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Original Article

Modifications of Atlantic salmon by-product oil for obtaining different ω -3 polyunsaturated fatty acids concentrates: An approach to comparative analysis

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ABSTRACT

Omega-3 polyunsaturated fatty acids (ω -3 PUFAs) rich 2-monoacylglycerols (2-MAG), omega-3 polyunsaturated free fatty acids (ω -3 PUFFAs) concentrate, and PUFA enriched acylglycerols were prepared from salmon frame bone oil (SFBO) by enzymatic alcoholysis, urea complexation, and enzymatic esterification, respectively. The yields of 2-MAG, ω -3 PUFFAs concentrate, and PUFA enriched acylglycerols were 40.25, 16.52, and 15.65%, respectively. ω -3 PUFFAs concentrate and PUFA enriched acylglycerols showed darker red color than SFBO and 2-MAG due to aggregation of astaxanthin pigment in ω -3 PUFFAs concentrate during urea complexation. The viscosity and specific gravity of SFBO and PUFA enriched acylglycerols showed similar values whereas 2-MAG and ω -3 PUFFAs showed significantly ($p < 0.05$) lower values. Stability parameters like acid value, peroxide value, free fatty acid value, and p -anisidine value of SFBO and ω -3 PUFFAs concentrates were within acceptable limits except extreme high acid value and free fatty acid value of ω -3 PUFFAs concentrate. Thermogravimetric analysis showed similar and higher thermal stability of SFBO and PUFA enriched acylglycerols than 2-MAG and ω -3 PUFFAs concentrate. The ω -3 PUFAs content in 2-MAG, ω -3 PUFFAs concentrate, and PUFA enriched acylglycerols was increased to 20.81, 52.96, and 51.74% respectively from 13.54% in SFBO. ω -3 PUFFAs concentrate and PUFA enriched acylglycerols showed higher DPPH and ABTS radical scavenging activity than SFBO and 2-MAG. The results obtained from this study suggest the production of PUFA enriched acylglycerols rich in ω -3 PUFAs supplements from fish oil for human and pet animals.

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1. Introduction

At present, modified oils and fats rich in ω -3 PUFAs are drawing great attention among the health cautious people for their functional or pharmaceutical roles. The market demand of ω -3 PUFAs rich fish oil is increasing due to its positive effects on human health and awareness of the nutritional value [1]. A number of studies confirmed the positive health effects of fish consumption in reducing coronary heart disease among the diverse populations [2]. Many of the researches worked on concentrating ω -3 and ω -6 PUFAs as human body is unable to synthesize those fatty acids sufficiently and needs to intake from external source with diet [3]. Fish oils typically contain α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) among the ω -3 PUFAs of which EPA (5 double bonds) and DHA (6 double bonds) are the common and widely researched [4]. The approaches for producing modified oils and fats rich in ω -3 PUFAs includes esterification, chemical hydrolysis, physical fractionation or chemical interesterification. But the chemical form of the final product is important to take into account, as the bioavailability of the ω -3 PUFAs vary depending on the existing forms such as in free fatty acids (FFAs), acylglycerols (AG), ethyl esters (EE), or phospholipids. Absorption of ω -3 PUFAs in EE form is poor in human body as pancreatic lipases are weak for EEs.

