



Fungal biomass production using Deproteinised Leaf Juice (DPJ) of *Trigonella foenum-graecum*

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Abstract: Isolation of leaf protein from the green foliage which releases Deproteinised Leaf Juice (DPJ). DPJ contains sugars, amino acids, minerals etc. Studies were conducted to investigate the possibilities to grow fungi on the deproteinised juice prepared from *Trigonella foenum-graecum* L. for the production of fungal biomass. Fresh green foliage of spinach was harvested early in the morning at a preflowering stage. It was pulped using pulper and subsequently pressed and the juice released due to the pressing was collected. The juice released during fractionation was employed for the preparation of LPC by head coagulation and the DPJ released after precipitation and isolation of proteins in juice was collected. The samples of DPJ were dried in hot air oven at 65^o C. The conventional GN medium was prepared. Simultaneously, the dry DPJ was dissolved in distilled water at various concentrations and used as a medium for growing fungi. The flasks were inoculated for 8 to 12 days after inoculation. The fungal biomass was harvested by filtration through Whatman filter paper. The mycelial biomass was dried along with the filter paper in an oven at 65±5^oC till constant weight. The yield of mycelial dry weight (MDW) was then recorded. During all experiments, the culture filtrate was collected after harvesting the biomass. During present investigation that for maximum production of mycelial biomass of fungi on deproteinized juice of *Trigonella foenum-graecum* L. in comparison with GN media.

Key Words: Fungal biomass; fungal medium; DPJ; etc.

INTRODUCTION

The plant parts, mostly the grains, in addition to roots, tubers and fruits are used by heterotrophic organisms as a sole source of food for survival. It has been observed that only a part of the food synthesized in leaf is transported to the edible portion and harvested for use in human nutrition. Thus the efficiency of using food synthesized in the leaves decreases when either grain or other plant parts are used. The efficiency of using plant food further decreases when food products of animal origin are considered. Only a part of food consumed by animal through leaves or foliage is available in the form of meat, milk, egg etc. It is therefore necessary to search the methods of harvesting food from plant with increased efficiency. Indian agriculture has made spectacular advancement in agriculture resulting in self-sufficiency in food grain production. However, a part of the population, living below poverty line, is still undernourished and/or malnourished. This malnutrition among these people is mainly due to deficiency of protein in their food. Protein deficiency and malnutrition among them is responsible for problems related to health, which may result in a serious social inequality. In order to overcome protein deficiency and malnutrition it is necessary to either increase the productivity of existing sources of protein e.g. pulses, milk, meat, eggs etc., or to investigate novel and/or non-conventional sources of protein. Attempts are being made to enhance the productivity of pulses through integrated fertilizer management and introduction of genetically modified varieties. However, sufficient amount of protein is not available to feed the increasing population. To overcome this situation, Prof. Pirie in 1942 proposed a system called as "Green Crop Fraction (GCF)" which has been advocated for extracting protein from green leaves for diversion of consumption by monogastrics.

In this process fresh, green leafy plant material is macerated to rupture the cells and pressed. These mechanical treatments (maceration and pressing) fractionate

