Food Chemistry 271 (2019) 372–379

Contents lists available at ScienceDirect

Food Chemistry

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

Lipase-catalyzed glycerolysis of anchovy oil in a solvent-free system: Simultaneous optimization of monoacylglycerol synthesis and end-product oxidative stability

David Palacios, Natividad Ortega, Nuria Rubio-Rodríguez, María D. Busto*

Department of Biotechnology and Food Science, University of Burgos, Plaza Misael Bañuelos, s/n, 09001 Burgos, Spain

ARTICLE INFO

Keywords:
Lipase PS-DI
Monoacylglycerols (MAG)
Oxidative stability
Polyunsaturated fatty acids (PUFA)
Solvent-free glycerolysis

ABSTRACT

The production of mono- and diacylglycerols rich in polyunsaturated fatty acids is achieved in this study, by solvent-free glycerolysis of anchovy oil with lipase PS-DI from *Burkholderia cepacia*. Attention is focused on the oxidative stability of the reaction products, determined in terms of induction time (I_t). The effects of glycerol/triacylglycerol molar ratio, enzyme concentration, and reaction temperature on mono- and diacylglycerol production and I_t are all assessed. The operating conditions that optimized monoacylglycerol yields and oxidative stability were a glycerol/triacylglycerol ratio of 3/1, 9.0% (w/w) Lipase PS-DI, a stirring rate of 200 rpm, and a reaction time of 4 h, at 45.8 °C, producing a content of 24.8% and 51.9% of mono- and diacylglycerols, respectively, over an I_t of 1.41 h. The glycerolysis conditions determined by simultaneous optimization strategy increased the oxidative stability of the glycerolysis products by 68%, which rose from 0.84 h (individual optimization) to 1.41 h.

1. Introduction

Mono- (MAG) and diacylglycerols (DAG) are non-ionic surfactants widely used as emulsifiers in many food products (e.g. margarines, sauces, dairy and confectionery products) (Kristensen, Xu & Mu, 2005; Pawongrat, Xu, & H-Kittikun, 2007). Additionally, they are also used in cosmetic, pharmaceutical, and textile products, due to their plasticizing and texturizing properties (Chang & Bodmeier, 1998; Valério, Rovani, Treichel, de Oliveira & Oliveira, 2010). The production of MAG on an industrial scale is currently performed by chemical glycerolysis of fats and oils using alkaline catalysts under a nitrogen gas atmosphere at very high temperatures. However, rather low yields are obtained with this procedure and the aggressive reaction conditions lead to severe fatty acid oxidation. The products tend to show a dark color and unwanted byproducts are generated that require additional purification (Feltes et al., 2012; Ghamgui et al., 2006).

Enzymatic catalysis has great potential as an alternative to chemical processes, due to properties such as substrate specificity, mild operational conditions, easy control, and the reusability of immobilized biocatalysts (Weber & Mukherjee, 2004). In practice, lipase-catalyzed glycerolysis is a complex process with several equilibriums from which it is difficult to obtain only one reaction product (Cheirsilp, Kaewthong, & H-Kittikun, 2007; Tan & Yin, 2005). Moreover, the high difference in polarity between glycerol (Gly) and tryacylglycerol (TAG) assists the formation of a heterogeneous reaction system with a limited mass transfer. Several alternatives, such as the addition of food additives as surfactants (Valério et al., 2009), the use of organic solvents (Cai, Gao, Liu, Zhong & Liu, 2016; Yang, Rebsdorf, Engelrud, & Xu, 2005), ionic liquids (Kahveci, Guo, Özçelik & Xu, 2010), and supercritical fluids (Moquin, Temelli, Sovová & Saldaña, 2006), have been followed to improve the miscibility of the glycerolysis reaction, thereby improving its yield. Despite the advantages of enzymatic catalysis, these technologies involve additional separation steps and recovery systems with high costs that currently hinder any commercial-scale application of this technique (Feltes et al., 2012; Fiametti, et al., 2009). Glycerolysis in a solvent-free system represents a promising option, in view of the increasing interest in developing simple and efficient separation processes. Solvent-free systems have many advantages such as reduced pollution, low costs, and simplicity in both processing and handling (Tanaka & Toda, 2000).

