The Journal of Animal & Plant Sciences, 21(4): 2011, Page: 844-849
ISSN: 1018-7081

COMPARATIVE ASSESSMENT OF VARIOUS AGRO-INDUSTRIAL WASTES FOR *SACCHAROMYCES CEREVISIAE* BIOMASS PRODUCTION AND ITS QUALITY EVALUATION AS SINGLE CELL PROTEIN

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ABSTRACT

People in 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 present study was planned to assess the feasibility of using agro-industrial wastes for *Saccharomyces cerevisiae* production and to evaluate protein quality of produced single cell protein (SCP) biomass. Potato peels contained significantly highest dry matter and carbohydrate content as compared to other wastes. Significantly higher ($P=0.00$) SCP biomass was produced using potato peels followed by carrot peels. On the basis of higher SCP biomass production, potato peels were selected for further biomass production. The SCP biomass contained 49.29±1.126% crude protein which was non-significant ($P=0.1710$) compared to commercially available *Saccharomyces cerevisiae.* The parameters for *in-vivo* protein quality assay in Sprague Dawley rats were; 93.68% true digestibility, 67.02% net protein utilization, 70.56% biological value, 4.55net protein ration, and 2.75 protein efficiency ratio, which are higher in comparison to most of cereal proteins. The present exploration depicted that *Saccharomyces cerevisiae* can be efficiently produced utilizing wastes and the produced biomass can potentially be used as protein source in various food formulations.

Keywords: *Saccharomyces cerevisiae*, protein quality, biomass production.

INTRODUCTION

Agricultural activities and food industry generate considerable quantities of wastes which are rich in organic matter and could constitute new materials for value added products. To this effect, their valorization by the biotechnical processes represents a solution of choice insofar biomass production. A large quantity of solid waste is generated from fruit processing industries. Among these, the orange production is predicted to reach 66.4 million tons by 2010 (Talebnia, 2008). Around half of this amount after extraction of the juice consists of orange peels, segment membranes and seed. The peels hold a range of carbohydrate polymers; makes it idyllic as a source of renewable energy through anaerobic digestion (Talebnia, 2008). Apple pomace, including peels and seeds constitute 25–35% of the quantity of the processed apples (Joshi, 1998; Maini and Sethi, 2000). Besides other nutrients, apple pomace is a rich source of pectin and is being used as a natural substrate for pectinase production.

Similarly, potatoes are one of the most important staple crops for human consumption. Potato peels are good sources of quality plant carbohydrate. Regarding citrus fruits, it is estimated that out of total annual domestic production (2.02 Million MT) 1.7 Million MT is available for consumption after post-harvest losses (PHDEC).

The continued population growth especially in developing and third-world countries is resulting in increased food demand in parallel and is posing serious threats to food security due to yawning gap in demand and supply (Anupama and Ravindera, 2000). Chronic malnutrition and hunger are typically most prevalent in developing countries. Malnutrition is a consequence of not taking appropriate amount or quality of nutrients comprising diet. The gap b/w demand and supply is expected to grow unless planned actions are taken to improve the situation. Therefore it is essential to search for un-conventional or novel proteins to supplement the available sources.

There have been studies as well as efforts to improve the protein quantity and quality of the finished food products by augmenting protein-rich cheaper ingredients in food formulations (Nasir and Butt, 2011; Hussain *et al.,* 2007). Although animal proteins are considered to be best quality proteins (Saima *et al*., 2008), however microbial protein also known as single cell protein grown on agricultural wastes is one of the important optional proteins because of higher protein content and very short growth cycle of microorganisms, thereby, leading to rapid biomass production (Bekatorou *et al*., 2006) Moreover, microbes are also able to grow on cheap nutrient sources resulting in economical, potentially supplemental protein biomass for balanced nutrition.

Yeast (*Saccharomyces cerevisiae*) is the most promising source to produce single cell protein using cheap raw materials. It is also easy to harvest due to bigger cell size and flocculation ability with lower amount of nucleic acids compared to bacteria (Wolf *et al*., 2003). The microbial protein has also been reported to contain better percentage of essential amino acids and better chemical score than soya protein (Lyutskanov, 1990).

The potential importance of yeasts in food formulations to improve human nutrition and to meet the demands in a world of low food production and rapidly growing population depicts the significant magnitude of yeast production. Keeping in view these facts and importance of single cell protein, the present study was planned to assess the potential utilization of some agricultural wastes to produce single cell protein and to determine the biological quality of produced biomass for food value-addition.

MATERIALS AND METHODS

Procurement of raw materials: Four agricultural products i.e. potatoes, apples, carrots and oranges samples were procured from the local market of Lahore. The peels were separated, oven dried, ground and sieved through 1-mm mesh screen. The samples thus prepared were packed in transparent Zip-lock polythene bags and stored at room temperature until further study.

