

Research Article

Single cell production by *Claveromyces fragilice* and *Fusarium oxysporum* in Kilka stick water

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Abstract

The production of single cell protein (SCP) is one of the cheap protein sources for use in aquaculture. In this study, *Claveromyces fragilice* and *Fusarium oxysporum* were used to produce SCP using Kilka stick water as the source of medium. Adaptation of *C. fragilice* and *F. oxysporum* to pure stick water was performed by increasing the concentration of stick water in distilled water. Treatments included 50% and 100% stick water, and a group without stick water as control. The pattern of the yeast and fungus growth was studied by spectrophotometry at 600 nm. The final product was analyzed for values of dry materials, amino acids profiles, total protein, moisture and ash. The results showed protein production by *C. fragilice* in 50% and 100% stick water was 55.35% and 57.47%, respectively, compared to 54.65% in control group ($P>0.05$). Protein production using *F. oxysporum* was 53.17% and $54.39\pm 0.45\%$ in 50% and 100% stick water, respectively compared to 49.71% in control group ($P>0.05$). The results showed that amino acids composition in produced SCP was comparable with the suggested profiles of requirement by FAO/WHO and NRC. Based on the obtained results, application of pure Kilka stick water is suitable for production of *C. fragilice* as the source of SCP.

Keywords: *Claveromyces fragilice*, *Fusarium oxysporum*, SCP, Stick water, Kilka fish meal.

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Introduction

Single cell protein (SCP) at the first time was introduced by Carl Wilson in 1944. The SCP is cell or protein derived from microorganisms grown on different sources of proteins and carbohydrates and is used as an alternative or supplementary source of food in the diet of animals and humans (Jamal *et al.*, 2007; Adedayo *et al.*, 2011). Various microorganisms such as bacteria, yeasts, algae can be used for SCP production (Kurbanoglu and Algur, 2002). Yeast and some fungi are ideal sources of feed or food because they have a fairly high content of protein which contains all of the amino acids. The amino acids are essential to human and animal's nutrition. In addition, an extremely important attribute of all fungal food is that they are virtually free of cholesterol. In this way the yeast/fungal food proteins are competitive with animal proteins (Moore and Chiu, 2001). In most cases, SCP was produced from food waste industry and there are few studies associated with production of SCP from aquatic waste materials. Aquatic organisms, due to their high protein and essential fatty acids have been extremely valuable and these wastes can be used as a substrate for the SCP production (Lunar *et al.*, 2006). One of the important environmental problems in Kilka meal factories is the stick water production, as a waste product, during meal production process (Mahdabi and Hosseini Shekarabi, 2018). Five hundred liters of stick water are produced for production of about 1000 kg of kilka meal.

Unfortunately, such stick water are released directly to the environment and because of the high fat and protein contents, the subsequent proteolytic and lipolytic activity by the bacteria can cause additional pollution in the environment (Kam *et al.*, 2012; Hadizadeh *et al.*, 2020). These wastes have rather protein and might be used as a substrate for the growth of yeast such as *Claveromycice fragilice* and fungus, *Fusarium oxysporum*. In the present study, we investigated the production of SCP from stick water of Kilka fish meal factory as medium for production of protein sources by *C. fragilice* and *F. oxysporum*.

Materials and methods

Microorganisms and media

C. fragilice and *F. oxysporum* were obtained from Iranian Research Organization for Science and Technology, which were prepared as a lyophilized stock. The lyophilized stocks of the yeast and fungus were cultivated in yeast extracts glucose broth (YGB) medium (Merck Germany) before being incubated at 30°C for 24 hours. The cells were harvested by centrifugation at 5000 rpm for 5 minutes.

Stick water

The stick water was obtained from Kilka fish meal factory (Shiner factory, Behshar, Mazandaran, Iran) and was kept at -20°C until used. The stick water was filtered by Whatman filter paper No. 1 and autoclaved at 121°C for 15 minutes (Hadizadeh *et al.*, 2020).

The characteristics of the stick water

The characteristics of different treatments of stick water were determined according to standard methods of American Public Health Association (APHA, 1998). The composition of stick water was measured using a photometer (Hitachi, Japan) using standard method of APHA (APHA, 1998). Crude lipid was measured according to the method described by Bligh and Dyer (1959). The percentage of dry weight, moisture, and ash were estimated by Association of Official Analytical Chemists method (AOAC) (AOAC, 2000). Also, the chemical and biochemical oxygen demands (COD and BOD) were determined according to the APHA (APHA, 1998). Total nitrogen was determined using Kjeldahl method (Kjeltec Analyzer Unit 2300).

