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preliminary communication

Production of γ-Decalactone by Yeast Strains under Different Conditions

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Summary

γ-Decalactone is a flavour compound that when obtained by biotechnological production using microorganisms is classified as natural. The aim of this study is to evaluate various conditions for γ-decalactone production by tropical yeast strains *Yarrowia lipolytica* CCMA 0242 and *Lindnera saturnus* CCMA 0243. The growth of and γ-decalactone production by *Y. lipolytica* CCMA 0242 were higher in castor oil than in glycerol. γ-Decalactone production in single batch or fed-batch fermentation did not differ significantly. The γ-decalactone production by *L. saturnus* CCMA 0243 was better at initial pH=5, while the production by *Y. lipolytica* CCMA 0242 was better at initial pH=6. The yeast *L. saturnus* CCMA 0243 produced more γ-decalactone than *Y. lipolytica* CCMA 0242 under the same fermentation conditions. The crude glycerol was not an alternative substrate for γ-decalactone production by *Y. lipolytica* CCMA 0242. Castor oil at volume fraction of 30 % showed better results as a substrate. The strain *L. saturnus* CCMA 0243 showed better results of γ-decalactone production. This yeast species can be considered an alternative producer of γ-decalactone in biotechnological processes.

Key words: castor oil, crude glycerol, *Lindnera saturnus*, microbial γ-decalactone, *Yarrowia lipolytica*

Introduction

Lactones are flavour compounds that can be directly obtained from fruits through chemical methods or biotechnological processes. Among lactones, γ-decalactone is the most widely produced and has a fruity peach flavour. Ricinoleic acid (12-hydroxy-octadec-9-enoic acid) is the main component (about 86 %) of castor oil and is used as the substrate in most production processes of γ-decalactone. This fatty acid is transformed into γ-decalactone by yeast strains *via* peroxisomal β-oxidation (*1–3*). Another substrate used in the production of microbial metabolites is crude glycerol, which is a by-product generated in the production of biodiesel. Crude glycerol has been explored as a substrate for microbial fermentation due to its wide availability and cost effectiveness (*4,5*).

In a single batch culture, after a few hours of fermentation, yeast can use the γ-decalactone as a carbon source

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when the substrate is completely consumed, resulting in its complete disappearance from the medium (*6,7*). An alternative to this problem is the production using fed-batch, in which the substrate is restored to the culture medium. This operation allows the achievement of higher cell density than that in single batch fermentation, and is used to obtain higher yields and productivity of the desired product by controlling the feeding of nutrients (*8*). In addition to the carbon source and its concentration, several other factors influence the production of primary and secondary metabolites during fermentation. The pH and agitation of the medium may interfere with the growth and production of γ-decalactone by yeasts. Oxygen transfer rate from the gas to the liquid medium can also be improved by increasing aeration and stirring rates, since it is known that oxygen may influence the activity of the enzymes of the peroxisomal β-oxidation pathway (*2,9*).

Yarrowia lipolytica is the most commonly used yeast for the conversion of ricinoleic acid to γ-decalactone, with higher yields being obtained due to its ability to grow on hydrophobic substrates and due to their efficient lipases, several cytochrome P450s, and acyl-CoA oxidase (*10*). The *Lindnera saturnus* CCMA 0243 (UFLA CES–Y677) strain was used for its ability to grow in glycerol (*5,11*). In this study, we hypothesised its ability to metabolise lipids, evaluating it for the production of γ-decalactone.

Thus, the objectives of this study are to evaluate the growth and production of γ-decalactone by *Y. lipolytica* CCMA 0242, using residual crude glycerol and castor oil as substrates at different volume fractions by single batch fermentation and fed-batch fermentation, and to evaluate the growth and production of γ-decalactone by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA 0243 at different pH values and agitation rates.

Material and Methods

Microorganisms and inoculum preparation

Yarrowia lipolytica CCMA 0242 and *Lindnera saturnus* CCMA 0243, obtained from the Culture Collection of Agricultural Microbiology, Department of Biology at the Federal University of Lavras, MG, Brazil, were used in this study. The *Y. lipolytica* CCMA 0242 strain was isolated from Brazilian kefir samples (*12*) and the *L. saturnus* CCMA 0243 strain was isolated from Brazilian cerrado soil (*13*). The *L. saturnus* CCMA 0243 strain showed good results metabolising glycerol and lipids (*5,11,13*).