Chemical analysis: The peel samples were analyzed for dry mater, crude protein, crude fat, crude fiber, ash and nitrogen free extract by following their respective procedures (AOAC, 2006).

Biomass production: The agro-industrial wastes (40 g of each waste) i.e., potato, orange, carrot and apple peels were used as a substrate for production of single cell protein. The industrial waste was degraded to convert cellulose content into more available sugars by chemical treatments with little modification to the procedure described by (Lenihan *et al.*, 2010). Fifty milliliters of 10 % (w/v) hydrochloric acid was added to the each waste (40g) in a 250 mL conical flask respectively. The solution was placed in water bath at 100°C for one hour. After being allowed to cool, it was filtered through wattman filter paper. The solution/broth that has been obtained was diluted 100 mL with distilled water. The broth was adjusted to pH 4.5 with 2.5 M sodium hydroxide. It was plugged with cotton wool. The solution was then autoclaved in an autoclave at 121°C for 20 minutes.

Saccharomyces cerevisiae solution (2 %) was prepared by dissolving one gram of *Saccharomyces cerevisiae* in 50 mL of distilled water. All flasks containing different sterilized media were cooled at room temperature and inoculated with 1.0 mL of *Saccharomyces cerevisiae* stock culture (Adoki, 2008). The inoculated flasks were then incubated in an incubator at 37 °C for 5 days. Biomass production was determined by filtering the sample through a 0.2-μm filter paper and then drying at 105 °C.

Functional properties: Functional properties such as water and oil absorption capacities, foaming properties (Narayana and Narasinga, 1982), least gelation concentration (Sathe and Salunkhe, 1981) and bulk density (Eggum, 1973) of single cell protein were determined according to their respective procedures.

Biological evaluation: Quality evaluation of single cell protein was done through feeding diets namely; single cell protein, casein and no-protein diets separately to three groups of rats (Table 1). Thirty weanling male Sprague Dawley rats were housed in animal room of the University of Veterinary & Animal Sciences, Lahore. After feeding on basal diet for one week, rats were randomly divided into three groups, ten in each. The groups were fed separately on isonitrogenous (10% protein) SCP and casein diets and no protein diets for 10 days (Babajide *et al*., 2008). Temperature (23±2 ºC) and relative humidity (50±5 %) with 12- hrs light-dark cycle were maintained throughout the experimental period. At the end of biological trial, overnight fasted rats were decapitated and their bodies were dried. The spilled diet, feces, urine and dried rats' bodies were subjected to nitrogen analysis. True digestibility, net protein utilization, biological value, net protein ratio, protein efficiency ratio and feed efficiency ratio were calculated as described by (Pellet and Young, 1980).

Statistical analysis: The data for chemical composition and biomass production was statistically analyzed using ANOVA technique under CRD (Cohort-CoStat-2003 software version 6.33) (Steel *et al*, 1997). Mean comparison was performed with Duncan's Multiple Range test and the level of significance was defined as $P \leq 0.05$. However, *t-test* was used for statistical comparison of the data for chemical composition of produced SCP biomass with commercially available biomass and protein quality of SCP biomass with casein protein.

RESULTS AND DISCUSSION

Composition of agro-industrial wastes: Chemical composition of wastes is summarized in Table 2. The composition of all the waste samples was significantly different for all the tested parameters i.e. crude protein, crude fat, crude fiber ash content and NFE. Orange peels contained highest content of crude protein and fat content and less content of ash and NFE as compared to potato waste peel samples. Apple waste was found to be the best source of available carbohydrates in contrast to protein and minerals (ash), which were lowest compared to other waste samples. Potato peels contained NFE and ash contents in significant amounts with comparable crude protein and lowest fat contents compared to other wastes which were evaluated in this study. This study was planned to assess the potential of various wastes for cost-effective yeast biomass production to be used as protein source in subsequent trials. The yeast biomass production depends on multiple factors and the most important of all are the carbohydrates and mineral contents. The findings of the present study for proximate chemical composition of wastes are corroborated with the results of various other studies (Veronika *et al*., 2009; Figuerola *et al*., 2005). However, there are other studies that have reported variable results from the findings of present investigation. In fact, these differences are expected owing to varietal, cultivar, environmental and soil condition differences (Barta, 2002).

