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Synthesis of aromatic ester by enzymatic reactions in supercritical carbon dioxide (CO2)

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Abstract
The study of the enzyme-catalysed synthesis of isoamyl acetate in supercritical carbon dioxide was performed. The effect of pressure and temperature on conversion, productivity and specific productivity rates, as well as on the initial velocity was evaluated. The commercial immobilized lipase from Candida Antartica Novozyme 435 was used as catalyst.

Key words:
isoamyl acetate, supercritical, lipase.

Introduction

Isoamyl acetate, also known as 3-methylbutyl ethanoate (CAS number: 123-92-2), is one of the most used aroma esters in the food industry [1] due to its strong odour of banana. It is applied in baked products, chewing gum, soft and hard candies, among others [2].

Lipases (glycerol ester hydrolases) are the most used enzymes to catalyze the synthesis of several low molecular esters, such as isoamyl acetate [3]. They are stable in pressurized fluids, specifically in supercritical (SC) carbon dioxide (CO_2), which increases their potential application in synthesis reactions. Among the fluids used as supercritical solvents, carbon dioxide is the most applied due to its low cost, nontoxicity, non-flammability, inertness, full recovery and moderate critical properties (Pc = 7.38 MPa, Tc = 304.2 K) [4, 5].

The focus of this study was to determine the effects of pressure and temperature on the enzyme-catalysed synthesis of isoamyl acetate in SC-CO_2, which was established in terms of conversion, productivity and specific productivity rates, as well as the initial velocity.

Results and Discussion

The reactants and products content were determined by gas chromatography. The results obtained are presented on Table 1.

The initial velocity shown for the experiments on Table 1 was measured at 5 minutes, and it decreased with an increase in the temperature for pressures 10 and 20 MPa. At 30 MPa the initial velocity decreases and then increases again. The higher velocities are usually result of milder conditions in the process, such as 40 MPa and 10 ºC, when there is no enzymatic inactivation.

From Table 1, it is observed that the ideal condition for this reaction is 20 MPa and 40 ºC, despite the lower initial velocity.

Romero *et al.* [3] studied the synthesis of isoamyl acetate using Novozym 435 in SC-CO_2. The authors affirmed that at a fixed temperature of 40 ºC, a change in the pressure from 8 to 30 MPa did not affect the conversion rate in a significant level. At a fixed pressure of 10 MPa and changing the temperature from 40 to 60 ºC, the same authors observed a decrease in the conversion rate, as well as in the reaction velocity. The optimal conditions obtained for this reaction were 10 MPa and 40 ºC.

Table 1. Results of conversion (X, %), productivity (P, g/h), specific productivity (SP, g/g.h) and initial velocity (IV, mmol/min) for the synthesis of isoamyl acetate in SC-CO_2. All experiments were conducted at fixed time (3 h).

P (MPa)	T (ºC)	X (%)	P (g/h)	SP (g/g.h)	IV (mmol/min)
10	40	39.9±6.3	0.5	2.9	2.9
10	50	55.5±19.3	0.7	4.0	2.8
10	60	46.3±5.6	0.6	3.3	1.6
20	40	81.4±2.0	1.1	5.9	1.6
20	50	39.6±9.6	0.5	2.8	1.5
20	60	45.3±4.5	0.6	3.3	0.6
30	40	48.2±13.1	0.6	3.5	1.7
30	50	68.7±6.7	0.9	4.9	1.4
30	60	47.0±14.4	0.6	3.4	2.2

Conclusions

Milder process conditions, such as 10 MPa and 40 ºC, led to higher initial velocities due to no inactivation of the enzyme. The highest esterification (81.4%), productivity (1.1 g/h) and specific productivity (5.9 g/g.h) were obtained for 20 MPa and 40 ºC.

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