
Utilization of *Carica papaya* peels as a substrate for single-cell protein production by selected *Aspergillus* species

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Abstract The results demonstrated a significant increase in crude protein content based on proximate analysis, the *C. papaya* peels inoculated with baker's yeast, had the highest increase of 56.57%, followed by the *C. papaya* peels inoculated with *A. oryzae* with 46.37% and *C. papaya* peels inoculated with *A. niger* with 19.31%. Meanwhile, *C. papaya* peels inoculated with *A. flavus*, and uninoculated *C. papaya* peels yielded the lowest crude protein content (CPC) with 14.17% and 0.00%, respectively. Even though the fungal inoculants did not surpass the ability of baker's yeast, they still gave a comparable result indicating their ability to produce high amount of protein which can be a low-cost alternative to using baker's yeast. Moreover, the significant increase in moisture, ash, fat and fiber content indicates the suitability of the fungal inoculants for SCP production. The findings highlighted the ability of these fungal inoculants to improve the nutritional profile of *C. papaya* peels, suggesting their potential as cost-effective alternatives for conventionally expensive protein sources. This approach offers a sustainable method for protein production and contributes to waste valorization, supporting environmental sustainability.

Keywords: Low-cost alternative, Proximate analysis, Solid-state fermentation, Sustainable protein production, Yeast

Introduction

Third-world countries have undergone rapid population expansion, which has raised the demand for food production and created a significant gap between supply and demand (Nasseri *et al.*, 2011). As a result, there is an increase in the number of people suffering from chronic malnutrition and hunger (Bacha *et al.*, 2011). According to Ahmad *et al.* (2009), this circumstance has also increased interest in creating novel food sources which are high in protein. One of the most critical steps in solving this problem was through the production of Single-Cell Proteins (SCPs) from fungi. SCPs generated from fungi have been

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used to replace costly traditional sources such as soymeal and fishmeal with higher protein content in staple foods to alleviate the protein deficit (Nasseri *et al.*, 2011).

There has been interest in using known filamentous fungi to produce SCP. This is mainly due to their exceptional ability to undergo post-translational changes and their exceptional secretion efficiency, which positions filamentous fungi as unique biological centers for manufacturing proteins. Interestingly, fungi have a useful characteristic: they can produce enzyme complexes that facilitate the conversion of various raw materials and industrial and agricultural byproducts into protein (Aggelopoulos *et al.*, 2013). According to a recent study by Valentino *et al.* (2016), fungi could be potential sources of SCP. This study also highlighted the connection between improved substrate proximate composition and higher protein content, which may affect animal nutrition and health favorably. Furthermore, it has been shown by Miller (2004) and Mishra and Arora (2004) that fungi can produce unique and fascinating bioactive secondary metabolites that have important uses in the industrial, agricultural, and pharmaceutical industries. Therefore, using certain filamentous fungi is a promising way to produce SCP.

When cultured on different carbon sources, microorganisms such as fungi, bacteria, yeast, and algae create a protein called SCP (Najafpour, 2007). SCP has different compositions depending on the particular microorganisms and growing media utilized. As a result, it includes water, ash, proteins, fats, carbohydrates, and other elements, including potassium and phosphorus (Ravindra, 2000). Additionally, according to Godoy *et al.* (2018), SCP is a high-protein resource for animal and human nutrition, providing a potential answer to the growing global food demand brought on by population growth (Najafpour, 2007). Yeast was firstly added to human meals, but the emphasis switched in using SCP in animal feeds because of the scarcity of fish and soybean meals in many nations (Daghir and Abdul-Baki 1977; Adnan *et al.*, 1997). An economically viable protein source that can address the global protein deficit and reduce the environmental impact of waste products is the production of SCP by fungal organisms using waste materials as substrates (Anupama and Ravindra 2000; Kurbanoglu and Algur 2002; Paraskevopoulou *et al.*, 2003). Furthermore, a range of agricultural leftovers are utilized as substrates for the production of SCP, such as rice bran, corn, sugarcane bagasse, and fruit and vegetable wastes (Bakshi *et al.*, 2016). Consequently, *Carica papaya* peels can be a valuable source of SCP when used as a substrate.

