Production of omega 3, 6, and 9 fatty acids from hydrolysis of vegetable oils and animal fat with *Colletotrichum gloeosporioides* lipase

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Received: 4 August 2017 / Revised: 5 October 2017 / Accepted: 12 October 2017 / Published online: 14 November 2017
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Abstract Hydrolysis of vegetable oils (Olive, corn, peanut, sesame, flaxseed, soy, canola, garlic, sunflower, almond, castor bean oils) and beef marrow bone oil by *Colletotrichum gloeosporioides* lipase was studied. The enzyme was capable of generating free fatty acids from all oils tested. The higher hydrolytic activity of the enzyme was towards olive (18.0 IU) and soybean (17.8 IU) oils. The average percentage of essential fatty acids generated from hydrolysis of the oils was 32.92% of omega 9 (as oleic acid C18:1), 26.24% of omega 6 (linoleic C18:2), and 5.86% of omega 3 (such as α -linolenic acid C18:3). Comparison between chromatographic profile of the oils and its enzymatic hydrolysate showed a good equivalence, stressing the applicability of these vegetable substrates under the action of lipase from *C. gloeosporioides* produce essential fatty acids, being more efficient production of α -linolenic acid from flaxseed oil, linoleic acid from sunflower oil, and oleic acid from olive.

Keywords *Colletotrichum gloeosporioides* lipase · Enzymatic hydrolysis · Omega 6 · Omega 3 · Vegetable oil

Introduction

The growth of the world population has been driven a demand for improvement of quality, quantity, and diversity of oil sources. To address this demand, it has been necessary to invest in the growth of major crops as well as in the boosting and popularization of consumption of oilseeds bearing lesser global expression [1]. In this context, world production of oilseeds has increased about of 1,552,590 tons in the last 10 years, reaching the sum of 3,867,932 tons in 2014 [2]. These natural oils have historically been applied in the food industry, animal nutrition, industry of detergents, and recently, in the production of biodiesel [3].

Vegetable oils are primarily composed of triglycerides containing fatty acids with different lengths (C8–C24) and degrees of saturation. These oils represent a renewable natural source of fatty acids [4]. One of the aspects of economic exploitation of vegetable oils and fats consists in producing concentrates of free fatty acids and, which are used as raw material in various industrial sectors such as emulsifiers, adhesives, surfactants, food, and personal care products [5]. In animal nutrition, addition of unsaturated fatty acids from plant sources in animal feed implies in healthier meat.

Poly and monounsaturated fatty acids generated from the hydrolysis of vegetable oils (such as omega 3, omega 6, and omega 9) have physiological properties such as promoting HDL levels increasing, reducing the risk of cardiovascular disease, besides their beneficial effects on atherosclerosis [6].

There are three main routes for the production of fatty acids from vegetable oils [7]. The first one consists in the mechanical separation of the oil from the seeds tissue utilizing high-pressure steam under high temperature (70 bar

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and 250 °C). This method, nevertheless effective, may lead to the concomitant extraction of undesirable products, such as bitter compounds. The second route consists in the use of alkaline chemical hydrolysis, which is also operative but have a high cost especially because the formation of side products (soap) that consequently requires further steps for product recovery. The third way to produce fatty acids from vegetable oils, and the less studied one, consists on enzymatic hydrolysis [7]. The latter offers the advantage of being held in mild conditions of pressure and temperature, easier product recovery [6, 8], less product loss caused by heating [9], besides fewer side reactions because the enzymes have chemo-, regio-, and enantioselectivity [10]. The biggest technological bottleneck to replace chemical by enzymatic catalysis for the production of fatty acids is to find enzymes with good yields, suitable catalytic specificity towards specific reactions, and low production cost [11]. Among the hydrolytic enzymes described in the literature, the potential of those produced by fungi of *Colletotrichum* genus has been emerging [12]. This fungus has been widely studied as a phytopathogen but other aspects have been observed [13]. It has been already reported the stability of a lipase from *C. gloeosporioides* in alkaline pH (pH 10) and its tolerance to some solvents (methanol, ethanol) that may be present in the reactions. In addition, this enzyme showed stability to substrates with different composition (residual soy oil, cocoa butter), as well as ability to carry hydrolysis and esterification reactions [14]. The objective of this work was to verify the hydrolytic ability profile of *C. gloeosporioides* lipase to several vegetable oils and animal fats of easy access to produce concentrates containing essential fatty acids.

