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A statistical approach for the production of lipid, biomass, and phenolic from a newly isolated *Pichia Kudriavzevii* strain from Caspian Sea fish

Mahshid Taremie
M. Sc. of Food Science and Technology, Faculty Of Agricultural Engineering, Shahrood University Of Technology, Shahrood, Iran, mahshid.tarami@gmail.com
Hamidreza Samadlouie *
Assistant Professor of Food Science and Technology, Faculty of Agriculture Engineering, Shahrood University Of Technology, Shahrood, Iran, hsamadlouie@yahoo.com
Shahrokh Gharanjik
Assistant Professor of Plant Biotechnology, Faculty of Agricultural Engineering, Shahrood University of Technology, Shahrood, Iran, gharanjik@shahroodut.ac.ir

Abstract

Introduction: A wide variety of oleaginous yeast and their productions have been used as dietary supplements in food and feed productions. Fatty acid profiles and lipid of oleaginous yeast strains were highly influenced by physic-chemical conditions of the medium.

Materials and methods: *Pichia kudriavzevii* isolated from the gill of pike perch was capable enough to produce a high quantity of lipid and polyunsaturated fatty acids (PUFA).One factor at time was used to investigate the effect of various selected substrates on the growth and production of lipid by *Pichia kudriavzevii* in batch fermentation, subsequently, the key substrates were optimized for lipid, biomass, and phenolic components production by using response surface methodology.

Results: The results of RSM indicated that maximum lipid (28 percentage of dry weight biomass) and phenolic component (2.25 mg gallic acid /g of dry biomass) were achieved in media that had60 g/l glucose and 9 g/l protein resources. Dry weight biomass which had the highest content of lipid produced the most phenolic components with the highest antioxidant activity (50 % Inhibition). *Pichia kudriavzevii'* lipid mainly consisted of palmitic acid (21.7%), oleic acid (33.2%) and linoleic acid (23.2 %), pointing that these fatty acids could be considered a reliable source of biodiesel production.

Discussion and conclusion: Discussion and Conclusion: The maximum content of phenolic components and lipid production were obtained in the same medium. To minimize the lipid oxidation, yeast was stimulated for high phenolic components production which had the highest antioxidant activity to prevent the accumulated lipid from oxidation. Overall, temperature reduction by varying methods had a significant impact on fatty acids profile of *Pichia kudriavzevii* lipid so that it could be regarded not only as biodiesel but also PUFAs sources.

Key words: Antioxidant Activity**,** Oleaginous Yeast**,** Lipid, Phenolic, *Pichia Kudriavzevii*, Polyunsaturated Fatty Acids, Response Surface Methodology.

*Corresponding Author

Introduction

Some kinds of microorganisms such as yeasts, bacteria, and fungi have a great ability to turn carbon sources like carbohydrates and other similar substrates into lipid. These microorganisms named as oleaginous microorganisms are able to produce high content of lipid in their biomass which is more than 20% of cell dry weight. Many practical ways were used to induce oleaginous yeast for more lipid accumulation. Metabolic engineering as well as optimization of physical and chemical medium composition could be considered the key approaches to induce oleaginous microorganism for more lipid production (1-3). Among oleaginous microorganisms, oleaginous yeasts had more advantages than other species. The accessibility of suitable oleaginous candidates, less fermentation time and intricate scale-up are features of these oleaginous species (4).Yeast lipids are very similar to the vegetable lipids so they can be generally known as promising sources of biodiesel production (5,6). Research on biodiesel production as promising sources of energy has been recently developed because of the possibility of fossil fuels depletion.Overall industry's target is to substitute novel resources of fuel like biodiesel with fossil fuels (7). However, PUFAs as functional fatty acids which have many crucial roles in brain functions cannot be synthesized in the human body (8), so finding a good and reliable source of these fatty acids have posed serious problem to costumers. After years of toil, oleaginous yeasts were discovered to produce lipid as a reliable source of biodiesel. On the other hand, PUFA usually produced by fungi and bacteria are more valuable than biodiesel (9).

Thus, in this study, oleaginous yeast was isolated from Caspian Sea fish and then it was identified by using the molecular method. Optimal media conditions for lipid and biomass production were screened by one-factor-at-a-time method and then these products and phenolic components were optimized by using response surface methodology. Finally, optimal condition for lipid production achieved by the optimization method was far evaluated by various temperature reduction rates to identify the effect of temperatures reduction on the fatty acid profiles of this new isolated oleaginous yeast.

