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Waste Cooking Oil as Substrate for Single Cell Protein Production by Yeast *Yarrowia Lipolytica*

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***Abstract* – Cooking oils are widely used in food preparation. During cooking, harmful compounds are formed in oils, therefore utilization of used cooking oils (waste cooking oils) is limited. Single cell protein (SCP) is dietary protein, which can be produced from various protein-rich microorganisms that are capable of utilizing industrial by-products such as waste cooking oil (WCO). In this study the utilization of industrial WCO (obtained from local potato chips manufacturer) as a carbon source for single cell protein production by yeast *Yarrowia lipolytica* was assessed. The medium containing 27.5 g/L WCO and C/N ratio of 5–10 for batch fermentations was determined to be the optimal composition for SCP production. In this study, the highest reported *Yarrowia lipolytica* biomass concentration (57.37 g/L) was achieved when WCO was used as the main carbon source. Protein concentrations were relatively low (12.6 %), which also affected the final protein yield (7.23 g/L). The resulting biomass accumulated low concentrations of toxic malondialdehyde (MDA) (2.32 mg MDA/kg) compared to concentrations initially detected in the WCO itself (30.87 mg MDA/kg). To the best of the authors knowledge this is the first study to report on MDA decrease via microbial fermentations.**

***Keywords* – Agricultural residues; animal feed; low-cost substrate; malondialdehyde; microbial protein; TBARS test; used cooking oil**

1. INTRODUCTION

Vegetable oils are widely used in cooking around the world. Vegetable oils are used in households, diners and food production at industrial scale. During frying, harmful compounds are formed in the oils [1] and for this reason frying oils must be changed regularly. These used cooking oils are called waste cooking oils (WCO). Approximately 10 million tons of WCO are generated worldwide each year [1]. Large amounts of WCO are discarded without proper treatment. Theoretically, one litre of oil can contaminate up to 1 million litres of water [2] and WCO is characterized by very high COD and BOD values, so WCO treatment is very important to reduce the negative environmental impact of these residues. In addition, more than 80 % of vegetable oils are consumed in households [3], [4], therefore it also puts a significant strain on municipal wastewater treatment systems, clogging pipelines and causing water pollution if these oils are not treated properly. In order not to incur additional costs in treatment of the generated WCO, a good approach is to use these oils as a raw material in the

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production of new products, thus not only generating additional income, but also recycling WCO, which in turn reduces the negative impact on the environment. WCO is two to three times cheaper than vegetable oils, so its use offers significant economic benefits [5]. WCO is currently used as a feedstock in animal feeds, soap production, oleochemical industries and biodiesel production [4], [6], [7]. However, the use of WCO as an additive in animal feeds has been banned in the European Union since 2002, as there is a risk that harmful compounds in WCO will end up in animal products and therefore in human diets [8], [9]. This ban is justified, because during frying, vegetable oils hydrolyze, oxidize and polymerize. Oxidation results in the formation of hydroperoxides, aldehydes, carnations, carboxylic acids, alkanes and alkenes in the oil [10]. Particularly harmful are the various aldehydic end-products that occur in frying oils during heating. They can cause a variety of health problems, such as atherosclerosis [11], ischemic heart disease, peripheral vascular disease etc. [12]. WCO can be purified using existing technological solutions that are widely used to refine raw vegetable oils, such as treatment with activated earth [13], bleaching, degumming and deodorizing processes [1], [14]. By using these methods, it is possible to get rid of most oxidation products, however, some harmful compounds such as malondialdehyde remain in the oil even after the above-mentioned purification measures [1], [15].

During heating, polyunsaturated fatty acids in oils are oxidized to form hydroperoxides, which, as oxidation continues, form secondary oxidation products such as malondialdehyde (MDA) [16], [17]. MDA is able to form adducts with proteins and DNA molecules, so MDA is recognized as a potent mutagen in humans [16], [18]–[20]. Determination of MDA concentration in fish and meat products is often used to determine the degree of lipid oxidation [16]. MDA concentrations are usually determined using spectrophotometric measurement of the violet adduct formed by the reaction of MDA with 2-thiobarbituric acid (TBA) [16], [21], this test is called the TBARS test. This method is often criticized for the non-specificity of the reaction, as compounds in products such as browning reaction products, sugar degradation products and proteins also react with TBA [1], thus increasing the measurement error [16], [22], [23]. For this reason, the possibility of replacing the TBARS test with more specific high-performance liquid chromatography (HPLC) analytical techniques has been discussed [1], [16], [24]. Although more accurate, HPCL assays are much more expensive, time consuming, and complex than classical colorimetric assays [16]. Papastergiadis *et al*. [16] compared the TBARS assay with HPLC analysis coupled with fluorescence detection. In these studies, there was no significant difference between the TBARS test and HPLC analysis results for products such as oils, raw, uncooked meat and fish products, while processed products such as nuts, meat, fish products and cheese showed significantly higher MDA concentrations in the TBARS tests than in HPLC analysis [16]. This suggests that TBARS analysis is useful and can be considered accurate when MDA is measured in raw products and oils. In this study, MDA was measured using TBARS test in oils and in raw yeast biomass, which is similar in macromolecular composition to meat products. Permissible MDA concentrations in meat products are below 2.0 mg MDA/kg of sample [25]. Lipids can be characterized as not rancid if MDA concentration is below 1.5 mg/kg of oil, slightly rancid from 1.6 to 3.6 mg/kg, and rancid above 3.7 mg/kg [25]–[27].

