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Rheological and Thermal Properties of Salmon Oil after *Aspergillus oryzae* Lipolysis

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Abstract: The aim of this research is physico-chemical characterization of salmon oil subjected to an enzymatic treatment in order to concentrate it in PUFA. Regio selective action of lipase resulting from *Aspergillus oryzae* (37°C, pH 7, SP 398: 40 KLU) make it possible to selectively hydrolyze the fatty acids mainly connected in position 1 and 3 on glycerol, thus modifying the physical properties of the reactional mixture. Viscosity and surface tensions were measured according to the temperature between -5 and 30°C over 48 h of hydrolysis. Two characteristic temperatures 12°C for the low lipolysis degree and 18°C for high values of DH. The enthalpic differential analysis between -60 and 40°C as well as measurements of the relaxation times T_1 and T_2 by protonic NMR made it possible to characterize the evolution of the reactional mixture according to the lipolysis. Finally analyses of viscosity according to a gradient of temperature ranging between 5 and 40°C or of the shear stress will make it possible to supplement knowledge on the modifications of structure of the reactional mixture. This work constitutes a preliminary stage with the optimization of the conditions of enrichment in salmon oil PUFA.

Key words: Salmon oil, *Aspergillus oryzae*, lipolysis, Newtonian fluid, DSC, viscosity

INTRODUCTION

Fish oil, which is a rich source of long-chain n-3 polyunsaturated fatty acids (PUFA), mainly EPA (C20:5n-3, eicosapentaenoic acid) and docosahexaenoic acid (C22:6n-3, DHA), has received much attention in the scientific and industrial communities because of its positive role in human health (Schmidt and Dyerberg, 1994; Dumay *et al*., 2006). The potential health benefits of fish oil include reduced risk of cardiovascular diseases, hypertension and atherosclerosis as well as inflammatory and autoimmune disorders. DHA (22:6n-3) is now recognized as a physiologically essential nutrient in the brain and retina for neural functioning and visual activity, respectively (Holub, 2001).

However, there is a potential of producing oil from by-products (Aidos *et al*., 2003; Ying *et al*., 2007). The by-products of fish processing, including heads, skins, frames and viscera, often end up in landfills or rendering plants.

Several of tons of fish processing waste is produced worldwide from salmon farming and could be converted into useful products such as salmon hydrolysate and oil (Wright, 2004). The hydrolysate is typically produced by maceration of viscera or skinny byproducts followed by enzymatic digestion of protein and removal of bones (Kristinsson and Rasco, 2000).

Salmon oil, a by-product of salmon processing, was used as a feedstock for biodiesel production via transesterification in a 2-step process (Mashad *et al*., 2008).

Several processes were use for the PUFA, the process consist of: concentration by centrifugation, extraction after saponification, concentration of PUFA by urea method, isolation of PUFA by preparative reverse phase HPLC, extraction and separation of PUFA with supercritical fluids and lipase-catalyzed reactions may be used for obtaining triacylglycerol enrichment of PUFA (Robles *et al*., 1998).

Enzymatic hydrolysis of oils produces a mixture of mono, di, triacylgerols and FFA. Most of the research in this area aims to increase the PUFA content of the remaining glycerols (Hishino *et al*., 1990; Shimade *et al*., 1994; Tanaka *et al*., 1992, 1993).

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Some authors reported that because n-3 PUFA is concentrated in the 2-position of TAG, hydrolysis of fish oil with 1, 3-specific lipase should produce PUFA-rich 2-MAG and 1 (3), 2-DAG (Tanaka *et al*., 1992).

The aim of this research is physicochemical characterization of salmon oil subjected to an enzymatic treatment in order to concentrate it in PUFA. For a possible industrial use of hydrolyzed salmon oil.

MATERIALS AND METHODS

Salmon heads (*Salmo salar*) oil were used to obtain oil by an enzymatic process according to the method described in previous papers (Linder *et al*., 2002; Xu *et al*., 2000).

A specific sn-1, sn-3 hydrolytic lipase from *Aspergillus oryzae* (Novozym® SP 398) was used for lipid hydrolysis. All enzymes were supplied by Novo Nordisk (Bagsvaard, Denmark).

Lipolysis kinetics: A reaction mixture containing 20 g of oil, 40 k Lipase Units (KLU) and 20 mL of distilled water was incubated at 37°C, pH 7.0 in a 200 mL erlenmeyer flask blanketed with nitrogen and kept sealed throughout the reaction. The vessels were agitated on an orbital shaker (G24 Environmental Incubator Shaker, New Brunswick Scientific Co., Inc. Edison N.J. USA) at 300 rpm. The course of the reaction was controlled by withdrawing 2 flasks at appropriate intervals over 48 h. In the first one, the reaction kinetics was stopped by the addition of 50 mL ethanol in the reactor and the liberation of fatty acids was determined by titration with 1.0 M NaOH. The hydrolysis percentage of the oil was measured according to the following equation:

$$\text{Hydrolysis (\%)} = \frac{\text{Acid value}_{(\text{hydrolyzed oil})} - \text{Acid value}_{(\text{unhydrolyzed oil})}}{\text{Saponification value}_{(\text{unhydrolyzed oil})} - \text{Acid value}_{(\text{unhydrolyzed oil})}}$$

where, acid value is expressed as the number of mg of KOH required to neutralize free fatty acids present in 1 g of oil; the saponification value is defined as the number of mg of KOH required to saponify 1 g of oil.

