

Bioconversion of Single Cell Protein from Underutilised Seaweed *Turbinaria* sp. using Natural Palmyrah Toddy Yeast

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Abstract

This study was aimed to produce Single Cell Protein (SCP) from underutilised, abundantly found marine seaweed *Turbinaria* sp. using natural yeast from palmyrah (*Borassus flabellifer*) toddy. *Turbinaria* sp. were collected, sun-dried, ground to powder form and *Turbinaria* solution was made by mixing distilled water and this was used as a growth medium throughout the experiment without adding any supplements. The experiments were performed in a shaking incubator with the fermentation medium of *Turbinaria* sp. 100 g/L (10%) inoculated with 10 mL natural palmyrah toddy. Fermentation was carried out at 29° C for 72 h and the crude protein content was determined using Kjeldahl apparatus (38.46%). When the growth temperature was optimised at 35° C, crude protein yield obtained was significantly increased by 1.36 times than the initial non-optimised condition with temperature 27° C. When fermentation time was optimised as 48 h (44.33%), crude protein yield was significantly increased by 1.14 times than the initial fermentation time (24 h-38.55%). When different combinations of *Turbinaria* sp. media and inoculum ratio (55:05, 50:10, 40:20, 30:30 and 20:40) were used as medium, crude protein yield was significantly increased by 1.44 times (50:10-43.72%) than the initial media inoculum ratio (55:05-30.21%). When initial pH of the media was changed (5.0, 6.0, 7.0, 8.0 and 9.0), significantly higher relative crude protein yield was obtained with the initial pH value 6.0 (*Turbinaria* medium-90.97%). Amino acid analysis revealed that the SCP produced from the *Turbinaria* medium had all the essential amino acids with comparatively higher amount of methionine (3.9%) and lower amount of threonine (0.2%). Vitamin B analysis revealed that SCP yield from *Turbinaria* medium contained thiamin (0.85 mg/100 g) and riboflavin (3.2 mg/100 g). After the optimisation of culture growing conditions and media composition, SCP production from the excessively grown, underutilised seaweed *Turbinaria*, was significantly increased by 1.13 times (from 38.46 to 43.72%) with palmyrah toddy mix.

Key words: crude protein, palmyrah toddy mix, single cell protein (SCP), Turbinaria sp.

1. Introduction

Due to the steep increasing global population, the need for food supply increases day by day for human and animals. Therefore, shortage of protein is becoming a serious challenge for both human and animals. To overcome this protein shortage, Single Cell Protein (SCP) is used as a protein supplement for both human and animals. The SCP is an edible protein which is extracted from dried cell biomass from various microorganisms. These dried biomass contain proteins, lipids, carbohydrates, nucleic acids, vitamins and minerals (Ware, 1997). The concept of using microorganism is an innovative way to solve the global food issue (Nasseri et al., 2011). Microorganisms like bacteria, yeast, marine microalgae and molds are used in SCP productions by utilising cheap materials as substrate (Srividya et al., 2013). The natural toddy can be used as a mixed culture of bacteria, yeast and fungus in the SCP production (Rajendran et al., 2018).

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Tappers climb on the palmyrah palm and sweet sap is obtained by tapping the inflorescence of palmyrah palm. Tappers store the sweet sap in the pot that hangs on the top of the palm. Unfermented sap is converted into alcoholic toddy by the wild yeast and bacteria that present in the toddy collecting pot (Theivendirajah, 1978). The northern region of Sri Lanka has various seaweeds abundantly along the coast (Coppejans et al., 2009). In this study, seaweed *Turbinaria* sp. was selected as source of SCP because of its high nutritional value and abundantly found around Jaffna peninsula (Jeyaseelan et al., 2012). Therefore it was decided to explore the possibility of producing single cell protein from one of the abundantly found under-utilised marine plant species *Turbinaria* sp. as substrate with the aid of natural palmyrah toddy yeast. The study was aimed to optimise the culture conditions to increase the SCP yield from *Turbinaria* sp. and to determine the compositions of vitamin B and amino acids in the SCP.

2. Materials and Methods

2.1 Collection of substrates

Seaweed *Turbinaria* sp. was collected and ground into fine powder. Fresh palmyrah toddy was obtained during October to February in the year 2019-2020.

2.2 Preparation of substrates

Ten grams of *Turbinaria* sp. powder digested in 100 mL of distilled water and extracted using muslin cloth. Filtered liquid of *Turbinaria* was used as growth medium.

