fermentation

MDPI

Article

Production of Single-Cell Protein from Fruit Peel Wastes Using Palmyrah Toddy Yeast

Punniamoorthy Thiviya [1], Ashoka Gamage [2], Ranganathan Kapilan [3], Othmane Merah [4,5,*] and Terrence Madhujith [6,*]

1 Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka; thiviya904@gmail.com
2 Chemical and Process Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya 20400, Sri Lanka; ashogamage@gmail.com
3 Department of Botany, Faculty of Science, University of Jaffna, Jaffna 40000, Sri Lanka; ranganat@ualberta.ca
4 Laboratoire de Chimie Agro-Industrielle (LCA), Institut National de la Recherche Agronomique, Université de Toulouse, CEDEX 4, 31030 Toulouse, France
5 Département Génie Biologique, IUT Paul Sabatier, Université Paul Sabatier, 32000 Auch, France
6 Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka
* Correspondence: othmane.merah@ensiacet.fr (O.M.); tmadhujith@agri.pdn.ac.lk (T.M.); Tel.: +33-53-432-3523 (O.M.); +94-71-341-2171 (T.M.)

Abstract: Single-cell protein (SCP) from agro-waste material has gained increased attention in the recent past as a relatively cheap and alternative protein source to meet the nutritional demand generated by the fast-growing population. Furthermore, bioconversion of these wastes into SCP such as value-added products reduce the environmental-related issues. In this study, locally available pineapple (*Ananas comosus*), watermelon (*Citrullus lanatus*), papaya (*Carica papaya*), sour orange (*Citrus medica*), banana (*Musa acuminata*) and mango (*Mangifera indica*) peel wastes were investigated for their suitability to produce SCP using palmyrah (*Borassus flabellifer*) toddy carrying natural mixed yeast and bacteria culture under liquid state fermentation system. Moreover, this study attempted to select the best substrate and the optimized process condition for SCP production to increase the protein yield. The physicochemical properties of selected fruit peels were analyzed. The sterilized peel extracts (10%, *v*/*v*) were inoculated with 5 mL of palmyrah toddy and allowed to ferment in a shaking incubator at 100 rpm for 48 h in triplicate At the end of fermentation, the sediments were collected by centrifugation at 1252× *g*, oven-dried, and the dry weight was taken to determine the protein content. The biomass yield ranged from 5.3 ± 0.6 to 11.7 ± 0.8 g/L, with the least biomass yield being observed with watermelon peels while the maximum yield was observed with papaya peels. Papaya peel generated a significantly higher ($p < 0.05$) amount of protein (52.4 ± 0.4%) followed by pineapple (49.7 ± 1.3%), watermelon (45.2 ± 0.7%), banana (30.4 ± 0.6%), sour orange (29.5 ± 1.2%) and mango (24.6 ± 0.2%) peels. The optimum condition for the fermentation of papaya waste was pH 5.0, 25 °C, and 24 h. Nucleic acid reduction treatment significantly reduces dry weight and protein content of biomass. It can be concluded that papaya peel waste is a suitable substrate for protein-rich cell biomass production using the natural toddy mixed culture of palmyrah.

Keywords: amino acid; fruit peel; liquid state fermentation; nucleic acid reduction; optimization; single-cell protein

Citation: Thiviya, P.; Gamage, A.; Kapilan, R.; Merah, O.; Madhujith, T. Production of Single-Cell Protein from Fruit Peel Wastes Using Palmyrah Toddy Yeast. *Fermentation* **2022**, *8*, 355. https://doi.org/10.3390/fermentation8080355

Academic Editors: Nirakar Pradhan, Joyabrata Mal and Donatella Cimini

Received: 16 June 2022
Accepted: 22 July 2022
Published: 26 July 2022

1. Introduction

The increasing population has created the demand for the formulation of innovative, unconventional, and alternative proteinaceous food sources [1]. Microorganisms have been explored in food production since antiquity [2]. On the other hand, globally significant amount of fruit waste is generated with increasing production which goes into waste.

Although fruit waste is biodegradable, improper management can constitute a public health risk and environmental crisis. Bioconversion of fruit waste into value-added products can minimize the effect of organic waste disposal on the environment [1]. Fruit waste is used to produce substances of economic importance, such as organic acids, single-cell protein (SCP), single-cell oils, enzymes, biocolors, flavors, aroma esters, cellulose, pectin, and other polysaccharides, antibiotics, biopesticides, and plant growth regulators can be used to generate bioethanol, biogas, and biohydrogen [3–5]. The present study aimed at studying the potential of bioconversion of locally available various fruit wastes into SCP in a liquid state fermentation system.

SCP has drawn much attention for its high efficiency in substrate conversion and high productivity. SCP production does not require large extent land and is independent of climatic and seasonal changes [6,7]. SCP contains a significant amount of protein, which accounts for 60–82% on a dry weight basis, essential amino acids lysine and methionine limiting in most plant and animal sources, and other nutritional components, including carbohydrates, fat, vitamins, and minerals [8]. Widespread usage of SCP human consumption is limited by high nucleic acid content and poor cell wall digestion, the two most important factors limiting the nutritional value of SCP [6].

SCP refers to the dead, dried microbial cell or total protein derived from pure or mixed microbial cell culture of bacteria, filamentous fungi, yeast, and algae, including unicellular algae and prokaryotic cyanobacteria, which grow on different carbon sources [8–11]. Despite the name suggesting single cell, biomass produced from fungi and some algae, which are multi-cellular, have also been considered as SCP [12].

SCP is widely used in animal feed and food applications, particularly in the food industry used as meat substitutes, texturizing agents, flavor enhancers, vitamin carriers, emulsifiers, and to improve the nutritive value of food products [8,13]. Moreover, SCP is commercially produced under different commercial names such as Quorn®, AlgaVia®, Marmite®, Vitam-R®, Pruteen®, Brovile®, and FermentIQ™ [14,15].

A wide variety of microorganisms and substrates, including agro-industrial wastes, various fruit wastes, cellulosic biomass, molasses, corn starch, dextrose, sucrose, soybean meal, brewery residues, industrial wastewater, biogas, ethanol, CO_2, are utilized for SCP production [16–18]. Of them, substrates rich in sugar are most widely used for SCP production [14].

Sri Lanka produced 0.97 metric tons (MT) of fruits in 2019, which contributed to 0.11% of world production [19]. The most common fruits consumed are banana, papaya, mango, and pineapple and their consumption has been increasing in the past decade [20]. Peels are the primary by-product representing nearly 30% of the total weight of fruit [21], and their extract mainly contains simple sugars such as sucrose, glucose, and fructose and a significant amount of minerals and nitrogen content [22]. Therefore, fruit peels can be used as carbon and energy sources for microbial growth, and thus, in SCP production. Since the cost of SCP and the economic viability of its production depend largely on substrate cost, waste from various fruits is a good alternative.

Palmyrah (*Borassus flabellifer*) palm is mostly grown in the north and eastern parts of Sri Lanka and other tropical parts of the world, including India, Burma, Thailand, Bangladesh, Vietnam, Malaysia, Indonesia, and East Africa [23,24]. Sap collected from tapping the inflorescence of the palmyrah is widely used for the production of meera (palm nectar/*pathaneer* in Tamil), toddy (fermented sap), and arrack (distilled spirit), wine, treacle, palm sugar, and jaggery [24]. Fermented sweet sap palmyrah (toddy), a local palm wine, is an alcoholic beverage produced by uncontrolled natural fermentation of sap by yeast and bacteria and contains an alcohol content of about 5% (*w*/*v*) [24]. Palmyrah toddy contains mixed cultures of yeast and bacteria, and commonly found microflora is identified as yeast, *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Kloeckera apiculata*, *Schizosaccharomyces pombe*, bacteria, *Bacillus cereus*, *Bacillus sphaericus*, and *Bacillus firmus*. The predominant and best alcoholic fermenter among yeasts is *S. cerevisiae* [25]. Further, palmyrah sap is rich in

reducing sugars and vitamins, particularly vitamin C, vitamin A, and niacin (B3), which are favorable for microorganisms to grow [26].

Mixed culture in SCP production improves production and the quality of biomass [27]. Some literature mentions the use of palmyrah toddy (mixed culture of yeast and bacteria or yeast isolates) in various products such as bio-ethanol, vinegar, and arrack [26,28]. Few mentions of SCP production using palmyrah toddy are found in the literature [29], and no literature focuses on fruit peel agro-waste. Furthermore, SCP production is influenced by the type of substrate and microorganism used, substrate concentration, availability of carbon and nitrogen sources, pH, temperature, aeration, agitation rate, and inoculum size [30].

In this regard, the present study aimed at exploring the possibilities of producing SCP from the mixed culture of palmyrah toddy through liquid state fermentation by using various locally available fruit peels as cheap energy sources. This study also attempted to select an alternate best substrate for SCP production and optimize fermentation conditions, including pH (3.0–5.5), fermentation temperature (25–40 °C), and fermentation time (24–168 h), in comparison with the control medium (glucose) to improve the protein yield with the potential substrate and to reduce the production cost.

