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Biotechnological Valorization of Cupuaçu By-Products: Solid-State Fermentation for Lipase Production by *Yarrowia lipolytica*

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Abstract: Lipases are enzymes that catalyze the hydrolysis of ester bonds of triacylglycerols at the oil–water interface, generating free fatty acids, glycerol, diacylglycerol, and monoacylglycerol, which can be produced from the fermentation of agro-industrial by-products rich in fatty acids, such as cupuaçu fat cake. In this study, *Yarrowia lipolytica* IMUFRJ50682 was used for lipase production from cupuaçu fat cake in solid-state fermentation (SSF) associated with soybean meal. The 2:1 ratio of cupuaçu fat cake/soybean meal increased the lipase activity of *Y. lipolytica* via SSF by approximately 30.3-fold compared to that in cupuaçu without supplementation. The optimal conditions for *Y. lipolytica* to produce lipase were obtained by supplementation with peptone, urea, and soybean oil (all at 1.5% *w*/*v*), reaching values of up to 70.6 U g^{-1}. These results demonstrate that cupuaçu fat cake associated with soybean meal can be used for lipase production and adds value to cupuaçu by-products. Furthermore, the proper processing of by-products can contribute to improving the economic viability of the biotechnological processing industry and help prevent the accumulation of waste and environmental pollution.

Keywords: by-products; soybean meal; sustainability; biotechnological processing; Amazon biome

Citation: Carvalho, A.S.S.; Rocha, R.d.C.P.e.; Sales, J.C.S.; Souza, C.E.C.d.; Lemes, A.C.; Coelho, M.A.Z. Biotechnological Valorization of Cupuaçu By-Products: Solid-State Fermentation for Lipase Production by *Yarrowia lipolytica*. *Fermentation* **2023**, *9*, 989. https://doi.org/10.3390/fermentation9110989

Academic Editor: Miguel Ladero

Received: 2 October 2023
Revised: 2 November 2023
Accepted: 15 November 2023
Published: 20 November 2023

1. Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are enzymes that catalyze the hydrolysis of ester bonds in triacylglycerols at the oil–water interface, generating free fatty acids, glycerol, diacylglycerol, and monoacylglycerol [1,2]. These enzymes can be obtained from various sources (animals, vegetables, and microbes) and are widely applied in different industrial sectors, such as the food, biotechnology, detergent, and textile industries, because of their ability to catalyze various reactions such as hydrolysis, esterification, and alcoholysis [3–5].

Both fungi and bacteria produce lipases [6,7]. In some cases, fungal lipases are secreted, which is advantageous for production. *Yarrowia lipolytica* is a versatile and non-conventional dimorphic yeast. It is generally recognized as safe (GRAS) by the Food and Drug Administration of the USA [8,9] and is considered a workhorse owing to its wide industrial applications [10]. *Y. lipolytica* can accumulate lipids and produce citric acid, isocitric acid, organic acids, lactones, erythritol, mannitol, and enzymes such as proteases and lipases [11–13]. It produces lipase in the presence of hydrophobic substrates, such as olive oil and oleic acid; nitrogen sources, such as peptone, casein, yeast extract, and tryptone; as well as several other substrates [14–17].

Microbial lipase production can be conducted through solid-state fermentation (SSF), which involves cultivation in the absence or near absence of free-flowing water [18–20].

This method results in minimal microbial contamination problems, especially bacterial contamination, owing to the low water content of the medium. Moreover, it has several advantages such as low protein breakdown (which is especially important if an enzyme is the target product), low energy requirements, low energy costs for sterilization, low fermenter volume, low catabolic repression, and high volumetric productivity. It also targets concentrated compounds, leading to lower costs related to downstream processes, high substrate concentration tolerance, and less wastewater [8,21]. However, a disadvantage of SSF is the difficulty in controlling parameters such as pH, temperature, and oxygen variation [22]; in addition, the product recovery may become more difficult [23].

In SSF processes for lipase production, by-products from oleaginous cultures are preferable because they already contain a certain amount of lipids that can act as inducers of enzyme production [24–26]. SSF facilitates the use of agro-industrial wastes as matrices for fermentation because they simulate the habitats of most microorganisms used in the process, such as nutrients, water, and growth support [18,27]. In addition to solving environmental problems through alternative uses instead of improper disposal, the use of agro-industrial by-products reduces the costs of biotechnological processes because they are abundant and inexpensive [28,29].

Several agro-industrial by-products from vegetable oil extraction have been used as substrates for lipase production through SSF, including canola oil cake, soybean meal [30], jatropha [31], palm oil cake [32], watermelon peels [33], andiroba [34], babassu oil cake [35], and sunflower soapstocks [36]. As a general characteristic in lipase production, agro-industrial by-product substrates must have a considerable lipid content and composition, since the induction of enzyme production depends on the type of lipid used as a carbon source, which is the major factor for the expression of lipase activity [8,37,38].

