Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/3038
DOI URL: http://dx.doi.org/10.21474/IJAR01/3038
ISSN NO. 2320-5407

RESEARCH ARTICLE

POTATO PEEL - A COST EFFECTIVE SUBSTRATE FOR PROTEIN ENRICHMENT AND WASTE MINIMIZATION.

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Manuscript Info

Manuscript History

Received: 30 November 2016
Final Accepted: 28 December 2016
Published: January 2017

Key words:-
Protein enrichment, Potato peel, *Saccharomyces cerevisiae*, Chemical analysis, Fermentation.

Abstract

Disposal of kitchen waste is universal and likewise the need for food to the growing human population is inevitable, a sure solution is immediately needed. The bioconversion of potato peel into certain valuable products like single cell protein (SCP) has the ability to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. The aim of using kitchen waste as substrate for the production of high nutritious food product may decrease environmental pollution due to utilization of kitchen waste as a substrate, up to some extent. However, using flour prepared from dried potato peels were inoculated with pure culture of *Saccharomyces cerevisiae* and then left for four days. Chemical analysis of the fermented potato peel mash revealed a significant ($p<0.05$) increase in the protein content of the potato peel mash when compared with unfermented potato peel .This result also shows that fermentation increased significantly the protein of the fermented potato peel mash, while a decrease in carbohydrate was observed. Moisture content of 125%, temperature of 30^0 and pH of 5.5 were maintained for growth of *S.Cerevisiae* on the mash. Therefore fermented peels could be a good source of protein enriched feed.

Introduction:-

Various forms of organic waste such as cellulose hemicelluloses, hydrocarbon and different types of agricultural waste were used in the production of protein enrich food[1]. Lignocelluloses wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose, and lignin. Potato (*Solanum tuberosum* L.) is one of the major world crops with world annual production of 180million tons on 2009 according to the Food and Agriculture Organization (FAO)[2].The Potato peels are rich in phytonutrients, carbohydrates, high in starch (8-28%) but with only 1-4% protein. Anon reported that potato starch is a large grained starch containing 25% amylose and 73% amylopectin and high phosphate content[3]. A large amount of potato peels are discarded during processing for chips by many industries. Protein enrichment of potato peels through inexpensive means is therefore desirable[4]. Solid state bio processing (SSB),an aerobic microbial transformation, has been successfully exploited for enzyme and food production, phenolic enhancement, fruity aroma production as well as many other uses. Culture conditions of bioprocessing are more similar to the natural habitat of filamentous fungi, which leads, in many cases, to higher efficiency, as well as lower generation of liquid waste[5].The materials that make up wastes should normally be recycled back into the ecosystem, e.g.:-straw, bagasse, citric acid, olive, and date waste, whey, molasses, animal

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manures and sewage. The amount of these wastes can be very high and may contribute to a significant level of pollution in watercourses[6]. A variety of microorganisms and substrate are used to produce protein rich food. Yeast is suitable for production because of its superior nutritional quality. The supplementation of cereals especially with yeast, make them a good source of protein for animal. The necessary factor considered for its production is the demonstration of toxic and carcinogenic compounds originated from substrates, biosynthesized by microorganisms or formed during processing. *Saccharomyces cerevisiae* and *Candida* has been used to bio convert agro-industrial waste into valuable protein sources. Of all the essential plant nutrients, Nitrogen is the most often limiting to potato production on sandy soils. Nitrogen fertilizer recommendations for irrigated soils are based on crop yield goal and previous crop. Nitrogen supplementation of the raw substrate with ammonium sulphate in solid state bio processing may stimulate growth or improve process efficiency[5].The objective of the present investigation were to study the protein enrichment of potato peel by the process of solid-state bio processing using *Saccharomyces cerevisiae* and its addition as a protein supplement to the diet of animals.

Material and Methods:-

Requirements:-Lyophilized culture, inoculating loop, incubator shaker, laminar Air Flow,Erlenmeyer flask (250ml), measuring cylinder, distilled water, autoclave, ethanol (70%), cotton, refrigerator, centrifuge, colorimeter, etc.

Preparation of YEPD media:-

Medium components:Pure culture of yeast (*Saccharomyces cerevisiae*) used was MTCC 170.It was revived on YEPD[5] (Yeast Extract 3.0g; Peptone 10.0g; Dextrose 20.0g; Agar 15.0g; distilled water 1.0 L) at 30^0 C for 48hrs and was stored at 4^0 C.

Procedure:-

- YEPD medium (50ml) was prepared in Erlenmeyer flask (250ml) and autoclaved at 15psi for 15min.
- Inoculum (2ml) developed was transferred to the 50ml YEPD medium prepared in a flask and kept for 30^0C for four days on rotatory shaker.
- Chemical analysis of the culture was done after every 24hrs of incubation for four days.