2. Materials and Methods

2.1. Materials and Chemicals

Pineapple (*Ananas comosus*), watermelon (*Citrullus lanatus*), papaya (*Carica papaya*), mango (*Mangifera indica*), sour orange (*Citrus medica*), and banana (*Musa acuminata*) peel were obtained from ripened fruits procured from local markets in Jaffna, the northern part of Sri Lanka. Palmyrah (*Borassus flabellifer*) toddy as a source of mixed culture of natural fermentative yeast and bacteria was collected (6–7 am) from mature palm using sterile vessels from Palm Products Distilleries, Thikkam, Jaffna, Sri Lanka. All the chemicals used were of analytical grade procured from Sigma Aldrich, Germany, and VWR chemicals, the USA.

2.2. Physicochemical Properties of Fruit Peels

The proximate composition of selected fruit peels was determined as per the method explained in AOAC official methods [31]. The yield percentage measures the dry weight in a 100 g fruit sample (sample dry weight/wet weight × 100). The moisture content of fruit peels was determined by drying samples in an oven (Memmert Oven UNB 100, Schwabach, Germany) at 105 °C until constant weight is obtained, crude protein content was determined using the Kjeldahl method, crude fat content was analyzed by continuous extraction in the Soxhlet apparatus (LabKits-SZF-06A, Hong Kong, China) at 75 °C for 6 h using petroleum ether as the solvent, and the ash content was determined by keeping the pre-weighed sample in a muffle furnace (Hobersal-HD-330PA, Barcelona, Spain) at 550 °C for 6 h. Total carbohydrate content was determined by difference [32]. All experiments were performed in triplicate, and the results were expressed on a dry weight basis, except for moisture content.

The total soluble solids content (TSS) and pH of each fruit peel were measured using a refractometer (Atago-DR-A1, Saitama, Japan) and pH meter (Ohaus-Starter 2100, Parsippany, NJ, USA), respectively. The reducing sugar content was estimated with 3,5-dinitrosalicylic acid (DNS) reagent by measuring absorbance at 540 nm in a spectrophotometer (UVmini-1240, Duisburg, Germany) [33].

2.3. Culture Media and Inoculum Preparations

The collected peel samples were cleaned and washed with distilled water, macerated separately using a laboratory blender, and filtered through a muslin cloth and a Whatman No 1 filter paper. The fruit peel medium and the control medium were prepared with the following composition at pH 5.0. The control medium was prepared with 10.0 g D-Glucose, 5.0 g $(NH_4)_2SO_4$, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.1 g $CaCl_2$ and 1000 mL

distilled water, whereas 1000 mL of fruit peel medium (10%, *v*/*v*) was prepared with 100 mL fruit juice, inorganic supplements (1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.1 g $CaCl_2$), and 900 mL distilled water [34]. Then the media were sterilized in an autoclave at 121 °C at 15 psi for 15 min and stored at 4 °C. A fresh palmyrah toddy sample was used as the source of natural mixed culture of yeast and bacteria. The viable cell count was determined by using a hemocytometer (Assistent, Sondheim, Germany).

2.4. Production of SCP Using Liquid State Fermentation Process

Fifty milliliters of sterilized fruit peel and control medium were transferred into a pre-sterilized Erlenmeyer flask (250 mL) in triplicate under sterile conditions. Each sterilized medium was inoculated with 5 mL of fresh palmyrah toddy sample ($1.63 \pm 0.15 \times 10^6$ cells/mL) after determining the mean viable cell count using a haemocytometer (Assistent, Sondheim, Germany). Then the fermentation was carried out in a shaking incubator (Lab Companion SI-600, Billerica, MA, USA) at a speed of 100 rpm for a specific time and temperature. After completion of fermentation, sediment was centrifuged (1252× *g* for 20 min, MSE-Minor, London, UK) and the residue was oven-dried at 50 °C for 16 h, and the mean dry weight of biomass was measured. Crude protein content was quantified using the Kjeldahl method taking 6.25 as the factor [34]. All the experiments were carried out in triplicate.

2.5. Selection of the Best Substrate for SCP Production

Sterilized diluted (10%, *v*/*v*) fruit peel extracts were used without adding any inorganic supplement to determine the best substrate for SCP production. Each fruit peel medium (50 mL) was inoculated with 5 mL of palmyrah toddy culture and fermented in a shaking incubator at 100 rpm for 48 h at 28 °C in three replicates. After 48 h of fermentation, sediment was centrifuged (1252× *g* for 20 min) and oven-dried (50 °C for 16 h) to determine the mean dry weight. The dried biomass was then analyzed for total nitrogen content by the Kjeldahl method, and crude protein was estimated by multiplying by 6.25. The results were compared to find the maximum yield of biomass and protein content to select the best substrate.

2.6. Optimization of Fermentation Condition and Comparison with Control Medium

Once the best substrate for SCP production was determined, the fermentation conditions were optimized: pH (3.0–5.5), fermenting temperature (25–40 °C), and time (24–168 h). The conditions were optimized by one factor at a time (OFAT) while keeping the other variables constant [35]. Dry biomass and the crude protein content were determined at each treatment and the results were compared. Optimization of pH, temperature, and time was compared with the control medium (glucose 10%, *w*/*v*) to study the potential of fruit peel as a substrate for SCP. All the experiments were carried out in three replicates in a shaking incubator at 100 rpm under specific fermentation conditions.

2.7. Effect of Nucleic Acid Reduction on SCP Production

Biomass harvested under optimized conditions (10% *v*/*v*, 25 °C, pH 5, 24 h) was treated for nucleic acid reduction. The reduction of nucleic acid from microbial cells with NaOH was carried out in triplicate [36,37]. For this, 5 g of dried microbial mass was treated with 20 mL of 1N NaOH. Then the nucleic acid content was reduced by placing the treated sample in a boiling water bath (100 °C) for 10 min and cooled in cold water. Then the solution was centrifuged at 1252× *g* for 20 min, and the sediment was tested for crude protein content using the Kjeldahl method.

2.8. Estimation of Amino Acid Content

Amino acid composition of biomass (1 mg) harvested under optimized conditions was determined using HPLC analyzer (Dionex UltiMate 3000, Thermoscientific, Waltham, MA, USA) after performing acid hydrolyzation with 6 N HCl at 110 °C for 24 h [38]. The results

were compared with the reference amino acid pattern suggested by FAO/WHO/UNU, 2007 [39].

2.9. Statistical Analysis

The results were reported as mean ± standard deviation of three replicates, and the statistical analyzes were performed using the Minitab 17 (Minitab Inc., State College, PA, USA) statistical package. The data were analyzed using one-way ANOVA, and Tukey's multiple comparison test was used to determine significant differences at $p < 0.05$.

3. Results and Discussion

3.1. Compositional Analysis of Substrates

Table 1 shows the physicochemical properties: moisture content, protein, fat, ash, and carbohydrate, and TSS, pH, and total reducing sugar () of fruit peel from pineapple, watermelon, papaya, mango, sour orange, and banana.

Table 1. Physicochemical properties of pineapple, watermelon, papaya, mango, sour orange, and banana peel.

Fruit Peel	Yield (%)	pH	TSS (%)	Reducing Sugar (%)	Moisture (%)	Ash (%) [1]	Fat (%) [1]	Protein (%) [1]	Total Carbohydrate (%) [1]
Pineapple	15.3 ± 0.9 ^{b}	3.7 ± 0.0 ^{e}	10.8 ± 0.0 ^{c}	2.6 ± 0.1 ^{b}	84.7 ± 0.9 ^{b}	4.5 ± 0.3 ^{c}	0.9 ± 0.1 ^{c}	6.9 ± 0.1 ^{c,d}	87.7 ± 0.4 ^{b}
Watermelon	5.0 ± 0.4 ^{c}	5.4 ± 0.0 ^{b}	3.2 ± 0.0 ^{f}	1.8 ± 0.1 ^{c,d}	95.0 ± 0.4 ^{a}	5.5 ± 0.2 ^{b,c}	1.5 ± 0.1^{b}	10.3 ± 0.3 ^{b}	82.6 ± 0.5 ^{c}
Papaya	8.4 ± 0.2 ^{c}	5.5 ± 0.0 ^{a}	6.5 ± 0.0 ^{e}	5.8 ± 0.1 ^{a}	91.6 ± 0.2 ^{a}	6.4 ± 0.4 ^{a,b}	1.1 ± 0.1 ^{b,c}	11.3 ± 0.6 ^{a}	81.2 ± 0.9 ^{c}
Sour orange	25.2 ± 2.5 ^{a}	4.1 ± 0.0 ^{d}	12.3 ± 0.0 ^{b}	1.2 ± 0.1 ^{d}	74.8 ± 2.5 ^{c}	6.1 ± 1.0 ^{a,b}	1.4 ± 0.1 ^{b,c}	7.2 ± 0.1 ^{c}	85.4 ± 0.9 ^{a}
Banana	26.1 ± 2.1 ^{a}	4.8 ± 0.0 ^{c}	7.1 ± 0.0 ^{d}	3.1 ± 0.4 ^{b}	73.9 ± 2.1 ^{c}	7.4 ± 0.3 ^{a}	2.6 ± 0.4 ^{a}	6.4 ± 0.2 ^{d}	83.6 ± 0.7 ^{a}
Mango	23.8 ± 0.2 ^{a}	4.1 ± 0.0 ^{d}	16.8 ± 0.0 ^{a}	2.4 ± 0.3 ^{b,c}	76.2 ± 0.2 ^{c}	4.2 ± 0.5 ^{c}	2.5 ± 0.3 ^{a}	6.2 ± 0.2 ^{d}	87.1 ± 0.8 ^{a}

Results are shown as mean ± standard deviation of three replicates, and different superscript letters in the same column are significantly different ($p < 0.05$). [1] Values are on a dry weight basis.