Several matrices such as Amazonian fruits have not been explored for lipase production. The Brazilian Amazon biome has one of the greatest diversities in the world, with fruits that can be exploited in the food and pharmaceutical industry [39,40]. Fruit by-products generated after processing can be used for biotechnological applications [41]. Thus, sustainable processes for the use and valorization of Amazonian products and their by-products should be established.

Cupuaçu is an Amazonian fruit with high nutritional value and is rich in active compounds, including polyphenols and flavonoids [42,43]. Cupuaçu stands out for having fruit pulp for consumption as juices and seeds that are used in fat extraction [44,45]. The fruit is 12–25 cm long and weighs 400–4500 g [46]. On an average, the fruit contains approximately 35 seeds [47] with an individual weight of 4–7 g [48]. This fruit is processed to obtain cupuaçu pulp, which is used in the preparation of juices, jellies, and other applications. This action generates by-products such as bark and seeds [49].

Brazil is the largest producer of cupuaçu, which occurs mainly in the northern region, particularly in the Amazonas, Pará, Acre, Rondônia, and Roraima. The country produces approximately 21.240 tons of fruit, which is of significant economic and social importance. Approximately 13.800 tons of byproducts that can be used in alternative processes are generated during the process [50]. The cupuaçu fat cake generated after partial lipid extraction still has a high residual lipid (21.3%), protein (14.9%), and carbohydrate (22%) content, which are ideal for biotechnological applications such as lipase production.

Lipase production using cupuaçu fat cake has already been studied with *Aspergillus ibericus* (maximum lipolytic activity of 11 U g^{-1}) using SSF processes [41]. However, no previous reports have been published on the production of lipases of biotechnological interest from cupuaçu fat cake using *Yarrowia lipolytica* to produce and optimize enzyme activity using SSF.

Thus, the aim of this study was to evaluate lipase production by *Y. lipolytica* IMUFRJ50682 using cupuaçu fat cake with a matrix supplemented with peptone, urea, and soybean oil to increase lipolytic activity.

2. Materials and Methods

Figure 1 shows the lipase production process by *Yarrowia lipolytica* IMUFRJ50682 via SSF (tray-type bioreactor) using cupuaçu fat cakes.

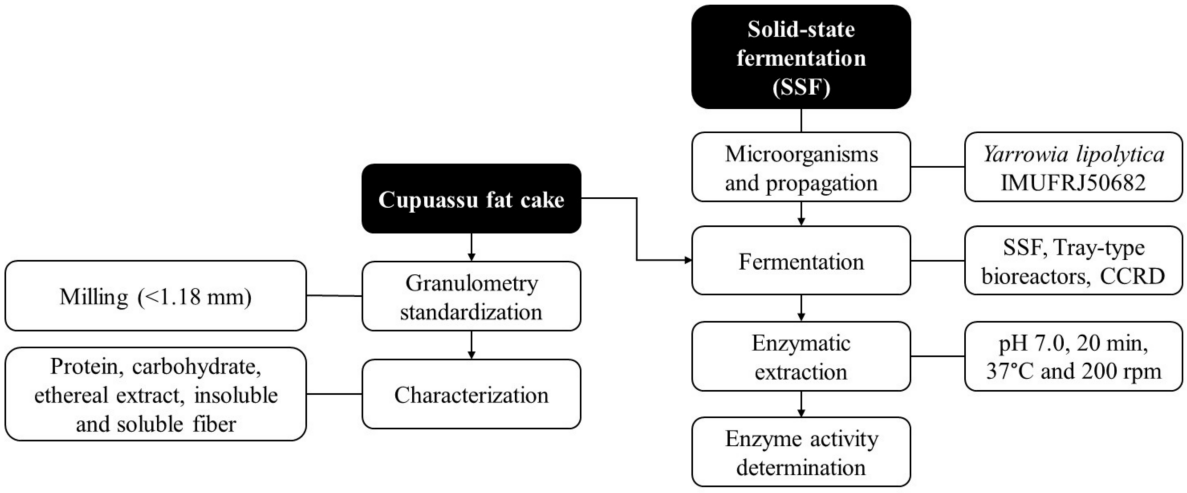

Figure 1. Process of lipase production by *Yarrowia lipolytica* IMUFRJ50682 using cupuaçu fat cake by-product via solid-state fermentation (SSF) at 28 °C in a tray-type bioreactor.

2.1. Materials

The cupuaçu fat cake obtained after the extraction of cupuaçu oil was kindly provided by Beraca Ingredientes Naturais® (Ananindeua, Brazil). *Y. lipolytica* IMUFRJ50682, isolated from an estuary in Guanabara Bay, Rio de Janeiro, Brazil, was used in this study and was described previously [51].

