

Full Length Research Paper

Acid-hydrolysed local Cowpea (*Vigna unguiculata*) as culture media for Laboratory Yeast (*Saccharomyces cerevisiae*)

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Cowpea (*Vigna Uguiculata*) seeds are nutritious components in the human diet; they contain 63.66 starch, 24.8 protein and 1.9% lipid-fat. Acid-hydrolysis of powdered cowpea extract leads to the breakdown of its complex molecules into simple materials. The hydrolysis of protein in the presence of an acid catalyst gives amino acids, which serves as nitrogen and carbon source for yeast growth. The acid-hydrolysed cowpea filtrates were now tested for yeast growth by culturing or growing the yeast on the media at a variable pH of 5.18 – 5.96. It was observed that test-tube labelled A and B has the highest mean absorbance of 0.810 and 0.882 at 0.5 and 1.0 g/ml cowpea filtrate level in relation to the normal yeast media. Hence, acid-hydrolysed cowpea at a concentration of 0.5 and 1.0 with pH of 5.56 and 5.58 which is cheap and commonly available can be used in growing yeast in the laboratory especially during industrial bumper production. Also, the amino acid content of the cowpea revealed highest in glutamic acid, aspartic acid, leucine and lysine by 1000, 700, 450 and 400% respectively.

Key words: Cowpea, *Vigna Uguiculata*, Culture media, Yeast, amino acids

INTRODUCTION

Cowpea (*Vigna unguiculata*), an annual legume, which belongs to the legume family (*Fabaceae-leguminosae*). It is also commonly referred to as southern pea, black eye pea, Crowder pea, lubia, biebe, coupe or frijole (Duke 1985, Singh *et al.*, 1997, Quine, 1997). Cowpea originated in African is widely grown in Africa, Latin-America, and Southern Asia and in the Southern United States. It is chiefly used as a grain crop, for animal fodder, or as a vegetable. It is used as a leguminous plant, as rotational cover crop which helps to meet cash crops' nitrogen needs. It is also helpful in the control of erosion and to improve soil fertility. It suppresses weeds

and can encourage population of beneficial insects to defend cash crops from insect pest. Cowpea has a high drought resistance which makes it valuable in un-irrigated fallow fields. Cowpea is also said to be of medicinal importance to man. Part of plant (roots, leaves and seeds) has been used to treat various medicinal ailments including painful menstruation, epilepsy, headaches, constipation, chest pain and bilharzias. In some parts of Nigeria, cowpeas are used in folk medicines, by grinding one or two seeds and mixing it with oil to treat stubborn boil (Altschul and Von 1973; Pfaff *et al.*, 1978) while the black-eyed cowpea is grown primarily in California and is marketed as California black-eyed peas (Almeyda and Martin 1980; Ehler and Hall, 1997). The international institute of tropical agriculture (IITA) has been working on the improvement of cowpea for more than 30 years and

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over 60 countries receive cowpea cultivars improved by IITA for testing and adaptation where needed. Cultivars are developed by the IITA in Ibadan. Bressani (1985).

Budding yeast are true fungi of the phylum *Ascomycetes*, class, *Saccharomycetes* also called *Hemiascomycetes*. Yeasts are characterized by a wide dispersion of natural habitats and are common on plant leaves and flower, soil and salt water. Yeasts are also found on the skin surface and in the intestine tract of warm blooded animals, where they thrive symbiotically or as parasites (Michael and Patrick, 1993; Adams, 1980; Yu, et al, 1998; Arroyo, 1981; Glen and Dilworth, 2006; Mortimer et al, 1992; Elizabeth et al, 1992). The most well known and commercially significant yeast are the related species and strains of *Saccharomyces Cerevisiae*. These organisms have long been utilized to ferment the sugars of rice, wheat, barley and corn to produce alcoholic beverages and in the baking industry to expand or raise and for some types of fermentation (Wien, and Drickamer, 1996; Singh and Rachie, 1985) Yeast is often taken as a vitamin supplement because it is 50% protein and is a rich source of B vitamins, niacin and folic acid. In brewing, *Saccharomyces carlsbergensis*, used after the Carlsberg Brewery in Copenhagen, where it was first isolated in pure culture. It is used in the production of several types of beers including lagers. *S. carlsbergensis* and for bottom fermentation. *S. cerevisiae* is also used for the production of top fermentation, in which the yeast rises to the surface of the brewing vessel Staton, (1966). The yeast's function in baking is to ferment sugars present in the flour or to add to the dough. This fermentation gives off carbon dioxide, (CO₂) and ethanol. The CO₂ is trapped within tiny bubbles and results in the dough expanding or rising. Kipps, (1970). The fermentation of wine is initiated by naturally occurring yeast presence in the vine yards. Many wineries still use nature strains, many uses modern methods of strain maintenance and isolation (Almeyda, and Martin, (1980)). It is therefore imperative to assess the microbial growth activity of cowpea seeds as culture medium on various fungal, bacteria and yeasts. The study aimed to investigate the Acid-hydrolysed local Cowpea (*Vigna unguiculata*) as a good alternative culture media for Laboratory Yeast (*Saccharomyces cerevisiae*).