The energy capacity of oils is about twice that of glucose, making WCO a very suitable substrate for culturing microorganisms that are able to use such substrates efficiently [28]. Due to its high energy capacity, low cost and wide availability, the use of WCO in microbiological fermentations is a promising alternative for the production of various value added products. In addition, the use of WCO in fermentations also ensures the treatment of

WCO, thus also reducing the strain on the environment. To date, the potential of WCO used as a substrate in microbiological fermentations has been reported for the production of single cell oils [2], [29]–[32] single cell proteins [32], [33], sophorolipids [13], carotene [4], erithryol [28], [34] lipase [32]–[36] and citric acid [28], [37].

Single cell proteins (SCP) are known as dietary single-cell microorganisms whose biomass or protein extracts are derived from pure or mixed microscopic algae, yeasts, mushrooms or bacterial cultures [38]. These microorganisms can be used as protein-rich foods or food ingredients or dietary supplements [39], but they are mainly used as food for human and animal consumption [40]. SCPs are a good alternative to replacing protein of agricultural origin, since SCP production is not characterized by high water consumption [41], it does not cover large areas of land, does not endanger environmental diversity [42], does not contribute to climate change and does not produce high greenhouse gas emissions [43], as it is the case with agriculture. To reduce the cost of production of SCP, it is essential to use biodegradable agro-industrial by-products and wastes such as WCO as a source of nutrients for the cultivation of microorganisms. All potentially applicable by-products for production of single cell proteins and single cell oils have been reviewed by Spalvins *et al*. [52]–[54].

Although WCO has the potential to process microbiological fermentations, existing oil degradation products such as peroxides, hydroperoxides, aldehydes, and ketones in WCO can negatively affect the metabolism of microorganisms and reduce the yield of fermentation products [13]. Therefore, when using WCO as a substrate in the production of unicellular proteins, it is necessary to check the concentrations of undesired compounds. If WCO is fermented to produce SCP, there is a risk that MDA will accumulate in the biomass of the cultured microorganisms and thus in the final product itself. Therefore, the SCP produced by fermenting WCO needs to be tested for the accumulation of MDA.

Among the various groups of microorganisms, such as bacteria, yeasts, fungi, and microalgae, yeasts are highly effective for growth on a variety of substrates, including a wide variety of agro-industrial by-products [44]. Yeasts are able to use sugars, glycerol, acetate and ethanol as carbon sources [45], but far fewer microorganisms are able to effectively use hydrophobic carbon sources such as oils. Yeast from genera such as *Cryptococcus*, *Trichosporon*, *Rhodosporidium*, *Geotrichum* and *Yarrowia* are known to be able to use hydrophobic substrates [46]. *Yarrowia lipolytica* is a widely studied microorganism in this respect, as it is not only able to efficiently use oils as the main carbon source, but its metabolic pathways allow for the utilization of hydrophobic substrates such as fatty acids, alkanes and triglycerides [46]. Extracellular lipases secreted by the yeast cleave lipids to free fatty acids, which are then diffused into cells at a favourable concentration gradient or transported using active transporters [47], [48]. Hydrophobic substrates are generally insoluble in water and form layer on the surface of the medium, making them inaccessible to cultured microorganisms. *Yarrowia lipolytica* is able to produce extracellular emulsifiers that increase the miscibility of hydrophobic substrates and thus make the relevant nutrients available to yeast [45], [49]. For this reason, the ability of *Yarrowia lipolytica* to use untreated WCO was also tested in this study. In other studies, where WCO is used as a substrate, the oil is usually emulsified by either adding oil emulsifying polysorbates to the media or ultrasonicating the medium, however, this increases the total cost of the medium. *Yarrowia lipolytica* is considered safe for human consumption, is tolerant to contamination, low oxygen concentrations, heavy metals and has extensive tools for genetic modification [37].