The mixture of the second flask was centrifuged at 5,000×g for 10 min in a J2-HS Beckman centrifuge (Beckman Instruments, Gagny, France) to recover the oil phase.

Fatty acid composition analysis: Fatty acid composition of each lipid classes was determined after transmethylation using borontrifluoride-methanol by gas chromatography. Lipid sample (5-40 mg) was heated at 100°C with 1.5 mL hexane and 1.5 borontrifluoride-methanom (8% solution) under nitrogen atmosphere in a Teflon-lined screw capped vial. A Perichrom™ 2000 system gas chromatograph (Perichrom, Saulx les Chartreux, France), equipped with a flame-ionization detector, was used for analyzing FAME. Chromatographic parameters were set as fellows: fused silica capillary column (30 m × 0.22 mm id.× 0.25 μm film thickness, BPX70 SGE Australia Pty. Ltd., analytical products); injector and detector temperatures 260°C; oven temperature programming: held 5 min at 145°C then ramped to 210°C at 2°C min^{-1} followed by a hold period of 10 min. Fatty acid were identified by comparison of their retention times with standard mixtures (PUFA1 from marine source and PUFA2 from animal source; Supelco, Bellfonte, P.A.).

Differential Scanning Calorimetry (DSC): Calorimetric evaluations of sample melting behavior were performed in a Perkin-Elmer (Model Pyris 1, Perkin Elmer Corp, Norwalk CT). All samples were tempered in the DSC cell according to the following conditions: samples were tempered at 50°C during 5 min, thus ensuring identical temperature histories; cooling from 20-60°C and holding 10 min. DSC analysis were performed from -60-40°C at a scan rate of 5°C min^{-1}. The onset, major peak maximum temperatures and enthalpy of melting (J g^{-1}) were analyzed from thermograms using the Pyris software (version 2.04, 1997).

Proton Nuclear Magnetic Resonance (H NMR): The NMR measurements (transverse relaxation T_2 and longitudinal relaxation T_1) were performed on a Brucker Minispec (Rheinstetten, Germany) operating at 20 MHz equipped with a liquid nitrogen temperature controller (BVT 3000 version 003 Brucker). The samples were equilibrated for 12 h at the desired measurement temperature using a thermostated bath, thus ensuring identical temperature memories. The acquisition parameters were: relaxationdelay, 3 sec; gain, 62 db; filter, 100 KHz; 14 data points, 4 acquisitions; 180-90°C Pulse separation, 5 ms; delay sampling window, 0.02 ms; sampling window, 0.05 ms.

Surface tension measurement: A thermostated Krüss tensiometer model K10 ST (Hamburg, Germany) was used for the measurement of the surface tension at atmospheric pressure, by the plate method of Wilhemy. Salmon oil samples (20 g) were equilibrated to the temperature ranging from 5-35°C in glycerol bath before transferring to the thermostated sample container. A digital temperature probe was plunged into the oil sample close to the platinum plate (0.5 cm). The tensiometer was checked daily by measuring the surface tension of distilled water at 20°C. Measurements were performed in 5 replicates of 2 independent essays.

Rheological measurements: Rheological properties of crude and hydrolyzed salmon oil by lipase SP 398 were performed under steady shear and dynamic conditions using a thermostated Stresstech Rheologica® apparatus equipped with a UP30 stried plate-plate device (gap 0.5 mm). Flow curves were determined as a function of hydrolysis degree and temperature. Experimental conditions were set as fellows: temperature gradient ranging from 45-5°C at 1°C min^{-1}, delay time 10 sec; stress 50 Pa.

RESULTS AND DISCUSSION

Lipolysis kinetics: The percentage of salmon oil hydrolyzed by the lipase as a function of time is showed in Fig. 1. The degree of hydrolysis after 48 h reached to a maximum of 57%.

Figure 1 shows the evolution of lipolysis kinetics (average of 3 repetitions) of salmon oil by a 1, 3-stereo specific lipase. It appears that the initial speed of the reaction remains high during the first 4 h.

A degree of lipolysis of 35% is reached after 10 h of reaction. In a general way, the optimal durations of lipolysis are included/understood enters 24 and 50 h of reaction (Tanaka *et al*., 1993; Shimada *et al*., 1997; Wanasundara and Shahidi, 1999; Moore and McNeill, 1996).

