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Single Cell Protein Production from Papaw and Banana Fruit Juices Using Baker's Yeast

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Abstract: There has been an increasing world population that results the need of increase in the food production. Increased protein demand led to the search for new and cheap protein supplements than conventional protein. Single Cell Protein (SCP) is one of the solutions for the quality protein. This study was aimed to produce SCP using baker's yeast from banana and papaw fruit juices in the Liquid State Fermentation (LSF) system. Fermentation media composed of papaw/banana fruit juices 100ml/L (10%), $MgSO_4$ 0.5g/L, NaCl 0.1g/L, $CaCl_2$ 0.1g/L and KH_2PO_4 1g/L. This was inoculated with 0.5g/50mL of baker's yeast (pure culture of *Saccharomyces cerevisiae*). Before optimization conditions, crude protein yielded 33.6% and 33.41% for papaw and banana as sole medium respectively after 24 hours of fermentation at 28°C with 100 rpm in a shaking incubator. The culture growing conditions of the fermentation processes were optimized individually, higher crude SCP yield was obtained after 72 hours of fermentation at 30°C using 40.36 % and 40.29% for papaw and banana media respectively. When the carbon source was replaced with 5% of each fruit juices, the crude protein yield was increased to 41.8% and 40.87% for papaw and banana medium respectively. Comparative study revealed that papaw medium yielded significantly higher protein content (1.244 times) than that of the banana medium (1.22 times) with baker's yeast, after the optimization. This study revealed that papaw and banana fruit juices could be used as effective carbon sources to produce SCP using baker's yeast.

Key words: Baker's Yeast · Banana Fruit Juice · Papaw Fruit Juice · Single Cell Protein

INTRODUCTION

Currently protein deficiency has become serious challenge worldwide and this emphasizes the search of new alternative protein from natural, easily available and cost effective sources. Single Cell Protein (SCP) is one of the solutions to meet the world protein demand. The SCP refers to dead, dry microbial cells from microbial cell culture [1]. SCP can be produced from different microorganisms like microalgae, fungi, yeast and bacteria [2]. Microorganisms utilize inexpensive carbon sources [3, 4] for growth to produce high quality protein. SCP is considered as high nutritive food because of higher protein, vitamins and essential amino acids [5] but major problems in SCP usage are high nucleic acid content and slower digestion. Nucleic acid content of SCP is the main factor that hindering its utilization as human food [6, 7]. SCP has been produced from Sugarcane bagasse [8, 9], pineapple waste [10], banana waste [11], vegetable oils [12], Chinese potato [13], papaya extract [14] and orange & cucumber peel [15, 16]. Diverse microorganisms such as *Candida utilis, Saccharomyces cerevisiae* [17, 18], *Penicillium janthinellum* and *Candida tropicalis* are capable of producing SCP under different culture conditions. Hence, the objective of the study was to produce SCP from cheap papaw and banana fruits using baker's yeast and to improve the yield by optimization of culture conditions and fermentation media. In this study, papaw and banana fruits were used as promising carbon sources for fermentation by baker's yeast.

MATERIALS AND METHODS

Collection of Substrate and Culture: Banana (Local variety- "Kathali") and Papaw (Variety- "Red lady") fruits used in this study were obtained from local market at Thirunelvelly, Jaffna, Sri Lanka. Baker's yeast was bought in the local food city.

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Preparation of Fruit Juice: Ripen banana and papaw fruits were taken and fruits were washed with sterile water and peeled off. Seeds were removed from sliced pulp and Pulp was macerated in a blender. The fruit juice was obtained from filtered with the use of Muslin cloth. The extracted juice was placed in a sterile container and both juices were autoclaved at 121°C for 15 psi and 15 minutes.