Both yeast strains were prepared in the same manner, according to Gomes *et al.* (*14*). An aliquot of stock culture in 20 % glycerol was transferred to the test tube containing yeast extract-peptone-dextrose (YEPD) broth (Himedia, Mumbai, India) maintained at 28 °C for 48 h. After this period, 10 % of the initial inoculum was transferred to larger volumes until reaching a final volume of 200 mL. They were kept under the same conditions at 150 rpm in an orbital shaker (model Excella E25; New Brunswick Scientific, Hamburg, Germany) until the end of the exponential growth phase. When this phase was reached, all the contents were centrifuged (model Rotina 380R; Hettich Zentrifugen, Tuttlingen, Germany) at 4 °C and 6000×*g* for 5 min and the cells were washed three times with sterile water before being placed in the new medium for conversion in a population of 7 log cell/mL.

In all assessments, the cell count was performed using a Neubauer chamber and methylene blue dye (*14*). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) except for crude glycerol, which was supplied by the Laboratory of Research on Oils, Fats and Biodiesel of Federal University of Lavras, Brazil.

γ-Decalactone production and analysis

After the inoculum preparation, both yeasts were submitted to fermentation to produce γ-decalactone. Fermentation was carried out in Erlenmeyer flasks of 125 mL with 50 mL of yeast nitrogen base (YNB) conversion medium (6.7 g/L; Himedia), NH_4Cl (2.5 g/L) and Tween 80 (2 g/L) (*2*). Sterile crude glycerol and castor oil were added separately in volume fractions of 10, 20 and 30 % (*i.e.* 5, 10 and 15 mL, respectively) (*15,16*). The pH value was adjusted to pH=6 using a digital pH meter (model B474; Micronal, São Paulo, Brazil) before the inoculation by the addition of NaOH (2 mol/L) and HCl (2 mol/L). The cultures were kept for 120 h, and samples were taken for analysis. During fed-batch fermentation, pulse feeding with 125 μL/L of castor oil or crude glycerol started 18 h after inoculation and was performed every 18 h until 120 h at all substrate volume fractions (10, 20 and 30 %) (*3*).

The best conditions for production of γ-decalactone, determined in the experiment above, were selected to evaluate the effects of pH and agitation speed on the growth of and γ-decalactone production by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA 0243. The fermentation was conducted as described in the first paragraph above.

γ-Decalactone was extracted from 2 mL of conversion medium with 2 mL of diethyl ether by ten gentle shakings. After 5 min, organic phase (ether) was separated and analysed by gas chromatography (GC) as described previously (*2*). The extracted samples were injected into the gas chromatograph (model 17A; Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (GC–FID), split/splitless injector, and a capillary column (length 30 m, diameter 0.25 mm and film thickness 0.25 μmol/L, DB-WAX model; J&W Scientific, Folsom, CA, USA) with N_2 as a carrier gas at a flow rate of 1.2 mL/min. The injector temperature was 200 °C and that of the detector was 250 °C. The oven temperature was maintained at 60 °C for 3 min, increased to 200 °C at a rate of 20 °C/min and kept at 200 °C for 10 min. Data were analysed using the program acquisition and integration GC class v. 2.5 (Shimadzu) and compared with the standard curve previously obtained for γ-decalactone compound (*2,11*).

Statistical analysis

Growth and production of γ-decalactone by both yeasts using different carbon sources and growth conditions were conducted in a randomised block design with three blocks, each block representing the experiment at different times. The fermentation was conducted at 29 °C and pH=6 with stirring at 150 rpm. The growth and the production of γ-decalactone were evaluated in a factorial

arrangement 2×3×2, with two carbon sources (crude glycerol and castor oil), three volume fractions of both carbon sources (10, 20 and 30 %), and two types of fermentation processes (single batch and fed-batch). For the evaluation of growth, samples were obtained after 24, 48, 72, 96 and 120 h of fermentation. The results of the yeast growth (cell number after each period of growth) were analysed according to a model containing the fixed effects of blocks, substrates, substrate volume fractions, type and time of fermentation, and their interactions. To evaluate the production of γ-decalactone, samples were collected after 120 h of fermentation. Production data were analysed by a model containing fixed effects of substrate concentration and type of fermentation.