Salmon oil was submitted to lipolysis with a specific sn-1, sn-3 lipase from *Aspergillus oryzae* to preserve the long chain n-3 PUFA, which are preferentially located at the sn-2 position on the glycerol backbone (Aursand *et al*., 1995; Nwosu and Boyd, 1997). Due to the several cis-cis unsaturations of PUFA, these molecules are strongly bent thus enhancing the steric hindrance effect. Therefore, the ester moiety of PUFA-containing acylglycerols is hardly split by a lipase, contrary to SFA and MUFA.

Fatty acid composition analysis: Fatty acids composition in oils stripes were analysed by GC throughout the lipolysis course (Table 1). Figure 2 shows the correlation between fatty acids classes and the lipolysis degree (DH) and Fig. 3 the correlation between ratio (DHA/EPA and PUFAn-3/PUFAn-6) and the lipolysis degree (DH). When lipolysis degree varies from 0-57%, SFA content grows from 23.24-25.82%, MUFA content from 30.58-31.81, on the other hand, PUFA n-6 content decreases from 6.65-4.99%.

Novozym SP 398 is a lipase 1, 3-stereo specific, it hydrolyzes in a preferential way the bonds esters in position 1 and 3 of glycerol. The experimental results confirm this property of selectivity (Fig. 2): SFA and

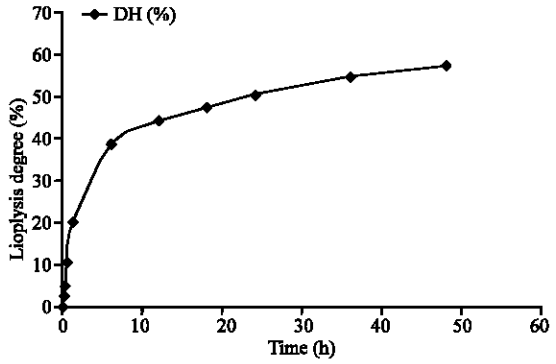

Fig. 1: Lipolysis degree of salmon oil after 48 h of hydrolysis by SP 398 lipase

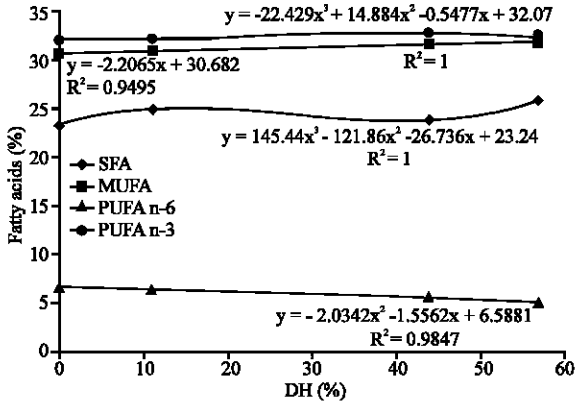

Fig. 2: Correlation between fatty acids classes and the lipolysis degree (DH)

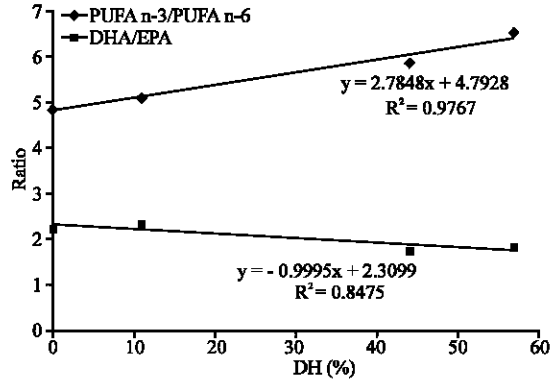

Fig. 3: Correlation between ratio (DHA/EPA and PUFAn-3/PUFAn-6) and the lipolysis degree (DH)

MUFA are esterified mainly on the positions sn-1 and sn-3 of the triacylglycerols (TAG) whereas PUFA are rather in position sn-2 (Ackman, 1982). PUFAn-3/PUFAn-6 has a good relation with lipolysis degree (Fig. 3). Broadly we note that DHA content increases when EPA content decreases.

Table 1: Fatty acids composition of salmon oil during lipolysis

Fatty acids	DH = 0%	DH = 11%	DH = 44%	DH =57%
C14:0	6.13	5.55	6.0	6.36
C16:0	15.31	15.30	14.0	15.26
C18:0	1.15	3.41	3.0	3.09
SFA	23.24	24.90	23.8	25.82
C16:1n-7	7.68	7.52	7.3	7.29
C18:1n-9	14.38	14.90	17.1	14.91
C18:1n-7	3.19	3.28		2.67
C20:1n-9	3.93	3.95	5.7	5.77
MUFA	30.58	31.01	31.8	31.81
C18:2n-6	3.68	3.35	2.2	2.12
C20:4n-6	1.46	1.48	1.5	1.57
PUFA n-6	6.65	6.29	5.6	4.99
C18:3n-3	1.04	0.96	1.1	0.80
C18:4n-3	1.73	1.70	2.0	1.88
C20:4n-3	11.30	11.35	9.6	10.15
C20:5n-3	3.94	3.77	5.4	5.30
C22:5n-3	4.23	4.53	3.9	4.04
C22:6n-3	8.78	8.80	9.5	9.56
PUFA n-3	32.07	32.16	32.8	32.44
Others	4.94	4.64	6.0	4.95
Σ n-3/Σ n-6	4.82	5.11	5.86	6.50
DHA/EPA	2.23	2.33	1.76	1.80

Atlantic salmon oil represent a natural source that generally have high levels n-3 PUFAs and low levels of n-6 fatty acids and may be considered as a healthpromoting product for human consumption by reducing n-6 and increasing n-3 intake (Torstensen *et al*., 2004).

