Lipase-Catalyzed Diacylglycerol Production Under Sonochemical Irradiation

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Introduction

Ultrasound as an environmentally benign method has found many interesting applications in organic chemistry [1, 2]. The chemical and physical effects of ultrasound arise from the cavitational collapse which produce extreme conditions locally and thus induce the formation of chemical species not easily attained under conventional conditions, driving a particular reactivity.

Diacylglycerols (DAG) are commonly used in different degrees of purity as additives for enhancing the plasticity of fats or as bases for the food, medicine and cosmetic industries. DAG are also used as estranger oils to separate materials from moulds and as an adjuster of fat crystals [3], precursors for organic synthesis of products such as phospholipids, glycolipids, lipoproteins, pro-drugs such as DAG-conjugated chlorambucil for treatment of lymphoma, (*S*)-(3,4-dihydroxyphenyl)alanine (LDOPA) for treatment of Parkinson's disease and many others. [4] More recently, DAG-rich oil has been used as a functional cooking oil, with a content of least 80% of 1,3-DAG [5].

Materials and Methods

Novozym 435, Lipozyme TL IM and Lipozyme RM IM were obtained from Novozymes. Mechanical stirring experiments were carried out in a reactor with thermostatic water bath, whose temperature could be maintained within ±0.5°C of the desired temperature. Ultrasound irradiations were carried out in a Branson 1210 equipment and the irradiations were performed within ±0.5 °C of the desired temperature.

Soybean oil hydrolysis by lipase in solvent-free system was investigated, in mechanical stirring bath and in ultrasonic bath. Batch reactions were carried out in 125 cm^3 conical flasks. Soybean oil, water and lipase were mixed in a conical flask at the desired reaction temperature. The reaction systems were mechanically stirred in a water bath or/and irradiated by ultrasound at the required power. All the reactions were performed at atmospheric pressure.

Results and Discussion

In this work, we select a solvent-free system and lipase catalyzed reaction, to investigate the effect of ultrasound irradiation on the characteristics of enzymatic hydrolysis of soybean oil as compared with the conventional mechanical stirring thermal method to the production of DAG. The parameters in consideration include temperature, reaction time and mechanical stirring rate.

Table 1: Hydrolisis of Soybean Oil under lipozyme TL IM catalysis under sonochemical and thermal methods

Entry	% Enzyme Lipozyme TL IM	Time	DAG Yield (%)[a] Sonochemical	DAG Yield (%)[a] Thermal
1	1	1h 30 min.	40.0	21.2
2	1	3 h	23.4	13.6
3	1	4h 30 min.	26.4	21.5
4	1	6 h	26.7	33.1
5	2	1h 30 min.	23.8	18.5
6	2	3 h	32.8	12.4
7	2	4h 30 min.	31.2	23.1
8	2	6 h	25.7	30.1
9	1	60 h	-	25.4
10	2	60 h	-	7.7

Table 2: Hydrolisis of Soybean Oil under lipozyme RM IM and Novozym 435 catalysis

Entry	% Enzyme	Enzyme	DAG Yield (%)[a]
1	1	Lipozyme RM IM	41.5
2	2	Lipozyme RM IM	34.9
3	1	Novozym 435	32.1
4	2	Novozym 435	30.2

Enzyme	Cycle	Yield (%) [a]	Cycle	Yield (%) [a]	Cycle	Yield (%) [a]
Lipozyme TL IM	1	40.0	2	38.5	3	36.5
Lipozyme RM IM	1	41.5	2	41.0	3	36.1
Novozym 435	1	32.1	2	30.5	3	28.7

Table 3: Recycle of the enzyme source on the hydrolisis of soybean oil

As shown in Table 1, entry 1, the best yield for DAG was achived when 1%w/w of Lipozyme TL IM was used with a reaction time of 1h 30 min. In absence of ultrasound irradiation the yield of DAG decreases dramatically and even after 60 hours of reaction (Entries 9 and 10, Table 1), no similar yield was obtained.

We have also evaluated the Lipozyme RM IM and Novozym 435 enzymes and they could also lead to good yields in short reaction times. The recycle of the enzyme was also done and the results sumarized on Table 4 show that even after 3 cycles the yield achived is very close to that obtained in the first cycle.

In conclusion we have developed an efficient protocol to diacilglycerol production by the hydrolisis of soybean oil under lipase catalyzed reaction and ultrasoun irradiation. The recycle of the enzyme was also performed and even after 3 cycles good yields could be obtained.

References

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