Food Research International 100 (2017) 572–578

Contents lists available at ScienceDirect

Food Research International

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

Substrates emulsification process to improve lipase-catalyzed sardine oil glycerolysis in different systems. Evaluation of lipid oxidation of the reaction products

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ARTICLE INFO

Chemical compounds studied in this article:
Glycerol (PubChem CID: 753)
Eicosapentaenoic acid (PubChem CID: 446284)
Docosahexaenoic acid (PubChem CID: 445580)
Aerosol OT (PubChem CID: 23673837)
Tween 80 (PubChem CID: 5281955)

Keywords:
Fish oil
Glycerolysis
Microemulsion
SC-CO_2
Peroxides adsorption

ABSTRACT

Mono- and diacylglycerols rich in omega-3 have a great interest due to their good bioavailability and oxidation stability compared with other kind of omega-3 concentrates. The main drawback in mono- and diacylglycerols production by glycerolysis is the immiscibility of the substrates, oil and glycerol. To improve mass transfer rates, avoiding the use of organic solvents, emulsification of both reactants as reverse micelles (glycerol-in-oil) was carried out previous to lipase-catalyzed sardine oil glycerolysis. Substrate emulsification yielded higher reaction rates compared to kinetics with no previous emulsification, but still lower than in organic solvents. To avoid the use of organic solvent, SC-CO_2 was used as reaction medium but no kinetic advantages were demonstrated in the pressure range from 15 to 25 MPa. By increasing temperature, from 40 to 90 °C, reaction rates increased both in a solvent-free system and in SC-CO_2 medium. It was also found that an increase in temperature does not lead to an increase in the final oxidation status of the reaction products. This behavior was due to the adsorption capacity of the Lipozyme 435 support, giving lower oxidation status at the highest temperature, 80–90 °C.

1. Introduction

The importance of omega-3 polyunsaturated fatty acids (n-3 PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in human nutrition and disease prevention is fully recognized scientifically (Kris-Etherton, Harris, & Appel, 2002; Riediger, Othman, Suh, & Moghadasian, 2009). n-3 PUFA supplements are available in different chemical forms. Among the different types of lipid derivatives containing n-3 PUFA concentrates, monoacylglycerols (MAG) and diacylglycerols (DAG) have good bioavailability and oxidation stability (Hernandez, 2014; Lawson & Hughes, 1988). Additionally, it must be also considered that dietary TAG are hydrolyzed in the small intestine to sn-2-MAG being the most favorable structure for n-3 PUFA to be adsorbed by intestinal mucosa (Bandarra et al., 2012). In addition, MAG or its mixtures with DAG account for 75% of the worldwide emulsifier production (Zhong et al., 2009). The well-known drawbacks of the conventional chemical glycerolysis technique (energy intensive, low yields (30–40%), oxidized products) have prompted a growing interest in the development of alternative processes for the production of MAG and DAG rich in n-3 PUFA. Enzyme-catalyzed reaction is an attractive alternative since the reaction can be carried out under mild conditions (Bornscheuer, 1995; Feltes, de Oliveira, Block, & Ninow, 2013).

To overcome the problem of the immiscibility of glycerol and oil, different approaches have been used in the literature to improve the contact between the reactants and hence reduce mass transfer limitations. Lipase-catalyzed glycerolysis has been carried out in different reaction media such as organic solvents (Damstrup et al., 2006), compressed fluids (Moquin, Temelli, King, & Palcic, 2005) and ionic liquids (Guo & Xu, 2006), in order to improve the mass transfer. Hovewer, cost, toxicity and energy required for solvent removal from the product mixture, are important aspects to be considered when dealing with conventional solvent systems (Prat, Hayler, & Wells, 2014). Recently, the use of different surfactants to increase the interfacial area, and ultrasound irradiation have been also proposed to reduce mass transfer limitations (Fiametti et al., 2012; Valério, Rovani, Treichel, De Oliveira, & Oliveira, 2010). Biocatalytic processing in microemulsion system has received attention in order to increase contact between substrates. The formation of a microemulsion of the reactants (glycerol-in-oil) as reverse micelles can help to improve mass transfer rates. Furthermore, lipases demonstrate high interfacial activity in micelle systems because the formation of the active site during the reaction occurs at the interface between the substrates and the enzyme. Several food grade surfactants are able to stabilize the micellar system improving system homogeneity (Carvalho & Cabral, 2000; Stamatis,

