

Full Length Research Paper

Production of single cell protein (SCP) and essentials amino acids from *Candida utilis* FMJ12 by solid state fermentation using mango waste supplemented with nitrogen sources

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In Burkina Faso, deficiency of amino acids in protein is becoming a major healthy public problem. This study was purposed to optimize essentials amino acids in single cell protein (SCP) by supplementing different nitrogen sources during fermentation of mango waste with *Candida utilis* FJM12. Analytical methods were used to determine biomass yield, chemical composition and amino acids profile of SCP. The principal component analysis (PCA) method was performed to identify the nitrogen source which exhibited best rate of SCP. The maximum biomass yield (6.48 ± 0.03 g/L) exhibited $9.65 \pm 0.36\%$ (w/w) of ash, while using yeast extract. The proximate composition of SCP revealed 56.40 ± 1.30 , 13.25 ± 0.11 , 3.80 ± 0.10 and $6.60 \pm 0.25\%$ (w/w), respectively for crude protein, lipids, carbohydrates, and nucleic acid content. PCA showed a strong correlation between yeast extract and ammonium sulphate and demonstrated their positive influence to increase the rate of SCP and essentials amino acids as compared to Food and Agriculture Organization (FAO) recommendation. These results demonstrated that *C. utilis* FJM12 could be suitable for essentials amino acids.

Key words: Mango waste, nitrogen source, *Candida utilis*, single cell protein (SCP), amino acids.

INTRODUCTION

Most residues could be converted into high-value materials through bio-components. Food security

problem concerns most of the developing countries (Tamrat, 2017). Fruit processing results in high amounts of by-products (peels, seeds) and represent an environmental pollution (Somda et al., 2011a). Burkina Faso and other developing countries have long been with problems of processing and preservation of locally produced agricultural food products (Somda et al., 2011a). Thus, finding new possible application area to further exploit these wastes for the production of high-value products have gained increasing interest (Malviya et al., 2010; Koubala et al., 2013). Waste products can be converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms (Kurbanoulu, 2001). So, mango residues coming from industrial area, market and site stockage can attain 50,000 tons per year in Burkina Faso (Somda et al., 2010, 2011a). Due to its important carbohydrates rate, mango waste can be a valuable fermentation substrate for both single cell protein (SCP) and essential amino acids production using yeasts.

Yeast SCP is a high nutrient feed substitute (Burgents et al., 2004). Among these, most popular are yeast species *Candida*, *Hansenula*, *Pichia*, *Torulopsis* and *Saccharomyces* (Bozakouk, 2002). *Candida utilis* has been frequently used in biomass production because of its ability to utilize a variety of carbon sources and to support high protein yield. It has been used for production of several industrial products both for human and animal consumption (Otero et al., 1998). Amino acids are critical to life and used as food or feed additives, in parenteral nutrition or as building blocks protein or for the chemical and pharmaceutical industries (Darshan and Priya, 2013; Kumagai, 2000; Wendisch et al., 2016).

The optimization of amino acids in SCP needs to apply an efficient process of fermentation as solid state fermentation. The Solid-State Fermentation (SSF) is defined as a fermentation process, which involves solid matrix in the absence or near absence of free water (Singhania et al., 2009). It has many advantages, such as high product yield with little risk of bacterial contamination, extended stability of products, low wastewater generation and low production costs (Zhao et al., 2008; Barrios-Gonzalez, 2012). SSF has received more interest from researchers and has been applied in various areas, such as biotransformation of crops and crop residues for microbial preparation, nutritional enrichment (Singhania et al., 2009) and production of a range of high value-added products (Quevedo-Hidalgo et al., 2013; Yu et al., 2014). Fermentative production of amino acids in the million-ton-scale has shaped modern biotechnology and its markets continue to grow steadily

(Wendisch et al., 2016). The SCP production could easily be improved by supplementing using organic or inorganic nitrogen source as yeast extract, peptone, ammonium sulphate and Ammonium nitrate. The use of cheap and readily available nitrogen source should be desirable as it lower cost of production.

Hence, this study focused on investigating the production of SCP and some different amino acids using mango waste supplemented with nitrogen sources through solid state fermentation.

