# PLANT ORIGIN LIQUID WASTE: A RESOURCE FOR SINGLE-CELL PROTEIN PRODUCTION BY YEAST

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**Abstract**

*Leaf protein was separated by heat coagulation (80°C) from leaf juices of four cruciferous plants: turnip (*Brassica campestris *L.), mustard (*Brassica nigra *Koch.), radish (*Raphanus sativus *L.) and cauliflower (*Brassica oleracea *L. var.* botrytis*). Three yeasts,* Saccharomyces cerevisiae, Torula utilis *and* Candida lipolytica, *were grown in deproteinized leaf juices (DLJ) of these plants. The yeast cells produced in these wheys were found to be rich in protein and vitamins. The chemical oxygen demand (COD) and biological oxygen demand (BOD) values of DLJ samples were reduced significantly by the growth of yeasts. Copyrigh*

*Key words:* Yeast, deproteinized leaf juice (DLJ), biomass, single-cell protein (SCP).

## INTRODUCTION

Increasing concern about pollution that occurs from agricultural and industrial wastes has stimulated interest in converting waste materials into commercially valuable products, especially single-cell protein (SCP) (Leman *et al.*, 1990). During production of vegetable protein from leaf juice, a liquid waste, deproteinized leaf juice (DLJ), is generated. This byproduct, composed of sugars, amino acids, lipids, minerals and vitamins, presents a serious treatment problem because of its high chemical and biochemical oxygen demands (COD and BOD) and low pH (Chanda *et al.*, 1984; Pirie, 1987). Microbiological transformation of these nutrients into useful biomass would be beneficial and the simultaneous reduction of COD and BOD levels of the liquid waste should be encouraged, to control pollution, as highlighted by a number of researchers (Quinn *et al.*, 1981; Mukherjee & Majumder, 1989; Jwanny *et al.*, 1990).

The present study was aimed at development of a yeast process for bioconversion of the vegetable byproducts and to reduce polluting water.

## METHODS

### Production of deproteinized leaf juice (DLJ)

The leaves of four cruciferous plants were selected as the starting material. Fresh leaves were collected from turnip (*Brassica campestris* L.), mustard (*Brassica nigra* Koch.), radish (*Raphanus sativus* L.) and cauliflower (*Brassica oleracea* L. var. *botrytis*). Leaf juice was extracted with a IBP Press and Pulper and protein was extracted from the leaf juice by heat coagulation at 80°C (Pirie, 1987).

### Analysis of DLJ and yeast biomass

Both DLJ and yeast biomass samples were analysed. Percent dry weight and ash content were determined. Total nitrogen was determined by a microkjeldahl method (Byers, 1967). Total carbohydrate was estimated according to the method of Whistler *et al.* (1962). Total lipid contents were determined following the method of Hudson and Karis (1973). Mineral contents were analysed following the methods described by Ward and Johnston (1962). Vitamin contents were determined following microbiological methods (Pearson, 1967).

### Strains and maintenance

Three non-pathogenic and non-toxic yeast strains were supplied by the National Collections of Industrial Microorganisms (NCIM), National Chemical Laboratory, Poona 411008, India: *Saccharomyces cerevisiae* NCIM 3095, *Torula utilis* NCIM 3055, *Candida lipolytica* NCIM 3229.

These strains were grown on Sabouraud's agar slants [sucrose 20, peptone 10, agar 20 (g/l); pH 7.2] at 28°C for 24 h and stored in a refrigerator. Then strains were transferred every 15 days and the cell dimensions were examined microscopically.

**Fermentation**

Cell suspensions were prepared from 24-h-old cultures in sterile, normal saline and were added aseptically to the 100 ml flasks containing DLJ (1 ml/25 ml of the substrate). Inoculum size was about $2 \times 10^4$ cells/ml (viable count); pH of the medium was 6.0 before autoclaving. Flasks were shaken in a rotary shaker (265 revolutions/minute with a throw of 1.89 cm) at 28°C. The incubation time required for maximum amount of biomass was determined by trial and found to be 96 h for all the strains tested.

