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RESEARCH ARTICLE

Effect of Various Oils on the Production of Lipase by *Rhodotorula mucilaginosa* and *Yarrowia lipolytica* Isolated from Spoiled Labneh

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ABSTRACT

The content of the medium is very important in the production of lipase enzyme. Microbial lipase production is carried out by adding carbon source to the compounds in the minimal medium. The use of olive oil is quite common in lipase production medium. In this study, it was aimed to increase lipase production by adding different oils instead of olive oil to the reference lipase production medium. For this purpose, corn oil, sunflower seed oil, walnut oil, hazelnut oil, safflower oil, avocado oil, organic sunflower seed oil, organic canola seed oil, organic flaxseed oil, organic soybean oil, pumpkin seed oil, black cumin oil, St. John's wort oil and sesame oil were evaluated in terms of lipase production. *Rhodotorula mucilaginosa* and *Yarrowia lipolytica* strains, which were isolated, purified, and identified, and which caused spoilage in labneh were studied in lipase production. When olive oil was used in lipase production, the lipase activity of *R. mucilaginosa* was determined as 7.50 U/ml, and that of *Y. lipolytica* was determined as 8.09 U/ml. It was determined that the lipase activity of *R. mucilaginosa* was increased by organic linseed oil as 10.67 U/ml, St. John's Wort oil as 10.17 U/ml, and organic sunflower oil as 9.67 U/ml, respectively. It was determined that the lipase activity of *Y. lipolytica* was increased by St. John's Wort oil as 13.50 U/ml, organic linseed oil as 10.84 U/ml, and organic sunflower oil as 10.33 U/ml, respectively. It was also observed that black cumin oil inhibited the growth of both yeast strains.

Keywords: Oils, Lipase production, *Rhodotorula mucilaginosa*, *Yarrowia lipolytica.*

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1. Introduction

Carboxylic ester hydrolases and lipases are known as lipolytic enzymes. The main difference between lipases (EC 3.1.1.3) and carboxylic esters is that lipases can act on insoluble esters. Carboxylic ester hydrolases act on soluble esters with relatively short fatty acid chains, whereas lipases act on substrates with long fatty acid chains. The substrates that lipases act on are emulsified substrates (Lock *et al.*, 2007). Unlike other esterases, lipases require an oil-water interface to be active (Yeşiloğlu, 2005).

Lipases have many industrial applications, including the production of lipid-derived flavors and fragrances, primarily in the food industry, solubilization of drugs, biocide production, biosensor modulation, biosurfactants, and treatment of olive mill wastewater in bioremediation (Benjamin & Pandey, 1998).

There are several approaches to alter enzyme-substrate selectivity and improve lipase production, one of which is the design of culture conditions to increase lipase production, in other words, medium engineering (Tsai *et al.*, 2006). Lipases are enzymes with significant specificity due to their substrate, functional group, regio-, stereo-, and chemo-selectivity (Dalla-Vecchia *et al.*, 2004; Castillo *et al.*, 2005; Yu *et al.*, 2005). By changing the medium components through medium engineering, it is possible to produce lipases with different specificities (Lanciotti *et al.*, 2005). Various components are present in the culture medium, some of which can be inducers for lipase production, while others can be suppressors. Examples of common inducers include long-chain fatty acids (such as olive oil) and organic nitrogen sources (Fickers *et al.*, 2004). Glucose and glycerol are among the common suppressors.

In this study, it was aimed to increase lipase production by using various oils other than olive oil added as a carbon source to the minimal medium medium, and the lipase production obtained with olive oil was compared with the effect of these oils on lipase production. For this purpose, corn oil, sunflower seed oil, walnut oil, hazelnut oil, safflower oil, avocado oil, organic sunflower seed oil, organic canola seed oil, organic flaxseed oil, organic soybean oil, pumpkin seed oil, black cumin oil, St. John's wort oil and sesame oil obtained from reliable companies in the market were evaluated in terms of lipase production. Yeast strains isolated and purified from spoiled labneh and identified as *Rhodotorula mucilaginosa* and *Yarrowia lipolytica* were used for lipase production.