2.2. Characterization of Cupuaçu Fat Cake

The physicochemical composition of the cupuaçu fat cake was determined by evaluating the protein, carbohydrate, ether extract, insoluble fiber, and soluble fiber contents (Table 1) according to the methodology described by the Association of Official Agricultural Chemists (AOAC) [52]. The protein content was determined using the Kjeldahl method with a conversion factor of 5.71, which is used to determine the protein content of soybeans. The carbohydrate content corresponds to the nitrogen-free extract (NIFEXT) and was determined by the difference, in addition to the other constituents presented, between a moisture value of 4.71% and an ash value of 7.24%. The ether extract content was determined using the Soxhlet method, which is defined as the sum of the substances extracted by ether. The insoluble fiber (NDF) content was determined using the Van Soest method, in which the fiber was separated from the other components of the cupuaçu cake by boiling with a detergent solution at neutral pH. The insoluble fiber (ADF) content was determined using the Goering and Van Soest method with the same principle as the previous method; however, an acidic detergent solution was used.

Table 1. Physicochemical composition of cupuaçu fat cake obtained after cupuaçu processing. * ADF is acid detergent fiber; ** NDF is neutral detergent fiber.

Components (%)	Cupuaçu Fat Cake
Proteins	14.93 ± 0.49
Ether extract	21.45 ± 0.44
Carbohydrates—Nifext	21.85 ± 0.32
Insoluble fibers—ADF *	23.45 ± 1.74
Insoluble fibers—NDF **	29.82 ± 0.79

2.3. Microorganisms and Propagation

Y. lipolytica IMUFRJ50682 was stored in yeast extract–peptone–dextrose (YPD) agar medium (*w*/*v*: 1% yeast extract, 2% peptone, 2% glucose, 3% agar) at 4 °C. Pure cultures of *Y. lipolytica* were obtained by subculturing in 200 mL YPD medium (*w*/*v*: 1% yeast extract, 2% peptone, 2% glucose) for 72 h at 28 °C and 160 rpm [30]. Samples were then collected, and the optical density was measured at 570 nm using a spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan). The optical density was used to calculate the cell growth, compared to a cell growth curve prepared previously, to standardize the cell concentration of starter yeast for SSF.

2.4. Solid-State Fermentation

A cupuaçu fat cake fermentation control was produced in tray-type bioreactors containing 10 g of substrate with adjusted granulometry (<1.18 mm), which had been previously separated using a stainless-steel sieve and autoclaved at 121 °C for 20 min. The moisture content was adjusted to 60% with distilled water, and an inoculum concentration of 0.69 mg dry biomass/g substrate was used. The bioreactors were incubated in a Bio-Oxygen Demand incubator (BOD) chamber at 28 °C under saturated moisture (99%) [30]. The same SSF was also conducted using a blend of cupuaçu fat cake with soybean meal to evaluate supplementation at a ratio of 1:2 (soybean meal/cupuaçu fat cake), with a final amount of 10 g of substrate. For both fermentations, the samples were removed at fixed intervals (0, 7, 10, 14, 24, 28, 36, 40, and 48 h), and lipase activity was evaluated using a titrimetric assay.

Moisture content and pH were monitored during fermentation. Moisture was monitored by drying 2 g of fermented matrix at 105 °C using a moisture analyzer balance (AND MX-50, Barueri, Brazil). Further, 0.5 g of fermented matrix was diluted in 3 mL of distilled water, and then the pH was measured using a pH meter (Tecnal, TR-107 PT100, Piracicaba, Brazil).

2.5. Central Composite Rotational Design

Fermentation was carried out using pre-established conditions for other by-products and using the screening performed by Rocha et al. [53], which allowed the selection of significant variables for lipase production by *Y. lipolytica* with cupuaçu; afterward, these conditions were evaluated using the Central Composite Rotational Design (CCRD). The CCRD was composed of six axial points and three repetitions at the central point, for a total of 17 assays. The independent variables included peptone (0.58–3.05%), urea (0.58–3.05%), and soybean oil (0.58–3.05). These three components have been used as supplements to induce lipase production. Lipase activity was measured using a spectrophotometric assay. The coded and actual values are listed in Table 2.

Table 2. Experimental design and responses for lipase production by *Y. lipolytica* obtained in the CCRD (coded and real values, respectively).

Assay	Peptone (%)	Urea (%)	Soybean Oil (%)	Lipase Activity (U g^{-1})
1	(−1) 0.58	(−1) 0.58	(−1) 0.58	47.38
2	(+1) 0.58	(−1) 0.58	(−1) 2.42	43.08
3	(−1) 0.58	(+1) 2.42	(−1) 0.58	51.72
4	(+1) 0.58	(+1) 2.42	(−1) 2.42	49.54
5	(−1) 2.42	(−1) 0.58	(+1) 0.58	37.10
6	(+1) 2.42	(−1) 0.58	(+1) 2.42	43.51
7	(−1) 2.42	(+1) 2.42	(+1) 0.58	37.70
8	(+1) 2.42	(+1) 2.42	(+1) 2.42	66.68
9	(−1.68) 0	(0) 1.50	(0) 1.50	40.61
10	(+1.68) 3.05	(0) 1.50	(0) 1.50	30.48
11	(0) 1.50	(−1.68) 0	(0) 1.50	45.05
12	(0) 1.50	(+1.68) 3.05	(0) 1.50	44.43
13	(0) 1.50	(0) 1.50	(−1.68) 0	59.53

Table 2. *Cont.*

Assay	Peptone (%)	Urea (%)	Soybean Oil (%)	Lipase Activity (U g^{-1})
14	(0) 1.50	(0) 1.50	(+1.68) 3.05	55.28
15	(0) 1.50	(0) 1.50	(0) 1.50	58.32
16	(0) 1.50	(0) 1.50	(0) 1.50	70.64
17	(0) 1.50	(0) 1.50	(0) 1.50	65.08

The surface response methodology (SRM) was used to obtain a model for lipase activity using the Statistica 10 software package, and non-explicit variables were fixed at the central points.