The influence of pH and agitation of the culture medium on the growth and production of γ-decalactone by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA 0243 were analysed in a completely randomised design with three replications. The results were evaluated in a factorial arrangement 2×2×2, using two yeast strains (*Y. lipolytica* and *L. saturnus*), two pH values (5 and 6) and two shaking speeds (150 and 200 rpm), *i.e.* eight combinations in total. For the growth evaluation, samples were taken after 24, 48, 72, 96 and 120 h of fermentation. The single batch fermentation was conducted at a temperature of 29 °C, and castor oil (30 %) was used as the substrate. The growth results were evaluated separately for each yeast, by a model containing fixed effects of pH, shaking and its interactions. The γ-decalactone production by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA was compared. Production data were analysed using the same model as described above (used to analyse the growth) plus the fixed effect of yeast species.

For all statistical analyses, the Tukey's test was used to compare the mean values, using Sisvar® software (*17*).

Results and Discussion

Growth and γ-decalactone production under different volume fractions of two carbon sources

Under the conditions evaluated in the first experiment, the yeast *L. saturnus* was not able to grow under any tested conditions. The growth of *Y. lipolytica* CCMA 0242 was different depending on the substrate, the type of fermentation, and fermentation time ($p<0.03$). The growth of this yeast was higher (mean value of 8.54 log cell/mL) in castor oil in all volume fractions evaluated than in glycerol (mean value of 8.32 log cell/mL) (Table 1, Fig. 1). The higher volume fractions of glycerol may have exerted a toxic effect on yeast cells. Crude glycerol has impurities, such as methanol, which can hinder the growth of some species of yeast (*18*). The *Y. lipolytica* CCMA 0242 population in fed-batch fermentation (8.46 log cell/mL) was higher than in single batch fermentation (8.40 log cell/mL) ($p<0.03$). The fed-batch fermentation process prevented the toxic effect of the substrate and products on the cells by controlling the supply of nutrients (*8*).

There was no significant difference ($p=0.42$) between the single batch and fed-batch fermentation in the production of γ-decalactone (data not shown). In another study, when different types of fermentations (single batch and fed-batch) were evaluated to produce γ-decalactone by *Y. lipolytica* W29, the production of the flavour was similar in both conditions (*16*). The yeast *Y. lipolytica* CCMA 0242 could produce γ-decalactone in both substrates (2). However, the production of γ-decalactone in a medium with 30 % castor oil (75.8 mg/L; Table 1) was 30 times higher than with 10 % crude glycerol (2.5 mg/L; the highest value was observed when glycerol was used as substrate). When comparing the production of γ-decalactone and the cell population in the medium, using the same type of substrate and its volume fractions (Table 1), it was found that the highest yield was achieved with the highest number of cells, using both carbon sources. In general, castor oil is the most suitable substrate and is used for the yeast growth and production of γ-decalactone (*15*).

Crude glycerol showed no potential as a substrate for the production of aroma by *Y. lipolytica* CCMA 0242 under the conditions used in this study. This by-product has been tested as a supplement for *Y. lipolytica* cultivation in the synthesis of various metabolites of industrial interest,

Table 1. Interaction between the effects of the volume fractions of castor oil and crude glycerol on the population of *Yarrowia lipolytica* CCMA 0242 and the production of γ-decalactone

Substrate	φ(substrate)/%			
	10	20	30	SEM
	N(log cell/mL)			
Castor oil	8.50^{Aa}	8.56^{Aa}	8.57^{Aa}	0.030
Glycerol	8.41^{Ab}	8.26^{Bb}	8.28^{Bb}	
	γ(γ-decalactone)/(mg/L)			
Castor oil	27.8^{Ba}	29.3^{Ba}	75.8^{Aa}	11.21
Glycerol	2.5^{Aa}	0.41^{Aa}	0.32^{Ab}	