2.3 Preliminary study

Filtered liquid of *Turbinaria* was used as growth medium. Fifty milliliters of *Turbinaria* medium was added to 250 mL conical flasks separately. Then 10 mL of natural palmyrah toddy was added to the medium. Erlenmeyer flasks of 250 mL were kept for liquid state fermentation in an orbital shaker at 100 rpm for 72 h at 29° C. After fermentation, the pellets were obtained by centrifugation at 4,000 rpm for 15 minutes and pellets were dried in the oven at 45° C for 24 h. Then the Kjeldahl method was used to determine the crude protein content (Plaza et al., 2013).

2.4 Optimisation of SCP production

(a) Effect of temperature

Optimisation of temperature was carried out at various temperatures such as 27° C, 30° C, 32° C, 35° C and 40° C.

(b) Effect of fermentation time

Fermentation was carried out for different days of 1 to 5 days at 35° C in 100 rpm shaking incubator.

(c) Effect of media inoculum ratio

Fermentation was carried out in different media inoculum ratio such as 55:05, 50:10, 40:20, 30:30 and 20:40 at 35° C for 48 h in 100 rpm shaking incubator.

(d) Effect of pH

Relative crude protein yield was compared in different pH (5, 6, 7, 8 and 9). Fermentation was carried out at 35° C for 48 h in 100 rpm shaking incubator. After each experiment, crude protein content was determined using Kjeldahl apparatus.

(e) Determination of amino acids composition of SCP

Amino acid composition was determined before and after fermentation process using amino acid analyser (Erdman et al., 1977).

(f) *Determination of vitamin B composition of SCP*

Vitamin B content was determined before and after fermentation using High Performance Liquid Chromatography (Giorgi et al., 2012)

2.5 *Statistical analysis*

All the experiments were done in triplicates and the mean values were used to plot the graphical representation. Statistical analyses were performed using Minitab 17.0 version. The data were analysed by one way ANOVA and paired T-test was used to determine significant differences at $p < 0.05$.

3. **Results and Discussion**

3.1 *Effect of temperature*

Incubation temperature is one of the main factors that have an effect on SCP production (Fatmeh et al., 2019). In this study, significantly higher SCP yield was obtained at 35° C at 100 rpm for 3days of fermentation (41.41%-Figure 1) than the other temperatures used. Effect of temperature on SCP yield reveals the protein content nearly 47.78% at 30° C by using sugar bagasse as the substrate (Razzaq et al., 2020). In another study, higher SCP yield was obtained at 35° C with *Aspergillus fumigatus* (Hezarjaribi et al., 2015). Even though SCP production at temperatures between 30-50° C under liquid state fermentation system is satisfactory, the SCP yield was significantly higher at 35° C after 3 days of fermentation. Since the crude protein yield was significantly higher in 35° C, this temperature was chosen for further studies.

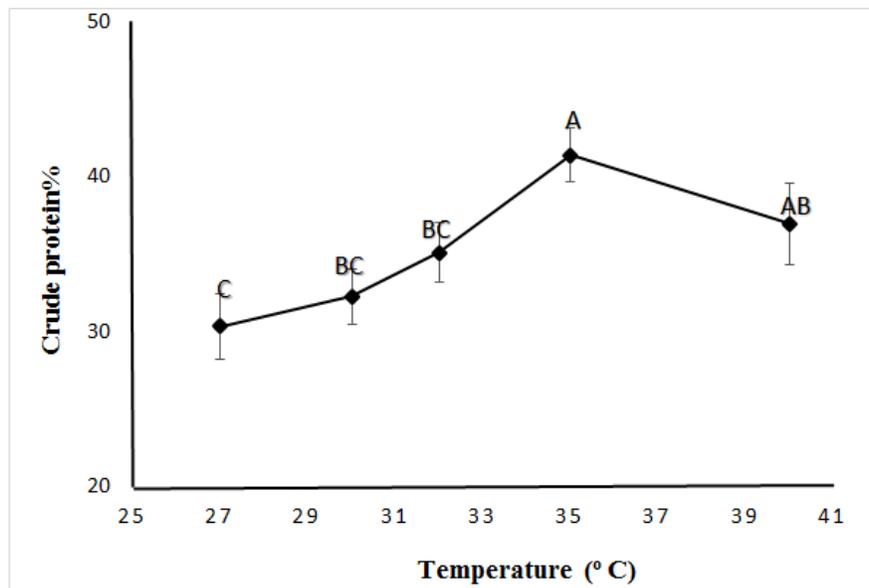


Figure 1. Effect of incubation temperature on SCP production from *Turbinaria* sp. substrate using natural palmyrah toddy yeast under liquid fermentation system.