Based on the physicochemical analysis, mango showed the highest value of TSS (16.8%) among the six selected fruit peels, while the lowest TSS was shown by watermelon (3.2%). In the six fruit peels analyzed, pH was recorded in the range of 3.7 to 5.4.

Sugars, particularly glucose, are common carbon sources for microorganisms [40]. Studies have shown that microbial biomass production is associated with the availability of reducing sugars [41]. Therefore, estimation of reducing sugar is essential to evaluate the potential of the fruit wastes as a substrate for fermentation. Results show that the analyzed fruit peels have reasonable amounts of reducing sugar ranging from 1.2 ± 0.1 g/100 g (sour orange) to 5.8 ± 0.1 g/100 g (papaya), making these biomass materials a suitable feedstock for bioconversion of these fruit peels into SCP. The reducing sugar content of banana, pineapple, and papaya peel was reported in the range of 1.30–4.54 mg/g by Saheed et al. [41].

The moisture content of fruit peels varied from 73.9 ± 2.1% to 95.0 ± 0.4%; the minimum level (73.9 ± 2.1%) was found in banana peel while the maximum (95.0 ± 0.4%) in watermelon (Table 1). The moisture content (84.7 ± 0.9%) of pineapple peel was comparable with other studies (82.7–82.9%) [42,43]. Morais et al. [43] reported higher values for moisture content of banana (89.8%) and papaya (86.8%).

Ash content of six peels tested was in the range of 4.2 ± 0.5 to 7.4 ± 0.3%, and the maximum ash content was recorded in banana peel (7.4 ± 0.3%) followed by papaya (6.4 ± 0.4) and sour orange (6.1 ± 1.0%) peels. Ash content of pineapple (4.5 ± 0.3%) and mango (4.2 ± 0.5%) peels was close to the values reported by Dias et al. [42] and Sánchez-Camargo et al. [44], respectively. Ash content of pineapple, papaya, banana, and watermelon was lower than the values reported by Morais et al. [43].

Six fruit peels contain a low amount of fat in the range of 0.9 ± 0.1% (pineapple) to 2.6 ± 0.4% (banana). A higher value has been reported for banana peel by Morais et al. [43]. The fat content of pineapple (0.9 ± 0.1%) was in agreement with

the findings of Dias et al. (0.99 ± 0.16%) [42], however, lower than the value reported by Romelle et al. (5.31 ± 0.74%) [21]. Mango contained 2.5 ± 0.3% of fat, which is similar to the findings of Ajila et al. [45] and higher than the value reported by Sánchez-Camargo et al. [44] for mango.

Furthermore, papaya peel contained a comparatively high quantity of protein (11.3 ± 0.6% on a dry weight basis), while watermelon and sour orange contained 10.3 ± 0.3% and 7.2 ± 0.1%, respectively. Mango showed the lowest protein content (6.2 ± 0.2%), which is at the same level as mentioned in some other studies [21,44,46], however, lower than the findings recorded by Garcia-Amezquita et al. [47] and Ajila et al. [45]. The protein content of pineapple peel was comparable with other studies (0.3–5%) [48]. The protein content of pineapple, banana, papaya, and watermelon was reported in the range of 7.3% (pineapple) to 16.9% (papaya) by Morais et al. [43].

Sour orange, banana, and mango peel contain a comparatively higher amount of carbohydrates, and the values are in accordance with other studies [42,47]. Proximate composition of fruit peels may vary with variety, origin, geographic location, seasonal variations, and maturity stage of the fruits [45,49]. Fruit wastes contain high carbohydrates, minerals, and other nutrients that support microbial growth [50]. Results of this study indicate that analyzed fruit peels contain adequate nutrient compositions that are useful carbon and nitrogen sources for microbial growth and can be a potential substrate for cost-effective biomass production.

3.2. Selection of the Best Substrate for SCP Production

The type of substrate influences microbial biomass production due to the variation in the rate of nutrient utilization as microorganisms react differently to each substrate [30,51,52]. Moreover, substrate type is one of the factors that affects the nutritional quality of microbial protein [53]. In this regard, the suitability of pineapple, watermelon, papaya, mango, sour orange, and banana peel for SCP production using the mixed culture of palmyrah toddy was determined based on the dry weight and its crude protein content of biomass (Table 2 and Figure 1).

Table 2. Dry weight and crude protein content of dry biomass produced from various fruit peels.

Fruit Peel	Dry Biomass (g/L)	Crude Protein Content (%)
Pineapple	9.40 ± 0.53 ^{a,b}	49.7 ± 1.3 ^{b}
Watermelon	5.33 ± 0.61 ^{c}	45.2 ± 0.7 ^{c}
Papaya	11.73 ± 0.81 ^{a}	52.4 ± 0.4 ^{a}
Sour orange	9.13 ± 0.64 ^{a,b}	29.5 ± 1.2 ^{d}
Banana	7.77 ± 1.88 ^{b,c}	30.4 ± 0.6 ^{d}
Mango	8.61 ± 0.90 ^{b}	24.6 ± 0.2 ^{e}

Results are shown as mean ± standard deviation of three replicates, and different superscript letters in the same column are significantly different ($p < 0.05$). Liquid state fermentation at 100 rpm with 10% (v/v) substrate concentration at pH 5.0, 28 °C for 48 h.

Based on the findings, biomass (11.73 ± 0.81 g/L) and protein contents (52.4 ± 0.4%) were significantly ($p < 0.05$) higher in papaya peel extract. Watermelon peel produced lesser biomass (5.33 ± 0.61 g/L), which may be due to its lower reducing sugar and high water contents that could not support the higher growth of yeast [51]. In comparison, mango-based medium reported a lesser protein content which accounted for 24.6 ± 0.2%.

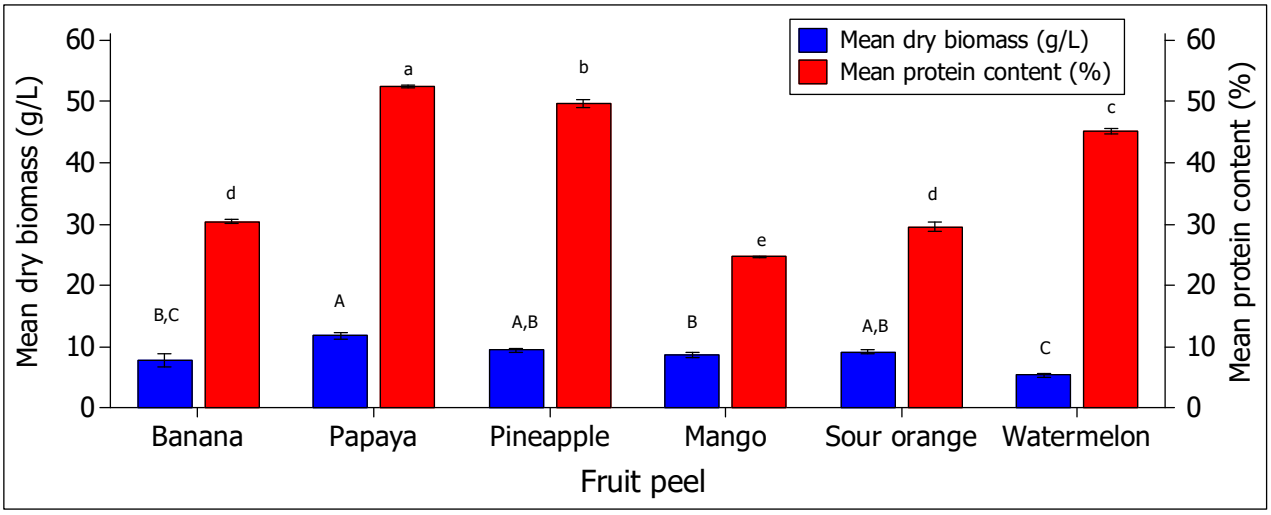


Figure 1. Effect of pineapple, watermelon, papaya, mango, sour orange, and banana peel on dry biomass and protein content of SCP produced from mixed culture of palmyrah toddy. Comparisons of mean were performed with Tukey's multiple comparison test at $p < 0.05$. Different uppercase and lowercase letters indicate the significant differences in dry biomass and protein content, respectively.

Mondal et al. [50] reported similar findings for protein content (30.5%) of biomass produced from orange peel using *S. cerevisiae* in submerged fermentation. A higher value was reported with *S. cerevisiae* grown on banana (59%) and mango (40%) peel in solid-state fermentation at 27 °C for 2 days [54]. Variation in the protein yield could result in high sugar content produced by hydrolysis of the solid substrate containing cellulose, starch, and pectin by microbial enzymes (cellulase, amylase, and pectinase) [55].