SEM=standard error of the mean
Mean values followed by the same capital letter in the same row and by the same lower case letter in the same column do not differ according to Tukey's test ($p<0.05$)

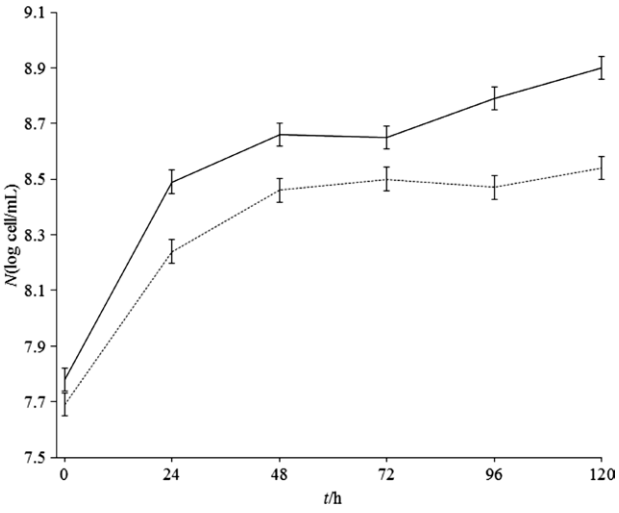

Fig. 1. Interaction between the effects of fermentation time and substrate used for the growth of *Yarrowia lipolytica* CCMA 0242. Castor oil is represented by continuous line and glycerol by dotted line. The bars represent the standard error of the mean

such as organic acids and lipids (*5*). The *Y. lipolytica* CCMA 0242 strain used in this study probably transformed the crude glycerol into other metabolites or used it as the carbon source for growth.

The production of γ-decalactone in the medium supplemented with castor oil at a volume fraction of 30 % was about 2.5 times higher (75.8 mg/L) than at other volume fractions, *i.e.* 10 and 20 % (27.8 and 29.3 mg/L, respectively) (Table 1). In the fermentation using 10 % glycerol as a carbon source, the production of γ-decalactone was approximately six times greater than at 20 or 30 % (0.41 and 0.32 mg/L, respectively) (Table 1). Although Lee *et al*. (*19*) reported that the substrates can inhibit cell growth, high castor oil volume fractions did not inhibit the growth of yeasts, remaining practically viable throughout the fermentation. These results are similar to those of Braga and Belo (*16*), according to which the aroma production by *Y. lipolytica* inoculated into different castor oil mass concentrations was higher when using the highest castor oil concentration (60 g/L). Most often γ-decalactone accumulation is directly related to the concentration of the substrate, since higher flavour production occurs at high substrate concentration (*9*).

According to our results, the best conditions for the production of γ-decalactone, among the evaluated possibilities, were to use castor oil as substrate at the volume fraction of 30 % in a single batch cultivation. These conditions were further used to evaluate the effects of pH and agitation during growth and production of γ-decalactone by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA 0243.

The influence of pH and agitation speed on the growth and production of γ-decalactone

There was no statistical difference in the pH and agitation speed during *Y. lipolytica* CCMA 0242 growth (Table 2). Oxygen is involved in the metabolic pathway of β-oxidation, participating in the production and reconsumption reactions of γ-decalactone (*20*). However, higher rates of agitation may cause metabolic changes and hydrodynamic stress in the cells, which may lead to changes in their morphology, metabolism and viability (*20,21*). Regarding aroma production, studies show that the increase in oxygen mass transfer rate (k_La) increased the rate of γ-decalactone production, however, the final concentration of aroma decreased (*21*).

The maximum growth of *L. saturnus* CCMA 0243 (8.94 log cell/mL) occurred after 24 h of cultivation and it was stable until 120 h ($p<0.01$ for the fermentation time effect; data not shown). Different agitation speed also influenced the yeast growth ($p<0.01$). The population of *L. saturnus* CCMA 0243 (8.87 log cell/mL) was 1.6 % higher at higher speed (200 rpm) than the population (8.73 log cell/mL) obtained at lower stirring speed (150 rpm; data not shown).

