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APPLICATION OF *CANDIDA VALIDA* AS A PROTEIN SUPPLEMENT

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Abstract : An investigation was carried out on the carbon and nitrogen sources needed for the growth of a yeast, *Candida valida* (syn. *C. mycoderma*) isolated from Ogi, a fermented edible corn product given to babies at weaning. The suitability of this organism as a protein supplement in foods was also determined. The yeast was grown in different carbon sources ;glucose, fructose, lactose, maltose, starch, dextrin, mannitol and ethanol and in different nitrogen sources like urea, amino acids, ammonium nitrate, sodium nitrate and ammonium sulphate, as part of the synthetic basal medium for seven days and the growth measured using the dry weight method. A significantly high increase in yield was observed when 1% fructose was used and maximum yield was obtained with 3.5 and 0.2% (w/v) of fructose and urea as carbon and nitrogen sources, respectively. Growing the fungus in 0.3% cane molasses, a natural substrate gave a significantly higher increase in yield than in a synthetic medium.

As a food supplement, other nutrient contents of this organism such as ash, crude fibre, lipids, and carbohydrates were also analysed in addition to the protein, free amino acids and energy values.

Key words: Protein supplement; Dry weight; Yield; Synthetic medium; Natural medium

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Introduction

The United Nations population experts project that there will be 8 billion people living on this planet by 2015 and 10.5 billion by 2110. This means that during the 35 year period (1980-2015), man must produce as much food as we have since the dawn of agriculture about 12,000years ago. World Health Organization estimates that 12,000,000 people die of hunger and starvation related diseases every year, half are children under the age of five (Miller, 1985). The current global economic meltdown coupled with the increasing world population have challenged science and technology to master the problem of supplying mankind with sufficient food, particularly protein (Willey et al. 2008). Conventional methods made to resolve this by increasing the productivity of agriculture, husbandry and fishery and decreasing losses in the manufacture and storage of raw materials and products have many constraints. Although cereals are the major source of protein, the protein from these is not as high in quality for human food as in meat protein which is still considered to be totally inadequate to meet the expected high protein demand. Thus the use of microorganism as a protein supplement appears attractive. Economically, this approach is more advisable because it takes 4.4kg of fodder protein to produce 1kg of food protein as milk or eggs and about 20kg of fodder protein to produce 1kg of beef protein (Prescott and Dunn 2004). Other advantages of microbial protein include the short generation time required to produce a rapid mass increase (Kuforiji et al, 2009). Besides having a high protein content containing amino acids, the cell matter is especially rich in most vitamin B group and therefore constitute potential vitamin enrichment for deficient diets (Jay 2005).

Most organisms involve in microbial protein production can utilize lignocellulosic and citrus wastes, sewage, five or six carbon sugars, disaccharides like molasses, whey and sulfite liquor and polysaccharides from grains, cassava, corn cobs and municipal solid wastes. For the

utilization of lignocellulose, a pretreatment is necessary and to the present time the only economic utilization of these is in mushroom production. These mushrooms contain lignocellulosic enzymes that can break down the complex compounds in these wastes and are cultivated for food in Asia and Africa (Poppe,2000: Kuforiji and Fasidi, 2008). Based on specifications, some microorganisms have been accepted as protein supplements, these include; algae-*Chlorella* and *Spirulina*; Bacteria-*Bacillus* and *Methylomonas*; Yeasts-*Candida* and *Saccharomyces* (Dubey 2009). Yeasts have the most favourable characteristics for use as a major source of food because of its nutritive value and *C. utilis* was incorporated into human food during the two World Wars. Generally, safety problems do not seem to provide any obstacles towards the use of these organisms (Nester et al. 2007).
Thus the objectives of this study are;

1) To isolate and identify an appropriate organism of high protein content from indigenous babies'weaning food produced from fermented corn (Ogi).
2) To determine the best carbon and nitrogen sources as primary nutrient sources for growth of the identified organism (synthetic medium).
3) To compare the yield of the organism in the above condition to that in cane molasses, a natural substrate.
4) To determine the proximate composition of the identified organism in synthetic medium and cane molasses so as to their suitability as supplement in foods.

Materials and Methods

1 Isolation and Identification of the yeast

Candida valida was isolated from babies' weaning food produced from fermented corn (Ogi), characterized and identified using Standard Mycological Methods (Proctor 1976; Smith 1969).

