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Full Length Research Paper

Production of *Saccharomyces cerevisiae* biomass in papaya extract medium

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Extracts of papaya fruit were used as substrate for single cell protein (SCP) production using *Saccharomyces cerevisiae*. A 500 g of papaya fruit was extracted with different volumes of sterile distilled water. Extraction with 200 mL of sterile distilled water sustained highest cell growth. Biochemical analysis of dry biomass revealed the following composition: 35.5% protein, 40.7% saccharide, 4.09% lipids, 0.04% magnesium, 9.63% moisture and 7.90% total ash. Nutrient found in papaya fruit extract were 9.8% saccharide, 0.1% crude protein and 7.3% total soluble sugars.

Key words: Papaya, *Saccharomyces cerevisiae,* single cell protein.

INTRODUCTION

Papaya (*Carica papaya* L.) is a sugar crop with soluble sacccharides in the form of glucose, fructose and sucrose (Solvaraj and Pal, 1982), and it is widely cultivated in several countries (Samson, 1980). In tropical climates, such as in Nigeria, the papaya trees continue bearing fruit throughout the year, and the fruit in turn follow the same pattern of maturity so that it is possible to pick the fruit at any desired stage of maturity (Cobley and Stele, 1976; Samson, 1980). It displays rapid growth and a high yield of 100 kg plant per year or 154,000 kg per hectare per year, even during the fourth year of growth (Allan, 1981). The average yield per hectare is about 22000 fruits weighing 34 tons (Allan, 1981).

Sugars represent that part of the fruits which is used by microorganisms for single cell protein and alcohol production (Oura, 1983). The potential of papaya as an energy crop was estimated by Ayanru et al. (1985) who showed that it has a capacity of generating ethanol by microbial conversion of sugars in the papaya fruit. Sugar crops like papaya fruit have the advantage over starch and cellulose waste in single-cell protein programmes, as the latter require extensive supply of sugars and digestion prior to their utilization for cell growth and biomass production.

The aim of this study was to investigate the growth of *S. cerevisiae* in papaya medium and the effects of different dilutions of the extract and inoculation size on the biomass production.

MATERIALS AND METHODS

Isolation of yeast

The isolate of *S. cerevisiae* was obtained from Raphia palms grown at the Nigerian Institute for Oil Palm Research, Benin City, and the malt extract medium was used for the isolation. Characterization and identification of the yeast was done based on the morphological and physiological characteristics of yeasts, described by Lodder (1970). Yeast inoculums were prepared by transferring growing *S. cerevisiae* colonies from one-week-old potato dextrose agar (PDA) slant to 10.0 mL dextrose broth (Oxoid) followed by incubation at 25^{o}C for 24 h.

Preparation of papaya fruit extract

A 500 g ripe papaya fruits were obtained from the New Benin market, Benin City. The fruits were washed with several changes of sterile water and peeled. Seeds and placenta were removed from the sliced pulp, cleaned initially with 2.0% solution of H_2SO_4, sliced into cubes, rinsed in sterile water, and pulverized into slurry using a sterile blender (Kenwood Blender Mixer, Licaudara, Model A515). The fruit extract was obtained from slurry filtered with the use of cheesecloth. The extract was placed into a sterile container and the

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sacchaide, crude protein and total soluble sugar were determined.

From the extract, 100 mL was measured into sterilized 250 mL conical flasks. To each was added glucose (2% w/v) and $(NH_4)_2HPO_4$ (0.25 % w/v) which served as a nitrogen source supplement. The conical flasks were plugged loosely with sterile cotton wool and aluminium foil. Sterilization was achieved by autoclaving at 1.0 kg/cm^2 pressure for 15 min at 121°C. On cooling, the medium in the flasks was inoculated with 1.0 mL of inoculums (3.4 x 10^3 cells/ml).

Aeration was achieved by shaking in an orbital shaker (Lan-line Instruments Inc.) at 200 rpm for 45 h at 25°C. The concentration of cells in the inoculums and inoculated extract was determined by inoculum size (4.2 x 10^3, 5.6 x 10^3 and 5.6 x 10^4 cells/mL) on the biomass production were investigated.viable colony count. Inoculated flasks were incubated at 25°C for 5 days. The effects of different diluted samples (extraction of papaya fruit with 200, 400 and 600 mL of distilled water) and varying

Determination of yield of dry biomass

At the end of incubation, the dry biomass yield of *S. cerevisiae* from the papaya medium was determined by harvesting the cells into a predried and preweighted aluminium pan and then dried to constant weight in an oven (Gallenkamp Oven B5 Model 0v 330, Gallenkamp, England) at 60°C for 48 h. The weight was taken using a Mettler electronic balanca H80, Gallenkamp, England.