Biomass yield and composition: Yeast biomass produced on different wastes (g/40g of waste) is presented in Figure 1. The biomass production on different wastes was significantly different ($P<0.01$). Momentously higher quantity of yeast biomass was obtained on potato wastes (5.29/40g of waste) followed by carrot waste while lowest amount of biomass was yielded on apple waste. Potato peels contained higher amount of available carbohydrates and minerals, while significantly lower content of fiber (Table 2) which might have favorably affected yeast biomass production. This type of composition has been reported to enhance biomass production (Lenihan *et al*., 2010). Though, orange peels contained the highest conc. of carbohydrates but supported less biomass production. This explains the importance of mineral content which were less in orange peels and hence resulted in lower growth of microbial biomass. Another possible reason of lower growth on orange peels might be due to higher crude fat content especially limonene (antimicrobial); makes hindrance in digestion process of microbes thus depriving yeast cells from essential nutrients (Talebnia, 2008).

Composition of produced biomass in comparison to commercial yeast biomass is presented in Table 3. There were no significant differences ($P>0.05$) in dry matter and proximate composition of produced yeast biomass compared to commercially available *Saccharomyces cerevisiae* (yeast). Though there was difference in chemical composition of various components of the yeast however, the differences were found to be non-significant. The findings suggest that experimentally produced yeast can efficiently be used in food formulations because of being economically cost effective and having reasonable quantity of protein content (49.29±0.126%). Thus it can be used to supplement existing sources of proteins and to enhance protein security for vulnerable groups in developing or under-developed countries. Similar findings have also been reported earlier by Paryad and Mahmoudi (2008).

Table 1. Formulation of diets (dry matter basis) per 100 grams.

Ingredients	Casein Diet	No Protein Diet	Single Cell Protein Diet
Corn oil	5.0	5.0	5.0
Mineral mixture	5.0	5.0	5.0
Vitamin mixture	1.0	1.0	1.0
Casein	11.0	0.0	0.0
Single cell protein	0.0	0.0	22.6
Corn starch	74.0	84.0	61.4
Pectin	5.0	5.0	5.0

Table 2. Proximate composition (Mean ± S. D) of various wastes samples

Peels	Proximate components (%)				
	Crude Protein	Crude fat	Crude fiber	Ash	NFE
Potato	5.14±0.22^{b}	1.10±0.305^{c}	3.47±0.571^{b}	7.96±0.71^{a}	82.32±1.68 a
Orange	10.13±1.77^{a}	4.09±0.25^{a}	7.24±0.90^{a}	3.64±0.82^{b}	74.88±3.11 b
Carrot	8.69±0.39^{a}	1.05±0.22^{c}	8.60±0.81^{a}	9.07±1.06^{a}	72.57±2.95 b
Apple	1.60±0.55^{c}	2.76±0.27^{b}	7.85±0.43^{a}	1.73±0.81^{c}	86.05±0.97 a

The values in a column with different letters are significantly different ($P\leq0.05$)

Functional properties of SCP biomass: Functional properties of ingredients are the important attributes which must be kept under consideration, while formulating recipes. These properties depend on many factors like protein, carbohydrates, interaction & quality of components and processing methods. These properties also depict the use & behavior of the substance to be used as ingredient in various food formulations; considerably affecting the quality and acceptability of end products. Results of various functional properties of produced yeast biomass are presented in Table 4 & 5. The results describe excellent water holding capacity (303.40±0.30 g/100g), oil absorption capacity (196.50±0.20 g/100g) and gelling properties (complete gelation at 0.70 g/20mL). Similar findings have been reported by Nirmala *et al*. (1992) for *Spirulina platensis spp*. These outcomes

Table 3. Composition (Mean±S.D) of commercial and produced yeast biomass on dry weight basis

Parameters	Commercial	Produced	P(t=0)
Moisture (%)*	2.40±1.385	4.25±2.684	0.1369 ns
Dry mater (%)*	97.60±1.385	95.75±2.685	0.1369 ns
Crude protein (%)	49.06±0.098	49.29±0.126	0.1710 ns
Crude fat (%)	2.95±0.635	2.77±0.822	0.4688 ns
Crude fiber (%)	2.72±0.218	3.18±0.047	0.0737 ns
Ash (%)	4.20±1.056	5.24±0.806	0.4194 ns
Nitrogen free extract (%)	41.08±1.338	39.51±0.510	0.2348 ns

ns = non-significant *values are on as such basis

Table 4. Functional properties of produced yeast SCP biomass

Parameter	Value (Mean±S.D)
Water holding capacity (%)	303.40±0.30
Oil absorption capacity (%)	196.50±0.20
Foaming capacity (%)	10.60±0.20
Loose bulk density (g/ mL)	0.65±0.01
Packed bulk density (g/ mL)	0.66±0.01

Table 5. Gelation potential of various concentrations of yeast SCP biomass

Concentration of SCP Biomass	Appearance
0.10 g/20mL	No gelation
0.20 g/20mL	No gelation
0.30 g/20mL	No gelation
0.40 g/20mL	No gelation
0.50 g/20mL	Partial gelation
0.60 g/20mL	Partial gelation
0.70 g/20mL	Complete gelation
0.80 g/20mL	Complete gelation

