Comparison of oil yield and quality obtained by different extraction procedures from salmon (*Salmo salar*) processing byproducts

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Abstract

The content and composition of lipids in different byproducts (skins, heads, and backbones) from mechanically processed farmed Atlantic salmon were determined and compared with that obtained from wild salmon. Three different procedures were used to establish the optimal conditions of oil extraction (at high temperature – 95°C, "cold" extraction at temperature not exceeding 15°C and enzyme assisted with Alcalase). "Cold" extraction at temperature not exceeding 15°C was very efficient, yielding almost 95% of the oil from skins. In the case of heads the obtained yield of about 71% was not lower than that from extraction performed at 95 °C or extraction supported by enzyme treatment. The peroxide value of oil isolated from the heads using "cold" extraction was at the same level as in oil of the enzyme assisted process, but 4 times lower than in oil extracted at high temperature. The results showed that the content of lipids from in the farmed salmon byproducts the content of lipids was about 45-55% higher than in byproducts of wild salmon , however the EPA+DHA content was 10-33% lower.

Practical applications: With "cold" extraction heating which is commonly used for oil recovery in the fish industry could be eliminated and thus the cost of the process would be lower and oxidative changes in the oil reduced. Furthermore, this method based on rules of "green chemistry" can be more attractive and alternative procedure of oil isolation from fatty fish byproducts than those using organic solvents. The fatty fish byproducts such as heads, skins and backbones may be used as a source of valuable oils rich in PUFA. The remaining material after oil isolation can be a source of collagen and gelatin used in the food, pharmaceutical and cosmetic industries and finally of minerals preparation (in the case of heads and backbones) used for enriching animal feed.

Introduction

The main characteristic of fish oil is the specifically high content of long-chain polyunsaturated fatty acids (PUFA) from the *n-3* family, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which is not the feature of lipids from other origins. Nowadays, consumption of these fatty acids is too small and it is the reason why supplementation of the diet with fish oil is recommended to reduce the deficiency of *n-3* PUFA.

EPA and DHA are highly susceptible to oxidation. Oxidized lipids lose their physiological functions and nutritive value. Moreover, their unpleasant smell also limits their application as food additives. In order to obtain oil suitable for human consumption it is necessary to use extraction methods that ensure high quality of the final product.

Fish oil is mainly obtained from whole fish or livers. However, some fish byproducts (especially from fatty fish processing) could serve as a source of good quality fish oil for human consumption [1-6]. The fat content and its composition depend on fish species and the kind of byproducts. The methods for extracting oil from byproducts are similar to those used for whole fish or fillets. Aidos *et al.* [4, 7-9] used steam rendering to recover the oil from byproducts of maatjes herring processing. Chantachum *et al*. [10] showed that optimum conditions for tuna head oil separation involved heating the samples at 85 °C for 30 minutes, followed by pressing. This method was also used to obtain oil from Alaska pink salmon heads and viscera [3, 11], pollock heads, viscera and skins [11] and Atlantic salmon byproducts, like heads, frame bones, skins and downgraded gutted fish [12]. However, high temperature extraction leads to low quality of the product. To improve the oxidative stability and quality of the product the oil must be cold filtered, bleached and deodorized under vacuum [13, 14]. A disadvantage of these processes is lower amount of long-chain PUFA in the refined oil than in the crude oil. Some authors propose using lower temperatures during the wet pretreatment

of the raw materials [15] or decreasing the pH value of liquid phase below 2 [3] to improve the quality of the products. The low pH deactivates enzymes that accelerate the development of unpleasant taste and odour. Barrier and Rousseau [16] patented a method of oil extraction from eviscerated headless and skinless fish mixed with water at temperature lower than 15 °C. Furthermore, enzymatic tissue pretreatment may be an efficient alternative technique for releasing lipids form fish meat and fishing industry byproducts. Fish oil extraction aided with enzymes is more efficient than classical extraction with organic solvents or wet rendering methods [17]. Dumay *et al*. [18, 19] have showed that it is possible to obtain valuable oil from sardine heads and viscera by commercially available proteases. Isolation of the oil supported by enzymes was successfully used to obtain oil from cod viscera and backbones [20, 21] or Nile perch and salmon heads [22]. The type of enzyme and the reaction conditions should be closely matched to the kind of byproducts. According to Gbogouri *et al*. [17] and Linder *et al*. [23] the most effective enzyme for oil isolation from Atlantic salmon heads was Alcalase®.

Solvent extraction and supercritical fluid extraction (SFE) ensure high quality of oil, however, these methods demand expensive equipment [24, 25, 26]. The solvent mixture must meet special requirements, because lipids in the fish tissue can differ in polarity. To avoid oxidation during extraction, the mixture of solvents used must also be effective at low temperatures. Chemical extraction with organic solvents is also used by some authors in laboratory practice to evaluate byproducts as a source of fish oil [5, 6]. SFE is rarely used for isolation of oil from fishing industry byproducts because of high costs. However, comparing to conventional fish oil extraction or enzymatic extraction, SFE may be useful for reducing oxidation [27]. Letisse *et al*. [28] showed that sardine oil obtained using SFE is purer but has lower content of PUFA than oil extracted by hexane.