MATERIALS AND METHODS

Strain and inoculum preparation

C. utilis FMJ12 was obtained from the culture collection of Laboratory of Microbiology and Microbial Biotechnology in the Department of Biochemistry and Microbiology, University Ouaga1 Pr Joseph KI-ZERBO, Burkina Faso. It was maintained at 4°C on yeast peptone dextrose (YPD) agar. Inoculum was prepared by inoculating a loop full of cells from 24 h old culture slant in conical flask containing 100 mL of YPD liquid medium at 30°C and 150 rpm for 24 h (Adan et al., 2011).

Media preparation for fermentation

An amount of 500 g of mango waste was dried at 105°C for 24 h (AOAC, 2016), then ground in a mortar and separated in a sieve shaker. The final waste particle size was approximately 2 mm in diameter. The nutrient broth liquid medium used was adapted from Adan et al. (2011) and then Darshan and Priya (2013). It contained in percentage (w/v): 0.5% MgSO₄, 0.5% KH₂PO₄, 0.01% FeSO₄, 0.12% Na₂SO₄, 5% glucose and was prepared in distilled water added with 0.2% of Tween-80. The medium for the fermentation procedure was prepared using mango waste 10% (w/v) of nutrients broth and final pH was adjusted to 7.00 ± 0.02.

Fermentation process

Fermentation medium was supplemented with organic nitrogen sources (peptone, yeast extract) and inorganic sources (Ammonium sulphate and Ammonium nitrate). Each nitrogen source was added separately at 1% (w/v) in growth medium. The mixtures were autoclaved at 121°C for 15 min. An inoculum of 10⁵ cells/mL was added at a ratio of 10 % (v/v) in to flasks and shaken at 150 rpm for 1 h before fermentation process. Flasks were kept in static incubator in solid state fermentation and maintained at 30°C for 72 h (Darshan and Priya, 2013).

Biomass of yeast cells

After 72 h of fermentation, the concentration of yeast cells in the fermenting matter was measured using the turbidimetric (absorbance at 600 nm) method and by determining dry weight of yeast cells. Cells were harvested by centrifugation at 16,000 rpm

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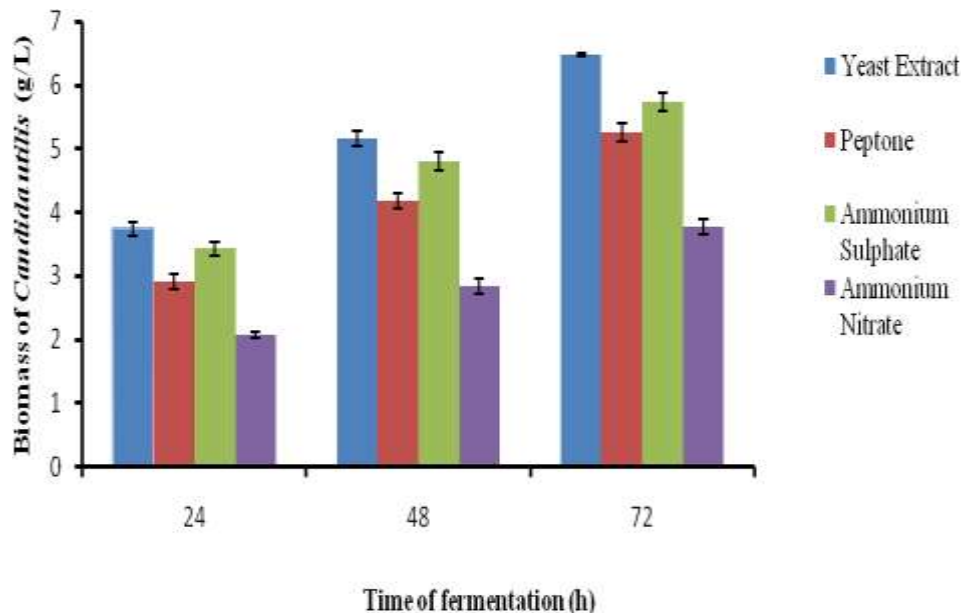


Figure 1. Biomass yield of *Candida utilis* FMJ12 according nitrogen source.

for 20 min washed twice with distilled water and dried in an oven at 50°C for 48 h. After 48 h, dry cells were weighed (Lagzouli et al., 2007b).

Chemical compounds of yeast cells

Total sugar content, dry matter and ash analysis were estimated by AOAC methods (2016). Total lipids were estimated by adapted methods of Kurbanoglu (2001). RNA and DNA levels were measured as described by Kurbanoglu (2001).

Total protein assay

Total protein content of yeast cells was measured by the micro-Kjeldahl method via multiplication of total nitrogen by 6.25 (AOAC, 2016).