2.6. Enzyme Extraction

For enzyme extraction, the materials from both fermentation set-ups (cupuaçu fat cake and cupuaçu fat cake supplemented with soybean meal) were used. Briefly, 50 mL of 50 mM potassium phosphate buffer (pH 7.0) was added to the bioreactor containing 10 g of fermented substrate and incubated in a rotary shaker for 20 min at 37 °C and 200 rpm. The crude enzyme extract was obtained after filtration of all contents using gauze, centrifuged at 3000 rpm for 5 min, and then used to determine lipase activity [30].

2.7. Screening for Lipase Activity

2.7.1. Spectrophotometric Assay

For the preliminary assays, the spectrophotometric method proposed by Pereira-Meirelles et al. [54] was used to measure lipase activity. The substrate *p*-nitrophenyl laurate (p-NPL) (0.504 mM) was prepared in phosphate buffer (50 mM, pH 7.0). The variation in absorbance owing to the hydrolysis of *p*-nitrophenyl laurate (p-NPL) to *p*-nitrophenol was measured using a spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan) at 410 nm. One unit (U) of lipase activity was defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-nitrophenol laurate per minute under the conditions mentioned above.

2.7.2. Titrimetric Assay

Lipase activity was measured using olive oil, as previously described by Freire et al. [55]. The reaction mixture was prepared using olive oil 5% (*w*/*v*) and gum arabic 5% (*w*/*v*) in 100 mM phosphate buffer 100 mM (pH 7.0) and emulsified in an Ultra Turrax (IKA, UltraTurrax, Staufen, Germany). Then, 19 mL of the reaction mixture and 1 mL of enzyme extract were incubated for 20 min at 200 rpm in a rotatory shaker at 37 °C. The reaction was stopped by the addition of 20 mL of a mixture containing acetone–ethanol (1:1). Free fatty acids released during hydrolysis were titrated using a 0.04 M NaOH solution in an automatic titrator (Metrohm, 916—Ti-Touch, São Paulo, Brazil). One unit of lipase activity (U) was expressed as the amount of enzyme that produces 1 µmol of fatty acid per minute under the assay conditions.

2.8. Statistical Analysis

Statistical analyses were performed using the Statistica 10 software. The obtained models were analyzed using analysis of variance (ANOVA) ($p < 0.1$), and significance was determined using Fisher's statistical test (F test).

3. Results and Discussion

3.1. Characterization of Cupuaçu Fat Cake

As shown in Table 1, the composition of the cupuaçu cake showed that they were an excellent substrate for lipase production using SSF. The cupuaçu cake consisted of 14.9% proteins, 21.8% carbohydrates, 53.27% insoluble fibers, and 21.4% ether extract, which included lipids and all other non-polar compounds that can be extracted using solvents such as phosphatides, steroids, pigments, fat-soluble vitamins, and waxes [56].

The substrate composition and its influence on the production of the target enzyme are well-described in the literature and are therefore important in the definition of a bioprocess. In relation to lipase, the relationship between enzyme production and microbial cell growth, as well as substrate consumption, was described by Pereira-Meirelles et al. [57]. In the first step, cell-bound lipases hydrolyze the inducing lipids present in the medium, generating the fatty acids necessary for metabolism and initiating cell growth. Subsequently, the concentration of fatty acids decreases; therefore, microorganisms secrete extracellular lipases to degrade lipids in the culture medium and provide fatty acids for metabolic maintenance [58].

Each component of the cupuaçu cake plays an important role in microbial metabolism. Protein in the medium is essential for microorganisms to produce enzymes by providing cells with the amino acids required for cellular metabolism and enzyme synthesis [59]. The residual oil content (ether extract) present in the cake induces lipase production and is essential for microorganisms to produce lipases when interacting with complex agro-industrial by-products [58]. In relation to the composition of fatty acids, Silva et al. [60] reported that cupuaçu fatty cakes consisted of approximately 39.73% saturated fatty acids, 47.83% monounsaturated fatty acids, and 12.44% polyunsaturated fatty acids. The main components of saturated, monounsaturated, and polyunsaturated fatty acids were stearic acid (24.21%), oleic acid (47.66%), and linoleic acid (6.97%), respectively.

