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USE OF DEPROTEINIZED JUICE OF DIFFERANT VEGETABLE LEAVES FOR THE GROWTH AND CELL BIOMASS, SINGLE CELL PROTEIN AND ALCOHOL PRODUCTION

K. G. Deshpande*, C. S. Dolas and Janjal P. H

Department of Post-Harvest and Biotechnology,
MGM College of Agricultural Biotechnology, Aurangabad, (MH) India.

ABSTRACT:

Using synthetic media for growing microorganism is very costly about, 500 gm of synthetic media cost up to Rs.2000-3000. Deproteinized juice (DPJ) produced by using vegetable waste can be used as natural media for growing microorganism and it is cheaper than synthetic media. DPJ produced by using leaves of vegetable, is free of proteins which are coagulated by heating the leaf extract juice at 90-95^{0}c. This DPJ juice can produce the growth and cell biomass, single cell protein and alcohol production.

KEYWORDS: Deproteinized juice, protein, alcohol, Microorganisms, Leaves, Vegetable.

***Corresponding Author: Dr. K. G. Deshpande** Ph. D.

Department of Post-Harvest and Biotechnology,

MGM College of Agricultural Biotechnology, Aurangabad, (MH) India.

1. INTRODUCTION

Using synthetic media for growing microorganism is very costly about, 500 gm of synthetic media cost up to Rs.2000-3000. Deproteinized juice (DPJ) produced by using vegetable waste can be used as natural media for growing microorganism and it is cheaper than synthetic media. Deproteinized juice makes full use of leaf nutrient. DPJ produced by using leaves of vegetable, is free of proteins which are coagulated by heating the leaf extract juice at 90-95^{0}c. It was reported earlier that this juice contents some nutrients such as Ca, Fe, Zn, Mg. which can support the growth of microorganism such as pseudomonas, yeast. The deproteinised leaf juice (DPJ) is rich in most of soluble plant nutrients. It was used for the cultivation of microorganism (Deshpande and Joshi, 1971). Studies undertaken on various aspects of DPJ by earlier workers in this laboratory indicated its use in microbial biotechnology particularly for growing fungi for the production of microbial products. Studies on microbial aspects indicated that the DPJ is a good medium for growing microbes including bacteria, fungi, yeast etc. for the single cell protein (SCP) and microbial metabolites. Apart from the initial investigation undertaken by Deshpande and Joshi (1971) it was pointed out by Ajaykumar and Mungikar (1990), that *Aspergillus Niger* and *Penicillium notatum* grows well on DPJ from various plants and they supported the value of DPJ as a growth medium for cultivating microorganisms. Gogle *et al.,* (2001) indicated that the DPJ can be used to produce fungal metabolites by growing proper fungal strains. Mungikar (2001) pointed out that the DPJ of lucern is comparable to the GN medium and it can be considered as a main source of nitrogen (N) to the fungi growing on it. Jadhav and Mungikar (2001) suggested the use of DPJ for microbial biomass production, while Sayyed and Mungikar (2001), advocated its use for the production of enzyme amylase. The conscious attempt to grow microbes for human diet started in Germany with Brevers yeast in 1910, and by 1914 about 10000 tons of dry yeast available for use in feeds.The nutritional requirement of various microorganisms may differ appreciably, but, all of them require C, N and P source as well as minerals and vitamins. The most important of these is a carbon source and many agricultural waste products are rich in carbon containing compounds. The utilization of such materials in SCP process serve two functions; reduction in pollution and creation of edible proteins.

2.MATERIALS AND METHODS

The design adopted for this experiment was Completely Randomized Design (CRD), total number of treatments were five, each replicated four times.

Treatment details:

T_1: Yeast extracts agar media, **T_2**: Radish leaf juice, **T_3**: Beetroot leaf juice, **T_4**: Cauliflower leaf juice, **T_5**: Cabbage leaf juice.

