Study of Inoculum size, Incubation temperature and Nucleic Acid Concentration on the Single Cell Protein produced by using Soymilk residue (Okara)

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Abstract-Okara, a soymilk residue is produced in huge quantities in soymilk industries. In the present investigation three combinations of *Okara* and wheat grits where prepared in the ratio of 3:1 (60% moisture content), 1:1 (40% moisture content), 1:3 (25 % moisture content) respectively. The combinations were inoculated with two fungal species viz. *Rhizopus oligosporus* and *Aspergillus oryzae*, with an inoculum size of 1.0×10^{3}, 1.0×10^{4} and 1.0×10^{5} cfu per g substrate. The combinations were incubated at different temperatures i.e. 20^{0} c, 25^{0} c and 30^{0} c. The combinations were finally analyzed for the effective inoculum size, incubation temperature and nucleic acid concentration. The results obtained depict that the best inoculum size for maximum SCP yield was 1.0×10^{3} cfu per g substrate. The best incubation temperature was found to be 25^{0} c. The maximum nucleic acid concentration was found to be 249 mg for *R. oligosporus* and 260 mg for *A. oryzae*.

Keywords: Inoculum size, Incubation temperature, Nucleic acid, SCP, *Okara*. *Rhizopus oligosporus* and *Aspergillus oryzae*.

INTRODUCTION

The term " Single Cell Protein" (SCP) was coined at Massachusetts Institute of Technology(MIT) to represent the cells of algae, bacteria, yeast and fungi grown for the protein contents (Schrimshaw[19],1975). Due to the increasing population and shortage of proteins, the world`s attention has been drawn to microbial sources of proteins. Hedenskog[17] et al., (1973) described some methods of processing the single cell protein. Huang[8] (1974) utilized acid brine for the production of food yeast. Bellamy[1] (1975) has studied the conversion of insoluble agricultural wastes to SCP by thermophilic microorganisms. Cooney[4] et al., (1975) produced SCP from methanol by using yeast. Bodwell[2] (1977) evaluated the proteins for humans. Chen and Peppler [3] (1977) highlighted the application of single cell protein in food. Ethanol was used as a substrate for the production of single cell protein by Laskin[13] (1977B). Dimmling[5] (1978) examined the raw materials for the production of SCP. Formation of single cell protein filament was detected by Huang[8] *et al*., (1978). Kharatyan (1978) explored the microbes used as foods for humans. Suitability of single cell protein as a feed for human beings has been studied by Kacmpfel[10] *et al*., (1995).A number of substrates are used for the production of SCP. Most of them include industrial and agricultural products/byproducts. Schuegerl and Rosen[18], (1997) have investigated the use of agricultural byproducts for fungal protein production

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Research on SCP has been stimulated by a concern over an eventual food crisis or food shortage that will occur if the world`s population is not controlled (Frazier[6],1995). Wills[22] (1999) highlighted some advantages of SCP over plant and animal sources of protein, which are as follows :

1. Microorganisms can provide rapid mass increase.
2. Microorganisms can easily be genetically modified to produce cell that bring about desirable results.
3. Microbial protein content is high.
4. For the production of SCP, raw material available in large quantities can be utilized.
5. SCP production can be carried out in a continuous culture, and therefore it is independent of climatic changes.

The nutritional status of SCP obtained from certain microorganisms was described by Singh[21] (2002). His report is depicted in table- 1

Table 1 – The nutritional status of SCP(%) obtained from some microorganisms.

Nutrient	Microorganisms			
	Paecilomyces varioti	Candida utilis	Methylophilus methylotrophus	Spirulina maxima
Protein	55	55	83	62
Fat	1	5	7	3
Ash	6	8	3	2

In the present investigation, *okara*, a byproduct of soymilk industry was used as the substrate. *okara* is produced in large quantities in soymilk industries, and poses a big disposal problem. Each kg of soybean processed for soymilk production yields 1.1 kg of *okara*. The production of soymilk is described in Fig. 1

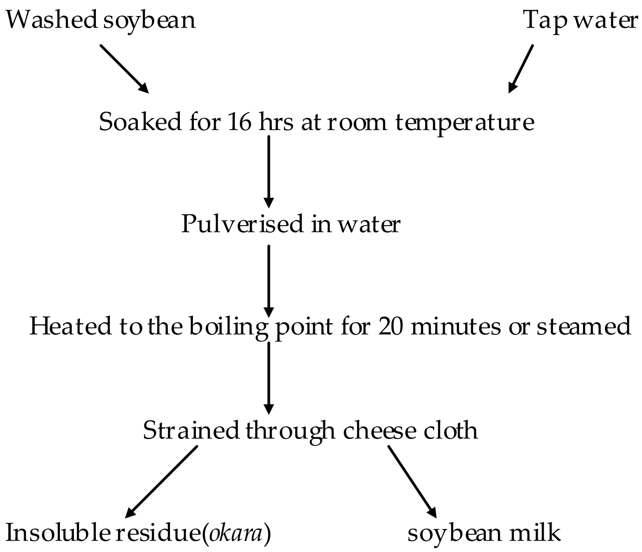

Fig.1 Showing protocol for soymilk production.