Materials and Methods

Isolation of Oleaginous Yeast: The gill of pike perch species from the Caspian Sea in Iran was selected to isolate the new oleaginous yeast. The surface of fish body was washed with alcohol 70%. Then, 1 g of fish gills was removed by a scalpel sterile blade. The samples were incubated at 25ºC in Yeast Glucose Chloramphenicol (YGC). The single-yeast-colony was isolated and kept in the refrigerator at 4ºC (10).

Medium Preparation: Seed culture (100 ml) containing glucose mono-hydrate (30 g/liter) and yeast extract (10 g/liter) was prepared in a 500 ml Erlenmeyer and then sterilized at 121ºC for 15 min. Afterward, the yeasts were inoculated at 26ºC and 170 rpm for 4 days. Then, 3-10% of seed cultures were added to the fermentation medium. $(NH_4)SO_4$ (2 g/liter), KH_2PO_4(3 g/liter), and $MgSO_4$(1.5 g/liter) were used as mineral elements in fermentation media(11); moreover, carbon and nitrogen sources, as well as pH, were examined as variables. Fermentation medium was incubated at 28ºC and 180 rpm for 4 days.

One-factor-at-a-time Method: One factor at the time was used to select the key substrates among the various substrates which had a substantial impact on lipid and biomass production. Carbon sources (Xylose, sucrose, glucose, and glycerol), pH (4, 6, and 8) and protein sources (Corn, soybean, Peptone and yeast extract) were applied as variable factors. Also, 30% glycerol, 50 g/l other carbon sources as

well as 5 g/l yeast extract as protein source were utilized. Moreover, pH 8, temperature 27 ˚c, and stirrer speed of 170 rpm and fermentation time of 4 days were also applied.

Analytical Methods: The dry weight of biomass, total lipids, the profile of fatty acids, phenolic component, and free radical-scavenging activity were determined as described in previous studies (11–15).

Identification of Yeast Strain: For the identification of the yeast species that produces the highest amount of lipid, yeast was first incubated in seed culture medium for 48 h; and then DNA was extracted using the DNA extraction kit (K721, Thermo, USA).The rDNA region NTS 2 (Ribosomal Non transcribed Spacer 2) was amplified using the forward (5'-GCATATCAATAAGCGGAGGAAAAG-3)and reverse primers (5'GGTCCGTGTTTCAAGACGG-3') in a thermocycler (16). The PCR product (680 base pairs (bp) length nucleotides) was extracted from gel and sent to the Takapu-Zist company sequencing. The obtained sequences were blasted in Gen Bank using NCBI website and the phylogenetic tree was drawn by using the Mega5 software.

Effect of Temperature Reduction on Fatty Acid Profiles: The effect of temperature reduction from 28 °C to 15 °C, which fell at 5 °C in each step, was investigated on *Pichia kudriavzevii* fatty acid profile. In sample 1, the temperature at the beginning of the fermentation process was 28 °C and at the end of the growth phase until the third day decreased to 24 °C and then reduced to 20 °C until the end of the fermentation process. In sample 2, the temperature was constant at 21 °C. In sample 3, the temperature at the beginning of the fermentation process was 21 °C until the growth stoppage, after that the temperature decreased to 15 °C until the fourth day. For further investigation, sample 4 was run; at the start of fermentation, the temperature was 25 °C and with stopping of the growth, the temperature decreased to 21 °C until third day, then the temperature stood at 15 °C.

Results

Identification of Isolated Yeast: The similarity of the NTS 2 sequence of isolated yeast with the other closely related species was obtained from Gen Bank. According to the phylogenetic tree (Fig. 1), the isolated strain was highly similar to the *Pichia kudriavzevii* species.