Methodology

Production of deproteinised juice from vegetable leaves of cauliflower, cabbage, beetroot, and radish. (Chanda and Chakrabarti 1996)

Procedure

- Collection of leaves of cauliflower, cabbage, beetroot, and radish leaves from local market.
- Leaves were washed with water and crushed in mortal and pestle to get pulp.
- Pulp was put on clean membrane cloth and squeezed and the juice was collected in container.
- 20-25 ml of water was taken in a suitable stainless steel container and it was allowed to boil and then slowly the juice was added with continous stirring.
- When the temperature reached between 90-95 ^{0}c, the contents was filterd through four fold musclin cloth.
- The filter was collected was allowed to cool,which is known as DPJ.

Morphological characteristics of *Saccharomyces cereviceae*

Active culture of *Saccharomyces cereviceae* was taken and suspension was prepared. About 20 ml of each vegetable were taken and added with agar plates. Inoculation of *S. cereviceae* by streaking on each agar plate was done kept and for incubation for 7 to 12 days. After 7 to 12 days morphological characters were noted.

Use of Natural media for growth of *Saccharomyces cereviceae*

Sterilization

The 25 ml of DPJ of each vegetable such as cabbage, cauliflower, radish and beetroot was poured in 100 ml conical flask. The flask was plugged with nonabsorbent cotton and autoclaved at 15 lbs for 30 min. The media were cooled at room temperature.

Inoculation

The autoclaved flask was transferred into inoculating chamber under the influence of UV light for surface sterilization. The fungi selected were S. cerevisiceae. The stock culture of fungi was collected from the Departmental culture collection, wherein the culture maintained on potato dextrose agar (PDA) medium. The inoculation was done under aseptic condition in UV chamber, for this purpose the inoculums in the form of spore suspension were prepared by adding 10 ml sterile distilled water to 7 days old spore culture of the fungi. Standardized DPJ was inoculated with 5 drops of spore suspension containing 5×10^2 spores per microscopic field. The inoculated flask was incubated at room temperature the growth of microbes was observed depending on nature of the experiment.

Collection of microbial biomass

After incubation of the flask and satisfactory growth of fungi, fungal biomass was harvested by filtration through whatman filter paper. The mycelial biomass retained on the filter paper was dried in oven at 65 ± 5^0C till constant weight. The yield of fungal biomass in terms of mycelia dry weight (MDW) was then recorded by subtracting weight of the filter paper. A blank or control flask was also processed simultaneously during all experiments wherein the flask remained uninoculated. The dry mycelia biomass in the form of mycelia dry weight (MDW) was corrected each time by subtracting dry weight obtained from non-inoculated flasks. After harvesting fungal biomass the optical density was measured.

Standardization of media by adding sugar and protein source

Protein source such as glucose, fructose, maltose, sucrose and carbohydrate source such as beef extract, yeast, KNO_3, meat extract etc. was added in DPJ at various concentrations such as 0.25, 0.5, 1, and 1.5% of each substrate and the media was autoclaved.

Biochemical test and morphological test

Biochemical test and morphological test of *Saccharomyces cereviceae* was tested by Cotton blue staining method.

Screening for cellulose

Cellulose mineral salt medium and cellulose agar medium was prepared

Detection of cellulose degrading organism

A set of tubes with a yeast cell. A filter paper was inserted in each tube in such a way that partof this paper was dipped in the medium the tube were incubated at 30^0cand were examined every day for cellulotyic activity.

Single cell production

The DPJ was used as a medium for yeast biomass production and the protein was estimated by foline lowery method. This method is about ten times sensitive by biurent method. The reagent called "Foline Ciocolteau" reagent is quite complex and contains phosphomolybolic assay and tungustale. The aromatic amino acids tyrosine and tryptophan present in protein react with these and produce dark blue colour. These colour formation is due to reduction phosphomolybdate by trycine and typtophane present in protein and reaction of alkaline copper with protein.