Carbohydrates and fibers provide nutrients for cell growth during fermentation. Thus, evaluating the carbohydrate composition is important; for instance, glucose can repress catabolic activity [61]. In addition, the by-products, especially fibers, act as a physical support for microorganisms and reduce the cost of the culture medium. SSF allows the use of agro-industrial by-products as alternative substrates to reduce the environmental problems associated with inappropriate waste disposal [34].

3.2. Lipase Production by Y. lipolytica Using Cupuaçu Fat Cake as Substrate and the Supplementation with Soybean Oil

The fermentation of cupuaçu fat cake by *Y. lipolytica* for lipase production was performed for 48 h. After analyzing the crude extract obtained, a low enzymatic activity was verified (<0.5 U g^{-1}). Figure 2 shows the lipase activity of *Y. lipolytica* IMUFRJ50682 during SSF using cupuaçu fat cake without supplementation. The maximum lipase activity (1.9 U g^{-1}) and productivity (0.2 U g^{-1} h^{-1}) were obtained after 10 h of fermentation. In our previous study, minimal or no lipolytic activity was noted before the 10 h mark [33].

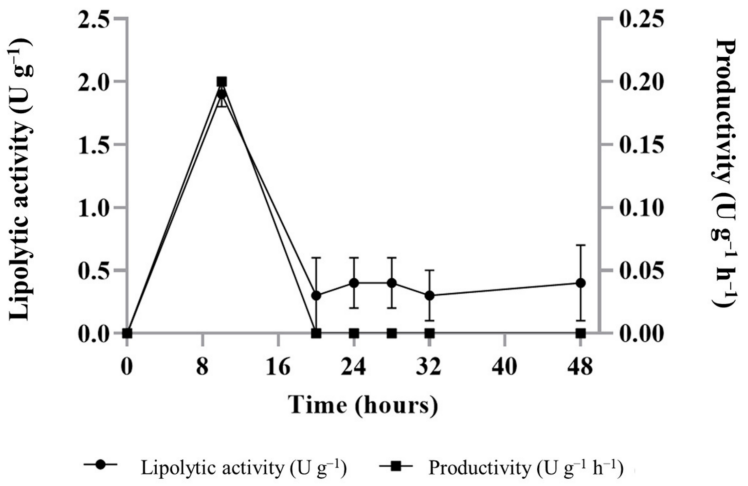

Figure 2. Lipase production by *Yarrowia lipolytica* using cupuaçu fat cake.

Although we expected a higher lipase activity owing to the high ether extract concentration of the cupuaçu fat cake, some factors may have influenced the activity, such as the presence of carbohydrates (21.85%) and the lower protein content (14.93%) (Table 1),

compared to the protein content of soybean meal (51.9%) [30]. The inhibitory effect of carbohydrates in oil cakes on lipase production has been previously reported [41]. The lipolytic activity measured in this study is represented by LIP2 (the main extracellular enzyme produced by *Y. lipolytica*). Since the medium contains a high C/N ratio, excess carbohydrates can delay lipase secretion [62].

Although this study did not quantify the carbon and nitrogen contents in the cupuaçu fat cake, the physicochemical composition suggests a low protein content (nitrogen source) and high carbohydrate and lipid contents (carbon source). However, in this study, we cannot say that all the carbohydrates present in the cupuaçu cake were assimilated by *Y. lipolytica*. Nonetheless, our results suggest that the assimilated carbohydrates may delay the secretion of lipase. A similar effect was observed in our previous studies using the same microorganism but different agro-industrial by-products as substrates. In lipase production using *Y. lipolytica* IMUFRJ 50682, a by-product with a low protein content (0.30 mg g^{-1} two-phase olive mill waste (TPOMW)) and another with a medium protein content (15 mg g^{-1} [wheat white—WB]) were noted. Furthermore, when only TPOMW was used, the lipolytic activity was low (0.79 U g^{-1}), but the combination of the two matrices increased the lipolytic activity to 9.69 U g^{-1} [62].

To induce lipase production, soybean meal was added (1:2 substrate ratio) to improve the protein composition of the fermentation matrix. Subsequently, medium optimization was performed using the variables that were previously studied by our research group and a fractional factorial design [53].

The association of the cupuaçu fat cake with soybean meal (1:2 substrate ratio) increased lipase production compared to the cupuaçu fat cake matrix used alone. The maximum lipase activity was observed after 40 h of fermentation (57.3 U g^{-1}). The pH increased from 6.0 to 7.8, probably because of degradation by the proteases produced by *Y. lipolytica* due to the release of ammonia from the deamination of amino acids [63]. No significant reduction in moisture was observed in the matrices (Figure 3), demonstrating that the system used for lipase production was effective in retaining moisture, which is an important factor for microbial growth and enzyme production [30].