This is not the first time when SCP production is attempted by using WCO as the main carbon source. Papanikolaou *et al*. [32] reported on simultaneous production of single cell oil, single cell protein and lipase from tallow by *Yarrowia lipolytica* [32], although

emulsifiers were used for the preparation of the medium. Yan *et al*. [33] reported on simultaneous SCP and lipase production by genetically engineered *Yarrowia lipolytica*, by using WCO as substrate [33], but this report lacked in-depth optimization of WCO medium, which is understandable since that was not the main focus of the study. To the best of authors' knowledge, this is the first study that focuses solely on SCP production by *Yarrowia lipolytica* when untreated WCO is used as the main carbon source.

2. MATERIALS AND METHODS

2.1. Waste Cooking Oil, Yarrowia Lipolytica

Waste cooking oil was kindly provided by the local potato chip manufacturer JSC "LATFOOD". For the preparation of chips, the company uses sunflower oil, therefore, to calculate the C/N ratios in the media, the proportion of fatty acids in sunflower oil was assumed to be as follows: palmitic acid 6.80 %, stearic acid 3.26 %, oleic acid 16.93 %, linoleic acid ($C_{18:2}$) 0.56 %, linoleic acid ($C_{18:3}$) 71.73 % [50].

Yarrowia lipolytica wild type, was kindly provided by Dr. biol. Vizma Nikolajeva from Microbial Strain Collection of Latvia, University of Latvia. The strain was cultured on malt extract agar plates at 28 °C for 1 week and then stored in fridge at 4 °C.

2.2. Medium Preparation

Three different oil samples were prepared for use in the cultivation experiments:

1. Refined sunflower oil (SFO) purchased from a local supermarket;
2. The second oil sample was prepared by treating the same store-bought sunflower oil by heating it at 170 °C for 48 hours (TSFO). The oil treatment in the laboratory was used according to the potato chip manufacturer's instructions on how often they change and at what temperature they heat the sunflower oil in their factory;
3. The aforementioned WCO an industrial sunflower oil (ISFO) provided by local potato chips manufacturer.

Three different control mediums were used in the culture experiments:

1. YEP medium consisted of yeast extract (10 g/L), peptone from meat (10 g/L), glucose (23.66 g/L, which is equivalent to 12.5 g/L of sunflower oil), sodium chloride (5 g/L). All components were dissolved in distilled water and autoclaved;
2. YNB medium consisted of yeast nitrogen base without amino acids and ammonium sulphate (1.7 g/L), sodium chloride (5 g/L), glucose (23.66 g/L), urea (1.39 g/L), for the C/N ratio of 15.
3. SFO and (4) TSFO mediums consisted of yeast nitrogen base without amino acids and ammonium sulphate (1.7 g/L), sodium chloride (5 g/L), corresponding SFO or TSFO oils (12.5 g/L), urea (1.39 g/L), for the C/N ratio of 15.

ISFO media consisted of yeast nitrogen base without amino acids and ammonium sulphate (1.7 g/L), sodium chloride (5 g/L), and ISFO oil concentrations and urea concentrations were changed to prepare media with different oil concentrations and C/N ratios.

The C/N ratios calculated for all media represent the mass ratio of carbon to nitrogen. For YNB, SFO, TSFO and ISFO media, all components except oils were dissolved in distilled water and filter sterilized using a 0.2 um filter. Oil samples were autoclaved and then added to the otherwise complete SFO, TSFO and ISFO mediums.

2.3. Cultivation Experiments

Pre-culture of *Yarrowia lipolytica* WT was prepared by introducing couple of colonies from agar plate in 50 ml flask containing 15 ml of liquid YEP medium. It was cultivated overnight under shaking at 200 rpm and 28 °C. 1 ml of overnight culture was centrifuged at 4000 rpm for 10 min, supernatant was decanted and cell pellet was resuspended in 1 ml of distilled sterile water, then centrifugation, removal of supernatant and resuspention in water was repeated so the most of the medium would be washed out. Cell concentration in the washed and resuspended sample was determined by counting the cells in hemocitometer and then adding appropriate volume so $1 \cdot 10^5$ cells/ml of medium would be added in each flask containing appropriate medium.

Cultivation experiments were performed in 50 ml flasks containing 15 ml of medium. Each control medium and the variants of the ISFO mediums were done in triplets. Cultivation was done under shaking at 200 rpm, 28 °C, for 5 days.