Among the six fruit peels studied, papaya peel extract can be considered the best substrate to produce SCP with the mixed culture of palmyrah toddy. Higher reducing sugar content and a reasonable amount of ash content in papaya peel would favor the higher biomass yield [56] (Table 1).

3.3. Optimization of Process Parameters in the SCP Production

Process parameters such as pH, fermentation temperature, and time were optimized for the SCP production in liquid state fermentation using the mixed culture of palmyrah toddy. Fermentation was carried out in glucose and papaya peel medium to study the potential of papaya peel as substrate.

3.3.1. Optimization of pH for SCP Production

Yeast can grow in a wide pH range of 2.5–8.5 and temperature between 2 and 45 °C. *S. cerevisiae* is an acidophilic microorganism, thus the optimum growth conditions are pH 4–6 and temperature 28–33 °C [57]. However, it can vary with the substrate type. Figure 2 compares the effect of initial pH on SCP production using two media under the same conditions. The biomass and protein content were increased up to pH 5.0 and then decreased with increasing pH in both papaya peel media and control media (Figure 2).

High dry biomass for papaya peel (23.93 ± 1.69 g/L) and control medium (22.54 ± 1.02 g/L) was recorded at pH 5.0. At the same time, lower biomass and protein contents were recorded at pH 3.0 in both media as metabolic activities of yeast were inhibited at lower initial pH conditions. Significantly ($p < 0.05$) higher protein content was observed at pH 5.0 for both media as per Tukey's multiple comparison test. The observation can be comparable with the control medium (Figure 2).

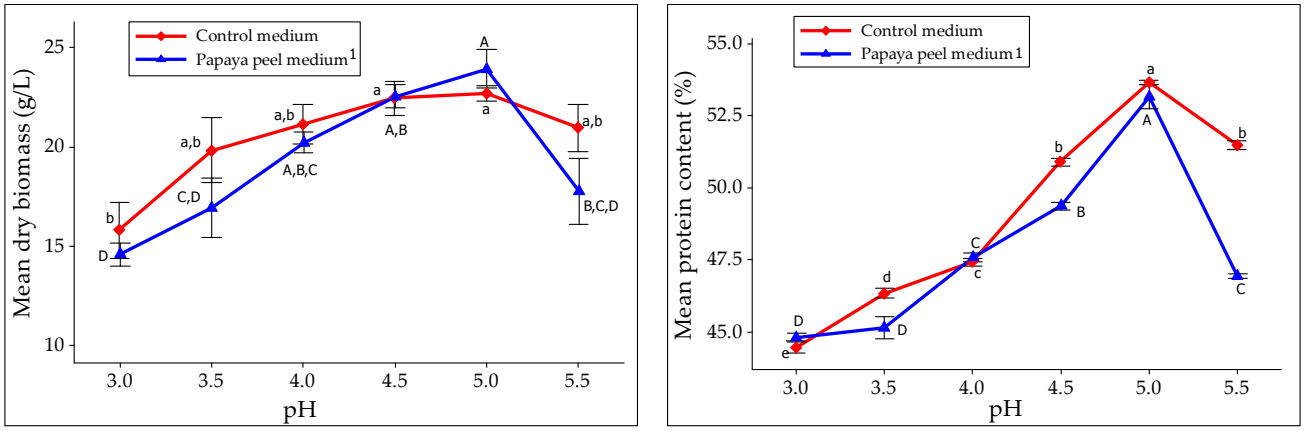


Figure 2. Effect of pH on the dry biomass and protein content of SCP produced from papaya peel and control media using mixed culture of palmyrah toddy in liquid state fermentation at 100 rpm with 10% (v/v) substrate concentration at 28 °C for 72 h: (**a**) effect of pH on dry biomass; (**b**) effect of pH on the protein content. Comparisons of mean were performed with Tukey's multiple comparison test at $p < 0.05$. Different uppercase and lowercase letters indicate the significant differences in papaya peel medium and control medium, respectively [1] [58].

Based on the results, optimum pH for SCP production using the mixed culture of yeast and bacteria from palmyrah toddy in papaya peel (10% (v/v) and the control medium was pH 5.0. Previous studies have also reported similar observations when using okara-wheat grit substrates to grow *Rhizopus oligosporus* and *Aspergillus oryzae* [59], pineapple peel for *S. cerevisiae* [60], and *Eichornia* and banana peel for *Aspergillus terreus* [61]. Rages and Haider reported pH 5.5 as the best initial pH for SCP production from *Yarrowia lipolytica* (formerly known as *Candida lipolytica*) using olive fruit wastes [62]. The pH of 7.0 was ideal for the growth of *S. cerevisiae* in orange peel [63].

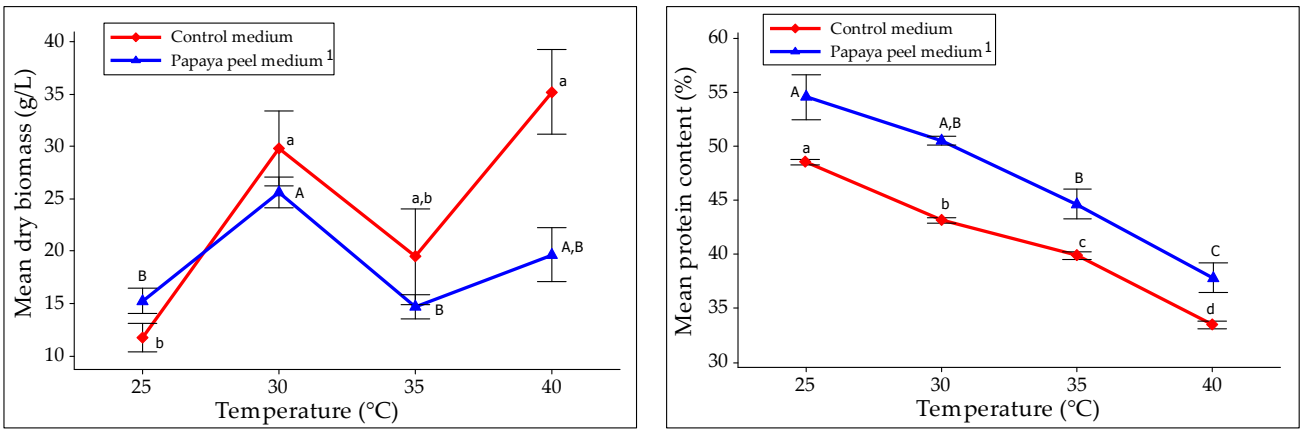
3.3.2. Optimization of Incubation Temperature for SCP Production

Incubation temperature is an important factor affecting microbial growth and thus the yield of biomass production [30]. Figure 3 show the effect of temperature on SCP produced from papaya peel extract and control medium.

Figure 3 depicts a similar observation in biomass yield and its protein content when the fermentation temperature was raised from of 25 °C to 30 °C in both media. In papaya peel medium, a significantly ($p < 0.05$) higher biomass was cropped at 30 °C (25.59 ± 2.58 g/L). When the temperature further increased above 30 °C, there was a drop in the yield of biomass (Figure 3), which could be related to the inactivation of metabolic enzymes and the disruption of cellular functions at high temperatures. Lower biomass was recorded at lower temperatures, probably due to the loss of membrane function inhibiting the uptake of key substrates [64]. Munawar et al. [65] also reported a similar observation with *Candida utilis* grown on fruit wastes yielded in liquid fermentation. Milala et al. [63] reported the maximum yield at 37 °C with *S. cerevisiae*, higher than the temperature reported in this study.

In contrast, protein content decreased with temperature, and the maximum value was obtained at 25 °C for 10% (v/v) papaya peel medium (54.5 ± 3.6%) (Figure 3). Tukey's multiple comparison test results suggest that 25 °C would be the optimum temperature for the mixed culture obtained from palmyrah toddy in papaya peel extract (10% v/v), which is in line with the results reported for *S. cerevisiae* harvested in pineapple peel [60]. The most common temperature used for the incubation of various microorganisms is 25–27 °C [30]. Kamal et al. [57] reported that 31 °C is the optimum for biomass and protein production from *Aspergillus niger* using banana peel. Jaganmohan et al. [52] also reported that 35 °C is

the optimum temperature for *A. terreus*, which is higher than the optimum temperature reported in this study.

