RESEARCH ARTICLE

Amino acid profile and protein quality assessment of a novel single cell protein as an animal feed supplement

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ABSTRACT - The study was intended to develop a single cell protein by solid state fermentation (SSF) technique using vegetable waste as the substrate. The SCP with high protein content and good nutritional value was developed to be supplemented in animal feed formulations. The fungal culture used for SSF, molecularly identified as *Penicillium oxalicum* NFCCI 2136, was an indigenous culture isolated from vegetable waste. The SCP showed a fairly high crude protein content of 38 per cent, a 197 per cent increase of protein content from the unfermented sample. The amino acid profile of the SCP was determined to assess its nutritional quality and it was compared with other Food and Agriculture Organization (FAO) standard reference proteins and conventional foods. The SCP was found to be rich in essential amino acids like leucine, methionine, tryptophan and phenylalanine. This was followed by computing the essential amino acid index, nutritional index, biological value and computed–protein efficiency ratio of the SCP. In addition, a multimycotoxin analysis of the SCP reported that the analyzed mycotoxins were not detected in the final product. The importance of this study lies in the fact that an indigenous fungal culture isolated from vegetable waste was used as protein enrichment, which can be suggested as an efficient method of solid waste management coupled with protein production.

KEY WORDS - Single cell protein, Solid state fermentation, Essential amino acid index, Nutritional index. Biological value, Computed –Protein efficiency ratio


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INTRODUCTION

Microorganisms can be cultivated on agro-industrial products with production of large amounts of cells rich in proteins that commonly contain all the essential amino acids, in addition to favourably high vitamin and mineral levels (Kuhad *et al.*,1997). It has been reported that Solid Sate Fermentation (SSF) is the most appropriate process in developing countries for the production of single cell protein, due to the advantage it offers (Carrizales and Jaffe, 1986). SSF resembles the natural habitat of microorganism and is, therefore, preferred choice for microorganisms to grow and produce useful value added products (Singhania *et al.*, 2009). The implication of a cheap and abundantly available raw material for SCP production using microbial species could be one of the best possible technologies for SCP commercialization. The amino acid composition is the most important factor in defining food or feed protein quality, followed by the digestibility of the protein and bioavailability of its amino acids (Sindayikengera and Wen-Shui, 2006). While considering the quality of SCP, the amount of protein and amino acid extracted from it needs to be determined, in order to verify it as a productive protein (Ahmadi *et al.*, 2010). Solid-state cultivation of *Aspergillus niger* and *Bacillus coagulans* were carried out to enrich the nutritional value of plant ingredients to use as aqua feed ingredients. The single-cell protein produced here contained all of the essential amino acids, and the amino acid profile assessment of the SCP was carried out to determine the nutritional characteristics of the SCP.
acids and it showed a good profile (Imelda et al., 2008). Ahmadi, 2010, reported that the microbial protein produced on treatment of wheat straw with Pleurotus florida indicated a ratio of essential amino acids to total amino acids as 65.6 per cent. Of the fungal cultures, members of the Basidiomycetes and Ascomycetes are supposed to be most efficient lignocelluloses converters in SSF (Moo-Young et al., 1983). Hence, a novel fungal culture indigenous to the system was selected for the present study, which aims at the best possible conversion of the substrate in the SSF system. It can also be appreciated as a very economical method for the conversion of vegetable waste into value added product. Since the reports regarding the amino acid profile analysis of SCP are limited, the present study is significant in evaluating the amino acid composition of the final product. In addition a complete analysis of the nutritional properties followed by a mycotoxin analysis was carried out for the characterization of the SCP to assess the presumptive nutritional value of SCP for being substituted in animal feed formulations.

