Food Chemistry 240 (2018) 286–294

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A lumped model of the lipase catalyzed hydrolysis of sardine oil to maximize polyunsaturated fatty acids content in acylglycerols

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ARTICLE INFO

Article history:
Received 1 November 2016
Received in revised form 20 June 2017
Accepted 8 July 2017
Available online 13 July 2017

Keywords:
Rhizomucor miehei-lipase
Water activity
Hydrolysis
PUFA concentration
Microaqueous systems

ABSTRACT

The aim of this work was to produce diacylglycerols (DAG) and monoacylglycerols (MAG) with a high content of polyunsaturated fatty acids (PUFA). *Rhizomucor miehei* lipase mediated-hydrolysis of sardine oil was conducted at several water activities. The system was mechanistically modeled to predict the time evolution of the concentration of triacylglycerols, DAG, MAG and free fatty acids (FFA) and the concentration of saturated, mono- and polyunsaturated fatty acids. The release of the first fatty acid from the triacylglycerol was independent on the unsaturation degree. Contrary, the hydrolysis of the second one was highly affected by the degree of unsaturation, PUFA being the fatty acids that showed the highest resistance to hydrolysis. MAG percentage was maximum (7 mol%) at lower water activities, while DAG content was favored at higher water activities (35 mol%), achieving a 2-fold concentration of DHA.

1. Introduction

Fish discards are defined as the fraction of organic material in the catch which is not retained for sale but returned to the sea (Kelleher, 2005). Discards present an environmental problem since they modify the marine trophic chains. Additionally, they imply the underutilization of important natural resources. In this context, discard practices have been prohibited by a recent European policy for fisheries (EU, 2013). Consequently, it is required the development of new techniques able to convert fish discards into valuable products. For instance, fish oil is an adequate substrate to produce modified oils with a high content of polyunsaturated fatty acids (PUFA). Among PUFA, omega-3 fatty acids and, more specifically, eicosapentaenoic and docosahexaenoic acids (EPA and DHA) play a beneficial role on the cardiovascular system and on the brain functions. A detailed description of the benefits that PUFA exert on the human health can be found elsewhere (Morales-Medina et al., 2013). Therefore, there is an increasing demand of oils with a high PUFA content in order to produce functional foods and nutraceuticals products (Lembke, 2013; Rubio-Rodriguez et al., 2010).

Concentration of PUFA can be conducted by physical or enzymatic methods (Lembke, 2013; Morales-Medina, De León, et al., 2015; Morales-Medina, García-Moreno, et al., 2015; Rubio-Rodriguez et al., 2010). In the physical approaches (i.e. supercritical fluid extraction, low temperature winterization), pre- and post-treatment steps are required. First, triacylglycerols (TAG) are converted into methyl/ethyl esters or free fatty acids (FFA) to increase the concentration rate of the process (Lembke, 2013). After physical concentration, the esters or FFA are re-esterified to improve the bioavailability of the PUFA (Lawson & Hughes, 1988; Small, 1991). Enzymatic methods are based on the use of highly selective lipases, which can be classified in two groups: random lipases that indistinctly react with the three positions of the glycerol backbone and sn-1(3)-specific lipases, that preferably react with the external bonds of the glycerol backbone (Hari Krishna & Karanth, 2002). The main advantage of the enzymatic techniques is that PUFA can be concentrated in the form of acylglycerols, with a special interest in the mono- (MAG) and diacylglycerols (DAG) fractions. MAG and DAG account for around 75% of the total production of emulsifiers that are applied in the food industry (Feltes, de Oliveira, Block, & Ninow, 2013). They are also widely employed in the pharmaceutical and cosmetic industries since they present no side effects after ingestion (Feltes et al., 2013). Hence, the production of MAG and DAG with high content of PUFA (mainly EPA or DHA) implies an added nutritional value. Additionally, in the case of the 2-MAG with high content of PUFA, they can be used as a substrate for the production of MLM structured lipids by re-esterification with medium chain fatty acids. These structured lipids show a faster absorption than the original oil and their daily intake might result in a lower accumulation of fat (Ruxton, Reed, Simpson, & Millington, 2007).

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http://dx.doi.org/10.1016/j.foodchem.2017.07.030

List of symbols and abbreviators

Symbol	Meaning
SFA	saturated fatty acids
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
TAG	triacylglycerols
FFA	free fatty acids
DAG	diacylglycerols
MAG	monoacylglycerols
1-MAG	1-monoacylglyerol
2-MAG	2-monoacylglycerol
TLC	thin layer chromatography
a_w	water activity
t	time, min
k_1, k_2	kinetic constant for the forward reactions of hydrolysis, 1/min
k_{-1}, k_{-2}	kinetic constant for the reverse reactions of hydrolysis, 1/(min·mol/mL)
k_{w1}, k_{w2}	kinetic constant for the forward reaction which includes water activity, 1/min
K_{eq1}, K_{eq2}	equilibrium constant of the hydrolysis reactions, mol/mL
$C_{i,\ global}$	concentration of the total and i fractions (i reffers to MAG, DAG, TAG and FFA), mol/mL
$C_{i,\ SFA}$	SFA concentration of the total and i fractions (i reffers to MAG, DAG, TAG and FFA), mol/mL
$C_{i,\ MUFA}$	MUFA concentration of the total and i fractions (i reffers to MAG, DAG, TAG and FFA), mol/mL
$C_{i,\ PUFA}$	PUFA concentration of the total and i fractions (i reffers to MAG, DAG, TAG and FFA), mol/mL