According to Fish Information & Services 2010 the Polish salmon market, mainly Atlantic salmon from Norwegian aquacultures, constitutes one of the largest in Europe. This

leads to formation of a large amount of fish byproducts - about 21000 t per year. Byproducts are usually converted into fodder meal, or if they are unsuitable for this purpose, they are directed to the landfills. However, the rational way is utilization of salmon byproducts as a raw material for obtaining collagen, gelatin and oil.

The aim of this study was to evaluate the potential of byproducts from mechanical processing of farmed salmon as a source of the health promoting lipids and to establish optimal conditions of oil extraction in respect to yield and quality of the product. Comparison of fatty acid composition of lipids in farmed and wild salmon byproducts was also made.

1. Materials and methods

1.1. Raw materials and reagents

The skins, heads and backbones from mechanically processed farmed salmons, kindly provided by MORPOL S.A. Poland were used. The particular raw materials with the residues of adhering tissues, scales, meat scraps and fat in partially frozen state were minced in a meat grinder (PA-22-M, Edesa HoReCa Ltd) with 5-mm diameter mesh, mixed, packed in approximately 250 g portions into polyethylene bags, sealed under nitrogen and stored at -20 °C not longer than 30 days before use.

Chloroform, methanol, ethanol, diethyl ether, phenolphthalein, sodium and potassium hydroxide were purchased from POCH S.A., Poland. Hexane and potassium iodide were purchased from Merck Poland Company. All chemicals used were analytical grade. For enzymatic extraction of oil Alcalase® AF 2.4L from Novozymes Company was used.

1.2. Analytical procedures

Total nitrogen and ash were determined according to AOAC methods [29] and lipid according to the Folch extraction procedure [30]. The phospholipids were determined as total phosphorous by using colorimetric method according to Totani *et al*. [31].

Primary oxidation products – hydroperoxides were determined as peroxide value (PV) according to the PN-EN ISO 3960:2005 Standard [32] and free fatty acids as the acid value (AV), according to the PN-EN ISO 660:1998 Standard [33].

The method of transesterification by methyl alcohol in the presence of alkaline catalyst at low temperature (according to PN-EN ISO 5509:2000) was used to convert fatty acids to methyl esters (FAME). The FAMEs were analyzed with Perkin Elmer Autosystem XL gas chromatograph, equipped with a 30 m DB-23 silica capillary column (J&W Scientific) of 0.25 mm ID and film coating thickness of 0.25μm. Helium carrier-gas column flow rate was 0.91 ml/min. A split-splitless (60:1) injector at 250 °C and flame-ionization detector (FID) at 250 °C were used. The column temperature, after an initial isothermal period of 5 min at 120 °C, was increased to 180 °C at a rate of 1.5 °C/min, and maintained for 25 min. The temperature was increased again to 210 °C and was maintained for 30 min.

1.3. Extraction of oil

Three procedures were used for oil extraction.

Procedure I ("high temperature")

Water at temperature 50°C was added to the frozen raw heads (1:1, w/v)-and the mixture was mixed with a hand blender (HR 1676/90, Phillips) about 5 minutes to form a homogenous pulp. During this procedure the temperature did not exceed 15°C. Then the pulp was heated at 95°C for 30 minutes under reduced pressure (0.02-0.04 MPa) with stirring, cooled under vacuum to room temperature, and centrifuged for 10 minutes at 8000x*g*. The oil from the upper phase was collected by using automatic pipette, the solid residues were discarded and the liquid phase was centrifuged again. The separated oil was collected and combined with the previously obtained fraction.

Procedure II ("cold" extraction)

Water at temperature 50°C was added to the frozen raw material (heads, skins and backbones, separately) (1:1, w/v) and the mixture was mixed with a hand blender (HR 1676/90, Phillips) about 5 minutes to form a homogenous pulp. During this procedure the temperature did not exceed 15°C. The pulp was then centrifuged 10 minutes at 8000xg. The oil-protein-water phase was separated from solid residues and was centrifuged again for 5 minutes at 8000xg. The separated oil was collected (by using automatic pipette) and weighed.

Procedure III

Extraction of oil from salmon heads was carried out according to Gbogouri *et al*. [17]. Minced salmon heads were mixed for 15 minutes with water (1:1 w/v) at 55 °C. The pH of the mixture was adjusted to 8.0 with 4 M NaOH and Alcalase® was added at substrate mass concentration of 5%. The enzymatic reaction was carried out at 55 °C for 2 hours under nitrogen with continuous stirring. The pH of reacting mixture was adjusted to 8.0 for every 15 minutes with 4M NaOH. After 2 hours the mixture was centrifuged for 30 minutes at 8000xg. The oil was collected from the upper phase and weighed.

All procedures of oil extraction were repeated 4-6 times. The results are averages from 4-6 replications ± standard deviation (SD).