2.4. Biomass, Protein and MDA Analysis

Biomass analysis was performed every day using a gravimetric method following a modification by Sestric *et al*. [51] in which the biomass was washed with hexane to separate excess oil from the biomass.

Before determining the protein concentration, biomass samples were lysed by adding 1 ml of 2.5 % SDS 0.2 M NaOH mixture to each sample (~0.01–0.05 g), then 0.5 mm glass beads (Retsch) were added to the samples, the samples were vortexed for 5 minutes, then placed in a water bath at 100 °C for 5 min and then vortexed again for 30 seconds. Protein concentration in biomass was determined using *Total Protein Kit*, *Micro Lowry*, Peterson's Modification (Sigma). Spectrophotometric measurements were performed using a UV-Vis spectrophotometer *BioMate 160* (Thermo Scientific).

MDA concentrations were measured for both SFO, TSFO, ISFO oil samples, and also for cell biomass samples at days 1 and 4, for samples cultured using the optimal SFO, TSFO and ISFO mediums. MDA concentration was determined using a lipid peroxidation assay kit (Sigma). Colorimetric measurements were performed using a UV-Vis spectrophotometer *BioMate 160* (Thermo Scientific).

3. Results and Discussion

3.1. Comparison with Controls

Protein yields of *Yarrowia lipolytica* cultures growing on YEP medium were approximately 2-fold higher than all other mediums when the same carbon concentrations were used, be it from glucose or oil (Fig. 1). Due to the unknown exact composition of yeast extract and peptone, the C/N ratio for YEP medium is unknown, but due to the high concentrations of proteins and free amino acids in these ingredients it is safe to assume that it is significantly lower than the C/N 15 used in other mediums.

There were significant differences between the three types of medium that used oil as the main carbon source (SFO, TSFO, ISFO) (Fig. 1). The treated sunflower oil, lagged significantly behind the other two mediums during the first three days of cultivation, on the fourth day the protein yield levelled off (Fig. 1). The delay observed in TSFO medium at the start of cultivation may be due to the presence of volatile growth inhibitory compounds in the medium, which over time might be neutralized by the yeast or partial or complete evaporation

of these compounds. It is also possible that these compounds only inhibited the synthesis of lipase and emulsifying compounds, so the oil was not as readily available for the yeast as it was for the other mediums, in any case determination of the actual reason behind this observation is beyond the scope of this study. Interestingly such an inhibitory effect was not observed for the samples from ISFO medium, where oil was treated very similarly (Fig. 1). Only difference being that ISFO contained trace amounts of potato chips which might serve as carbon source in the first days of the cultivation and after that lipases and emulsifying compounds were synthesized in appropriate concentration for the yeast to switch the nutrient sources.

In the first three days, untreated sunflower oil medium (SFO) showed significantly faster protein accumulation than TSFO and ISFO media (Fig. 1), which indirectly indicates the effect of inhibitory compounds on *Yarrowia lipolytica* cultures in TSFO and ISFO.

For SFO, TSFO, ISFO and YNB mediums, the same ingredient base and C/N ratios were used to compare the effect of carbon source on protein production. The protein yield did not differ significantly from ISFO media (Fig. 1), from which it can be concluded that *Yarrowia lipolytica* is equally effective in using both WCO and glucose as a carbon source, which indicates the advantages of WCO, as it is not only significantly cheaper, but also has 1.89 times higher energy capacity than glucose.

