

Impact of Lipase-Mediated Hydrolysis of Castor Oil on γ -Decalactone Production by *Yarrowia lipolytica*

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Abstract γ -Decalactone is an industrially interesting peach-like aroma compound that can be produced biotechnologically through the biotransformation of ricinoleic acid. Castor oil (CO) is the raw material most used as the ricinoleic acid source. The effect of different CO concentrations on the γ -decalactone production by *Yarrowia lipolytica* was investigated in batch processing, and 30 g L⁻¹ was found to be the optimal oil concentration. Under these conditions, cells were able to produce lipase but at low activity levels, which might limit ricinoleic acid release and consequently, the γ -decalactone production rate. Thus, the enzymatic hydrolysis of CO by commercial lipases was studied under different operating conditions. Lipozyme TL IM was found to be the most efficient and the optimal hydrolysis conditions were pH 8 and 27 °C. The use of hydrolyzed CO in the aroma production allowed a decrease in the lag phase for γ -decalactone secretion.

Keywords Castor oil · Enzymatic hydrolysis · γ -Decalactone · Lipase · *Yarrowia lipolytica*

Introduction

Lactones are aroma compounds widely used in the flavoring industry, naturally present in fruits and in some fermented food products [1]. These compounds may be

obtained directly from fruits and by chemical synthesis, but increasing demand by consumers for natural flavor compounds has encouraged the development of natural lactones production by microbial processes, leading to aroma compounds that are classified as natural by European and US food legislation [2].

γ -Decalactone is one of the most produced lactones, with a fruity note important in the formulation of peach, mango, apricot, strawberry and chocolate aromas [3]. It can be biotechnologically produced through the biotransformation of ricinoleic acid (12-hydroxyoctadec-9-enoic acid), a hydroxylated C₁₈ fatty acid that, which in its esterified form, is the major constituent (about 86 %) of castor oil (CO). CO is a natural and non-toxic oil obtained from the seed of the castor plant, *Ricinus communis* [4]. This oil is commonly used in the production of γ -decalactone and its triglycerides need to be hydrolyzed in order to release ricinoleic acid. There are several methods described to promote hydrolysis yielding high conversions. Usually, ricinoleic acid is produced by saponification followed by acidification. Although the reaction conditions are mild (70–100 °C), the product obtained has an unacceptable odor and coloration and contains a high quantity of the by-product, Na₂SO₄, which is difficult to remove [5]. The high-temperature and high-pressure processes of manufacturing fatty acids are not suitable for castor oil hydrolysis since they lead to the formation of an undesirable product named ricinoleic acid estolide and may cause denaturation of the product [5–7]. With this in mind, a lipase-catalyzed enzymatic hydrolysis obviates these drawbacks as it operates at moderate temperatures (ca. 35–40 °C) and produces ricinoleic acid which is odorless and light-colored [7, 8].

Yarrowia lipolytica is one of the yeast species able to carry out the biotransformation of ricinoleic acid into

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γ -decalactone [9]. The process involves the substrate degradation through the peroxisomal β -oxidation, leading to the formation of 4-hydroxydecanoic acid, which cyclizes into γ -decalactone [10]. This yeast is also known to be a lipase producer [11–13]. Since these enzymes catalyze the hydrolysis of triacyl-glycerides into glycerol and free fatty acids, their presence in the medium may improve the availability of fatty acid substrates to the microorganism. However, lipase activity may be influenced by the activity of proteases, since these enzymes are involved in the digestion/degradation of proteins [14].

With the aim of improving the productivity of the aroma production process, in a first approach, the present work reports the influence of different castor oil concentrations on the production of γ -decalactone by *Y. lipolytica*. The production of lipase and protease was monitored during these experiments. Since the process revealed itself to be very slow, probably due to an inefficient hydrolysis of the oil, its enzymatic hydrolysis was investigated. In previous works [15], the ability of *Y. lipolytica* to produce lipase was used in order to assess the influence of this endogenous enzyme upon γ -decalactone production. Different *Y. lipolytica* strains and culture conditions were studied and the results revealed that the induction of lipase production by this yeast did not improve the aroma production, but reduced the lag phase of the process. Therefore, in the present work, the use of different commercial lipases from other microbial sources than *Y. lipolytica* to hydrolyze castor oil were tested. The influence of using hydrolyzed castor oil in the aroma production was studied, as well as the use of the commercial enzyme in the biotransformation of non-hydrolyzed CO.

