LIPASE-CATALYZED SYNTHESIS OF 1,3-DIACYLGLYCEROL FROM LARD OIL AND FATTY ACID PROFILE CHANGE

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Abstract – Diacylglycerol (DAG) was synthesized by esterification of lard oil and glycerol with Novozyme 435 as catalyst, and the effects of reaction temperature, mass ratio of glycerol to lard oil on the synthesis of DAG were studied. The optimal reaction conditions were as follows: dosage of Novozyme 435 9% (based on the total mass of substrates), reaction temperature 55℃, mass ratio of glycerol to lard oil 44%. Under these conditions, the DAG content reached 46% after reacting for 16 h. Gas chromatography analysis showed that there was no significant difference in fatty acid composition between lard-based DAG oil and lard oil. Thus, the lard-based DAG oil has both the characteristics of lard and the special nutritional characteristics of the DAG.

Key Words – lard-based DAG oil, enzymatic synthesis, fatty acid

I. INTRODUCTION

Diacylglycerol (DAG), the esterification product of glycerol and two fatty acids, has been confirmed as having certain nutritional benefits such as the ability to reduce serum triacylglycerol (TAG) concentration, body-weight and visceral fat, so it is thought to prevent obesity and weight-related disorders [1]. Thus it has received extensive attention in recent years and been used extensively in food industry, pharmaceutical industry, chemical engineering industry and so on.

As a big consumer of pork, China has rich resources of lard oil. However, a number of studies have demonstrated that long-term high intake of animal fats and oils will lead to weight gain and elevate blood lipids. Therefore, with the increased public interest in fitness and healthy dietary habits, the lard food consumption is declining, resulting in lard resources over-production.

In this work, two major factors that affecting the product of lard-based DAG oil (the mass ratio of glycerol to lard oil and the reaction temperature) were controlled to find the optimum reaction conditions. And the fatty acid composition of DAG were determined by gas chromatography (GC). There was little information available about the lard-based DAG oil, therefore, the objective of this study was to fill this void and to expand new realm for lard oil.

II. MATERIALS AND METHODS

Mateials: Lard oil was purchased from local supermarket; Commercial immobilized lipase Novozyme 435 was purchased from Novozymes A/S (Bagsvaerd, Denmark); A Supelco 37 component fatty acid methyl ester (FAME)

mix was ordered from Sigma-Aldrich (Taufkirchen, Germany). Glycerol, n-hexane, isopropanol, potassium hydroxide and methyl alcohol were supplied by Anhui agriculture product Engineering Laboratory.

Methods: Esterification of glycerol was performed in 25 mL Erlenmeyers where the immobilized lipase Novozyme 435 (9% of total mass of substrates) was packed. Then those Erlenmeyers were put in a constant temperature air bath oscillator for 16h. The mass ratio of glycerol to lard and the reaction temperature were controlled and their levels were 40%-50% and 50–60℃.

The acylglycerol composition was determined using a high performance liquid chromatography (HPLC; Waters, USA). Analysis of methyl esterified samples were performed in an Agilent gas chromatograph equipped with a capillary column HP-88 (100 m×0.25 mm, 0.2 μm). Statistical analysis was performed through subjection of data to analysis of variance (ANOVA) using SPSS statistical software.

III. RESULTS AND DISCUSSION

It has been reported that the reaction temperature mainly affects the activity of the enzyme and the higher temperature was beneficial to the synthesis of DAG when the Novozyme 435 was catalyzed [2]. The effect of reaction temperature on the synthesis of DAG was investigated under the condition that mass ratio of glycerol to lard oil 30% and dosage of Novozyme 435 9% of the total mass of the substrates. The HPLC data showed that as the reaction temperature increased from 50 - 60℃, the rate of DAG ascended first, and finally tended to be stable, the peak appeared at 55℃. Thus, a reaction temperature of 55℃ was selected for subsequent testing.

The effect of mass ratio of glycerol and lard oil on DAG synthesis was studied under the the reaction temperature of 55℃, the addition of Novozyme 435 was 9% of the total mass of the substrates. The HPLC results showed that the content of DAG peaked when the mass ratio of glycerol to lard oil was 44% and then began a slow decline. This indicated that the appropriate increase in the amount of glycerol is beneficial for the esterification reaction. Studies have shown that excess glycerol increases the viscosity of the reaction system and improves the thermal stability of the enzyme [3]. When the mass ratio of glycerol to lard was more than 44%, the DAG content in the product was not significantly increased, that may be due to excessive glycerol lead to excessive reaction system viscosity and affect the esterification reaction. Therefore, the 44% was selected for follow-up study.

The results of GC analysis showed that twenty fatty acids were found in lard oil and DAG oil respectively, and the main fatty acids were shown in Table 1.

Table 1 fatty acid composition of lard-based DAG oil and lard oil

Fatty acid composition (%)	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
DAG oil	1.16±0.01	20.74±0.05	1.31±0.00	10.25±0.02	38.03±0.11	22.01±0.05
lard oil	1.11±0.01	20.69±0.04	1.29±0.01	10.33±0.02	38.26±0.11	21.99±0.01

As seen in this table, the fatty acid composition of DAG oil was very close to that of raw material, which indicated that there was no selectivity to fatty acids when catalyzed by Novozyme 435. That also means the synthesis of 1,3-DAG did not reduce the nutritional of DAG oil.

IV. CONCLUSION

The study showed that the optimum conditions for the synthesis of lard-based DAG oil were as follows: dosage of Novozyme 435 9% (based on the total mass of substrates), reaction temperature 55℃, mass ratio of glycerol to lard 44%. Under these conditions, the DAG content reached 46% after reacting for 16 h. There was no significant difference between the composition of fatty acid of lard-based DAG oil and the lard oil. This result not only provides a theoretical basis for the further study of functional lard oil, but also provides new opportunities for the application of lard oil.

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