Production of single cell protein using pineapple, sour orange, and sour mango peel

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ABSTRACT

Protein deficiency has become a major challenge worldwide with the fast growing world population, thus, it is important to explore novel alternative methodologies to produce protein to meet the nutritional demand. In this backdrop, locally available pineapple, sour orange and sour mango peel wastes were studied for their suitability to produce single cell proteins using natural palmyrah (Borassusflabellifer) toddy yeast under liquid state fermentation system. Pineapple, sour orange and sour mango peel were collected, cleaned, washed and blended separately and their physico-chemical properties such as total soluble solid (TSS), pH, reducing sugar, moisture content, protein, fat and ash content were determined. The extract of fruit peels were filtered and diluted to 10% and autoclaved separately. The sterilized peel extracts were inoculated with 5 mL natural palmyrah toddy yeast ((1.625±0.15) × 10^6 cells/mL) and allowed for fermentation at 100 rpm for 48 h. The sediments were collected by centrifugation, oven dried and the dry weight was measured to determine the protein content. The biomass yield ranged from 8.61-9.40 g/L with the least biomass yield was observed with sour mango while the maximum yield was observed with pineapple. Pineapple peel generates significantly higher amount of protein (49.7± 1.3%) followed by the sour orange and sour mango peel (29.5 ± 1.2% and 24.6 ± 0.2% respectively). It is concluded thatnatural and locally available pineapple peel waste are good substrate for the production of protein-rich cell biomass using fermentation by natural toddy yeast of palmyrah.

Key words: Liquid state fermentation, Palmyrah toddy yeast, Pineapple peel, Single cell protein, Sour mango peel, Sour orange peel

INTRODUCTION

Single cell protein (SCP) is the dead, dried microbial cell protein or total protein extracted from pure microbial cell cultures, such as algae, bacteria, filamentous fungi, or yeasts which grow on different substrates. SCP has applications in animal feed and human food supplements. It is used for the production of animal feed for fattening calves, poultry, pigs, and fish. It is widely used in the food industry as aroma carriers, vitamin carriers, emulsifying aids, and to improve the nutritive value of baked products, soups, ready-to-serve meals, and diet recipes (Suman, *et al.*, 2015).

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Microbial protein has become popular for its high protein content (60-82% of dry cell weight), high efficiency in substrate conversion, high productivity, and requirement forless space (Nasseri, *et al.*, 2011). Besides high protein content, SCP also contains fats, carbohydrates, nucleic acids, vitamins, and minerals. SCP has a good nutrition profile, as it is rich in certain essential amino acids such as lysine and methionine, which are limiting in most plant and animal foods (Suman, *et al.*, 2015). High nucleic acid content and low cell wall digestibility are the two most important factors limiting the nutritional value of yeast for human consumption. Purine in nucleic acid is metabolized into uric acid, and its high concentration may result in gout and renal calculi. Therefore nucleic acid content of SCP must be reduced below 2% (Nasseri, *et al.*, 2011).

Microorganisms used for the production of SCP are bacteria (*Cellulomonas and Alcaligenes*), algae (*Spirulina and Chlorella*), molds (*Trichoderma, Fusarium, and Rhizopus*), and yeast (*Candida and Saccharomyces*) (Nasseri, *et al.*, 2011). Though bacteria has high protein content and short generation time over yeast and mold, it has own limitations such as poor public acceptance, difficulty in harvesting, and high nucleic acid content. Yeast is suitable for SCP production because of its good nutritional profile, easeof harvesting, and ability to grow at low pH, and has a long history of use in traditional fermentation (Nasseri, et al., 2011; Qiang & Iong-zong, 2016).

A wide variety of raw materials can be used for SCP production. Whey, orange peel residue, sweet orange residue, sugarcane bagasse, paper mill waste, rice husk, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, grape waste, and mango waste are some examples (Mensah & Twumasi, 2017). Carbohydrate based substrates are the most widely used raw materials for SCP production due to the fact that sugars are natural microbial substrates (Ugalde & Castrillo, 2002).

Nearly 40% of fruit production goes into waste in Sri Lanka, mainly due to improper supply chain and value chain activities (Institute of Post Havest Technology , 2014). The non-edible portion of fruits, which accounts for about 10–60% of the total weight of the fresh produce,is discarded as waste. Postharvest losses of some fruits stand even higher (*e.g.*, mango 30-50%, banana 20%, orange 30-50%) (Ruvini, *et al.*, 2018). Improper management of these wastes constitutes a public health risk and an environmental problem. SCP derived from various waste materials via microbial fermentation can play a vital role in waste management (Spalvins, *et al.*, 2018). There have been very few studies reported exploring the use of fruit wastes for SCP production. This study was aimed at selecting the best substrate for liquid state SCP production among pineapple, sour orange, and sour mango peel wastes.