Protein recovery by sonication

Suspension of 2.5% of dry yeast cells in 50 mL of distilled water was sonicated in a sonicator Q125 (Qsonica-LLC, USA) at a fixed power of 600 W, frequency of 20 kHz and amplitude of 50%. Total cycle time for ultrasonic treatment was 10 min. The pulse duration and pulse intervals were 1 min each. The jar was immersed in an ice-water bath to avoid a temperature increase during sonication. Cell debris and particles were removed by centrifugation at 11500xg for 10 min and crude protein was stored at -5°C (Mirzaei et al., 2015).

Amino acids analysis

Crude protein obtained after sonication was precipitated using ammonium sulphate and acetone. Microwave assisted acid hydrolysis was explored to speed up hydrolysis. Crude amino acids

were extracted by using centrifugation at 10,000 rpm for 15 min. Amino acid analysis was carried out after hydrolysis with 6 N HCl at 110°C for 24 h in a Biotronic LC-5001 Amino Acid Analyser (Germany) according to the method of Kurbanoglu (2001).

Statistical analysis

Statistical analysis was carried using Statistica V7.1. Correlation between different parameters was determined and p-value ($p < 0.05$ or 0.0001) was considered statistically significant. Associations between nitrogen source and SCP production were performed through Pearson's correlation.

Principal Component Analysis was performed in order to identify the best nitrogen source which exhibited higher rate of SCP. Principal component analysis and principal coordinate analysis plots were generated by regrouping of variables.

RESULTS AND DISCUSSION

Figure 1 shows the biomass yield of *C. utilis* FMJ12 when grown in different medium. The maximum biomass yield was obtained after 72 h with supplementation of yeast extract (6.48 ± 0.03 g/L) as nitrogen source followed by Ammonium sulphate (5.74 ± 0.15 g/L), Peptone (5.25 ± 0.14 g/L) and closed by Ammonium nitrate (3.77 ± 0.12 g/L). The results obtained by supplementing yeast extract were higher to those reported by Ouedraogo et al. (2017) and Jaganmohan et al. (2013) as 4.68 (g/L) but lower than those (7.23 g/L) reported by Kurbanoglu (2001). It has been remarked that biomass of *C. utilis* FMJ12 was increased with addition of yeast extract to the medium and would be strongly supporting efficient growth. The source of nitrogen plays a vital role in the improvement of

Table 1. Proximate composition of biomass of *Candida utilis* FMJ12.

Component (%)	Yeast extract	Peptone	Ammonium sulphate	Ammonium nitrate	P-value
Ash	9.65±0.36	7.72±0.47	8.23±0.38	6.27±0.29	<0.01
Total protein	56.40±1.30	45.12±1.19	50.76±1.17	30.84±1.15	<0.01
Total lipids	13.25±0.11	7.95±0.13	10.60±0.21	4.64±0.35	<0.01
Carbohydrates	3.80±0.10	2.28±0.13	2.85±0.12	1.14±0.10	<0.01
Nucleic acid	6.60±0.25	4.29±0.23	5.28±0.20	3.33±0.17	<0.01

efficiency and economics of microbial fermentation (Nancib et al., 2001).

The proximate composition of biomass obtained from the fermentation of *C. utilis* FMJ12 using organic and inorganic sources of nitrogen is shown in Table 1. The percentages of ash in dry cells were significantly different ($p < 0.01$) and ranged respectively as 6.27±0.29, 7.72±0.47, 8.23±0.38, and 9.65±0.36% (w/w) for ammonium nitrate, peptone, ammonium sulphate and yeast extract. These results were emphasized as found by Nasser et al. (2011) who demonstrated that ash of yeast ranged from 5 to 10%. The rate of ash obtained using Yeast extract was close to the result (8.4%) recorded by Husseiny et al. (2016).

Crude protein was produced with significant difference under influence of organic and nitrogen source ($p < 0.01$). The mean values were located between 30.84±1.15 and 56.40±1.30% (w/w). Data showed that the maximum yield of protein was achieved by the addition of organic nitrogen source as Yeast extract to the production medium. The protein reached after supplementing of yeast extract was higher than the value found by Gao et al. (2007) in yeast (53%) and similar with value of Rajoka et al. (2006) in *C. utilis* (56.34%) and yet lower than that found by Rajoka (2005) in *Cellulomonas biazotea* (60%) and Somda et al. (2017) in *Saccharomyces cerevisiae* SKM10 (79.14%). The mean values of lipids content significantly ranged ($p < 0.01$) from 4.64±0.35 to 13.25±0.11% (w/w). The result obtained using yeast extract as organic nitrogen source was higher than crude lipid content recorded by Parajo et al. (1995), Kurbanoglu (2001) and Husseiny et al. (2016) who reported, respectively 9, 5.4, and 5.05% in SCP of yeast.