Batch culture

To investigate growth of yeast and fungus, we used a control treatment without stick water and treatments with 50% and 100% stick water as the medium. Five percent of each treatment was cultivated. Yeast/fungus growth process was investigated at different times. Yeast and fungus were grown at temperature 30°C and pH 4.5 for 5 days. A volume of 100 mL of each yeast and fungus treatment was used in three replicates. Sample collection was performed under aseptic condition before being centrifuged for 5-10 minutes at 5000 rpm. The harvested cells were treated at 105°C for 2 hours before being transferred to Desiccator. Total weight of SCP production and

levels of protein, moisture, ash, dry matter and amino acid profiles were measured.

Amino acids analysis

The amino acid profiles of *C. fragilice* and *F. oxysporum* samples at maximum growth time were centrifuged for 5-10 minutes at 5000 rpm prior to being washed up with de-ionized water to remove soluble from residual nitrogen salts. The amino acids analysis was carried out by the Pico-Tag technique at three steps: (a) Hydrolysis of protein or peptide samples to yield free amino acids, (b) pre-column derivatization of the samples with PITC, and (c) analysis by reverse phase HPLC (SPELCO SIL, LC-DABS, USA). The chromatographic separation on the hydrolysates was performed using a reverse phase Pico-Tag column (4.6×120 mm) C18 at 38 °C and UV detector Vis at 436 nm (Ovissipour *et al.*, 2009). The chemical score of the SCP was computed according to Kam *et al.* (2012). The results were compared with the essential amino acid (EAA) profile as described by FAO/WHO (1990) and NRC (1993)

Results

The results of the stick water analysis are summarized in Table 1. The highest amount of protein productions using *C. fragilice* in 50% and 100% stick water were 55.35% and 57.4%, respectively compared to 54.65% in control group ($p<0.05$). Also, the protein productions by *F. oxysporum* were 54.39%, 53.17% in 50% and 100% stick water, respectively compared to 49.71%,

respectively ($p<0.05$). The other chemical composition (ash and moisture) of the treatments are given in

Tables 2 and 3. In addition, the amino acids profiles of the SCP products are shown in Table 4.

Table 1: Analysis of stick water used in this study.

Parameter	Value*
Potassium (mg L ⁻¹)	1454±126
Calcium (mg L ⁻¹)	2155±156
Na (mg L ⁻¹)	110±8.6
Nitrate (mg L ⁻¹)	231±10.8
Nitrite (mg L ⁻¹)	0.65±0.003
Ammonium (mg L ⁻¹)	0.16±0.001
COD (mg L ⁻¹)	6300±586.3
BOD (mg L ⁻¹)	2520±185.4
Total lipid (mg 100g ⁻¹ dry sample)	0.075±0.004
Protein (% in DWB)	68.25±1.04
pH	6.45±0.6
TS (mg L ⁻¹)	4.5±0.06
TSS (mg L ⁻¹)	47±0.01
TDS (mg L ⁻¹)	4.32±0.02
TVA (mg L ⁻¹)	0.311±0.001
VSS (mg L ⁻¹)	0.075±0.002

* Values are the means ± standard deviation of three determinations. COD= chemical oxygen demand, BOD=biological oxygen demand, TSS= Total suspended solid, TDS= Total dissolved solid, TVA= total volatile acid, VSS=volatile suspended solid, TS= total solid

Table 2: Chemical composition of dry matter produced from *Claveromyces fragilice* in different treatments of stick water.

Protein%	Ash%	Dry mater%	Moisture%	Biomass (g L ⁻¹)	Treatment%
54.65±0.35 ^c	3.68±0.028 ^a	90.15±0.20 ^c	4.85±0.25 ^a	4.16	Control
55.35±0.24 ^b	3.36±0.053 ^a	94.36±0.35 ^a	3.25±0.045 ^b	4.85	50% stick water
57.47±0.11 ^a	3.41±0.17 ^a	94.30±0.8 ^a	2.85±0.057 ^c	5.39	100% stick water

* Means with different letters in a row are significantly different ($p<0.05$)

Table 3: Chemical composition of dry matter produced from *Fusarium oxysporum* in different treatments of stick water.