Differential Scanning Calorimetry (DSC): The thermo grams of salmon oils are represented on Fig. 4 and 5. At 0% salmon oil presents two characteristic peaks located in the low melting points respectively to ±43.2°C (one set = -50.6°C; $\Delta H = 11.6$ J g^{-1}) and -9.5°C (one set = -13.4°C; $\Delta H = 16.3$ J g^{-1}). One observes during the hydrolysis by lipase, a significant evolution of the fusion enthalpy, in particular by the appearance of many peaks in the zone of the means and high melting points. This enthalpy evolution of the profile appears immediately after attack of stereospecific lipase, leading to the release of free fatty acids saturated, preferentially located with position 1 and 3. For example one can quote the melting points of C14:0 (58.5°C), C16:0 (63-64°C) and C18:0 (69-70°C).

To 11% of hydrolysis, one observes a reduction in the enthalpy of fusion of the peaks at low melting points compared to initial oil (one set = -61°C; maximum peak = -47.5°C; $\Delta H = 8.34$ J g^{-1}) and an appearance of peaks at average melting points in negative temperature: (onset = -36.7°C; maximum peak = -22.2°C; $\Delta H = 8.0$ J g^{-1}) and in positive temperature (onset = -2.8°C; maximum peak = 4.8°C; $\Delta H = 2$ J g^{-1}) and (onset = 15.8°C; maximum peak = 19.9°C; $\Delta H = 2.1$ J g^{-1}). Between 40 and 57% of hydrolysis, one observes a significant increase in the peaks at high melting points (onset = +8.5°C; maximum peak = +22.2°C; $\Delta H = 13.0$ J g^{-1}).

Proton Nuclear Magnetic Resonance (H NMR): Examples of correlation between longitudinal relaxation time (T_1) and the hydrolysis degree (DH) are shown in Fig. 6. The effect of temperature was also examined between -30-20°C. The proton mobility showed a strong dependence of the temperature.

T_1 of initial oil evolves/moves 42 ms with -30°C at 133 ms for a temperature of 18°C. If one takes into account the effect of the degree of hydrolysis, the T_1 passes at the end of 48 h (DH = 57%) 44 ms to -30°C-163 ms to 18°C.

No significant variation of the T_1 was observed at -30°C. This result is indicative of the low mobility of the molecules at low temperature. However, the effect of hydrolysis was most pronounced when the temperature increased. The T_1 value of the initial oil was 102 ms against 122 ms after the lipase action leading to a DH = 60%. Each point in the correlation corresponds to the mean of 10 NMR measurements.

For each temperature, there is a linear correlation between T_1 and DH. T_1 increases with the DH. At low temperature the correlation is not clear. The relaxation time T_2. Both T_1 and T_2 are influenced by the temperature.

The T_2 can be measured by fitting the free induction decay (FID) acquired directly after a 90°C pulse. The effects of temperature and the lypolysis degree over the transversal relaxation time (T_2) are represented on Fig. 7. No significant difference was noticed on comparing the NMR relaxation results. Same evolution is observed that with T_1, T_2 believes linearly with DH.

Surface tension measurement: The measured values of surface tension against air of the salmon oil at various stages of hydrolysis by lipase are given in Fig. 8. As the degree of lipolysis increased the values of surface tension increases too. For each temperature, it increases in an exponential way up to 20% and remains constant up to 57% of lipolysis owing to the liberation of naturally occurring surface-active materials, such as mono-and diacylglycerols and free fatty acids which increase during the kinetics.

Data are essentially similar in magnitude and fall within a narrow range of 30.5-31.6 mNm^{-1} at 35°C and 31.6-32.6 at 20°C. However, at lower temperature the surface tension showed a wide spread of values of 32.9-34.8 mNm^{-1}.

The monoacylglycerols are nonionic, but considerably more polar than the triglycerides and exhibited a strong surface activity at the oil/water interface and they are widely used as food emulsifiers (Ho and Chow, 2000).

The effect of surface-active substances such as monoglyceride (chain length, concentration, saturated and insaturated fatty acid) has been reported (Ho and Chow, 2000).