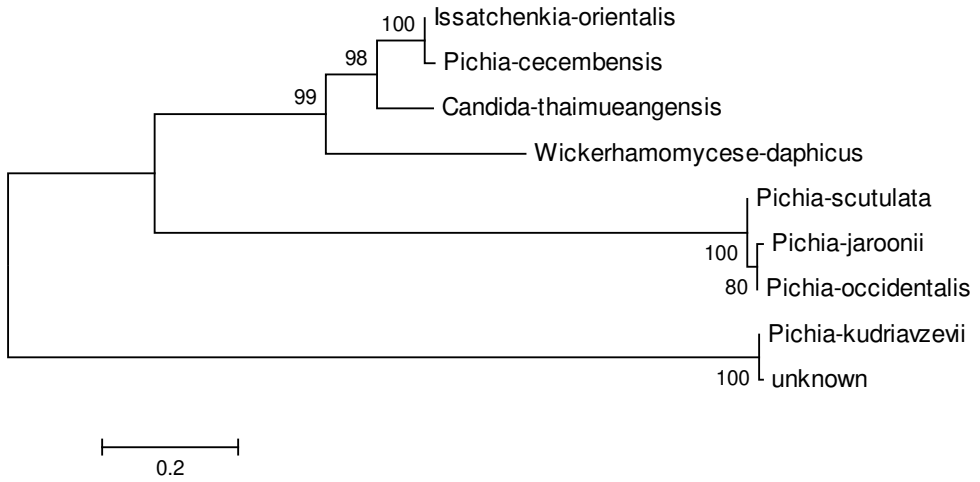

Fig. 1- The phylogenetic tree of the isolated yeast strain (unknown) with 8 similar species based on nearest neighbor interchange analysis of the NTS2 region (The bootstrap level: 1000 pseudo-replications)

Effects of pH on Biomass and Lipid Production by *Pichia Kudriavzevii*: The effect of pH as a chemical factor on lipid and biomass production was proved (17). As can be seen in Table 1, the optimal pH for lipid accumulation was higher than that of pH for biomass production. The highest lipid accumulation was observed at pH 8.0, while the highest dry weight biomass was achieved at pH 4 and 6. Thus, pH 8 was used for the rest of this experiment.

Table 1- The Effects of pH on the Lipid and Dry Weight Biomass of *Pichia Kudriavzevii*

pH	dry weight biomass	lipid
4	0.76±0.12	10.2±0.9
6	0.76±0.09	11.5±1
8	0.7±0.1	14.2±0.7

Effect of Different Carbon Sources on Lipid and Biomass Production: The oleaginous yeast strains are able to utilize a wide range of carbon sources such as hexoses and pentoses such as galactose, xylose, mannose, and cellobiose as well as glycerol sources (22, 23). Isolated yeast was cultivated in four different carbon resources and harvested at the middle of the stationary growth phase according to optical density (not shown). *Pichia kudriavzevii* was able to grow on each tested carbon sources. Among carbon substrates, glucose strongly influenced the cell biomass (Table 2).

According to Table 2, the highest lipid accumulation was obtained in glucose and sucrose substrates. So these carbon sources were used in the remaining of this experiment.

Table 2- The Effects of Various Carbon Resources on the Lipid and Dry Weight Biomass of *Pichia Kudriavzevii*

Carbon sources	Dry weight Biomass (%medium)	Lipid (% of dry weight biomass)
glycerol	1.28±0.1	14.7±0.8
sucrose	0.66±0.08	24.3±1.1
xylose	1±0.11	12.4±0.9
glucose	0.54±0.03	23.8±1.4

Effect of Different Protein Resources on Lipid and Biomass Production: As the glucose and sucrose had the greatest impact on lipid production, the effects of the various sources of proteins with the mentioned carbon substrates were investigated for biomass and oil production. Effects of protein source on biomass and lipid production of *Pichia kudriavzevii* were presented in Table 3. Among the protein sources with sucrose (Fig. 2a), corn supported the maximum biomass (1.2 percentage of media), followed by soybean (0.77 percentage of media), peptone (0.44 percentage of media) and yeast extract (0.46 percentage of media). Among the protein sources used for the lipid production, soybean and glucose supported the maximum lipid content (26 percentage of biomass), followed by soybean and sucrose (20 percentage of biomass).Peptone and yeast extract were less able to produce biomass and lipid in comparison with soybean source, so this substrate was chosen as a suitable nitrogen source.

Table 3- Effects of Various Nitrogen Resources on the Lipid and Dry Weight Biomass Production of *Pichia Kudriavzevii*

Protein sources	Glucose		sucrose	
	Lipid (% of dry weight biomass)	Dry weight Biomass (% of medium)	Lipid (% of dry weight biomass)	Dry weight Biomass (% of medium)
Soybean	26±2.1	0.64±0.1	20.13±3	0.77±0.21
corn	16.26±1.6	1.1±0.12	16.5±2.1	1.2±0.19
peptone	4.41±0.8	0.36±0.08	4.26±1.3	0.42±0.06
Yeast extract	9.13±1.1	0.44±0.14	10.43±0.9	0.46±0.02

Effects of the Mineral Elements on Lipid and Biomass Production by *Pichia Kudriavzevii*: Mineral elements such as magnesium, potassium, and calcium, etc. are normally essential for yeast growth and lipid accumulation at the low concentrations (20). The results indicated that the absence of mineral elements led to reductionin total dry weight biomass content and lipid production (Table 4).