Materials and methods

Chemicals

All chemicals were of analytical grade and they were obtained from Vetec Química fina LTDA (Rio de Janeiro, Brazil). Castor bean oil has kindly donated by Bioóleo LTDA (Bahia, Brazil). Other oils were obtained in local stores from Belo Horizonte city, MG (Brazil).

Enzyme pool preparation

A lipolytic strain of *C. gloeosporioides* deposited at Food Microbiology Laboratory of Universidade Federal de Minas Gerais was used. It was preserved in potato dextrose agar (PDA). For obtaining enzyme pool, the fungus was transferred to PDA plates and cultivated for 5 days at 25 °C. Spores were harvested and an inoculum with concentration of 10^7 spores per milliliter was prepared

using a Neubauer chamber. This suspension was inoculated to a medium containing (g/L): peptone [10], $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.6), KH_2PO_4 (1.0), NH_4NO_3 (1.0), and olive oil (4 mL) at pH 6.0 for liquid substrate fermentation ($112 \times \text{g}$, 30 °C). After 48 h, the broth obtained was filtered on Whatman (number 1) membrane. Biomass was separated and the filtrate (enzyme pool) without fungal cells was used in the experiments. This pool was stored at -80 °C until further analysis.

Total protein content

Total protein content was determined to calculate specific activity according to the amount of protein present in enzyme pool. Bradford reagent (160 μL) was added in microplates containing *C. gloeosporioides* enzyme pool (40 μL). The mixture was gently stirred, left at rest for 15 min in the dark and then, absorbance was read at 595 nm [15]. A standard curve was built with bovine serum albumin at room temperature for comparison.

Lipase activity

A *p*-nitrophenylpalmitate (*p*NPP) solution in isopropanol (3 mg/mL) was mixed (1:9) with TRIS–HCl at pH 8.0 (0.09 M), arabic gum (2 mg/mL), and triton X-100 (14 mg/mL). The enzyme pool was added to this mixture and absorbance was determined at 410 nm against a blank (without enzyme). After 10 min at 37 °C, absorbance of the reaction mixture was determined at 410 nm. In order to calculate lipase activity, a standard curve using the reaction product, *p*-nitrophenol (*p*NP), as a solution in isopropanol was used. The amount of enzyme required to release one μmol of *p*NP per L of enzyme pool added to the test, by min, was defined as one unit of lipase activity (U/mL min). This methodology was adapted from literature [16].

Enzymatic hydrolysis

To evaluate the *C. gloeosporioides* lipase hydrolytic action over different oils titrations tests were carried out as described by literature [17]. Olive, corn, peanut, sesame, flaxseed, soy, canola, garlic, sunflower, almond, castor bean oils along with beef marrow were used as substrates. The reactions were initiated at 30 °C, with 5.0 mL of substrate constituted by an oil emulsion (25% v/v) in polyvinyl alcohol (2% v/v), 5 mL of buffer TRIS–HCl (0.1 mol/L), at pH 8.0. Enzyme pool (1 mL) was added in two ways: in its natural form or in its denaturated form (boiled at 100 °C). Reactions were carried out for 10 min, under orbital shake (45 cycles/min), and were stopped by adding 10 mL of a mixture containing acetone/ethanol (1:1). Yield of fatty acids released in the enzymatic

hydrolysis was determined by titration with NaOH 0.05 N using thymolphthalein (0.2%). Results were expressed IU (micromols of fatty acids released by min, per mL, under test conditions). The mixture obtained from enzymatic reaction was stored under refrigeration until further analysis.