2. Material and Method

2.1. Yeast Strains

Species identification of yeast strains isolated and purified from spoiled labneh was performed with universal primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3'). As a result, it was determined that the first of the two yeast species isolated and purified was *Rhodotorula mucilaginosa* and the other was *Yarrowia lipolytica*.

2.2. Lipase Production Medium

There are many media for lipase production. However, the media we used as a reference in our study contains 12 NaH_2PO_4, 2 KH_2PO_4, 0.3 $MgSO_4.7H_2O$, 0.25 $CaCl_2$, 0.005 $FeSO_4.7H_2O$, 0.015 $MnSO_4.7H_2O$, 0.03 $ZnSO_4.7H_2O$ in grams per liter (Hatzinikolaou *et al.*, 1996). After sterilization in an autoclave at 121 °C and 1 atm pressure for 15 minutes, lipase production was determined by adding various oils as a carbon source under sterile conditions when the temperature dropped below 65 °C.

2.3. Oils Used for Lipase Production

After the minimal medium content was autoclaved, various oils were used as the carbon source required for lipase production. In addition to the natural extra virgin olive oil frequently used in studies, corn oil, sunflower seed oil, walnut oil, hazelnut oil, safflower oil, avocado oil, organic sunflower seed oil, organic canola seed oil, organic flaxseed oil, organic soybean oil, pumpkin seed oil, black cumin oil, St. John's wort oil and sesame oil were evaluated in terms of lipase production. These oils were supplied from reliable companies in the market and the composition of the oils is shown in Table I.

2.4. Inoculation and Incubation

In order to inoculate microorganisms into lipase production medium, firstly stock cultures were prepared for *Rhodotorula mucilaginosa* and *Yarrowia lipolytica* strains. Pure cultures in Potato Dextrose Agar (PDA) medium were inoculated into potato dextrose broth medium (100 ml medium in 250 ml Erlen Meyers) and incubated at 30°C for 48 hours. After incubation, microorganism counts in liquid stock cultures was determined by petri plate method, and liquid stock cultures were adjusted for both yeast strains to be 25×10^7 yeast/ml for each strain. 1 ml of stock yeast cultures were inoculated into lipase production medium, and incubation was carried out at 30°C for 48 hours for each experimental study.

2.5. Determination of Dry Weight of Strains After Incubation

To determine the dry weights of *R. mucilaginosa* and *Y. lipolytica* strains, first, the production media were centrifuged at 5000 rpm for 15 minutes. After centrifugation, the upper part was used for determination of lipase activity, while the dry weight of the biomass that settled to the bottom was determined. The strains, completely free of medium and oil residues, were dried in an oven at 40°C for 7 days and then their dry weights were measured and graphed.

