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Comparative assessment of fruit waste as potential substrates for the production of Single cell protein by *Saccharomyces cerevisiae* and *Spirulina*

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Abstract:
People in one-third world and developing countries are suffering from menace of protein deficiency in their diets resulting in serious protein-energy malnutrition problems. The situation demands exploration of new un-conventional protein sources to fortify human food. The protein from microbes is cheap, easy to obtain in crude form, nutritive and can be made available as a food or feed additive to increase its nutritional value. The disposal of wastes is a serious problem and their deposition poses health hazard for all the living beings. The bio conversion of fruit waste by microbial fermentation into single cell protein has potential use for solving the worldwide protein deficiency in food. The present study was planned to assess the feasibility of using fruit wastes for producing single cell protein (SCP) by Saccharomyces cerevisiae and Spirulina. The comparative assessment revealed that apple waste generates higher amount of protein as compared to cucumber waste. The present exploration depicted that Saccharomyces cerevisiae can be an efficient producer of Single cell protein (SCP) utilizing Apple wastes as a substrate supplemented in Glucose fruit hydrolysates medium. It showed the highest production of protein with the concentration of 1.85mg/ml. Thus the present findings help in Single Cell Protein production from inexpensive, cheap, readily available agro waste material.
KEYWORDS: Single cell protein, Fruit wastes, Saccharomyces cerevisiae , Spirulina

INTRODUCTION

Proteins are building blocks of all the living beings. They are needed in growth and development. They are needed in much more quantities than any other metabolite and their destruction or degradation ultimately extinguishes the flame of life. The protein comes from a number of vegetables, cereals and fruits, often not affordable by a common man and therefore microbial proteins can be an alternative source to feed economically down trodden communities in the world in general and India in particular.

Single Cell Protein (SCP) serves as the most important steps for this goal and is an innovative way to successfully solve the food problem at global level. The term Single Cell Protein refers to dead, dry microbial cells or total proteins extracted from pure microbial cell culture and is produced using a number of different microorganisms including bacterium, fungus and algae. It can also be refered as biomass, bioprotein or microbial protein. Besides high protein content (about 60-82% of dry cell weight), Single Cell Protein also contains fats, carbohydrates, nucleic acids, vitamins and minerals. Another striking feature with Single Cell Protein is that it is rich in certain essential amino acids like lysine, methionine which are limiting in most plant and animal foods. This protein can be used as additive added to the main diet instead of sources known very expensive (Gour et al.,2015).

India is the second major producer of fruits and vegetables in the world. It contributes 10% of world fruit production. According to India Agricultural Research Data Book 2004, the total waste generated from fruits and vegetables comes to 50 million tons per annum (Adoki et al., 2008). Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth (Yabaya et al., 2008).Thus fruit processing wastes are useful substrates for production of microbial proteins. The utilization of fruit wastes in the production of Single Cell Protein will help in controlling pollution and also in solving waste disposable problem to some extent in addition to satisfy the world shortage of protein rich food (Barton,1999)

Different types of microbes such as bacteria, fungi, mold, algae and yeasts can be used as the sources of Single Cell Protein. Algal single Cell Protein has limitations such as the need for warm temperatures and plenty of sunlight in addition to carbon dioxide, and also that the algal cell wall is indigestible. Bacteria are capable of growth on a wide variety of substrates, have a short generation time and have high protein content. Yeasts are probably the most widely accepted and used microorganism for Single Cell Protein (Singh et al., 2009). These include strains of *Candida utilis, C. arborea, C. pulcherrima and Saccharomyces cerevisiae* (Saquido et al.,1981).

The present study was focused on yeast and algal single cell protein rather than bacterial and fungal single cell protein. Over the last few years, a lot of research has been done for reprocessing and reuse of different fruit wastes for the conversion of valuable and nutritive products. Therefore the present investigation aimed to assess the feasibility of using apple and cucumber fruit peels as the potential substrate for *Saccharomyces cerevisiae* and *Spirulina* single cell protein (SCP) biomass.