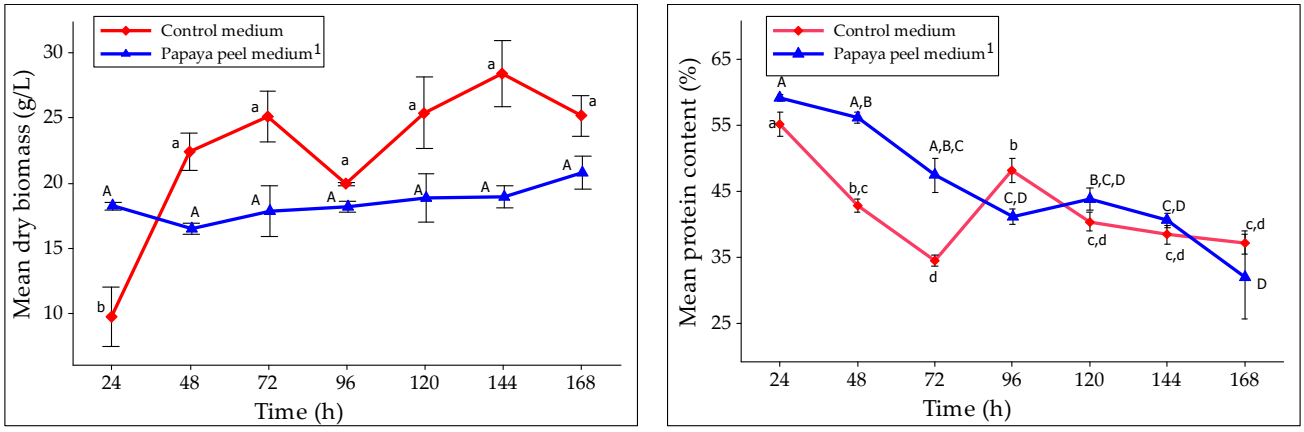


(**a**) (**b**)

Figure 3. Effect of fermentation temperature on dry biomass and protein content of SCP produced from papaya peel and control media using mixed culture of palmyrah toddy in liquid state fermentation at 100 rpm with 10% (*v*/*v*) substrate concentration at pH 5.0 for 72 h: (**a**) Effect of fermentation temperature on dry biomass; (**b**) Effect of fermentation temperature on the protein content. Comparisons of mean were performed with Tukey's multiple comparison test at $p < 0.05$. Different uppercase and lowercase letters indicate the significant differences in papaya peel medium and control medium, respectively [1] [58].

3.3.3. Optimization of Incubation Time for the SCP Production

The effect of fermentation time on SCP production from papaya peel extract and control medium was studied, and the results are presented in Figure 4.



(**a**) (**b**)

Figure 4. Effect of fermentation time on the dry biomass and protein content of SCP produced from papaya peel and control media using a mixed culture of palmyrah toddy in liquid state fermentation at 100 rpm with 10% (*v*/*v*) substrate concentration at pH 5.0 and 25 °C: (**a**) effect of fermentation time on dry biomass; (**b**) effect of fermentation time on the protein content. Comparisons of mean were performed with Tukey's multiple comparison test at $p < 0.05$. Different uppercase and lowercase letters indicate the significant differences in papaya peel medium and control medium, respectively [1] [58].

The highest biomass production for papaya peel extract and control medium was recorded after fermentation of 168 h (20.79 ± 2.21 g/L) and 144 h (28.43 ± 4.39 g/L), respectively. Tukey's multiple comparison test showed no significant difference in the mean

biomass obtained with papaya peel medium, and it fell in line with the biomass production using *Rhodococcus opacus* [66]. In contrast, maximum biomass production of *C. utilis* was found after 4 days of incubation in liquid state fermentation at 30 °C and 120 rpm [65]. The decrease in biomass yield after 144 h in the control medium may be attributable to nutrient depletion in the growth media [67]. However, there was a slight increase in biomass yield at 168 h, probably due to the increase in available soluble sugar [56].

Significantly ($p < 0.05$) higher protein content was recorded after 24 h of fermentation in papaya peel medium (59.1 ± 0.8%) and the control medium (55.2 ± 3.1%) and decreased over time. This phenomenon is probably due to the culture aging and autolysis caused by nutrient depletion or higher initial inoculum [68,69]. Similarly, in *C. utilis*, the highest protein content was observed at the early stage of microbial growth (after 24 h of growth) and decreased afterward as they store carbohydrates such as starch and glycogen during aging [70]. Ojokoh and Uzeh [69] observed that viable cell counts were decreased after 1 or 2 days of fermentation in papaya peel medium.

Longer fermentation time was reported in other studies when using various fruit wastes. Rages and Haider obtained the maximum protein production after 4 days of fermentation when *Y. lipolytica* (formerly known as *C. lipolytica*) was grown on olive fruit wastes [62]. Maximum biomass and protein content were recorded after 6 days of fermentation when *A. niger* and various fruit waste media, including banana, watermelon, pineapple, and orange, were used at 28 °C and 120 rpm to produce SCP [67].

From the economic standpoint, the optimum fermentation time to grow the mixed culture of palmyrah toddy in papaya peel extract is 24 h, which could result in lower energy cost, manpower hours, and high production.

3.3.4. Effect of Optimization on Biomass and Protein Content

Optimal conditions for fermentation using the mixed culture of palmyrah toddy were pH 5.0, 25 °C, and 24 h for papaya peel medium (10%, v/v). Furthermore, the results show that optimization of process parameters of papaya peel medium generates significantly ($p < 0.05$) lower biomass production (23.15 ± 2.31 g/L) than the control medium (27.75 ± 0.93 g/L) (Table 3). Though, a significantly higher protein content was observed with papaya peel medium (56.1 ± 0.4%) than the control medium (54.3 ± 0.6%) (Table 3 and Figure 5). The above results established that papaya peel extract and natural mixed culture of palmyrah toddy could be used to produce SCP in liquid state fermentation. Moreover, the present study also confirms the previous findings as they generate a high amount of protein than the control medium under their optimized condition.

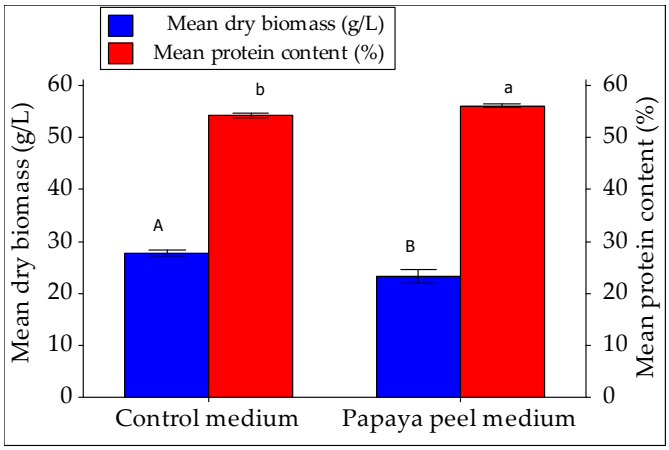


Figure 5. Dry weight and protein content of biomass produced in papaya peel medium and control medium under optimization. Comparisons of mean were performed with Tukey's multiple comparison test at $p < 0.05$. Different uppercase and lowercase letters indicate the significant differences in dry biomass and protein content, respectively. A,B: Dry biomass; a,b: Protein content.

Table 3. Dry weight and the protein content of dry biomass produced in papaya peel medium and control medium under optimized conditions.

Condition	Dry Biomass (g/L)	Crude Protein Content (%)
Control medium	27.75 ± 0.93^{A}	54.3 ± 0.6^{b}
Papaya peel medium	23.15 ± 2.31^{B}	56.1 ± 0.4^{a}

Results are shown as mean ± standard deviation of three replicates, and different superscript letters in the same column are significantly different ($p < 0.05$).

3.3.5. Effect of Nucleic Acid Reduction

Generally, bacterial and fungal species have a higher nucleic acid content of 9–16% [9]. SCP with high nucleic acid content was only approved for animal nutrition and recommended for animals with a short life span [71]. Human consumption of more than 2 g of nucleic acid equivalent per day elevates the serum uric acid level from purine metabolism and may lead to gout and renal calculi [9,14]. Therefore, for human consumption, the nucleic acid contents of SCP must be reduced below 2% [6]. Alkaline hydrolysis is a compromise method that consists of an incubation at pH 9.5 followed by a heat shock, which precipitates protein [14]. Moreover, the breakdown of cell walls and nucleic acid reduction would increase the digestibility and palatability [72,73].

Dried weight and the protein content of the biomass obtained before and after the nucleic acid treatment were determined and compared (Table 4 and Figure 6). Nucleic acid reduction treatment affected a significant ($p < 0.05$) reduction in the yield of dried biomass and protein content of biomass harvested under optimized conditions (Table 4).

Table 4. Dry weight and protein content of dry biomass before and after nucleic acid treatment.

Condition	Dry Biomass (g/L)	Crude Protein Content (%)
Before nucleic acid reduction treatment	23.2 ± 2.3^{A}	56.1 ± 0.4^{a}
After nucleic acid reduction treatment	16.7 ± 1.2^{B}	45.3 ± 0.3^{b}

Results are shown as mean ± standard deviation of three replicates, and different superscript letters in the same column are significantly different ($p < 0.05$).