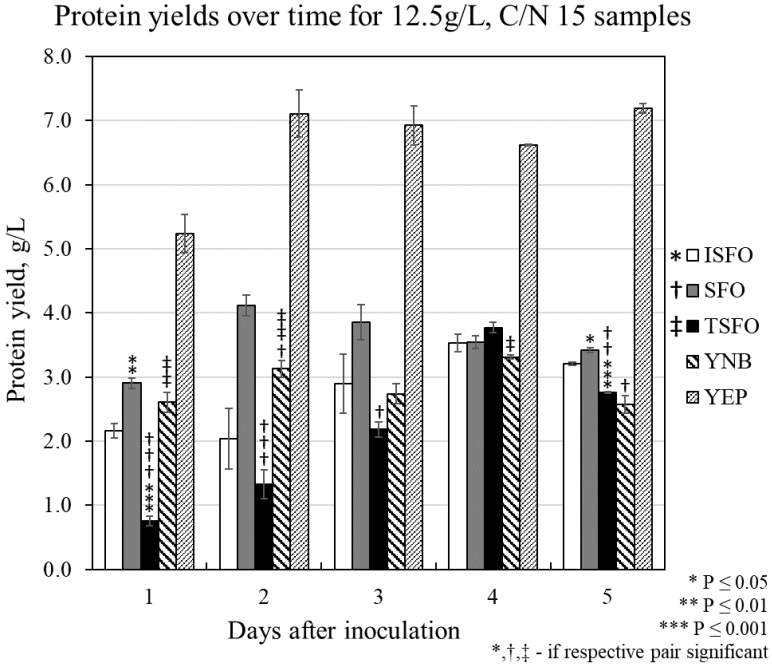

Fig. 1. Change in Yarrowia lipolytica protein yields over time when cultivated on control samples (YEP, YNB, SFO, TSFO) and test samples (ISFO). YEP – yeast extract peptone medium (optimal), containing 23.66 g/L glucose, which is equivalent to 12.5 g/L of sunflower oil, unknown C/N ratio due to peptone and yeast extract. YNB – yeast nitrogen base medium (minimal), containing 23.66 g/L glucose, C/N ratio 15. SFO, TSFO, ISFO – same composition as YNB medium only with glucose replaced by 12.5 g/L of untreated, treated or industrial (WCO) sunflower oil respectively, C/N ratio 15. Error bars – standard deviation. Significance test – *t*-Test (*,†,‡ represent the respective pairing if statistically different, e.g. if column has † on top of it, it means that SFO result is statistically different to result in respective column), all pairings with YEP results were significant (not shown). Cultivation was done in 50 ml flasks containing 15 ml medium, 200 rpm, 28 °C, triplets.

3.2. Optimal WCO Concentration

Comparing the protein yields in the mediums varying with the WCO concentrations, the highest protein yield (6.02 g/L) was reached on the fourth day at 27.5 g/L WCO concentration (Fig. 2). For the 20 g/L and 35 g/L WCO samples, the results were similar at 5.48 g/L and 5.26 g/L, respectively (Fig. 2). The direction of the curve for the 35 g/L samples suggests that it is possible that the protein yield could increase with prolonged cultivation, so it was tested by measuring the change in biomass concentration over six days (Fig. 3). The results showed that at sixth day culture in this medium also undergoes a death phase (Fig. 3), therefore a higher increase in protein yield cannot be expected if cultivations are prolonged past 4 days. Interestingly, higher protein yields in the first days of cultivation are achieved in mediums with lower WCO concentrations (Fig. 2, 12.5 g/L WCO on day 1, 20 g/L WCO on day 2 and 3), which suggests that yeast initiates exponential growth only when the oil is sufficiently emulsified or the ratio of synthesized extracellular lipases to WCO concentration in the medium has reached a certain limit. This observation is also supported by visual changes in the consistency of the media over time (Fig. 4). Overall, 27.5 g/L can be considered optimal WCO concentration for SCP production in batch fermentations, although no statistically significant differences were observed between the 20 g/L, 27.5 g/L, 35 g/L samples on the fourth or the fifth day (Fig. 2), therefore there is reason to believe that oil concentrations can be varied in the range of 20–35 g/L without significantly compromising the SCP yields.