MATERIALS AND METHODS

- **Collection and Preparation of substrates**

The cucumber and apple fruit wastes were collected from the local market of Nagpur region, Maharashtra and washed several times with sterile distilled water. The peels were separated, oven dried (at 40-50°C), ground and sieved through mesh screen.The sample thus prepared were packed in transparent sterilized polythene bags and stored for further studies.

- **Microorganism**

Microorganisms used to ferment fruit waste were *Saccharomyces cerevisiae* and *Spirulina (algae)* were procured by the Laboratory and maintained for further studies.

- **Preparation of Fruit Hydrolysate**

The fruit peels were used as substrate for production of SCP. Fruit peels were degraded to convert cellulose content into more available sugars by chemical treatments. A 50 ml of 10% (w/v) HCl was added to each waste (4gm) in conical flask containing apple and cucumberwaste. The mixture/solution was placed in water bath at 100°C for one hour and was allowed to cool. Further the mixture was filtered through Whatman filter paper no.1. The filtrates were diluted with sterile distilled water and autoclaved at 121°C for 15 minutes. The sterile solution was used as carbon and nitrogen source for biomass production. These fruit hydrolysate were used as the substrates for microorganisms.

- **Inoculum Preparation**

Saccharomyces cerevisiae and *Spirulina* cultures were suspended in nutrient broth medium separately and incubated at 28°C for 24hours. This culture suspension was used as an inoculum.

- **Fermentation and harvesting of single cell protein**

Submerged fermentations were carried out in Erlenmeyer flasks with three different media namely **Supplemented Fruit Hydrolysate (SFH) (Table 1), Glucose supplemented Fruit Hydrolysate (GFH) (Table 2) and Fruit Hydrolysate medium (FHM) using apple and cucumber wastes.** A 98 ml of each medium was transferred into 250 ml Erlenmeyer flask and sterilized at 121^{0}C for 15 minutes. A 2ml inoculum of *Saccharomyces cerevisiae* was aseptically transferred into each flasks containing three trial mediums. All the flasks were incubated at 28^{0}C for 6 days followed by determination of biomass.With reference to *Spirulina*, similar procedure was opted for submerged fermentation using **apple and cucumber wastes** supplemented in **Zarrouk medium(Table 3,4)**

- **Centrifugation and recovery**

After fermentation, the medium was filtered and the filtrate was centrifuged at 12000 rpm for 10 minutes. Further supernatant was utilized for Protein estimation (assay).

- **Bioconversation of fruit waste and proximate analysis of Protein (Estimation)**

The amount of protein was estimated by the standard method of Lowry et al., using Bovine serum Albumin as standard. In this method, a set of 10 test tubes were prepared according to the standard protocol **for estimation of protein (Table 5).** The set of test tubes were allowed to stand at room temperature for 10 minutes followed by the addition of 0.5ml of Folin–Ciocalteu reagent with constant mixing. Further test tubes were incubated for 30 minutes at room temperature and optical density was measured at 670 nm.

- **STANDARD BSA CURVE**

The concentration of protein was calculated from standard BSA curve (Table 6)

RESULTS AND DISCUSSION:

People are becoming health conscious and consume large quantities of fruits and fruit juices leading to the accumulation of fruit wastes. The disposal of wastes is a serious problem and their deposition poses health hazard for all the living beings. These wastes can be used as a substrate for the growth of microbes. A variety of fruit wastes have been used as substrates for the production of SCP by various researchers.

Kamel (1979) reported the use of dates as a potential substrate for the production of single cell protein. Sweet orange residues have been used for SCP production by Nwabueze and

Ogumtimein (1987). Rahmat et al. (1995) used apple pomace for the production of single cell protein from *Kloechera apiculata* and *Candida utilis* so as to improve stock feed. Pineapple cannery effluent has been utilized for SCP production by Nigam (1998). Essien et al. (2005) utilized banana peel as a substrate for mould growth and biomass production.