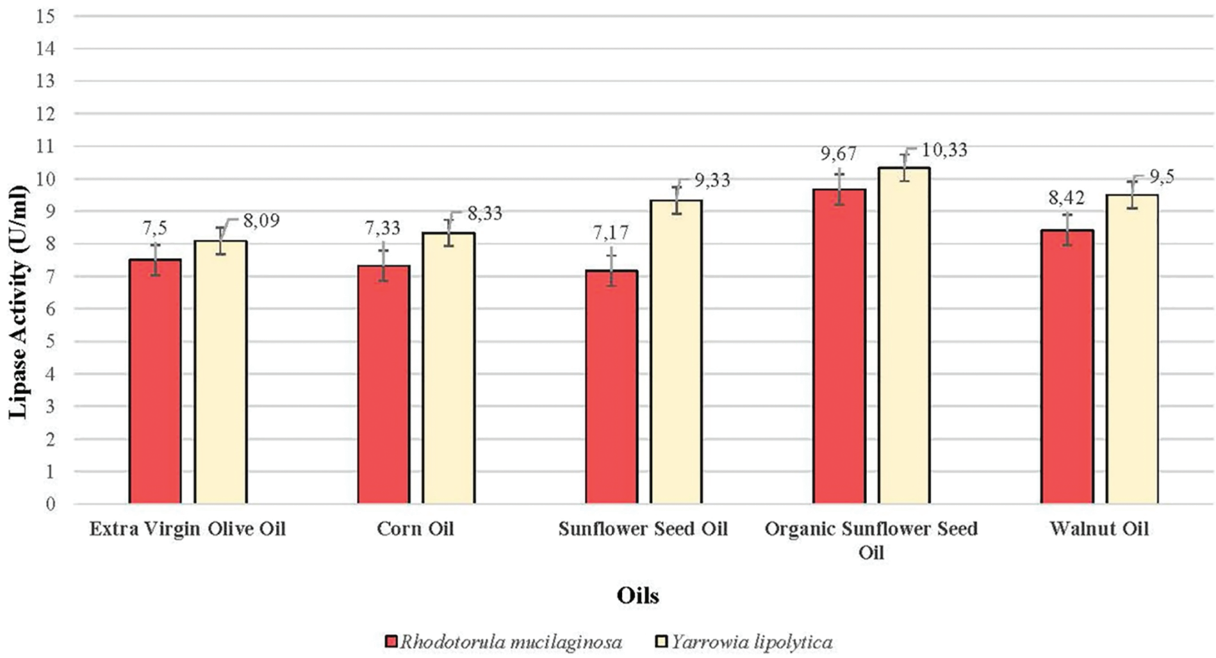
TABLE I: Composition of Oils Used for Lipase Production

Oils	Compositions of oils (gr/100gr, w/w)		
	Monounsaturated fats	Polyunsaturated fats	Saturated fats
Natural extra virgin olive oil	72	11	17
Corn oil	28	51	12
Sunflower seed oil	32	49	10
Organic sunflower seed oil	36.43	55	8.57
Walnut oil	22.8	63.3	9.1
Hazelnut oil	77	15	8
Safflower oil	21	69	10
Avocado oil	61.1	25.6	13.3
Organic canola seed oil	58.9	29.6	7.1
Organic flaxseed oil	22.86	70.00	7.14
Organic soybean oil	22.8	57.7	15.6
Pumpkin seed oil	34.29	47.86	17.14
St. John's wort oil	72.97	10.99	16.04
Sesame oil	40	43.57	15.71
Black cumin oil	24.29	58.57	16.43

2.6. Determination of Lipase Activity by Titration

After lipase production, the titration method was applied for determination of lipase activity (Sugihara *et al*., 1991). Lipase activity was performed by titration in four stages. In the first stage, 1 ml oil, 4.5 ml 50 mM pH 5.6 acetate buffer, 0.5 ml 0.1 M $CaCl_2$, and 1 ml filtrate were added. After incubation to obtain filtrate, culture media were centrifuged at 5000 rpm for 15 minutes. In the second stage, the media prepared in the first stage was incubated at 30°C for 30 minutes at 200 rpm cyclic shaking speed. This stage is the stage where the oil added to the media reacts with the lipase enzyme in the filtrate and fatty acids are released as a result. In order to terminate this reaction, 20 ml of 99% ethyl alcohol was added to the media after incubation in the third stage. The last and fourth stage is the stage of measuring lipase activity by titration. After the third stage, the medium was titrated to pH 10.5 with 50 mM KOH. Lipase activity was calculated by replacing the spent KOH in the formula, and the amount of fatty acid released was determined as unit activity: 50 × Spent KOH amount (ml)/30 = U/ml. In the determination of lipase activity, distilled water was used instead of the filtrate, and the obtained value was obtained by subtracting it from the measurements made with the filtrate. One lipase activity unit was defined as the activity that released 1 μmol of fatty acid under the above conditions.