Culture revival of Saccharomyces cerevisiae(MTCC no. : 170):-

Medium components:- Pure culture of yeast (*Saccharomyces cerevisiae*) used was MTCC 170 .It was revived on YEPD[5] (Yeast Extract 3.0g; Peptone 10.0g; Dextrose 20.0g; Agar 15.0g; distilled water 1.0 L) at 30^0 C for 48 hrs and was stored at 4^0C.

Procedure:-

- Pure culture was obtained in lyophilized form from MTCC (microbial type culture collection), IMTECH.
- Culture was revived in Yeast extract potato dextrose (YEPD) broth medium consists of Yeast extract 3.0g; Peptone 10.0g; Dextrose 20.0g; Agar 15.0g; Distilled water 1.0L.
- Culture was spread to the YEPDA medium plates using spreader under sterile conditions and incubated at 30 ^{0}C for 24hrs. Optical density of the culture was checked in colorimeter at 680nm.
- Sub culturing was done to maintain culture for further use.

Preparation of YEPD media supplemented with ammonium sulphate:-

Medium components:- YEPD medium for *Saccharomyces cerevisiae* production was prepared[5].It consists of (g/l): Yeast extract 3.0g; Peptone 10.0g; Dextrose 20.0g, ammonium sulphate 2.5%, distilled water 1.0L.

Procedure:-

- YEPD medium (50ml) was prepared in Erlenmeyer flask (250ml), and ammonium sulphate (1.25g/50ml) was added and autoclaved at 15psi for 15min.
- Inoculum (2ml) developed was transferred to the 50ml YEPD medium prepared in a flask and kept at 30^0C for four days on rotatory shaker.
- Chemical analysis of the culture was done after every 24hrs of incubation for four days.

Substrate:-
The potato peels used in this study was obtained from nearby fast food facilities. The peels were washed thoroughly with sterile distilled water, cut into tiny bits and dried in an oven at 60^0C for 48 hrs. The dried peels were powdered by using Pestle and Mortar, subsequently sieved with 0.5mm screen mesh[4].

Preparation of potato peel media in YEPD broth:-
Medium components:-
Dried potato peel powder (10g), specific medium for *Saccharomyces cerevisiae* production was prepared[5] .It consists of (g/l): Yeast extract 3.0g; Peptone 10.0g; Dextrose 20.0g, distilled water 1.0L.

Procedure:-
- YEPD medium (50ml) was prepared in Erlenmeyer flask (250ml) and autoclaved at 15psi for 15min. Inoculum (2ml) developed was transferred to the above prepared medium in a flask and kept at 30^0C for 17-18hrs.Dried potato peels were weighed (10g). Moisture level (125% (v/w)) was adjusted and autoclaved at 121^0C for 15min.
- Under aseptic conditions, YEPD growth media was transferred to flask containing driedpotato peels which was then kept for fermentation at 30^0C on rotatory shaker (150rpm) for four days.
- Chemical analysis of the culture was done after every 24hrs of incubation for four days.

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- Dried potato peels were weighed (10g). Moisture level (125% (v/w)) was adjusted. Ammonium sulphate was added (2.5%) in the flask containing dried potato peels and autoclaved at 121^0C for 15min.
- Under aseptic conditions, YEPD growth media was transferred to flask containing dried potato peels which was then kept for fermentation at 30^0C on rotatory shaker (150rpm) for four days.
- Chemical analysis of the culture was done after every 24hrs of incubation for four days.

Solid State Bio processing:-
Solid state bioprocessing was carried out in 250ml Erlenmeyer flasks containing potato peel(10g).Flasks were aseptically inoculated with inoculum suspension of *Saccharomyces cerevisiae* (2x 10^8 CFU/ml).Flasks were covered with sterilized gauze covers and statically incubated at 30^0C in a laboratory incubator for 4dayswith intermittent manual shaking. Samples, as whole flasks in duplicate were withdrawn after each 24hrs.Thetime necessary to reach the peak of protein concentration was evaluated[5].

Result:-

Chemical analysis of fermented potato residue:-
Moisture content:-
Fermented potato residue was dried at 60^0C until its weight remained constant. The weight difference after drying was considered the moisture content.

Moisture Content:-

Item	Moisture content (%)
Unfermented potato peel	**71.7%**
Fermented potato peel	**85.51%**

Water holding capacity:-
Potato peel residue was soaked in water at 4^0C for 24hrs and centrifuged at 14000 rpm for 1hr, the supernatant was discarded, and the amount of moisture of the residual pellet was determined as water-holding capacity.