2. Results and discussion

2.1. Chemical composition of raw materials and characteristic of the oil

The richest source of oil among the examined byproducts of both farmed and wild salmon, are skins (Table 1). They constituted above 20% of lipids while 14.8% and 15.6% w ere present in heads and backbones, respectively. Higher content of total lipids in salmon heads (amounted to about 20%) reported Linder *et al*. [23] and Gbogouri *et al*. [17]. The byproducts from wild salmon contained less oil than byproducts from farmed salmon - generally about 50% less than in the same type of farmed salmon byproducts. The lipid content in all types of byproducts from wild salmon was similar and reached about 8%. These

results are consistent with those obtained by Hamilton *et al*. [24] – skin-on fillets from farmed salmon had more lipids (16.6%) than from wild salmon (6.4%). As would be expected the content of mineral compounds (ash) in the heads and backbones was higher than in the skins for both farmed and wild salmon. For example, the farmed salmon heads contained 4.2% of ash, which can be explained by the presence of bones and gristle, while the farmed salmon skins contained only 2.3% of ash. The same situation was observed in the case of wild salmon, where backbones contained 4.6% of ash and skins contained only 1.5%. Simultaneously, what is obvious, the content of proteins was negatively correlated with the content of minerals (ash).

The content of phospholipids in farmed salmon heads and backbones was 30% higher than in skins (Table 2). It results from the fact that the former are rich in nervous tissue (brain and spinal cord), of which phospholipids are important components.

The level of peroxides in the oil isolated from skins and backbones of farmed salmon by using Folch procedure did not exceed 0.9 mEq O_2/kg, while in the oil from heads it was about 3 times higher (Table 2). The presence of some amount of blood in the heads is probably responsible for such results. Especially the gills are richly supplied with blood vessels in order to act as a respiratory organ. The autoxidation of hemoglobin is an important reaction responsible for the ability of hemoglobin to accelerate lipid oxidation. Release of the oxygen from oxyhemoglobin leads to formation of methemoglobin and superoxide anion radical [35, 36]. Next, the superoxide radical is rapidly dismutated to oxygen and hydrogen peroxide. Hydrogen peroxide can react with previously formed methemoglobin what causes the formation of a ferryl protein radical – known as an initiator of lipid oxidation [35, 37]. Furthermore, when a considerable amount of peroxides is present, iron can be released from hemin and participate in oxidation of lipids [36, 38].

198 The free fatty acids content (expressed as mg KOH/g) from particular types of farmed
199 salmon byproducts was low and did not exceed 1.3 mg KOH/g (Table 2). It indicated that
200 significant hydrolytic changes in lipids did not occur during processing and extraction
201 procedure.

202 The fatty acid compositions of wild and farmed salmon byproducts lipids are shown in
203 Table 3.

204 The vast majority of fatty acids present in oil of both farmed and wild salmon were
205 unsaturated, 70-75% of total fatty acids. Gbogouri *et al*. [17] reported similar value (about
206 74%) for the oil isolated by chemical extraction (Folch method) from farmed salmon heads.
207 The content of saturated fatty acids (SAFA) oils of all salmon byproducts ranged from 18.6%
208 to 23.9% and it was the lowest value in the skins, both from wild and farmed salmon (Table
209 3). The most abundant SAFA in the oils from different fish byproducts was palmitic acid (C
210 16:0), while the amount of oleic acid (C 18:1 n-9) was the largest among monounsaturated
211 fatty acids (MUFA).

212 MUFA content was lower, whereas the PUFA content was higher, in lipids of
213 byproducts from wild salmon than farmed salmon (Table 3). EPA and DHA were the major
214 components among the PUFA in all oils of farmed and wild salmon. However, EPA+DHA
215 content in the oils ~~lipids~~ of farmed salmon byproducts (16.4 – 18.9%) was 10-33% lower than
216 that in the oils of wild salmon (21-24.6%). On the other hand, Blanchet *et al*. [39] showed that
217 the content of EPA+DHA was about 17% higher in oil of meat from farmed than from wild
218 salmon. Probably such differences may be due to the different feed, which highly influences
219 the lipid content and their fatty acid composition. Additionally differences can occur in the
220 same fish depending on the period of development, reproduction and spawning. Therefore, if
221 the farmed and wild salmon used in the experiments are from different stages of development
222 the results can be varied. Furthermore, the amount of EPA+DHA in the oil obtained from

wild and farmed salmon byproducts was lower than in oils from other fish species like menhaden (26.4%), sardines (27.5%) and tuna (28%) [40, 41, 42]. Nevertheless, the farmed salmon byproducts constitute an abundant source of EPA+DHA.