Several oils and fats have been used as starting materials for MAG production. However, over the last decade, fish oils have received a lot of attention because of their content in n-3 polyunsaturated fatty acids. PUFA are health-beneficial fatty acids, because of their role in the regulation of inflammation, cholesterol metabolism, and brain functions (Nicholson, Khademi & Moghadasian, 2013). Nevertheless, even

* Corresponding author.
E-mail address: dbusto@ubu.es (M.D. Busto).

https://doi.org/10.1016/j.foodchem.2018.07.184
Received 24 October 2017; Received in revised form 13 April 2018; Accepted 25 July 2018
Available online 27 July 2018

frozen PUFA easily reacts with oxygen and forms hydroperoxide and free radicals (Miyashita, 2014), due to the high number of its double bonds. Moreover, the volatile compounds during oxidation lead to flavor deterioration reducing their acceptance among consumers. More than an organoleptic problem, lipid oxidation also has nutritional and health implications. In fact, the increased use of n-3 PUFA in the food industry has resulted in new challenges concerning the avoidance or at least the minimization of lipid oxidation (Jacobsen, 2015). Nevertheless, the measurement of lipid oxidation is a difficult task, due to its complex oxidation process that depends on many factors. Numerous analytical methods for determining lipid oxidation in foods have been developed, based on the measurement of oxygen adsorption, the peroxide value, the formation of free radicals and primary and secondary oxidation products, as well as the evaluation of the oil stability index (Shahidi, Wang & Wanasundara, 2017). Rancimat is a method that companies have frequently used to determine the oil stability index. It determines the conductivity of volatile oxidation products dissolved in water. The time that elapses until the point when volatile acids are detected in the measuring vessel is referred to as the induction time (I_t) or the oil stability index (Dabrowski, Konopka, Czaplicki & Tariska, 2017). I_t characterizes the oxidation stability of oils and fats: the lengthier the I_t induction time, the more stable the sample.

Several studies have dealt with the lipase-catalyzed synthesis of MAG and DAG enriched in PUFA in non-conventional media (Fiametti et al., 2009; He et al., 2017; Pawongrat et al., 2007). However, none of these studies considered the oxidative stability of the reaction products during the design of the process.

Based on these considerations, the aim of this work will be to synthesize MAG and DAG enriched in PUFA, in a solvent-free glycerolysis system using the immobilized Lipase PS–DI, thereby maintaining the oxidative stability of the reaction end-products at a maximum. To that end, the effects of reaction temperature, the Gly/TAG substrate molar ratio, and, enzyme concentrations in MAG and DAG production will all be studied using response surface methodology (RSM). The induction time (I_t), will also be determined to obtain the reaction conditions that minimize end-product oxidation levels. Likewise, the desirability function approach of Derringer will be applied for simultaneous optimization of MAG yields and oxidative stability.

2. Materials and methods

2.1. Materials

Anchovy oil used in glycerolysis reactions, with a PUFA content of 34.6% (7.6% eicosapentaenoic acid, EPA, and 12.7% docosahexaenoic acid, DHA), was supplied by Denomega Nutritional Oils (Leknes, Norway). Lipase from *Burkholderia cepacia*, Lipase PS–DI, commercially immobilized on diatomite, was provided by Sigma-Aldrich Co (St. Louis, MO, USA). The MAG, DAG, and TAG standards for HPLC and Glycerol (99%) were obtained from Sigma-Aldrich Co (St. Louis, MO, USA). Isooctane, methyl tert-butyl ether and acetic acid of HPLC grade were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Silica gel 60 extra pure was supplied by Merck-Millipore (Madrid, Spain).