Protein%	Ash%	Dry mater%	Moisture%	Biomass (g L ⁻¹)	Treatment%
49.7±0.21 ^a	3.41±0.054 ^a	95.61±0.31 ^c	3.59±0.051 ^a	6.11	Control
53.17±0.29 ^a	3.60±0.040 ^a	96.29±0.17 ^b	3.29±0.031 ^b	6.54	50% stick water
54.39±0.45 ^b	3.69±0.11 ^a	98.61±0.54 ^a	2.11±0.036 ^c	7.28	100% stick water

* Means with different letters in a row differ significantly ($p<0.05$)

Table 4: Profile of amino acids in single cell protein products obtained from growing *C. fragilice* and *F. oxysporum* in 50% and 100% stick water of Kilka meal processing plant.

Amino acid	<i>C. fragilice</i> (g 100g ⁻¹)	<i>F. oxysporum</i> (g 100g ⁻¹)	Fish meal (g 100g ⁻¹)	Reference Protein ¹	Reference Protein ²
Aspartic acid	4.35	4.73	8.60	-	-
sapaartic acid					
Glutamic acid	4.85	6.36	13.4	-	-
lutamic acid					
Serin	4.41	3.79	4.10	-	-
Glycine	4.55	3.06	9.30	-	-
Threonine	3.51	2.74	3.8	0.9	3.9
Histidine	2.26	1.49	2	1.6	2.1
Alanine	4.29	3.50	6.3	-	-
Proline	6.29	4.17	5.5	-	-
Arginine	1.75	2.26	6.1	-	-
Tirosine	3.17	3.11	2.8	-	-
Valine	3.65	2.88	4.5	1.3	3.6
Methionine	1.44	1.45	2.4	1.7	3.1
Isoleucine	2.25	2.17	3.8	1.3	2.5
leucine	3.54	2.34	6.4	1.9	3.3
Phenylalanine	2.81	2.25	3.4	-	6.5
Lysine	3.19	3.09	6.7	-	-
Cysteine	-	-	0/9	-	-
Σ AA	27.57	24.19	37.99	-	-
Σ NAA	28.74	25.16	50	-	-
Σ NAA/ ΣAA	0.95	0.94	0/75	-	-

¹Chemical score calculated with FAO/WHO reference protein as the base.

²Chemical score calculated with amino acid requirements as per NRC (requirements of common carp).

Discussion

The value of biomass production using *C. fragilice* in both 50% and 100% stick water was insignificantly higher (4.85-5.39 g/L) than control group (4.16 g/L). Also, there was no significant difference in total biomass between the treatments. Similarly, the biomass production using *F. oxysporum* (6.54 - 7.28 g/L) was insignificant among the treatments and between treatments and control one. However, the biomass concentration was higher in *F. oxysporum* grown in stick water than the *C. fragilice*, indicating a better condition for growing *F. oxysporum* on the medium containing stick water as a carbon source. Also, there was no difference in the level of protein production by two yeast and fungus in the stick water, but both yeast and

fungus produced higher protein levels at both stick water concentrations than control groups. The maximum biomass production of yeast and fungus was seen at the nearly end of the fermentation period. The diminution in biomass may be due to either the exhaustion of the carbon source or the cell autolysis, which is dis-agreement with the findings in other studies regarding SCP production by microorganisms (Zhang *et al.*, 2008). Other authors have investigated many bacterial biomasses growing in different substrates (Mahat and MacRae, 1992; Nigam, 1998, 2000; Lee and KyunKim, 2001; Kurbanoglu and Algur, 2002; Schultz *et al.*, 2006; Jamal *et al.*, 2007; Zhang *et al.*, 2008). The maximum amount of biomass production of 7.28 g L⁻¹ in this study was higher than the

average reported for *Candida utilis* (5.1 g L⁻¹), and *Trichoderma viride* WEBL0702 (5.54 g L⁻¹) grown on molasses, and winery waste water treatment, respectively (Nigam, 2000; Zhang *et al.*, 2008). However, the maximum amount of biomass production was 11.48 g L⁻¹ in *Mucor hiemalis* in presence of wheat flour and *C. utilis* (7 g L⁻¹) grown from pineapple cannery effluent (Lee and Kyun Kim, 2001; Jamal *et al.*, 2007). Such differences might be due to the type of bacterium and fungus or used mediums (Mahat and MacRae, 1992; Konlani *et al.*, 1996; Nigam, 2000).