3.2 *Effect of fermentation time*

Quantity of SCP increased with increasing fermentation time from the 1st day till the end of 2nd day (Figure 2). After 2 days of fermentation, crude protein of SCP production started to decline. Significantly higher SCP yield was obtained on the 2nd day of fermentation (44.33%-Figure 2), thus 2 days of fermentation time was chosen for further studies. Similar observations were made in an experiment carried out with papaya juice (Rajendran et al., 2018), sugarcane bagasse using yeast (Samadi et al., 2016) and with pineapple waste using yeast (Dhanasekaran et al., 2011).

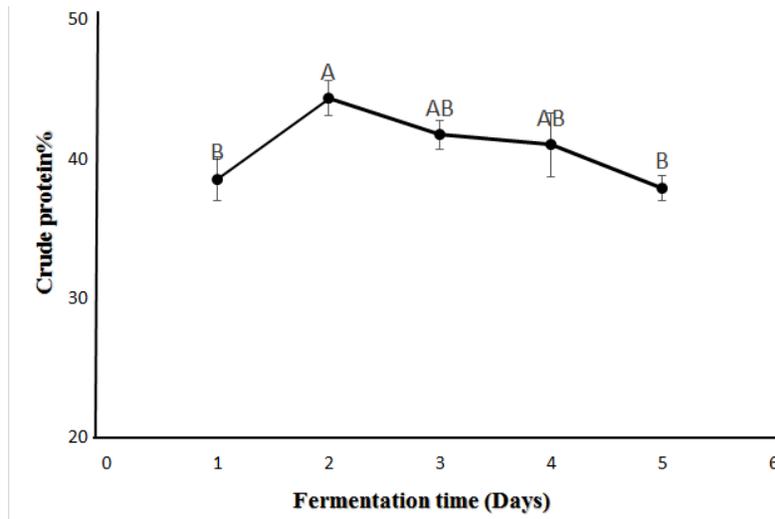


Figure 2. Effect of fermentation time on SCP production from *Turbinaria* sp. substrate using natural palmyrah toddy yeast under liquid fermentation system.

3.3 Effect of media inoculum ratio

SCP yield from the container that had inoculum ratio of 55:05 was significantly lower (30.20%) than the others. There were no significant differences in the SCP yield when media inoculum ratios were maintained as 50:10, 40:20 and 30:30 (Figure 3). The amount of crude protein quite higher in the ratio of 50:10. Therefore 50:10 ratio was selected for further optimisation. In another study, maximum yield obtained at an inoculum size of 10% (V/M) in the SCP production through microbial conversion of wheat bran for animal feed (Yunus et al., 2015).

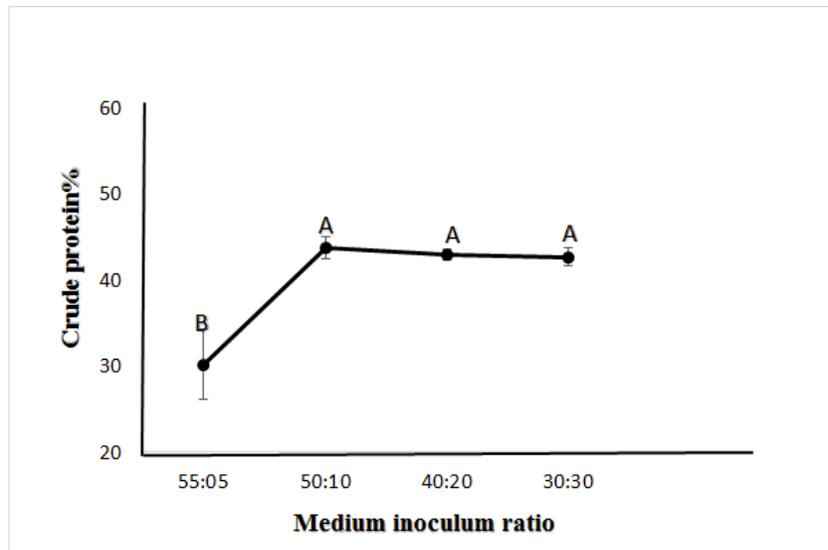


Figure 3. Effect of medium inoculum ratio on SCP production from *Turbinaria* sp. substrate using natural palmyrah toddy yeast under liquid fermentation system.

3.4 Effect of pH

Since pH of the media will affect the growth and metabolic activities of the fermenting microorganisms, it was decided to perform an SCP production experiment with media of different pH values. There were no significant differences in the SCP yield produced when the pH of the media was 5.0, 6.0 and 7.0 (Figure 4). Similarly, there were no significant differences in the SCP yield, when the

pH of the media was set at 8.0 and 9.0 (Figure 4). In another study, SCP production was significantly higher at pH 6.0 when rice was used as substrate in the presence of *Candida utilis* (Rajoka et al., 2003).