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http://dx.doi.org/10.1016/j.foodres.2017.07.048
Received 18 May 2017; Received in revised form 18 July 2017; Accepted 19 July 2017
Available online 21 July 2017

Xenakis, & Kolisis, 1999). Nevertheless, it must be taken into account that some food grade surfactants have chemical functions that could be modified by lipases. For instance, the lipase Novozym 435 presented activity at particular conditions towards some surfactants as soy lecithin and Tween in glycerolysis reactions (Camino Feltes, Villeneuve, Baréa, de Oliveira, & Ninow, 2012). To avoid this problem, other synthetic surfactants, such as sodium (bis-2-ethyl-hexyl) sulfosuccinate (aerosol-OT or AOT), have been used. AOT has been reported to form micelles in a great number of nonpolar substances and several other polar solvents such as glycerol (Fiametti, Rovani, Oliveira, Corazza, Treichel, & Oliveira, 2009). In this case, good results have been obtained in glycerolysis systems when adding more that 7.5% of AOT (Fiametti et al., 2009). However, the high amount of this surfactant may generate problems during removal processes (Stamatis, Xenakis, & Kolisis, 1994).

Another alternative to organic solvents is the use of the supercritical fluids (SCFs) as reaction medium. Supercritical carbon dioxide (SC-CO_2) is probably the most used SCF due to its additional benefits (non-toxic, non-flammable, readily available at high purities and low costs, and relatively mild critical conditions) that are appealing when choosing environmental replacement for organic solvents (Matsuda, 2013; Rezaei, Temelli, & Jenab, 2007). SC-CO_2 has liquid-like density but gas-like viscosity resulting in high mass transfer being a clean alternative to replace organic solvents. Enzymatic concentration of n-3 PUFA in supercritical fluids (SCFs) is an interesting option for the prevention of oxidation during processing of fish oil (Lin, Chen, & Chang, 2006; Roh, Kim, & Choi, 2015). Besides, SC-CO_2 can be easily separated from the reaction products by simple depressurization and allows fractionation of the reaction products. Some previous studies of enzymatic reactions of different lipid sources in SC-CO_2 have been reported in the literature. However, in case of enzymatic glycerolysis, other compressed fluids such as propane, *n*-butane, and acetone, have been used (Esmelindro et al., 2008; Tai & Brunner, 2011; Valério et al., 2010). Some studies of glycerolysis of vegetable oils in SC-CO_2 at high temperatures can be found but with no enzymatic catalyst (Moquin et al., 2005; Temelli, King, & List, 1996).

In a previous work, a detail kinetic study of glycerolysis of sardine oil using Lipozyme® 435 form *Candida antarctica* B as biocatalyst in an optimized amount of tert-butanol was performed (Solaesa, Sanz, Beltrán, & Melgosa, 2016). Tert-butanol helped to create a homogeneous phase and to reduce mass transfer limitations. However, organic solvents present different environmental concerns. In this work, to improve contact between substrates, avoiding the use of organic solvents, emulsification of glycerol and oil before glycerolysis reaction was considered. Glycerolysis reaction has been performed in a solvent free system at atmospheric pressure and in SC-CO_2 as reaction medium with previous substrates emulsification. The effect of adding a surfactant, AOT or Tween 80, to stabilize the emulsion, on glycerolysis performance has been also studied. Glycerolysis has been determined at different operating temperatures at atmosphere pressure, 0.1 MPa, and in SC-CO_2 medium in the pressure range from 15 to 25 MPa. Since n-3 PUFA are highly susceptible to oxidation; the oxidative status of the final reaction products was evaluated through the peroxide and anisidine values. Reaction yields and the oxidation values of the reaction products were compared for both systems.