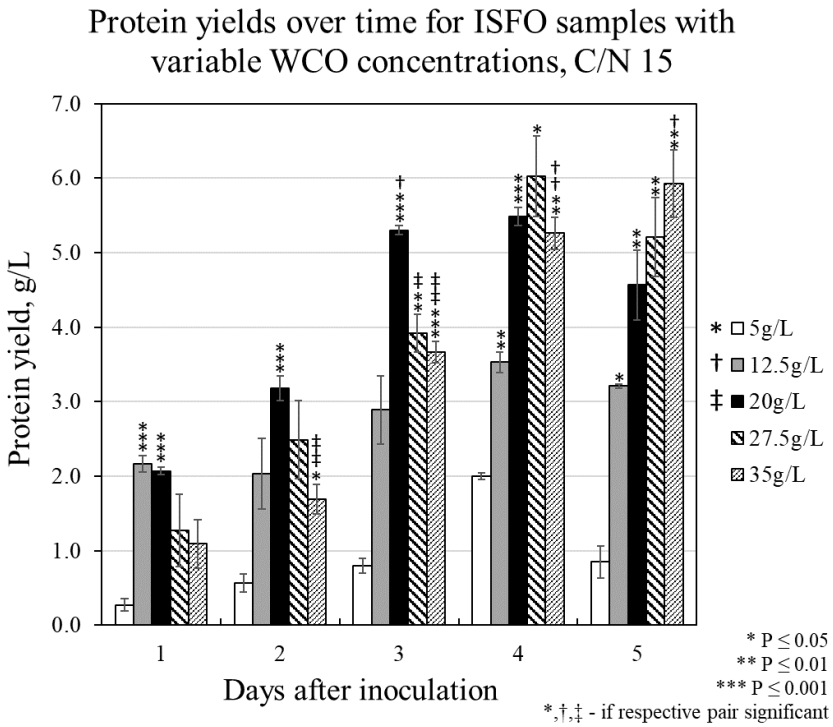

Fig. 2. Change in *Yarrowia lipolytica* protein yields over time when cultivated in mediums containing variable concentrations of industrial (WCO) sunflower oil. All mediums had C/N ratio of 15. Error bars – standard deviation. Significance test – *t*-Test (*,†,‡ represent the respective pairing if statistically different). All samples cultivated in 50 ml flasks containing 15 ml medium, 200 rpm, 28 °C, triplets.

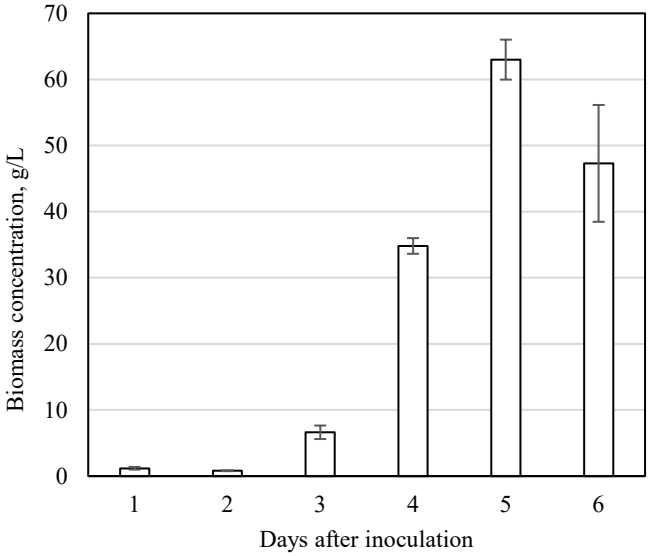

Fig. 3. Biomass concentration over time for ISFO medium containing 35g/L WCO, C/N ratio 15. Error bars – standard deviation. All samples cultivated in 50 ml flasks containing 15 ml medium, 200 rpm, 28 °C, triplets.

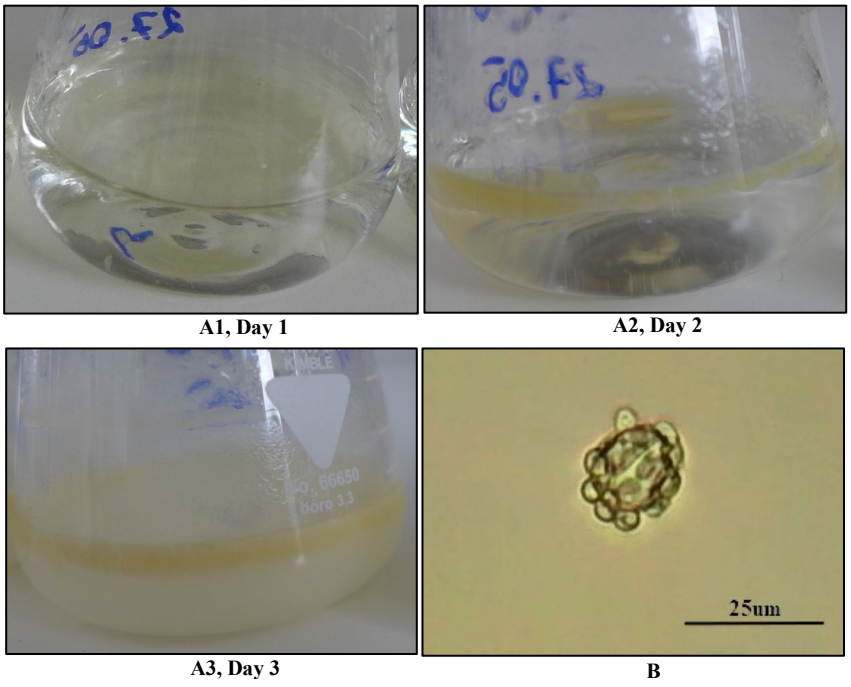

Fig. 4. A) WCO emulsification by *Yarrowia lipolytica* over time; B) *Yarrowia lipolytica* around an emulsified WCO droplet (400x magnification), second day after inoculation.