As can be seen in Table 4, the equal amount of yeast extract and soybean as protein sources at 5 g/l with glucose as carbon sources and mineral elements were suitable substrates for lipid production via one factor at a time method.

Table 4. Effects of the Mineral Elements on the Lipid and Dry Weight Biomass Percentage of *Pichia Kudriavzevii*

sample	Mineral element		Distilled water	
	Dry weight Biomass (% of medium)	Lipid (% of dry weight biomass)	Dry weight Biomass (% of medium)	Lipid (% of dry weight biomass)
soybean	0.792±0.2	20.16±0.8	0.744±0.1	19.62±1.1
corn	1.05±0.16	15.23±1.2	0.914±0.3	9.84±0.7
yeast extract	0.456±0.1	14.91±1	0.344±0.09	11.04±1.5
yeast extract + soybean	0.428±0.04	27.36±2	0.31±0.1	24.1±2.2
yeast extract + corn	0.79±0.15	22.15±0.93	0.65±0.14	20±0.6

Optimization of Carbon and Protein Content using Response Surface Methodology: This study showed that two independent factors, carbon and protein sources had significant effects on biomass and lipid production. The experimental runs and results of the CCD were shown in Table 6. Ten runs in a single block were used to study the effects of these two factors on biomass, phenolic component, and lipid production.

Different amounts of glucose as carbon source and yeast extract with equal content of soybean as protein sources were used for the optimization of dry weight biomass, lipid and phenolic production by oleaginous yeast using response surface methodology.

The results indicated that biomass concentrations were ranged from 0.64 to 0.93 percent of the media. Lipid content differed from 8.6 to 28.1 percent of dry biomass. And phenolic compounds varied from 0.05 to 2.2 mg/g (Table 6). The result indicated that a good fit (R2 = 0.99) for lipid production was given by the quadratic regression relationship: Lipid = $33.12281+4.68314 \times A\ 7.94903 \times B-0.15020 \times A \times B-0.083872 \times A^2+1.43317 \times B^2+0.010330 \times A^2 \times B\ \ 0.044839 \times A \times B^2$. The results showed that lipid production was significantly influenced by carbon (F=897.23 and p< 0.0011). The highest content of lipid (28.12 percentage of biomass) was obtained at the high amount of glucose (66.21 g/L) and low content of yeast extract (7.5 g/l). The medium supporting the highest content of phenolic components was identical to the medium which induced the highest content of lipid. The results showed that phenolic production was significantly influenced by nitrogen content (F=208.53 and p < 0.0048) (Table 5). The highest content of phenolic

components (2.2 mg gallic acid /g of dry biomass) was obtained at 66 g/L glucose and 7.5 g/l yeast extract. A good fit (R2 = 0.99) was also observed for the biomass production: Biomass = 3.11140+0.12972 × A+0.75389 × B 0.023168 × A × B-1.03219E-003 × A^2 0.027744 × B^2+1.40959E-004 × A^2 × B+6.63202E-004 × A × B^2.

Table 5- ANOVA Parameters of the Models Fitted for Biomass and Oil Response

Source	Mean Square			F Value			p-value Prob> F		
Model	phenolic	biomass	lipid	phenolic	lipid	biomass	phenolic	biomass	lipid
	0.651308	0.130538	508.702	67.77227	344.0081	72.77352	0.0146	0.0136	0.0029
A-A	1.29605	0.01805	189.540	134.8612	897.2329	70.43902	0.0073	0.0139	0.0011
B-B	2.004002	0.02	163.624	208.5276	774.5517	78.04878	0.0048	0.0126	0.0013
AB	0.469225	0.0016	64.3204	48.82547	304.4753	6.243902	0.0199	0.1297	0.0033
A^2	0.02248	0.000145	9.46286	2.339177	44.79462	0.56446	0.2658	0.5308	0.0216
B^2	0.048381	0.000788	61.0280	5.034275	288.8902	3.073171	0.1540	0.2217	0.0034
A^2B	1.863662	0.012574	67.5303	193.9244	319.6703	49.06768	0.0051	0.0198	0.0031
AB^2	0.126727	0.007731	35.3419	13.18664	167.2991	30.1717	0.0682	0.0316	0.0059
Residual	0.00961	0.000513	0.4225						
Lack of Fit	0.016021	0.000313	0.36125	5.006406	5.897959	1.5625	0.2676	0.4296	0.2487
Pure Error	0.0032	0.0002	0.06125						
Cor Total		0.13105	509.124						