Medium Preparation and Fermentation: Papaw and banana medium were prepared with the following composition approximately (Papaw/banana juice 100ml/L, KH_2PO_4- 1.0g, $MgSO_4.7H_2O$-0.5g, NaCl-0.1g, $CaCl_2$-0.1g and distilled water 900ml). Each of the medium was transferred into sterile bottles and autoclaved at 121°C for 15 psi and 15 minutes. Then 50 mL of each medium was distributed into 250 mL separate Erlenmeyer flasks with triplicates. This was inoculated with 0.5 g of baker's yeast. Then inoculated flasks were placed in a shaking incubator at 100 rpm. After completion of each batch of fermentation, the fermented liquid was poured into centrifuge tube and it was centrifuged at 4000 rpm for 20 minutes [15]. Sediment was collected and oven dried at 50°C for 16 hours. Dry weight was measured and protein estimation was done according to the method of Kjeldahl [19]. Crude protein value was expressed as N*6.25.

Preliminary Study: In order to produce SCP, 50 mL of banana and papaw fruit juices (10% concentration of sole medium) were distributed in Erlenmeyer flasks separately. This experiment was carried out in triplicates. 0.5g of baker's yeast was inoculated into each medium under sterile condition. Then fermentation was carried out for 24 hours at 28°C with 100 rpm speed in a shaking incubator. Crude protein contents were determined by the method of Kjeldahl.

Optimization for SCP Production

Effect of Fermentation Time: Fermentation was carried out with papaw and banana medium at 28°C and samples were taken from 1, 2, 3, 4, 5, 7 days of fermentation. Crude protein contents were measured using Kjeldahl method.

Effect of Fermentation Temperature: To determine the optimum temperature, fermentation was carried out with papaw and banana medium for 3 days at differ temperature in range of 20 to 40°C with an increment of 5°C. Crude protein contents were determined using Kjeldahl method.

Effect of Different Concentrations of Fruit Juices: Fermentation was carried out with different concentrations of fruit juices (1, 2, 3, 4, 5, 10, 20, 50 and 100%) and crude protein contents were determined using Kjeldahl method.

Statistical Analysis: All experiments were carried out in triplicates and the average values were used to plot the graphical representations. Means and standard error of the mean were calculated for each parameter measured for the three biological replicates of experiments in each case. Statistical analysis was done by Completely Randomized Design (CRD) method using the SAS-8 statistical package. The means of results were compared using Duncan's Multiple Range Test (DMRT at 0.05α).

RESULTS AND DISCUSSION

Production of Single Cell Protein: In this study, 33.6 ± 0.02% and 33.41 ± 0.05% of crude protein yield were obtained for papaw and banana as sole medium respectively. Based on this results it is possible to explain that baker's yeast was able to produce relatively high amount of protein in sole papaw and banana fruit juice medium without any nutrient supplements. In order to increase the crude protein yield, optimization studies using different culture growing conditions were used.

Effect of Fermentation Time: The highest amount of crude protein (Papaw medium - 40.18% and banana medium - 39.38%) was obtained after 3 days of fermentation. The results of this study are showed in Figure 1 and 2. Crude Protein was increased with increasing fermentation time from 1 day to 3 days in both medium and then started to decline. The limited availability of nutrients and reduction of carbon source during continued fermentation possibly forced the microbial cells to consume the produced protein [9].

The dry biomass content was increased with increasing fermentation time. Biomass production was highest on 7th day of fermentation for both papaw and banana medium (Table 1). Based on the results, dry bio mass produced from banana medium (0.59g) was significantly higher than papaw medium (0.45g) on 7th day of fermentation.

Nevertheless maximum crude protein yield was obtained on 3rd day of fermentation. Therefore 3 days of fermentation time was chosen for further studies.

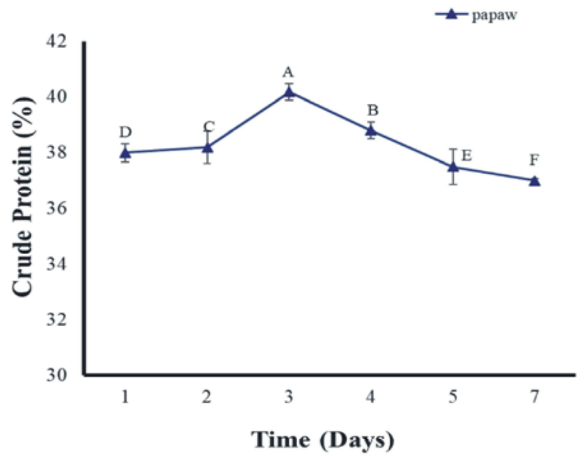

Fig. 1: Effect of fermentation time on SCP production from baker's yeast at 28°C, under the liquid fermentation system. Mean significant differences were tested for papaw medium using DMRT at α level 0.05. Capital letters indicated the significant difference between means of crude protein of papaw medium.