2 Carbon nutrition

The carbon nutrition study of the yeast was determined . The basal growth medium consisted of $(NH_4)_2SO_4$ 2.0 g; $MgSO_4.7H_2O$ 0.2g; NaCl 5.0g; $FeSO_4$ 0.01g ; K_2HPO_4 0.5g and distilled water to 1 litre mark. 10 g of each of the carbon sources; glucose, fructose, mannitol , ethanol , sucrose, dextrin, xylose, maltose, lactose and soluble starch was added to the basal medium in each conical flask. Growth of the organism was assessed using the dry weight method (Staples 1976).
For this organism, fructose proved to be the best carbon source, hence, different concentrations of fructose (2.0 – 6.0) was dissolved in 100 ml of the basal medium. The mixture was sterilized at 110^oC for 10 mins.

3 Nitrogen nutrition

The nitrogen nutrition was determined by dissolving 0.4 g of the total nitrogen in urea, ammonium sulphate , ammonium nitrate , asparagines, glutamine, methionine and other amino acids in 1 litre of the basal medium (excluding ammonium sulphate). Growth of the organism was assessed using the dry weight method.
Urea proved to be the best nitrogen source, hence the concentration of this was varied from 0.05 -0.4 % to determine that which gave the optimum growth.

4 Growth of the Yeast in cane molasses- a natural substrate

Different concentrations of cane molasses was prepared by dissolving 0.01- 0.4g in 100ml of the basal medium for microorganism using different carbon sources. The mixture was sterilized at 110^oC for 1h. It was cooled and 10ml of the yeast suspension was inoculated into the medium.
In all cases the period of incubation was 7 days at 28^oC.

5 Chemical Analysis of the Yeast Samples;

5.1. Moisture content

Moisture was determined after drying the sample at 105^oC for 24 h (Staples 1976).

5.2. Ash and Crude Fibre

The ash content of the yeast sample was determined by incinerating the dried fungus of known weight at 600^oC for 12h in a Gallenkamp furnace.
The crude fibre was determined by treating the defatted yeast sample with 0.112 M H_2SO_4 and 0.313 M NaOH (Pearson 1976).

5.3. Total lipids

The lipid content in 2 g of each sample was determined as in AOAC (1980).

5.4. Protein

Crude protein was estimated by determination of total nitrogen in 2 g of each sample by the Kjedahl's method, using a factor of 6.25 (Nielsen 2002).

5.5. Carbohydrates

The anthrone method was used for the estimation of total carbohydrates. The concentration of glucose was read off from the standard curve of glucose prepared as described by AOAC (1980).

5.6. Free Amino Acids

This was determined by first hydrolyzing the samples in 6 M HCl and incubating in vacuo at 110^oC for 2h. A standard curve using serially diluted leucine was obtained (Barker 1971).

5.7. Energy values of *Candida valida*

The energy values of the yeast were calculated on the basis of their content of crude protein, fat and carbohydrate by using the factors 17, 37 and 17 kJg^{-1}, respectively (Europa 1990).

6. Statistical Analysis

Samples were in three replicates and the means were quoted with their standard errors (SE). Statistical analyses were carried out using Analysis of Variance (Sokal and Rohlf 1995).