2.2. Influence of different extraction procedures on the oils characteristics

Three different procedures were used to isolate oil from salmon byproducts. The yield of the oil obtained by procedure I "high temperature", which is typical for industrial rendering of oil, reached only about 70% (Table 4) of the content of oil established by using Folch method (Table 1). The reason for the relatively low oil yield can be the high content of phospholipids in salmon heads, which stabilize emulsions and render separation of the oil more difficult. Moreover, according to Chantachum *et al*. [10], such high temperature as 95 °C used during extraction can additionally impede the oil release. They found that packed unfolded proteins and trapped lipid droplets were formed under these conditions [10]. The temperature of oil isolation has also a great influence on the oxidative stability of the oil. The oil obtained from heads by using procedure I had the highest PV, about 9 $mEqO_2$/kg of the oil (Table 4), while the oil obtained by chemical (Folch method) extraction from the same type of byproducts showed 3.5 times lower PV (Table 2). Similar results were reported by Skâra *et al*. (2004) who found that the PV of oil extracted at high temperatures from mixed salmon byproducts (heads, frame bones, skins and down-graded gutted fish) reached about 10 $mEqO_2$/kg of the oil. The content of free fatty acids in the oil obtained by using procedure I (Table 4) was only 8% higher than in the oil extracted by the Folch method (Table 2). This suggests that the temperature has no large impact on hydrolysis of the oil, although the calculated differences were statistically significant.

In the next step, the procedure of oil extraction was modified and as the raw material, besides heads, skins and backbones were also used. The homogenized pulp was not heated during extraction. The temperature in the process did not exceed 15 °C when procedure II of

extraction (“cold” extraction) was used. This was possible to ensure, because the raw material in partially frozen state was used in experiments. The results showed that the yield of oil depended on the type of byproducts (Table 4). The highest yield of oil was extracted from skins, nearly 95%, while for heads it amounted to 71% and was similar to that obtained when procedure I of extraction was used. The PVs of the oil isolated from all types of byproducts by procedure II (Table 4) were lower or similar to the oil extracted by Folch method (Table 2 and Table 4). The content of free fatty acids in oils isolated from all types of byproducts was even lower (for instance in skins AV was two times lower) than in the oil obtained by Folch method. The content of phospholipids in oils from all types of byproducts was much lower than in the oil isolated by using Folch method (Table 4). Polar lipids can be bound to proteins, but using solvent mixture of chloroform-methanol in Folch method allowed their release from complexes and thus the extracted oil had higher phospholipids content than the oils extracted only by using water (II extraction). However, the amount of phospholipids in the oil isolated from salmon heads (0.15%) by procedure II (cold extraction) was higher than in the oil obtained by procedure I - at high temperature (0.02%) because during heating of the pulp, phospholipids participate in forming of stable emulsions what lowers their separation by centrifugation.

The influence of temperature on the oil quality is clearly visible in the case of oils from salmon heads. The PV of oil isolated from heads by using procedure II was 4 times lower and the AV was about 80% lower than in the oil isolated at high temperature (procedure I).

Extraction supported by enzyme Alcalase® (procedure III) was used to improve the yield of oil isolation from salmon heads. Some authors reached satisfactory yield of fish oil by using enzymes, for example Gbogouri et al. [17] obtained 92% yield of oil isolation from salmon heads by using Alcalase®. However, in our work the amount of oil extracted from

salmon heads in the presence of Alcalase® was similar to that obtained by using the procedure I and procedure II and reached about 70% (Table 4). The PV of the oil (1.6 $mEqO_2/kg$ of the oil) was the same as for the oil obtained by using "cold" extraction (procedure II) and lower than in the oils isolated from salmon heads by chemical extraction (Folch method) and procedure I (Table 4). The amount of free fatty acids was lower than that in the oil obtained by Folch method (Table 2) and in the oil obtained by using procedure I, but higher than in the oil extracted by using procedure II (Table 4). The oil isolated from the heads by using enzymes was characterized with high content of phospholipids (1.47%), the value of which was about 10 times higher than in the oils isolated by other procedures (Table 4). The enzymes most probably contribute to releasing of phospholipids from the membranes. From the biological point of view, the presence of phospholipids in oil and their consumption is desirable [18].

In the oil isolated from all types of byproducts by the "cold" extraction (procedure II), the MUFA content (Table 5) was higher than in the oils obtained by using Folch method (Table 3 and 5). The PUFA and EPA+DHA content in the oil isolated from skins (33.3% and 15.4% respectively) was slightly lower than in the oil obtained by Folch method (34.8% and 16.9% respectively), but these were statistically significant differences. In the oil from salmon heads (Table 5), the EPA+DHA content (16.7%) was about 20% lower in comparison with the oil extracted by Folch method (18.9%), but only about 10% lower from the oil obtained according to the procedure I or by using Alcalase®.

In general it can be concluded that the content of PUFA and EPA+DHA in oils from „cold" extraction of byproducts is very close to those obtained by using procedures I ("high temperature") and III ("by enzymes").

Conclusions

This study has shown that the byproducts from mechanically processed salmon could serve as a source of oils rich in PUFA.

The "cold" extraction of oil from salmon byproducts shows some advantages in comparison to procedures commonly used in the fishing industry. This process conducted at low temperatures allows achieving high yield of oil and simultaneously inhibits lipid oxidation and thus ensures higher oil quality than in the oil obtained at high temperatures. This is especially important for the oil designed as a food supplement to enrich the diet in PUFAs. Furthermore, with elimination of the heating step the cost of the process is significantly reduced.

With "cold" extraction it is possible to obtain similar oil yield and amount of EPA + DHA to the ones obtained in the case of extraction supported by enzymes, but the former is more suitable for this purpose. Use of enzymes has special requirements e.g. exactly defined, usually enhanced temperature, what additionally makes higher the costs of oil isolation.

