

## 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 ( $C_4$ - $C_{11}$ ) 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,  $H_2SO_4$ , 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  $C_4$  to  $C_{11}$ ) 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*-butyl butyrate, *iso*-butyl 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 lyophilic 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  $n_D^{20}$  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

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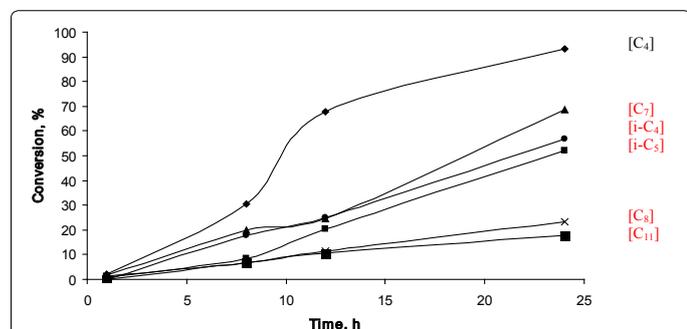
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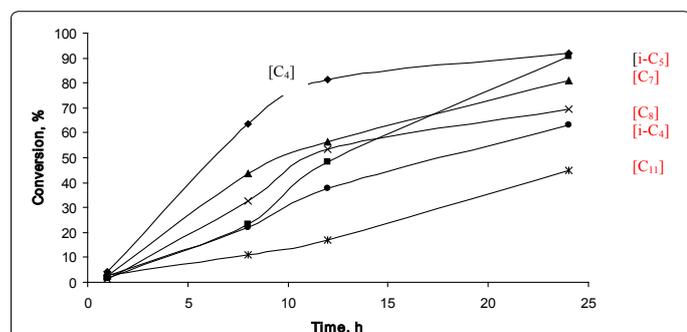
acetylation was complicated. The lowest conversion was observed for undecyl alcohol at all used ranges of amount of enzyme and time of the process (Figures 1-3). The maximum effect (67%) was achieved only after 36 hours of reaction. The presence of a branched radical in *iso*-amyl and *iso*-butyl alcohols 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 in acidity caused decreasing of 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  $\nu_{C=O}$  appeared at 1180-1176  $cm^{-1}$  region. Those stretching vibrations is absent in the spectrums of initial substrates. The high-intensity valent stretching vibration of carboxylic bond  $\nu_{C=O}$  of butyric acid esters are at 1735-1737  $cm^{-1}$  region. The shift of the stretching vibrations  $\nu_{C=O}$  in esters in comparison with  $\nu_{C=O}$  in butyric acid to 30-32  $cm^{-1}$  and the value of refractive index  $n_D^{20}$  corresponding to reference data are additional confirmation of the formation of esters.

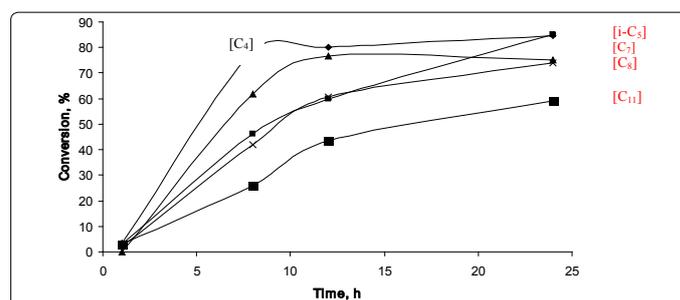
The obtained data show that enzymatic synthesis for the lower alcohols - C4 and C5 - proceed most effectively and with a high yield. It is these esters are produced and used in industry on a larger scale in comparison with esters of higher alcohols. So, replacement of chemical methods of synthesis of the lower alcohols esters on enzymatic one



**Figure 1:** The dependence of conversion of process of an esterification on time for alcohols *n*-butyl ( $C_4$ ), *iso*-butyl (*i*- $C_4$ ), *iso*-amyl (*i*- $C_5$ ), *n*-heptyl ( $C_7$ ), *n*-oktyl ( $C_8$ ), *n*-undecyl ( $C_{11}$ ). Reaction conditions: the ratio acid: alcohol – 1: 2, amount of enzyme – 5 mg / 0.1  $\mu$ mol of acid, the reaction environment – hexane.