Lipid accumulation of algae, yeasts, and other microbes were affected by varying degrees of carbon/nitrogen content (9). The biomass content of *Pichia kudriavzevii* was highly affected by the protein levels (Samples 1 and 8, Table 2). High content of glucose (60 g/l) induced lipid and phenolic component production, on the other hand, more than 30 g/l glucose content had a deterrent effect on biomass production. The lowest glucose and protein concentration had a negative effect on both biomass and phenolic components (Sample 1, Table 6).

The simultaneous increase in proteins and glucose which were leveled at the high quantity had a positive effect on lipids and phenolic components production (Samples 4 and 10, Table 2), while at the low glucose concentration, for any increase in protein level, lipid production fell (Samples 8 and 1, Table 2).The result was in contrast to previous findings which protein content had a negative effect on lipid production, the amount of carbon must be taken into consideration.

Table 6- Results of FCCCD using Two Variables Showing Observed and Predicted Responses

			Lipid(%)		biomass(%)		phenolic component (mggallic acid /g)	
Sample	Glucose (g/l)	Yeast Extract (g/l)	Predicted	Observed	Predicted	Observed	Predicted	Observed
1	30	5	18.3	18.51±1.5	0.58	0.59±0.1	0.995	0.95±0.02
2	45	7.5	21.47	21.65±1	0.73	0.72±0.08	1.26	1.22±0.03
3	66.2132	7.5	28.33	28.12±0.8	0.64	0.64±0.03	2.20	2.25±0.05
4	60	10	22.48	22.7±0.6	0.87	0.88±0.07	2.05	2.1±0.035
5	45	7.5	21.47	21.65±0.5	0.73	0.74±0.06	1.3	1.26±0.1
6	45	11.03553	5.1	4.91±0.4	0.85	0.85±0.09	0.053	0.098±0.003
7	23.7868	7.5	8.8	8.65±1.3	0.83	0.83±0.04	0.595	0.64±0.09
8	30	10	9.1	9.32±1.1	0.92	0.93±0.095	0.82	0.78±0.06
9	45	3.964466	23.2	23±0.9	0.65	0.65±0.03	2.145	2.1±0.05
10	60	5	15.6	15.85±0.6	0.61	0.62±0.08	0.945	0.9±0.1

Lipid, Biomass and Phenolic Production: The interaction effects between two factors (glucose and protein) on lipid, biomass, and phenol production were shown by response surface plots and contour plots (Figure 2).

The interaction effects of the two parameters (glucose and protein) on lipid, biomass, and phenol production were examined by RSM plots and contour plots, as shown in Figure 2., the maximum lipid production (27.3% of biomass) and biomass (0.92 percentages of media) was obtained in the media containing 60 g L^{-1} glucose and 8 g L^{-1} yeast extract and 30 g L^{-1}glucose 10 g L^{-1} yeast extract respectively as it was predicted by the model. The present result indicated that optimization made an improvement in lipid production to 28 percent, compared to (21) who reported *Pichia kudriavzevii* was able to accumulate 23 percent lipid. The observed lipid, biomass, and phenolic production were 28, 0.95 percent and 2.2 mg gallic acid /g of dry biomass, respectively. The results well agreed with the predicted value and verified them. The maximum content of phenolic components and lipid production also obtained in medium containing 60 g L^{-1} glucose, 8 g L^{-1} yeast extract, the medium was the same for both products. The culture condition had a high impact on the total phenol content of *Pichia kudriavzevii*.

Antioxidant Activity: The results indicated that the biomass of *Pichia kudriavzevii* had a high antioxidant activity (Table 7). The lowest antioxidant activity was observed in the dry weight biomass with the lowest lipid content, whereas sample 3 with the highest lipid content supported the highest antioxidant activity (50%), followed by sample 9 with 23 percent lipids in dry weight biomass (45%), and then sample 4 with 23 percent of lipid in dry weight biomass (44%) (Table 7). The total phenolic content results showed a good correlation with the antioxidant activity and lipid content of biomass.

