Production of Single Cell Protein from Banana Peel, Papaya Juice and Whey and Estimation of Protein Concentration

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ABSTRACT

Objectives: This study was aimed to produce single cell protein from possible substrate to yield high protein concentration.

Methods: All three types of samples viz banana peel, papaya juice and whey were examined for the estimation of protein concentration. The study was carried out in the Microbiological Laboratory of the Department of Microbiology, D.A.V. College, Dhobighat, Lalitpur from October 19, 2019 to November 29, 2019. The fermentation process was carried out at 32°C for 5 days and physiochemical analysis of medium was performed every 24 hours. After fermentation, the microbial biomass was extracted and protein concentration was estimated colorimetrically by modified Biuret method.

Results: Out of three samples, papaya juice had the highest yeast cell count of 136.25×10^4cells/ml followed by whey extract producing 124×10^4cells/ml and, the least cell was by banana peel of 104×10^4cells/ml. The protein concentration was estimated by the modified biuret's test. The SCP produced from papaya juice was highest with protein concentration of 23.5% followed by whey extract of 22% and, the lowest protein concentration was of SCP produced from banana peel with 16.04%.

Conclusion: The result of this study suggests that production of SCP from whey extract is considered best in terms of better yield and low cost.

Keywords: Single cell protein, Fermentation, Baker's yeast, Whey extract, Modified biuret's test

INTRODUCTION

Proteins make up about 15% of the total mass of the human body and are essential to us in many ways. They are involved in the build-up of tissue as well as in oxygen transfer in our bloodstream (hemoglobin) and the enzymes that power almost all the chemical reactions occurring in our bodies. Proteins come in many shapes and forms and are built up from amino acids. Many of these amino acids are synthesized de-novo (from scratch), but some (the essential amino acids) must be supplied through our diet. There are 9 essential amino acids, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine tryptophan, and valine (Rose, 1982). The best food for supplying the body with these essential components is through meaty protein-rich food sources such as fish, meat, and egg. Currently the rapid increase in food demand for growing population and protein deficiency has been a serious challenge worldwide. This emphasizes the search of new alternative food source having less environmental foot print, higher yield and lower cost.

Date of Submission: October 10, 2022 **Date of Acceptance:** November 20, 2022
Published Online: December 31, 2022 **DOI:** https://doi.org/10.3126/tujm.v9i1.50391

One of the possible substitute is Single cell proteins (SCP), which is a name for extracted or dried microbial cells originating from fungi, algae, yeast, actinomycetes and bacteria also collectively known as 'microbial protein'. The term 'Single Cell Protein' (SCP) was coined by C.L. Wilson in 1966 and has been used widely since that time in referring to microbial cells grown in large industrial systems as a protein source for food and feed applications (Scrimshaw,1968). However, this term refers to whole microbial biomass, i.e. to a complex mixture of proteins, nucleic acids, carbohydrates, lipids, minerals, vitamins, and other cell constituents. Furthermore, the term SCP applies not only to single-cell organisms, such as yeast, bacteria, and unicellular algae but also to coenocytic multicellular molds (Hamdan & Senez, 1992). The use of microorganisms as a protein source in human foods and animal feed has long been established. In the early days, microorganisms played an important role in producing fermented foods, such as bread, cheese, wine, yogurt, and other useful compounds. Single Cell Protein (SCP) has been under active investigation for more than 30 years, as an ingredient in animal feeds, and yeast protein has been used in varying degrees in foods and fodder.

Microorganisms utilize inexpensive carbon sources for growth to produce high quality protein (Mahmood, 2012; Yakoub & Dahot, 2010). SCP is considered as high nutritive food because of higher protein, vitamins and essential amino acids (Galvez et al.,1990) but major problems in SCP usage are high nucleic acid content, slower digestion and high cost. Nucleic acid content of SCP is the main factor that hindering its utilization as human food (Anupama, 2000). SCP has been produced from very high-quality protein from low-grade waste material with the help of microbes (Adedayo et al 2011). Carbohydrates most commonly used substrate for single-cell production, due to its renewable character (Ugalde & Castrillo, 2002). There are many other substrates for the production of SCP, conventional substrate includes fruit, molasses, starch, and vegetable waste while unconventional substrates are natural gas, methanol, ethanol, petroleum by-products, and lignocellulosic biomass (Bekatorou et al 2006).