Overall, the values of dry matter and ash were increased in the treated groups compared to control groups, while levels of moisture were decreased. In general, the protein content in production of the SCP should be between 39 to 73% (Gao *et al.*, 2007). Many fungal species are used as protein rich food (Frazier and Westhoff, 1990; Anupama, 2000; Zhang *et al.*, 2008). For instance, the production of fungal biomass protein produced by *A. niger* in winery waste- water was 36.6 % (Zhang *et al.*, 2008). The results of this study showed that the SCP produced by *C.fragilice* and *F. oxysporum* contained all the essential amino acids (EAA). The composition of the EAA of *L. acidophilus* and *A. niger* appears to be comparable with the protein value recommended by the FAO standard and the SCP from other sources (Erdman *et al.*, 1977; Anupama, 2000). The maximum amino acid produced by *C. fragilice* biomass in 100% stick water was proline, while that produced by *F.*

oxysporum was glutamic acid. Among non-essential amino acids (NEAA) in *C. fragilice* and *F. oxysporum* biomass, glutamate and aspartate were in the highest concentrations. The EAA profiles of the SCP produced by these yeast and fungus are comparable with FAO reference protein as well as with some other food proteins sources such as fish meal, carp and FAO/WHO standard. Methionine is known to be the limiting amino acid in SCP (Shipman *et al.*, 1975; Fabregas and Herrero, 1985; Kim and Lee, 2000). The essential amino acids such as leucine, methionine, and lysine in SCP are very important for the growth of marine fish. For example, lysine is reported to be able to stimulate the growth of marine animals (Stottrup and Mc Evoy, 2003; Gao *et al.*, 2007). Many fish require high levels of good protein in their diets, but the use of dietary protein for growth depends ultimately upon the availability of a suitable balance of amino acids (Barroso *et al.*, 1999; Kim and Lee, 2000; Janbakhsh *et al.*, 2018). The amount of crude proteins of *C. fragilice* and *F. oxysporum* biomass grown on stick water in this study is comparable with the highest levels in many corresponding microorganisms reported in the literature. The amount of crude protein produced by *Rhodo pseudomonas palustris* was 72–74%, and its amino acid profile was comparable with the FAO guideline (Kim and Lee, 2000). *Cellulomonasbi azotea* cultivated in perennial grass produced biomass with 60% protein and 10% RNA with all desired amino acids (Rajoka *et al.*, 2006).

In conclusion, the results of the present study showed that *C. fragilice* and *F. oxysporum* can be used successfully to produce SCP in stick water as the substrate. Maximum biomass productions of 7.28 g L⁻¹ and 5.39 g L⁻¹ by *F. oxysporum* and *C. fragilice* could be feasible to develop biotechnological treatment process for use of stick water as the source of medium for production of SCP with a cost-effective price.

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References

- Adedayo, M.R., Ajiboye, E.A., Akintunde, J.K. and Odaibo, A., 2011.** Cell proteins: As nutritional enhancer. *Advance Applied Science Research*, 2(5), 396-409.
- Anupama R., P., 2000.** Value-added food: Single cell protein. *Biotech Advance*, 18, 459-479.
- AOAC.,2000.** Official methods of analysis of the association of official analytical chemists. Gaithersburg.
- APHA.,1998.** Standard methods for the examination of water and waste water. Washington, DC.
- Barroso ,J.B., Perag, J., Garcia Salguo, L., de laHiguera, M. and Lupiane, J.A., 1999.** Variations in the kinetic behavior of the NADPH-production systems in different tissues of the trout when fedon an amino-acid-based diet at different frequencies. *International Journal of Biochemistry and Cell Biology*, 31, 277–290.
- Bligh, E.G.and Dyer, W.J., 1959.** A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–917.
- Erdman, M.D., Bergen, W.G. and Reddy, C.A., 1977.** Amino acid profiles and presumptive nutrition an assessment of single-cell protein from certain Lactobacilli. *Applied Environmental Microbiology*, 33, 901–905.
- Fabregas, J. and Herrero, C., 1985.** Marine microalgae as a potential source of single cell protein (SCP). *Applied Microbiology and Biotechnology*, 23, 110–113.
- FAO/WHO.,1990.** Energy and protein requirements. Report of Joint FAO/WHO/UNU Expert Consultation. World Health Organization Technical Report Series 724.Geneva, Switzerland: FAO/WHO and United Nations University. pp. 116–129.
- Frazier, W.C. and Westhoff, D.C., 1990.** Food microbiology. New Delhi, India: Tata McGraw Hill Publishing Co. Ltd.
- Gao, L., Chi, Z., Sheng, J., Ni, X. and Wang, L., 2007.** Single cell protein production from Jerusalem artichokeextract by a recently isolated marine yeast *Cryptococcus aureus* G7a and its nutritive analysis. *Applied Environmental Microbiology*, 77, 825–832.
- Hadizadeh, Z., Mehrgan, M.S. and Hosseini Shekarabi, S.P., 2020.** The potential use of stickwater from a kilka fishmeal plant in *Dunaliella salina* cultivation. *Environmental*