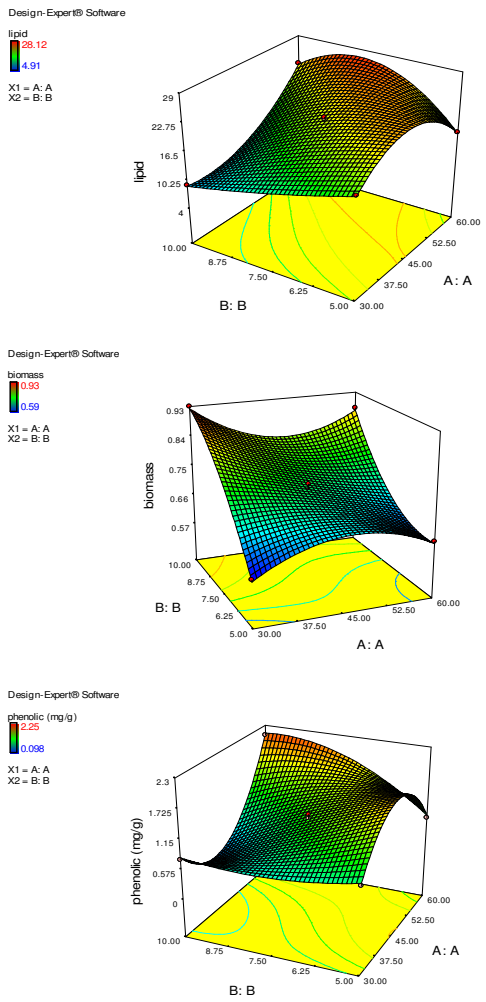

Fig. 2- Three-dimensional Response Surface Plot of Lipid, Biomass and Phenol

Table 7- DPPH Scavenging Activity (% Inhibition) of Different Samples of the Dry Weight of *PichiaKudriavzevii* and BHD

Samples	phenolic component (mg/g)	DPPH Scavenging Activity (% Inhibition)	lipid
1	0.95±0.02	38±1.9	18.51±1.5
2	1.22±0.03	37±0.5	21.65±1
3	2.25±0.05	50±1.21	28.12±0.8
4	2.1±0.035	44±2.3	22.7±0.6
5	1.26±0.1	35±0.8	21.65±0.5
6	0.098±0.003	30±1.8	4.91±0.4
7	0.64±0.09	31±1.5	8.65±1.3
8	0.78±0.06	40±0.75	9.32±1.1
9	2.1±0.05	45±0.51	23±0.9
10	0.9±0.1	38±2.12	15.85±0.6
BHT		75±0.44	

Effects of Temperature on Lipid Composition: Fatty acid profile of *Pichia kudriavzevii* at 28 °C was shown in Table 8 (sample0). Oleic acid (33.2%) and linoleic (23.2%) fatty acids was found predominate in *Pichia kudriavzevii'* lipid. Linolenic acid (3.60) was also observed in lipid of *Pichia kudriavzevii.* In common, de novo synthesized yeast lipid consists of C16 and C18 fatty acids. Palmitic acid (C16:0) founded the 15-25% w/w, of total lipids, palmitoleic (Δ9 C16:1) is produced lower than 5% w/w. Also, stearic acid (C18:0) is produced generally very low in the yeast lipid (5-8% w/w). Oleic acid (Δ9 C18:1) as a predominant fatty acid accumulated higher than 70% w/w, while linoleic (Δ9, 12C18:2) is produced in the second position (15-25% w/w). Unsaturated fatty acids like α-linolenic acid C18:3 -Δ9,12,15 and other high long PUFA are synthesized negligibly in lipid accumulation (19,22,23). The present results indicated that *Pichia kudriavzevii* fatty acids had a high similarity to the other oleaginous yeasts. The dominant fatty acids including palmitic acid (C16:0), stearic acid (C18:0) oleic acid (C18:1) and linoleic acid (C18:2), were accounted for about 84% of the total fatty acids. Demirbas (24) reported that the quality of biodiesel was affected by palmitic acid (C16:0) and oleic acid (C18:1). In this study, the high quantities of the C16:0 and C18:1 (about 54% of the total fatty acids) was observed, implying that *P. kudriavzevii* was a suitable source of biodiesel production. Similar results were reported by Sankh et al (21). Many studies indicated that reduction in temperature had a positive impact on PUFA in lipid. Thus, it could be stated temperature reduction could affect the profile of fatty acids of *P. kudriavzevii.* In sample 1 (Table 8), unsaturated fatty acid increased in *P. kudriavzevii* lipid. Linoleic acid content increased concomitantly with a more pronounced decrease in the saturated fatty acid like C12:0, C14:0 and C16:0 (Palmitic acid). Linolenic acid in this treatment reached 4.5 percent of the lipid. In next treatment (Sample 2), oleic acid increased to 36 percent of lipid. In comparison with the previous results which had a negative effect on production of linoleic and palmitic acid, linolenic acid was the highest (4.8 percentage of the lipid), being 0.3 percent more than previous treatments. In this step (Sample 3), the oleic acid content stood at the highest amount (39.5 percentage of lipid). Reduction of temperature that applied in Sample 4 had a negative effect on commercial fatty acids such as linoleic and linolenic.