Production of γ-decalactone by different yeast species

The interaction between the production of γ-decalactone by each yeast and the fermentation time was especially remarkable at 96 h, when a higher aroma production was observed (512.5 mg/L by *L. saturnus* CCMA 0243 and 214.8 mg/L by *Y. lipolytica* CCMA 0242; Fig. 2). After 96 h of fermentation, the production of γ-decalactone by both yeast strains decreased. A possible explanation may be related to the availability of oxygen in the medium, since it is an intervening factor in the metabolic pathway involved in the reactions of production and reconsump-

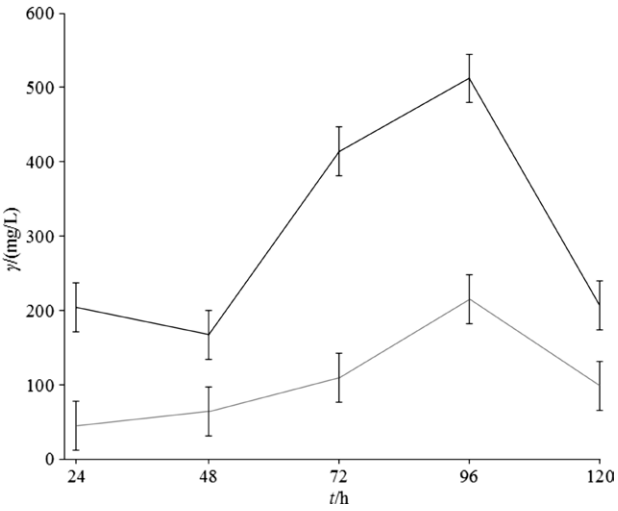

Fig. 2. Interaction between the effects of fermentation time and the production of γ-decalactone by *Yarrowia lipolytica* CCMA 0242 (dotted line) and *Lindnera saturnus* CCMA 0243 (continuous line) using 30 % (by volume) castor oil. The bars represent the standard error of the mean

Table 2. Interaction between the effects of the pH and fermentation conditions on the production of γ-decalactone by the yeasts *Yarrowia lipolitica* CCMA 0242 and *Lindnera saturnus* CCMA 0243

Y	pH	A	T	Y×pH	Y×A	Y×T	pH×A	pH×T	A×T	Y×pH×A	Y×pH×T	Y×A×T	A×pH×T
p-value													
<0.01	0.99	0.28	<0.01	0.04	0.40	<0.01	0.13	0.33	0.68	0.58	0.49	0.99	0.96
				γ(γ-decalactone)/(mg/L)									SEM
pH				*Yarrowia lipolytica* CCMA 0242						*Lindnera saturnus* CCMA 0243			
5				84.4^{Ba}						323.2^{Aa}			20.71
6				128.3^{Ba}						278.5^{Aa}			

Y=yeast, A=agitation speed, T=fermentation time, SEM=standard error of the mean
Mean values followed by the same capital letter in the same row and by the same lower case letter in the same column do not differ according to Tukey's test ($p<0.05$)

tion of aroma (*20*). The oxidation pathway may lead to the accumulation of other compounds (3-hydroxy-γ-decalactone, dec-2-en-4-olide and dec-3-en-4-olide) derived from 4-hydroxydecanoic acid, the direct precursor of γ-decalactone. The accumulation of these different decalactones in the medium gives an indication of various activities of the enzymes of the pathway. The γ-decalactone accumulation increases when acyl-CoA oxidase activity decreases (*7*). The activities of the peroxisomal β-oxidation enzymes, acyl-CoA oxidase and 3-hydroxyacyl-CoA dehydrogenase, may be influenced by oxygen, which is necessary for the regeneration of the cofactors FAD+ and, more indirectly NAD+ (*22*).

Yarrowia lipolytica is the most commonly used yeast for the conversion of ricinoleic acid to γ-decalactone, with higher yields due to its ability to grow on hydrophobic substrates (*10*). However, the production of γ-decalactone by *L. saturnus* CCMA 0243 was 2.38 times higher than by *Y. lipolytica* CCMA 0242. In a previous work, *L. saturnus* CCMA 0243 achieved a ten times higher γ-decalactone production using crude glycerol under different experimental conditions (*11*). Given these results, this yeast species can be considered an alternative producer of γ-decalactone in biotechnological processes.