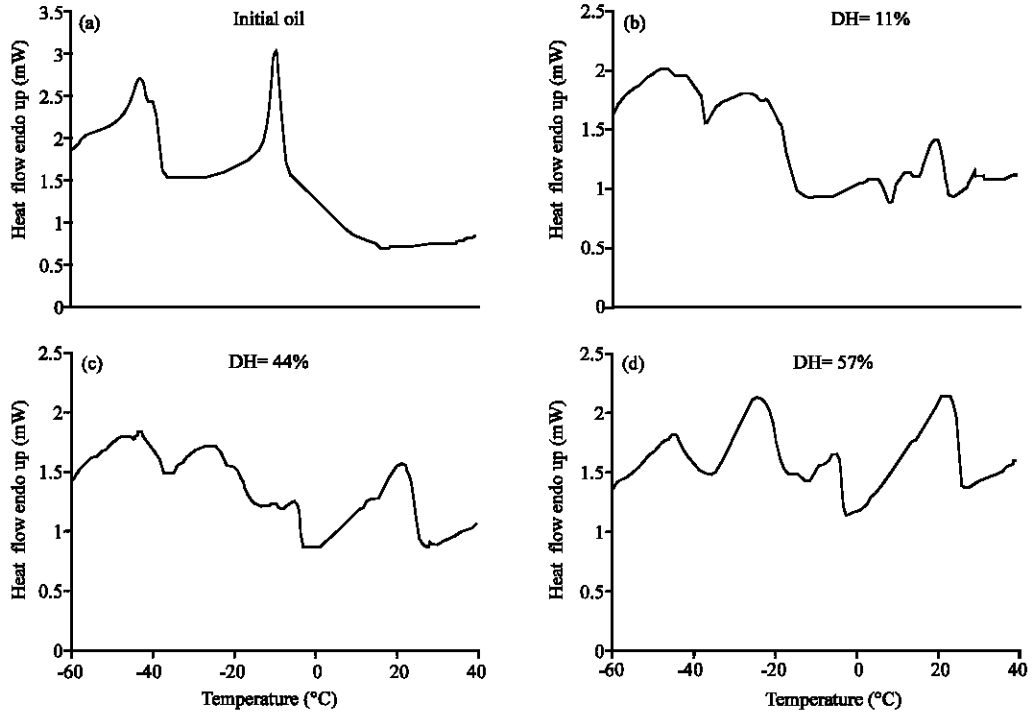

Fig. 4: Differential scanning calorimetry melting thermograms of salmon oil hydrolyzed by the lipase SP398

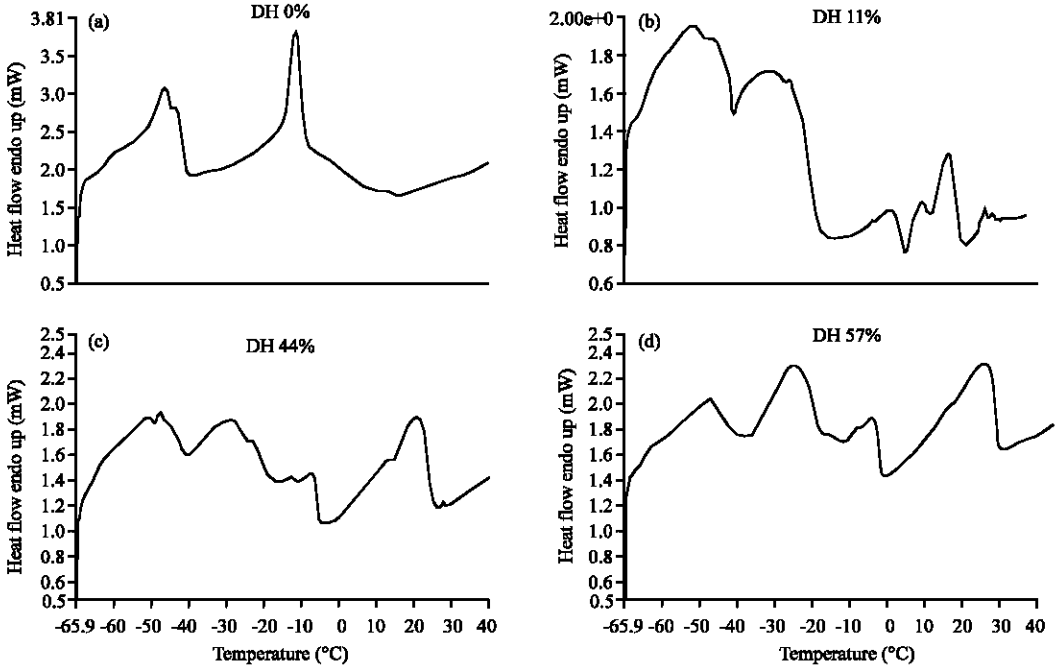

Fig. 5: Differential scanning calorimetry melting thermograms of salmon oil after SP398 lipolysis

Rheological measurements: The viscosities, expressed in mPas, were determined at temperature ranging from 5-37°C for different hydrolysis degree (0, 11, 44 and 57%) were presented in Fig. 9.