Among the various methods for ω -3 PUFAs concentrate production, distillation requires high temperature which cause oxidation, polymerization, and isomerization of double bonds [5,6]. Low temperature crystallization cannot produce highly concentrated ω -3 PUFAs [7]. Use of high pressure in supercritical fluid extraction process cause high capital cost, which limit the use in processing companies [5]. This study was based on the efficient, easy, and cheap methods for ω -3 PUFAs concentrate production to compare various parameters of the final products. Production of 2-MAG enriched in EPA and DHA from enzymatic alcoholysis of fish oil catalyzed by *sn*-1,3 specific lipase is a simple method [8]. The fatty acids of 1,3 positions are converted to ethyl esters and 2 positioned fatty acids remained in the acylglycerol fraction. The extent of ω -3 PUFAs in the 2-MAG depends on their extent in 2 position of glycerol backbone. The final product is 2-MAG, which is considered as good but the problem of its applicability during low content of ω -3 PUFAs in the original oil and interchange of fatty acids from 2 position of glycerol, resulting production of ethyl esters. Urea complexation is considered as the most efficient and simplest method where ω -3 PUFAs are obtained in the form of free fatty acids (FFAs). It is a simple, inexpensive, robust, quick, and environmental friendly technique [9,10]. PUFAs in FFAs form are absorbed more efficiently than PUFAs in triacylglycerols (TAG) or EE form, but they may have irritant effects and are highly prone to auto-oxidation [11]. Preparation of triacylglycerols using ω -3 PUFAs and glycerol by enzymatic esterification can solve these obstacles. Acylglycerols, especially TAG, are natural, highly bioavailable, and easy to use in industrial formulations [12]. A recent study concluded that supplementation of omega-3 fatty acids in the form of TAG for 6 months increased EPA and DHA in red blood cells significantly compared with providing FA-EE form [13]. A

numbers of physico-chemical, catalytic/bio-catalytic techniques can be used for TAG production [5,14]. Isomerization and oxidation reactions cause losses of ω -3 PUFAs in traditional physico-chemical methods of TAG formation [15]. On the other hand, there is a number of advantages of using lipases as those ensure mild reaction conditions, low energy consumptions, limited undesirable side products which reduces purification and separation steps [16].

Atlantic salmon (*Salmo salar*) is rich of lipids, health beneficial omega-3 fatty acids, and high quality proteins. The characteristic pink color of Atlantic salmon is due to the carotenoid pigment astaxanthin, which is lipid soluble and has numerous health benefits including antioxidant, anti-inflammation, anti-cancer, anti-tumor, and anti-diabetic effects [17–21]. Salmon by-products are the materials left after removal of directly consumable main products (e.g., fillets) in the salmon processing/filleting industries. By-products are the secondary products not suitable for direct human consumption and need additional processing steps to use. Fish processing may generate up to 50% of the whole fish weight as by-products including heads, frame bones, skin, and viscera [22] which are generally discarded as processing leftovers or used for the production of animals feed or fertilizers. Utilization of fish by-products for fish oil production has many advantages; primarily it increases the overall value of the catch, reduce waste disposal/treatment cost, and ultimately lowers environmental pollution.

Although some researchers worked to produce ω -3 PUFAs concentrate from fish oil by various methods, there is very limited information of the final ω -3 PUFAs concentrate regarding physio-chemical, thermal, and bio-potential analyses. For the direct consumption and utilization of ω -3 PUFAs concentrate as food ingredients, it is important to know those above mentioned properties. In the present study, ω -3 PUFAs rich 2-MAG, ω -3 PUFFAs concentrate, and PUFA enriched acylglycerols were produced from SFBO by different methods such as lipase catalytic alcoholysis, urea complexation, and lipase mediated esterification, respectively. The main objective of the study was to evaluate the yield, physico-chemical properties, thermogravimetric analysis, fatty acids composition, and biological (radical scavenging) activities of the final ω -3 PUFAs concentrates obtained by different methods.

2. Materials and methods

2.1. Materials and reagents

Salmon frame bone oil (SFBO) was collected from Seawell Co., Ltd. (frozen salmon was imported from Norway), Haeundae-gu, Busan, Republic of Korea. Lipase Novozymes-435 (*Candida antarctica*) and Lipozyme RMIM (*Rhizopus miehei*) immobilized in macroporous anion exchange resin was bought from Novozymes A/S, Denmark. Fatty acid methyl esters (FAME), astaxanthin standard, *p*-anisidine, DPPH, ABTS, ascorbic acid, trolox, and butylated hydroxytoluene (BHT) were obtained from Sigma–Aldrich Co., St. Louis, Missouri, USA. Urea purity >99.00% was purchased from Junsei Chemical Co., Ltd., Tokyo, Japan. Analytical or HPLC graded solvents only were used in this study.

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