MATERIALS AND METHODS

Sample Collection.

The cowpea seeds, (1500g) brown variety (*Vigna unguiculata*) were randomly selected from Abraka market in Delta State, Nigeria between 1:00pm to 4:00pm.

Unwanted materials like stones and chaffs were selected and the cowpea seeds were grinded to powder by the use of attrition mill and sieved through a sieve cloth with about 0.25mm pore size. The powder was preserved for one week at room temperature for subsequent assays.

Determination of proximate analysis of cowpea.

Proximate analysis that describes the basic nutrient composition of food in terms of moisture, protein, lipid-fats, crude fibre, Ash (minerals) and carbohydrate was determined. Yeast peptone D-glucose (YPD media) or yeast media (YM) was thereafter prepared (Stanbury, et al., 1995).

Preparation of Substrate (Cowpea Flour) Acid Hydrolysis of Cowpea Sample

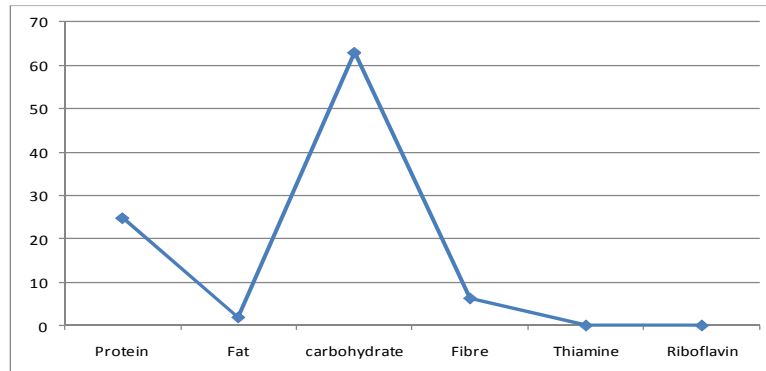
10g of cowpea flour powdered cowpea was measured into two sets of different 250ml conical flask. 99.5ml of distilled water was added to the sample and 0.5ml of concentrated H₂SO₄. Then 0.5ml of distilled water was added to have a total of 100ml of distilled water present in sample. The conical flask containing the mixture was covered with aluminium foil and autoclaved at 121°C for 30 minutes and allowed to cool at room temperature and then filtered through filter paper. The residue was discarded and the various filtrates were transferred approximately in the sterile container (test tubes) that has been labelled A-G. The filtrates were kept for analysis.

Innoculation of The Filtrate With Yeast Media

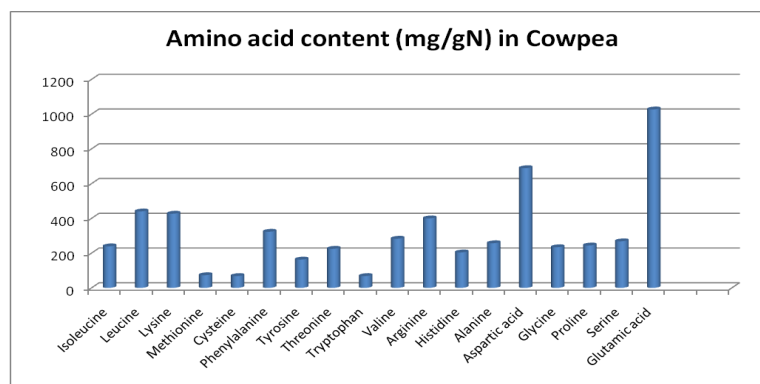
The pH of the autoclaved filtrates present in test tube labelled A-G was then inoculated with 1ml of yeast culture or yeast media which allow the yeast to grow for 24-30 hours in an incubator. 0.1ml of antibiotics (Ampiclox) was added to each test-tube. It was then made up to 100ml. the pH was determined and absorbance/optical density were taken at 600nm for yeast growth.

Statistical Analysis

Treated groups were compared to control group by student's-t-test using Microsoft excel. All data were expressed as mean ± SD of number of experiments (n = 5). A value of P < 0.05 was considered to indicate a significant difference.



Graph 1: nutrient content of matured cowpea seed.



Graph 2: Amino acid content (mg/gN) of protein present in cowpea.

RESULTS

Shown in graph 1 above is the nutrient content of matured cowpea seed.

The proteins consist of 90% water insoluble globulins and 10% water soluble albumins. Cowpea contains high Carbohydrate 63% and protein 24.8% while Thiamine, Riboflavin and Niacin are significantly low.

Although much variation occurs, cowpea is deficient in cystine, methionine and tryptophan. It is rich in lysine, thiamine, tryptophan and isoleucine (Ahlgran 1982). Total sugars range from 13.7 – 19.7% and they include the following: 1.5% sucrose, 0.4% raffinose, 3.1% verboscose and 2.0% stachyose. Starch varies 50.6% to 28.7% amylase and 11.4% to 36.6% amylopectin. Digestibility of cowpea is increased by grinding the seed into fine powder. Seeds contain a trypsin inhibitor, a chymotrypsin inhibitor and cyanogens in concentration of 2mg/100ml extract. Cooking improves the nutritive value perhaps because the activity of trypsin inhibitors and/or the amount of other toxins are decreased by heat.