Results & Discussion

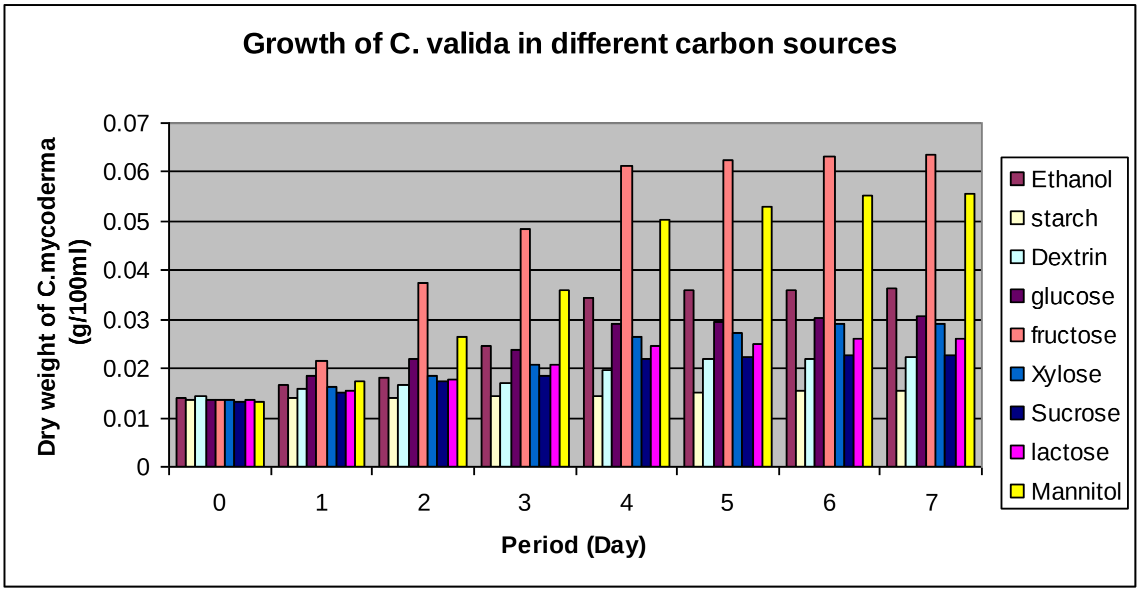
The *Candida valida* isolated from babies' weaning food product made from fermented corn, Ogi, showed increase in dry weight when grown in a synthetic basal medium containing each of the carbon source (Fig. 1). The organism showed the greatest increase of about 3.7 times for fructose, while the least of only 0.14 times was obtained for starch (Fig. 1). The sample statistics of F distribution (FS) showed that the carbon sources had a significant effect on growth of the organism (Sokal and Rohlf 1995). Fructose was reported as being readily utilized by yeasts and normally taken up by the constitutive hexose transport system in the same way as glucose (Sols et al, 1971). Before the utilization of starch, however, there must be the enzyme amylase to hydrolyse it. This may account for the minimal growth in the medium containing starch as a carbon source. Organisms tend to grow better at a particular concentration of their requirements, hence the concentration of fructose was varied (Fig.2). The cell yield of *Candida valida* increased maximally at a concentration of 3.5 %. Higher concentrations had an inhibitory effect on growth of this organism hence there was decrease in the yield of cells which may be attributed to the 'crabtree effect' (Sols et al. 1971).

For efficient growth in a medium, a utilizable source of the element nitrogen must be present in order that organisms can synthesize amino acids and thus proteins and certain vitamins. *C. valida* exhibited the highest growth rate (about 31.5 times increase) in urea at a concentration of 0.2 % followed by a decrease when higher concentrations of 0.3 and 0.4% urea were used (Figs. 3& 4). Urea, in addition to being a nitrogen source, also contribute carbon and energy sources. Morris (1858) however revealed that there is no optimum amount of nitrogen for a culture since the demand is in the first instance on the carbon supply, thus, any factor may change the apparent optimum concentration of the nitrogen sources.

Pure chemicals are generally not used for 'Single Cell Protein' production on an industrial scale because of the high cost of materials. Natural substrates like cane molasses are commonly used. There was an increase in dry weight of the organisms as the concentration in cane molasses was increased from 0.01 -0.3 % (Fig.5). This was followed by a decrease when a higher concentration of 0.4 % was used. *C. valida* showed the greatest and least increase of about 11.7 and 9.3 times at concentrations of 0.3 and 0.4 %, respectively (Fig. 5). When the dry weight of *C.valida* grown in 0.3 % of cane molasses were converted to an equivalent of 1 % (i .e. the dry weights obtained multiplied by 3.3), *C. valida* was found to have initial dry weight of 0.015 gml^{-2} (day 0) and final dry weight of 0.0705 g /100 ml (day 7) . Thus, the equivalent growth of the organism with cane molasses was calculated to be higher than the equivalent growth in 1 % fructose (Fig.1).