In optics to know the rheological properties of these oils, we studied the influence of the temperature and hydrolysis degree on viscosity. When the temperature increases, viscosity decreases exponentially (Fig. 9) some

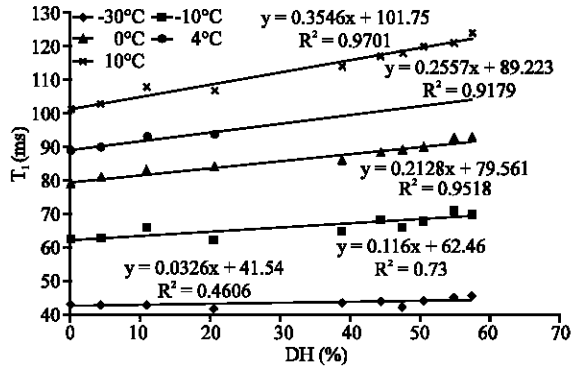

Fig. 6: Correlation between longitudinal relaxation time (T_1) and the hydrolysis degree (DH)

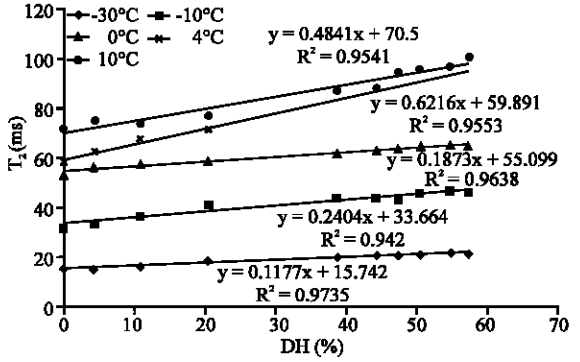

Fig. 7: Correlation between transversal relaxation time (T_2) and the hydrolysis degree (DH)

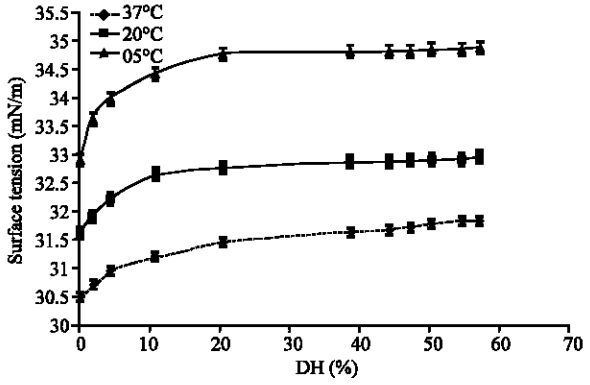

Fig. 8: Surface tension of salmon oil at 37, 20 and 4°C during hydrolysis by lipase SP 398

is the hydrolysis degree (Igwe, 2004; Dzondo *et al*., 2005; Nzikou *et al*., 2006, 2007). Viscosity varies between 26.93 and 81.69 mPas when temperature decreases of 45-5°C for crude oil. After lipolysis of salmon oil the oil viscosity decreases of 181.87-26.25 mPas at 11%, 396.26-25.15 mPas (44%) and 398.66-21.11 mPas (57%).

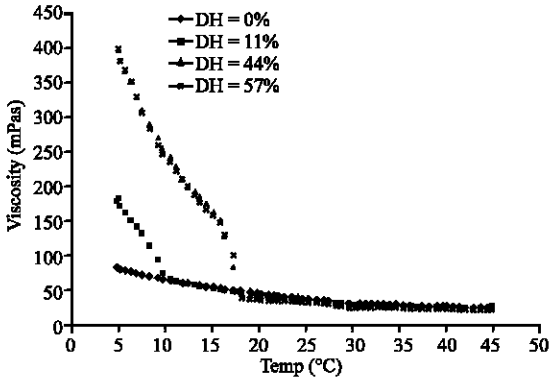

Fig. 9: Viscosities of hydrolysed salmon oil by the lipase SP398 as a function of temperature

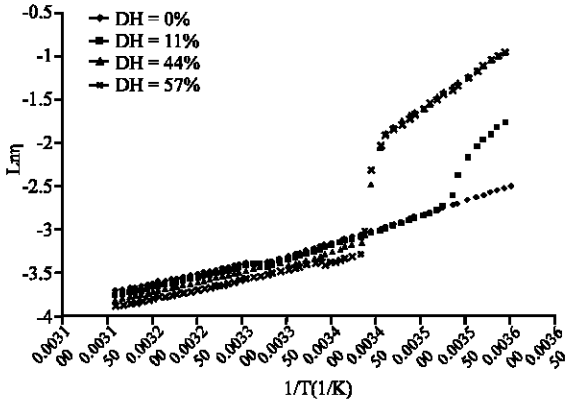

Fig. 10: Arrhenius plot of hydrolysed salmon oil

From 11% of lipolysis, there is a rupture of the curve of viscosity to 12°C. Same rupture appears with 18°C for 44 and 57%.

Between 18 and 45°C, oils have the same rheological behavior; they are Newtonian since viscosity decrease in an exponential way.