Table 1 shows the various composition of cowpea with its respect to proximate analysis. Carbohydrate and Protein show the highest value signifying conducive medium for yeast growth.

DISCUSSION

From the proximate analysis of cowpea, the compositions are lipid 2.2%, protein 25.39%, Ash 3.66%, fibre 2.35%, carbohydrate 66.35%. The cowpea flour contains a great percentage of carbohydrate and protein which can serve as nutrient source for yeast growth. Upon the hydrolysis of cowpea with H_2SO_4 , the monomers of protein and carbohydrate content are released with amino acids and glucose respectively. The molecular mechanism of acid catalysed hydrolysis of cowpea flour which contains protein and carbohydrate involves the cleavage of the peptide and glycosidic bonds (Arias et al. 1997).

The result obtained in test tube C shows that there was a significant difference in the content of both the cowpea

Table 1: various composition of cowpea

% Cowpea Contents	Mean \pm S.E.M
Moisture	0.84 \pm 0.00913
Lipid-fat	0.22 \pm 0.013
Fibre	8.44 \pm 0.0071
Ash	0.026 \pm 0.0012
Protein	25.38 \pm 0.049
Carbohydrate	66.35 \pm 0.173
Energy Kcal	387.0 \pm 0.66

Table 2: Yeast Growth

Test tube	Control	A	B	C	D	E	F	G (YPD)
Absorbance \pm SD	0.0115 \pm 0.001	0.810 \pm 0.0044	0.882 \pm 0.0041	0.099 \pm 0.00	0.133 \pm 0.00071	0.192 \pm 0.00	0.088 \pm 0.00	0.680 \pm 0.0041
p ^H	5.64	5.56	5.58	5.48	5.42	5.18	5.42	5.96

and the poultry extract except for the energy content of both in which there was no significant difference ($P > 0.05$) (Baldwin *et al.* 2010).

From the results obtained, for yeast growth, table 2, in all the test-tubes; A to F and G (normal YPD media) using different concentrations of cowpea filtrates, it was observed that the mean absorbance is higher in the test-tube labelled A and B as 0.812 and 0.882 at a concentration of cowpea filtrate of 0.5 and 1.0g/ml and the G (normal YPD media) as 0.680. This high value of absorbance that was taken at 600nm shows a high growth rate of yeast (*saccharomyce cerevisiae*). This is in accordance with the evolutionary findings of (Geoffrey *et al.* 1995) who observed that the best method for measuring the optical density of a yeast suspension and the way of evaluating yeast growth is spectrophotometry, and that the higher the absorbance, the better the yeast growth.

The production of yeast is pivotal for the industrial use of product and development of an economic culture necessary to obtain a high quality of biomass. The optimal growth of yeast as shown by studies are best done in the presence of carbon and nitrogen sources, hence, glucose serve as an important carbon source for yeast growth with a dual role in biosynthesis and energy production and for microbial fermentation processes (Jennifer *et al.* 2002; Anthony 2005). This was also confirmed in this study; cowpea was used as nitrogen and carbon sources for the laboratory development of yeast culture media.

The in vitro study of yeast growth is potentially affected by the pH level of the growth media. Studies on the

growth of American type culture collection (ATCC) and field strains of (*M. pachydermatis*) was optimal between the pH value of 4.0 and 8.0, and inhibited at the range of 1.0 – 3.0 and 9.0 – 10.0 (Cook, 1985; M. Raines, 1992,1995; Nordstrom, 2000; Ahlgran,1982; Campbell, and Duffus, (1988); Patrick,1994; Sare *et al.* 1983) This study also observed that in the test-tube labelled A, B and G that have pH of 5.56 – 5.96 revealed agreement with the pH optimum growth range of *M. pachydermatis* as 4.0 and 8.). This therefore suggests that the pH ranging from 5.56 – 5.96 in the yeast culture media is suitable for yeast growth which results from the acid hydrolyze cowpea.

CONCLUSION

Acid-hydrolysed cowpea can serve as a good nutrient source for the growth of yeast and for culturing yeast (*S. cerevisiae*) in the laboratory at concentration of 0.5 and 1.0 g/ml with a pH of 5.56 and 5.58 respectively. In nutshell, acid-hydrolysed cowpea which is a cheap source of nitrogen and carbon can serve as an alternative for the Yeast Peptone D-glucose media in the development of laboratory yeast culture media. Thus, acid-hydrolysed local cowpea, which is cheap, can be used in growing yeast in the laboratory especially during industrial bumper production. However, to improve this research work, different culture conditions such as temperature, aeration and exposure to CO₂ should be carefully monitored as they might predispose different effects on the yeast growth.

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