Obtaining free fatty acids by chemical hydrolysis and their methyl esters derivatives

Fatty acids composition of oils studied in this work was first determined using conventional physical–chemical hydrolysis, for comparison purposes. The chemical hydrolysis was used as a control experiment in the present work. The methodology consisted on alkaline hydrolysis, followed by methylation of the free fatty acids obtained, and analysis of fatty acids methyl esters profile by gas chromatography, and was accomplished as previously described [18], with minor modifications, as following described. An amount of 10 mg of oil was dissolved in 100 μ L of a solution containing (1:1 v/v) ethanol (95%) and potassium hydroxide (1 mol/L). After vigorous shaking for 10 s, the oil was hydrolyzed for 5 min in a microwave apparatus (Panasonic Piccolo) (80 W power). The mixtures obtained after hydrolysis were cooled. Then, 400 μ L of HCl 20%, 4 μ g NaCl, and 600 μ L of ethyl acetate were added to the mixtures. After vigorous shaking (10 s) mixtures were left at rest for 5 min. Then, 300 μ L of the organic layer were removed, dried by evaporation, to yield the free fatty acids separated from the mixture [19]. Free fatty acids were methylated with 100 μ L of a solution of BF_3 in methanol (14%) and heated for 10 min in water bath at 80 °C. Then were diluted with 400 μ L of methanol and stored for further analysis (EN 14103, 2003). After this procedure, 1 μ L of the resulting solution of samples from chemical hydrolysis was separated for injection into the gas chromatograph.

Processing fatty acids from enzymatic hydrolysis

Samples containing fatty acids from enzymatic hydrolysis were extracted and methylated as previously described for samples from chemical hydrolysis. However, prior to gas chromatography analysis, 100 μ L of each sample was mixed with an internal standard constituted by methyl pentadecanoate ($\text{C}_{15:0}$; 9.2 mg/mL). After agitation, 1 μ L of this solution was separated for injection into the gas chromatograph.

Analysis of fatty acids profile by gas chromatography

All hydrolyzed and methylated oil samples were submitted to gas chromatography analysis in a HP7820A gas

chromatograph equipped with gas flame ionization detector. EZchrom Elite compact (Agilent) was used as data acquisition program. The column used was HP-INNO-WAX (Agilent) (15 m $\times$ 0.25 mm $\times$ 0.25 μ m) with the following gradient of temperature: 120 °C at 0 min, increasing 7 °C/min up to 220 °C. Injector split operated at 1/50 (250 °C) that was also detector temperature. Hydrogen gas was used as carrier, with flow rate of 2 mL/min. Injection volume was 1 μ L. The identification of peaks was accomplished by comparison of data obtained to retention times of a chromatogram, run in the same conditions, of a methylated fatty acids standard (SUPELCO37).

Statistical analysis

Lipase hydrolytic action over different oils were analyzed by ANOVA (one-way, 95% of confidence) and Tukey's multiple comparison tests. Correlation (Pearson r) between chemical and enzymatic fatty acids profile was also evaluated. Graphpad prisma 5.02 was used in data evaluation.

Results and discussion

The enzyme pool obtained from *C. gloeosporioides* presented lipolytic activity of 0.742 U/mL and a specific activity of 4.68 U/mg protein. These data reveals that most of the enzymes present in the enzyme pool have hydrolytic activities, as expected. The hydrolytic action of this lipolytic preparation was evaluated on eleven different vegetable oils and on a fat of animal origin (beef marrow). Results are presented in Table 1. The major hydrolytic efficiencies were observed for olive oil, reaching 100% of efficiency and hydrolytic activity of 18.0 IU, followed by soybeans oil (98.9%; 17.8 IU), and canola (88.4%; 15.9 IU) (Table 1). The lowest degrees of hydrolysis activity were detected when flaxseed (46.3%; 8.3 IU) and castor bean oil (36.8%; 6.6 IU) oils were used as enzyme substrates (Table 1).

A central feature for large scale use of lipase in oleochemical industry consists in the possibility of obtaining fatty acids from renewable raw materials like natural oils and fats [7] such as sunflower oil, coconut, corn, olive, rice bran, as well as tallow, and bone. The type of fatty acids obtained in such process depends on the natural oil composition. In order to rate the importance of the hydrolytic activity of *C. gloeosporioides* lipases, the fatty acids profile of the oils used in this study was determined. Oils were subjected to chemical hydrolysis followed by esterification with methanol and it showed high concentration of unsaturated long chains fatty acids (Table 2).