Experimental Procedures

Strains, Media and Culture Conditions

Yarrowia lipolytica W29 (ATCC20460) was cultured for 48 h on a YPD medium (30 g L⁻¹ agar, 20 g L⁻¹ glucose, 20 g L⁻¹ peptone, 10 g L⁻¹ yeast extract) at 27 °C and used to inoculate (to a cellular concentration of 1.2×10^8 cells mL⁻¹) a 500-mL baffled Erlenmeyer flask containing 200 mL of YPD medium (20 g L⁻¹ glucose, 20 g L⁻¹ peptone, 10 g L⁻¹ yeast extract). Flasks were shaken at 140 rpm and 27 °C for 24 h until the cultures reached the late logarithmic growth phase, with a final concentration of 1.0×10^9 cells mL⁻¹, and the glucose was completely consumed (confirmed by the DNS method [16]).

The source of ricinoleic acid used was castor oil. In a first approach, different concentrations of CO were tested. Thus, the composition of the biotransformation medium was 6.7 g L⁻¹ Yeast Nitrogen Base (YNB) with amino

acids, 2.5 g L⁻¹ NH₄Cl, 10, 30 or 50 g L⁻¹ CO and 1, 3 or 5 g L⁻¹ Tween 80, respectively. For these experiments, the biotransformation medium components were directly added to the YPD medium with the grown cells, as an emulsion, in order to start the biotransformation stage.

After selecting the optimal substrate concentration, assays were performed in order to improve the productivity of the process, by using CO enzymatically hydrolyzed (according to the methodology described in the sequence) or by adding a commercial lipase to improve the hydrolysis of the oil during the aroma production. In the first case, 10 g of lipase (35 U) was added to the flask containing the biotransformation medium (200 mL) and it was incubated at 140 rpm and 27 °C for 48 h. At this point, the biotransformation medium was inoculated with cells pre-grown in 200 mL YPD medium (in a 500-mL baffled Erlenmeyer flask, 140 rpm and 27 °C for 24 h), that were separated from the medium by centrifugation (6000 g, 5 min). The initial cellular concentration of the biotransformation stage was 1.2×10^8 cells mL⁻¹. In the second case, no previous CO hydrolysis was performed and the biotransformation medium with non-hydrolyzed CO was inoculated as above described for the first case, but 10 g of commercial lipase (35 U) was added simultaneously with medium inoculation.

All chemicals were purchased from Sigma-Aldrich (Sintra, Portugal), except for CO that was purchased from Interfat, S.A. (Barcelona, Spain).

Analytical Methods

Samples were collected at appropriate intervals for analysis of cell concentration and viability, aroma compound and ricinoleic acid quantification, lipase and protease activities.

Cell concentration was estimated using a Neubauer-improved counting chamber. For viability, the methylene blue method was used [17].

γ -Decalactone and ricinoleic acid were extracted from 2 mL broth with 2 mL diethyl ether. The organic phase was analyzed by GC (Varian 3800 instrument, Varian, Inc., Palo Alto, CA, USA) as described in previous works [18].

Total esterase (lipase adhered to cell surface and extracellular) activity was determined by a spectrophotometric method using *p*-nitrophenyl-butyrate (*p*-NPB) as substrate. One unit of activity was defined as the amount of enzyme that produces 1 μ mol of *p*-nitrophenol per minute under assay conditions [19].

Total protease activity was quantified at 37 °C for 40 min, as described elsewhere [18], using 0.5 % (w/v) azocasein as substrate in 50 mM acetate buffer at pH 5.0. One unit of activity was defined as the amount of enzyme that caused an increase of 0.01 of absorbance relative to the blank per minute, under assay conditions.

Enzymatic Hydrolysis of Castor Oil

Several commercial lipases were tested for CO oil hydrolysis: CALB L from *Candida antarctica*, Lipozyme TL IM from *Thermomyces lanuginosus* and Lipolase 100T from *Aspergillus oryzae* (enzymes kindly supplied by Novozymes, Bagsvaerd, Denmark).

The reaction mixture consisted of 30 g L⁻¹ CO, 3 g L⁻¹ Tween 80, 3 mL of 10 mM potassium phosphate buffer (pH 6, 7 and 8) and 23.2 U of enzyme. These components were placed in a 25-mL Erlenmeyer flask, with a working volume of 7 mL. The reaction was carried out at 27 or 37 °C, in an orbital shaker, at 200 rpm, for 50 h. Samples were removed over time and 4 mL acetone/ethanol (1:1 v/v) was added to stop the reaction. Liberated fatty acids were titrated with 0.1 M alcoholic KOH (ethanol 98 %), using phenolphthalein as the indicator.

The percentage of CO hydrolysis was calculated as indicated by Eq. 1:

$$\text{Hydrolysis (\%)} = \frac{M \cdot MM \cdot (V - V_B)}{C_{\text{oil}} \cdot V_s \cdot SV} \times 100 \quad (1)$$

where M (mol L⁻¹) is the molarity of KOH; MM (g mol⁻¹) is the molar mass of KOH; V (mL) is the KOH volume spent in the sample titration; V_B (mL) is the KOH volume spent in the blank titration; C_{oil} (mg mL⁻¹) is the oil concentration in the sample; V_s (mL) is the titrated sample volume; and SV is the saponification value (177 mg KOH g⁻¹, according to the supplier).