RESEARCH METHODS

Solid state fermentation and data analysis:
The fungal culture isolated from vegetable waste was molecularly identified and used for SSF with vegetable waste as the substrate. SSF was carried out using 50gm of vegetable waste taken in a 500ml conical flask. The fermentation was carried out for a period of 12 days under an optimized condition of pH 5.5, temperature 30°C, with 0.5 per cent urea as the nitrogen source, at a moisture content of 40 per cent and with an inoculum size of 205 x 10⁴ spores/ml. The effects of different growth factors were studied for the maximum product ion of the SCP. The fermentation was maintained at 60°C. The total run was programmed for 60 min. For the amino acid analysis was done with non-switching flow method and fluorescence detection after post-column derivatization with ophthaldehyde. In the case of proline and hydroxyl proline, imino group is converted to amino group by hypochlorite. Amino acid standard (Sigma chemical Co., St. Louis, USA) was also run to calculate the concentration of amino acids in the sample. The amount of each amino acid is expressed as g/100g protein.

Calculation of chemical score, essential amino acid index, biological value and nutritional index of the single cell protein:
Chemical score was calculated using the formula FAQ,1968 (Food and agriculture organization,1968). Calculation of a chemical score (CS) was based on the proportion of the amino acid in the feed protein compared with that of ideal protein (Tom Brody,1999). Essential amino acid index (EAA index) was calculated according to the procedure of Oser (1951), taking into account the ratio of EAA in the test protein relative to their respective amounts in whole egg protein.

The biological value (BV) was calculated using the formula of Oser (1959).

Nutritional index (NI) was calculated using the formula of Crisan and Sands (1978).

The Computed–Protein efficiency ratio (C-PER) was calculated according to the method of Satterlee et al. (1979).

Mycotoxin analysis:
The SCP was subjected to multimycotoxin analysis to rule out the possibility of mycotoxins in the fermented substrate. The mycotoxins looked for were Aflatoxins B₁, B₂, G₁, and G₂, Ochratoxins, Zearalenone, T-2 toxins and citrinin. The procedure adopted for multimycotoxin analysis was
Quantitative Thin Layer Chromatography method for the analysis of aflatoxin, ochratoxins, zearalenone, T-2 toxins, sterigmatocystin in food stuffs (Chandrasekharan, 1999).

**RESEARCH FINDINGS AND ANALYSIS**

The findings obtained from the present investigation are presented below:

The fungal culture selected for the production of SCP was molecularly identified to be *Penicillium oxalicum NFCCI* 2136. The crude protein content of the substrate (vegetable matter), before fermentation was found to be 12.8 per cent and it increased to 38 per cent after SSF, showing a percentage increase of 197 per cent from the initial value.

**Amino acid characterization of the SCP:**

Single cell protein produced by *P. oxalicum* contained an essential amino acid profile featuring an appreciable amounts of methionine (5.8g/100 g of protein) and tryptophan (3.76 g/100 g protein) but devoid of lysine and cysteine. In Table 1 the amino acid composition of the SCP from *P. oxalicum* is compared with that of the FAO reference protein (FAQ/WHO/UN, 1985), whey protein concentrate (WPC80, 80% protein based on dry weight), sodium caseinate (88.03% protein based on dry weight) (Sindayikengera and Wen-Shui, 2006), human milk (Hambraeus, 1982) and conventional foods (Erdman et al., 1977).

The results showed that the concentration of essential amino acids in SCP from *P. oxalicum* was equal to or greater than those in the FAO reference protein, suggesting that it can be used as a good protein supplement for animals. Leucine and methionine in SCP were having high values of 10.1 and 5.8g/100g protein, respectively, when compared with FAQ ref proteins. Phenyl alanine (9.7g/100g) showed comparable value with whole egg while Threonine (3.9g/100g) showed same value with soy protein suggesting the SCP as being valuable in terms of nutritional value. Percentage of Valine (4.8 g/100g) was slightly lower than other ref proteins except WPC80 and FAQ ref protein. Lysine one of the most limiting essential amino acid in cereals, but present in higher concentration in animal protein sources, was found to be lacking in this SCP. So, in the present study lysine was not reported in the amino acid profile, which is one of the disadvantage of this SCP.

**Chemical score, essential amino acid index, biological value and nutritional index:**

The chemical score of EAA like Leu, Phe+Tyr and Trp were found to be higher than WPC80 and SC.

The 1st, 2nd and 3rd limiting amino acids of the single cell protein were Lysine, Isoleucine and Valine, respectively. The results are tabulated in Table 2.