Reagents:

Std. solution of protein (bovine albumin)

Reagent **A**: alkaline sodium bicarbonate

Sodium carbonates 2% in 0.1 N sodium hydroxide

Reagent **B**: copper sulphate Sodium potassium tartarate solution

0.5% copper sulphate in 1% sodium potassium tartarate

Reagent **C**: alkaline solution

Mix 50 ml of solution A with 1 ml of solution B

Reagent **D**: Foline Ciocaleteau reagent

This is commercially available and is diluted with equal volume of water.

Procedure

Std. stock solution of protein (bovine albumin) was prepared as 20 mg/100ml. then different dilution were prepared from the stock. To each 1ml of sample, 5 ml of alkaline solution (Reagent C) was added contents were mixed thoroughly on cyclomixer and allow standing for 15 min at room temp. Then 0.5 ml of diluted Folin ciocaleteau (Reagent D) was added with shaking. The mixture was kept at room temp. For 30 min. absorbance of color compound complex formed was read at 660 nm of spectrophotometer. A graph of concentration of protein v/s O.D was plotted. From the graphs, the conc. Of protein in unknown sample was estimated.

Ethyl alcohol fermentation using *Saccharomyces cerevisiceae*

Ethyl alcohol is produced by enzymatic action on sugar or starch by fermentation. The substance rich in starch are potato, maize, rice, barley .sugar present in molasses of cane juice. Molasses is the dark viscous residue left behind on crystallization with sucrose. sugar cane juice contains sucrose for fermentation when an yeast culture of *saccharomyces cerevisiceae*. Varity ellipsoids is added, it gives a high yield of alcohol. It is also called as distilleries yeast. The enzyme invertase breaks down sucrose to glucose and fructose.

$$C_{12}H_{22}O_{11} \text{ (Invertase)- } C_6H_{12}O_6 + C_6H_{12}O_6.$$

Glucose and fructose are converted to alcohol by zymase produced by yeast Due to evolution of Co_2; effervesces are seen indicating the progress of fermentation. Alcohol is extracted by fractional distillation. Alcohol is detected by iodoform test. Estimation is done with a specific gravity method with which the percentage of alcohol is obtained by refining the AOAC table.

Industrially alcohol is employed as a solvent .it is used as a raw material for chemical synthesis and for production of gasoline. DPJ juice, paraffin wax, distillation apparatus, specific gravity bottle.

Preparation of yeast inoculums

50 ml of DPJ juice was taken in a flask the flask were autoclaved at 10/b for 20 min. After cooling, a pure culture of *Saccharomyces cereviceae* was inoculated. Flasks were incubated on shaker for 24 hrs.

A sterilized DPJ juice fermentation

1000ml of DPJ in each plant such as beetroot, radish, cabbage, cauliflower was pasteurized at 8^0c for 30 min. After cooling, 5% preprodused inoculums was added. The flask was sealed with wax and incubated for a week at a room temperature.

Distillation of fermented liquor

The liquor of each flask was distilled separately using a distillation apparatus. The temperature for distillation was about 78.5^0c i.e. the temperature for alcohol evaporation. The condense vapors was collected in a flask which was later used for detection and estimation.

Detection of alcohol by iodo form test

Reagents : Distillated alcohol, NaOH, Layal's iodine: - I_2- 1gm, KI- 2 gm, D/W- 300 m

Procedure

About 3 ml of distillate was taken in a flask pH was made alkaline with NaOH. A few drop of hyles iodine was added. The contents were heated for 5-10 min. a smear of this was made on a glass slide and the formation of iodine crystals was seen under microscope.

Estimation of alcohol by specific gravity method

Procedure

1. Weight of empty specific bottle- X gm
2. Weight of with water- Y gm
3. Weight of bottle with distillate- Z gm
4. Weight of water- Y-X gm
5. Weight of distillate- Z-X gms

FORMULA

Specific gravity of distillate = weight of distillate / weight of water

After the calculating specific gravity, the percentage of alcohol was estimated from the AOAC table.