Results and Discussion

Effect of Castor Oil Concentration on γ -Decalactone Production

The cellular growth during the CO biotransformation is low and appeared to be independent of the substrate concentration, ranging in all cases from 9×10^8 cells mL⁻¹ to 2×10^9 cells mL⁻¹ that represents only 0.3 log₁₀ increase of cell density. No effects of CO concentration on cells were observed since the cells viability remained practically constant at 100 %, in all cases.

Figure 1 indicates a maximum γ -decalactone concentration of $1,839 \pm 295$ mg L⁻¹, obtained at 160 h, with 30 g L⁻¹ of CO. The maximum aroma concentrations of the compound, obtained with 10 and 50 g L⁻¹ substrate were similar between them (approximately 1,350 mg γ -decalactone L⁻¹). However, the process was slower when higher oil concentrations were used, with a lag phase of around 100 h before significant amounts of γ -decalactone were detected in the medium. In contrast, when methyl ricinoleate was used, the maximum of aroma

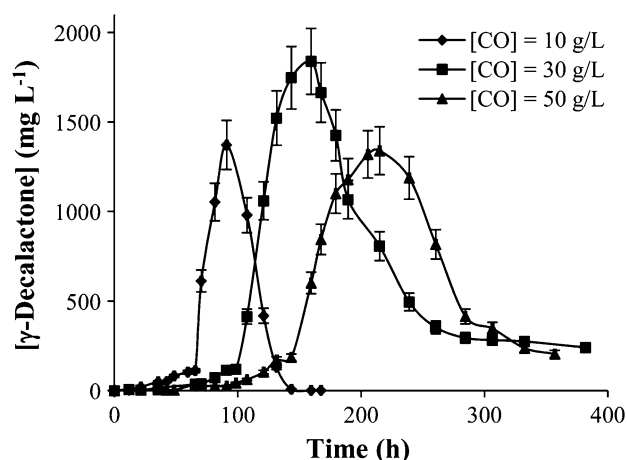


Fig. 1 Time course of γ -decalactone accumulation in biotransformation media with different concentrations of CO. Data are the mean values from two samples, each from two independent experiments

production was attained before or close to 100 h of operation time, depending on the substrate concentration [18].

With a substrate concentration of 10 g L⁻¹, after reaching the peak of aroma concentration, its concentration started to decrease, until it had completely disappeared from the medium. This is due to the ability of yeasts to consume γ -decalactone as a carbon source [20]. The consumption of the aroma started when no more ricinoleic acid was detected in the medium (data not shown). The complete disappearance of the lactone from the medium was not observed when 30 and 50 g L⁻¹ CO were used; in those cases a concentration of the lactone around 200 mg L⁻¹ still remained.

Despite high concentrations of γ -decalactone being achieved, the process was very slow, which may be caused by the use of CO as the substrate, thus a limitation by the inefficient hydrolysis of the triglycerides may be hypothesized. For this reason, extracellular enzymes, such as lipase and protease, that are known to be produced by *Y. lipolytica*, were analyzed in samples from the biotransformation media.

CO induced lipase excretion and, consequently, protease production, since this enzyme is induced by the presence of extracellular proteins [21, 22]. The induction of both enzymes was observed for all the CO concentrations used (Fig. 2).

For the range of concentrations tested, the increase in oil concentration did not improve the lipase activity (Fig. 2a). In fact, the maximum lipase activity (1,415 U L⁻¹) was achieved using the lowest CO concentration tested, which was 10 g L⁻¹. Lakshmi et al. [23] studied the effect of different substrates and concentrations on lipase production by the yeast *Candida rugosa* and concluded that there was an optimum substrate concentration above which no