Summarizing, it can be stated, that the "cold" extraction could be an attractive solution for isolating the oil from fatty fish byproducts. This procedure allows achieving the oil from fish skins, backbones and heads with high yield and quality in a simple and cheap way.

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Conflict of interest

There are none financial and commercial conflicts of interest.

3. References

[1] Rubio-Rodriguez, N., de Diego, S.M., Beltran, S., Jaime, I., Sanz, M.T., & Rovira J. Supercritical fluid extraction of fish oil from fish by-products: A comparison with other extraction methods. *J. Food Eng.* 2012, *109*, 238-248.

[2] Kim, S.-K., & Mendis, E. Bioactive compounds from marine processing byproducts – a review. *Food Res. Int.* 2006, *39*, 383-393.

[3] Wu, T.H., & Bechtel, P.J. Salmon by-product storage and oil extraction. *Food Chem.* 2008, *111*, 868-871.

[4] Aidos, I., van der Padt, A., Boom, R.M., & Luten J.B. Upgrading of maatjes herring byproducts: production of crude fish oil. *J. Agri.Food Chem.* 2001, *49*, 3697-3704.

[5] Byun, H-G., Eom, T-K., Jung, W-K., & Kim S-W. Characterization of fish oil extracted from fish processing by-products. *J. Food Sci. Nutr.* 2008, *13*, 7-11.

[6] Khoddami, A., Ariffin, A.A., Bakar, J., & Ghazali, H.M. (2009). Fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (*Sardinella lemuru*). *WASJ* 2009, *7*, 127-131.

[7] Aidos, I., Lourenço, S., van der Padt, A., Lutenand, J.B., & Boom R.M. Stability of crude herring oil produced from fresh byproducts: Influence of temperature during storage. *J. Food Sci.* 2002, *67*, 3314-3320.

[8] Aidos, I., Masbernat-Martinez, S., Luten, J.B., Boom, R.M., & van der Padt A. Composition and stability of herring oil recovered from sorted byproducts as compared to oil from mixed byproducts. *Journal of Agriculture and Food Chemistry* 2002, *50*, 2818-2824.

[9] Aidos, I., Kreb, B., Boonman, M., Luten, J.B., & van der Padt A. Influence of production process parameters on fish oil quality in a pilot plant. *J. Food Sci.* 2003, *68*, 581-587.

344 [10] Chantachum, S., Benjakul, S., & Sriwirat N. Separation and quality of fish oil from
345 precooked and non-precooked tuna heads. *Food Chem.* 2000, *69*, 289-294.
346 [11] Oliviera, A.C.M., & Bechtel, P.J. Lipid composition of Alaska pink salmon
347 (*Oncorhynchus gorbuscha*) and Alaska walleye pollock (*Theragra chalcogramma*)
348 byproducts. *J. Aquat. Food Prod. Tech.* 2005, *14*, 73-91.
349 [12] Skâra, T., Sivertsvik, M., & Birkeland, S. (2004). Production of salmon oil from filleting
350 byproducts - Effects of storage conditions on lipid oxidation and content of omega-3
351 polyunsaturated fatty acids. *J. Food Sci.* 2004, *69*, 417-421.
352 [13] J.B. Crowther, B.H. Booth, D.S. Blackwell: World Patent WO/2001/041582 (2001).
353 [14] M. Takao: US Patent 4.623.488 (1986).
354 [15] P. Bladh: US Patent 4.344.976 (1982).
355 [16] P. Barrier, J. Rousseau: US Patent 6.214.396 (2001).
356 [17] Gbogouri, G.A., Linder, M., Fanni, J., & Parmentier, M. Analysis of lipids extracted
357 from salmon (*Salmo salar*) heads by commercial proteolytic enzymes. *Eur. J. Lipid Sci.*
358 *Technol.* 2006, *108*, 766-775.
359 [18] Dumay, J., Allery, M., Donnay-Moreno, C., Barnathan, G., Jaouen, P., Carbonneau, M.
360 E., & Bergé, J.P. Optimization of hydrolysis of sardine (*Sardina pilchardus*) heads with
361 Protamex: enhancement of lipid and phospholipid extraction. *J. Sci. Food Agriculture*
362 2009, *89*, 1599-1606.
363 [19] Dumay, J., Donnay-Moreno, C., Baranthan, G., Jaouen, P., & Berge, J.P. Improvement
364 of lipid and phospholipid recoveries from sardine (*Sardina plichardus*) viscera using
365 industrial proteases. *Process Biochem.* 2006, *41*, 2327-2332.
366 [20] Šližyte, R., Rustad, T., & Storrř, I. Enzymatic hydrolysis of cod (*Gadus morhua*) by-
367 products. Optimization of yield and properties of lipid and protein fractions. *Process*
368 *Biochem.* 2005, *40*, 3680-3692.

[21] Daukšas, E., Falch, E., Šližyte, R., & Rustad, T. Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (*Gadus morhua*) by-products. *Process Biochem.* 2005, *40*, 2659-2670.