Papaya, scientifically named *C. papaya*, is a herbaceous succulent plant in the Caricaceae family. Although it originated in the tropical parts of the United States, it is currently commonly grown in other tropical nations due to its year-

round availability and mouthwatering fruit that resembles melon (Banerjee *et al.*, 2006). Numerous chemicals are abundant in *C. papaya*, such as flavonols, tannins, terpenes, carotenoids, pectin, papain, chymopapain, carpaine, nicotine, and vitamin C and E (Tona *et al.*, 1998; Kantham *et al.*, 2011). According to phytochemical analysis, papaya peel is an excellent source of protein, β -carotene, minerals, and fiber content (Pavithra *et al.*, 2017). Furthermore, it has been shown that agricultural residues, such as papaya peel, can encourage the growth of microorganisms needed to produce SCP, which improves feed quality (Anupama and Ravindra 2000; Ravindra, 2001; Sharma and Arora, 2015).

Therefore, this study focused on the potential of selected *Aspergillus* species in enriching the crude protein content of *C. papaya* peels through Solid State Fermentation (SSF).

Materials and method

Sample collection

Culture samples of *A. niger*, *A. oryzae*, and *A. flavus* were obtained from Philippine Center for Postharvest Development and Mechanism (PHilMech) – Science City of Muñoz, Nueva Ecija, Philippines. Peels of ripe *C. papaya* were obtained at Dampulan, Jaen, Nueva Ecija, Philippines. The collected *C. papaya* peels were washed thoroughly with tap water and then sun dried for seven days. The sample was then pulverized using a blender and was put in a ziplock bag. Then, 250 g of the pulverized samples were sent to the Department of Agriculture – Regional Field Office III – City of San Fernando, Pampanga for proximate analysis of the nutritional content, such as the crude protein, ash, fiber, moisture, and crude fat content. This served as the initial Crude Protein Content (CPC) of the substrate.

Preparation and sterilization of culture media

Thirty-nine grams of Potato Dextrose Agar (PDA) was suspended in 1L of distilled water. The mixture was stirred continuously until it was thoroughly homogenized. The media was dispensed in bottles and tubes. It was then autoclaved at 15 psi at 121°C for 30 minutes (Fajardo, 2018). The prepared media in the test tubes were slanted and left to cool. Then, each of the fungal inoculants were inoculated into the test tube slants and incubated for seven days.

Single-cell protein production

SCP production was subdivided into six processes, the preparation of the subcultures, preparation of the substrate, SSF, harvesting and drying of the samples and the proximate composition. For the preparation of the subcultures, fungal inoculants namely: *A. niger*, *A. flavus* and *A. oryzae* were subjected to subculture in the slanted media and were grown for seven days. Then, 20 ml of distilled water was added to the cultures and were adjusted to 5.0×10^6 spores per ml using a hemocytometer. For the preparation of the substrate, two hundred fifty grams of sterilized powdered *C. papaya* peels were placed in a clean wide-mouth glass container, and two hundred seventy-five milliliters of distilled water were added to the substrate to obtain 60 to 65% moisture content. This was covered and sealed with a cling wrap and was sterilized using an autoclave at 15 psi at 121°C for thirty minutes (Valentino *et al.*, 2016).

Experimental treatments

Five treatments were used for SCP production. Each treatment was replicated thrice. *C. papaya* peels were inoculated with *A. flavus* in Treatment 1, *C. papaya* peels were inoculated with *A. niger* in Treatment 2, *C. papaya* peels were inoculated with *A. oryzae* in Treatment 3, *C. papaya* peels were inoculated with a baker's yeast in Treatment 4, and as for the Treatment 5, the *C. papaya* peels remained uninoculated.

Solid state fermentation

Twenty ml of the adjusted spore suspension of different fungi was aseptically transferred to the 250 g of substrate. The fungal enriched *C. papaya* peels were covered and sealed with a cling wrap and was stirred to facilitate the mixing of cultures every 48 hours. This was done to prevent the settling of the spores at the bottom of the cultures. The inoculum was allowed to acclimatize in the substrate for twenty days at room temperature (Fajardo, 2018).

Harvesting and drying of samples

After twenty days of SSF, the fungal-enriched *C. papaya* peels were sterilized at 15 psi for 30 minutes to prevent the fungi from further proliferation. The freshly sterilized *C. papaya* peels were then spread out on clean containers and sun-dried for seven days. Then, the sun-dried peels were pulverized using a blender to create a fine powder consistency (Fajardo, 2018).

