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Acidity reduction of mammalian fat by enzymatic esterification

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Abstract

This work studies the mammalian fat acidity reduction, through enzymatic esterification with ethanol for converting FFA into esters. The fat samples collected in a Portuguese company were characterized for their acid value, iodine value, density, kinematic viscosity and moisture content. Four commercial enzymes were tested as catalyst. Lipozyme CALB L contributed to highest acidity reduction. It was selected for the parametric study of the best operating conditions: 45 ºC of temperature, enzyme/fat and ethanol/FFA mass ratios of respectively0.0060 and 3.25wt/wt, which reduced 67 % the acidity in just one reaction step, after 3 h of reaction time.

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*Keywords:*Acidity reduction;Enzymatic esterification; Free fatty acids; FFA; Lipozyme CALB L; Mammalian fat

1. Introduction

Animal fats obtained from by-products of the meat processing industry are normally used in the production of animal feed, soap or biodiesel, due to their low cost and availability. The use of these fats in the animal feed industry requires a high quality standard, in particular to have a low free fatty acid (FFA) content or acidity, otherwise they have a lower market value and will be used preferentially for biodiesel production [1]. Due to an inadequate

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industrial processing of the raw material and in particular during its transportation and storage, the fat can suffer chemical processes such as oxidation, hydrolysis, hydrogenation or fermentation, which causes the release of FFAs and confer the acidic character of fats [2].

FFAs are responsible for the deterioration of the product and rancidity, causing undesirable changes in color, flavor, aroma and consistency of the fatty material. There are two types of rancidity: hydrolytic and oxidative. In the hydrolytic rancidity, there is a breakdown of ester bonds due to the action of enzymes or chemical agents. The oxidative rancidity occurs in lipids that have unsaturated fatty acids or free unsaturated fatty acids. The double bonds can form free radicals that react with atmospheric oxygen leading to the formation of other compounds such as aldehydes, peroxides, alcohols, acids and ketones. The reactions that incite this type of rancidity are auto-oxidation and photo-oxidation. There are several factors that influence the decomposition of fats, such as exposure to light, storage temperature, moisture content, amount of oxygen present and fat composition. To avoid deterioration of products by oxidation, it is usual to add antioxidants, since these are substances capable of preventing degradation of fats. Therefore, to increase the quality of fats it is necessary to find a way to stabilize the FFAs present and convert them into more stable compounds, such as esters.

Various options are available to reduce the acidity and increase the fat commercial value[3], being the enzymatic esterification one of the most interesting [4,5] due to its several advantages when compared to purely chemical processes [6,7]: it can be applied to oils and fats from various sources, such as mammalian and poultry fat or fish oil; lipases are more selective to the reactants and can effectively catalyze the esterification reaction of fats with high acidity; it is not necessary to use strong acids or alkalis as catalysts; the reaction occurs at milder temperatures (<60 °C) and at atmospheric pressure, in comparison to the alternative chemically catalyzed reactions, allowing preserving the fat properties with reduced energy input and costs [8]. Also, the enzymatic catalyzed reaction reduces product losses, in comparison to the alkali catalyzed reaction, in which saponification of FFAs occurs. On the other hand, the use of ethanol instead of methanol is less hazardous for human health and the environment, while generating water as by-product [9,10]and ethanol can be obtained from renewable sources[11], such as residual biomass or an industry's by-product [12]. Hence, in this work, the enzymatic esterification was the method selected to perform the study of the acidity reduction of mammalian fat.

To the authors best knowledge no studies were found in the literature concerning the reduction of acidity on animal fats by esterification and using enzymes as catalyst. Although not directly comparable to this work, the study of Véras et al. [13] looked at biodiesel production through simultaneous esterification and transesterification, without co-solvents and avoiding inhibition of the enzyme by ethanol and glycerol, concluding that the enzymatic production of biodiesel performed faster than most processes presented in literature, even those that include stepwise addition of alcohol or co-solvents.

2. Methods

2.1. Enzyme selection and esterification reaction

Four commercial lipase enzymes from Novozymes: Novozym 435, Lipozyme CALB L, Lipozyme TL 100 L and Lecitase Ultra, known to be able to catalyze the esterification reaction of oil and fats, were tested in this study, which activity and optimum temperature range are shown in Table 1.

Table 1. Characteristics of the lipases tested.

Enzyme	Activity	Optimum Temperature
Lecitase Ultra	10 000 LU/g	35-60 °C
Lipozyme CALB L	5 000 LU/g	30-60 °C
Lipozyme TL 100 L	100 000 LU/g	20-50 °C
Novozym 435	10 000 PLU/g	30-60 °C

To perform the esterification reaction, it was first estimated the minimum amount needed of each enzyme, considering their activity. Then, it was determined the amount of alcohol required for the esterification reaction,