The EAAI and BV of SCP was found to be higher than WPC80 and SC, suggesting its good nutritional quality. The C-PER value of SCP was found to be closer to SC while NI value was lower than WPC80 and SC, since the protein content

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>SCP</th>
<th>WPC80</th>
<th>SC</th>
<th>Egg</th>
<th>Soya protein</th>
<th>Beef protein</th>
<th>Human milk</th>
<th>FAQ/Ref Std</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile</td>
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<td>4.97</td>
<td>4.59</td>
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<td>5.8</td>
<td>5.3</td>
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<td>10.66</td>
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<td>8.8</td>
<td>7.6</td>
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<tr>
<td>Met+Cys</td>
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<td>2.7</td>
<td>3.6</td>
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<td>8</td>
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<tr>
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<td>5.64</td>
<td>7.1</td>
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<td>5.5</td>
<td>5.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Trp</td>
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<td>1.73</td>
<td>1.04</td>
<td>1.33</td>
<td>1.4</td>
<td>1.2</td>
<td>Nah</td>
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<td>1.73</td>
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<td></td>
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<td></td>
<td></td>
<td>5.54</td>
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</table>

Mycotoxin analysis:

The multimycotoxin analysis of the SCP, indicated that aflatoxins (AFs), ochratoxin A (OTA), T-2 toxin, citrinin and zearalenone (ZEA) were not present in detectable limits in the SCP produced by \textit{P. oxalicum}. Ishibashi (1998) reported that incorporating appreciable amount of threonine in the feed will help to increase the weight of the eggs produced by egg laying hens. Phenylalanine in the SCP showed comparable value with whole egg, while threonine showed same value with soy protein suggesting the SCP as being valuable in terms of nutritional value. The Leu, Met, Phe and Trp levels of the SCP showed higher values than soy protein. Since soy protein is widely used in animal diets, this finding is highly advantageous in terms of the economic aspects of formulating animal feed.

The proportion of glycine in the SCP was significant \textit{i.e.} 4.9 g/100 g of protein, much higher than casein and equivalent to whey reference protein. Glutamic acid, another non-essential amino acid in the SCP is also having a commendable value as per the results obtained from the amino acid analysis of the SCP. Lysine is one of the most limiting essential amino acids in cereals, but present in higher concentration in animal protein sources such as beef and egg (Erdman et al., 1977). In the present study, lysine was not reported in the amino acid profile, which is one of the disadvantage of the SCP produced by \textit{P. oxalicum} fungal strain. This advantage of lysine deficiency can be better overcome by supplementation with synthetic amino acids and other cheaper protein sources rice in lysine.

The methods utilized to determine protein quality include chemical score, protein efficiency ratio, biological value and the protein digestibility corrected amino acid score (Gertjan, 2000). The chemical score of Leu, Phe+Tyr and Trp of SCP were having higher values than WPC 80 and SC. The limiting amino acid typically determines the capacity that the protein has for being utilized by the body (Munaver, 1959). The limiting amino acids in the present study were lysine, isoleucine and valine.

The safety, acceptability, and toxicology of SCP are factors to be considered while formulating SCP feed formulations. Animal feeds with ingredients such as oilseed cakes, peanut, cottonseed and coconut cake or corn grits often contain mycotoxins. Feed conversion to animal protein formulations. Animal feeds with ingredients such as oilseed cakes, peanut, cottonseed and coconut cake or corn grits often contain mycotoxins. Feed conversion to animal protein feed to animal protein feed. Another amino acid of significance in SCP is methionine and the SCP produced in the present study was having an ideal methionine concentration of 5.8g/100g of protein. It is noted that sulphur containing amino acid like methionine can produce eggs with stronger shells and improve the production of feather on laying hens (Kalinowski et al., 2003). The importance of arginine, glutamine, proline or leucine lies in the fact that, dietary supplementation of these amino acids to weaning piglets enhance their growth performance. Arginine or glutamine is also effective in increasing milk production by lactating cows (Guoyao, 2010). Valine and threonine which are very essential for the immune system were also seen in significant proportion in the SCP. Ishibashi (1998) reported that incorporating an appreciable amount of threonine in the feed will help to increase the weight of the eggs produced by egg laying hens. Phenylalanine in the SCP showed comparable value with whole egg, while threonine showed same value with soy protein suggesting the SCP as being valuable in terms of nutritional value. The Leu, Met, Phe and Trp levels of the SCP showed higher values than soy protein. Since soy protein is widely used in animal diets, this finding is highly advantageous in terms of the economic aspects of formulating animal feed.