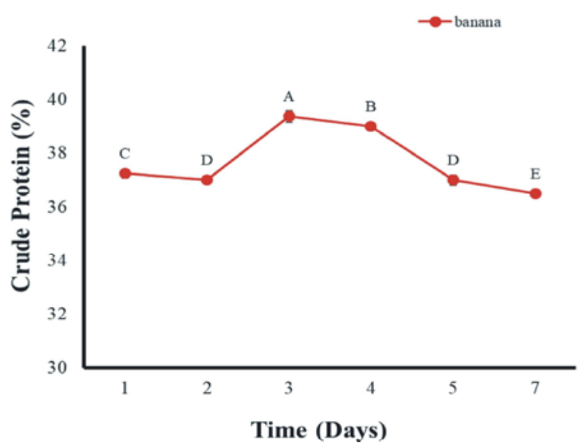

Fig. 2: Effect of fermentation time on SCP production from baker's yeast at 28°C, under the liquid fermentation system. Mean significant differences were tested for banana medium using DMRT at α level 0.05. Capital letters indicated the significant difference between means of crude protein of banana medium.

Table 1: Effect of fermentation time on dry biomass (g) production from baker's yeast

Days	Papaw medium (g)	Banana medium (g)
1	0.192	0.234
2	0.251	0.272
3	0.266	0.298
4	0.314	0.352
5	0.393	0.420
7	0.450	0.591

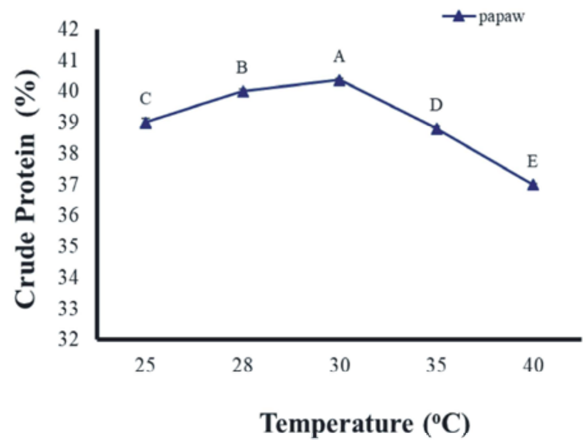

Fig. 3: Effect of temperature on SCP production from papaw fruit juice using baker's yeast in 3 days of fermentation time, under the liquid fermentation system. Mean significant differences were tested for papaw medium using DMRT (α=0.05). Capital letters indicated the significant difference between means of crude protein of papaw medium at differ temperatures.

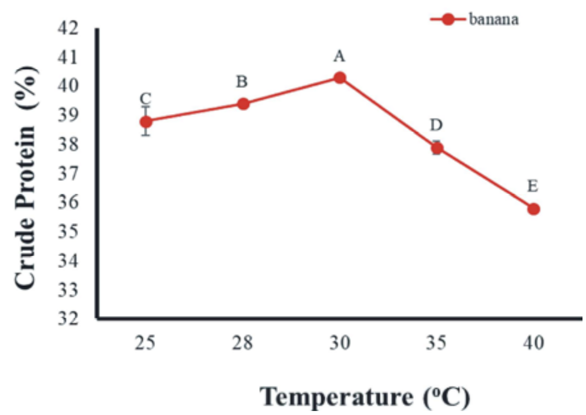

Fig. 4: Effect of temperature on SCP production using banana fruit juice from baker's yeast in 3 days of fermentation time, under the liquid fermentation system. Mean significant differences were tested for banana medium using DMRT (α=0.05). Capital letters indicated the significant difference between means of crude protein of banana medium at differ temperatures.

