Enzymatic Synthesis of Fatty Esters by Lipase from Porcine Pancreas

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Abstract

An enzymatic synthesis of aliphatic esters of butyric acid in the waterless environment of organic solvent hexane was carried out with use of the enzyme preparation lipase from porcine pancreas. Aliphatic alcohols with different length of hydrocarbon radical (C₄-C₉) were used as substrates. Conversion of the esters depending on time of the process and concentration of the enzyme was defined at 30 °C of the process. It was found that an increase in length of the hydro carbonic radical of alcohol and branching of a chain complicated enzymatic synthesis. For the lower alcohols the conversion reach more than 90%.

Keywords: Catalyst; Statistica; Enzyme; Acetylation; Carboxylic

Introduction

Among flavouring substances possessing a characteristic smell, one of the most common classes of organic compounds are esters, which have found application in the production of perfume and cosmetic products, soaps, synthetic detergents and in other areas. The register of esters of aliphatic and aromatic ranks, the terpenoid of a cyclic and acyclic structure used as flavoring substances in EU countries includes 793 substances, in the Russian Federation - 666 substances [1]. Esters determine the aroma of many fruits, berries, flowers and are popular flavoring food additives. It was developed a variety of chemical methods for synthetic esters producing – esterification and transesterification with the acid and alkaline catalysts, the catalytic acylation of alcohols by carboxylic acids anhydrides and halogen anhydrides, interaction of acids with olefins at elevated pressure and acid catalysis, and other methods [2]. From all known chemical methods of receiving esters the most common method of an esterification of acids by alcohols requires the use of strong acids (HCl, H₂SO₄, arylsulfonic acids and another acids) as catalysts, temperature of process 70-150 °C. The increased temperatures and presence of strong aggressive acids in the reaction environment undoubtedly creates problems of ecological and technological character (existence of sour waste, decrease of product purity, strict requirements to the equipment, increase of power expenses).

This work is devoted to enzymatic method of obtaining of aliphatic esters. As enzymes for the synthesis lipases are increasingly frequently being used. They have already found application in fat-and-oil industry for implementation of reactions of transesterification of triacylglycerides, and now it become applicable for the synthesis of esters of different structures in aqueous and non-aqueous environments [3-7]. The replacement of the known chemical methods of synthesis of esters on enzymatic has undoubted advantages (technological, environmental, economic), because it allows to carry out the synthesis at lower temperatures, in the absence of elevated pressure and corrosive environment.

In our work as substrates butyric acid and aliphatic alcohols of various length of hydro carbonic chain (from C₄ to C₉) and as an enzyme the pancreatic lipase were used. The process was carried out in a non-aqueous environment.

Aliphatic esters of lower aliphatic acids, in particular butyrate, are the food flavorings and a part of the perfume composition (ethyl butyrate, n-butylic butyrate, iso-butylic butyrate, iso-amyl butyrate, hexyl butyrate, n-octyl butyrate). They are present in many plant essential oils and synthesized by chemical methods.

Materials and Methods

Used substrates – aliphatic alcohols (n-butanol, iso-butanol, iso-pentanol, n-heptanol, n-oktanol, n-undecanol) and butyric acid – were reagent grade. An enzyme – a commercial Lipase preparation from porcine pancreas, Type II lyophillic dried. Production – U.S. Activity – 100-500 units/mg protein, specific activity of 107 units/mg of protein. The activity of the enzyme preparation was determined using a standard method based on the hydrolysis of olive oil – method Ota, Yamada, - described [8].

As the solvent it was used hexane (reagent grade), boiling point 69 °C.

Molar ratio of butyric acid: alcohol – 1: 2.

The synthesis was carried out at 30 °C, the duration of the process varied from 1 to 30 hours.

The amount of enzyme introduced into the reaction mixture was 5-30 mg/0.1 μmol of acid.

Control of process of esterification was carried out by titration of residual acid with a 0.1 N alcoholic solution of NaOH (in 80% alcohol) in the presence of 0,05 ml of 1% alcohol solution of phenolphthalein as an indicator until a constant pink color obtaining. All experiments were made in six replicates. The data were processed by means of applied software package Statistica. The relative error was not more than 7%. After the process was complete the enzyme was filtered, an excess of alcohol and hexane remove by vacuum distillation. IR spectrum of the obtained esters was recorded on the Lumex spectrometer (Infra-LUM). Refractivity nD was detected on the Refractometer IRF-22.