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The safety, acceptability, and toxicology of SCP are factors to be considered while formulating SCP feed formulations. Animal feeds with ingredients such as oilseed cakes, peanut, cottonseed and coconut cake or corn grits often contain mycotoxins. Feed conversion to animal protein is always reduced by the presence of mycotoxins. In addition, mycotoxins have a negative effect on animal health and fertility of the animal is also affected (Bhat and Miller, 1991). In view of the above facts, a multimycotoxin analysis of the SCP produced in the present study, was being carried out. The results indicated that, these toxins were not present in detectable limits in the SCP produced by \textit{P. oxalicum}. These findings have added value towards the safety aspects of the SCP produced in the present work and the feasibility of it being supplemented with animal feed formulations.

Table 2: Chemical score of SCP, WPC80 and sodium caseinate

<table>
<thead>
<tr>
<th>Essential Amino acid</th>
<th>SCP</th>
<th>WPC80</th>
<th>SCb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile</td>
<td>7</td>
<td>79.37</td>
<td>78.76</td>
</tr>
<tr>
<td>Leu</td>
<td>138</td>
<td>127.68</td>
<td>114.40</td>
</tr>
<tr>
<td>Lys</td>
<td>14</td>
<td>145.09</td>
<td>137.13</td>
</tr>
<tr>
<td>Met+Cys</td>
<td>135</td>
<td>152.74</td>
<td>65.89</td>
</tr>
<tr>
<td>Phe+Tyr</td>
<td>124</td>
<td>61.34</td>
<td>114.83</td>
</tr>
<tr>
<td>Thr</td>
<td>96</td>
<td>141.99</td>
<td>89.93</td>
</tr>
<tr>
<td>Val</td>
<td>81</td>
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<tr>
<td>Trp</td>
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<td>113.97</td>
<td>73.61</td>
</tr>
<tr>
<td>1st LAA</td>
<td>Lys</td>
<td>Val</td>
<td>Met+Cys</td>
</tr>
<tr>
<td>2nd LAA</td>
<td>Ileu</td>
<td>Phe+Tye</td>
<td>Trp</td>
</tr>
<tr>
<td>3rd LAA</td>
<td>Val</td>
<td>Ile</td>
<td>Ile</td>
</tr>
</tbody>
</table>

Table 3: Nutritional indicates (%) of SCP, WPC80 and sodium caseinate

<table>
<thead>
<tr>
<th>Essential amino acid</th>
<th>SCP</th>
<th>WPC80</th>
<th>SCb</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAA index</td>
<td>83</td>
<td>72.28</td>
<td>77.80</td>
</tr>
<tr>
<td>Predicated BV</td>
<td>78.77</td>
<td>67.09</td>
<td>73.10</td>
</tr>
<tr>
<td>NI</td>
<td>31.54</td>
<td>58.42</td>
<td>69.34</td>
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<tr>
<td>C-PER</td>
<td>2.164</td>
<td>4.85</td>
<td>3.06</td>
</tr>
</tbody>
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\(^{a,b}\) From Sindayikengera (2006), LAA-Limiting amino acids

\(^{a}\) From Sindayikengera (2006)
proper food. The results of the present study suggested that the fungal strain *Poxalicum* isolated from vegetable waste was very efficient in converting the substrate into single cell protein by SSF technique. The SCP was showing high protein content, amino acid profile, good nutritional value and reported to be safe in mycotoxin analysis which would make it an ideal supplement in animal feed formulations.

**Abbreviations:**

SCP, Single cell protein; SSF, Solid State Fermentation; EAAI, Essential amino acid index; NI, Nutritional index; BV, Biological value; C-PER, Computed–Protein efficiency ratio; WPC, whey protein concentrate; SC, sodium caseinate.

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**LITERATURE CITED**


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