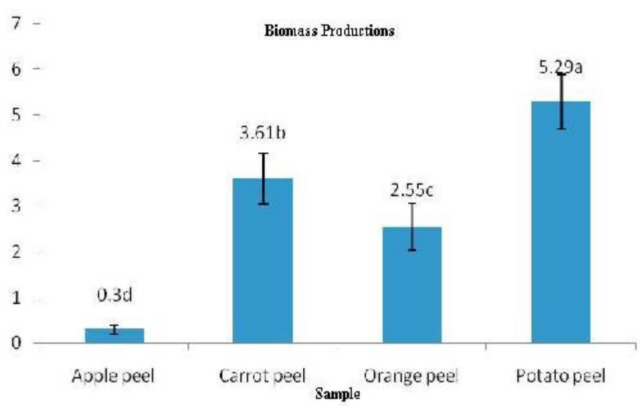

Figure 1. SCP biomass production (g/40g peels±SD) on various agro-industrial waste peels
The bar values with different letters are significantly different *(P≤0.05*)

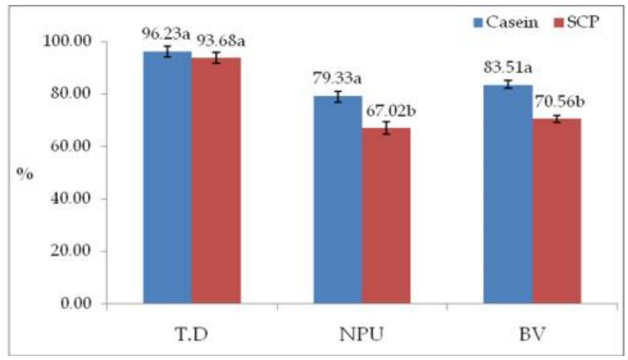

Figure 2. *In-vivo* total digestibility, net protein utilization, and biological value of produced SCP biomass and casein protein
The bar values (for individual parameters) with different letters are significantly different (*P≤0.05*)

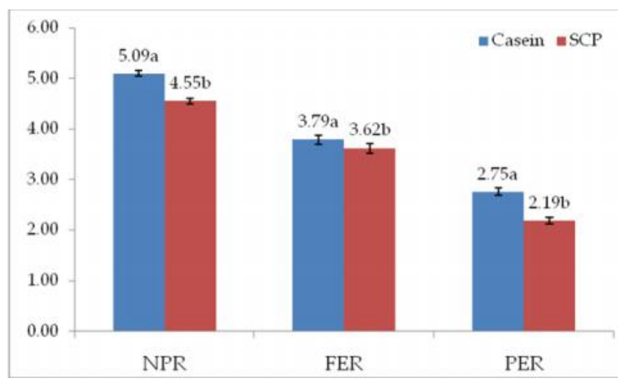

Figure 3. *In-vivo* net protein ratio, feed efficiency ratio and protein efficiency ratio of produced SCP biomass and casein protein
The bar values (for individual parameters) with different letters are significantly different (*P≤0.05*)

show the potential of SCP biomass in food products which require high water absorption capacity (like bread), increased oil retention capacity (like fried products) and more gelling capabilities (like jams). The lower bulk density of SCP biomass also portrays its potential in weaning food products where lower density of ingredients is desirable. Overall, the functionality of SCP biomass can be efficiently utilized in variety of products i.e. meat, sausages, bread, and cakes, *jalaibi, samosas* and *pakoras.*

Biological evaluation of SCP biomass: The mean values of for various protein quality biological parameters are presented in Figure 2 & 3. True digestibility (TD) of yeast biomass was found to be non-significant with (P>0.05) casein protein. Conversely, net protein utilization (NPU), biological value (BV), net protein ratio (NPR), protein efficiency ratio (PER) and feed efficiency ratio (FER) of SCP biomass were significantly lower ($P<0.05$) compared to standard casein protein. However, the results of these quality parameters of SCP are quite comparable with casein in quantitative terms. The findings of TD, NPU for yeast biomass in this study are higher than values ($82.12^b \pm 1.06$, $60.21^b \pm 0.91$, respectively) reported by Zepka *et al.* (2010) for single cell protein produced by *Aphanothece microscopica Nageli Microalgae.* The findings of the present study for casein protein quality are also consistent with those reported by Nasir. (2009) in sprawg Daley rates.

Conclusion: Potato waste (peels) was found to be the best source of nutrients to produce yeast biomass followed by carrot and orange wastes. The produced yeast biomass exhibited excellent functional properties and good *in-vivo* protein quality depicting its potential to be used in variety of food formulations to improve the quality and acceptability of finished foods. The information derived from this study can be used by food processors, biotechnologists and nutritionist to produce and incorporate yeast biomass as food ingredient.

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