Proximate composition

Two hundred fifty grams of the pulverized peels were sent to the Feed Chemical Analysis laboratory, Department of Agriculture – Regional Field Office III, City of San Fernando, Pampanga for proximate analysis of the nutritional content, such as the crude protein, ash, fiber, moisture, and crude fat content. The fungal enriched *C. papaya* peels CPC served as the final CPC. Whereas the CPC of the uninoculated *C. papaya* peels served as the initial CPC (Valentino *et al.*, 2016).

Statistical analysis

Analysis of Variance (ANOVA) was used in the study and was laid out in a Completely Randomized Design. Data was evaluated using comparison among means by Tukey's Honest Significant Difference (HSD) Test. All tests of significance were conducted at 5% probability level.

Results

Increase in crude protein content

Result showed that crude protein content is increased (Table 1). Among the treatments, the positive control, which was the *C. papaya* peels inoculated with baker's yeast, had an increase of 56.57%, followed by the *C. papaya* peels inoculated with *A. oryzae* with 46.37% and *C. papaya* peels inoculated with *A. niger* with 19.31%. While *C. papaya* peels inoculated with *A. flavus*, and uninoculated *C. papaya* peels yielded the lowest CPC with 14.17% and 0.00% respectively. The results revealed that the fungal enriched *C. papaya* peels was significantly increased in CPC, especially with *C. papaya* peels inoculated with *A. oryzae*, but when compared to the positive control, *C. papaya* peels inoculated with baker's yeast yielded a favorable result compared to the fungi.

Table 1. Crude protein content of the inoculated and uninoculated *C. papaya* peels

Treatments	Initial CPC	Final CPC	% Increase in CPC
<i>C. papaya</i> peels + <i>A. flavus</i>	10.20 ± 0.75	11.57 ± 0.40 ^c	14.71
<i>C. papaya</i> peels + <i>A. niger</i>	10.20 ± 0.75	12.17 ± 2.78 ^{bc}	19.31
<i>C. papaya</i> peels + <i>A. oryzae</i>	10.20 ± 0.75	14.93 ± 2.61 ^{ab}	46.37
<i>C. papaya</i> peels + baker's yeast	10.20 ± 0.75	15.97 ± 1.15 ^a	56.57
Uninoculated <i>C. papaya</i> peels	10.20 ± 0.75	10.20 ± 0.75 ^c	00.00

Values are presented in mean ± standard deviation. Means with the same superscript letter within a column are not significantly different (p-value<0.05).

Proximate composition

The proximate composition of treated and untreated *Carica papaya* peels was evaluated, including ash, fiber, moisture, and fat content (Table 2). Ash content, which measures the overall mineral content, was highest in *C. papaya* peels inoculated with *A. flavus* at $20.10 \pm 1.76\%$, indicating significant mineralization by the fungi. The negative control (uninoculated *C. papaya* peels) had an ash content of $16.27 \pm 1.76\%$, while other treatments had varying lower ash contents. On the other hand, crude fiber content was highest in *C. papaya* peels inoculated with baker's yeast at $13.23 \pm 0.49\%$. This was followed by *A. oryzae*, *A. flavus*, *A. niger*, and uninoculated *C. papaya* peels. While moisture content was highest in *C. papaya* peels inoculated with baker's yeast at $14.13 \pm 2.75\%$, followed by *A. flavus*, *A. niger*, *A. oryzae*, and uninoculated *C. papaya* peels. And lastly, crude fat content increased in all inoculated *C. papaya* peels, with *C. papaya* peels inoculated with *A. niger* having the highest at $3.50 \pm 0.26\%$.

Table 2. Proximate composition such as ash, crude fiber, moisture, and crude fat content of the inoculated and uninoculated *C. papaya* peels

Treatments	% Ash content	% Crude fiber content	% Moisture content	% Crude fat content
<i>C. papaya</i> peels + <i>A. flavus</i>	20.10 ± 1.76^a	10.33 ± 0.25^{bc}	11.90 ± 2.42^a	2.57 ± 0.98^{bc}
<i>C. papaya</i> peels + <i>A. niger</i>	11.57 ± 3.14^c	9.43 ± 2.90^{bc}	11.80 ± 5.22^a	3.50 ± 0.26^a
<i>C. papaya</i> peels + <i>A. oryzae</i>	10.13 ± 0.64^c	12.03 ± 1.76^{ab}	9.83 ± 2.38^a	3.37 ± 0.45^{ab}
<i>C. papaya</i> peels + baker's yeast	13.27 ± 1.94^{bc}	13.23 ± 0.49^a	14.13 ± 2.75^a	1.87 ± 0.15^c
Uninoculated <i>C. papaya</i> peels	16.27 ± 1.76^b	8.87 ± 0.31^c	8.83 ± 1.030^a	1.93 ± 0.15^c

Values are presented in mean $\pm$ standard deviation. Means with the same superscript letter within a column are not significantly different (p-value<0.05).