**Figure 2:** The dependence of conversion of process of an esterification on time for alcohols *n*-butyl ( $C_4$ ), *iso*-butyl (*i*- $C_4$ ), *iso*-amyl (*i*- $C_5$ ), heptyl ( $C_7$ ), *n*-oktyl ( $C_8$ ), *n*-undecyl ( $C_{11}$ ). Reaction conditions: the ratio acid: alcohol – 1: 2, amount of enzyme – 10 mg / 0.1  $\mu$ mol of acid, the reaction environment – hexane.



**Figure 3:** The dependence of conversion of process of an esterification on time for alcohols *n*-butyl ( $C_4$ ), *iso*-amyl (*i*- $C_5$ ), *n*-heptyl ( $C_7$ ), *n*-oktyl ( $C_8$ ), *n*-undecyl ( $C_{11}$ ). Reaction conditions: the ratio acid: alcohol – 1: 2, amount of enzyme – 20mg / 0.1  $\mu$ mol of acid, the reaction environment – hexane.

| R                         | Conversion, % | Time, h | Amount of enzyme mg/0.1 $\mu$ mol of acid | Data of IR spectrum     |                         | $n_D^{20}$ |
|---------------------------|---------------|---------|---|-------------------------|-------------------------|------------|
|                           |               |         |   | $\nu_{C=O}$ , $cm^{-1}$ | $\nu_{C-O}$ , $cm^{-1}$ |            |
| <i>n</i> - $C_4H_9$       | 93            | 24      | 5   | 1735.5                  | 1179.1                  | 1.4050     |
| <i>i</i> - $C_5H_{11}$    | 91            | 24      | 10  | 1735.6                  | 1180.5                  | 1.4160     |
| <i>n</i> - $C_7H_{13}$    | 87            | 30      | 10  | 1737.2                  | 1178.3                  | 1.4215     |
| <i>n</i> - $C_8H_{15}$    | 74            | 24      | 20  | 1736.9                  | 1176.7                  | 1.4230     |
| <i>n</i> - $C_{11}H_{23}$ | 67            | 36      | 20  | 1737.8                  | 1177.6                  | 1.4310     |

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

**Table 1:** Conditions for maximum conversion of the esters of  $CH_3(CH_2)_2COOR$  and some of their characteristics.

would be effective and demanded. The same interest has an enzymatic esterification of high alcohols because the chemical esterification for them is retarded. In our experiments conversion of high alcohols at enzymatic synthesis is enough high (74- 67%) in the used conditions. Thus, our studies showed that the pancreatic lipase can be used as a catalyst for obtaining aliphatic esters, so enzymatic synthesis could be an alternative to the known chemical methods of esters synthesis.

## References

- Smirnov EV (2008) Food flavorings. Reference book. Profession, Sankt-Petersburg.
- Barry MT, Ian F (1979) The Synthesis and Reactions of Organic Compounds. Carboxylic Acids. Phosphorus Compounds. In Comprehensive Organic Chemistry (2nd edn.), Sutherland JO (Ed.), Pergamon Press ZTD, United Kingdom.
- Gubicza L, Kabiri-Badr A, Keoves E, Belafi-Bako K (2001) Large-scale enzymatic production of natural flavour esters in organic solvent with continuous water removal. J Biotechnol 84: 193-196.
- Güvenç A, Kapucu N, Mehmetoglu Ü (2002) The production of isoamylacetate using immobilized lipases in solvent-free system. Process Biochem 38: 379-386.
- Güvenç A, Kapucu N, Bayraktar E, Mehmetoglu Ü (2003) Optimization of the enzymatic production of isoamylacetate with Novozym 435 from Candida Antarctica. Chemical Engineering Communications 190: 948-961.
- Xiao Z, Hou X, Lyu X, Zhao JY, Xi L, et al. (2015) Enzymatic synthesis of aroma acetoin fatty acid esters by immobilized Candida antarctica lipase B. Biotechnol Lett 37: 1671-1677.
- Gamayurova VS, Zinov'eva ME, Kalacheva NV, Snaider KL (2015) Enzymatic Synthesis of Polyethylene Glycol-400 Esters. Catalysis in Industry 7: 239-243.
- Gracheva IM, Grachev YP, Mosichev EG, Borisenko EG, Bogatkov SV, et al. (1982) Laboratornyi practicum (Handbook) on enzyme preparation technology. Moscow, Pystchevaya promyshlennost.
- Genesh J (1971) Titration in non-aqueous media. Mir, Moscow, Russia.
- Vojtkevich SA (1994) 865 flavoring substances for perfumes and household chemicals. Moscow, Russia.