green crop into two fractions; protein rich plant juice for monogastrics (non-ruminants) and fibrous crop for the ruminant animals. The leaf extract or juice contains protein, sugar, salts, lipids and vitamins along with the moisture in the plant. When the juice is heated to over 80^o C or acidified to pH 4, green protein rich curd referred as Leaf Protein Concentrate (LPC) is produced. The LPC can be separated from deproteinized juice (DPJ) brown whey like liquid by filtration through cotton cloth. In this way green foliage can be fractionated mechanically into three fractions: first-fibrous pressed crop, second-leaf protein concentrate and third-deproteinized juice (Wilkins *et al.*, 1977; Pirie 1978, Jadhav and Mungikar, 1998, 2001. Jadhav, 2009a, 2009b, 2014 and 2015). A comparative study of MSA tools based on sequence alignment fetchers and platform independency to select the appropriate tool desired (Sayyed and Sarkhawas, 2011). A comparative study of protein structure visualization tools for various display capabilities (Ansari & Ilyas, 2011). A comparative study of different properties provided by protein structure visualization tools (Ilyas and Ansari, 2013). LPC: a good source of cyanocobalamin (B12), ascorbic acid (Vitamin C) and folic acid (Vitamin B9) (Ilyas and Badar, 2010a). Estimation of thiamine, riboflavin and pyridoxine from LPC of some plants (Ilyas and Badar 2010b). Production of amylase of DPJ of four different plants (Sayyed, 2013). Study of LPC and PCR prepared from radish (*Raphanus sativus* Linn.) (Sayyed, 2011). Effect of additives on chlorophyll content in wet LPC prepared from juice of *Medicago sativa* Linn. (Sayyed, 2010). Changes in chlorophyll content of Lucerne leaf juice during storage. (Sayyed and Mungikar, 2003). Deproteinised Leaf juice as a medium for fungal growth and for production of protease (Josephin and Sayyed, 2005). Use of Deproteinised Leaf Juice (DPJ) in microbial biotechnology (Sayyed and Mungikar, 2005). The impact of mathematical tools on cellular and molecular Biology and the importance of leaf protein to solve protein deficiency (Shaikh and Sayyed,

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Trigonella foenum-graecum L. belong to family Fabaceae, commonly known as 'Methi'. This plant grown throughout the Country and generally known as 'Fenugreek'. An evergreen tree with thin branches, elliptical leaves ovoid in color. The plant grows up to a height of one fit and has a deep root system. It has a high protein content of around 18-22% and is a good source of vitamins and minerals. Dry matter yields are likely to be 14t/ha. It will require adequate phosphate and potash levels to achieve this and a pH of 6 is considered the minimum.

During, Green Crop Fractionation fresh (GCF), green foliage is mechanically fractionated to isolate protein in leaf from indigestible fibrous or cell wall material. The foliage is initially macerated, and subsequently pressed. The leaf juice was heated to about 95^o c for 15 min. the Leaf protein concentrate (LPC) was removed by filtration through cotton plug. The brown coloured liquid (DPJ) was collected, dried and preserved (Wilkins. *et al.*, 1977; Pirie 1978).

The leaf juice related due to the pressing is then employed for the preparation of a product called leaf protein concentration (LPC), which is considered as protein – mineral – vitamin rich concentrate suitable for human nutrition. The pressed crop residue (PCR) left after extraction of juice is suitable in animal nutrition, while the Deproteinised Leaf juice (DPJ) remaining after isolating LPC from leaf juice is a byproduct of GCF more efficient (Beula Josephin and Mungikar, 2003).

The pressed crop residue (PCR), also known as fibrous residue, left after the extraction of juice still contained from 9 to 16% crude protein in its dry matter (DM), depending on the species used for fractionation. This can be successfully used as a food for cattle (Raymond and Harris, 1957; Bryant *et al.*, 1983). The raw unfractionated juice can be fed to pigs or used as milk replace to pre-ruminant calves (Prasad *et al.*, 1977a, 1977b; Mungikar *et al.*, 1978). Leaf protein concentrate (LPC) contain from 40 to 70 % protein on DM Basis, along with appreciable quantities of β -carotene (pro-vitamin A), vitamin E and minerals. The LPC can be used as a protein-vitamin-mineral supplement in poultry, calf (Joshi *et al.*, 1983, 1984) or ever human nutrition (Pirie, 1978; shah, 1983). The nutritional value of LPC extracted from green crop foliage is comparable to that of protein isolated of animal origin and superior or seed protein (Morris, 1977). Fibrous pressed crop (PCR) residue, left after the extraction of leaf protein is suitable feed for cattle (Connel and Houseman, 1977). Economic advantages could be gained in agriculture with commercial production of leaf protein (Wilkins *et al.*, 1977).