Bacteria, yeasts, fungi, and algae are used to produce biomass. The choice of microorganisms depends on numerous criteria such as the growth of microorganisms should be fast and a broader range of materials may be considered as suitable substrates. The other criteria may be nutritional (energy value, protein content, amino acid balance), technical (the type of culture, type of separation, nutritional requirements). Candia utilis, Sccharomyscetes cerevisiae, penicillium janthinellus, Bacillus cerus and Asperigillus niger are capable of producing SCP under different culture conditions (Mondal et al 2012; Azam et al 2014). *Saccharomyces cerevisiae* was known as the most widely used eukaryotic microorganism for biological studies and applications as well as single-cell protein production (Rajagopalan & Krishnan, 2008). It has fermentative capabilities to utilize sucrose, glucose, fructose, and maltose as carbon sources and produce alcohol under anaerobic conditions (Thammasittirong et al 2013). It is sensitive to high amounts of glucose, also termed Crabtree-positive, exhibiting aerobic ethanol production in the presence of excess glucose (Nishino et al 2015). It can be grown in either liquid medium or on solid-state fermentation cultures. The nutritional requirement of this microorganism is including dextrose (glucose) as a carbon source and salts that supply nitrogen, phosphorus, and trace metals.

Hence, the main objective of this study was to produce SCP from papaya juice, whey extract and banana peel using baker's yeast and estimation of protein concentration and, to determine which of the substrate is efficient in terms of yield, protein concentration and cost.

MATERIALS AND METHODS

A cross sectional study was carried out in the Microbiology laboratory of the department of microbiology, DAV College, Dhobighat, Lalitpur from October 19, 2019 till November 29, 2019. The production of single cell protein was done from various substrates which were studied, compared, and analyzed using physicochemical parameters.

Collection of substrates and culture: Papaya, banana were collected from ason market and whey was collected from dairy market of ason. The bakers yeast was brought from big mart, jhamsikhel.

Preparation of medium: The papaya was washed, peeled, cut and blended then passed through 6-fold muslin cloth. Banana peel was taken and kept on 10% KOH and heated at temperature of 100°C for 1 hour and cooled (Kamal & Mondal, 2019) then finally filtered through 6-fold muslin cloth. The whey was boiled at 100°C for 15 minutes and filtered through 6-fold muslin cloth. Then 1gm of KH_2PO_4, 0.4gm of NaCl and 0.1gm of $CaCl_2$ were added to papaya juice and banana peel filtrate (Nasseri et al 2011). 0.8gm of $(NH_4)_2SO_4$ was added to whey extract described by Claydon & Bechtle (1971). Each of the medium was transferred into sterile conical flask and, they were sterilized by autoclaving at 121°C at 15 psi for 15 minutes.

Inoculation of Baker's Yeast: About 0.5g of baker's yeast was inoculated on each conical flask containing banana peel medium, papaya juice medium and, whey medium. To prevent contamination, the conical flask was tightly sealed by cotton plug.

Fermentation: The submerged fermentation process was carried out in an incubator maintained at the temperature 32°C. Periodic shaking of the flasks was done at intervals of 24 hours.

Physiochemical analysis of the medium: At every 24 hours interval measurement of pH, TSS and cell counts were performed described by Funtes (2014) and calculated using following formula.

Cells/ml= (no. of cells counted on squares ×dilution factor)/(no of squares) × 104

Storage of fermented product: After fermentation, the flasks were stored at refrigerated temperature to reduce microbial contamination and stop further fermentation of the medium.

Extraction of microbial biomass: After completion of the batch of fermentation, the fermented liquid was poured into a centrifuge tube and centrifuged at 4000rpm for 20 minutes. The sediment was collected and the supernatant was discarded. Again distilled water was added and centrifuged at 4000 rpm for 20 minutes. Then the supernatant was discarded and the sediment was oven-dried at 50°C for 16 hours then cell dry weight was measured.

Weighing of dried microbial cell: The dried microbial cell/ *Saccharomyces cerevisiae* was weighted by using a weighing balance and calculated.