3.3. Optimal C/N Ratio

It is rational to propose that the media with the lowest C/N ratio are most likely to achieve the highest protein yields, as the media will have higher nitrogen concentration, which is a limiting element for protein synthesis. In this case, it was surprising that this was only partially confirmed, with the highest protein yield (4.30 g/L) reached on third day of medium with C/N 5, while medium with C/N 10 reached the second highest protein yield (4.14 g/L) on the fourth day (Fig. 5). Also on the third day, no statistically significant difference was observed between mediums with C/N ratios 5, 10 and 15 (Fig. 5). It is possible that higher protein yields could be reached if C/N ratios would be lower than 5, but since no significant difference was observed between C/N 5 and 10 ratio samples on any of the days, the most likely optimal C/N ratio for SCP production using this WCO is in the range of 5 to 10.

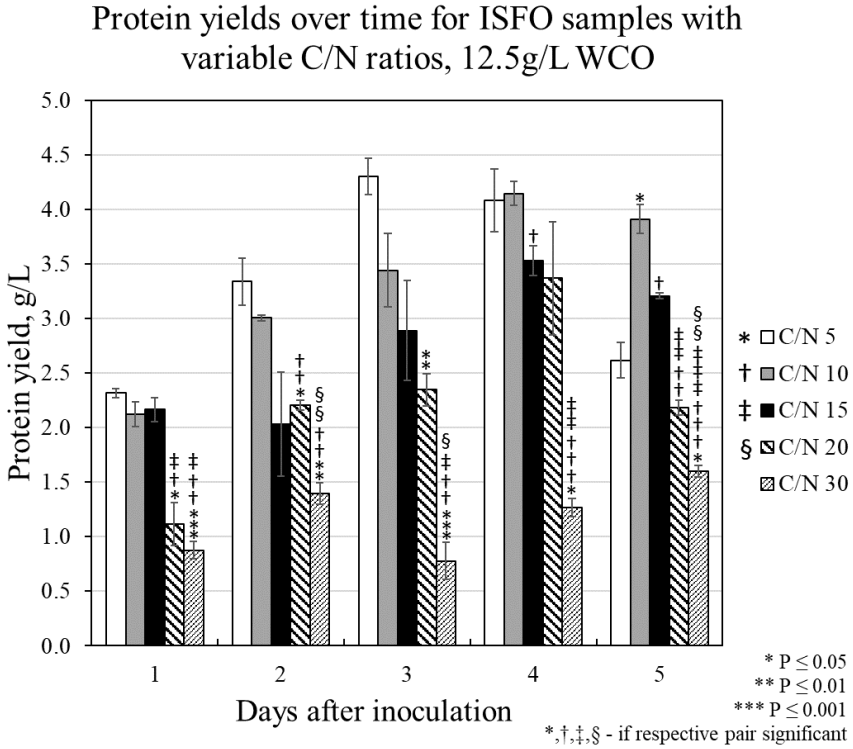

Fig. 5. Change in *Yarrowia lipolytica* protein yields over time when cultivated in mediums containing variable C/N ratios. All mediums had the same concentration of industrial (WCO) sunflower oil (12.5 g/L). Error bars – standard deviation. Significance test – *t*-Test (*,†,‡,§ represent the respective pairing if statistically different). All samples cultivated in 50 ml flasks containing 15 ml medium, 200 rpm, 28 °C, triplets.

3.4. Changes in MDA Concentrations

Initial MDA concentrations for TSFO and ISFO oil samples were high, 32.31 and 30.87 mg MDA/kg, respectively (Table 1). Any oils with MDA concentration above 3.7 mg/kg of product are considered rancid. As the highest protein yield was reached on the fourth day, MDA measurements were also performed on samples harvested on the fourth day using the optimal medium composition (27.5 g/L oil, C/N10). MDA analysis was also performed on

the respective samples on the first day of cultivation to compare changes in MDA concentration over time as the biomass increased exponentially. Yeast biomass cultivated on untreated sunflower oil (SFO) showed safe levels of MDA on the fourth day of cultivation, 0.14 mg MDA/kg of biomass (Table 2). For meat products, safe concentrations of MDA are considered to be <2.0 mg MDA/kg of product. Yeast biomass cultivated on both TSFO and ISFO oils exceeded this limit at 2.42 and 2.32 mg MDA/kg biomass, respectively (Table 2). Consequently, SCP obtained by cultivating yeast on WCO cannot be considered safe for use in animal feeds. Of course, SCP is usually fed in a mixture with other feed ingredients, thus reducing the total concentration of MDA in the feed, but this would not be recommended given the toxicity of MDA. The sharp decrease in MDA concentration in yeast biomass samples can be explained by the fact that part of MDA accumulated in yeast cells only partially and part of MDA remained in the medium, or MDA reacted with DNA and protein molecules in yeast cells. Adduct formation and toxic effects of MDA on yeast cells may also explain the marked delay in culture development observed in TSFO medium (Fig. 1), as significantly higher concentrations of MDA were detected in TSFO samples on the first day than in other samples (Table 2). As the biomass cultivated on WCO showed a significant reduction in MDA concentration, from 9.75 to 2.32 mg MDA/kg, it is reasonable to suggest that adapting the use of WCO substrate in larger volumes using a bioreactor and thus achieving significantly higher yeast biomass concentrations could reduce MDA to safe levels. To the best of the authors' knowledge this is the first study to report on MDA decrease via microbial fermentations. Further research is needed not only to optimize the production of various fermentation products when using WCO, but also to understand the biochemical mechanism that ensures the reduction of MDA concentration in the microbial biomass.