In the current investigation, comparative assessment of two fruit wastes (apple and cucumber) as the potential substrates for the production of Single cell protein by *Saccharomyces cerevisiae* and *Spirulina* was taken into consideration. This study revealed *Saccharomyces cerevisiae* to be an efficient producer of Single cell protein (SCP) utilizing Apple wastes as a substrate supplemented in Glucose fruit hydrolysates medium. It showed the highest production of protein with the concentration of 1.85 mg/ml while lowest production of protein with the concentration of 0.68 mg/ml was showed by *Saccharomyces cerevisiae with cucumber waste* as a substrate supplemented in Fruit hydrolysates medium (Table 7 Graph 1)

Based on fermentation observations the highest protein content was recorded on that biomass where *Saccharomyces cerevisiae* was used as inoculum. Among the two micro organisms *S.cerevisiae* is effective in utilization of the carbon source from the fruit waste when compared to *Spirulina* These findings were in agreement with the findings of Kumar et al., (2008). *Spirulina* showed the highest production of protein with the concentration of 1.80 mg/ml with apple waste as a substrate supplemented in Glucose fruit hydrolysates medium while the lowest production of protein at the concentration of 0.50 mg/ml (Table 8 Graph 2) was showed with cucumber waste as a substrate supplemented in Fruit hydrolysates medium. In algae *spirulina*, higher yield of protein was found when grown on Glucose Supplemented apple fruit peels Hydrolysates (GSAFPH) medium. Glucose Supplemented Fruit Hydrolysate (GFH) medium were used for fermentation gave optimum results. These findings were in agreement with the finding of Khan and Dahot (2010). Khan et al.(2009) investigated the production of fungal single cell protein using *Rhizopus oligosporus* grown on fruit wastes. The study revealed that papaya fruit waste generates highest amount of protein per 100g of substrate used, followed by cucumber peelings, pomegranate rind, pineapple fruit skin and watermelon skin respectively with 59.5 mg, 57.3 mg, 51.6 mg, 48.0 mg and 43.2 mg crude protein respectively.

Thus yeast served as the good source for Single cell protein production. It has been reported that yeast is a probiotic component i.e. microorganisms which are good to human and animal body. This probiotic component can be utilized for single cell protein production thus, consumption of protein produced by yeast is good for health and is a great supplement for protein source .This proteinaceous product can be refined and further processed to obtain a pure single cell protein which can be used as food and feed. Similarly the agricultural waste, fruit waste and other vegetable waste from markets and industries can be used for single cell protein production thus greatly useful in controlling pollution and enables recycling of waste which are being throwed. Yeast is currently the most commonly used organism in the production of biomass, probably because it is already accepted both in human food and animal feed industries. Yeast based processes are the most advanced towards commercial production, followed by bacterial processes. Yeast may have many convenient characteristics, such as the ability to use a wide variety of substrates like hexose and pentose. In this study yeast showed promising result for single cell protein production.

CONCLUSION:

Hence according to the present investigation it may be concluded that higher yield of Single cell protein from *Saccharomyces cerevisiae* was possible by submerged fermentation of both substrates. The degree of SCP production depends on the type of substrate used and also on the media composition. For *Saccaharomyces cerevisiae,* apple waste was better substrate followed by cucumber waste. The addition of glucose provided available carbon source for the organisms thereby enhancing SCP production. The present finding reveals that apple and cucumber peels were used as potential source for product with higher protein content by utilizing various ingredients present in them and there is a possibility of converting fruit wastes to proteinaceous feed and food. Thus fruit wastes should be exploited properly as a substrate for the production of cellular biomass of edible yeast instead of dumping them. So they can be used as food / feed supplement with least expenditure of money.

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Table 1: Supplement Fruit Hydrolysate medium (SFH)

Sr. No.	Ingredients / Composition	Gms / Litre
1	$(NH_4)_2SO_4$	2.000
2	KH_2PO_4	1.000
3	$MgSO_4.7H2O$	0.500
4	NaCl	0.100
5	$CaCl_2$	0.100
6	Fruit Hydrolysate	40.00
7	Final PH	5.5
8	Distilled water	1.000

Table 2: Glucose Fruit Hydrolysate medium (GFHM)

