a dry environment (solid form).

LABELLING

The label states that the product complies with the voluntary monograph for Salmon Protein Type 2, and gives information as to the relevant storage conditions and maximum storage time.

REFERENCES

Aksnes, A., Hope, B., Jönsson, E., Björnsson, B.T. Albrektsen, S. (2006) Size-fractionated fish hydrolysate as fed ingredient for rainbow trout (*Oncorhynchus mykiss*) fed high plant protein diets. I: Growth, growth regulation and feed utilization. *Aquaculture* 261, 305-317.

Bidlingmeyer, B. A., Cohen, S. A., Tarvin, T. L., Frost, B. (1987) A new, rapid, high sensitive analysis of amino-acids in food type samples. *J. AOAC.* 70, 241-247.

ISO 6496 - 1999 Dry matter

ISO 5983 - 1997 Raw protein

ISO 5984 - 2002 Ash

ISO ISO 21528-1 -2004 Enterobacteriaceae

Nielsen, P.M., Petersen, D., Dambmann, C. (2006) Improved method for determining food protein degree of hydrolysis. *J. Food Sci.* 66, 642-646.

<http://lib3.dss.go.th/fulltext/Journal/Journal%20of%20food%20science/2001%20v.66/no.5/jfsv66n5p0642-0646ms19991109%5B1%5D.pdf>

NMKL no 071 - Salmonella

NordVal no. 012 - Total aerobic germs.

NordVal no. 016 - Yeasts and moulds

Nordval no 023 - Salmonella

NS 9402 – Lipid

Skrede, A. (1979) Utilization of fish and animal byproducts in mink nutrition. IV. Fecal excretion and digestibility of nitrogen and amino acids by mink fed cod (*Gadus morhua*) fillet or meat-and-bone meal. Acta Agric. Scand. 29, 241-256.