Effect of Fermentation Temperature: Significantly lower and higher crude protein yield (papaw medium– 34.08% and 40.36%, banana medium – 33.8% and 40.29% - Figure 3 and 4) were obtained at 40°C and 30°C respectively after 3days of fermentation with baker's yeast. At higher temperature (at 40°C), SCP yield was lower and this may be due to the partial deactivation of

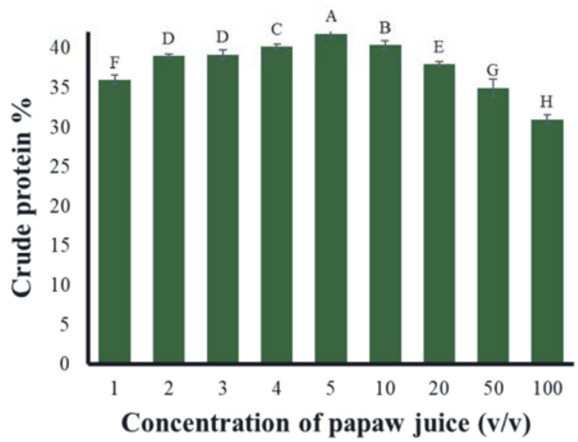

Fig. 5: Effect of different concentrations of papaw fruit juice on SCP production from baker's yeast in 3 days of fermentation at 30°C, under the liquid fermentation system. Mean significant differences were tested using DMRT at α level 0.05. Same letters indicated no significant difference between means of crude protein at differ concentrations of papaw juice medium.

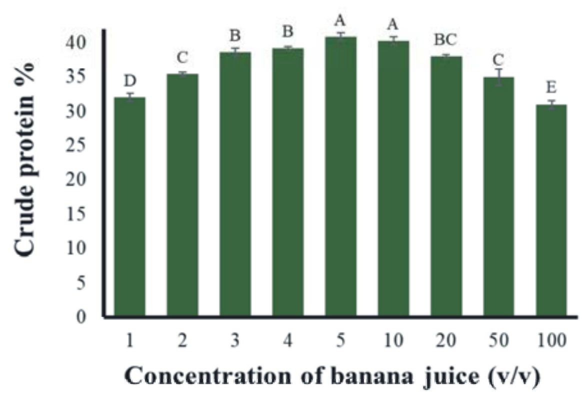

Fig. 6: Effect of different concentrations of banana fruit juice on SCP production from baker's yeast in 3 days of fermentation at 30°C, under the liquid fermentation system. Mean significant differences were tested using DMRT at α level 0.05. Same letters indicated no significant difference between means of crude protein at differ concentrations of banana juice medium.

enzymes involved in metabolic reaction within microbial cells [9]. Based on the above results, 30°C of fermentation temperature was selected as optimum fermentation temperature for highest SCP production.

Effect of Concentration of Carbon Sources: When 5% of the respective juices were used significantly higher crude protein yield was obtained (papaw medium – 41.8% and banana medium – 40.87% - Figures 5 and 6). Amount of crude protein yield increased with the increasing fruit juice concentrations from 1% to 5% and after that amount of crude protein showed a reducing trend and the reduction may be due to the substrate suppressive effect. Based on the above results, 5% of fruit juice concentration was selected as optimum fruit juice concentration for highest SCP production for both juices.

The crude protein yield significantly increased by optimization of fermentation time (72 hrs.), temperature (30°C) and fruit juice concentration (5%).

CONCLUSION

Given these facts, SCP could be produced with papaw and banana fruit juices independently as carbon source using baker's yeast. The conditions optimized were fermentation time (72 hrs.), temperature (30°C) and concentration of carbon source (5%) for both the fruit juices. After optimization of the culture growing conditions and media composition, SCP production was significantly increased from 33.6 to 41.8% (1.244 times) for papaw medium and 33.41 to 40.87% (1.22 times) for banana medium. Thus, single cell protein can be produced from cheap and bountiful source of papaw and banana fruit juices separately using baker's yeast.

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