**Measurement of growth**

Growth of the yeasts was measured by dry cell weight (DCW) of the harvested cells. DCW was obtained following centrifugation at 3000 rpm for 30 min (International Portable Refrigerated Centrifuge model PR-2 Serial No. A 2723 X-1, International Equipment Company, Boston, MA, USA) and drying the cell mass at 80°C overnight.

**Determination of COD and BOD**

COD was measured by diluting the sample (1:1000) with redistilled water (Ballinger, 1979). For BOD measurement the samples were diluted to 1:1, 1:10, 1:100 and 1:1000 ratio as required (Taras *et al.*, 1971).

## RESULTS AND DISCUSSION

Table 1 shows the compositions of DLJ samples, which contained only soluble nitrogen. The true protein content was negligible. Paper chromatographic studies showed that DLJ contained soluble carbohydrates and other constituents which could be utilized by fungi.

Table 2 shows the biomass produced by three yeast strains in four different DLJ samples. Growth of *Saccharomyces cerevisiae* was best in radish DLJ, while for *Torula utilis* and *Candida lipolytica*, turnip DLJ was slightly better. Biomass was poorest in cauliflower DLJ with all yeast strains. This might have been due to the fact that cauliflower DLJ contained only 0.24% of sugar, 0.12% total nitrogen and 0.05% of lipid, contents which were much lower than those of other DLJ samples (Table 1). *Saccharomyces cerevisiae* in turnip, mustard and radish DLJ and *Torula utilis* in mustard and radish DLJ samples showed yields above 0.8 g/100 ml, which can be compared with that obtained by Paradez-Lopez and Camagro (1973), who reported

**Table 1. Chemical composition of four DLJ samples[a]**

| Chemical composition (g/100 ml) | ±S.D. | *Brassica nigra* | *Brassica campestris* | *Raphanus sativus* | *Brassica oleracea* var. *botrytis* |
|---|---|---|---|---|---|
| Ash | 0.01 | 1.70 | 1.65 | 1.00 | 1.00 |
| Dry wt. | 0.05 | 3.70 | 3.60 | 2.30 | 2.00 |
| Total carbohydrates | 0.05 | 0.90 | 0.80 | 0.80 | 0.24 |
| Total N | 0.05 | 0.24 | 0.25 | 0.22 | 0.12 |
| Total lipid | 0.05 | 0.32 | 0.40 | 0.10 | 0.05 |
| Minerals (mg/100 ml) | | | | | |
| Inorganic P | 0.5 | 6.3 | 2.0 | 2.75 | 6.5 |
| Ca | 5.0 | 210.0 | 160.0 | 170.0 | 160.0 |
| Na | 3.0 | 95.6 | 315.0 | 285.6 | 76.5 |
| K | 5.0 | 163.7 | 83.5 | 90.0 | 269.5 |
| Fe | 0.05 | 0.16 | 0.08 | 0.062 | 0.051 |
| Vitamin (mg/100 ml) | | | | | |
| Riboflavin | 5 | 15 | 50 | 30 | 50 |
| Niacin | 50 | 130 | 307 | 300 | 327 |
| Pantothenic acid | 20 | 125 | 175 | 62 | 400 |

[a]Each value is the mean of five samples.

**Table 2. Biomass produced by three yeast strains in four different DLJ samples[a] (g/100 ml)**

| Test organism | *Brassica campestris* (Turnip) | *Brassica nigra* (Mustard) | *Raphanus sativus* (Radish) | *Brassica oleracea* var. *botrytis* |
|---|---|---|---|---|
| *Saccharomyces cerevisiae* | 0.94 | 0.89 | 1.00 | 0.61 |
| *Torula utilis* | 1.25 | 0.96 | 1.10 | 0.50 |
| *Candida lipolytica* | 0.75 | 0.70 | 0.60 | 0.15 |

[a]Each value is the mean of five tests. S.D. ±0.05.

production of 0.8 g biomass/100 ml of alfalfa byproduct with *Candida* sp. Production of biomass by *S. cerevisiae* in a number of DLJ samples was reported in a note by the present authors (Chanda *et al.*, 1980).

Table 3 shows percentage reductions of COD and BOD, as well as efficient utilization of C and N sources.