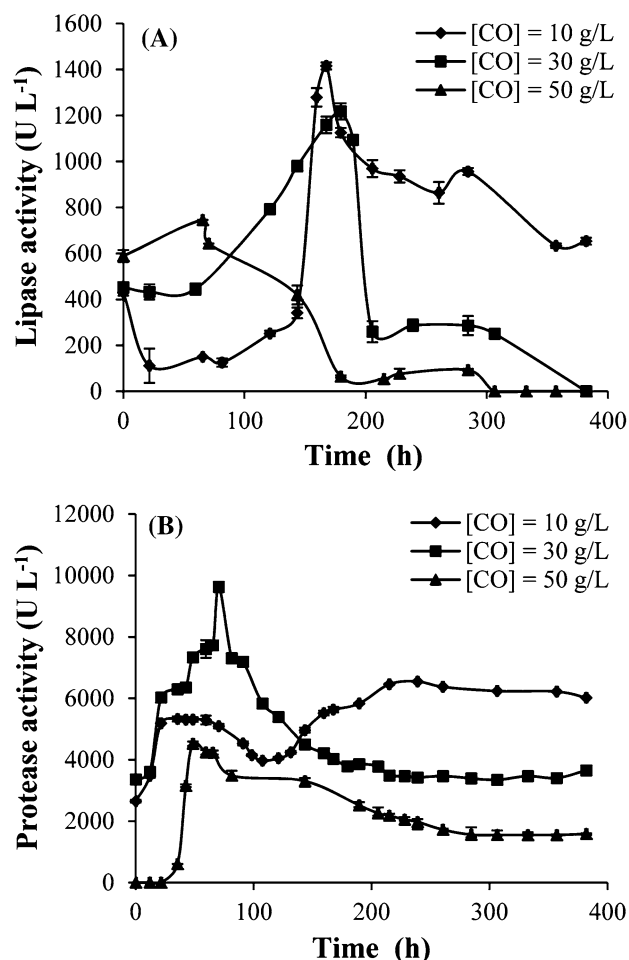


Fig. 2 Lipase (a) and protease (b) activity profiles in biotransformation media with different concentrations of CO. Data are the mean values from three samples, each from two independent experiments

increase in lipase activity occurred. In their work, the maximum lipolytic activity was achieved using 10 g L⁻¹ of all the substrates studied. Also Gomes et al. [18] tested different concentrations of methyl ricinoleate as substrate and the maximum lipase activity was obtained with the lowest oil concentration tested, which was 10 g L⁻¹ substrate. This was most likely due to the excess of substrate in the medium. According to Pereira-Meirelles et al. [24], when the oil is abundant, lipases in the cell wall or membrane are able to hydrolyze the oil at a rate high enough and thus lipases are not secreted unless the substrate becomes scarce and more lipase is needed to promote the substrate uptake and ensure cell subsistence.

Nevertheless, the levels of lipase activity would probably be greater if lipases were not subjected to the action of protease degradation. In fact, high concentrations of proteases were detected in the biotransformation media (Fig. 2b).

With the exception of the medium with 50 g L⁻¹ CO, where the lipase activity remained lower, the peaks of

lipolytic activity were achieved after the maximum accumulation of γ -decalactone occurred (Fig. 1), meaning that the extracellular lipase was not induced soon enough to improve the oil hydrolysis and subsequent metabolism. This may be attributed to an excess of substrate that inhibits lipase excretion [24], suggesting that the release of ricinoleic acid from castor oil could be improved by the addition of an extracellular lipase which would accelerate the process and therefore increase productivity.

Enzymatic Hydrolysis of Castor Oil

The enzymatic hydrolysis of oils may be influenced by several external factors, including pH and temperature. The optimal values of these parameters for CO hydrolysis were determined by conducting the same reaction in phosphate buffer at different pH values (6, 7 and 8) and temperatures (27 and 37 °C). The efficiency of different commercial lipases (i.e., CALB L from *Candida antarctica*, Lipozyme TL IM from *Thermomyces lanuginosus* and Lipolase 100T from *Aspergillus oryzae*) on the enzymatic hydrolysis of the oil was also tested.

To study the effect of temperature on CO hydrolysis, two different temperatures were tested using the commercial lipases: 37 °C (one of the most widely used temperatures for the determination of lipolytic activity [23–27]) and the optimal temperature given by the supplier (70 °C for Lipozyme TL IM and 55 °C for Lipolase 100T). We found that under the reactions conditions used, 37 °C was the best temperature for the lipolytic activity. Indeed, 232.2 ± 18.1 U mg⁻¹ of Lipozyme TL IM and 233.3 ± 41.5 U mg⁻¹ of Lipolase 100T were obtained at 37 °C, whereas 87.4 ± 23.2 U mg⁻¹ of Lipozyme TL IM and 141.3 ± 24.9 U mg⁻¹ of Lipolase 100T were determined at 70 and 55 °C, respectively. These differences in the enzymatic activities between the values reported herein and the ones given by the supplier, may be caused by the use of different methods to determine lipolytic activity.

The optimal temperature for CALB L is between 26 and 37 °C [28], so no further temperature trials were needed. Hence, 37 °C was selected for the following hydrolysis tests regardless of the lipase used.

The ionization (i.e., proton association/dissociation) of free substrate and free lipase, as well as that of the lipase-substrate complex are affected by changes in pH [29]. Thus, the resulting effect of varying the pH of the phosphate buffer (6, 7 and 8) on the rate of CO hydrolysis, at 37 °C, was analyzed for each lipase and it is presented in Fig. 3. From this figure one can observe that the maximum hydrolysis yield (85.3 %) obtained after 30 h with Lipozyme TL IM, was similar for all the pH values studied.