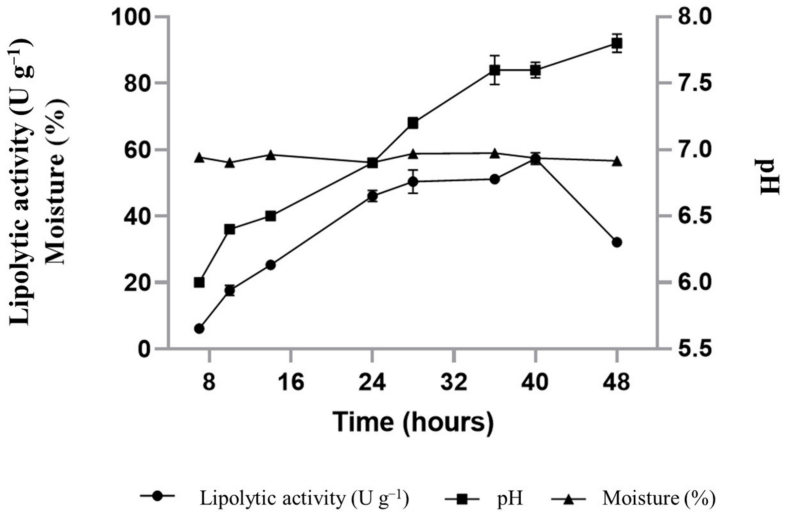

Figure 3. Time course evaluation of lipase production using cupuaçu fat cake and soybean meal (1:2).

Mixing different by-products can be beneficial to microorganisms due to differences in the nutritional composition of these materials, for example, a mixture of wheat bran and clay butter; a mixture of wheat bran (6.25 g), coconut oil cake (2.5 g), and wheat rawa (1.25 g); and a mixture of gingelly oil cake and wheat bran (3:1, *w*/*w*) which produced lipase activities of 308 U g^{-1}, 620.8 U g^{-1}, and 2867.18 U g^{-1}, respectively [64–66].

The production of lipases by *Y. lipolytica* through SSF has also been reported [33]. The lipase production by *Y. lipolytica* was evaluated using soybean meal and watermelon peel as

substrates. The addition of watermelon peel resulted in an approximately 31% increase in lipase activity, reaching 75.22 U g^{-1}. Nascimento et al. [67] also examined the production of lipases by *Y. lipolytica* via SSF using yeast extract, bactopeptone, and soybean oil, achieving activities of 32 U g^{-1} in tray bioreactors.

Furthermore, several other studies reported the production of lipase using SSF, which included the use of *Y. lipolytica* NCIM 3589 with sugar cane bagasse, wheat bran, and rice bran (up to 9.3 U g D.W^{-1}) [68]; niger seed (*Guizotia Abyssinica*) oil cake [69] and palm kernel (*Elaeis guineensis*) cake [70] (up to 26.42 and 18.58 U g D.W^{-1}, respectively); and mustard oil cake with the final production of 57.89 U g D.W^{-1} (hydrolytic activity) [71].

The strain NRRL Y-1095 also was used to produce lipase using a crude olive oil cake, reaching values of up to 40 U g D.W^{-1} (hydrolytic activity) for the biomass pretreated with a base (NaOH 3% w/v) [72]. In another study, cottonseed cake and soybean cake supplemented with sludge were used with the strain IMUFRJ 50682 and hydrolytic activities (in p-nitrophenyl dodecanoate) of 102 and 139 U g D.W^{-1} were observed, respectively [73].

Additionally, studies using two-phase olive mill waste and wheat bran obtained maximum lipase activity values of 486 U g D.W^{-1} at 96 h, similar to the study of Souza that used soybean cakes (maximum activity of 93.9 U g D.W^{-1}) and compared it to canola cakes (highest lipolytic activity of 72.6 U g D.W^{-1}) without supplementation.

All reported studies demonstrate the importance of evaluating the conditions of the fermentation process and investing in the use of agro-industrial by-products as nutritional and low-cost raw materials to produce lipases from *Y. lipolytica*.

3.3. Optimization of Lipase Production Using a CCRD (2^3)

A CCRD was performed to calculate the ideal urea, peptone, and soybean oil concentrations in the supplementation solution. The choice of variables to be studied was based on our previous study [53]. The values of moisture and inoculum size were kept constant at 60% and 3.0 mL, respectively. The results of these 17 assays are presented in Table 2. The experimental matrix was evaluated using analysis of variance (ANOVA) with a 90% confidence interval ($p < 0.1$) (Table 3). The ANOVA showed that the experimental data created a coefficient of determination (R^2) of 0.85.

Table 3. ANOVA for the quadratic model of the response surface for lipase production.