The high moisture content of *okara* (Shurtleff and Aoyagi[20], 1979) acts as a drawback in its utilization. Nutritionally *okara* is rich and it contains 79.6 % moisture, 19.91% protein, 8.37 % fat, 2.87% starch and 9.53 % carbohydrate. In addition to this it is also contains major minerals like calcium, iron, copper and zinc. Taking into consideration the nutritional quality of *okara*, Righelato and Elsworth[17] (1970) successfully utilized it for the production of some fermentation products. Kinoshita[12] *et al* ., (1985) have also used *okara* for the production of riboflavin and lipase. Matsuo[15] , (1997) has reported the in vivo antioxidant activity of *okara* by *Aspergillus oryzae*. Matsuo (loc. Cit) has further reported the consumption of *okara* fermented with *Actinomucor elegans* (meitauza). Ma[16] *et. al*., (1997) has studied the isolation and characterization of protein from soymilk residue (*okara*). The fibrinolytic activity of natto produced from *okara* fermented by *Bacillus subtilis* has been investigated by Miyamura[16] *et al*., (1998). Yousufi[23] *et al*., 2003 have investigated the production of SCP using *okara*-wheat grit combinations controlling its moisture content.

Keeping into consideration the above evidences on the utility of *okara*, the present investigation was undertaken.

MATERIAL AND METHODS:

The material used in the present investigation include *okara*, wheat grits were mixed in ratios of 3:1 (150 g *okara* / 50 g wheat grits), 1:1 (100 g *okara* / 100 g wheat grits) and 1:3 (50 g *okara* / 150 g wheat grits), so as to prepare three combinations with moisture content, 60 %, 40 % and 25 % respectively. The wheat grits not only reduced the moisture content but also provided fermentable carbohydrates. The combinations were filled in Petri plates, which were autoclaved at standard temperature, pressure and time. After autoclaving, the combinations were aseptically inoculated with two fungal cultures of *R. oligosporus* and *A. oryzae* separately. The inoculum size of the inoculum was 1.0×10^{3} , 1.0×10^{4} and 1.0×10^{5} cfu per g substrate. The combinations were then incubated at different temperatures i.e. 20^{0} c, 25^{0} c and 30^{0} c. After incubation, small amount of samples were removed from each combination. A part of the sample was used for fresh weight analysis, whereas another part was dried and used for dry weight analysis. The samples were subjected to determination of the nucleic acid concentration using diphenylamine reagent.

RESULT AND DISCUSSION:

The best inoculum size for maximum SCP yield was found to be 1.0×10^{3} (Table-2).

Table-2 Effect of inoculum sizes on SCP yield.

Organism	Okara / wheat grits (g)	SCP yield (g per 100 g substrate) Inoculum size (per g substrate)		
		1.0×10^{3}	1.0×10^{4}	1.0×10^{5}
R.. oligosporus	150 / 50(60.1) 100/100(40.1) 50 / 150(25.1)	19.88 18.98 18.92	16.97 17.31 17.23	13.96 14.77 13.99
A.. oryzae	150 / 50(60.5) 100/100(40.1) 50 / 150(25.1)	22.50 20.30 21.45	17.96 18.27 17.19	13.79 14.97 14.55

Note: Initial moisture contents are given in parentheses

The maximum SCP yield was obtained with the inoculum size of 1.0×10^{3} In case of *R. oligosporus* the maximum SCP yield was found to be 19.88 % (150 / 50 combination) whereas in case of *A. oryzae* the maximum SCP yield was found to be 22.50 % (150/50 combination).

The best incubation temperature for maximum SCP yield was found to be 25^{0} C (Table-3).

Table-3 Effect of incubation temperature in SCP yield.

Organism	Okara / wheat grits (g)	SCP yield (g per 100 g substrate) Incubation temperature		
		20^{0} C	25^{0} C	30^{0} C
R. oligosporus	150 / 50(60.1) 100/ 100(40.1) 50 / 150(25.1)	19.88 18.98 18.92	15.23 17.31 17.23	13.96 14.77 13.99

A. oryzae	150 / 50(60.5) 100/ 100(40.1) 50 / 150(25.1)	22.50 20.30 21.45	18.30 18.27 17.19	13.79 14.97 14.55

Note: Initial moisture contents are given in parentheses

The maximum SCP yield was obtained at 25⁰ C temperature, in case of *R. oligosporus* it was found to be 15.23 % (150/50 combination) and in case of *A. oryzae* it was found to be 18.30 % (100/100 combination).

The nucleic acid concentrations obtained in different combinations are depicted in Table -4.

Table-4 Nucleic acid concentration in SCP yield.

Organism	Okara / wheat grits (g)	SCP yield (g per 100 g substrate)	
		Fresh weight basis(in mg)	Dry weight Basis (in mg)
R. oligosporus	150 / 50(59.9)	218	245
	100/100(40.1)	225	249
	50 / 150(25.1)	240	246
A. oryzae	150 / 50(60.5)	226	250
	100/100(40.1)	247	228
	50 / 150(25.0)	245	260

Note: Initial moisture contents are given in parentheses

The maximum nucleic acid concentration achieved for *R. oligosporus* was 249 mg (100/100 combination) on dry weight basis and for *A. oryzae* it was found to be 260 mg (50/150 combination) on dry weight basis. According to Food and Agricultural Organisation (FAO) 2 gram nucleic acid per day from SCP for an adult has been given as safe practical limit. Since the results in Table-4 shows that nucleic acid concentration is far below the tolerance limit, therefore the SCP produced can be considered safe for dietary intake. The over all result is that *Okara* was successfully utilized to produce SCP.

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