The hydrolysis of CO promoted with Lipolase 100T, at pH values of 7 and 8 was rather identical, reaching its

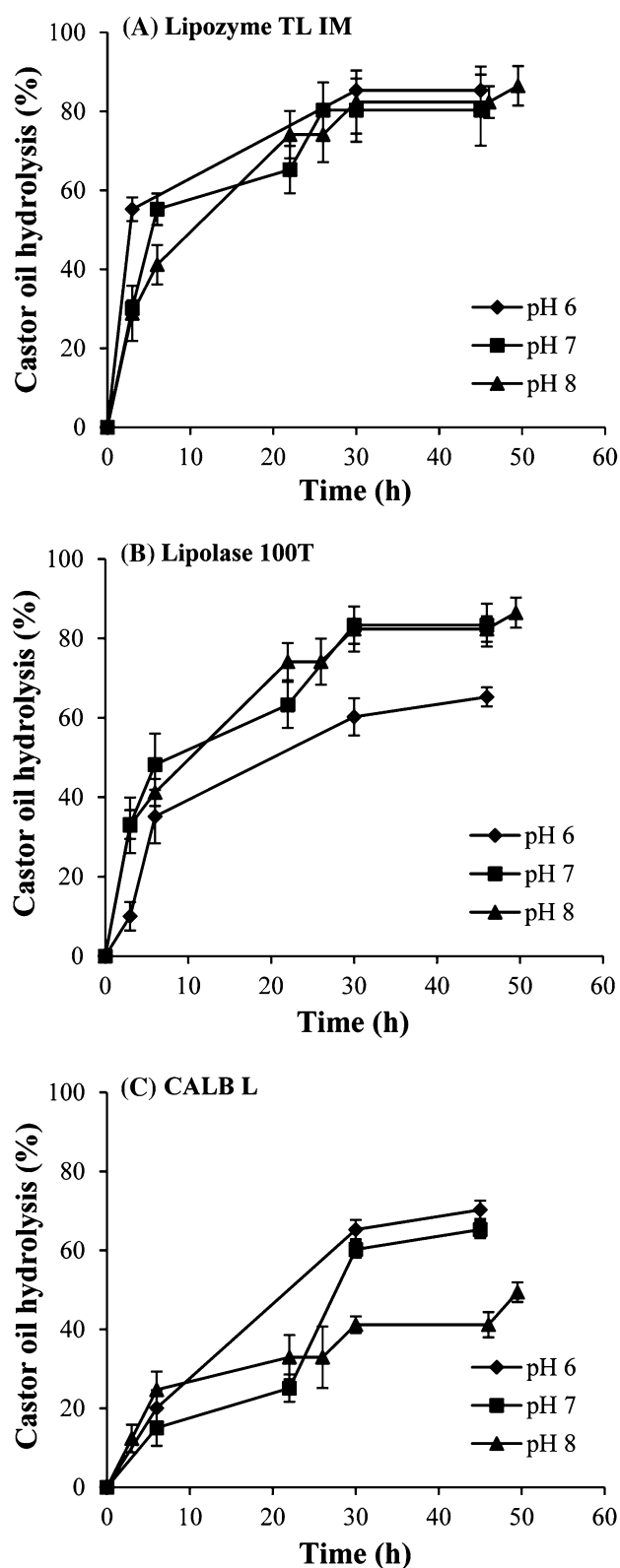


Fig. 3 Impact of pH on the rate of CO hydrolysis, at 37 °C. Data are the mean values of three independent experiments. Data are the mean values from two samples, each from two independent experiments

highest yield of 83.3 % after 30 h of reaction. pH 6 leads to an oil hydrolysis yield of 65.2 %. Puthli et al. [4] also tested the hydrolysis of CO using a conjugated Lipolase enzyme (28 U mL^{-1}) but obtained a maximum hydrolysis percentage of 61 %, at pH 7, after 72 h of reaction.

Among the commercial lipases tested, CALB L was found to be the least efficient in the hydrolysis of CO, since it led to a maximum hydrolysis yield of only 65 %, after 48 h, at pH 7. The lower efficiency of CALB L may be related with the fact that it was the only free enzyme presented as a solution tested, compared to the others that were immobilized thus presenting higher stability and activity [30, 31].

In all cases, after 6 h of reaction, the hydrolysis reaction rate slowed down, indicating that the enzymatic hydrolysis rate of CO was not linear with time. A possible explanation for this behavior may be that, in the initial part of the reaction, there are more interactions between oil and enzyme and consequently the hydrolysis rate is higher. However, as the reaction time proceeds, higher amounts of fatty acids resulting from the oil hydrolysis accumulate and consequently the substrate concentration decrease, also leading to a decrease in CO hydrolysis rate [4].