METHODOLOGY

Materials

Pineapple, sour orange and sour mango peel were collected from local markets in Jaffna, Sri Lanka. Palmyrah toddy was collected from mature palm in Thikkam, Jaffna, Sri Lanka.

Physico-Chemical Analysis of Pineapple, Sour Orange, And Sour Mango Peel

Cleaned and washed pineapple, sour orange and sour mango peels were weighed and the moisture, protein, fat, and ash content were determined using AOAC (2006) methods. The total soluble solids

(TSS) and pH were determined by refractometer and pH meter respectively. The reducing sugar content was determined by a spectrophotometric method (Miller, 1959).

Preparation of Fruit Waste Medium

The collected pineapple, sour orange, and sour mango peels were separately cleaned with distiled water, macerated using the blender into a slurry, and filtered through Whatman No 1 filter paper. The solid content and the pH of the extracts were determined using a refractometer (Atago – DR-A1, Japan) and a digital pH meter (Ohaus - Starter 2100, USA) respectively. The extracts were diluted to 10% using distilled water and sterilized in an autoclave at 121^{0}C at 15 psi for 15 min, seperately. These sterilized diluted peel extracts were designated as Fruit Waste Medium (FWM),and was used as substrate to select the best fruit peel for the SCP production. Fresh palmyrah toddy samples were used as the source of natural yeast, *Saccharomyces cerevisiae*. The viable cell count was determined using a haemocytometer.

Production of SCP in Liquid State Fermentation

Sterilized pineapple, sour orange, and sour mango peel substrates (50 mL) were transferred into pre-sterilized conical flasks in triplicate under sterile conditions. Then the each 50 mL of sterilized medium was inoculated with 5 mL of fresh palmyrah toddy sample ((1.63 ±0.15)×10^{6}cells/mL) and allowed for fermentation in shaking incubator (Lab companion SI-600, USA) at a speed of 100 rpm for 48 h at 28^{o}C. After 48 h, the content was centrifuged (4000 rpm for 20 min) and the residue was oven dried (50^{o}C for 16 h) and weighed. Protein content was determined on the basis of total nitrogen content (N×6.25) using Kjeldahl method as per the protocol explained in AOAC, 2006 (Dhanasekaran, et al., 2011).

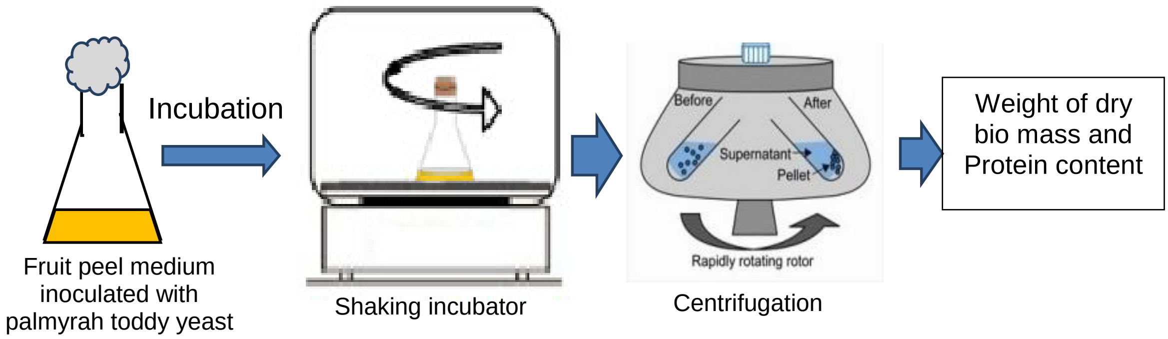

Figure 1. Flow diagram for SCP production from fruit peel

Statistical Analysis

All the experiments were carried out in triplicates and the results were presented as the average of triplicates ± standard deviation. Minitab 17 (Minitab Inc., State College, PA, USA) statistical package was used to execute all statistical analysis. The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine significant differences at $p < 0.05$.

RESULTS AND DISCUSSION

The physico-chemical properties such as pH, the total soluble solid, reducing sugar, moisture, fat, protein, and ash content of the pineapple, sour orange, and sour mango peel have been presented in the Table 1.

The composition of all the waste samples was significantly different for tested parameters such as moisture, protein, fat, ash, and reducing sugar content. Pineapple peel exhibited the highest moisture content, protein and reducing sugar content and less fat as compared to other peel sample. Sour orange contains higher amounts of protein (7.2±0.1%), while sour mango contains higher amounts of total soluble solids (16.8%) compared to other wastes. Overall results of compositional analysis indicated that the selected pineapple, sour orange, and sour mango peel can be used as potential substrates for microbial growth, due to their reasonable carbohydrate and mineral content.