Table 8- Effect of Temperature Schedule on the Profile of Fatty Acids of *Pichia Kudriavzevii* Lipid

fatty acid	Sample 0	Sample 1	Sample 2	Sample 3	Sample 4
C12:0	1.636±0.01	0.081±0.02	0.290±0.03	0.161±0.03	0.294±0.02
C14:0	4.536±0.2	0.487±0.1	1.545±0.1	1.271±0.041	2.343±0.032
C16:0	21.682±0.32	17.93±0.032	14.926±0.4	16.005±0.4	15.292±0.22
C16:1	3.606±0.14	5.284±0.2	3.632±0.37	4.330±0.2	4.623±0.05
C17:0	1.046±0.03	0.232±0.01	0.410±0.04	0.188±0.07	2.073±0.03
C17:1	0.282±0.08	0.725±0.02	0.539±0.02	0.572±0.036	1.200±0.02
C18:0	6.904±0.12	5.123±0.4	6.014±0.1	4.997±0.4	10.992±0.15
C18:1(n-6)C	33.224±0.7	32.272±0.6	36.787±0.35	39.509±0.7	35.770±0.27
C18:2(n-6)C	23.213±0.4	32.603±0.3	30.040±0.46	27.670±0.52	21.511±0.16
C18:3(n-3)ω3	3.216	4.588±0.05	4.808±0.05	4.108±0.3	3.402±0.02
C20:0	0.000	0.160±0.01	0.091±0.02	0.176±0.02	0.290±0.01

Discussion

The gill of pike-perch fish was identified as the best source of oleaginous yeast with the highest ability to produce lipids. This species was named *Pichia kudriavzevii.* Only one study has explored *Pichia kudriavzevii* as oleaginous yeast so there has been very little information about its fatty acid profiles and the effect of culture conditions on these profiles. Sankh et al (21) reported that the isolated oleaginous yeast, *Pichia kudriavzevii MTCC 5493*, from rotten fruits could accumulate 23% lipid in dry weight biomass in the appropriate culture condition and in regards to the profile of fatty acid, lipid of *Pichia kudriavzevii* was a suitable source of biodiesel production. recent research indicated that *Pichia kudriavzevii* also can be considered a good source of other high-value products such as lipid, ethanol, and D-xylonate (25–27). Regards the former research (21), which enhanced the lipid of *Pichia kudriavzevii* to 23 percent, present research made a 5 percent improvement in lipid production (28 percent lipid in dry weight biomass). An increase in phenolic content coincided with a subsequent increase in antioxidant activities and lipid contents in dry weight biomass. The result indicated that the culture conditions had a significant effect on the total phenol content of *P.kudriavzevii*. It could be concluded that yeast stimulates to produce high phenolic components with the highest antioxidant activity to protect the accumulated lipid, so the phenolic components could be produced as a defensive system against lipid oxidation in the vulnerable high lipid-content cells.