The most common enzymatic methods employed to produce 2-MAG are alcoholysis, glycerolysis and hydrolysis. Alcoholysis is a reaction between an ester (i.e. triacylglycerol) and an alcohol such as methanol or ethanol. This reaction has been proposed for the production of 2-MAG with a maximum theoretical yield of 33 mol% (Munio, Robles, Esteban, Gonzalez, & Molina, 2009). Glycerolysis is a variation of alcoholysis which employs glycerin and results in a much higher yield. Effectively, processes with a yield of MAG higher than 90% have been reported, resulting in the conversion of almost all TAG into MAG (Pawongrat, Xu, & H-Kittikun, 2007). Consequently, to increase the PUFA content, a concentration with acetone at low temperature is required (Pawongrat et al., 2007). On the other hand, hydrolysis is the reaction between TAG and water to produce DAG, MAG, FFA and glycerin. This reaction can be conducted in both aqueous (i.e. emulsions) and organic systems. For the latter case, several works have been conducted to produce PUFA concentrates in the form of free fatty acids (Gámez-Meza et al., 2003) or acylglycerols (Kahveci & Xu, 2011; Wanasundara & Shahidi, 1998a; Wanasundara & Shahidi, 1998b; Wanasundara, Wanasundara, & Shahidi, 2005). Furthermore, the influence that enzyme to substrate ratio, reaction time and temperature exerted on the final composition of the non-hydrolyzed TAG has been described (Wanasundara & Shahidi, 1998a; Wanasundara & Shahidi, 1998b). However, this approach is not efficient to produce MAG, since the excess of water leads to a high content of PUFA in the DAG and TAG fractions with negligible amount of MAG (Hoshino, Yamane, & Shimizu, 1990; Kahveci & Xu, 2011). Hence, the content of DAG and MAG could be increased by controlling the water content in the organic system. Water content affects the enzyme structure and the conditions of the system (i.e. by facilitating the reagent diffusion or influencing the equilibrium) (Hari Krishna & Karanth, 2002). Especially in microaqueous systems (Xu, 2000), water content is measured as water activity, which is a dynamic parameter that measures the energy status of water. It is defined as the vapor pressure of water divided by that of pure water at the same temperature. Water activity is considered the most convenient parameter to characterize the water level of microaqueous systems because it determines the distribution of water among the phases. Although several works have focused on the kinetic study of alcoholysis or acidolysis to produce modified triacylglycerols (Pacheco, Crapiste, & Carrín, 2010; Zhao, Lu, Bie, Lu, & Liu, 2007), there are no studies dealing with the kinetic behavior of fish oil hydrolysis in microaqueous systems. Additionally, a model of the partition of fatty acids among the acylglycerols and FFA fractions has not been presented in the literature. Further research is required for a better understanding of the effect of water activity on the kinetic of the hydrolysis, on the selectivity of lipases and on the composition of the acylglycerols and free fatty acids produced.

The aim of this work was to study the production of di- and monoacylglycerols with a high content of polyunsaturated fatty acids (PUFA) (more specifically, EPA and DHA). To that end, it was modeled the influence of water activity on the hydrolysis of sardine oil in microaqueous systems using lipase from *Rhizomucor miehei*. A mechanistic model comprising two second-order reversible reactions was developed.

2. Materials and methods

2.1. Oil, lipases and chemicals

Refined sardine oil with 21.5 wt% of EPA and 10.4 wt% of DHA was provided by Industrias Afines S.L (Mos, Spain). The lipases employed were Lipozyme RM IM, an immobilized lipase from *Rhizomucor miehei* (Sigma-Aldrich, St Louis, MO, USA) and Novozyme 435 from *Candida antarctica* (Novozymes, Bagsvaerd, Denmark). Nonadecanoic acid, employed as standard, was purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were of analytical grade.

2.2. Enzymatic hydrolysis of triacylglycerols

Hydrolysis was conducted at three different levels of water activitiy (a_w: 0.33, 0.6 and 0.8). Water activity (a_w) of the media was controlled employing mixtures of salts with different degrees of hydration (Zacharis, Omar, Partridge, Robb, & Halling, 1997). A mixture of sodium acetate anhydrous and tri-hydrate (0.5 g:0.5 g) was employed to set the water activity at 0.3. In the case of $a_w = 0.6$, the mixture of salts were $NaHPO_4 * 2{\cdot}H_2O$ and $NaHPO_4 * 7{\cdot}H_2O$ (0.5 g:0.5 g), while for $a_w = 0.8$, a mixture of $NaHPO_4 * 7{\cdot}H_2O$ and $NaHPO_4 * 12{\cdot}H_2O$ (0.5 g:0.5 g) was used. Additionally, to ensure that water activity of the media was effectively controlled, it was monitored during the entire reaction time employing a LabMaster a_w (Novasina, Lachen, Switzerland).

Each pair of salts was mixed in 25 mL of hexane and stored in 50 mL Erlenmeyer amber flasks for 48 h at 40 °C and 300 rpm until equilibrium was obtained. Then, 2.5*g* of refined oil and 0.25*g* of