The Arrhenius's equation was used to determine the activation energy from the viscosity results.

$$\eta = Ae^{-Ea/RT}$$

A = The frequency factor called also exponential pre factor energy.

Ea = Barrier to cross before the elementary. flow can begin in kJ mol^{-1}.

R = 8.31 $Jmol^{-1}$ K^{-1} (perfect gas constant).

T = Absolute temperature (K).

In a plot (Fig. 10) of ln η against 1/T, $-E_a/R$ is the slope from which E_a was evaluated. Activation energies of oils are given in Table 2.

Table 2: Activation energy in kJmol^{-1} for fatty acids classes in salmon oils

	$E\alpha_1$	$E\alpha_2$	$E\alpha_3$
DH = 0%	-22.55	0.00	0.00
DH = 11%	-22.26	-111.88	0.00
DH = 44%	-18.95	-365.68	-58.09
DH = 57%	-18.21	-401.09	-60.64

Activation energies of the various classes of fatty acids contained in these oils were given in Table 2.

To 0%, there is a fatty acids class with energy of activation of -22.55 kJ mol^{-1}. We have 2 fatty acids classes for 11% whereas there are three fatty acids classes with following energies of activation: -18.6, -383.34 and -60 kJ mol^{-1}.

For the first class of fatty acid, activation energy increases to -22.55 at -18.21 kJ mol^{-1} when lipolysis degre varies from 0-57%. For the two others, it decreases.

CONCLUSION

After lipasic hydrolysis, the salmon oil was characterized on the physicochemical level. Surface tensions were measured according to the temperature between -5 and 30°C over 48 h of lipolysis. It does not vary to DH = 20%. The study of the rheological behavior of oils between 5 and 45°C revealed the existence two characteristic temperatures 12°C for the low DH and 18°C for high values of DH. The differential enthalpic analysis between -60 and 60°C as well as measurements of the relaxation times T_1 and T_2 by protonic NMR made it possible to characterize the evolution of the reactional mixture according to the lipolysis. Finally, analyses of viscosity according to a gradient of temperature ranging between 5 and 45°C or of the shear stress will make it possible to supplement knowledge on the modifications of structure of the reactional mixture.

REFERENCES

Ackman, R.G., 1982. Fatty acid composition of fish oils. In: Nutritional evaluation of long-chain fatty acids in fish oils. Eds. S. Barlow and Stansby. Academic Press London, pp: 25-88.

Aidos, I., R. Schelvis-Smit, M. Veldman, J.B. Luten, A. van der Padt and R.M. Boom, 2003. Chemical and sensory evaluation of crude oil extracted from herring by-products from different processing operations. J. Agric. Food Chem., 51 (7): 1897-1903. PMID: 12643649.

Aursand, M., L. Jorgensen and H. Grasdalen, 1995. Positional Distribution of ω3 fatty acids in marine lipid triacylglycerols by high-resolution ^{13}C nuclear magnetic resonance spectroscopy. J. Am. Oil Chem. Soc., 72: 293-297. DOI: 10.1007/1-4020-3910-7, 10.1007/1-4020-3910-7_108.

Dumay, J., C. Donnay-Moreno, G. Barnathan, P. Jaouen and J.P. Bergé, 2006. Improvement of lipid and phospholipid recoveries from sardine (*Sardina pilchardus*) viscera using industrial proteases. Process Biochem., 41: 2327-2332.

Dzondo, M.G., J.M. Nzikou, E. Matouba, A. Etoumongo, M. Linder and S. Desobry, 2005. Characterisation and nutritional interest of safou pulp oil. Process Biochem., 40 (1): 307-312. DOI: 10.1016/j.procbio.2004.01.003.

El-Mashad, H.M., R. Zhang and J.R. Avena-Bustillos, 2008. A two-step process for biodiesel production from salmon oil. Biosyst. Eng., 99: 220-227. INIST: 4708,354000161948890080.

Hishino, T., T. Yamane and S. Shimizu, 1990. Selective hydrolysis of fish oil by lipase to concentrate n-3 polyunsaturated fatty acids. Agric. Biol. Chem., 54: 1459-1467.

Ho, C.C. and M.C. Chow, 2000. The effect of the refining process on the interfacial properties of palm oil. J. Am. Oil Chem. Soc., 7: 191-199.

Holub, B.J., 2001. Docosahexaenoic Acid in Human Health. In: Shahidi, F. and J.W. Finley (Eds.). Omega-3 fatty acids, chemistry, nutrition and health effects ACS Symposium series 788 m ACS Press, 166 (5): 54-65. http://www.cmaj.ca/misc/terms.shtml. http://www.cmaj.ca/cgi/external_ref?access_num=2804830&link_ type=MED.

Igwe, I.O., 2004. The effects of temperature on the viscosity of vegetables oils in solution. Indust. Crops Prod.,19: 185-190. DOI: 10.1016/j.indcrop.2003.09.006.

Kristinsson, H.G. and B.A. Rasco, 2000. Fish protein hydrolysates: Production, biochemical and functional properties. Critical Rev. Food Sci. Nutr., 40 (1): 43-81.