3.RESULTS AND DISCUSSION

Effect of substrate concentration added to the DPJ of various vegetable on fungal biomass production: The DPJ prepared from different vegetable was used for growing different vegetable although the DPJ of vegetables such as (cabbage, cauliflower, beetroot and radish) where containing nutrient material attempts were made to determine where they could be complete media for growing yeast or not for this purpose these DPJ was standardized by using carbohydrate such as Glucose, fructose, sucrose and maltose in single replication. The carbohydrates were added in the DPJ at cabbage the high growth at yeast (in terms of optical density) was found in 0.50% addition of glucose. Indicating that glucose is best suitable carbohydrate for DPJ of cabbage for growing yeast .In case of cauliflower 1.5% glucose is added highest growth of yeast was found indicating that sucrose is best suitable for the growth of yeast of DPJ of cauliflower In case of radish 1.00% glucose was found to be suitable for maximum growth of yeast on DPJ radish. Indicating that fructose is suitable carbohydrate. In case of beetroot 1.00% glucose was best suitable for growing yeast.Yeast is one of the important micro-organisms having its own value in demos tic as well as industrial purpose, especially in the preparation of food products. During present research project, efforts were made to measure the productivity of yeast biomass when the byproduct DPJ

was employed as a growth medium. In order to enhance the growth of yeast, the DPJ was reconstituted by adding carbon sources in the form of glucose, maltose, fructose, sucrose. The main objective was to evaluate the possibility of using DPJ for the production of single cell protein in the form of yeast. The growth of yeast cell on standard medium was greater than to the fresh DPJ.

Fig 1. Vegetable leaves.

Fig 2. Slurry of leaves

Fig. 3. Deproteinised juice (after filtration)

Detection of cellulose degrading organism

The growth of cellulose decomposing bacteria was detected by the breakdown of the filter paper at the air –liquid interphase. Some cellulose decomposing fungi produced black pigment thus break down of filter paper may be accomplished by black colouration of paper.

Optical Density to express Biomass of Yeast Cell

The Table 1 gives information about the optical density to express biomass of yeast cell on conventional YEPDA as well as standard DPJ from fresh vegetable leaves of cabbage, cauliflower, radish, and beetroot**.** The statistical analysis shown those T1 and T4 values were significant where as T_2, T_3 T_5 not significant when compared to C.D. T_4 was found significant over rest of all. Indicating that yeast grown well on DPJ of cauliflower giving good biomass (optical density was 1.22 nm.) T_1 was also found significant thus in synthetic media of YEPDA biomass was found to be greater with optical density 1.29 nm. Which is lesser than T_4 Biomass grown on cauliflower DPJ was greater than Biomass grown on synthetic media i.e. YEPDA

T/R	TOTAL	MEAN
YEPDB (T_1)	5.14	1.29
RADISH (T_2)	4.52	1.13
BEETROOT (T_3)	4.44	1.11
CAULIFLOWER (T_4)	4.89	1.22
CABBAGE (T_5)	4.89	1.22

S.E= ± 0.01004 C.D= ± 0.04173 'T' value= 2.94

Table:1 Optical density of yeast cell biomass.

FOLIN LOWERY METHOD

Standard protein solution: - 20 mg /ml

Stock solution: - 200 micro gm /ml

After adding reagent C the contents were mix thoroughly on cyclomixer and allowed to stand for

Sr. no	Conc. in micro.gm/mi	Amount of stock solution	Amount of D/W ml	Reagent C(ml)	Reagent D(ml)	O.D 670 nm
1	20	0.1	0.9	5	0.5	1.11
2	40	0.2	0.8	5	0.5	1.15
3	60	0.3	0.7	5	0.5	1.15
4	80	0.4	0.6	5	0.5	1.16
5	100	0.5	0.5	5	0.5	1.16
6	120	0.6	0.4	5	0.5	1.18
7	140	0.7	0.3	5	0.5	1.20
8	160	0.8	0.2	5	0.5	1.20
9	180	0.9	0.1	5	0.5	1.24
10	200	1.0	00	5	0.5	1.24
	Un known	-	-	-	-	0.87

15 min at room temp. After adding reagent D the mixture was kept at room temperature for 30 min.