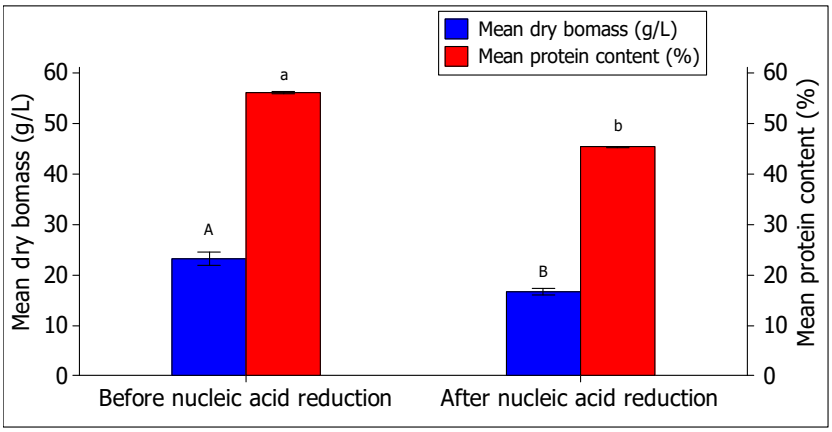


Figure 6. Effect of nucleic acid reduction treatment on the dry biomass and protein content of SCP produced from papaya peel using mixed culture of palmyrah toddy. Comparisons of mean were performed with Tukey's multiple comparison test at $p < 0.05$. Different uppercase and lowercase letters indicate the significant differences in dry biomass and protein content, respectively. A,B: Dry biomass; a,b: Protein content.

Loss in biomass may be due to the diffusion of degraded nucleic acid components out of the cells, which results in 35–38% biomass loss [71]. Hedenskog and Ebbinghaus reported a strong decrease in the ribonucleic acid (RNA) content of yeast at higher pH 8–9 after heating at 80–90 °C, whereas the addition of NaCl resulted in further reduction of

RNA [74]. Alkaline treatment using NH_4OH also improves the in vitro digestibility of *Candida utilis* biomass in addition to the nucleic acid reduction [75].

Furthermore, 70–80% of total nitrogen is represented by amino acids, while the rest occurs in nucleic acids [6]. Since the protein content was determined based on total nitrogen content (N×6.25), nucleic acid reduction treatment resulted in the reduction of protein content. This study also reported a significant ($p < 0.05$) reduction in mean dried biomass (28.0%) and mean protein content (19.3%), and still, it can be a good source of protein (Table 4).

3.3.6. Nutrient Analysis

The nutritive value of SCP varies with the microorganisms used and the substrate on which the microorganisms grow. The method of harvesting, drying, and processing conditions also affect the nutritive value of the finished product [76].

Composition of major amino acids is compared with the amino acid requirement of an adult in Table 5 [39]. Biomass produced using palmyrah toddy culture on papaya peel wastes contained 56.1% protein with a good amino acid profile comparable to those required by the FAO for the daily human diet. Leucine (29.2 g/mg) and arginine (22.5 g/mg) are present in higher concentration. This biomass also contains a considerable amount of methionine (14.4 g/mg), which is limiting in many plant and animal sources.

Table 5. Comparison of amino acid composition of SCP produced on papaya peel based medium with amino acid requirement.

Description	Amino Acid										
	His [1]	Ile [1]	Leu [1]	Lys [1]	Met + Cys [1,2]	Phe + Tyr [1,3]	Thr [1]	Trp [1]	Val [1]	Glu	Arg
SCP from papaya peel based medium	8.5	14.6	29.2	8.2	14.4	6.5	5.5	9.2	14.7	12.6	22.5
Amino acid requirement (FAO/WHO/UNU, 2007 [39])	15	30	59	45	22	38	23	6	39	NR	NR

[1] Essential amino acid. [2] Sulphur amino acids (methionine + cysteine). [3] Aromatic amino acids (phenylalanine + tyrosine). mg/g protein. Three-letter abbreviation denotes the amino acids; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Cys, cysteine; Phe, phenylalanine; Tyr, Tyrosine; Thr, threonine; Trp, tryptophan; Val, valine; Glu, glutamate, Arg, Arginine. NR, Not Reported.

The results indicate that the obtained microbial protein can be a suitable protein supplement as food or feed. The final product should be nutritious and should also pass all toxicity tests to be commercialized as a food product. Strain identification and long-term feeding trials should be carried out to identify toxicological effects with special emphasis on carcinogenicity.

4. Conclusions and Future Perspectives

The present findings reveal that papaya, watermelon, pineapple, banana, mango, and sour orange peel waste can be used as an effective and alternate carbon source for SCP production. Papaya peel (11.73 ± 0.81 g/L yield with 52.4 ± 0.4% of protein content) is the best substrate among the six peels tested for SCP production using the natural mixed culture obtained from palmyrah toddy through liquid fermentation. Optimum conditions to ferment papaya peel extract (10%, *v*/*v*) using the mixed culture of palmyrah toddy were pH 5.0, 25 °C, and 24 h. After optimization, the protein content of biomass produced from papaya peel increased slightly (52.4 ± 0.4% to 56.1 ± 0.4%) while the biomass increased by 2-fold (11.73 ± 0.81 to 23.15 ± 2.31 g/L). SCP produced from papaya peel medium exhibited a higher protein content (56.1 ± 0.4%) than that of the control medium (54.3 ± 0.6%) under optimized conditions. Nucleic acid reduction treatment has a significant effect on dried biomass and protein content reduction in SCP (28.1% and 19.3% of reduction, respectively). Furthermore, amino acid content of SCP produced from papaya peel is comparable with amino acid requirement recommended by FAO/WHO/UNU. According to the findings

of this study, papaya peel extract can be the best candidate for SCP production using the natural mixed culture of palmyrah toddy culture in liquid state fermentation.

The OFAT approach has limitations as it consumes more time, and there is difficulty in studying the interaction of each factor on SCP production. Though high protein yield was recorded under this optimized condition, the use of statistical optimization tools such as response surface methodology (RSM) enhances the process of SCP production by evaluating the interactions among factors and responses. Generally, agricultural wastes are limited in nitrogen content thus supplementation with nitrogen sources can further increase the protein content of SCP [77]. Compared to monoculture, co-cultures enhance biomass productivity by efficient saccharification and utilization of substrate, or by removal of inhibitory by-products. Co-culture may reduce the fermentation time and eliminate the substrate treatment resulting in cost reduction [78]. Therefore, the mixed culture of palmyrah toddy can be a good source for SCP production. Further, the high amount of protein content (56.1%) of obtained biomass indicates the potential of natural palmyrah toddy culture for commercial application. Since no toxicological studies have been made, it is recommended to carry out further work directed to this area.

Author Contributions: Original draft, P.T., R.K. and T.M. Writing—review and editing, P.T., A.G., R.K., O.M. and T.M. All authors have read and agreed to the published version of the manuscript.

Funding: National Research Council (NRC), Sri Lanka (Grant No: NRC 2016/071).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors wish to acknowledge the financial support granted by the National Research Council (NRC), Sri Lanka (Grant No: NRC 2016/071).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Spalvins, K.; Zihare, L.; Blumberga, D. Single Cell Protein Production from Waste Biomass: Comparison of Various Industrial by-Products. *Energy Procedia* **2018**, *147*, 409–418. [CrossRef]
2. Seviour, R.J.; Harvey, L.M.; Fazenda, M.; McNeil, B. 5—Production of Foods and Food Components by Microbial Fermentation: An Introduction. In *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*; McNeil, B., Archer, D., Giavasis, I., Harvey, L., Eds.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing: Sawston, UK, 2013; pp. 97–124, ISBN 978-0-85709-343-1.
3. Abdel-Shafy, H.I.; Mansour, M.S.M. Solid Waste Issue: Sources, Composition, Disposal, Recycling, and Valorization. *Egypt. J. Pet.* **2018**, *27*, 1275–1290. [CrossRef]
4. Dhillon, G.S.; Kaur, S.; Brar, S.K. Perspective of Apple Processing Wastes as Low-Cost Substrates for Bioproduction of High Value Products: A Review. *Renew. Sustain. Energy Rev.* **2013**, *27*, 789–805. [CrossRef]
5. Panda, S.K.; Ray, R.C.; Mishra, S.S.; Kayitesi, E. Microbial Processing of Fruit and Vegetable Wastes into Potential Biocommodities: A Review. *Crit. Rev. Biotechnol.* **2018**, *38*, 1–16. [CrossRef] [PubMed]
6. Nasseri, A.T.; Rasoul-Amini, S.; Morowvat, M.H.; Ghasemi, Y. Single Cell Protein: Production and Process. *Am. J. Food Technol.* **2011**, *6*, 103–116. [CrossRef]
7. Sharif, M.; Zafar, M.H.; Aqib, A.I.; Saeed, M.; Farag, M.R.; Alagawany, M. Single Cell Protein: Sources, Mechanism of Production, Nutritional Value and Its Uses in Aquaculture Nutrition. *Aquaculture* **2021**, *531*, 735885. [CrossRef]
8. Suman, G.; Nupur, M.; Anuradha, S.; Pradeep, B. Single Cell Protein Production: A Review. *Int. J. Curr. Microbiol. App. Sci.* **2015**, *4*, 251–262.
9. Anupama; Ravindra, P. Value-Added Food: Single Cell Protein. *Biotechnol. Adv.* **2000**, *18*, 459–479. [CrossRef]
10. El-Sayed, A.-F.M. Alternative Dietary Protein Sources for Farmed Tilapia, Oreochromis Spp. *Aquaculture* **1999**, *179*, 149–168. [CrossRef]
11. García-Garibay, M.; Gómez-Ruiz, L.; Cruz-Guerrero, A.E.; Bárzana, E. SINGLE CELL PROTEIN | The Algae. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Tortorello, M.L., Eds.; Academic Press: Oxford, UK, 2014; pp. 425–430, ISBN 978-0-12-384733-1.
12. Sadler, M.J. Fungal Protein. In *New and Developing Sources of Food Proteins*; Hudson, B.J.F., Ed.; Springer US: Boston, MA, USA, 1994; pp. 343–362, ISBN 978-1-4615-2652-0.