Discussion of Results

Results of the conducted researches are shown in Figures 1-3 and in the Table 1. The analysis of the presented data shows that with increase in length of the hydro carbonic radical of alcohol process of enzymatic
acetylation was complicated. The lowest conversion was observed for undecyl alcohol at all used ranges of amount of enzyme and time of the process (Figures 1-3). The maximum effect (67%) was achieved only after 36 hours of reaction. The presence of a branched radical in iso-amyl and iso-butyl alcohols complicated the process of acylation at low amounts of enzyme preparation usage. With increasing of quantity of enzyme preparation in reaction mixture a conversion level of iso-amyl alcohol was practically leveled with n-butyl alcohol conversion, which showed the highest yield (93%, 24 hours). Such character of change of reactivity of alcohols to an esterification can be explained by the fact that with length increasing and branching of the hydrocarbon radical of the alcohols the acidity of the hydroxyl proton decreases. This change increased the efficiency of deprotonation -- one of the necessary stages of esterification [2,9,10]. As a result acetylation by alcohols with long or branched hydrocarbon radicals is slowed in comparison with short normal radicals.

IR spectra clearly confirm the formation of esters, because stretching vibrations of ester bonds $\nu_{С=О}$ appeared at 1180-1176 cm$^{-1}$ region. Those stretching vibrations is absent in the spectra of initial substrates. The high-intensity valent stretching vibration of carboxylic bond $\nu_{С=О}$ of butyric acid esters are at 1735-1737 cm$^{-1}$ region. The shift of the stretching vibrations $\nu_{С=О}$ in esters in comparison with $\nu_{С=О}$ in butyric acid to 30-32 cm$^{-1}$ and the value of refractive index $n_D$ corresponding to reference data are additional confirmation of the formation of esters.

The obtained data show that enzymatic synthesis for the lower alcohols - C4 and C5 - proceed most effectively and with a high yield. Some of their characteristics.

Figure 1: The dependence of conversion of process of an esterifikation on time for alcohols n-butyl (C4), iso-amyl (i-C5), n-heptyl (C7), n-oktyl (C8), n-undecyl (C11). Reaction conditions: the ratio acid: alcohol – 1: 2, amount of enzyme – 5 mg / 0.1 μmol of acid, the reaction environment – hexane.

Figure 2: The dependence of conversion of process of an esterifikation on time for alcohols n-butyl (C4), iso-butyl (i-C4), iso-amyl (i-C5), heptyl (C7), n-oktyl (C8), n-undecyl (C11). Reaction conditions: the ratio acid: alcohol – 1: 2, amount of enzyme – 10 mg / 0.1 μmol of acid, the reaction environment – hexane.

Figure 3: The dependence of conversion of process of an esterifikation on time for alcohols n-butyl (C4), iso-amyl (i-C5), n-heptyl (C7), n-oktyl (C8), n-undecyl (C11). Reaction conditions: the ratio acid: alcohol – 1: 2, amount of enzyme – 20 mg / 0.1 μmol of acid, the reaction environment – hexane.

Table 1: Conditions for maximum conversion of the esters of CH$_3$(CH$_2$)$_n$COOR and some of their characteristics.

| R     | Conversion, % | Time, h | Amount of enzyme mg/0.1 μmol of acid | Data of IR spectrum | n$_D$ |
|-------|---------------|---------|-------------------------------------|---------------------|-------|
| n-C$_4$H$_9$ | 93 | 24 | 5 | 1735.5 | 1179.1 | 1.4050 |
| i-C$_5$H$_{11}$ | 91 | 24 | 10 | 1735.6 | 1180.5 | 1.4160 |
| n-C$_5$H$_{11}$ | 87 | 30 | 10 | 1737.2 | 1178.3 | 1.4215 |
| i-C$_7$H$_{15}$ | 94 | 24 | 20 | 1736.9 | 1176.7 | 1.4230 |
| n-C$_7$H$_{15}$ | 67 | 36 | 20 | 1737.8 | 1177.6 | 1.4310 |

*High-intensity valent stretching vibration of carboxylic bond $\nu_{С=О}$ of butyric acid is 1705.9 cm$^{-1}$

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