Water holding Capacity:-

Item	Water holding capacity (%)
Unfermented potato peel	85.02%

Determining pH level:-

PH level were estimated in a suspension of sample (0.5g) in distilled water (10ml). Statistical analysis of data was determined by Analysis of Variance (ANOVA) and Tukey test was applied with accepted significance of $p<0.05$.PH level was kept constant at 5.5 throughout the fermentation.

Protein content in wet biomass:-

Protein content was calculated by Folin Lowry method.

Table1:-Protein content of wet biomass of different media during fermentation.

Media	24 Hours (µg/ml)	48 Hours (µg/ml)	72 Hours (µg/ml)	96 Hours (µg/ml)
YEPD	2.2	3.5	6.2	6.2
YEPD+AS	14.2	17.7	24	26.6
YEPD+PP	21.3	23.1	27.55	30.2
PP+AS	28.4	34.6	40.8	45.3

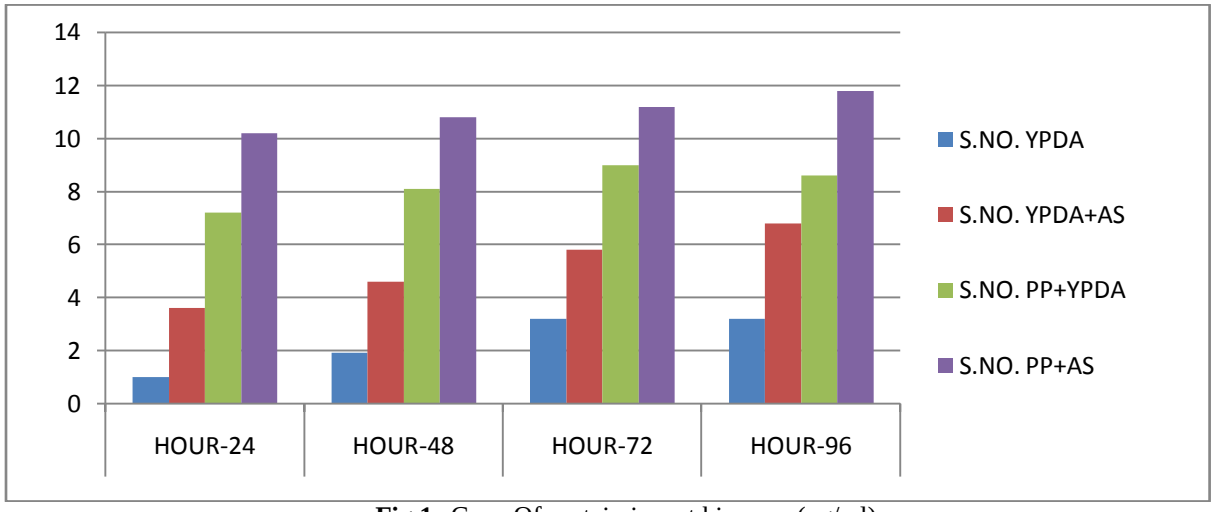

Fig 1:-Conc Of protein in wet biomass (ug/ml)

The protein content of YEPDA was lower than the protein content in the substrate supplemented with ammonium sulphate during solid state fermentation for four days by *Saccharomyces cerevisiae*. But there was constant increase of protein content in the potato peel supplemented with and without ammonium sulphate as compared to synthetic media. With low concentration (2.2µg/ml) of protein in synthetic media during first 24hr of fermentation, effect of nitrogen supplementation was examined as shown in fig-1. Supplementation with 2.5% of ammonium sulphate, results in the enrichment of protein level (45.3µg/ml) of potato peel as compared to concentration (21.3µg/ml) of potato peel without ammonium sulphate during solid state fermentation by *Saccharomyces cerevisiae*. This shows that ammonium sulphate have been utilized as a source of nitrogen which results in the increase in protein content of the potato peel during fermentation.