- Science and Pollution Research*, 27(2), 2144-2154.
- Jamal, P., Alam, Md.Z. and Umi, N., 2007.** Potential strain to produce bio protein from cheaper canadum diets using an organically certified protein. *Aquaculture*, 257, 393-399.
- Janbakhsh, S., Hosseini Shekarabi, S.P. and Shamsaie Mergan, M., 2018.** Nutritional value and heavy metal content of fishmeal from the Southwest Caspian Sea. *Caspian Journal of Environmental Sciences*, 16(4), 307-317.
- Kam, S., AbedianKenari, A. and Younesi, H., 2012.** Production of single cell protein in stick water by *Lactobacillus acidophilus* and *Aspergillus niger*. *Journal of Aquatic Food Product Technology*, 21, 403-417.
- Kim, J.K., and Lee, B.K., 2000.** Mass production of *Rhodospseudomonas palustris* as diet for aquaculture. *Aquacultural Engineering*, 23, 281-293.
- Konlani, S., Delgenes, J.P., Moletta, R., Traore, A. and Doh, A., 1996.** Optimization of cell yield of *Candida krusei* SO1 and *Saccharomyces* sp. LK3G cultured in sorghum hydrolysate. *Bioresource Technology*, 57, 275-281.
- Kurbanoglu, E.B. and Algur, O.F., 2002.** Single cell protein production from ram horn hydrolysate by bacteria. *Bioresource Technology*, 85, 125-129.
- Lee, B.K. and Kyun Kim, J., 2001.** Production of *Candi dautilis* biomass on molasses in different culture types. *Aquacultural Engineering*, 25, 111-124.
- Lunar, A.N., Craig S.R. and Mclean, E., 2006.** Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. *Aquaculture*, 257, 393-399.
- Mahdabi, M. and Hosseini Shekarabi, S.P., 2018.** A comparative study on some functional and antioxidant properties of kilka meat, fishmeal, and stickwater protein hydrolysates. *Journal of Aquatic Food Product Technology*, 27(7), 844-858.
- Mahat, M.S. and MacRae, I.C., 1992.** *Rhizopus oligosporus* grown on natural rubber waste serum for production of single cell protein: Apreliminary study. *World Journal of Microbiology and Biotechnology*, 8, 63-64.
- Moore, D. and Chiu, S.W., 2001.** Fungal products as food. Bio-Exploitation of Filamentous Fungi. Hong Kong. Chapter 10. pp. 223-251.
- Nigam, J.N., 1998.** Single cell protein from pine apple cannery effluent. *World Journal of Microbiology and Biotechnology*, 14, 693-696.
- Nigam, J.N., 2000.** Cultivation of *Candida langeroniin* sugar cane bagasse hemicellulos ichydrolyzate for the production of single cell protein. *World Journal of Microbiology and Biotechnology*, 16, 367-372.
- NRC., 1993.** National research council-nutrient requirements of fish. Washington, DC: National Academy of Sciences. 3, 124-128.

- Ovissipour, M., Abedian, A.M., Motamedzadegan, A., Rasco, B., Safari, R. and Shahiri, H., 2009.** The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from the Persian sturgeon (*Acipenser persicus*) viscera. *Food Chemistry*, 115, 238–242.
- Patel, R.S., Ghormade, V. and Deshpande, M.V., 2000.** Chitinolytic enzymes: An exploration. *Enzyme Microbiology and Technology*, 26, 473–483.
- Rajoka, M.I., Khan, S.H., Jabbar, M.A., Awan, M.S. and Hashmi, A.S., 2006.** Kinetics of batch single cell protein production from rice polishing with *Candida utilis* in continuously aerated tank reactors. *Bioresource Technology*, 97, 1934–1941.
- Schultz, N., Chang, L., Hauck, A., Reuss, M. and Syldatk, C., 2006.** Microbial production of single cell protein from deproteinized whey concentrates. *Applied Microbiology and Biotechnology*, 69, 515–520.
- Shipman, R.H., Kao, I.C. and Fan, L.T., 1975.** Single cell protein production by photosynthetic bacteria cultivation in agricultural by products. *Biotechnology Bioeng*, 17, 1561–1570.
- Stottrup, J. and McEvoy, L.A., 2003.** Live feeds in marine aquaculture. Oxford, United Kingdom: Blackwell Science Ltd, 115–119.
- Zhang, Z.Y., Jin, B., Bai, Z.H. and Wang, X.Y., 2008.** Production of fungal biomass protein using micro fungi from winery waste water treatment. *Bioresource Technology*, 99, 3871–3876.