The major fatty acids present in oils were determined by comparison with retention times of fatty acids present in a

Table 1 Hydrolytic activity of lipase from *C. gloeosporioides* on different substrates

Oil	% de hydrolysis	Hydrolytic activity (UI/mL enzymatic preparation) ¹
Olive	100.0 ± 3.6	18.0 ± 0.7 ^a
Soy	98.9 ± 6.6	17.8 ± 1.2 ^a
Canola	88.4 ± 13.8	15.9 ± 2.5 ^{ab}
Almond	88.4 ± 0.0	15.9 ± 0.3 ^{abc}
Beef marrow	84.2 ± 3.6	15.1 ± 0.7 ^{abcd}
Sunflower	84.2 ± 7.9	15.1 ± 1.4 ^{abcd}
Sesame	77.9 ± 3.6	14.0 ± 0.7 ^{bcde}
Peanut	71.6 ± 4.8	12.9 ± 0.7 ^{bcdef}
Garlic	71.6 ± 3.6	12.9 ± 0.9 ^{bcdefg}
Corn	65.3 ± 4.8	11.7 ± 0.9 ^{efg}
Flaxseed	46.3 ± 3.6	8.3 ± 0.7 ^h
Castor bean	36.8 ± 1.8	6.6 ± 0.3 ^h

¹Different letters represent a statistically significant difference with 95% confidence

commercial standard and compared quali and quantitatively with data on fatty oils profiles from oil sources profiled by the National Nutrient Database for Standard Reference from United States Department of Agriculture (USDA) for olive (reference number 04053), soybean (04044), canola (04582), sunflower (04506), sesame (04058), peanut (04042), corn (04518), and flaxseed (042231) oils [20]. This comparison is important to point out seasonal variations since oils contents can vary the fatty acids composition according to several phenomena such as genetic variety, climate issues during cultivation, harvesting time, and extraction process.

The garlic oil utilized in this study contained the same composition of major fatty acids (linoleic, oleic, and palmitic) of the garlic oil (reference number 11215) described by USDA [20], however in different order of majority (linoleic > palmitic > oleic). It was also observed for the almond oil studied contained the fatty acids linoleic, oleic, and palmitic, in different concentrations than those described for the same oil (reference number 04529) in USDA database (oleic > linoleic > palmitic). Almond oil profile found in this work corroborates data described for oil of almond from Argentina [21].

The major fatty acids of beef marrow were oleic (57.16%) and palmitic acid (16.02%), a result similar to that was found for the same animal fat [22]. Linoleic, oleic, and palmitic acids, in that order, were the acids in greater concentration in soybean (52.41; 23.17; 10.81%), sunflower (57.64; 28.16; 6.82%), sesame (41.57; 41.13; 9.28%), corn (46.62; 32.72; 12.88%), almond (49.3; 26.05; 10.63%), and garlic (38.47; 21.07; 8.30%) oils as shown in Table 2.

Chromatographic profiles of fatty acids esters from the oils hydrolyzed with *C. gloeosporioides* lipase were compared to the esters profiles of the same samples when

submitted to chemical hydrolysis. Chemical hydrolysis was used here as a control experiment.

The effectiveness of the enzymatic reaction was therefore assured. Long chain fatty acids (comprising more than 12 carbon atoms) were the most abundant ones detected after enzymatic hydrolysis, the same results obtained using chemical hydrolysis (Table 3). Castor bean oil showed the highest concentration of fatty acids with long carbonic chain (99%), followed by canola (99.7%), and olive (99.7%) oils. Enzyme hydrolytic action on all oils generated primarily unsaturated fatty acids (Table 3). Castor bean oil (92.7%), followed by canola (87.3%), sunflower (83.8%), and flaxseed (83.1%) oils generated the highest yields of these fatty acids (Table 3).

Many different foodstuffs have a varied composition of beneficial fatty acids but meat derivatives and dairy products have high saturated fatty acids compositions [23]. The beef marrow, though of animal origin, presented, after enzymatic hydrolysis, a profile with high percentage of unsaturated chain fatty acids, generating around 37% of oleic acid (C18:1), as can be seen in Table 2, which is a good feature for an animal fat.