The production of γ-decalactone by *Y. lipolytica* CCMA 0242 was lower (214.8 mg/L) than previously reported ones (263 mg/L (*23*) and 220 mg/L (*3*)). However, γ-decalactone production in the present study was higher than in some other published studies, where 168 mg/L (*14*) and 87 mg/L (*2*) of γ-decalactone were obtained using the same species. These results can be explained by the different strains and different production conditions used.

The use of different shaking speed did not influence the production of γ-decalactone by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA 0243 (Table 2). γ-Decalactone production by *L. saturnus* CCMA 0243 was better at initial pH=5, and by *Y. lipolytica* CCMA 0242 at initial pH=6. The production of γ-decalactone at different initial pH values (4.5, 5.6 and 6.7) by the yeast *Y. lipolytica* showed the best results (1.284 mg/L at pH=6.7) (*9*).

Conclusion

This study has shown that by using castor oil at volume fraction of 30 % better results in the production of γ-decalactone by *Yarrowia lipolytica* CCMA 0242 were achieved. Crude glycerol was not efficient as an alternative substrate for γ-decalactone production by *Y. lipolytica* CCMA 0242. There was no difference in the production of γ-decalactone in two types of fermentation (single batch or fed-batch). *Lindnera saturnus* 0243 CCMA was successful in converting the castor oil to γ-decalactone, therefore this yeast species can be considered as an alternative source for the production of γ-decalactone. Different pH conditions during the conversion interfered with the production of γ-decalactone by *Y. lipolytica* CCMA 0242 and *L. saturnus* CCMA 0243.

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Conflict of interest

The authors have no conflict of interest to declare.

References

1. Maume KA, Cheetham PSJ. The production of γ-decalactone by fermentation of castor oil. Biocatal Biotransform. 1991;5: 79–97. https://doi.org/10.3109/10242429109014857
2. Gomes N, Teixeira JA, Belo I. The use of methyl ricinoleate in lactone production by Yarrowia lipolytica: aspects of bioprocess operation that influence the overall performance. Biocatal Biotransform. 2010;28:227–34. https://doi.org/10.3109/10242422.2010.493208
3. Moradi H, Asadollahi MA, Nahvi I. Improved γ-decalactone production from castor oil by fed-batch cultivation of Yarrowia lipolytica. Biocatal Agric Biotechnol. 2013;2:64–8. https://doi.org/10.1016/j.bcab.2012.11.001
4. da Silva GP, Mack M, Contiero J. Glycerol: a promising and abundant carbon source for industrial microbiology. Biotechnol Adv. 2009;27:30–9. https://doi.org/10.1016/j.biotechadv.2008.07.006
5. Souza KST, Schwan RF, Dias DR. Lipid and citric acid production by wild yeasts grown in glycerol. J Microbiol Biotechnol. 2014;24:497–506. https://doi.org/10.4014/jmb.1310.10084
6. Pagot Y, Le Clainche A, Nicaud JM, Waché Y, Belin JM. Peroxisomal β-oxidation activities and γ-decalactone production by the yeast Yarrowia lipolytica. Appl Microbiol Biotechnol. 1998;49:295–300. https://doi.org/10.1007/s002530051172
7. Waché Y, Aguedo M, Choquet A, Gatfield IL, Nicaud JM, Belin JM. Role of β-oxidation enzymes in γ-decalactone production by the yeast Yarrowia lipolytica. Appl Environ Microbiol. 2001;67:5700–4. https://doi.org/10.1128/AEM.67.12.5700–5704.2001
8. Lee SL, Cheng HY, Chen WC, Chou CC. Effect of physical factors on the production of γ-decalactone by immobilized cells of Sporidiobolus salmonicolor. Process Biochem. 1999; 34:845–50. https://doi.org/S0032-9592(99)00010-2
9. Gomes N, Teixeira JA, Belo I. Empirical modelling as an experimental approach to optimize lactone production. Catal Sci Technol. 2011;1:86–92. https://doi.org/10.1039/C0CY00017E
10. Waché Y, Aguedo M, Nicaud JM, Belin JM. Catabolism of hydroxyacids and biotechnological production of lactones by Yarrowia lipolytica. Appl Microbiol Biotechnol. 2003;61:393–404. https://doi.org/10.1007/s00253-002-1207-1
11. Soares GPA, Souza KST, Vilela LF, Schwan RF, Dias DR. γ-Decalactone production by Yarrowia lipolytica and Lindnera saturnus in crude glycerol. Prep Biochem Biotechnol. 2017;47. https://doi.org/10.1080/10826068.2017.1286601
12. Miguel MGCP, Cardoso PG, Magalhães KT, Schwan RF. Profile of microbial communities present in tibico (sugary kefir) grains from different Brazilian states. World J Microbiol Biotechnol. 2011;27:1875–84. https://doi.org/10.1007/s11274-010-0646-6
13. Carvalho FP, de Souza AC, Magalhães-Guedes KT, Dias DR, Silva CF, Schwan RF. Yeasts diversity in Brazilian Cerrado