Since the best oil hydrolysis yields achieved with the immobilized lipases were similar at pH 7 and 8, this last value was chosen because it is near of the equivalence point of phenolphthalein, the indicator used in the titrations.

In a final attempt, since the biotransformation process was performed at 27 °C, the same temperature was tested on the oil hydrolysis rate, in comparison with the optimum temperature of 37 °C, determined for both immobilized enzymes. Figure 4 shows that Lipozyme TL IM led to a hydrolysis percentage of 95.4 % after 26 h, at 27 °C. The maximum hydrolysis rate obtained using Lipolase 100T (82.3 %, achieved in 30 h, at 37 °C) was lower, so Lipozyme TL IM was the lipase chosen to be used in the biotransformation experiments.

Impact of Castor Oil Enzymatic Hydrolysis on γ -Decalactone Production

Two different aroma production strategies were investigated and compared with the usual biotransformation of ricinoleic acid. The first methodology consisted in performing a hydrolysis of the oil with an exogenous lipase Lipozyme TL IM before starting the biotransformation; The second strategy consisted in the addition of the same lipase to the biotransformation medium with the aim of improving the hydrolysis of CO.

The results of γ -decalactone production are presented in Fig. 5 and it is shown that the addition of Lipozyme TL

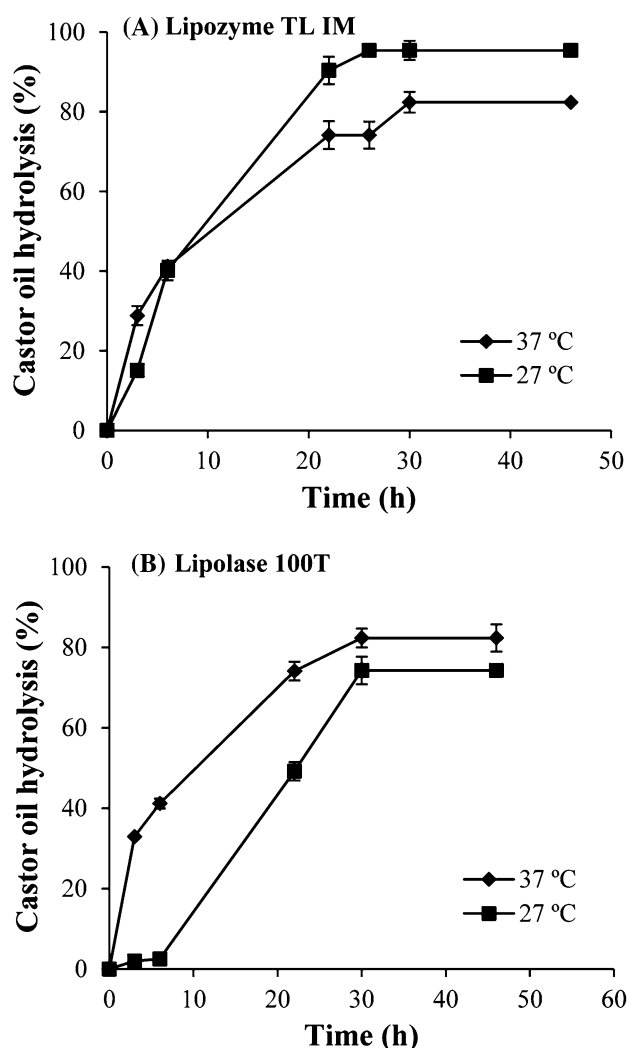


Fig. 4 Effect of temperature on CO hydrolysis rate, at pH 8. Data are the mean values from two samples, each from two independent experiments

IM, during the biotransformation, or at an earlier stage, decreased the lag phase for the production of γ -decalactone from CO by *Yarrowia lipolytica*. However, although the maximum production of lactone was reached earlier in the experiments with Lipozyme TL IM, the accumulated concentration was lower (ca. $1,000 \text{ mg L}^{-1}$, at 137 h, in both cases) than that obtained in the control assay, where no lipase was added ($1,544 \text{ mg L}^{-1}$, at 190 h). This is related to the fact that, by adding a lipase to enhance the hydrolysis of CO, there is an increased release of fatty acids [particularly ricinoleic acid (90 %), linoleic acid (4.2 %), oleic acid (3 %), stearic acid (1 %) and palmitic acid (1 %)] that may be toxic for the yeasts, leading to a lower production of γ -decalactone [20].