After determining the fatty acids present in the oils using conventional chemical hydrolysis, the same oils were hydrolyzed using *C. gloeosporioides* lipase, to test its hydrolytic potential. In general, there was a positive correlation between the two methods of hydrolysis (as shown by Pearson *r* in Table 2), showing that this lipase can be used without loss of normal characteristics of the oils (Table 2). Enzyme action resulted in obtaining hydrolysates rich in fatty acids essential for human consume. Hydrolysates rich in omega 9 (as oleic acid, C18:1) were predominant after enzymatic hydrolysis of olive (72.3%), canola (56.9%), and sesame (37.6%) oils. Hydrolyzed material rich in omega 6 (linoleic acid, C18:2) were

Table 2 Profile of fatty acids esters obtained after chemical hydrolysis (C) and enzymatic hydrolysis (E) with lipase of *C. gloeosporioides* over lipid substrates

Acids	Olive		Soy		Canola		Almond		Beef marrow		Sunflower	
	C	E	C	E	C	E	C	E	C	E	C	E
C 10:0	-	0.1 ± 0.0	-	-	-	0.4 ± 0.01	-	0.1 ± 0.0	-	-	-	0.6 ± 0.0
C 12:0	-	0.3 ± 0.0	-	-	-	0.4 ± 0.01	-	0.4 ± 0.01	-	-	-	0.3 ± 0.01
C 14:0	-	0.1 ± 0.0	-	-	-	-	0.1 ± 0.0	0.3 ± 0.01	-	1.17 ± 0.0	2.6 ± 0.05	0.3 ± 0.01
C 15:0	-	0.0	-	-	-	-	-	-	0.37 ± 0.0	7.1 ± 0.14	-	-
C 16:0 (palmitic acid)	10.73 ± 0.2	12.2 ± 0.2	10.81 ± 0.21	13.3 ± 0.26	4.91 ± 0.1	6.7 ± 0.13	10.63 ± 0.21	13.5 ± 0.26	16.02 ± 0.3	15.3 ± 0.3	6.82 ± 0.13	8.7 ± 0.17
C 16:1	0.74 ± 0.01	0.8 ± 0.0	-	-	0.33 ± 0.01	0.4 ± 0.01	0.14 ± 0.0	-	6.86 ± 0.1	5.7 ± 0.11	0.14 ± 0.0	-
C 17:0	-	0.1 ± 0.0	-	-	0.17 ± 0.0	-	-	-	0.70 ± 0.0	1.4 ± 0.03	-	-
C 18:0	3.55 ± 0.1	3.9 ± 0.1	4.04 ± 0.08	4.8 ± 0.09	2.75 ± 0.05	3.7 ± 0.07	4.18 ± 0.08	5.7 ± 0.11	4.61 ± 0.1	6.9 ± 0.13	3.60 ± 0.07	4.5 ± 0.09
C 18:1 (omega-9)	75.87 ± 1.5	72.3 ± 1.4	23.17 ± 0.45	22.4 ± 0.44	60.59 ± 1.18	56.9 ± 1.11	26.05 ± 0.51	32.9 ± 0.64	57.16 ± 1.1	36.8 ± 0.72	28.16 ± 0.55	26.6 ± 0.52
C 18:2 (omega-6)	5.98 ± 0.1	5.6 ± 0.1	52.41 ± 1.02	44.9 ± 0.88	18.87 ± 0.37	15.0 ± 0.29	49.30 ± 0.96	33.3 ± 0.65	1.94 ± 0.0	1.2 ± 0.02	57.64 ± 1.12	49.8 ± 0.97
C 18:3 (omega-3)	0.64 ± 0.01	0.5 ± 0.0	7.05 ± 0.014	5.8 ± 0.11	8.27 ± 0.16	6.3 ± 0.12	5.86 ± 0.12	2.7 ± 0.05	0.83 ± 0.0	-	0.29 ± 0.01	0.3 ± 0.01
C 20:0	-	0.4 ± 0.0	0.1 ± 0.0	0.7 ± 0.01	0.24 ± 0.0	0.5 ± 0.0	0.18 ± 0.0	0.7 ± 0.01	0.98 ± 0.0	1.5 ± 0.03	0.12 ± 0.0	0.4 ± 0.01
C 20:1	-	-	-	-	-	-	-	-	-	-	-	-
C 22:0	-	-	-	-	-	-	-	-	-	-	-	-
C 18:1-OH	-	-	-	-	-	-	-	-	-	-	-	-
Other	2.49 ± 0.0	3.8 ± 0.1	2.36 ± 0.05	7.5 ± 0.15	3.87 ± 0.08	10.0 ± 0.2	3.66 ± 0.07	10.3 ± 0.02	9.36 ± 0.2	18.7 ± 0.37	3.24 ± 0.06	8.4 ± 0.16
Identified	97.51	96.2	97.48	92.5	95.89	90.0	96.16	89.7	89.66	79.8	96.64	91.6
Total	100	100	100	100	100	100	100	100	100	100	100	100
Pearson (r)	-	0.999	-	0.996	-	0.998	-	0.943	-	0.974	-	0.998