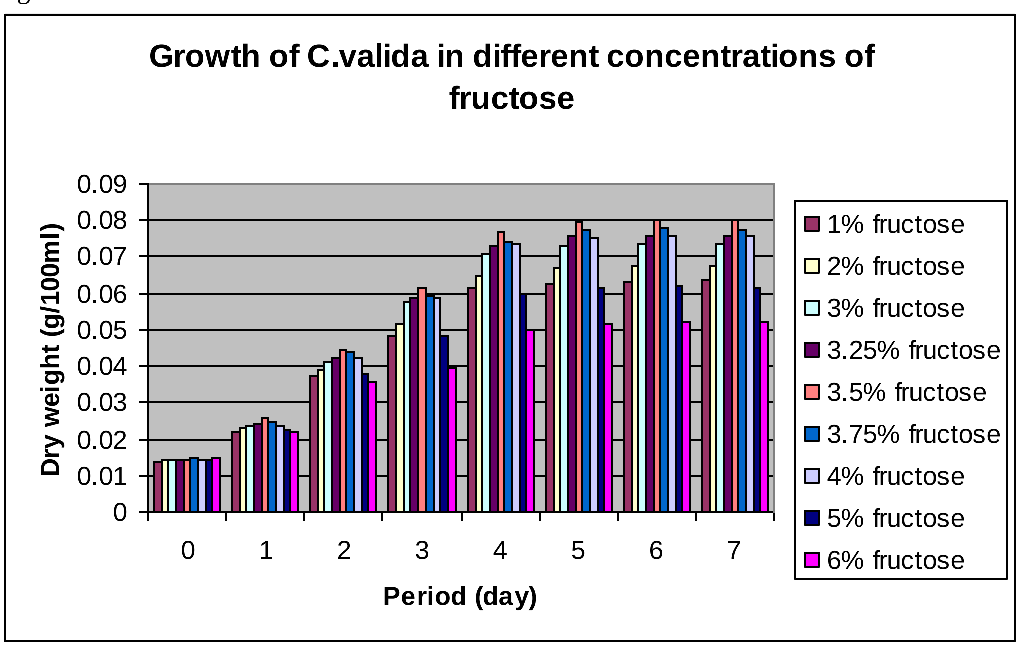
The use of an organism for food or as a supplement requires that it contains essential nutrients, thus, the proximate composition of the organisms in a synthetic medium and cane molasses were analysed (Table 1).About one half of the dry weight of yeasts generally contain crude protein and this consists of about 80% amino acids, 12% nucleic acids and 8% ammonia, with about 7% of the total nitrogen occurring as free amino acids (Peppler 1970). Cane molasses as a substrate, gave a higher protein content which may be due to the presence of urea and other nitrogen sources in it. The free amino acids can easily be utilized unlike the proteins which are normally associated with yeast cell wall. Furthermore, the nitrogen content on which the protein value was based can best be regarded as only a rough index of the cells nutritive value, since it is known that considerable part of the total microbial cell nitrogen is found in purine and pyrimidine bases of nucleic acids and small amounts, in addition, in glucosamine, choline and so on (Kihlberg 1972). Although microorganisms could not be considered as a main source of carbohydrate in food, yet there is a calorific contribution as also expressed by the energy values of 1376.6 and 1354.7 kJg^{-1}in synthetic medium and in cane molasses, respectively (Table 1). Kuforiji and Aboaba 2009 reported that *C. tropicalis* had higher energy values of 1504.81 and 1373.91 kJ/g in synthetic medium and in cane molasses, respectively when compared to *C.valida*. The low lipid content implies the yeast is non-fattening and the crude fibre content suggest the roughage ability of this fungus as there is a general agreement among clinical investigators that etiologies of constipation, diverticular diseases of the colon and cardiovascular and lipid metabolism disease may be related to the chronic consumption of fibre depleted diets, while, high fibre ingestion may decrease the digestibility of dietary protein, minerals, fats and energy because of their absorptive properties (Amen and Spiller 1978). Miller (1978) suggested that yeasts grown as food / feed supplement should have 45 -49 % protein, 4 -7 % fat, 26-37 % carbohydrate and 5-10 % ash on a dry weight basis. It was observed that the protein and ash contents of *C. valida* were below the acceptable levels in both media, while the carbohydrate and lipid contents were within the range. Cane molasses proved to be a better medium for the cultivation of this organism, while, the synthetic medium could be modified to give a better biomass of higher nutritional value. In all, the yeast, *C. valida* has potentials for being used as protein supplement based on the proximate composition. The global economic meltdown portrays increase incidence of malnutrition in developing countries, thus, in addition to sourcing for food rich in proteins at low cost like mushrooms, more efforts must be geared at using yeasts to obtain the needed components at affordable price (Kuforiji and Fasidi 2008). Azolay et al (1980) reported that *C.tropicalis* has been used for protein enrichment of cassava and corn with the resultant mixture having protein content of above 20%. This represents a balanced diet for either animal or human food.