Variation Source	Sum of Squares	Degrees of Freedom	Means Square	F-Value	F-Critical	Adj. R^2
Regression	1764.90	9	196.10	4.63	2.73	0.85617
Residual	296.49	7	42.36			
Lack of fit	220.27	5	44.05	1.16	9.29	
Pure error	76.22	2	38.11			
SQ total	2061.39	16				

An adjusted model for predicting the maximum lipase activity was obtained, as described in Equation (1), where X_A corresponds to peptone (%), X_B to urea (%), and X_C to the SM (%) contents.

$$\text{Lipase activity (U g}^{-1}) = 36.848 + 21.489 \times X_A - 11.771 \times X_A{}^2 + 18.180 \times X_B - 7.890 \times X_B{}^2 - 5.646 \times X_C - 2.434 \times X_C{}^2 + 1.914 \times X_A \times X_B + 6.185 \times X_A \times X_C + 3.646\, X_A \times X_C \quad (1)$$

Three-dimensional response surface plots were constructed to determine the optimal level of each variable for lipase production (Figure 4A–C). The response surfaces indicated that very high concentrations of urea, soybean oil, and peptone negatively affected lipolytic activity. Peptone and urea have restricted values in a range around 1.5% of the mean activity of 66.6 U g^{-1} according to Table 2. However, soybean oil has greater flexibility, with optimum values between 1.5 and 3.0%, demonstrating that *Y. lipolytica* is less sensitive to this parameter, as identified using a fractional factorial design [53].

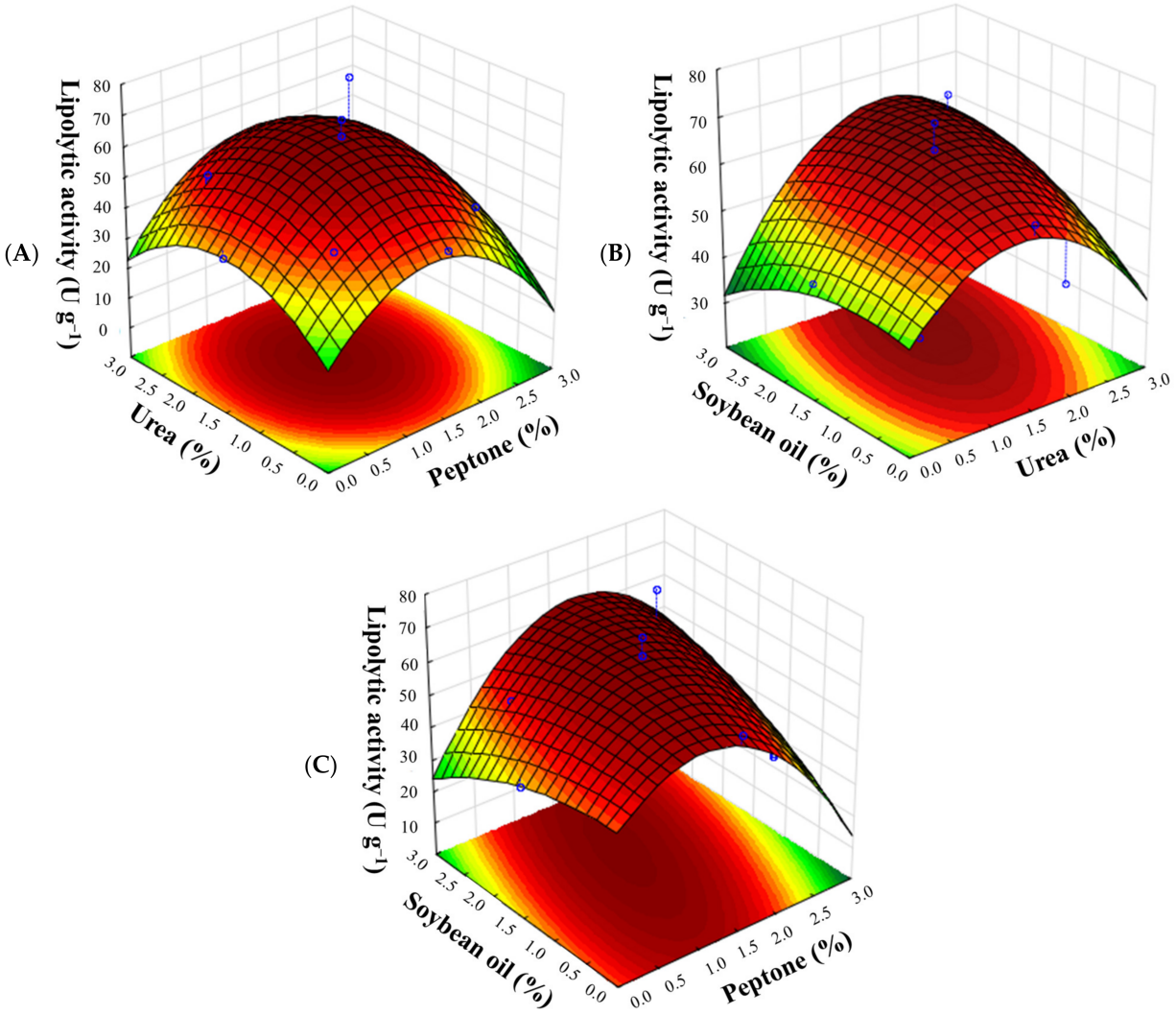

Figure 4. Response surface plot indicating the effects of interactions between (**A**) urea and peptone, (**B**) soybean oil and urea, and (**C**) soybean oil and peptone on lipase activity.