Dry cell weight = weight of centrifuge tube with sediment – the weight of centrifuge tube

Determination of protein concentration: The protein content of the biomass was measured colorimetrically following the modified biuret's method described by Herbert et al (1971), and Kamal & Mondal (2019).

% Protein = (X(mgL-1)×OD of sample)/(weight of sample(g))×100

Here, X = amount of protein (mg/L) from standard curve equation

RESULTS

Changes in pH during fermentation: The initial pH of the banana peel, papaya juice, and whey extract mediums were 6.1, 5.8, and 5.2 which gradually decreased to 4.4, 4.2, and 4.3 respectively till 5th day. The result of this study is shown in figure 1.

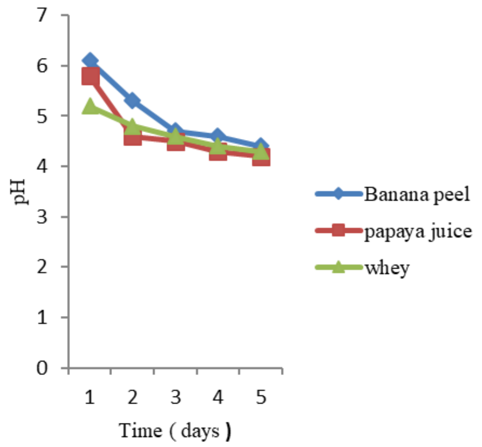

Figure 1. Change in pH during fermentation.

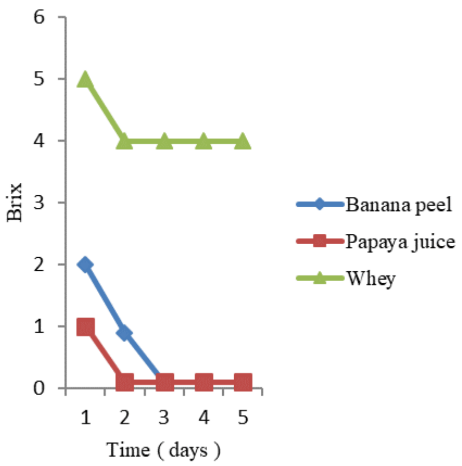

Figure 2. Changes in TSS during fermentation.

Changes in TSS during fermentation: The initial TSS of banana peel, papaya juice, and whey was 2, 1, and 5 which decreased to 0.1, 0.1, and 4 respectively till the 5th day of fermentation as shown in figure 2.

Changes in Microbial cell/ *Saccharomyces cerevisiae* count during fermentation: The initial *Saccharomyces cerevisiae* count in banana peel, papaya juice, and whey extract medium was 41.25×104 cells /ml, 44.25×104 cells/ml, and 43×104 cells/ml which rapidly increased and reached to the top of 104×104cells/ml, 136.25×104 cells/ml and 124×104cells/ml then decreased to 52×104 cells/ml, 104.5×104 cells/ml and 98.75×104 cells/ml till 5th day of fermentation as shown in figure 3. The highest cell count was obtained on the 3rd day of fermentation.

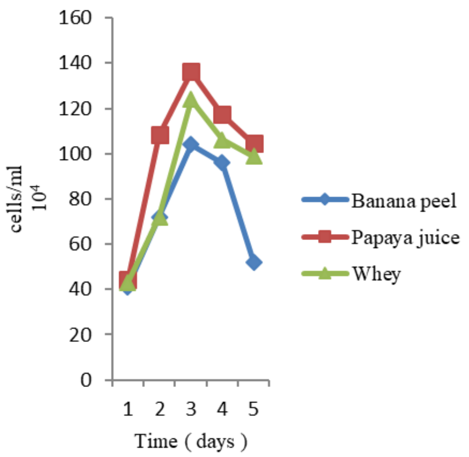

Figure 3. Chnages in Microbial cell/ *Saccharomyces cerevisiae* during fermentation