2.2. Enzymatic glycerolysis

Glycerolysis was done in a screw-capped crystal flask by mixing different amounts of Gly with 6 g of anchovy oil to prepare molar ratios of 1/1, 2/1, and 3/1, according to the experimental design. Previously, the adsorption of Gly onto silica gel had been performed, as described Berger, Laumen & Schneider (1992), to overcome the solubility problems of glycerol in organic phase and to prevent its aggregation on immobilized enzymes (Castillo, Dossat, Marty, Condoret & Combes, 1997). The enzymatic reaction was initiated by adding the Lipase PS–DI. The water content of the immobilized enzyme had previously been adjusted over saturated LiCl solution (Pawongrat et al., 2008) in a desiccator at 25 °C for 24 h (a_w = 0.113). The reaction mixture was incubated, under dark conditions, at different temperatures (40, 50, and 60 °C) with shaking at 200 rpm, over 4 h, based on previous studies performed in our laboratory. At the end of the reaction period, the immobilized lipase was removed by centrifugation (5,000 rpm for 5 min) and the products were recovered for further analysis.

2.3. Quantitative analysis of reaction products

Reaction products were quantified by using a HPLC-ELSD system from Agilent 1200 Series, with a Lichrospher 100 Diol column (4 × 250 mm, 5 μm) at 35 °C and 0.35 MPa. The reaction mixture was diluted in isooctane and separated using an elution gradient. The mobile phase was a mixture of isooctane and methyl tert-butyl ether/acetic acid (99.9/0.1, v/v) with a flow rate of 1 mL/min (Solaesa, Sanz, Falkeborg, Beltrán & Guo, 2016).

The reaction products are presented as mass fraction (%, w/w, based on the total oil) of MAG, DAG, TAG, and FFA. All data were the mean of two experiments with a deviation within 2.7%.

2.4. Oxidative stability

Oxidative stability in terms of induction time (I_t) was determined with a Rancimat apparatus 743 (Metrohm, Herisau, Switzerland). Samples of 2.0 g were oxidized at 110 °C with a constant air flow of 9 L/h. Determination of I_t was based on the conductimetric detection of volatile oxidation products. The time that elapsed until these oxidation products appeared was the I_t (Dabrowski et al., 2017). All data were the mean of two experiments with a deviation within 2.9%.

2.5. Experimental design, statistical analysis, and multiple response optimization

Response surface methodology (RSM) was used for modelling the enzymatic glycerolysis and to optimize the reaction conditions that maximize MAG and DAG yields. RSM is a powerful tool that permits a simultaneous study of several factors in a reduced number of experiments taking account of interactions between factors. A three-factor factorial design with two face-centered cube central levels was applied. The factors chosen were reaction temperature, substrate molar ratio (Gly/TAG), and enzyme concentration. Seventeen runs consisting of 8 factorial points, 6 star points, and 3 center points were performed as per the experimental design (Table 1). The response variables were MAG, DAG, and TAG contents, and induction time (I_t). The optimization of the conditions and the response surfaces were calculated using Statgraphics Centurion XVI (version 16.2.04). This software package was also used to fit the second-order model to the independent variables, by using the following equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i}^{i<j} \sum_{j} \beta_{ij} X_i X_j + e \quad (1)$$

where y is the dependent variable (response) to be modelled, X_i and X_j are the independent variables (factors), β_o, β_i, β_{ii} y β_{ij} are regression coefficients, and e is the error term. The model was simplified by means of analysis of variance (ANOVA) in which statistically significant ($p > 0.05$) terms were discarded.

Derringer's desirability function (Candioti, de Zan, Cámara, & Goicoechea, 2014) was used to establish the experimental conditions (factor levels) and, simultaneously, the optimal value for MAG production and oxidative stability (I_t). Individual responses are transformed into desirability functions with this method and then combined into a single one called global desirability (D) using the following equation:

$$D = (d_1^{r1} d_2^{r2} \cdots d_k^{rk})^{1/\Sigma rk} \quad (2)$$