Nevertheless, the productivity values obtained in each of the three media tested (7.70 ± 1.15 , 7.89 ± 1.18 and

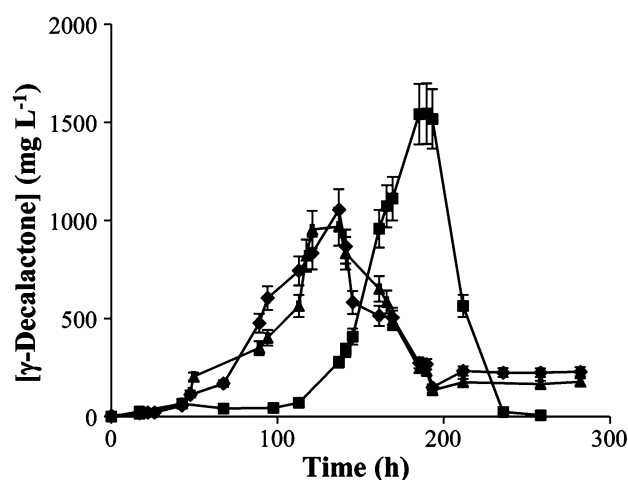


Fig. 5 Time course of γ -decalactone accumulation in biotransformation media with 30 g L^{-1} CO as a result of different production strategies: with Lipozyme TL IM (black diamonds); with CO previously hydrolyzed by Lipozyme TL IM (black triangles); without lipase addition (control) (black squares). Data are the mean values from two samples, each from two independent experiments

$8.33 \pm 1.24 \text{ mg L}^{-1} \text{ h}^{-1}$, respectively for medium with Lipozyme TL IM, with CO previously hydrolyzed by Lipozyme TL IM and without lipase addition, respectively) were not significantly different, which indicated that by the action of Lipozyme TL IM it is possible to produce γ -decalactone in less time keeping identical values of aroma productivity. In fact, a gain of around 50 h in the overall batch process is obtained.

It is worth noting that the maximum accumulation of γ -decalactone obtained in the control assay ($1,544 \text{ mg L}^{-1}$ in 190 h) is slightly lower than that obtained in the previous experiments ($1,839 \text{ mg L}^{-1}$ in 160 h). That lower accumulation of lactone is attributed to the fact that cells were centrifuged and resuspended in the biotransformation medium, in the control assay. This step was necessary in order to compare the results with those where lipase was involved. However, with this procedure, cells take longer time to adapt to the new medium, influencing not only the concentration of lactone produced but also the production time.

Conclusions

A CO concentration of 30 g L^{-1} was found to be the optimal one for the production of γ -decalactone.

Different extracellular commercial lipases, pH and temperature values were investigated for CO hydrolysis. Lipozyme TL IM, at pH 8 and 27°C , revealed themselves to be the most efficient lipase and operating conditions, to hydrolyze CO.

The effects, on the lactone production, of using CO previously hydrolyzed by the selected lipase or adding that lipase to the biotransformation medium, were evaluated and compared with experiments performed without enzymatic hydrolysis of the oil. The process was faster when lipases were involved, but γ -decalactone concentrations were lower, resulting in similar productivity values to those without using lipases.