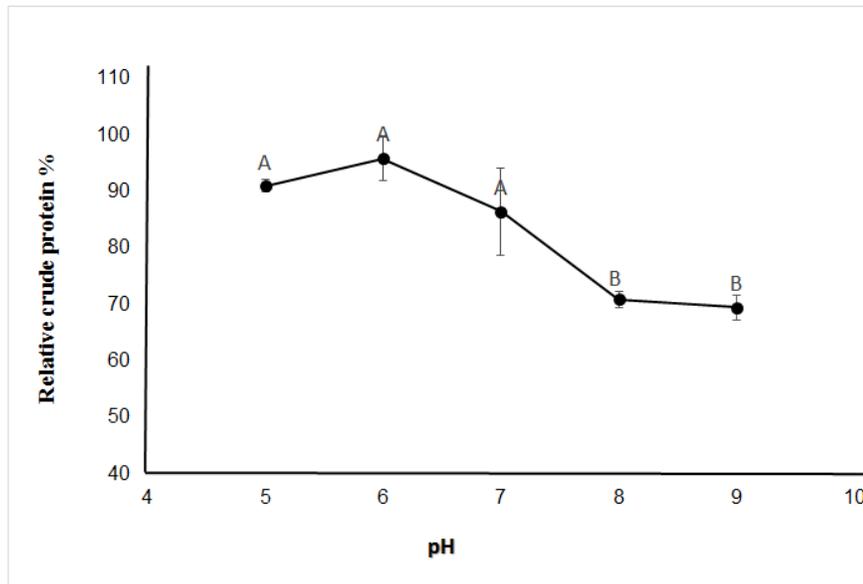


Figure 4. Effect of pH on SCP production from sea weed *Turbinaria* sp. as substrate using natural palmyrah toddy yeast under liquid fermentation system.

3.5 Determination of amino acid composition

Amino acid analysis after fermentation revealed that amino acids methionine and leucine were found to be in significantly higher quantities in *Turbinaria*, whereas threonine and lysine were found to be in significantly lower quantities than other amino acids. Similar observations were obtained in the study of SCP production from banana juice (Rajendran et al 2018). Amount of all the essential amino acids have been increased after fermentation compared to the amount before fermentation. Quantities of amino acids such as tryptophan, methionine, leucine and arginine were significantly increased after fermentation. Bioconversion of substrates into microbial enzymes may lead to the increase of some amino acids during fermentation (Oseni and Akindahunsi, 2011). There were no significant difference in the amount of lysine, threonine, valine, isoleucine, glutamate, phenylalanine and histidine before and after fermentation (Table 1).

Table 1: Amino acid composition (% dry matter) obtained from SCP produced from the seaweed *Turbinaria* sp. as medium.

Amino acids	Amount (% dry matter)	
	(Before fermentation)	(After fermentation)
Lysine *	0.2	0.4
Tryptophan *	1.2	2.5#
Threonine*	0.2	0.2
Methionine *	1.9	3.9#
Valine *	1.8	2.4
Isoleucine *	0.7	1.0
Leucine *	2.2	2.8#
Glutamate	0.9	1.1
Phenylalanine*	0.2	0.6
Histidine*	0.3	0.5
Arginine	1.6	2.1#

Essential amino acids are denoted using*

Significant increase is denoted using#

3.6 Determination of vitamin B composition

Vitamin B components such as thiamin and riboflavin were found in the SCP from Sea weed *Turbinaria* where as other vitamin components were absent or in trace levels that cannot be measured. Thiamin and riboflavin type vitamin B was reported in the SCP produced from dextrose medium using *Candida tropicalis* (Wijeyeratne and Jayathilaka, 2000). There were no significant difference in the level of thiamin and riboflavin before and after fermentation (Table 2).

Table 2: Thiamin and Riboflavin content (mg/100 g) of SCP produced from seaweed *Turbinaria* sp. as medium.

Vitamin B	Weight	
	(Before fermentation)	(After fermentation)
Thiamin	0.75	0.85
Riboflavin	2.25	3.20

4. Conclusion

Single cell protein was produced from abundantly available marine weed *Turbinaria* sp. using natural yeast found in palmyrah toddy mixed culture under optimised conditions. Optimisation of condition such as fermentation time (72 h), temperature (35° C), source of inoculum (50:10), pH of the media (6) increased the yield by 1.136 times. After the optimisation of the culture growing conditions and media composition, SCP production was significantly increased from 38.46 to 43.72% (1.13 times). SCP produced from marine weed *Turbinaria* contained all the essential amino acids to human as well as vitamin B components thiamin and riboflavin. In that, amount of amino acids such as tryptophan, methionine, leucine and arginine show significant increase after fermentation.

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