2. Materials and methods

2.1. Materials

Refined sardine oil was provided by Industrias Afines S.L. (Spain) with 18.3% of EPA and 7% of DHA and a water content of 0.2% (Solaesa, Bucio, Sanz, Beltrán, & Rebolleda, 2014). Glycerol was purchased from Sigma Aldrich with a purity of ≥ 99.5% and a water content of 0.18%. The food grade lipase Lipozyme 435 from *Candida antarctica* B (immobilized on a macroporous hydrophobic acrylic resin), was donated by Novozymes A/S (Bagsvaerd, Denmark). Carbon dioxide (99.9%) was supplied by Air Liquide S.A. (Spain). Polyoxyethylene sorbitan monooleate (Tween 80) and sodium bis (2-ethylhexyl) sulfosuccinate (Aerosol AOT or AOT), used as food grade surfactants, were purchased by Sigma Aldrich. All other chemicals used in different analyses were of analytical or HPLC grade.

2.2. Emulsification process

Microemulsions of the glycerolysis system of sardine oil were prepared at a fixed mole ratio of 3:1 (glycerol:oil) since this mole ratio was found as the optimum in a previous kinetic study (Solaesa, Sanz, Beltrán, and Melgosa, 2016). A high-speed blender (Miccra D9 equipped with a DS-20/PF EMR rotor–stator) at different speeds, from 16,000 to 35,000 rpm, was used by pulses during 3 min. To prepare the surfactant-free emulsion as reverse micelles, the appropriate amount of glycerol (10 g) was added drop by drop to the suitable amount of oil (30 g) while being completely mixed at high speed. Dispersed (glycerol) and continuous (sardine oil) phases were identified by the dilution test (Mize et al., 2013). Furthermore, different concentrations (0.5, 1 and 1.5% in glycerol or oil as indicated in Table 2) of two food grade surfactants, AOT and Tween 80, were tested in order to improve the stability of the emulsion. A defined quantity of each surfactant was dissolved in oil or in glycerol, depending on its solubility. The characterization of the emulsions was performed 10 min after emulsification to avoid any creaming or coalescence effect. Particle size distribution (PSD), mean droplet diameter and polydispersity index (PDI) of samples were measured by dynamic light scattering (DLS), using a Zetasizer Nano ZS apparatus (Malvern Instruments Ltd., UK) to evaluate the best conditions to produce a stable emulsion with small (or the smallest) droplet size.

2.3. Lipase-catalyzed glycerolysis of sardine oil in different systems

A comparative study of lipase-catalyzed glycerolysis in different systems was carried out. All the experiments were conducted in a batch mode keeping constant the enzyme concentration at 5 wt% (by weight of substrates) and the substrate mole ratio (3:1, glycerol to oil) according to previous work (Solaesa, Sanz, Beltrán, and Melgosa, 2016). Table 1 summarizes all glycerolysis reactions that have been done in this work. Experiments 1–6 have been carried out at atmospheric pressure in a solvent free system in a 100 mL jacketed batch reactor. First of all, experiments 1 and 2 were carried out to evaluate the effect of previous substrates emulsification on reaction rate. Experiments 3 and 4 were performed with emulsified substrates stabilized by adding a food grade surfactant, AOT and Tween 80 respectively, at the optimum

Table 1
Summary of the reaction conditions for lipase-catalyzed sardine oil glycerolysis reactions carried out in this work.

Exp.	Reaction medium	Pressure (MPa)	Temperature (°C)	Emulsification
1	Solvent free	0.1	50	No
2		0.1	50	Yes
3		0.1	50	Yes[a]
4		0.1	50	Yes[b]
5		0.1	80	Yes
6		0.1	90	Yes
7	SC-CO_2 as solvent	15	50	Yes
8		20	50	Yes
9		25	50	Yes
10		15	40	Yes
11		15	65	Yes
12		15	80	Yes
13		15	90	Yes

[a] AOT as surfactant.
[b] Tween 80 as surfactant.