Linder, M., E. Matouba, J. Fanni and M. Parmentier, 2002. N-3 PUFA enrichment of salmon oil as obtained by lipolysis, filtration and enzymatic re-esterification. Eur. J. Lipid Sci. Technol., 104 (8): 455-462. DOI: 10.1002/1438-9312(200208)104:8<455:AID-EJLT455>3.0.CO;2-Q.

Linder, M., J. Fanni and M. Parmentier, 2001. Enzyme-catalysed enrichment of n-3 polyunsaturated fatty acids of salmon oil: Optimisation of reaction conditions. OCL, 8: 73-77. INIST: 22926, 35400009782641.0160.

Moore, S.R. and G.P. McNeill, 1996. Production of triglycerides enriched in long-chain n-3 poly-unsaturated fatty acids from fish oil. J. Am. Oil Chem. Soc., 73 (11): 1409-1414. DOI: 10.1007/BF02523504.

Nwosu, C.V. and L.C. Boyd, 1997. Positional distribution of fatty acids on triacylglycerols of menhaden (*Brevoortia tyrannis*) and salmon (*Salmo salar*) oils. J. Food Lipids, 4: 65-74. DOI: 10.1111/j.1745-4522.1997.tb00081.x.

Nzikou, J.M., M. Mvoula-Tsieri, L. Matos, E. Matouba, A.C. Ngakegni-Limbili, M. Linder and S. Desobry, 2007. *Solanum nigrum* L. seeds as an Alternative source of Edible lipids and Nutrinent in Congo Brazzaville. J. Applied Sci., 7 (8): 1107-1115. http://www.ansijournals.com/jas/2007/1107-1115.pdf. http://www.adsabs.harvard.edu/.

Nzikou, J.M., M. Mvoula-Tsieri, E. Matouba, J.M. Ouamba, C. Kapseu, M. Parmentier and S. Desobry, 2006. A study on gumbo seed grown in Congo Brazzaville for its food and Industrial applications. Afr. J. Biotechnol., 5 (23): 2469-2475. http://www.academicjournals.org/AJB.

Robles, M.A., E.M. Grima, G.A. Giménez and M.J.I. Gonzalez, 1998. Downstream processing of algal polyunsaturated fatty acids. Biotechnol. Advances, 16 (3): 517-580. DOI: 10.1016/S0734-9750(97)00083-9.

Schmidt, E.B. and J. Dyerberg, 1994. Omega-3 fatty acids: Current status in cardiovascular medicine. Drugs, 47: 405-424.

Shimada, Y., K. Maruyama, A. Sugihara, S. Moriyama and Y. Tominaga, 1997. Purification of Docosahexaenoic acid from tuna oil by a 2-step enzymatic Method: Hydrolysis and selective esterification. J. Am. Oil Chem. Soc., 74 (11): 1441-1446. INIST: 204, 354000079-62187.0150.

Shimade, Y., K. Maruyama, S. Okazaki, A. Sugihara and Y. Tominaga, 1994. Enrichment of polyunsaturated fatty acids with Geotrichum candidum lipase. J. Am. Oil Chem. Soc., 71: 951-954.

Tanaka, Y., T. Funada, J. Hirano and R. Hashizume, 1993. Triglyceride specificity of *Candida cylindracea* lipase: Effect of docosahexaenoic acid on resistance of triglyceride to lipase. J. Am. Oil Chem. Soc., 70: 1031-1034.

Tanaka, Y., J. Hirano and T. Funada, 1992. Concentration of docosahexaenoic acid in glyceride by hydrolysis of by hydrolysis of fish oil with *Candida cylindracea* lipase. J. Am. Oil Chem. Soc., 69 (12): 1210-1214.

Torstensen, B.E., L. Frøyland, R. Ørnsrud and Ø. Lie, 2004. Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. Food Chem., 87: 567-580. DOI: 10.1016/j.foodchem.2004.01.009.

Wanasundara, U.N. and F. Shahidi, 1999. Concentration of omega-3 polyunsaturated fatty acids of seal blubber oil by urea complexation: Optimisation of reaction conditions. Food Chem., 65: 41-49. INIST: 17810, 35400007437073.0070.

Wright, I., 2004. Salmon by-products. Aqua Feeds: Formulation and Beyond, 1 (1): 10-12. http://www.feedware.com/aqua/magazine/index.html.

Xu, X., A. Skands, G. Jonsson and J. Adler-Nissen, 2000. Production of structured lipids by lipase-catalysed interesterification in an ultrafiltration membrane reactor. Biotechnol. Lett., 22: 1667-1671. http://www.ingentaconnect.com/content/klu/bile/2000/00000022/00000021/00281751.

Ying, Z., T. Madhujith, N. Mahfouz and F. Shahidi, 2007. Compositional characteristics of muscle and visceral oil from steelhead trout and their oxidative stability. Food Chem., 104: 602-608. DOI: 10.1016/j.foodchem.2006.12.036.