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References

- Lin S-J, Lee S-L, Chou C-C (1996) Effect of various fatty acids components of castor oil on the growth and production of γ -decalactone by *Sporobolomyces odoratus*. J Ferment Bioeng 82:42–45
- Neto RS, Pastore GM, Macedo GA (2004) Biocatalysis and biotransformation producing γ -decalactone. J Food Sci 69:677–680
- Lee SL, Lin S-J, Chou CC (1995) Growth and production of γ -decalactone by *Sporobolomyces odoratus* in jar fermenters as affected by pH, aeration and fed-batch technique. J Ferment Bioeng 80(2):195–199
- Puthli MS, Rathod VK, Pandit AB (2006) Enzymatic hydrolysis of castor oil: process intensification studies. Biochem Eng J 31:31–41
- Goswami D, Sen R, Basu JK, De S (2009) Maximization of bioconversion of castor oil into ricinoleic acid by response surface methodology. Bioresour Technol 100:4067–4073
- Lakshminarayana G, Subbarao R, Sastry YSR, Kale V, Rao TC, Gangadhar A (1984) High pressure splitting of castor oil. J Am Oil Chem Soc 61:1204–1206
- Rathod VK, Pandit AB (2009) Effect of various additives on enzymatic hydrolysis of castor oil. Biochem Eng J 47:93–99
- Sharon C, Furugoh S, Yamakido T, Ogawa HI, Kato Y (1998) Purification and characterization of a lipase from *Pseudomonas aeruginosa* KKA-5 and its role in castor oil hydrolysis. J Ind Microbiol Biotechnol 20:304–307
- Aguedo M, Beney L, Waché Y, Belin J-M (2003) Mechanisms underlying the toxicity of lactone aroma compounds towards the producing yeast cells. J Appl Microbiol 94:258–265
- Blin-Perrin C, Molle D, Dufosse L, Le-Quere J-L, Viel C, Mauvais G, Feron G (2000) Metabolism of ricinoleic acid into γ -decalactone: β -oxidation and long chain acyl intermediates of ricinoleic acid in the genus *Sporidiobolus* sp. FEMS Microbiol Lett 188:69–74
- Lopes M, Gomes N, Gonçalves C, Coelho MAZ, Mota M, Belo I (2008) *Yarrowia lipolytica* lipase production enhanced by increased air pressure. Lett Appl Microbiol 46:255–260
- Pignède G, Wang H-J, Fudalej F, Seman M, Gaillardin C, Nicaud J-M (2000) Autocloning and amplification of LIP2 in *Yarrowia lipolytica*. Appl Environ Microbiol 66(8):3283–3289
- Aloulou A, Rodriguez JA, Puccinelli D, Mouz N, Leclaire J, Leblond Y, Carrière F (2007) Purification and biochemical characterization of the LIP2 lipase from *Yarrowia lipolytica*. Biochim Biophys Acta 1771:228–237
- Najjar A, Robert S, Guérin C, Violet-Asther M, Carrière F (2011) Quantitative study of lipase secretion, extracellular lipolysis and lipid storage in the yeast *Yarrowia lipolytica* grown in the presence of olive oil: analogies with lipolysis in humans. Appl Microbiol Biotechnol 89:1947–1962
- Braga A, Gomes N, Belo I (2012) Lipase induction in *Yarrowia lipolytica* for castor oil hydrolysis and its effect on γ -decalactone production. J Am Oil Chem Soc. doi:10.1007/s11746-011-1987-5
- Gonçalves C, Rodriguez-Jasso RM, Gomes N, Teixeira JA, Belo I (2010) Adaptation of dinitrosalicylic acid method to microtiter plates. Anal Methods 2:2046–2048
- Bonora A, Mares D (1982) A simple colorimetric method for detecting cell viability in cultures of eukaryotic microorganisms. Curr Microbiol 7:217–221
- Gomes N, Teixeira JA, Belo I (2010) The use of methyl ricinoleate in lactone production by *Yarrowia lipolytica*: aspects of bioprocess operation that influence the overall performance. Biocatal Biotransform 28(4):227–234
- Gomes N, Gonçalves C, García-Román M, Teixeira JA, Belo I (2011) Optimization of a colorimetric assay for yeast lipase activity in complex systems. Anal Methods 3:1008–1013
- Waché Y, Aguedo M, Choquet A, Gatfield I, Nicaud JM, Belin JM (2001) Role of β -oxidation enzymes in γ -decalactone production by the yeast *Yarrowia lipolytica*. Appl Environ Microbiol 67(12):5700–5704
- Fukushima Y, Itoh H, Fukase T, Motai H (1989) Continuous protease production in a carbon limited chemostat culture by salt tolerant *Aspergillus oryzae*. Appl Microbiol Biotechnol 30:604–608
- Srinivasan M, Dhar SC (1990) Factors influencing extracellular protease synthesis in an *Aspergillus flavus* isolate. Acta Microbiol 37(1):15–23
- Lakshmi BS, Kanguane P, Abraham B, Pennathur G (1999) Effect of vegetable oils in the secretion of lipase from *Candida rugosa* (DSM 2031). Lett Appl Microbiol 29:66–70
- Pereira-Meirelles FV, Rocha-Leão MHM, Sant'Anna Jr GL (2000) Lipase location in *Yarrowia lipolytica* cells. Biotechnol Lett 22:71–75
- Corzo G, Revah S (1999) Production and characteristics of the lipase from *Yarrowia lipolytica* 681. Bioresour Technol 70:173–180
- Amaral PFF, Rocha-Leão MHM, Marrucho IM, Coutinho JAP, Coelho MAZ (2006) Improving lipase production using a perfluorocarbon as oxygen carrier. J Chem Technol Biotechnol 81:1368–1374
- Reagent Chemicals ACS Specifications (1993) American Chemical Society, 8th edn, Washington DC
- Brigida AIS, Amaral PF, Gonçalves LR, Coelho MAS (2007) Characterization of an extracellular lipase from *Yarrowia lipolytica*. In: Proceedings of European Congress of Chemical Engineering, Copenhagen, pp 855–856
- Tipton KF, Dixon HB (1979) Effects of pH on enzymes. Methods Enzymol 63:183–234
- Nasratun M, Said HA, Noraziah A, Abd Alla AN (2009) Immobilization of lipase from *Candida rugosa* on chitosan beads for transesterification reaction. Am J Appl Sci 6:1653–1657
- Sharma R, Chisti Y, Banerjee UC (2001) Production, purification, characterization and applications of lipases. Biotechnol Adv 19:627–662