Acids	Sesame		Peanut		Garlic		Corn		Flaxseed		Castor bean	
	C	E	C	E	C	E	C	E	C	E	C	E
C 10:0	-	-	-	-	-	-	-	-	-	-	-	-
C 12:0	-	0.4 ± 0.01	-	1.0 ± 0.02	0.16 ± 0.0	0.4 ± 0.01	-	0.5 ± 0.01	-	0.2 ± 0.01	-	0.1 ± 0.01
C 14:0	-	0.3 ± 0.01	-	0.2 ± 0.01	0.83 ± 0.02	0.2 ± 0.0	-	-	-	0.1 ± 0.01	-	-
C 15:0	-	-	-	-	0.36 ± 0.01	3.4 ± 0.07	-	-	-	-	-	-
C 16:0 (palmitic acid)	9.28 ± 0.18	12.2 ± 0.24	10.26 ± 0.2	13.5 ± 0.26	8.30 ± 0.16	11.6 ± 0.23	12.88 ± 0.25	16.0 ± 0.01	6.74 ± 0.13	8.5 ± 0.17	2.71 ± 0.05	1.7 ± 0.03
C 16:1	0.24 ± 0.0	0.4 ± 0.01	-	-	0.51 ± 0.01	-	0.20 ± 0.0	-	0.13 ± 0.01	0.1 ± 0.01	-	-
C 17:0	-	-	-	-	0.37 ± 0.01	-	-	-	-	0.1 ± 0.01	-	-
C 18:0	4.24 ± 0.08	6.6 ± 0.13	4.19 ± 0.08	6.3 ± 0.12	3.87 ± 0.08	5.0 ± 0.01	2.71 ± 0.05	3.6 ± 0.07	5.39 ± 0.11	6.2 ± 0.02	2.02 ± 0.04	1.5 ± 0.03
C 18:1 (omega-9)	41.13 ± 0.8	37.6 ± 0.73	43.29 ± 0.85	30.5 ± 0.6	21.07 ± 0.41	24.2 ± 0.47	32.72 ± 0.64	31.2 ± 0.01	19.23 ± 0.37	18.7 ± 0.37	5.96 ± 0.12	4.9 ± 0.1
C 18:2 (omega-6)	41.57 ± 0.81	28.4 ± 0.55	31.89 ± 0.62	32.5 ± 0.63	38.47 ± 0.75	39.5 ± 0.77	46.62 ± 0.01	38.9 ± 0.76	13.76 ± 0.27	12.5 ± 0.24	15.84 ± 0.31	13.4 ± 0.26
C 18:3 (omega-3)	0.50 ± 0.01	0.7 ± 0.01	0.23 ± 0.0	2.6 ± 0.05	4.00 ± 0.08	3.5 ± 0.02	1.35 ± 0.03	1.2 ± 0.02	52.62 ± 1.03	45.9 ± 0.9	1.18 ± 0.02	0.8 ± 0.02
C 20:0	0.12 ± 0.0	0.9 ± 0.02	-	1.1 ± 0.02	0.35 ± 0.01	0.8 ± 0.0	0.17 ± 0.01	0.5 ± 0.01	-	0.6 ± 0.01	1.80 ± 0.04	1.9 ± 0.04
C 20:1	-	-	1.77 ± 0.04	3.1 ± 0.06	-	-	-	-	-	-	6.35 ± 0.12	4.3 ± 0.08