13. Hezarjaribi, M.; Ardestani, F.; Ghorbani, H.R. Single Cell Protein Production by Saccharomyces Cerevisiae Using an Optimized Culture Medium Composition in a Batch Submerged Bioprocess. *Appl. Biochem. Biotechnol.* **2016**, *179*, 1336–1345. [CrossRef]
14. Ugalde, U.O.; Castrillo, J.I. Single Cell Proteins from Fungi and Yeasts. In *Applied Mycology and Biotechnology*; Khachatourians, G.G., Arora, D.K., Eds.; Agriculture and Food Production; Elsevier: Amsterdam, The Netherlands, 2002; Volume 2, pp. 123–149.
15. Wikandari, R.; Manikharda; Baldermann, S.; Ningrum, A.; Taherzadeh, M.J. Application of Cell Culture Technology and Genetic Engineering for Production of Future Foods and Crop Improvement to Strengthen Food Security. *Bioengineered* **2021**, *12*, 11305–11330. [CrossRef] [PubMed]
16. Jones, S.W.; Karpol, A.; Friedman, S.; Maru, B.T.; Tracy, B.P. Recent Advances in Single Cell Protein Use as a Feed Ingredient in Aquaculture. *Curr. Opin. Biotechnol.* **2020**, *61*, 189–197. [CrossRef] [PubMed]
17. Mensah, J.K.M.; Twumasi, P. Use of Pineapple Waste for Single Cell Protein (SCP) Production and the Effect of Substrate Concentration on the Yield. *J. Food Process Eng.* **2017**, *40*, e12478. [CrossRef]
18. Thiviya, P.; Gamage, A.; Kapilan, R.; Merah, O.; Madhujith, T. Single Cell Protein Production Using Different Fruit Waste: A Review. *Separations* **2022**, *9*, 178. [CrossRef]
19. FAO. *World Food and Agriculture—Statistical Yearbook 2021*; FAO Statistical Yearbook—World Food and Agriculture; FAO: Rome, Italy, 2021; ISBN 978-92-5-134332-6.
20. Weerahewa, J.; Rajapakse, C.; Pushpakumara, G. An Analysis of Consumer Demand for Fruits in Sri Lanka. 1981–2010. *Appetite* **2013**, *60*, 252–258. [CrossRef]
21. Romelle, F.D.; Rani, A.; Manohar, R.S. Chemical Composition of Some Selected Fruit Peels. *Eur. J. Food Sci. Technol.* **2016**, *4*, 12–21.
22. Abdullah; Mat, H.B. The Characteristic of Pineapple Waste from Canning Industry. *Adv. Sci. Lett.* **2017**, *23*, 5691–5693. [CrossRef]
23. Rayavarapu, B.; Tallapragada, P.; Usha, M.S. Statistical Optimization of γ-Aminobutyric Acid Production by Response Surface Methodology and Artificial Neural Network Models Using Lactobacillus Fermentum Isolated from Palm Wine. *Biocatal. Agric. Biotechnol.* **2019**, *22*, 101362. [CrossRef]
24. Vengaiah, P.C.; Murthy, G.N.; Sattiraju, M.; Maheswarappa, H.P. Value Added Food Products from Palmyrah Palm *(Borassus flabellifer* L). *J. Nutr. Health Sci.* **2017**, *4*, 105. [CrossRef]
25. Theivendirarajah, K.; Chrystopher, R.K. Microflora and Microbial Activity in Palmyrah (Borassus Flabellifer) Palm Wine in Sri Lanka. *Mircen J.* **1987**, *3*, 23–31. [CrossRef]
26. Limtong, S.; Am-In, S.; Kaewwichian, R.; Kaewkrajay, C.; Jindamorakot, S. Exploration of Yeast Communities in Fresh Coconut, Palmyra, and Nipa Palm Saps and Ethanol-Fermenting Ability of Isolated Yeasts. *Antonie Van Leeuwenhoek* **2020**, *113*, 2077–2095. [CrossRef] [PubMed]
27. Yadav, J.S.S.; Bezawada, J.; Ajila, C.M.; Yan, S.; Tyagi, R.D.; Surampalli, R.Y. Mixed Culture of Kluyveromyces Marxianus and Candida Krusei for Single-Cell Protein Production and Organic Load Removal from Whey. *Bioresour. Technol.* **2014**, *164*, 119–127. [CrossRef] [PubMed]
28. Balakumar, S.; Arasaratnam, V. Comparison of Industrial Scale Ethanol Production from a Palmyrah-Based Carbon Source by Commercial Yeast and a Mixed Culture from Palmyrah Toddy. *J. Inst. Brew.* **2009**, *115*, 105–110. [CrossRef]
29. Rajendran, S.; Kapilan, R.; Vasantharuba, S. Papaw Fruit Juice as Source for Single Cell Protein Production Using Natural Palmyrah Toddy Yeast. *Ceylon J. Sci.* **2018**, *47*, 379–386. [CrossRef]
30. Reihani, S.F.S.; Khosravi-Darani, K. Influencing Factors on Single-Cell Protein Production by Submerged Fermentation: A Review. *Electron. J. Biotechnol.* **2019**, *37*, 34–40. [CrossRef]
31. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, USA, 2006.
32. BeMiller, J.N. Carbohydrate Analysis. In *Food Analysis*; Nielsen, S.S., Ed.; Food Analysis; Springer: Boston, MA, USA, 2010; pp. 147–177, ISBN 978-1-4419-1478-1.
33. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
34. Dharumadurai, D.; Subramaniyan, L.; Subhasish, S.; Nooruddin, T.; Annamalai, P. Production of Single Cell Protein from Pineapple Waste Using Yeast. *Innov. Rom. Food Biotechnol.* **2011**, *8*, 26–32.
35. Kheiralla, Z.H.; El-Gendy, N.S.; Ahmed, H.A.; Shaltout, T.H.; Hussein, M.M.D. One-Factor-at-a-Time (OFAT) Optimization of Hemicellulases Production from Fusarium Moniliforme in Submerged Fermentation. *Energy Sources Part A Recovery Util. Environ. Eff.* **2018**, *40*, 1877–1885. [CrossRef]
36. Abou-Zeid, A.-Z.A.; Khan, J.A.; Abulnaja, K.O. On Methods for Reduction of Nucleic Acids Content in a Single-Cell Protein from Gas Oil. *Bioresour. Technol.* **1995**, *52*, 21–24. [CrossRef]
37. Herbert, D.; Phipps, P.J.; Strange, R.E. Chapter III Chemical Analysis of Microbial Cells. In *Methods in Microbiology*; Norris, J.R., Ribbons, D.W., Eds.; Academic Press: London, UK, 1971; Volume 5, pp. 209–344.
38. Fountoulakis, M.; Lahm, H.-W. Hydrolysis and Amino Acid Composition Analysis of Proteins. *J. Chromatogr. A* **1998**, *826*, 109–134. [CrossRef]
39. Joint FAO/WHO/UNU. *Protein and Amino Acid Requirements in Human Nutrition*; World Health Organization: Geneva, Switzerland, 2007; ISBN 978-92-4-120935-9.
40. Saejung, C.; Salasook, P. Recycling of Sugar Industry Wastewater for Single-Cell Protein Production with Supplemental Carotenoids. *Environ. Technol.* **2020**, *41*, 59–70. [CrossRef] [PubMed]