(A)



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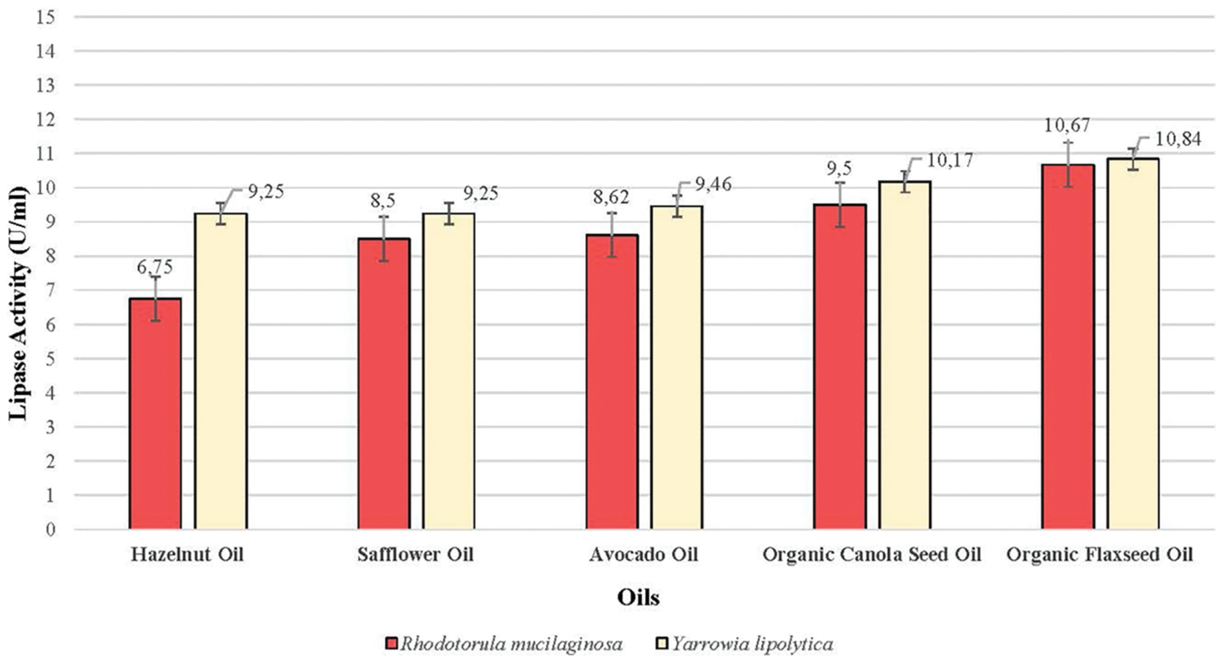

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(C)