Fig. 1



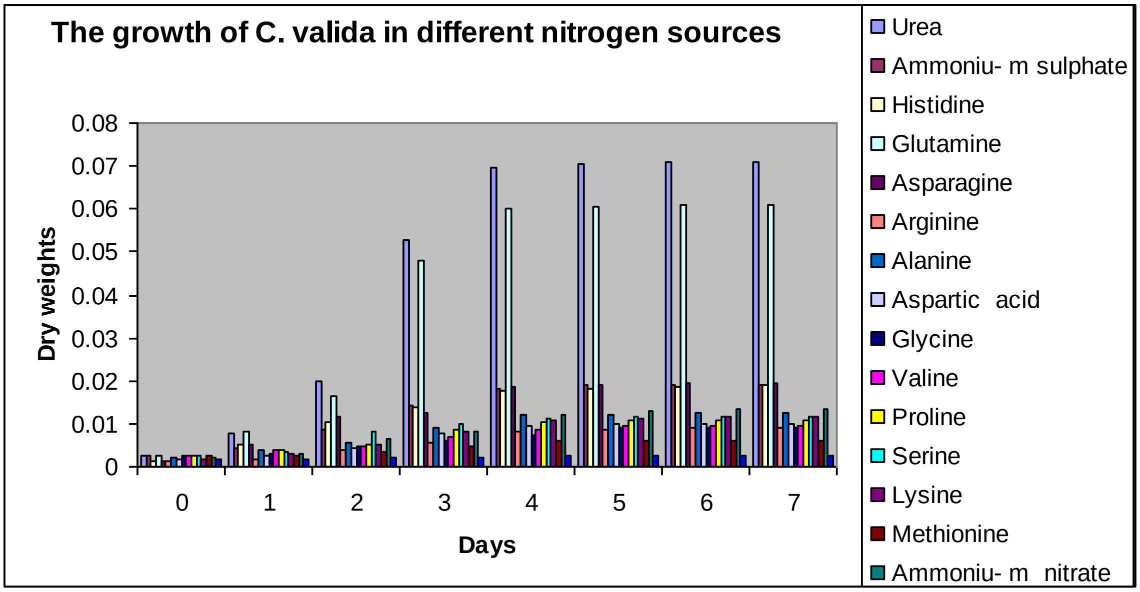
Values are means of three replicates $F_{0.05}$=0.010; F=$_{0.01}$=0.013.Mean difference higher than $F_{0.05}$ & $F_{0.01}$ are significant at 5 and 1% levels, respectively.

Fig.2



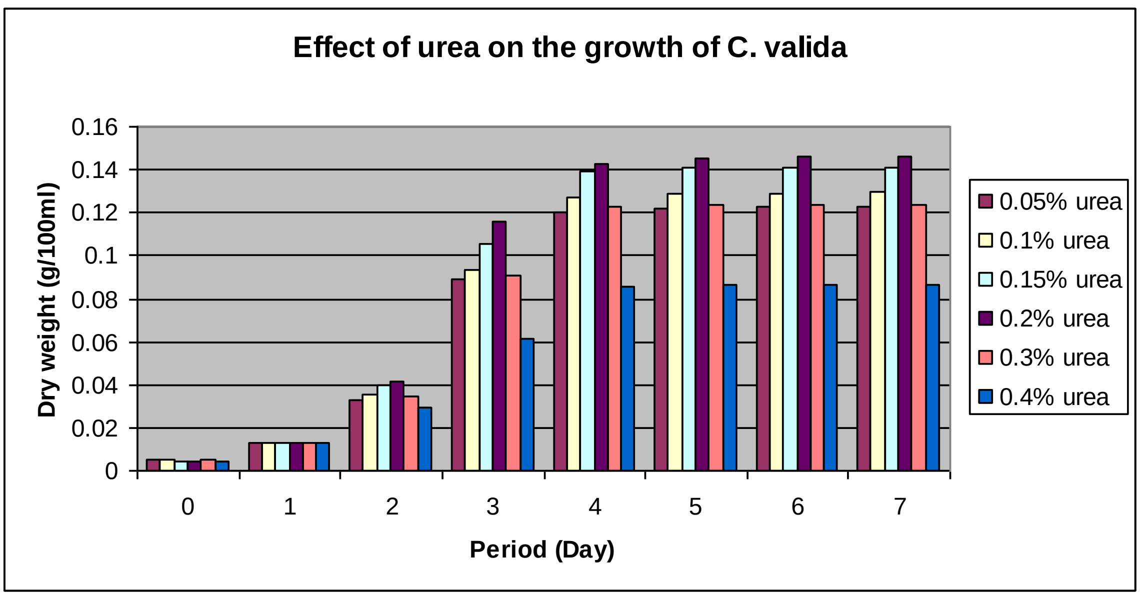
Values are means of three replicates $F_{0.05}$=0.023; $F_{0.01}$=0.031.Mean difference higher than $F_{0.05}$ & $F_{0.01}$ are significant at 5 and 1% levels, respectively.