In line with previous researches, there has been a significant correlation between antioxidant activity and phenolic content (28–30). Many famous Fruits like grapes, apples, and berries have 2–3 mg polyphenols per grams of fresh weight (31). The phenolic components in dry weight of biomass of *Pichia kudriavzevii* were considerable so this species could be considered a promising source of phenolic components. The effect of temperature reduction on fatty acid profiles of *Pichia kudriavzevii* had been investigated. Thus, 28^{o}C induced high content of short length fatty acid like C12, C14, and C16;0 (Palmitoleic acid) so a reduction in unsaturated fatty acids in this condition was expected. Temperature reduction from 28 to 21 had a positive effect on unsaturated fatty acid like linoleic and linolenic acid. In this condition, saturated fatty acids decreased (Samples1 and 2). The highest amount of linolenic acid was obtained at 21°C. Similar research indicated that oleaginous yeast lipid made up a higher PUFA content and the highest productivity of α-linolenic acid was attained once a reduction in temperature condition was applied (32). The amount of palmitoleic and stearic acids increased with more temperature reduction less than 21°C as a result of the unsaturated fatty acids reduction. Similar research showed that temperature influenced the composition of fatty acids of *Rhodotorula glacialis* DBVPG 4785 lipid. C16 andC18 slightly decreased with reduction in temperature from 20 to 15 °C, while C16:1, C18;1 and C18;3 slightly increased. The highest content of PUFA (37.1 percentage of lipid) was achieved in sample 1 (28°C-24°C-21°C), so that it could be considered a good source of PUFA and the lowest one (25 percentage of lipid) was in sample 4 (25°C-21°C-15°C). Samples 0 (28°C) and 3 (21°C -15°C) had the highest content of C16:0 and C18:1 so these treatments could be regarded as the best resources of biodiesel production with 54.9 and 55.5 percent of these two fatty acids, respectively. On the other hand, temperatures reduction was not able to make considerable alteration in the fatty acid profile of *Pichia kudriavzevii* lipid.

Conflict of interest

All authors have no conflict of interest to declare.

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روش آماری برای تولید روغن، توده زیستی و ترکیبات فنولیک از گونه Pichiakudriavzevii جدا شده از ماهی دریای خزر

طارمی، مهشید: دانش آموخته گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود ایران mahshid.tarami@gmail.com

صمدلویی، حمیدرضا: استادیار گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران hsamadlouie@yahoo.com

قرنجیک، شاهرخ: استادیار بیوتکنولوژی گیاهی،دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران gharanjik@shahroodut.ac.ir

چکیده

مقدمه: طیف وسیعی از گونه های مخمر روغنی به عنوان افزودنی در غذای انسان و دام استفاده می شود. روغن و ساختار اسیدهای چرب گونه های مخمر روغنی تحت تاثیر ترکیبات شیمیایی و فیزیکی محیط کشت می باشد.

مواد و روش ها: گونه مخمری Pichiakudriavzevii جدا شده از آبشش صوفدارای توانایی قابل توجه ای در تولید چربی و اسیدهای چرب غیر اشباع بلند زنجیره دارد. روش یک عامل در یک زماناستفاد شد تا تاثیر هر یک از سوبستراهای مهم بر روی تولید رشد و روغن از گونه P.kudriavzevii بررسی شود و در نهایت سوبستراهای کلیدی برای تولید روغن، توده زیستی و ترکیبات فنولیک به روش آماری سطح پاسخ بهینه سازی شد.

نتایج: نتایج سطح پاسخ نشان داد که بیشترین میزان چربی (۲۸ درصد وزن خشک توده زیستی) و ترکیبات فنولیک (۲,۲۵ میلی گرم گالیریک اسید در گرم وزن خشک توده زیستی) در محیطی بدیت آمد که حاوی ۶۰ گرم در لیتر گلوکز و ۹ گرم در لیتر پروتئین بود. توده زیستی که حاوی بیشترین میزان چربی بود حداکثر میزان ترکیبات فنولیک با بیشترین میزان آنتی اکسیدان را داشت (۵۰ درصد بازدارندگی). روغن P.kudriavzevii به صورت عمده از پالمیتیک (۲۱,۷ درصد)، اولئیک اسید (۳۳,۲ درصد) و لینولئیک اسید (۲۳,۲ درصد)که نشان می دهد روغن این گونه قارچی منبع مناسبی برای بیودیزل می باشد.

بحث و نتیجه گیری: بیشترین میزان ترکیبات فنولیک و روغن در محیط کشت مشابه ای بدست آمد. برای به حداقل رساندن فساد اکسیداتیو، مخمر تحریک شده تا حداکثر میزان فنولیک اسید با حداکثر فعالیت آنتی اکسیدانی را تولید کند. در نهایت پروفایل اسید چرب P.kudriavzevii با کاهش دما تغییر کرده از این رو روغن این گونه نه تنها به عنوان بیودیزل بلکه به عنوان منبع غنی اسیدهای چرب غیراشباع می باشد.

واژه‌های کلیدی: فعالیت آنتی اکسیدانی، مخمرهای روغنی، چربی، فنولیک، Pichiakudriavzevii، اسیدهای چرب غیراشباع بلند زنجیره، سطح پاسخ

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