Ultrasound irradiation promoted efficient solvent-free lipase-catalyzed production of mono- and diacylglycerols from olive oil

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ABSTRACT

This work reports the enzymatic production of mono- and diacylglycerols under the influence of ultrasound irradiation, in a solvent-free system, with and without the presence of surfactants at a constant temperature of 65 °C, glycerol to oil molar ratio of 2:1 and a commercial immobilized lipase (Novozym 435) as catalyst. For this purpose, two operation modes were adopted: the use of a sonotrode (ultrasonic probe), without agitation, varying reaction time, irradiation amplitude (25–45% of the total power) and type of surfactant, and a mechanically stirred reactor (600 rpm) under ultrasound irradiation in a water bath, testing different surfactants. Results show that very satisfactory MAG and DAG yields, above 50 wt.%, can be obtained without the use of surfactant, at mild irradiation power supply (~130 W), with no important enzyme activity losses verified, in a relatively short reaction time (2 h), and low enzyme content (7.5 wt.%). Also, reaction kinetic results show that contents of MAG + DAG as high as ~65 wt.% can be achieved at longer times (6 h), indicating a promising route for producing MAG and DAG using ultrasound irradiation.

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1. Introduction

Monoacylglycerols (MAG) are nonionic surfactants and emulsifiers containing hydrophilic and hydrophobic parts in molecules with widespread use in food industry, with applications in dairy products, margarines, bakery products and sauces. In addition, due to their excellent lubricant and plasticizing properties, MAG are used in textile processing, production of plastics and formulations of oils for different types of machinery [1–4].

Diacylglycerols (DAG) are esters of glycerol in which two of the hydroxyl groups are esterified with fatty acids (FA). DAG, particularly 1,3-DAG, have been recently claimed to be able to reduce postprandial serum triacylglycerols (TAG) levels, and may have several beneficial effects on human health, such as obesity and lipemia prevention [5–7]. Today, DAG is marketed as functional cooking oil under the brand names "Healthy Econa Cooking Oil" in Japan and "Enova Oil" in the USA [8–10].

Currently, large-scale production of MAG and DAG has been performed by chemical glycerolysis of fats and oils at high temperatures, in the presence of inorganic alkaline catalysts. Because of the high reaction temperature (200–250 °C), dark-colored, burn-tasted products are formed. Moreover, the chemical catalysis process is energy intensive, provides low yields (30–40%) and there is the need of product post-purification by molecular distillation [11–13].

At this point, it may also be opportune to mention that due to the establishment of world-wide biodiesel government programs, huge amounts of glycerol surplus are expected to occur in the near future, which means an unavoidable driving force for the development of new technologies devoted to the transformation of such by-product, e.g. MAG and DAG production, from biodiesel industrial processing.

Lipase-catalyzed systems have been employed to produce MAG and DAG, especially in organic medium [1–4], in solvent-free systems [14–16], with free or immobilized enzymes [1–4,14–16], in ionic liquids [17,18] or using compressed fluids as reaction media [5,19]. Enzymatically, MAG and DAG can be produced by direct esterification, glycerolysis, interesterification, partial hydrolysis, or a combination of partial hydrolysis and esterification [15,20,21]. Application of enzymes in the field of MAG and DAG production has attracted great attention due to several attractive features, such as mild reaction conditions, selectivity and "green" reaction systems [10]. In particular, biocatalysis through the use of lipases has become an increasingly important research field in recent years, and has been considered a practical alternative to

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chemical methods in the production of MAG, DAG and fine chemicals in the near future [22,23]. Though the main disadvantage of the use of lipases in industrial applications is at present the high cost of enzyme production, recent advances in enzyme technology, such as the use of solvent-tolerant lipases and immobilized lipases – making catalyst re-utilization possible, have allowed for the development of cost-effective systems [24]. To overcome the commonly poor dissolution of both hydrophilic (glycerol) and lipophilic (triglyceride) substrates involved in lipase-catalyzed glycerolysis for MAG and DAG production, surface-active agents have been employed, aimed at the production of MAG and DAG, with free [25] or immobilized lipases [2,3,5] in reverse micelle systems [26–30].

Another approach towards improving glycerolysis efficiency would be the use of ultrasonic irradiation, a technique that has been recently identified as an excellent tool for improvement of chemical, physical and biological processes, particularly in highly viscous and immiscible reaction systems [10]. In fact, ultrasound as an environmentally benign method has found many interesting applications in organic chemistry and biochemical engineering/biotechnology [31–40]. The chemical and physical effects of ultrasound arise from cavitational collapse which produces extreme conditions locally and thus induces the formation of chemical species not easily attained under conventional conditions, driving a particular reactivity [31]. When cavitation bubbles collapse near the phase boundary of two immiscible liquids, the resultant shock wave can provide a very efficient stirring/mixing of the layers. As a consequence, cavitation phenomenon can increase reaction rates in heterogeneous reactions and hence may become a useful tool in enzymatic reactions [41,42].