Fig.3



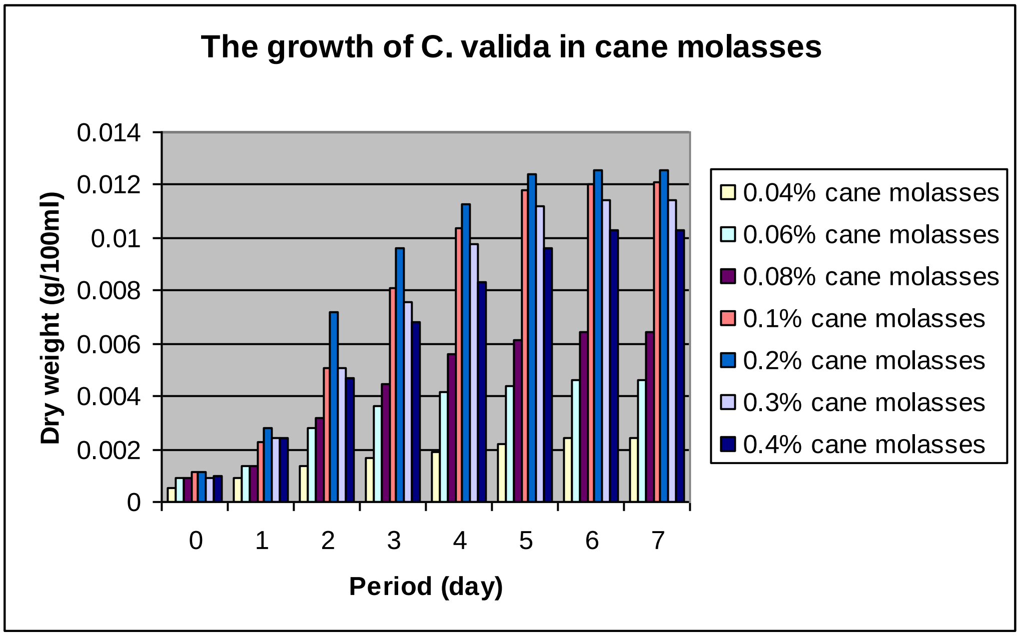
Values are means of triplicate determination. $F_{0.05}$=0.011; $F_{0.01}$=0.015. Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

Fig. 4



Values are means of three replicates. $F_{0.05}$=0.056; $F_{0.01}$=0.074.Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and 1% levels, respectively.

Fig.5



Values are means of triplicate determination.$F_{0.05}$=0.003; $F_{0.01}$=0.004.Mean difference higher than $F_{0.05}$ and $F_{0.01}$ are significant at 5 and1% levels, respec

Table 1.
Proximate composition of *Candida valida* in a synthetic medium and in cane molasses

Growth medium	Moisture (%)	Ash	Cru-de fibre	Lipids	Protein	Carbohy drate	Energy Value (kJg^{-1})	Free amino acid (gl^{-1})
i) Synthetic	4.7	2.0	3.7	4.4	42.6	28.8*	1376.6*	$46x10^{-3}$
ii) Cane molasses	4.9	3.0	4.0	3.9	44.3*	26.9	1354.7	42×10^{-3}

Values of ash, crude fibre, lipids, protein and carbohydrate are in % dry weight.
Values are means of triplicate determinations. $F_{0.05}$=1.16.
Mean $\pm$SE differences higher than $F_{0.05}$ are significant at 5% level (*)

Conclusion

Thus, it can be concluded that *C. valida* is potentially suitable as a protein supplement as shown in the proximate composition. Higher yield of *C valida* can also be obtained by cultivation on cane molasses instead of using synthetic media.

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