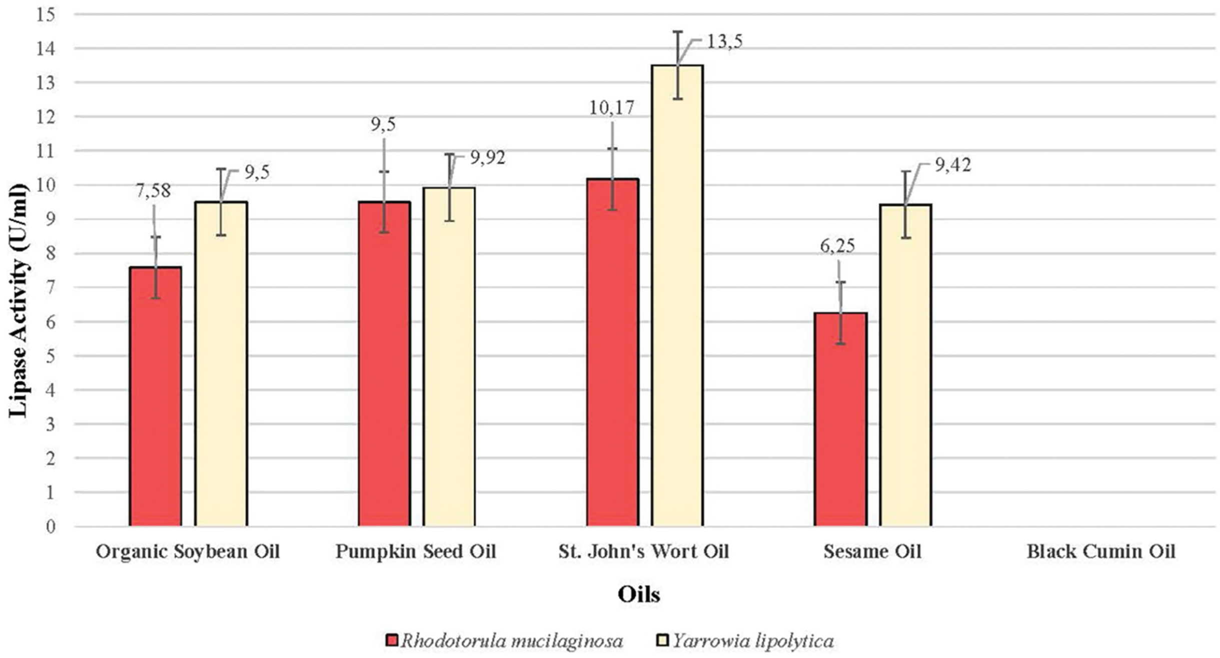

Fig. 1. Effect of various oils on lipase production by *R. mucilaginosa* and *Yarrowia lipolytica*: A) extra virgin olive oil, corn oil, sunflower seed oil, organic sunflower seed oil, walnut oil, B) hazelnut oil, safflower oil, avocado oil, organic canola seed oil, organic flaxseed oil, C) organic soybean oil, pumpkin seed oil, St. John's wort oil, sesame oil, black cumin oil.

3. Results

Lipase production of *Rhodotorula mucilaginosa* and *Yarrowia lipolytica* yeast strains isolated and identified from spoiled labneh was investigated with various oils added to the medium as carbon sources. First of all, the effect of olive oil, which is frequently used in lipase production, on lipase production was determined and calculated as 7.50 U/ml for *R. mucilaginosa* and 8.09 U/ml for *Y. lipolytica*, respectively. Starting from this reference point, various oils other than olive oil were added to the lipase production medium and the effect of these oils on lipase production was determined. According to the obtained results, it was determined that the lipase activity of *R. mucilaginosa* was increased by 10.67 U/ml of organic flaxseed oil, 10.17 U/ml of St. John's wort oil and 9.67 U/ml of organic sunflower oil, respectively, while the lipase activity of *Y. lipolytica* was increased by 13.50 U/ml of St. John's wort oil, 10.84 U/ml of organic linseed oil and 10.33 U/ml of organic sunflower oil, respectively. Lipase production results of all oils used in the study are given in Fig. 1. In addition, the effects of the oils used in the study on the growth of *R. mucilaginosa* and *Y. lipolytica* were also determined. After incubation, their dry weights were calculated and graphed (Fig. 2). After incubation in lipase production medium, the highest biomass increase was detected in media containing organic flaxseed (2.88), organic canola seed (2.02) and organic sunflower oils (1.73) per g/L for *R. mucilaginosa*. The highest biomass increase of *Y. lipolytica* occurred in media containing sesame oil (4.10), sunflower oil (3.96), and hazelnut oil (3.43) per g/L, respectively. The biomasses of *R. mucilaginosa* and *Y. lipolytica* in lipase production medium containing olive oil were determined as 1.01 and 2.19 g/L, respectively. It was observed that black cumin oil, one of the oils used in lipase production, seriously suppressed the growth of both yeast strains. As a result of this suppression, lipase production could not be measured.