soils: study of the enzymatic activities. Afr J Microbiol Res. 2013;7:4176–90.
https://doi.org/10.5897/AJMR2013.5752

14. Gomes N, Waché Y, Teixeira JA, Belo I. Oil-in-water emulsions characterization by laser granulometry and impact on γ-decalactone production in Yarrowia lipolytica. Biotechnol Lett. 2011;33:1601–6.
https://doi.org/10.1007/s10529-011-0593-9
15. Gomes N, Teixeira JA, Belo I. Fed-batch versus batch cultures of Yarrowia lipolytica for γ-decalactone production from methyl ricinoleate. Biotechnol Lett. 2012;34:649–54.
https://doi.org/10.1007/s10529-011-0824-0
16. Braga A, Belo I. Production of γ-decalactone by Yarrowia lipolytica: insights into experimental conditions and operating mode optimization. J Chem Technol Biotechnol. 2014;90: 559–65.
https://doi.org/10.1002/jctb.4349
17. Ferreira DF. Sisvar: a computer statistical analysis system. Ciênc Agrotec. 2011;35:1039–42.
http://doi.org/10.1590/S1413-70542011000600001
18. Ooi TL, Yong KC, Hazimah AH, Dzulkefly K, Wan Yunus WMZ. Glycerol residue: a rich source of glycerol and medium chain fatty acids. J Oleo Sci. 2004;53:29–33.
https://doi.org/10.5650/jos.53.29
19. Lee SL, Cheng HY, Chen WC, Chou CC. Production of γ-decalactone from ricinoleic acid by immobilized cells of Sporidiobolus salmonicolor. Process Biochem. 1998;33:453–459.
https://doi.org/10.1016/S0032-9592(98)00013-2
20. Gomes N, Aguedo M, Teixeira J, Belo I. Oxygen mass transfer in a biphasic medium: influence on the conversion of methyl ricinoleate into γ-decalactone by the yeast Yarrowia lipolytica. Biochem Eng J. 2007;35:380–6.
https://doi.org/10.1016/j.bej.2007.02.002
21. Braga A, Mesquita DP, Amaral AL, Ferreira EC, Belo I. Aroma production by Yarrowia lipolytica in airlift and stirred tank bioreactors: differences in yeast metabolism and morphology. Biochem Eng J. 2015;93:55–62.
https://doi.org/10.1016/j.bej.2014.09.006
22. Bakker BM, Overkamp KM, van Maris AJA, Kötter P, Luhik MAH, van Dijken JP, Pronk JT. Stoichiometry and compartmentation of NADH metabolism in Saccharomyces cerevisiae. FEMS Microbiol Rev. 2001;25:15–37.
https://doi.org/10.1111/j.1574-6976.2001.tb00570.x
23. Aguedo M, Gomes N, Garcia EE, Waché Y, Mota M, Teixeira JA, Belo I. γ-Decalactone production by Yarrowia lipolytica under increased O_2 transfer rates. Biotechnol Lett. 2005;27: 1617–21.
https://doi.org/10.1007/s10529-005-2517-z