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**Research Article**

# Single cell production by *Claveromycice frajilice* and *Fusarium oxysporum* in Kilka stick water

Babazadeh M.[1]; Soltani M.[2, 3]*; Kamali A.[1]; Saediasl M.R.[4]

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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, *Claveromycice frajilice* and *Fusarium oxysporum* were used to produce SCP using Kilka stick water as the source of medium. Adaptation of *C. frajilice* 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. frajilice* 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±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 to the obtained results, application of pure Kilka stick water is suitable for production of *C. frajilice* as the source of SCP.

**Keywords**: *Claveromycice frajilice*, *Fusarium oxysporum*, SCP, Stick water, Kilka fish meal.

1-Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran
2-Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3-Freshwater Fish Group and Fish Health Unit, Veterinary and Life Sciences, Murdoch University, Australia
4-Department of Food Science, Islamic Azad University, Sabzevar Branch, Khorasan Razavi, Iran
*Corresponding authors Emails: msoltani@ut.ac.ir

## 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 litters 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 frajilice* 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. frajilice* and *F. oxysporum.*

## Materials and methods

*Microorganisms and media*

*C. frajilice* 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$^{o}$C for 24 hours. The cells were harvested by centrifugation at 5000 rpm for 5 minuts.

*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).



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*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 determind 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. *frajilice* 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 (SUPELCOSIL, 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. frajilice* 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%,



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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 *Claveromycice frajilice* 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)



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**Table 4: Profile of amino acids in single cell protein products obtained from growing *C. frajilice* and *F. oxysporum* in 505 and 100% stick water of Kilka meal processing plant.**

| Amino acid | *C. frajilice* (g $100g^{-1}$) | *F. oxysporum* (g $100g^{-1}$) | Fish meal (g $100g^{-1}$) | Reference Protein[1] | Reference Protein[2] |
|---|---|---|---|---|---|
| Aspartic acid sapaartic acid | 4.35 | 4.73 | 8.60 | - | - |
| Glutamic acid lutamic acid | 4.85 | 6.36 | 13.4 | - | - |
| 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 | - | - |

[1]Chemical score calculated with FAO/WHO reference protein as the base. [2]Chemical score calculated with amino acid requirements as per NRC (requirements of common carp).

## Discussion

The value of biomass production using *C. frajilice* 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 biomas 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. *frajilice*, 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



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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.frajilice* and *F. oxysporum* contianed 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. frajilice* biomass in 100% stick water was proline, while that produced by *F. oxysporum* was glutamic acid. Among non-essential amino acids (NEAA) in *C. frajilice* 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. frajilice* 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).



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In conclusion, the results of the present study showed that *C. frajilice* 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. frajilice* 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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