[22] Mbatia, B., Adlercreutz, D., Adlercreutz, P., Mahadhy, A., Mulaa, F., & Mattiasson, B. Enzymatic oil extraction and positional analysis of ω-3 fatty acids in Nile perch and salmon heads. *Process Biochem.* 2010, *45*, 815-819.

[23] Linder, M., Fanni, J., & Parmentier, M. Proteolytic extraction of salmon oil and PUFA concentration by lipases. *Mar. Biotechnol.* 2005, *15*, 70-76.

[24] Moffat, C.F., McGill, A.S., Hardy, R., & Anderson, R.S. The production of fish oils enriched in polyunsaturated fatty acids containing triglycerides. *JAOCS* 1993, *70*, 133-138.

[25] Dunford, N.T., Temmeli, F., & LeBlanc, E. Supercritical CO_2 extraction of oil and residual proteins from Atlantic mackerel (*Scomber scombrus*) as affected by moisture content. *J. Food Sci.* 1997, *62*, 289-294.

[26] Regost, C., Jakobsen, J.V., & Røra, A.M.B. Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Res. Int.* 2004, *37*, 259-271.

[27] Rubio-Rodriguez, N., de Diego, S.M., Beltran, S., Jaime, I., Sanz, M.T., & Rovira, J. Supercritical fluid extraction of the omega-3 rich oil contained in hake (*Merluccius capensis–Merluccius paradoxus*) by-products: Study of the influence of process parameters on the extraction yield and oil quality. *J. Supercrit. Fluids* 2008, *47*, 215-226.

[28] Letisse, M., Rozieres, M., Hiol, A., Sergent, M., & Comeau, L. Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier: I. Optimization of extraction conditions. *J. Supercrit. Fluids* 2006, *38*, 27-36.

393 [29] Helrich K. (Ed.): Official methods of analysis (15th ed.), Association of Official
394 Analytical Chemists, Virginia (USA) 1990.
395 [30] Undeland, I., Härröd, M., & Lingnert, H. (1998). Comparison between methods using
396 low-toxicity solvents for the extraction of lipids from herring (*Clupea harengus*). *Food*
397 *Chem.* 1998, *61*, 355-365.
398 [31] Totani, Y., Pretorius, H.E., & du Plessis, L.M. Extraction of phospholipids from plant
399 oils and colorimetric determination of total phosphorous. *JAOCS* 1982, *59*, 162-163.
400 [32] PN-EN ISO 3960:2005. Animal and vegetable fats and oils. Determination of peroxide
401 value.
402 [33] PN-EN ISO 660:1998. Animal and vegetable fats and oils. Determination of acid value
403 and acidity.
404 [34] Hamilton, M.C., Hites, R.A., Schwager, S.J., Foran, J.A., Knuth, B.A., & Carpenter,
405 D.O. Lipid composition and contaminants in farmed and wild salmon. *Environ. Sci.*
406 *Technol.* 2005, *39*, 8622-8629.
407 [35] Richards, M.P., Modra, A.M., & Li, R. Role of deoxyhemoglobin in lipid oxidation of
408 washed cod muscle mediated by trout, poultry and beef hemoglobins. *Meat Sci.* 2002, *62*,
409 157-163.
410 [36] Everse, J., & Hsia, N. The toxicities of native and modified hemoglobins. *Free Radical*
411 *Bio. Med.* 1997, *22*, 1075-1099.
412 [37] Harel, S., & Kanner, J. Muscle membranal lipid peroxidation initiated by hydrogen
413 peroxide-activated metmyoglobin. *J. Agri.Food Chem.* 1985, *33*, 1188-1192.
414 [38] Puppo, A., & Halliwell, B. Formation of hydroxyl radicals form hydrogen peroxide in the
415 presence of iron. Is haemoglobin a biological Fenton reagent? *Biochem. J.* 1988, *249*,
416 185-190.

[39] Blanchet, C., Lucas, M., Julien, P., Levesque, B., & Dewailly E. (2005). Fatty acids composition of wild and farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Lipids* 2005, *40*, 529-531.

[40] Tanaka, Y., Hirano, J., & Funada, T. Concentration of docosahexaenoic acid in glyceride by hydrolysis of fish oil with *Candida cylindracea* lipase. *JAOCS* 1992, *69*, 1210-1214.

[41] Chen, T-Ch., & Ju, Y-H. Enrichment of eicosapentaenoic acid and docosahexaenoic acid in saponified menhaden oil. *JAOCS* 2000, *77*, 425-428.

[42] Ganga, A., Nieto, S., Sanhuez, J., Romo, J., Speisky, H., & Valenzuela A. Concentration and stabilization of *n-3* polyunsaturated fatty acids from sardine oil. *JAOCS* 1998, *75*, 733-736.