Analytical procedure

The total saccharide was determined according to Pearson (1982) using anthrone method. Total soluble sugar was determined by the method of AOAC (1980). Moisture content was determined as described by Osborne and Voost (1978), based on the principle of drying to constant weight. Protein was determined by a modified biuret method described by Herbet et al. (1971). The procedure of Kates (1991) was used for the determination of the lipid content. The E.D.T.A. titrimetric method was used for the estimation of calcium (APHA, 1975). Estimation of inorganic phosphate was done according to Cooper and Simmons (1975). The E.D.T.A. titrimetric method was also used for the determination of magnesium content (Cooper and Simmons, 1975). Ash content was obtained by igniting the samples in a muffle furnace at 55°C as previously described by Pearson (1982).

Table 1. The viable cell counts of *S. cerevisiae* in papaya medium during 5 days of incubation. The initial inoculum contained 4.6 x 10^3 cells/mL.

Days of incubation	cfu
1	6.49 x 10^7
2	7.10 x 10^7
3	6.40 X 10^6
4	8.16 x 10^5
5	6.27 x 10^4

Each value reported is an average of triplicate counts.

RESULTS

The viable cell counts of the yeast isolate in the fruit pulp extract is presented in Table 1. Inoculum containing 4.2 x 10^3, 5.6 x 10^3 and 5.4 x 10^4 cells/mL as initial inoculum gave peak cell densities of 8.50 x 10^5, 1.20 x 10^8 and 1.17 x 10^7 cfu/mL after 2 days of incubation (the peak point of growth). It is seen that growth increased up to 4 h and declined thereafter (Figure 1). The medium extracted with 200 mL sterile distilled water produced highest cell growth (Table 2). Growth in the undiluted sample and samples extracted with 400 mL and 600 mL sterile distilled water peaked after 1 or 2 days (Figure 2). Data on the dry biomass harvested from the papaya medium are shown in Table 3. The results of the chemical analysis of the papaya fruit extract are presented in Table 4. The results of chemical analysis of the dry yeast cells produced from *S. cerevisiae* are presented in Table 5.

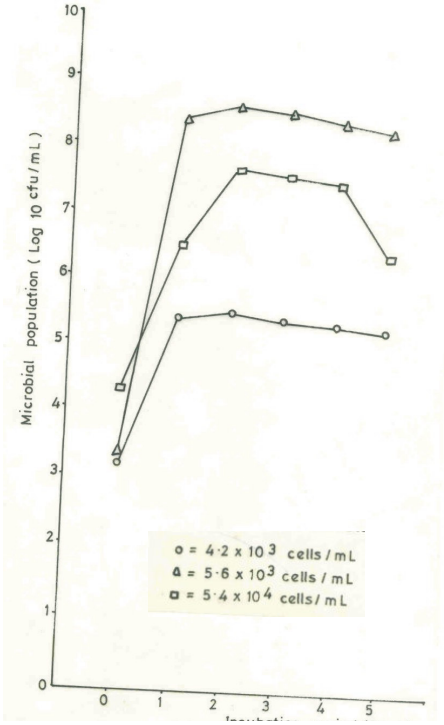

Figure 1. Growth of *S. cerevisiae* in papaya fruit extract with varying amounts of initial inoculum.

DISCUSSION

The mean viable cell counts of *S. cerevisiae* in papaya medium were found to decrease after 1 or 2 days (Table 1). The decrease in growth can be attributed to limiting nutrients and oxygen, arising from their exhaustion. Autolysis is enhanced by the exhaustion of nutrients and oxygen (Kays and vanderzant, 1980).

The investigation revealed that increase an initial inoculum size up to 5.6 x 10^3 cells/mL yield cell growth. Concentrations above this value, however, produced

Table 2. Cell counts of *S. cerevisiae* in undiluted and diluted papaya medium extracted with different volumes of sterile distilled water. The initial inoculum contained 5.2 x 10^5 cells/mL.