Table 2 continued

Acids	Sesame		Peanut		Garlic		Corn		Flaxseed		Castor bean	
	C	E	C	E	C	E	C	E	C	E	C	E
C 22:0	-	-	3.79 ± 0.07	1.8 ± 0.04	-	-	-	-	-	-	-	0.2 ± 0.01
C 18:1-OH	-	-	1.72 ± 0.03	0.7 ± 0.01	-	-	-	-	-	-	-	44.0 ± 0.86
Other	2.91 ± 0.06	12.5 ± 0.24	2.86 ± 0.06	6.6 ± 0.13	21.71 ± 0.42	11.5 ± 0.22	3.35 ± 0.07	8.1 ± 0.16	2.13 ± 0.04	7.1 ± 0.14	64.15 ± 0.2	27.2 ± 0.53
Identified	96.96	87.5	89.86	93.4	77.94	87.7	96.48	91.9	97.87	92.9	27.70	72.8
Total	100	100	100	100	100	100	100	100	100	100	100	100
Pearson (r)		0.981		0.970		0.94		0.993		0.998		0.998

obtained mainly from sunflower (49.8%), soybean (44.9%), garlic (39.5%), and corn (38.9%) oils, while omega 3 representant (α -linolenic acid, C18:3) was more common in flaxseed oil (45.9%).

As already mentioned, there was a good equivalence of data obtained from chemical hydrolysis and enzymatic hydrolysis (Table 2), stressing the applicability of this enzyme in producing pools of fatty acids from vegetal substrates (Fig. 1). Interestingly, part of oleic (Fig. 1) and linoleic (Fig. 2) acids present in triglycerides were not converted into free fatty acids through the action of the enzyme pool from *C. gloeosporioides*. In almond and sesame oils come about the greatest differences between linoleic acid contents in the hydrolysate after chemical and enzymatic hydrolysis. For the oleic acid, the largest difference occurred with beef marrow and peanut oil. Therefore, enzyme ability to hydrolyse fatty acids is not related to the type of fatty acid, since the same fatty acid, in different substrates, had different rates of conversion into free fatty acids. Therefore, this feature may be related to the position that these fatty acids occupy in the triglyceride skeleton, as previously demonstrated with a sn-1,3 specific lipase from *Rhizopus delemar* [24].

There is a consensus on the efficiency and low environmental impact of use of enzymatic processes in the oleochemical industry for the production of fatty acids; however, implementation of such processes in replacement of chemical catalysis is difficult because of the high cost of enzymes production [11]. Studies towards more efficient enzymes and with a lower cost of production are essential to change this reality.

Colletotrichum gloeosporioides lipolytic potential was already reported in the literature [12, 14] and the present study broadens the scope of its applications. Hydrolytic activity of *C. gloeosporioides* enzymes pool studied in our work is high, compared to the literature. Lower hydrolysis yields on soybean oil using commercial lipases from *Candida rugosa* and *Thermomyces lanuginosa* (70 and 50% of hydrolysis, respectively) have been showed [25], which reinforce the importance of *C. gloeosporioides* lipase.

Enzyme source is decisive in the specificity of enzymes [8]. *C. gloeosporioides* shows to possess an enzyme pool that accept a wide range of substrates, corroborating early reports [12, 14]. The lipase from *C. gloeosporioides* was able to hydrolyze edible oil substrates, with a diverse composition of triglycerides (both in size and in saturation level).

Substrate preference is very common in enzymes. Rather than a limiting property, this selectivity improves the scope of applications for a given enzyme according to the substrates. As an example, the literature has been showed similar behavior when studying a commercial

Table 3 Analysis of the types of fatty acid esters found after hydrolysis of lipid substrates for lipase of *C. gloeosporioides*

Substrates	% C10–C12 medium	% C14–C22 long	% saturated fat acids	% unsaturated fat acids
Olive	0.3	99.7	17.7	82.3
Soy	0.8	99.2	21.1	78.9
Canola	0.3	99.7	12.7	87.3
Almond	0.6	99.4	23.2	76.8
Beef marrow	3.5	98.4	47.1	54.8
Sunflower	1.0	99.0	16.2	83.8
Sesame	0.4	99.6	23.3	76.7
Peanut	1.1	98.9	25.7	74.3
Garlic	0.4	99.6	24.1	75.9
Corn	0.6	99.4	22.5	77.5
Flaxseed	0.4	99.6	16.9	83.1
Castor bean	0.1	99.9	7.3	92.7

phospholipase from *Aspergillus oryzae* which gave greater hydrolysis yield on soybean oil than sunflower oil [26].