Concerning carbohydrates content, it was found significantly different ($p < 0.01$) and ranged from 1.14±0.10 to 3.80±0.10% (w/w), which are lower to the percentage obtained in *Hansenula* species (24%) by Shojaosadati et al. (1999) and *S. cerevisiae* (26%) by Husseiny et al. (2016). Nucleic acid contents of SCP was found to range from 3.33±0.17 to 6.60±0.25% (w/w) which is significantly lower than the values reported by Kurbanoglu (2001) as 7.47% and higher than Ibrahim Rajoka et al. (2005) at 2.75%. On the other hand, the high RNA contents are reported to be toxic for human consumption, while harmless for most animals (Kurbanoglu, 2001). While

most microorganisms contain nucleic acid between 6 and 15%, the low content in *C. utilis* is a very interesting result for animals feed.

The comparison of the obtained data presented in Table 1 demonstrates that the best bioconversion of mango waste to high yield of protein was obtained by supplementing Yeast extract to basic medium. The mango waste contained mineral elements which could help to increase growth and stability of fungi strains. Amino acid profile of SCP produced by *C. utilis* FMJ12, was determined and data indicated that it could be compared favourably with FAO standards (Table 2). Data recorded in Table 2 showed that, the biomass cells had 19 kinds of essential amino acids. It is apparent from the results that the addition of nitrogen sources efficiently affects the SCP and essential amino acids productivities by *C. utilis* FMJ12. Amino acid concentrations as isoleucine, leucine, lysine, phenylalanine, threonine, and Tryptohan were somewhat higher than the FAO reference protein and could be beneficial for nutritional need. Among the amino acids, glutamic acid (16.57%) and cysteine (10.16%) were the most abundant produced after supplementing with yeast extract. Addition of yeast extract and ammonium sulphate to the production medium resulted in the highest amount of SCP essential amino acids as lysine, leucine, isoleucine, phenylalanine, methionine, threonine, and valine.

The amounts of SCP and essentials amino acids obtained after optimizing the medium by yeast extract represent more 2-fold increase as compared to amounts recommended by FAO. These results are in agreement with the results by Paraskevopoulos et al. (2003) who found that the maximum SCP production by yeast and other organisms was obtained after supplementing medium with Yeast extract and Ammonium sulphate. Also, Zhang et al. (2008) and Husseiny et al. (2016) reported that using ammonium sulphate as nitrogen source give the highest yield of SCP by *A. oryzae* and *S. cerevisiae*. It was reported that the potential nutritional value of SCP is determined with amount of lysine and methionine amino acids (Kurbanoglu, 2001). In agreement with the present results, Zheng et al. (2005) and Rajoka et al. (2006) recorded that, the biomass obtained from *C. utilis* contained all the essential amino acids for human nutrition. These results show that strain

Table 2. Amino acids composition of SCP from *Candida utilis* FMJ12.

Amino acids (g/100 g) of SCP	Nitrogen source				
	Yeast extract	Peptone	Ammonium sulphate	Ammonium nitrate	FAO* standards
Isoleucine	5.28±0.2	0.21±0.01	3.15±0.11	1.07±0.02	2.20
Leucine	6.73±0.21	0.26±0.11	1.69±0.10	1.10±0.11	2.20
Lysine	5.81±0.21	0.20±0.02	3.76±0.22	1.17±0.03	1.60
Methionine	1.67±0.01	0.00	0.48±0.11	0.03±0.01	2.20
Phenylalanine	4.62±0.30	0.17±0.01	3.15±0.04	1.07±0.02	2.20
Threonine	4.62±0.22	0.20±0.01	1.99±0.11	0.07±0.01	1.00
Tryptophan	2.75±0.01	0.05±0.01	1.69±0.13	0.06±0.01	0.50
Valine	6.05±0.01	0.23±0.01	3.23±0.11	1.09±0.01	1.60
Cysteine	10.16±0.10	4.62±0.21	7.35±0.33	5.30±0.20	2.20
Aspartic acid	4.62±0.20	0.40±0.01	2.24±0.01	0.15±0.01	1.85
Serine	3.87±0.11	0.19±0.01	1.43±0.11	0.78±0.21	1.80
Glutamic acid	16.57±0.12	2.52±0.12	10.10±0.10	4.20±0.10	1.82
Proline	3.08±0.01	0.18±0.01	2.40±0.12	0.16±0.01	1.84
Glycine	3.92±0.22	0.25±0.01	3.39±0.13	1.10±0.02	1.85
Alanine	7.59±0.20	0.27±0.04	3.37±0.14	1.05±0.01	1.81
Tyrosine	2.64±0.11	0.10±0.01	1.59±0.23	0.06±0.01	2.80
Histidine	3.78±0.31	0.62±0.02	1.35±0.11	0.35±0.01	1.85
Glutamine	4.84±0.21	2.79±0.13	2.40±0.10	0.08±0.01	1.83
Arginine	4.82±0.13	0.13±0.02	2.00±0.01	1.05±0.02	1.78