The compositions of yeast biomass obtained from four fermented DLJ samples are compared in Table 4 with that of food yeast available in the market (Gopalan *et al.*, 1976). In comparison with the SCP obtained from the DLJ samples and commercial samples, the former show higher values of proteins and vitamins but carbohydrate contents are much lower than that of the recorded value of marketed yeasts.

The leaf juices were all from edible plants. The total nucleic acid content of yeast cells is usually in the range of 8–10%, which is non-toxic for feed use (Sista & Srivastava, 1981). Davis (1973) also pointed out that in terms of animal nutrition, the nucleic acid content of SCP from yeast is totally irrelevant. Based on these references it was supposed that the present SCP produced was non-toxic.

In developing countries like India, where per capita availability of conventional sources of protein has come down and an animal feed-compounding industry is not well established, the development of single-cell protein (SCP) could be suggested as a complementary route to augment food and fodder production (Kahlon *et al.*, 1990). In India, dried yeast is used as feed for all classes of farm animals (Banerjee, 1988).

The composition of DLJ suggests that it may serve as a good nutrient medium for microbes, but at the same time its direct disposal to the agricultural fields may cause phytotoxicity to crops (Pirie, 1987). Fermentation of this waste, before disposal, may solve the disposal problem (Leman *et al.*, 1990). Besides the production of SCP (Chen & Peppler, 1977) and reduction of pollution parameters, fermentation may produce some other byproducts which may have some other industrial importance.

**Table 3. Utilization of sugar and nitrogen and reduction of COD and BOD of DLJ samples by yeasts[a]**

| DLJ samples | Test organisms[b] | Utilization of sugar (percent) | Utilization of nitrogen (percent) | COD of DLJ (mg/l) | Reduction (percent) | BOD of DLJ (mg/l) | Reduction (percent) |
|---|---|---|---|---|---|---|---|
| Turnip | a | 98 | 57 | | 52 | | 80 |
| | b | 95 | 59 | 28500 | 56 | 19000 | 82 |
| | c | 96 | 60 | | 47 | | 75 |
| Radish | a | 99 | 61 | | 51 | | 78 |
| | b | 98 | 59 | 25240 | 54 | 16800 | 79 |
| | c | 97 | 57 | | 47 | | 79 |
| Mustard | a | 99 | 62 | | 60 | | 97 |
| | b | 98 | 60 | 30100 | 68 | 20050 | 98 |
| | c | 99 | 58 | | 52 | | 95 |
| Cauliflower | a | 97 | 60 | | 50 | | 78 |
| | b | 98 | 59 | 20225 | 52 | 12900 | 78 |
| | c | 99 | 58 | | 46 | | 74 |

[a]Each value is the mean of 5 cultures.
[b]a = *S. cerevisiae*, b = *T. utilis*, c = *C. lipolytica*.

**Table 4. Composition of yeast biomass harvested from DLJ samples after 96 h growth**

| Composition[a] (g/100 g of dried samples) | Types of yeast | | | Standard values[b] of food yeast |
|---|---|---|---|---|
| | *S. cerevisiae* | *T. utilis* | *C. lipolytica* | |
| Moisture | 10.6 | 6.2 | 14.6 | 7.8 |
| Crude protein (Total N × 6.25) | 45.6 | 54.3 | 50.5 | 35.7 |
| Lipids | 8.0 | 8.1 | 7.0 | 1.8 |
| Ash | 10.3 | 7.6 | 5.5 | 8.4 |
| Carbohydrates | 23.2 | 21.7 | 20.6 | 46.3 |
| Vitamins (mg/100 g of dried samples) | | | | |
| Riboflavin | 60.0 | 70.0 | 65.0 | — |
| Thiamine | 2.0 | 3.0 | 2.0 | 3.2 |
| Niacin | 32.5 | 35.0 | 32.0 | 27.0 |
| Pantothenic acid | 120.0 | 110.0 | 135.0 | — |
| Pyridoxin | 30.0 | 32.0 | 35.0 | — |
| Folic acid | 10.0 | 15.0 | 11.0 | — |

[a]All values are means of five samples from five different cultures of each yeast.
[b]From Gopalan *et al.* (1976).

So fermentation may be economically attractive compared with other methods of disposal, as suggested by Moon and Hammond (1978).

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