Peptone and urea have been studied as nitrogen sources for lipase production by *Y. lipolytica*, and activities of 109 and 59 U g^{-1}, respectively, were found [74]. The increase in lipase production by *Y. lipolytica* is associated with the qualitative and quantitative content of bioactive peptides from casein hydrolysates that induce lipase production [75]. In addition, *Y. lipolytica* has been studied in waste such as soybean hulls supplemented with yeast extract, bactopeptone, and soybean oil, and mustard oil cake with urea, obtaining 32 U g^{-1} and 57.89 U g^{-1} of lipase activity, respectively [67,71]. Other microorganisms have already been used in the production of lipase such as *Trichoderma* and *Aspergillus brasiliensis* using oil palm empty fruit bunches supplemented with glucose, urea, and olive oil, and malt bagasse supplemented with soybean oil which reached lipase activities of 0.390 U g^{-1} and 9.1 U g^{-1}, respectively [76,77].

Based on the information presented, the production of lipase can be stimulated by supplementing the culture medium, and adapting to the nutritional needs of the microorganism using different components and agro-industrial by-products. In addition, the use of by-products can emphasize the value of plant materials from specific regions, such as the Amazon region, by stimulating sustainable exploitation and contributing to the preservation of important biomes and the biotechnological production of materials with high added value [34,78,79].

To popularize and enable this type of process, it is important to overcome the challenges regarding the determination and standardization of the composition, cost estimation, and processing of agro-industrial by-products, which are indeed significant in the context of sustainable and economically viable practices, considering the variability in by-products, complexity of the supply chain, energy consumption, regulatory aspects, economic viability, processing capacity, and flexibility.

The composition of agro-industrial by-products can vary widely depending on factors such as the type, seasonality, quantity, and geographic location. This variability complicates the production of consistent products. However, modern techniques can be adopted to help determine the composition and quality of the by-products. The complexity of the supply chain makes the integration of by-product collection and processing into existing supply chains both intricate and expensive. Collaborative efforts among agriculture, food processing, and waste management can help streamline supply chains. Sharing resources such as transportation and storage facilities can reduce costs and improve logistics. Furthermore, the processing of by-products often requires energy-intensive processes, which can increase operational costs and environmental impacts. Therefore, it is important to investigate energy-efficient processing technologies that can reduce energy consumption. Additionally, it is important to consider renewable energy sources for power-processing units. Another aspect that requires consideration is regulatory compliance. Meeting regulatory requirements can be a barrier, especially if by-products are used for human consumption. Therefore, it is important to work closely with regulatory agencies to understand and comply with safety and quality standards [28,34,80].

Processing new by-products can be economically challenging because of the initial investment required and competition with established low-cost products. Therefore, it is important to explore value-added products or co-products that can be generated from these by-products, and consequently, market these high-value ingredients to increase revenue [81]. In addition, the processing of large quantities of heterogeneous raw materials may require investments in flexible and scalable processing units. Therefore, it is essential to invest in modular processing equipment that can be adjusted for different raw material types and quantities [28,34].

In summary, addressing the challenges associated with agro-industrial by-products requires a multifaceted approach that involves technological innovation, collaboration, regulatory compliance, and a focus on economic viability. Overcoming these barriers can lead to more sustainable and profitable agro-industrial practices, while reducing waste and environmental impacts.

4. Conclusions

This study introduced an alternative approach for enhancing the value of agro-industrial by-products through SSF. Specifically, we explored the use of a cupuaçu fat cake in combination with soybean meal as a fermentation medium to produce lipases using *Y. lipolytica* IMUFRJ 50682. By supplementing the SSF with urea (1.5% w/v), peptone (1.5% w/v), and soybean oil (1–3% w/v), we observed a notable improvement in lipase production, resulting in a 16% increase (66.6 U g^{-1}). This study showed that lipase production using by-products from the Amazon region combined with soybean meal is possible. We also propose the application of fermented solids and lipase enzymes in various desirable reactions for the food industry.

Author Contributions: Conceptualization, A.C.L. and M.A.Z.C.; methodology, A.S.S.C., R.d.C.P.e.R., J.C.S.S. and C.E.C.d.S.; formal analysis, A.S.S.C., R.d.C.P.e.R., J.C.S.S. and C.E.C.d.S.; investigation, A.S.S.C., R.d.C.P.e.R., J.C.S.S. and C.E.C.d.S.; resources, A.S.S.C., R.d.C.P.e.R., J.C.S.S. and C.E.C.d.S.; data curation, A.S.S.C., R.d.C.P.e.R., J.C.S.S. and C.E.C.d.S.; writing—original draft preparation, A.S.S.C., R.d.C.P.e.R., J.C.S.S. and C.E.C.d.S.; writing—review and editing, A.C.L. and M.A.Z.C.; supervision, A.C.L. and M.A.Z.C.; project administration, A.C.L. and M.A.Z.C.; funding acquisition, A.C.L. and M.A.Z.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES—Finance Code 001); the Conselho Nacional de Desenvolvimento Científico (CNPq); and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

Conflicts of Interest: The authors declare no conflict of interest.

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