FAO: Food and Agriculture Organization.
Source: *<http://www.fao.org>.

Table 3. Pearson's matrix correlation of nitrogen source using for amino acids production.

Nitrogen source	Yeast extract	Peptone	Ammonium sulphate	Ammonium nitrate	FAO
Yeast extract	1				
Peptone	0.628*	1			
Ammonium sulphate	0.928*	0.693*	1		
Ammonium nitrate	0.843*	0.766*	0.897*	1	
FAO	0.067	0.149	0.0721	0.195	1

*Correlation is significant at the 0.05 level.

C. utilis FMJ12 was suitable for single-cell protein production.

The Pearson's matrix correlation of nitrogen source influencing amino acids production is shown in Table 3 and shows positive correlations ($p=5\%$) among parameters studied. The highest positive correlation was observed between the presence of yeast extract and ammonium sulphate ($r=0.928$) followed by ammonium sulphate and Ammonium nitrate ($r=0.897$), then yeast extract and ammonium nitrate. The strong correlation between yeast extract and ammonium sulphate has demonstrated their positive influence on increase of the rate of SCP and amino acids as compared to FAO recommendation.

Table 4 contains the relevant results of coordinate of nitrogen sources influence to SCP and essential amino acids production. Analysis of principal components exhibited the variability of influence of four nitrogen sources on SCP and essentials amino acids production. The cumulative values of the variance of the first three principal components (F1, F2 and F3) for the parameters were 100%, with Eigen-values range between 0.41 and 3.41 (Table 4). Principal component F1 had an Eigen value of 3.41 and contributed to 68.24% of the variation of the parameters. This principal component (F1) is associated positively to isoleucine, leucine, lysine, and valine production. Principal components F2 and F3 had respective Eigen-values of 0.99 and 0.41, accounting for

Table 4. Coordinate of nitrogen source and their contribution to SCP and essentials amino acids production.

Parameter (Nitrogen source)	Principal components		
	F1	F2	F3
Yeast extract	0.92	-0.13	-0.28
Peptone	0.83	0.039	0.54
Ammonium sulphate	0.96	-0.12	-0.18
Ammonium nitrate	0.96	0.029	-0.0079
FAO	0.18	0.98	-0.082
Eigen value	3.41	0.99	0.41
Variance (%)	68.24	19.87	8.30
Cumulative (%)	96.41	98.82	100.00