Optical Density of growth of yeast cells grown on DPJ by adding various conc. of protein at 670 nm Standard Protein

The following table gives information on optical density of growth of yeast cell grown at DPJ by adding various concentration of carbon source at 670nm standard protein .result obtained were as follows .the statistical analysis show T4 value were significant where asT_1, T_2,T_3 T_5 not significant when compared to C.D . T_4 was found significant over rest of all. Indicating that yeast grown well on DPJ of cauliflower giving good protein optical density that is 1.96 nm. T_1 was also found significant. By using Folin lowery estimation the unknown concentration of protein produced was found to be significant in the cauliflower DPJ T_4 than rest of all media. It was indicated T_4 by the O.D that is 1.96 at 670 nm. The T_3 was also found significant over T_1 T_2.

Table No 3. Standard Protein Mean values by Folin lowery method.

T/R	TOTAL	MEAN
YEPDB (T_1)	7.47	1.87
RADISH (T_2)	7.40	1.85
BEETROOT (T_3)	7.83	1.96
CAULIFLOWER (T_4)	7.85	1.96
CABBAGE(T_5)	**7.57**	1.89

S.E= ± 0.017 C.D= ± 0.05 'T' value= 2.131

Percentage of Alcohol produced by the fermentation of DPJ of different vegetables by *Saccharomyces cereviceae* by specific gravity method.

Table 4 gives the percentage of alcohol produced by *sacchromyceae cerevisiceae* grown on different DPJ method. Detection of alcohol by Iodoform test and the obtained result were Crystals yellow, hexagonal Crystals were insoluble in ethanol and ether melting point was 119^0c. After the calculating specific gravity, the percentage of alcohol was estimated from the AOAC table. The statistical analysis shows that treatment value not significant when compared to C.D. T_1 was found significant over rest of all. Indicating that the alcohol percentage was well on the control medium giving good alcohol percentage 9.49 mg/ml.T_3 was also significant thus in synthetic media of (YEPDA).by alcohol percentage found to be 7.37 mg/ml. which was lesser than the T_2. That means in DPJ of cabbage produced was having higher alcohol percentage than alcohol percentage of rest of treatment.

Table No 4: Percentage of Alcohol production

T/R	TOTAL	MEAN
YEPDB	**37.96**	**9.49**
RADISH	**30.02**	**7.51**
BEETROOT	**38.66**	**9.67**
CAULIFLOWER	**22.79**	**5.70**
CABBAGE	**29.48**	**7.37**

S.E=0.66456 C.D=2.76269 'T'value-2.941

Fig 4. Yeast grown on DPJ

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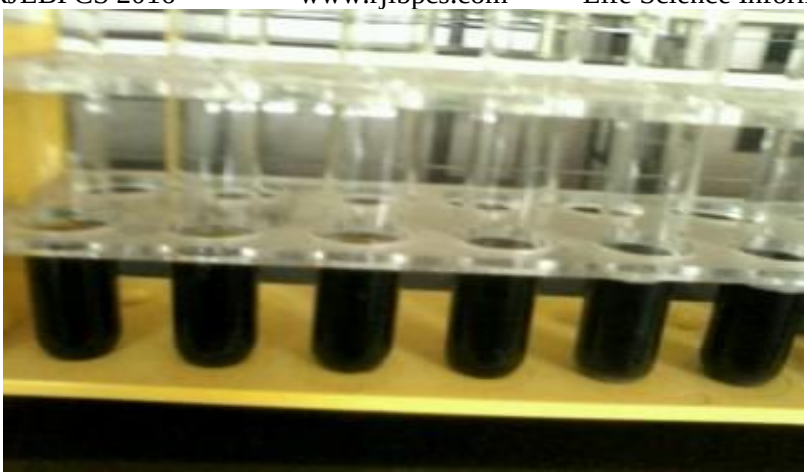

Fig 5. Folin lowery protein estimation method

Fig 6. Alcohol distillate

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