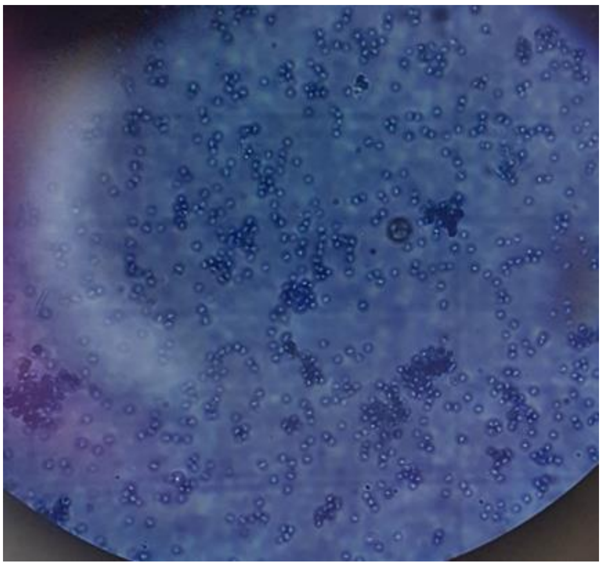

Figure 4. Cell count of *S. cerevisiae* cells/ml using Neubauer chamber

Determination of protein concentration: The protein concentration of *Saccharomyces cerevisiae* produced from the banana peel, papaya juice, and whey extract medium was 16.04%, 23.5%, and 22% respectively. The highest protein concentration of SCP was isolated from papaya juice with 23.5 % followed by whey extract with 22% and banana peel with 16.04 % of protein. The result of this study is shown in figure 4.

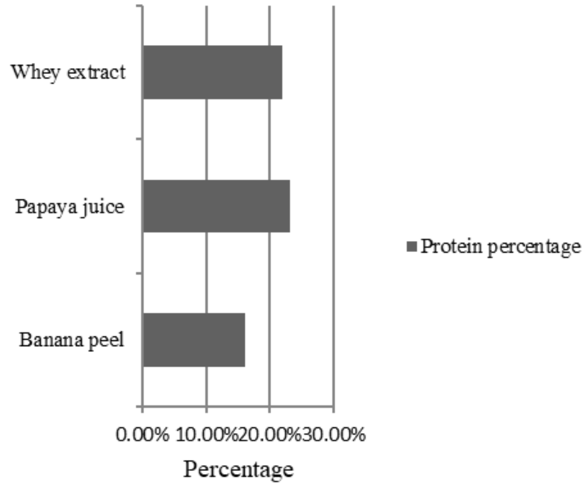

Figure 5. Determination of protein concentration

DISCUSSION

Various physiochemical analyses were performed during production of SCP. One of the analysis was measurement of pH. During fermentation, there was a gradual decrease in pH. The initial pH of banana peel, papaya juice, and whey extract was 6.1, 5.8, and 5.2 respectively. After 24 hours of fermentation, there was no significant change in pH but after 2 days of fermentation, the pH started to decrease gradually and reached 5.3, 4.6, 4.8 for banana peel, papaya juice, and whey extract respectively. Leahu et al., (2013) studied the physicochemical parameters of papaya and banana. The decrease in pH was due to an increase in acidity. Coote & Kirsop (1976) studied the factors responsible for the decrease in pH during fermentation and reported the pH continues to drop for a variety of reasons. Yeast cells take in ammonium ions (which are strongly basic) and excrete organic acids (including lactic acid). Carbon dioxide, which gives a relatively weak acid in solution, and the organic acids are the major acidic molecules known to be excreted by yeast during fermentation. It has been suggested by the studies of Burger et al (1952) that the removal of buffering substances is partly responsible for the change in pH during fermentation, the inference being that the acidic substances of the substrate then give rise to a higher hydrogen ion concentration in the less well-buffered solution.