Protein content in dry biomass:-

Table2:-Protein content in dry biomass of different media after fermentation

Media	96 Hours (µg/g)
YEPD	7.1
YEPD+AS	29.3
YEPD+PP	34.6
PP+AS	49.7

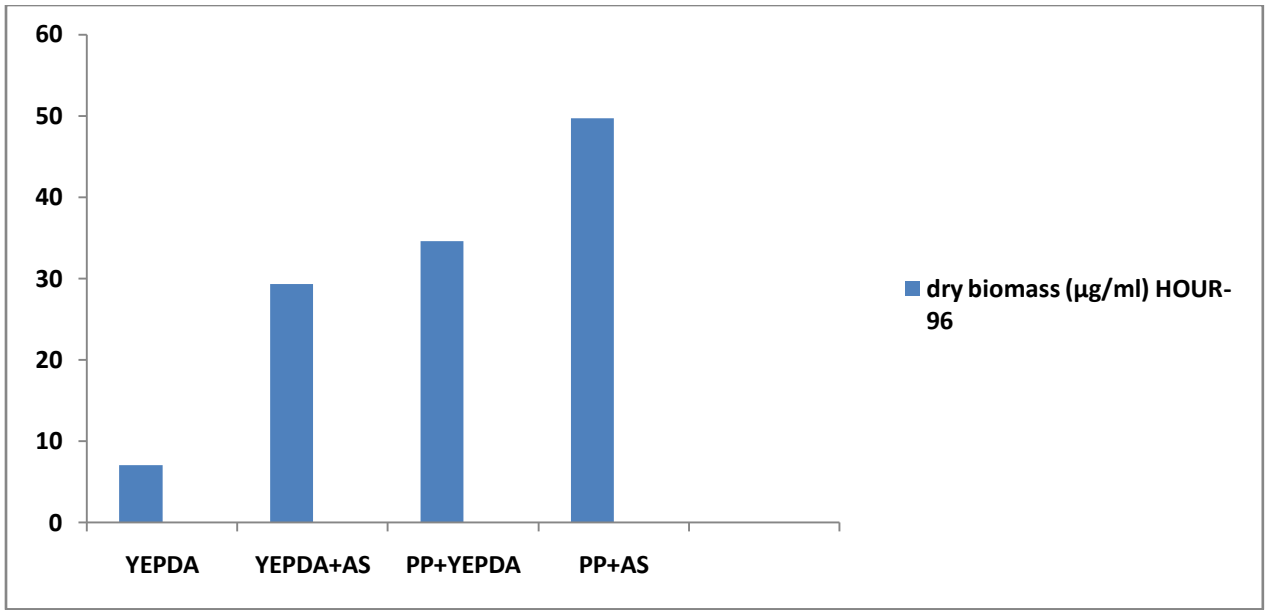

Fig 2:-Conc of protein in dry biomass (96-hrs) (ug/ml)

The final dried biomass of substrate after 96hrs of fermentation results in the overall increase in the protein content as compared to wet biomass. Protein content in the final bioprocessed product reached 49.7µg/ml when supplemented with ammonium sulphate. Without supplementation of ammonium sulphate, bio processed sample reached around 34.6µg/ml in the dried biomass. This shows that *Saccharomyces cerevisiae* have utilized ammonium sulphate, source of nitrogen for protein enrichment.

Discussion:-

The results obtained from this study revealed that fermentation can bring about desirable changes in the nutrient composition of potato peels. From this study, yeast showed potential to increase the proteincontent of the potato peel mash. The yeast *S. cerevisiae* demonstrated the best ability to enrich the peel mash in four days. The peels when fermented with *S. cerevisiae* had an improvement to18.62%[4]. This implied that yeast had significant ($P< 0.05$) effect on the protein content. The increase in the crude protein observed could be attributed to the additional crude protein (extracellular enzymes)such as amylases production from the fungal mycelia[9-12] and thus secreted into the supernatant of fermenting mash inorder to make use of the starches as a carbon source[13].Furthermore, increase in the growth of the microorganisms in the fermenting potatopeel mash may possibly account for increase in the protein content of the fermented peel mash as reported by some other workers[14,15]. Similar results using sweet potato have been reported by[8, 16]. An optimum temperature and pH range of 25^0C and 5.5 respectively supported the highest crude protein formation when *S. cerevisiae* was grown on the potato peel mashes. This finding is in agreement with that of[17] who reported a temperature range between 25^0C and 30^0C to be favorable for the growth of mostyeast. Similar findings were also reported by[14]. This observation further confirms that the increase in crudeproteins observed is as a result of an increase in cell mass generated by the organism.Therefore, the commercial utility of protein enrichment of potato peel residue with *Saccharomyces cerevisiae* by solid state fermentation for animal feed appears to be promising as described by[18]. The water holding capacity of potato peel residue was 85.02%. The moisture content of the unfermented potato peel substrate was 71.7% which was lower than the water holding capacity. Whereas, the moisture content of fermented potato peel was (85.51%) lower than the moisture content in potato peel supplemented (86.89%) with ammonium sulphate. Similar findings were also reported by[7].

Conclusion:-

The results obtained in the study have shown that growing of yeast on potato peel mash can enrich its protein content. This could be attributed to the ability of *Saccharomyces cerevisiae* to utilize ammonium sulphate as a source of nitrogen in protein enrichment, apart from this, to make use of potato peel carbohydrate as a source of carbon. The increase in the microbial biomass in the potato peel supplemented with ammonium sulphate account for the increase in the protein content by yeast during fermentation.

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