438 Table 1. Chemical composition of raw material

Type of salmon byproducts	Total lipids [%]		Total proteins [%]		Ash [%]	
	farmed	wild	farmed	wild	farmed	wild
Heads	14.8±0.68^{b}	8.0±0.15^{b}	14.00±0.13^{c}	16.3±0.50^{c}	3.8±0.45^{a}	3.5±0.24^{b}
Skins	20.2±0.64^{a}	8.6±0.05^{a}	21.19±0.14^{a}	22.9±2.40^{a}	2.3±0.11^{b}	1.5±0.08^{c}
Backbones	15.6±0.39^{ab}	7.3±0.02^{c}	16.69±0.05^{b}	18.0±0.14^{b}	4.2±0.14^{a}	4.6±0.38^{a}

439 Results are expressed as means of four measurements ± SD. The values in the columns marked with
440 different letters (a - c) differ significantly ($p<0.05$).

441

442 Table 2. Chemical characteristic of oil isolated according to Folch procedure from different

443 types of farmed salmon byproducts

Type of byproducts	PV	AV	Phospholipids
	[mEq O_2/kg]	[mg KOH/g]	[% of total lipids]
Heads	2.56±0.02[a]	1.23±0.04[a]	3.1±0.1[a]
Skins	0.88±0.01[b]	0.98±0.02[c]	2.0±0.0[b]
Backbones	0.68±0.00[c]	1.12±0.05[b]	3.3±0.2[a]

444 Results are expressed as means of four measurements ± SD. The values in the columns marked with

445 different letters (a - c) differ significantly ($p<0.05$).

446

447

448 Table 3. Fatty acid composition (% of the total fatty acids) of oils extracted by Folch
449 procedure from various salmon byproducts

Fatty acid	Wild salmon			Farmed salmon		
	backbones	heads	skins	backbones	heads	skins
SAFA						
C 14:0	3.2±0.0	3.6±0.1	3.2±0.0	4.2±0.4	3.7±0.1	3.9±0.0
C 15:0	0.4±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
C 16:0	15.6±0.2	16.9±0.2	15.0±0.1	11.7±0.3	11.4±0.2	11.9±0.0
C 17:0	0.1±0.0	0.1±0.0	0.1±0.0	0.5±0.0	0.5±0.0	0.5±0.0
C 18:0	2.8±0.1	2.9±0.0	2.5±0.1	2.5±0.0	2.7±0.1	2.5±0.0
C 20:0	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.0	0.5±0.0	0.5±0.0
ΣSAFA	22.1±0.1^{b}	23.9±0.1^{a}	21.1±0.0^{c}	19.7±0.2^{d}	19.1±0.1^{e}	18.6±0.0^{f}
MUFA						
C 14:1	0.3±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C 16:1	0.3±0.0	0.4±0.0	0.4±0.0	0.2±0.0	0.2±0.0	0.2±0.0
C 16:1 n-7	4.0±0.1	4.3±0.1	4.0±0.0	4.7±0.2	4.6±0.1	4.7±0.0
C 17:1	0.5±0.0	0.5±0.0	0.5±0.0	0.4±0.0	0.4±0.0	0.4±0.0
C 18:1 n-9	21.8±0.0	23.4±0.2	23.0±0.2	22.8±0.1	20.7±0.0	22.7±0.1
C 18:1 n-7	2.6±0.0	2.7±0.1	2.5±0.0	2.9±0.0	2.9±0.1	2.8±0.0
C 20:1 n-9	1.2±0.1	1.4±0.0	1.2±0.1	5.0±0.2	4.8±0.0	4.9±0.1
C 22:1 n-11	0.0±0.0	0.0±0.0	0.0±0.0	4.4±0.2	4.2±0.0	4.1±0.0
C 22:1 n-9	0.2±0.0	0.2±0.0	0.2±0.0	0.7±0.0	0.6±0.0	0.6±0.0
C 24:1	1.2±0.0	1.2±0.0	1.1±0.0	0.6±0.0	0.6±0.0	0.6±0.0
ΣMUFA	32.1±0.1^{f}	34.4±0.1^{d}	33.0±0.0^{e}	41.8±0.2^{a}	39.1±0.0^{c}	41.1±0.0^{b}
PUFA						
C 18:2 n-6	4.3±0.0	4.4±0.0	4.5±0.0	6.7±0.0	6.1±0.0	6.8±0.0
C 18:3 n-3	2.8±0.0	2.7±0.1	2.8±0.0	2.7±0.0	2.3±0.0	2.7±0.0
C 18:4 n-3	1.9±0.0	1.7±0.0	1.8±0.0	1.3±0.1	1.4±0.0	1.4±0.0
C 20:2 n-6	0.9±0.0	0.9±0.0	0.9±0.0	0.7±0.0	0.7±0.0	0.7±0.0
C 20:3 n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0
C 20:3 n-3	0.4±0.0	0.4±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.0
C 20:4 n-6	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.8±0.0	0.7±0.0
C 20:4 n-3	2.0±0.0	1.8±0.0	2.0±0.0	1.3±0.0	1.5±0.0	1.4±0.0
C 20:5 n-3	6.6±0.1	5.9±0.0	6.4±0.1	7.5±0.0	8.6±0.2	7.8±0.0
C 22:5 n-3	3.1±0.0	2.7±0.0	3.2±0.0	3.5±0.1	3.9±0.0	3.7±0.0
C 22:6 n-3	18.0±0.2^{a}	15.1±0.1^{b}	17.7±0.2^{a}	8.9±0.1^{e}	10.3±0.1^{c}	9.1±0.0^{d}
ΣPUFA	40.7±0.2^{a}	36.3±0.0^{b}	40.4±0.1^{a}	33.7±0.1^{c}	36.1±0.0^{c}	34.8±0.0^{d}
EPA+DHA	24.6±0.1^{a}	21.0±0.0^{c}	24.1±0.1^{b}	16.4±0.0^{f}	18.9±0.1^{d}	16.9±0.0^{e}

450 Results are expressed as means of six measurements ± SD. The values in the rows marked with
451 different letters (a - c) differ significantly ($p<0.05$).