41. Saheed, O.K.; Jamal, P.; Karim, M.I.A.; Alam, M.Z.; Muyibi, S.A. Utilization of Fruit Peels as Carbon Source for White Rot Fungi Biomass Production under Submerged State Bioconversion. *J. King Saud Univ. Sci.* **2016**, *28*, 143–151. [CrossRef]
42. Dias, P.G.I.; Sajiwanie, J.W.A.; Rathnayaka, R.M.U.S.K. Chemical Composition, Physicochemical and Technological Properties of Selected Fruit Peels as a Potential Food Source. *Int. J. Fruit Sci.* **2020**, *20*, S240–S251. [CrossRef]
43. Morais, D.R.; Rotta, E.M.; Sargi, S.C.; Bonafe, E.G.; Suzuki, R.M.; Souza, N.E.; Matsushita, M.; Visentainer, J.V. Proximate Composition, Mineral Contents and Fatty Acid Composition of the Different Parts and Dried Peels of Tropical Fruits Cultivated in Brazil. *J. Braz. Chem. Soc.* **2017**, *28*, 308–318. [CrossRef]
44. Sánchez-Camargo, A.d.P.; Gutiérrez, L.-F.; Vargas, S.M.; Martinez-Correa, H.A.; Parada-Alfonso, F.; Narváez-Cuenca, C.-E. Valorisation of Mango Peel: Proximate Composition, Supercritical Fluid Extraction of Carotenoids, and Application as an Antioxidant Additive for an Edible Oil. *J. Supercrit. Fluids* **2019**, *152*, 104574. [CrossRef]
45. Ajila, C.M.; Bhat, S.G.; Prasada Rao, U.J.S. Valuable Components of Raw and Ripe Peels from Two Indian Mango Varieties. *Food Chem.* **2007**, *102*, 1006–1011. [CrossRef]
46. Wongkaew, M.; Kittiwachana, S.; Phuangsaijai, N.; Tinpovong, B.; Tiyayon, C.; Pusadee, T.; Chuttong, B.; Sringarm, K.; Bhat, F.M.; Sommano, S.R.; et al. Fruit Characteristics, Peel Nutritional Compositions, and Their Relationships with Mango Peel Pectin Quality. *Plants* **2021**, *10*, 1148. [CrossRef]
47. Garcia-Amezquita, L.E.; Tejada-Ortigoza, V.; Campanella, O.H.; Welti-Chanes, J. Influence of Drying Method on the Composition, Physicochemical Properties, and Prebiotic Potential of Dietary Fibre Concentrates from Fruit Peels. *J. Food Qual.* **2018**, *2018*, e9105237. [CrossRef]
48. Banerjee, S.; Ranganathan, V.; Patti, A.; Arora, A. Valorisation of Pineapple Wastes for Food and Therapeutic Applications. *Trends Food Sci. Technol.* **2018**, *82*, 60–70. [CrossRef]
49. Pathak, P.D.; Mandavgane, S.A.; Kulkarni, B.D. Waste to Wealth: A Case Study of Papaya Peel. *Waste and Biomass Valorization* **2019**, *10*, 1755–1766. [CrossRef]
50. Mondal, A.K.; Sengupta, S.; Bhowal, J.; Bhattacharya, D.K. Utilization of Fruit Wastes in Producing Single Cell Protein. *Int. J. Sci. Environ. Technol.* **2012**, *1*, 430–438.
51. Mahnaaz, K.; Khan, S.S.; Zafar, A.; Arshiya, T. Production of Fungal Single Cell Protein Using Rhizopus Oligosporus Grown on Fruit Wastes. In *Biological Forum*; Satya Prakashan: Delhi, India, 2009; Volume 1, pp. 26–28.
52. Spalvins, K.; Ivanovs, K.; Blumberga, D. Single Cell Protein Production from Waste Biomass: Review of Various Agricultural by-Products. *Agron. Res.* **2018**, *16*, 14931508. [CrossRef]
53. Bratosin, B.C.; Darjan, S.; Vodnar, D.C. Single Cell Protein: A Potential Substitute in Human and Animal Nutrition. *Sustainability* **2021**, *13*, 9284. [CrossRef]
54. Khan, M.; Khan, S.S.; Ahmed, Z.; Tanveer, A. Production of Single Cell Protein from Saccharomyces Cerevisiae by Utilizing Fruit Wastes. *Nanobiotech. Univers.* **2010**, *1*, 127–132.
55. Yabaya, A.; Ado, S.A. Mycelial Protein Production by Aspergillus Niger Using Banana Peels. *Sci. World J.* **2008**, *3*, 9–12. [CrossRef]
56. Tropea, A.; Ferracane, A.; Albergamo, A.; Potortì, A.G.; Lo Turco, V.; Di Bella, G. Single Cell Protein Production through Multi Food-Waste Substrate Fermentation. *Fermentation* **2022**, *8*, 91. [CrossRef]
57. Jach, M.E.; Serefko, A.; Ziaja, M.; Kieliszek, M. Yeast Protein as an Easily Accessible Food Source. *Metabolites* **2022**, *12*, 63. [CrossRef]
58. Thiviya, P.; Kapilan, R.; Madhujith, T. Bioconversion of Fruit Wastes of Papaya, Watermelon, and Banana into Single Cell Protein Production. *Trop. Agric. Res.* **2021**, *32*, 503–514. [CrossRef]
59. Yousufi, M.K. Impact of PH on the Single Cell Protein Produced on Okara-Wheat Grit Substrates Using Rhizopus Oligosporus and Aspergillus Oryzae. *IOSR J. Environ. Sci. Toxicol. Food Technol.* **2012**, *1*, 32–35. [CrossRef]
60. Umesh, M.; Thazeem, B.; Preethi, K. Valorization of Pineapple Peels through Single Cell Protein Production Using Saccharomyces Cerevisiae NCDC 364. *Appl. Food Biotechnol.* **2019**, *6*, 255–263. [CrossRef]
61. Jaganmohan, P.; Daas, B.P.; Prasad, S.V. Production of Single Cell Protein (SCP) with Aspergillus Terreus Using Solid State Fermentation. *Eur. J. Biol. Sci.* **2013**, *5*, 38–43. [CrossRef]
62. Rages, A.A.; Haider, M.M. Alkaline Hydrolysis of Olive Fruits Wastes for the Production of Single Cell Protein by Candida Lipolytica. *Biocatal. Agric. Biotechnol.* **2021**, *33*, 101999. [CrossRef]
63. Milala, M.A.; Yakubu, M.; Burah, B.; Laminu, H.H.; Bashir, H. Production and Optimization of Single Cell Protein from Orange Peels by Saccharomyces Cerevisiae. *J. Biosci. Biotechnol. Discov.* **2018**, *3*, 99–104. [CrossRef]
64. Nedwell, D.B. Effect of Low Temperature on Microbial Growth: Lowered Affinity for Substrates Limits Growth at Low Temperature. *FEMS Microbiol. Ecol.* **1999**, *30*, 101–111. [CrossRef]
65. Munawar, R.; Irfan, M.; Nadeem, M.; Syed, Q.; Siddique, Z. Biosynthesis of Single Cell Biomass of Candida Utilis by Submerged Fermentation. *Pak. J. Sci.* **2010**, *62*, 1–5.
66. Mahan, K.M.; Le, R.K.; Wells, T., Jr.; Anderson, S.; Yuan, J.S.; Stoklosa, R.J.; Bhalla, A.; Hodge, D.B.; Ragauskas, A.J. Production of Single Cell Protein from Agro-Waste Using Rhodococcus Opacus. *J. Ind. Microbiol. Biotechnol.* **2018**, *45*, 795–801. [CrossRef]
67. Oshoma, C.E.; Eguakun-Owie, S.O. Conversion of Food Waste to Single Cell Protein Using Aspergillus Niger. *J. Appl. Sci. Environ. Manag.* **2018**, *22*, 350–355. [CrossRef]
68. Gervasi, T.; Pellizzeri, V.; Calabrese, G.; Di Bella, G.; Cicero, N.; Dugo, G. Production of Single Cell Protein (SCP) from Food and Agricultural Waste by Using Saccharomyces Cerevisiae. *Nat. Prod. Res.* **2018**, *32*, 648–653. [CrossRef]

69. Ojokoh, A.O.; Uzeh, R.E. Production of Saccharomyces Cerevisiae Biomass in Papaya Extract Medium. *Afr. J. Biotechnol.* **2005**, *4*, 1281–1284. [CrossRef]
70. Zayed, G.; Mostafa, N. Studies on the Production and Kinetic Aspects of Single Cell Protein from Sugar Cane Bagasse Saccharified by Aspergillus Niger. *Biomass Bioenergy* **1992**, *3*, 363–367. [CrossRef]
71. Ritala, A.; Häkkinen, S.T.; Toivari, M.; Wiebe, M.G. Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. *Front. Microbiol.* **2017**, *8*, 2009. [CrossRef] [PubMed]
72. Bajpai, P. *Single Cell Protein Production from Lignocellulosic Biomass*; Bajpai, P., Ed.; SpringerBriefs in Molecular Science; Springer: Singapore, 2017; ISBN 978-981-10-5873-8.
73. Linder, T. Making the Case for Edible Microorganisms as an Integral Part of a More Sustainable and Resilient Food Production System. *Food Sec.* **2019**, *11*, 265–278. [CrossRef]
74. Hedenskog, G.; Ebbinghaus, L. Reduction of the Nucleic Acid Content of Single-Cell Protein Concentrates. *Biotechnol. Bioeng.* **1972**, *14*, 447–457. [CrossRef] [PubMed]
75. Parajó, J.C.; Santos, V.; Domínguez, H.; Vázquez, M. NH4OH-Based Pretreatment for Improving the Nutritional Quality of Single-Cell Protein (SCP). *Appl Biochem. Biotechnol.* **1995**, *55*, 133–149. [CrossRef]
76. Adedayo, M.R.; Ajiboye, E.A.; Akintunde, J.K.; Odaibo, A. Single Cell Proteins: As Nutritional Enhancer. *Adv. Appl. Sci. Res.* **2011**, *2*, 396–409.
77. Oshoma, C.E.; Eguakun-Owie, S.O.; Obuekwe, I.S. Utilization of Banana Peel as a Substrate for Single Cell Protein and Amylase Production by Aspergillus Niger. *Afr. Sci.* **2019**, *18*, 143–150.
78. Bajpai, P. Use of Mixed Cultures. In *Single Cell Protein Production from Lignocellulosic Biomass*; Bajpai, P., Ed.; SpringerBriefs in Molecular Science; Springer: Singapore, 2017; pp. 37–40. ISBN 978-981-10-5873-8.