Other lipases from fungi have also shown diversity of substrates, such as *Rhizopus oryzae* [27]. The diversity of substrates of lipase metabolized by enzymes from the fungus *C. gloeosporioides* suggests that these oils participate in the metabolism of this microorganism as a source of carbon and energy. These enzymes play an important role in the utilization of carbon sources, facilitating microbial survival even in very unkind environments [28]. In this context, low cost oils could be applied as a source of carbon in lipase production by *C. gloeosporioides*, aiming to reduce the concentration of traditional components of means of cultivation, which are more costly. The bioconversion of agriculture waste, for example, in the production of lipase and other products will be a priority within biotechnology in the next years [11].

The results on fatty acid compositions of the oils herein studied demonstrated the potential of the enzyme pool utilized to produce concentrates containing high amounts of essential fatty acids, important for human health. Omega 3 such as α -linolenic acid (C18:3) and omega 6 such as linoleic acid (C18:2) are essential fatty acid because mammals are unable to metabolically introduce double bonds at positions omega 3 and 6 of fatty acids carbonic chains [29]. Therefore, the source of these nutrients comes exclusively from food. Omega 9 (such as oleic acid C18:1) is also essential, but can be synthesized from reserves of omega 3 and 6. Action of *C. gloeosporioides* enzymes on selected substrates was effective in generating concentrates containing omega 9. The best substrates in this case were olive, canola (with good yields of hydrolysis), and sesame oils. Other authors also reported olive oils with high concentration of oleic acid after hydrolyzed by lipase, with good yields [5].

Fig. 1 Analysis of the concentration of C18:1 (omega 9, oleic acid) (%) generated by chemical (dark gray) and enzymatic (light gray) hydrolysis using lipase from *C. gloeosporioides*, in different oils. 1, Olive; 2, Soy; 3, Canola; 4, Almond; 5, Beef marrow; 6, Sunflower; 7, Sesame; 8, Peanut; 9, Garlic; 10, Corn; 11, Flaxseed; 12, Castor bean. Pearson $r = 0.9387$, 95% confidence

Fig. 2 Analysis of the concentration of C18:2 (Omega-6 linoleic acid) generated by chemical (dark gray) and enzymatic (light gray) hydrolysis using lipase from *C. gloeosporioides*, in different oils. 1, Olive; 2, Soy; 3, Canola; 4, Almond; 5, Beef marrow; 6, Sunflower; 7, Sesame; 8, Peanut; 9, Garlic; 10, Corn; 11, Flaxseed; 12, Castor bean. Pearson $r = 0.9656$, 95% confidence

In the present study it was observed that for the generation of omega 6 fatty acids, the best substrates were sunflower, soybean (with higher degrees of hydrolysis), garlic, and corn oils. For omega 3 production from enzymatic hydrolysis, the best substrate was flaxseed oil, on which the enzyme converted 50% of the substrate into α -linolenic acid.

Lipase of different microbial sources, plants or animals have different specificity for oils and fats hydrolysis [8]. They can be 'not specific', 'sn-1,3 specific' or 'sn-1,2 specific', depending on the amino acids that make up the active site. This opens opportunity for further studies with enzymes from *C. gloeosporioides* since specificity is a very important quality in enzymes, allowing the preparation of structured triglycerides, that have a huge market nowadays [30]. It is worth mentioning that the fatty acids in triglycerides that have not been converted into free fatty acids are also of great importance in the food industry, as a food additive. This is because the fatty acids in the form of acylglycerols (monoacyl and diglycerides) are considered equivalent by the human body as well as the free form and, unlike these, have more resistance to oxidative deterioration [31].

In conclusion, the lipase from *C. gloeosporioides* proved to be able to generate hydrolyzed rich in fatty acids from substrates of different origins, being particularly efficient on olive and soybean oils. In addition, the enzyme was very active in the hydrolysis of long chain and unsaturated fatty acids.

The lipase was particularly able to efficiently generate α -linolenic acid from flaxseed oil, linoleic acid from sunflower oil, soybean, garlic, and corn, and oleic acid from olive, canola, and sesame.

Studies on the specificity as well as immobilization of lipase from *C. gloeosporioides* can help in increasing its income from hydrolysis as well as to direct the best substrates for the production of free fatty acids for industrial use.

Acknowledgements The authors acknowledge grants and scholarships from the Brazilian Funding Agencies CAPES and CNPq. All authors have no conflict of interest with any of the funding sources.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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