4. Discussion

Yarrowia lipolytica is a dimorphic, aerobic and non-pathogenic yeast. The prominent features of this yeast include its ability to grow in hydrophobic environments, its relatively large phylogenetic distance compared to other yeasts, its unique genome structure and sharing common features with higher eukaryotes. The ability to grow in hydrophobic environments allows this yeast to use triglycerides and fatty acids as carbon sources in its metabolism (Gonçalves *et al*., 2014; Park & Ledesma-Amaro, 2023). *Y. lipolytica*, which has an important place in biotechnological applications, can cause food spoilage in some cases and the psychrotrophic nature of some strains can also be a factor in food spoilage at low temperatures. *Y. lipolytica* can cause a surface color change, which is an undesirable situation in cheeses (Zinjarde, 2014). It can be said that the industrial importance of *Yarrowia lipolytica* is based on lipase production. For this reason, there are many publications on *Y. lipolytica* lipase in the literature.

Rhodotorula mucilaginosa is considered one of the red yeasts. It owes this title to the formation of pinkish-red colonies due to the production of significant amounts of carotenoid pigments inside the cell (Li *et al*., 2022). *R. mucilaginosa* is a hyphaeless, unicellular, saprophytic eukaryote. They can be found widely in ecological environments, as well as in contaminated and polluted environments, and can even be found in the rhizosphere soil of plants as endophytes (Zhao *et al*., 2019; Saha & Seal, 2015). Although most strains of *R. mucilaginosa* are considered reliable, some have low pathogenicity and are considered opportunistic pathogens. However, just like *Y. lipolytica*, lipase production studies from *R. mucilaginosa* are also quite numerous.

Many media are used in lipase production studies from microbial sources. These media generally contain nutrients such as peptone, $MgSO_4{\bullet}7H_2O$, K_2HPO_4, KH_2PO_4, $CaCl_2$, $(NH4)_2SO_4$, $FeCl_3{\bullet}H_2O$, $FeSO_4{\bullet}7H_2O$, NaH_2PO_4, $NaNO_3$, $MnSO_4{\bullet}7H_2O$, $ZnSO_4{\bullet}7H_2O$ (Hatzinikolaou *et al*., 1996; Benjamin & Pandey, 1996; Iftikhar *et al*., 2008; Açıkel *et al*., 2011; Shafqat *et al*., 2015; Soleymani *et al*., 2017; Adetunji & Olaniran, 2018; Pramitasari & Ilmi, 2021). However, in most of these media, 1% or 2% olive oil is added to the basal medium for lipase production.

As mentioned above, there are many lipase production studies from *Y. lipolytica* and *R. mucilaginosa*. In a recent study, lipase production from *Yarrowia lipolytica* was studied and it was found an activity of 634.32 U/L (Santos *et al*., 2022). In another study, Darvishi *et al*. (2011) used olive oil in lipase production medium and detected 356 U/mL lipase activity from the *Y. lipolytica* strain that had been mutated. Yu *et al*. (2007) detected 5720 U/ml activity in the *Y. lipolytica* lipase they purified. In another study, maximum lipase activity was obtained as 13.0 U/mL with 70 g/L (1:1) peptone mixture and an initial pH of 5.0 (Gonçalves *et al*., 2013). Darvishi *et al*. (2009) investigated the effects of different oils on lipase production of *Y. lipolytica* DSM 3286 strain and determined lipase activity as 34.6 ± 0.1 U/mL in olive oil-containing medium. In their