52

453 Table 4. Characteristics of the oils isolated from salmon byproducts using three different
454 procedures of extraction.

Procedure of extraction	Type of byproducts	Yield [%]	PV [mEqO$_2$/kg]	AV [mgKOH/g]	Phospholipids [% of total lipids]
I	Heads	71.1±0.4^{c}	9.2±0.6^{a}	1.34±0.03^{a}	0.02±0.00^{d}
II	Heads	71.5±1.1^{c}	2.5±0.2^{b}	0.18±0.01^{e}	0.15±0.01^{c}
	Skins	95.2±2.2^{a}	0.8±0.1^{d}	0.43±0.01^{cd}	0.13±0.01^{c}
	Backbones	82.7±1.7^{b}	0.7±0.1^{d}	0.85±0.02^{b}	0.29±0.06^{b}
III	Heads	72.1±0.9^{c}	1.6±0.1^{c}	0.70±0.02^{c}	1.47±0.11^{a}

455 Results are expressed as means of six measurements ± SD. The values in the columns marked with
456 different letters (a - c) differ significantly ($p<0.05$).

457

458 Table 5. Fatty acid composition (% of the total fatty acids) of oils isolated from salmon
459 byproducts using three different procedures of extraction

	Procedure of extraction				
Fatty acid	I	II			III
	heads	heads	skins	backbones	heads
SAFA					
C 14:0	4.1±0.1	4.1±0.2	4.1±0.2	4.0±0.0	4.6±0.1
C 15:0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
C 16:0	11.7±0.2	11.6±0.2	11.4±0.5	11.2±0.0	12.2±0.1
C 17:0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0
C 18:0	2.6±0.1	2.6±0.1	2.5±0.0	2.5±0.0	2.6±0.0
C 20:0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0
ΣSAFA	19.7±0.2^{b}	19.6±0.2^{b}	19.3±0.4^{bc}	19.0±0.0^{c}	20.7±0.1^{a}
MUFA					
C 14:1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C 16:1	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
C 16:1 n-7	5.0±0.1	4.4±0.1	4.7±0.3	4.7±0.0	5.1±0.1
C 17:1	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
C 18:1 n-9	21.4±0.2	23.9±0.2	23.4±0.6	23.1±0.2	21.7±0.3
C 18:1 n-7	3.0±0.1	2.8±0.0	2.9±0.1	2.7±0.1	3.0±0.1
C 20:1 n-9	4.6±0.1	5.0±0.1	5.0±0.1	5.3±0.3	4.6±0.1
C 22:1 n-11	4.0±0.0	4.4±0.1	4.2±0.1	4.5±0.0	3.9±0.1
C 22:1 n-9	0.6±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.6±0.0
C 24:1	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.5±0.0
ΣMUFA	39.9±0.2^{b}	42.5±0.2^{a}	42.2±0.5^{a}	42.3±0.2^{a}	40.1±0.2^{b}
PUFA					
C 18:2 n-6	6.4±0.1	7.2±0.0	7.1±0.2	6.8±0.0	6.4±0.2
C 18:3 n-3	2.4±0.1	2.9±0.0	2.8±0.2	2.7±0.0	2.5±0.1
C 18:4 n-3	1.4±0.0	1.3±0.0	1.4±0.0	1.4±0.0	1.4±0.0
C 20:2 n-6	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0
C 20:3 n-6	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
C 20:3 n-3	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
C 20:4 n-6	0.7±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0
C 20:4 n-3	1.4±0.0	1.3±0.0	1.4±0.1	1.4±0.0	1.4±0.0
C 20:5 n-3	8.3±0.2	7.1±0.1	7.3±0.4	7.4±0.0	8.0±0.2
C 22:5 n-3	3.6±0.0	3.1±0.0	3.4±0.5	3.4±0.0	3.4±0.1
C 22:6 n-3	9.1±0.2	8.0±0.1	8.1±0.3	8.3±0.0	8.7±0.2
ΣPUFA	34.5±0.2^{a}	32.7±0.1^{c}	33.3±0.5^{bc}	33.2±0.0^{bc}	33.6±0.2^{b}
EPA+DHA	17.4±0.2^{a}	15.1±0.1^{d}	15.4±0.3^{cd}	15.7±0.0^{c}	16.7±0.2^{b}

460 Results are expressed as means of six measurements ± SD. The values in the rows marked with
461 different letters (a - c) differ significantly ($p<0.05$).