Sr. No.	Ingredients / Composition	Gms / Litre
1	$(NH_4)_2SO_4$	2.000
2	KH_2PO_4	1.000
3	$MgSO_4.7H2O$	0.500
4	NaCl	0.100
5	$CaCl_2$	0.100
6	Fruit Hydrolysate	40.00
7	Final PH	5.5
8	Glucose	2.000
9.	Distilled water	1.000

Table 3: Composition of the Zarrouk Medium

Sr. No.	Ingredients / Composition	Gms / Litre
1	$NaHCO_3$	18.00
2	K_2HPO_4	0.500
3	$NaNO_3$	2.500
4	NaCl	1.000
5	K_2SO4	1.000
6	$MgSO_4$. 7 H2O	0.200
7	$CaCl_2$	0.040
8	$FeSO_4$. 7 H2O	0.010
9.	EDTA	0.080
10.	Solution A(ml)	1.000

Table 4: Composition of solution A (Zarrouk Medium)

Sr. No.	Ingredients / Composition	Gms / Litre
1	H_3BO_3	2.860
2	$MnCl_2$. 4 H2O	1.810
3	$ZnSO_4$	0.220
4	$CuSO_4$	0.080
5	$CaCl_2$	0.100
6	Na_2MoO_4	0.010
7	Distilled Water	1.000

Table 5: Standard protocol for estimation of proteinTable 6: Standard Protein Assay

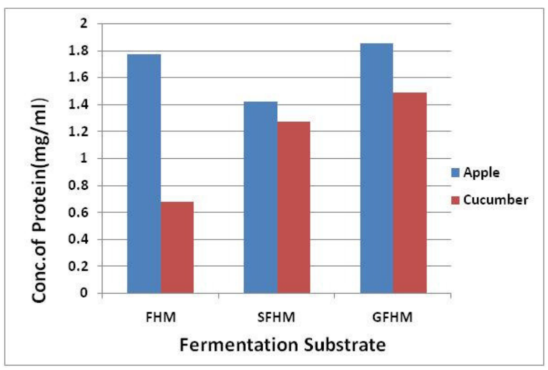
Sr. No	Reagent	Blank	T1	T2	T3	T4	T5	T6	T7	T8	Unknown(SFH,GFH& FHM)
1.	BSA(ml)	-	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	-
2.	BSA unknown	-	-	-	-	-	-	-	-	-	0.8
3.	Distilled water(ml)	2	1.8	1.6	1.4	1.2	1	0.8	0.6	0.4	1.2
4.	Alk.$CuSO_4$ (ml)	5	5	5	5	5	5	5	5	5	5

Table 7: Concentration of protein produced by *S.cerevisiae*

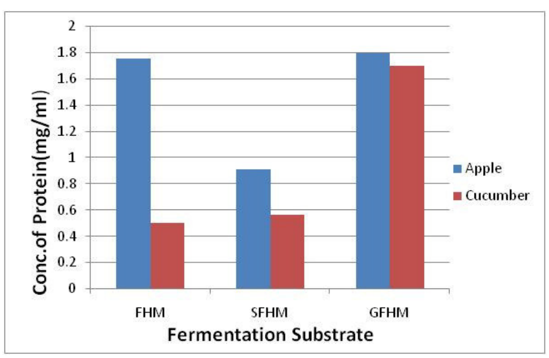
Test tube	Optical Density(nm)
Blank	0.00
T1	0.15
T2	0.26
T3	0.41
T4	0.58
T5	0.70
T6	0.87
T7	0.85
T8	0.82

Table 8: Concentration of protein produced by *Spirulina*

Fruit wastes	Fermentation Medium	Concentration of protein (mg/ml)
Apple	Fruit Hydrolysate Medium	1.76
	Supplemented Fruit Hydrolysate Medium	0.91
	Glucose Fruit Hydrolysate Medium	**1.80**
Cucumber	Fruit Hydrolysate Medium	**0.50**
	Supplemented Fruit Hydrolysate Medium	0.56
	Glucose Fruit Hydrolysate Medium	1.70



Graph1: Effect of nutrient supplementation on protein production in *S.cerevisiae* for apple and cucumber



Graph 2: Effect of nutrient supplementation on protein production in *Spirulina* for apple and cucumber

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