Table 1: Physico-chemical properties (on dry weight basis) of pineapple peel, sour orange and sour mango peel.

Physico-chemical property	Fruit peel		
	Pineapple	Sour mango	Sour orange
pH	3.7	4.1	4.1
TSS (%)	10.8	16.8	12.3
Reducing sugar (g/L)	27.8 ± 0.9[a]	25.3 ± 2.6[a]	12.3 ± 1.3[b]
Moisture content (%)*	84.7 ± 0.9[a]	76.2 ± 0.2[b]	74.8 ± 2.5[b]
Ash content (%)*	4.5 ± 0.3[a,b]	4.2 ± 0.5[b]	6.1 ± 1.0[a]
Fat content (%)*	0.9 ± 0.1[c]	2.5 ± 0.3[a]	1.4 ± 0.1[b]
Protein content (%)*	6.9 ± 0.1[a]	6.2 ± 0.2[b]	7.2 ± 0.1[a]

Results are expressed as mean of triplicate ± standard deviation.

*Values are in dry weight basis

The values in the same row with different superscripted letters are significantly different (P≤0.05).

Most of the findings of the present study for physico-chemical composition of fruit wastes are similar to the results of various other studies (Imran, *et al*., 2013; Moraisa, *et al*., 2017). The variation in the findings of the present investigation may be due to varietal, cultivar, environmental, and soil condition differences (Barta, 2002).

Selection of the Best Substrate

This study was planned to assess the potential of various fruit wastes for cost effective biomass production. Pineapple, sour orange, and sour mango peel extract were used as fermentation media to harvest dry biomass. The dry mass and protein content were recorded (Table 2).

There is no significant difference in the dry biomass yield and the results obtained were in the range 8.61-9.40 g/L (Table 2). The least biomass yield was obtained for sour mango and the maximum yield was obtained for pineapple. The highest crude protein yield was observed in the medium containing pineapple (49.7 ± 1.3%), followed by sour orange (29.5 ± 1.2%) and sour mango (24.6 ± 0.2%) (Table 2). Based on the results, pineapple peel extract can be a reasonably suitable substrate for SCP production. The higher reducing sugar content in pineapple peel waste compared to other media favors the higher yield of SCP (Table 1).

Table 2: Dry biomass content and crude protein content of SCP from pineapple, sour orange and sour mango peel medium.

Peel type	Dry biomass (g/L)	Crude protein content (%)
Pineapple	9.40 ± 0.53[a]	49.7 ± 1.3[a]
Sour orange	9.13 ± 0.64[a]	29.5 ± 1.2[b]
Sour mango	8.61 ± 0.90[a]	24.6 ± 0.2[c]

Results are expressed as mean of triplicate ± standard deviation
Means in the same column that do not share the same superscripted letter are significantly different.

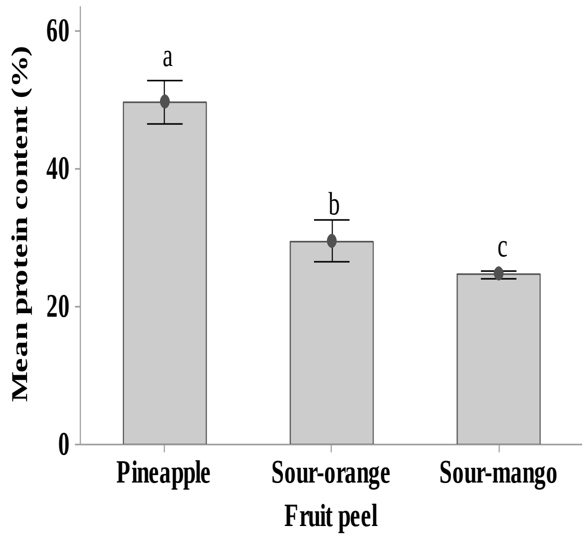

Figure 2: Crude protein content of SCP from pineapple, sour orange, and sour mango peel medium

Some of the previous studies carried out adding supplements such as nitrogen, carbon, and glucose sources were used for the biomass production on waste materials (Mondal, *et al*., 2012). No such supplements were used in the present study to grow yeast culture on selected fruit peel, thus this process of SCP production can be considered cheaper. The above results established that pineapple, sour orange, and sour mango peel extract and palmyrah toddy yeast can be used to produce SCP. This biomass could be recommended as a food or feed after appropriate food quality testing. Since fruit waste were used as substrate in the present study, it plays a vital role in waste management (Suman, *et al*., 2015).

CONCLUSION

Locally available pineapple peel waste can be suggested as the best substrate among the pineapple, sour orange, and sour mango peel for the production of SCP using natural palmyrah toddy yeast through fermentation. SCP produced using pineapple peel as the substrate resulted in 49.7 ± 1.3% of protein with a yield of 9.40 ± 0.53 g/L.

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