Daysof incubation	Undiluted sample	Diluted Samples		
		200 mL	400 mL	600 mL
1	9.20 x 10^7	1.02 x 10^8	7.22x10^5	6.72x10^4
2	8.64 x 10^7	1.25 x 10^8	4.37x10^5	3.26x10^4
3	7.50 x 10^6	9.30 x 10^7	5.29x10^4	4.28x10^3
4	4.60 x 10^5	8.24 x10^7	3.50x10^3	8.49x10^2
5	4.00 x 10^5	8.26 x 10^6	3.17x10^3	5.20x10^2

Each value reported is an average of triplicate counts.

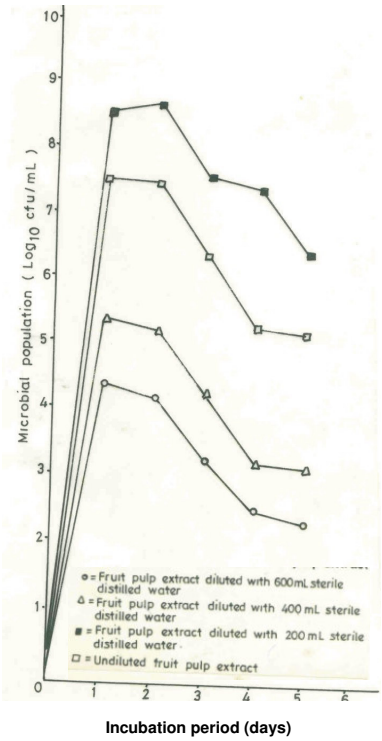

Figure 2. Growth of *S. cerevisiae* in papaya fruit pulp extracts.

Table 3. Dry mass of *S. cerevisiae* harvested from papaya medium per 100 mL.

Number of harvest	Dry mass of cells (g)
1	0.56
2	0.47
3	0.59
4	0.39
Total	2.01
Average	0.52

disproportionate increase in growth of *S. cerevisiae* (Figure I). Reade and Gregory (1975) reported that

Table 4. Percentage chemical composition of papaya fruit extract.

Constituents	Percent
Moisture content	82.3
Protein	0.1
Saccharide	9.8
Total soluble sugars	7.3
Total ash	0.2
Lipids	0.3
Calcium	0.01
Inorganic phosphate	0.01
Magnesium	-

Table 5. Percentage chemical composition of dry yeast cells produced from *S. cerevisiae*.

Constituents	Percent
Moisture content	9.63
Protein	35.5
Saccharide	40.7
Total soluble sugars	7.90
Total ash	0.16
Lipids	0.004
Calcium	4.5
Inorganic phosphate	4.09
Magnesium	-

autolysis is likely to be increased with high initial inoculum. This is because there is the existence of disproportionate amount of nutrients, as well as lower conversion of efficiency. According to Chikwendu (1987), at lower inoculum level cells are larger and further indicated that this type of growth arises when competition for available nutrients is not great among the cells present.

The growth of *S. cerevisiae* in the papaya fruit extract prepared with 200 mL sterile distilled water increased

over that of the undiluted fruit extract, and samples prepared with 400 and 600 mL sterile distilled water at the peak of growth (Table 2, Figure 2). Ayanru et al. (1985) observed a similar effect of dilution of papaya juice in ethanol production, indicating that increased cell growth with dilution was due to more favorable osmotic pressure and sugar concentration effects. Reade and Gregory (1975) have also ascribed decreased cell growth at high substrate concentration level to early autolysis. The poor cell growth in extract prepared with 400 mL and 600 mL sterile distilled water may be attributed to reduction of available sugars and in the concentration of growth factors.

The yield of dry mass of cells from the papaya medium ranged from 0.39 to 0.59 g with an average of 0.52 g (Table 3). The results of the chemical analysis of papaya fruit extract established that it is a good energy and nutrient source for cell mass formation with a high saccharide content of 9.8% (Table 4). The results of chemical analysis of the dry yeast cells show that the yeast protein was of high nutritional value (Table 5). The level of crude protein of 35.5% obtained from the dry biomass recommends the product as a potential food and feed supplement when compared to the lower limits of 8% for cattle and poultry feed (Han and Anderson, 1974).

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