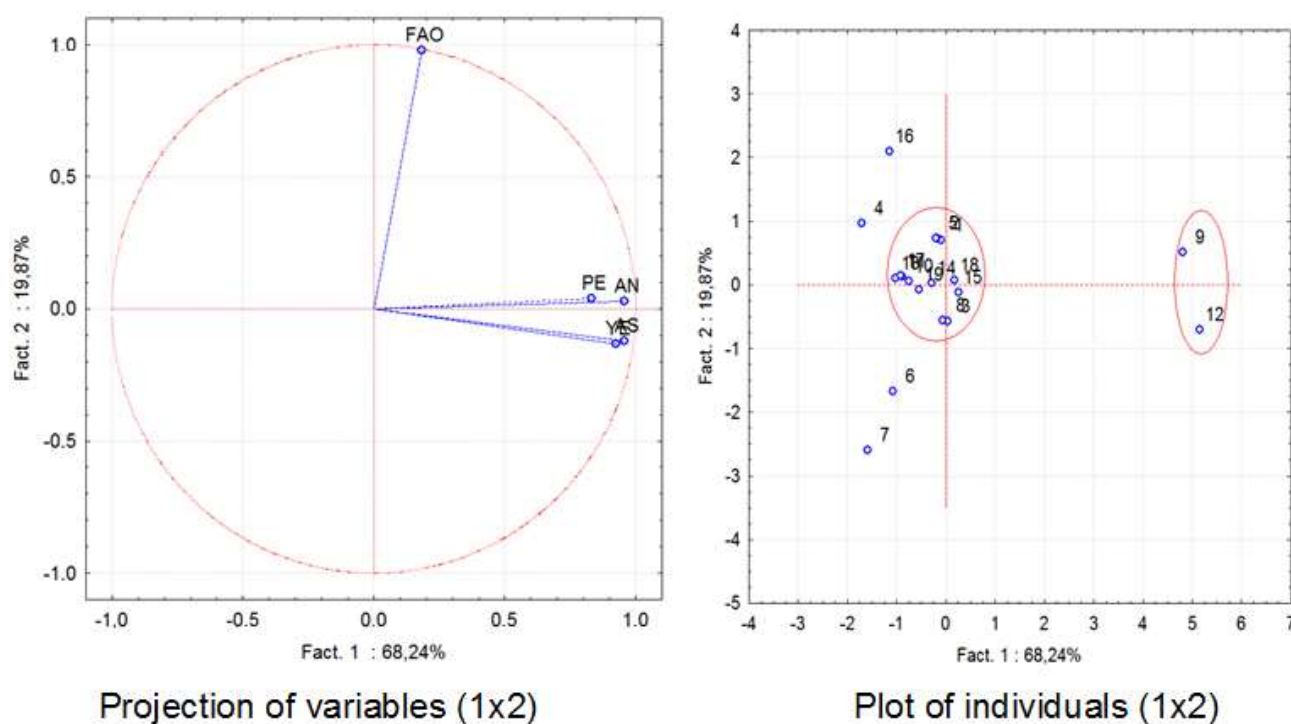


Figure 2. Principal Component Analysis (PCA) of amino acids produced in fermentation medium supplemented with different nitrogen sources. The explained variance (%) is reported for each principal component, and dotted ellipses represent the 95% confidence limits for amino acids. AN, Ammonium nitrate; AS, ammonium sulphate; PE, peptone; YE, yeast extract. 1, isoleucine; 2, leucine; 3, lysine; 4, methionine; 5, phenylalanine; 6, threonine; 7, tryptophan; 8, valine; 9, cysteine; 10, aspartic acid; 11, serine; 12, glutamic acid; 13, proline; 14, glycine; 15, alanine; 16, tyrosine; 17, histidine; 18, glutamine; 19-arginine.

19.87 and 8.30% to the total variation and were associated positively with the rate of Cysteine and Glutamic acid.

Principal component analysis (PCA) of amino acids produced in fermentation medium supplemented with different nitrogen sources are shown in Figure 2. The results with influence of nitrogen source and amino acids data in Figure 2 confirm that PCA can find a reduced set of variables that are useful for understanding the

experiments. The projection and score-plot resulting from PCA achieved by combining F1 (68.24% explained variance) and F2 (19.87% explained variance) is as shown in Figure 2. The first two components account for over 90% of the cumulative allowed for most of the information to be visualized in two dimensions. The production of cysteine was positively correlated by supplementation of peptone or ammonium nitrate in medium of fermentation and in opposite glutamic acid it

was correlated by yeast extract and ammonium sulphate. The loading plot revealed that the variance associated to Isoleucine, Leucine, Lysine, and Valine had the largest weight in F1. It was shown that the production of Isoleucine and Leucine influenced by Peptone or Ammonium nitrate were negatively correlated with Lysine and Valine influenced by Yeast extract and Ammonium sulphate. The results demonstrated that, the maximum yield of essential amino acids was strongly correlated with the presence of Yeast extract.

Conclusion

Finally, the possibility to use of mango waste, as a low cost agro-industrial biomass source for production of SCP by *C. utilis* FMJ12 was demonstrated in this study. This approach could also be used to minimize the environmental pollution. The amino acid profile of the produced SCP was comparable to FAO standards, therefore advocating their use in food applications. It is however, recommended that further large-scale studies be carried out in addition to extensive toxicological and acceptability tests.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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