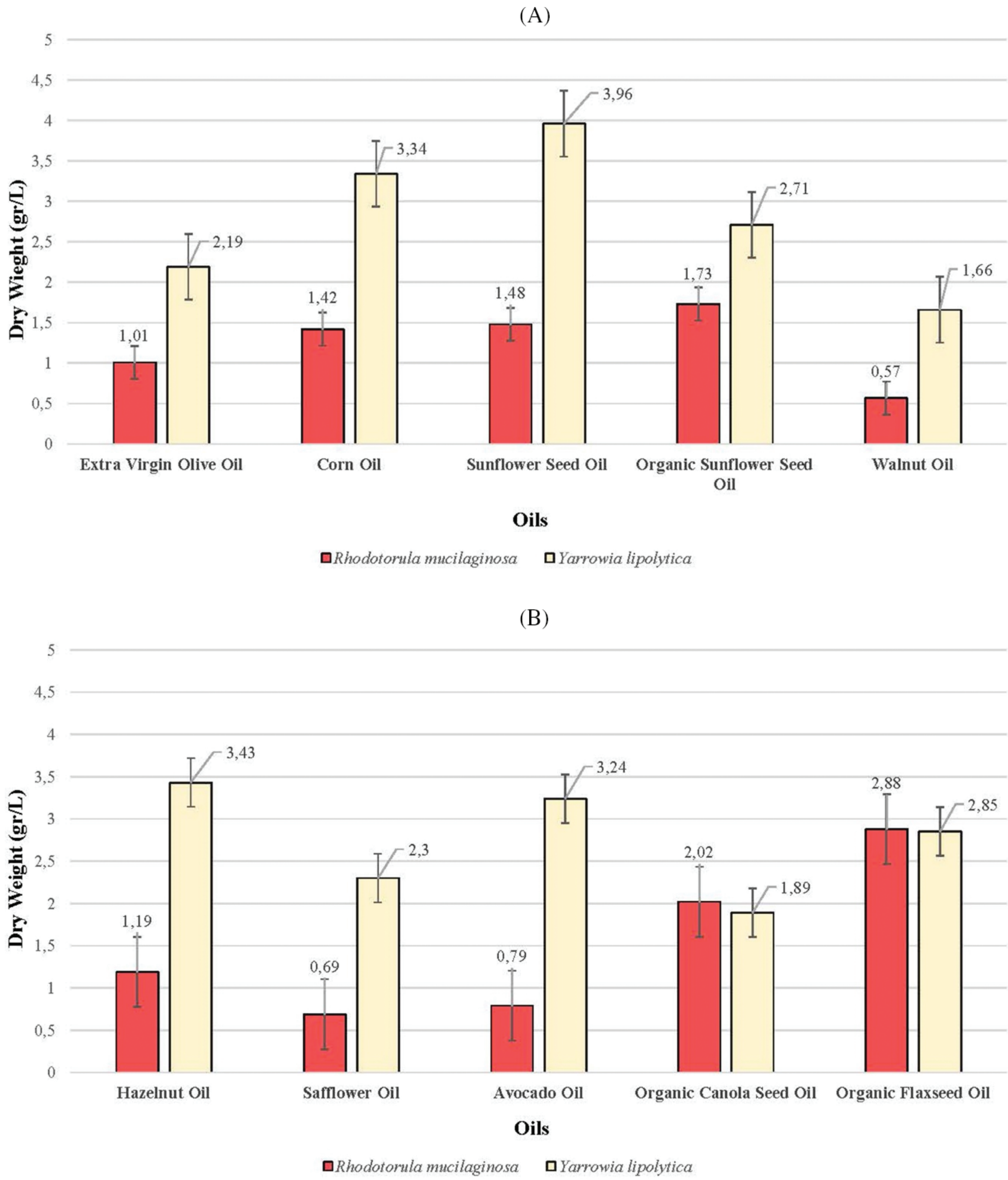

Fig. 2. Continued

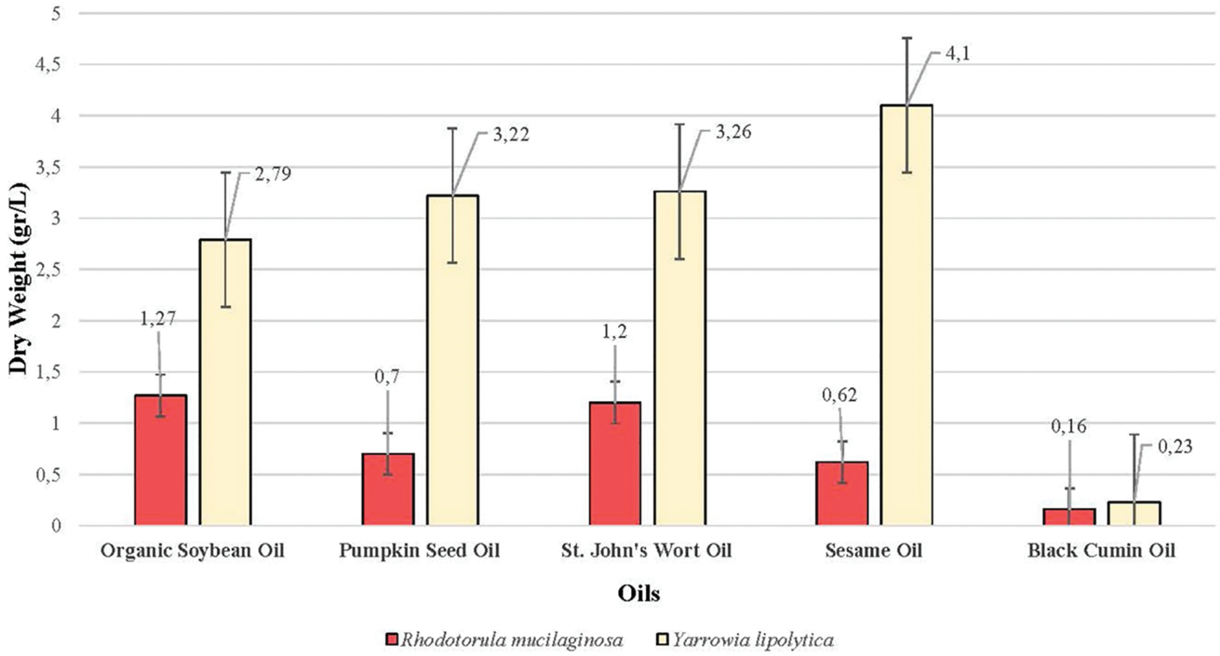

Fig. 2. Effect of various oils on the biomass of *R. mucilaginosa* and *Y. lipolytica* (Dry weight, gr/L): A) extra virgin olive oil, corn oil, sunflower seed oil, organic sunflower seed oil, walnut oil, B) hazelnut oil, safflower oil, avocado oil, organic canola seed oil, organic flaxseed oil, and C) organic soybean oil, pumpkin seed oil, St. John's wort oil, sesame oil, black cumin oil.

research, they used wheat germ oil, sweet almond oil, bitter almond oil, castor oil, sesame oil, soybean oil, olive oil, canola oil, walnut oil, and coconut oil. It appears that these high lipase production values are achieved either as a result of optimization of environmental conditions or mutation of the yeast. In a study conducted with *Rhodotorula mucilaginosa*, 3% molasses, and 1% olive oil were studied in the production environment, and lipase activity was determined as 10.3 U/ml (Hammamchı & Cihangir, 2017). In a study on *Rhodotorula mucilaginosa* MTCC 8737 strain, molasses was used in lipase production medium and maximum lipase activity was obtained as 72 U/ml (Potumarthi *et al*., 2008). Nuylert and Hongpattarakere (2013) determined the maximum lipase production of R. mucilaginosa P11I89 as 272.72 U/L.

When compared with studies with both *Y. lipolytica* and *R. mucilaginosa*, we can recommend the use of organic flaxseed oil instead of olive oil for *R. mucilaginosa* and St. John's Wort oil for *Y. lipolytica* in lipase production media.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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