There was a gradual decrease in TSS during fermentation. Then after a point the TSS remained constant till the end of fermentation. . The TSS initially measured was 2, 1, and 5 Brix for banana peel, papaya juice, and whey extract respectively. After 1 day of fermentation, there were no significant changes but it started to decrease from the second day of fermentation to 5.3, 0.1, and 4 Brix for banana peel, papaya juice, and whey extract respectively. The TSS remained constant for papaya and whey from the second day of fermentation till the fifth day. The TSS of banana peel decreased up to 4.4 till the fifth day of fermentation. As demonstrated by (Abrol & Joshi ,2011) the total soluble solids(TSS) value, as measured using a refractometer, is an approximate measure of the sugar content in the substrate, whereas the reducing sugar value has higher precision. The sugar content measured as the total soluble solid (TSS) and reducing sugar content decreased as the fermentation time increased. When the TSS value remains constant it indicated completion of fermentation as there is no sugar remaining for utilization by microorganisms for the production of fermentation products. The higher decrease in TSS during initial fermentation is attributed to the higher ferment ability of different substrates because of more availability of sugar and less ethyl alcohol in the medium. With the increase in time, however, the ethanol content increased exerting an inhibitory effect on the ferment ability (Nishino et al 1985). Therefore, the trend of ethanol increase or TSS fall during fermentation is natural as discussed earlier in other wines also (Amerine et al 1980).

From 24 hours of fermentation, there no was significant increase in cell count of *Saccharomyces cerevisiae* for all the substrates. There was rapid increase in cell count during fermentation and reached to the top and then started to decrease significantly, Since the *Saccharomyces cerevisiae* cells/ml was resulted highest in the third day of fermentation in all of the substrate i.e Banana peel, papaya juice, and whey extract. where the *Saccharomyces cerevisiae* cell count was the highest in all substrates that are 104×104 cells/ml for banana peel, 136.25×104 cells/ml for papaya juice, and 124×104 cells/ml for whey extract.

The third-day fermentation is used for the production of single cell protein because the cell count is highest on the third day of fermentation and we can yield the highest amount of protein from each microbial cell. Yeast grows exponentially after a brief lag phase. They absorb dissolved oxygen and use various minerals that exist within the substrate to build up cell walls. Yeast then begins splitting, increasing cell count for the conditions they are in. The sugars and assimilable nutrients are also quickly consumed resulting in carbon and nutrient deficit, allowing the cell to remain in a dormant state, a common response to nutrient depletion in yeast cells then cell count starts to drop (Gibson et al 2007).

After the fermentation, the protein concentration from the various substrates was calculated. The protein concentration for banana peel was calculated as 16.04%, papaya juice was 23.5% and, 22% for whey extract. The papaya juice had the highest protein concentration of 23.5% followed by whey extract with 22% and the lowest was of the banana peel with 16.04% of protein. These changes in protein concentration were due to the presence of a large amount of sugar and nutrients in papaya juice than other substrates which allow the yeast cell to multiply rapidly and produce a large number of cells.

Among all the three substrates used whey is the most suitable type of substrate used for the production of single cell protein in terms of cost and quality. Even though the papaya juice yields a large number of yeast cells than whey extract the cost of papaya juice is higher than whey. Papaya is also seasonal fruit and can only be found at a certain period. Whey is a waste product from the dairy industry and can be available easily. It produced more yeast cells than banana peel as well. So whey extract is the comparatively more suitable substrate used for SCP production.

CONCLUSION

In this study, it was explored that the *Saccharomyces cerevisiae* or bakers yeast can be used for the production of Single cell protein using various substrates i.e banana peel, papaya juice, and whey extract. With the analysis of cost and production efficiency, the whey extract can be used for the production of Single-cell protein since they are low in cost and available easily than papaya juice and banana peel. It produced a comparatively high yeast cell count by fermentation. Therefore, the production of single cell protein using whey extract as the substrate can be the upcoming protein feed using *Saccharomyces cerevisiae*. Since the *Saccharomyces cerevisiae* are widely used in the food industry people wouldn't be scared of consuming the Single Cell Protein produced from them. Also, in the light of protein shortage, microorganisms offer many possibilities for protein production. They can be used to replace totally or partially the valuable amount of conventional vegetable and animal protein feed. For this, the development of technologies to utilize the waste products and food products would play a major role in the production of SCP.

ACKNOWLEDGEMENTS

We would like to express our sincere immense gratitude towards D.A.V. College for providing us opportunity and the laboratory facilities needed for the completion of this study. We would also like to thank our Co ordinator of the Department of Microbiology, D.A.V. Mr. Shashi Bhushan Chaturwedi for his continued support and encouragement without which, this would not have been possible. Lastly, we would like to appreciate our teachers who provided us constant guidance and support during our work.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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