Though the application of ultrasound irradiation to enzymatic reactions is not well explored [43–46], it can be used to reduce mass transfer limitations in enzymatic reactions [41–47], as it is known to perturb weak interactions and to induce conformational changes in protein structures [48,49]. Nevertheless, one should notice that the influence of ultrasound irradiation on enzymatic reactions has been less extensively studied [43,47,50–54], and can be grouped into two main groups. The first approach makes use of ultrasound as an enzymic pretreatment to reduce particle size. This is especially relevant when using enzyme powders to catalyze reactions in organic media [55–57], and in such cases, reduction in particle size and consequent increase in the catalytic surface area are useful to reduce mass transfer limitations. The second approach involves the use of ultrasound throughout the reaction, where the cavitation energy is thought to accelerate the reaction rate. The mechanism by which this occurs is unclear, but perhaps, by increasing the movement of liquid molecules, the substrates access to the active site is increased [50].

While it has been shown that the second approach can accelerate enzymatic reactions [43,47], other reports have demonstrated the occurrence of enzyme inactivation [51–53]. In general, enzymes are known to be more stable in nearly anhydrous organic solvents [55–57], therefore it is not surprising that all reported cases of rate enhancements resulting from ultrasonic treatment are those involving enzymic catalysis in organic media [43,47]. Recently, the enhancement of enzyme activity of Novozym 435 by ultrasound irradiation has been reported in the literature [58].

The performance of ultrasound irradiation in enhancing emulsification of immiscible reaction media was demonstrated in the production of biodiesel by transesterification of triglycerides in homogeneous [35,59–79], heterogeneous catalysts, earth metal oxides and lipases [58,80].

While a relatively abundant number of papers on biodiesel production under sonochemical irradiation can be found in the open literature, there is no corresponding development regarding MAG and/or DAG. In fact, to the best of our knowledge, only one report

on MAG and DAG production through glycerolysis mediated by ultrasound irradiation was found, but using a base-catalyst, in organic solvent medium [10]. Another focused on enzymatic hydrolysis of triglycerides to produce DAG [81].

In this context, this work is aimed to report the production of MAG and DAG through enzymatic glycerolysis of olive oil in solvent-free system under ultrasound irradiation and reflects the efforts of our research group to develop new processes for enzyme-catalyzed glycerolysis [82–89]. Effects of addition of different surfactants, reaction temperature and ultrasonic irradiation modes, namely ultrasonic with and without agitation, on MAG and DAG production were investigated.

2. Materials and methods

2.1. Materials

The substrates used in glycerolysis reactions were commercial olive oil (Arisco, Brazil), glycerol (Merck, 99.5%) and n-hexane (Quimex, 99.5%), which was used for removing and washing the enzymes at the end of each experimental run. The acid value (mg KOH/g) and water content (wt.%) (Karl Fischer titration method, DL 50, Mettler-Toledo) of the olive oil were determined as 0.11 and 0.04, respectively. A commercial immobilized lipase (Novozym 435) from *Candida antarctica* (immobilized on a macroporous anionic resin, 1.4 wt.% water) was purchased from Novozymes (Araucária, PR, Brazil) and presented an enzyme activity of around 55 U/g, determined as the initial rates in esterification reactions between lauric acid and propanol at a molar ratio of 3:1, temperature of 60 °C and enzyme concentration of 5 wt.% in relation to the substrates. One unit of activity (U) was defined as the amount of enzyme necessary to consume 1 μmol of lauric acid per minute [90].

Polyoxyethylene sorbitol trioleate (Tween 85, Fluka), polyoxyethylene glycol sorbitan monostearate (Tween 65, Fluka), Triton X-100 (sucrose ester, Difco, purity of 98%, water content lower than 2%) and AOT (sodium (bis-2-ethyl-hexyl) sulfosuccinate, Aerosol-OT or AOT, Sigma–Aldrich, 99%) were used as surfactants. All surfactants, except for Triton X-100, were of analytical grade. These surfactants were chosen based on previous results of MAG and DAG production from enzymatic reactions carried out by our research group [88,89]. For the GC analysis, n-heptane, derivatizing MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) and chromatographic standards of glycerol, monoolein, diolein, triolein and oleic acid were purchased from Sigma–Aldrich.

2.2. Experimental procedure

Enzymatic ultrasound-assisted glycerolysis reactions were carried out in two modes, using an ultrasonic bath and a sonotrode. In the first case, mechanically stirred (IKA-RW 20 digital stirrer) Erlenmeyer type flasks (50 mL) were immersed in the thermostatic ultrasonic bath (Unique apparatus) (temperature accuracy of ± 0.5 °C) and the stirring rate was kept constant for all experimental conditions at 600 rpm [83,84]. The ultrasonic bath was equipped with a transducer having longitudinal vibrations, operating frequency of 37 kHz and a maximum rated power output of 132 W, which was kept at the highest deliver value for all experiments. The ultrasonic transducer (surface area of 282.2 cm^2) is fitted at the bottom of the bath horizontally along the length of bath. According to Deshmane et al. [38], the advantage of using such a system is that if offers a much larger effective cavitational area as compared to conventional immersion-based axial transducers and hence results in uniform cavitational activity distribution in the ultrasonic bath.