TABLE 1. MDA CONCENTRATIONS IN OIL SAMPLES

Oil sample	mg MDA/kg of sample
SFO	1.50
TSFO	32.31
ISFO	30.87

Note: SFO – untreated sunflower oil, TSFO – treated sunflower oil, ISFO – industrial (WCO) sunflower oil

TABLE 2. CHANGE IN MDA CONCENTRATIONS OVER TIME IN *YARROWIA LIPOLYTICA* BIOMASS

Medium	Day 1		Day 4	
	g biomass/L	mg MDA/kg	g biomass/L	mg MDA/kg
SFO	8.89	6.32	50.75	0.14
TSFO	2.56	18.02	52.22	2.42
ISFO	6.81	9.75	57.88	2.32

Note: Yeast cultivated in optimal mediums containing 27.5 of appropriate oil and C/N ratio 10

In this study, the highest reported *Yarrowia lipolytica* biomass concentration (57.37 g/L) was reached when cultivated on medium containing WCO as the main carbon source. Papanikolaou *et al*. [32] and Yan *et al*. [33] achieved 30.5 g/L and 17.9 g/L, respectively. The protein concentrations obtained in this study were relatively low for optimal ISFO medium (12.6 %), while Yan *et al*. [33] reported protein concentrations ranged from 45 to 54 %. Therefore, it can be concluded that by selecting and replacing wild type with better adapted mutants or genetically engineered strains, it is possible to significantly increase the *Yarrowia lipolytica* protein yields. In addition, as no significant differences were observed in the ability of *Yarrowia lipolytica* to efficiently utilize both glucose and WCO, fermentation scale-up to higher volume bioreactors with controlled pH and oxygen concentrations would

allow much higher biomass and protein concentrations to be achieved. By using WCO as a substrate in this way, it would be possible to achieve similar biomass concentrations as reported by other research groups. Yan *et al*. [33] reported on achieving a record high biomass concentration of 151.2 g/L by engineered *Yarrowia lipolytica* in a 10 L bioreactor using molasses as a main carbon source.

4. CONCLUSIONS

Waste cooking oil is a cheap and energy-dense by-product that can be used in microbiological fermentations to produce a range of value-added products, including single cell protein. *Yarrowia lipolytica* is able to use WCO effectively without the need to emulsify WCO prior fermentation with either emulsifiers or ultrasonification. Thus, the use of *Yarrowia lipolytica* can significantly reduce the cost of using WCO. Single cell proteins are microbial biomass that can be used as a protein-rich raw material for animal and human consumption. During the heating of WCO, a number of toxic compounds are formed, so when WCO is used as a substrate in the production of SCP, this study investigated the obtainable SCP yields and the accumulation of toxic compounds in the resulting microbial biomass.

In terms of protein yield, no significant differences were observed between carbon sources (glucose and industrial sunflower oil) from which it can be concluded that WCO from potato chip processing is a very promising and cheap carbon source for cultivating *Yarrowia lipolytica* with almost twice the energy capacity as glucose (1.89 times).

The medium containing 27.5 g/L WCO and C/N ratio of 5–10 for batch fermentations was determined to be the optimal composition for SCP production. In this study, the highest reported *Yarrowia lipolytica* biomass concentration (57.37 g/L) was achieved when WCO was used as the main carbon source. Protein concentrations were relatively low (12.6 %), which also affected the final protein yield (7.23 g/L). The resulting biomass accumulated low concentrations of toxic malondialdehyde (MDA) (2.32 mg MDA/kg) when compared to concentrations initially detected in the WCO itself (30.87 mg MDA/kg). If use of WCO in fermentations are further developed by reaching MDA concentrations below 2.0 mg/kg, the resulting SCP would be safe for use in animal feeds, which would provide significant economic benefits, while at the same time significantly reducing the negative environmental impacts by treating WCO.

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