Discussion

Crude protein content in fungal biomass can be significantly enhanced under fermentation conditions. This can be accomplished through SSF, a crucial step in transforming lignocellulosic materials into SCP. In SSF, solid lignocellulosic substrates such as agricultural residues and organic waste materials containing cellulose, hemicellulose, lignin, pentoses, and sugar can be used for fungal growth, due to the fungal heterotrophic growth characteristics. These substrates can be metabolized and converted to biomass, incorporating atmospheric carbon dioxide, by fungi responsible for their growth (Gabres,

2016). A variety of fungal species, such as *Aspergillus*, *Trichoderma*, and *Rhizopus* species, are known to grow well and perform well in biomass conversion of lignocellulosic biomass via efficient carbohydrate and other substrate simplification, followed by assimilation as carbon and energy substrates to enable their protein synthesis (Oduguwa *et al.*, 2019).

With the growing fungi consuming the available nitrogen sources in the substrate and utilizing them in their cellular proteins, the CPC of the fungal biomass increases. Under SSF conditions, environmental parameters such as moisture content, temperature, pH, and aeration are optimized to maximize protein production (Paredes-Lopez *et al.*, 2015). The resulting protein-rich fungal biomass can subsequently be harvested and processed to SCP. The high crude protein content makes SCP a valuable nutritional supplement, potentially serving as an alternative to conventional protein sources in animal feed and human diets. The efficiency of this process, combined with the use of low-cost substrates, highlights the potential of SSF to produce SCP in a sustainable and economically viable manner (Bhattacharya and Raha, 2002). Moreover, the increased crude protein content through SSF is recognized in producing SCP from fungi. This process not only enhances the nutritional value of the fungal biomass but also provides a method for converting waste materials into valuable protein products, supporting both environmental sustainability and food security (Sadh *et al.*, 2018).

On the other hand, the significant increase in moisture, ash, fat, and fiber content indicates that the fungal inoculants were suitable for SCP production. Ash content is a crucial indicator of the mineral composition within the substrate, with higher levels signifying increased mineral concentration. This enrichment of essential minerals, such as phosphorus, potassium, calcium, and magnesium, provides vital nutrients necessary for fungal growth and metabolism, consequently supporting the possibility of SCP production (Mussatto *et al.*, 2012).

Moreover, elevated levels of crude fiber indicate the effective breakdown of complex organic compounds within the substrate, leading to the concentration of fiber components. This breakdown enhances the substrate's nutritional profile, making it more suitable for SCP productions (Martin, 2012).

Furthermore, increased moisture levels indicate the ability of fungi to retain water within the substrate, promoting microbial growth and metabolic activity. This active metabolic process drives biochemical transformations, such as the breakdown of organic matter, essential for SCP production. Moreover, higher moisture content fosters favorable conditions for fungal proliferation and sporulation, facilitating increased synthesis of proteins and biomass. The metabolic activity prompted by increased moisture content highlights how well

fungal inoculation utilizes substrate resources to produce SCP (Rodrigues *et al.*, 2022).

Lastly, elevated crude fat levels contribute to improved crude protein yield in SCP production processes, highlighting the positive correlation between lipid content in growth medium and protein synthesis efficiency in specific fungi during microbial fermentation for SCP. Furthermore, balancing nutrients in the growth medium, including modifying crude fat content, has enhanced overall biomass yield, increasing the crude protein content (Dong *et al.*, 2020; Kumar and Ray, 2014).

Overall, the study found that fungal inoculants, including *A. oryzae*, *A. niger*, and *A. flavus* can be a possible source of SCP. Although fungal inoculants did not outperform baker's yeast in terms of efficiency, they still have the potential to be a probable source of SCP since these fungi possess enzymatic capabilities that effectively break down complex substrates like *C. papaya* peels into protein-rich biomass (Kumar and Ray, 2014). Additionally, the proximate composition analysis highlights the significance of optimizing fungal fermentation strategies for SCP production, emphasizing the roles of mineralization, fiber breakdown, moisture retention, and lipid accumulation in creating low-cost nutrient-rich biomass for industrial and nutritional needs.

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Conflicts of interest

The authors declare no conflict of interest.

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