The efficiency of agriculture land utilization increases with GCF applied to the conventional farming (Jones and Houseman, 1975). Deproteinised juice generally contains 50 to 70% of the original extracted juice DM (Dry Matter), and evaporation (concentration) to around 50% DM basis provides the molasses-type product suitable for incorporation with fiber extract and resultant ensiled product or dehydrated and cubed product. The low DM concentrations of DPJ arising from fractionation suggest that concentration to remove excess water would be of benefit for the economic utilization of this by-product.

The biochemical composition of extracted DPJ is subject to variation due to crop forage species as raw material, seasonal sampling (harvest timing), general agronomic factors in forages species growth and composition. The mechanical and heat fractionation/extraction process employed. In addition to providing an additional nitrogen input to fiber extracts to improve extracted fiber silage/ baleage ruminant feed quality, concentrated DPJ also contains appreciable levels of water-soluble carbohydrate and minerals of benefit to animals' nutrition

The plants surrounding us are green because of the presence of chlorophyll pigments in the chloroplast. These important cell organelles chloroplast is located in all green tissues and organ, especially the leaves. It plays an important role in geobiological cycle, wherein it absorbs solar energy and utilization it for the manufacturing of food for all, utilizing water and carbon dioxide from soil and air respectively. The metabolic process, wherein the food is prepared in the form of simple carbohydrate is called photosynthesis the most important phenomenon in plant the simple carbohydrate (e.g. Phosphoglyceraldehyde-a3C compound and subsequently glucose a 3+3=6C compound)is then converted to other complex subsequently like sucrose, maltose, starch, cellulose, nucleic acid, and other nitrogen containing molecules including secondary metabolites like alkaloids, phenols, sterols etc., fatty acid, fats, oils, lipids, waxes, cutin, suberin and other derived fats, vitamins like Thiamine (B1), Riboflavin(B2), Pyridoxamine (B6), Cyanocobalamine (B12), β - carotene (pro- vitamin – A), Ascorbic acid (C) tocopherol (E); growth regulating substance e.g. auxins, gibberellins, cytokinen, ethylene, abscisic acids etc. other metabolites through several metabolic process are continuously in process in living and active plant. Fresh green foliage of fenugreek was fractionated using IBP equipments. The leaf juice released was collected and employed for the preparation of LPC by heat coagulation, it was observed that the heat coagulation method was most suitable for commercial production of LPC as it is simple, less expensive and gives a product of desirable value for commercial scale production of LPC in small scale industry.

Suitability of DPJ as a silage additive and in animal nutrition has been advocated. In addition, it has been reported that if this by- product is recycled back to the soil; it acts as manure for crop plants like Lucerne, Sorghum, maize, bajara etc. however if it is made available to the

plants in excess, it may be phytotoxic leading to lower yield and damage to the crop plant.

DPJ is used along with PCR in animal nutrition (Joshi *et al.*, 1983), for irrigation as a fertilizer source (Ream *et al.*, 1977, 1983; Dakore and Mungikar, 1986; Ajaykumar and Mungikar, 1990, Jadhav and Mungikar, 1998) or for growing useful microorganism (Deshpande and Joshi, 1969; Pirie, 1971, 1978; Ajaykumar and Mungikar, 1990a 1990b; Baviskar *et al.*, 1999).

The DPJ is rich in soluble nutrition of the cell and contain enough carbohydrates and nitrogen containing compounds, along with vitamins and minerals to support the growth of microorganism. Earlier studies in this laboratory have pointed out its suitability as a medium for growing bacteria, yeast and filamentous fungi. It was pointed out that the DPJ could be used in microbial technology for the production of single cell protein, alcohol, nitrogen fixing organism, enzymes, toxins, organic acids, antibiotics etc. by cultivation suitable (Beula Josephin and Mungikar, 2003).

Importance

Trigonella foenum-graecum L. is one of the most common vegetables grown throughout the Country. Fenugreek is a leguminous annual plant that grows to around 60cm tall. The leaves are similar to clover in shape. Flowers are pea-shaped and yellow or white and appear in the leaf axils. Like other legumes, the seeds are held in pods. Fenugreek pods are thin and crescent-shaped. The light brown seed harvested from the dried pods has a strong curry flavor. The seeds can be lightly roasted and ground and used as flavoring, especially in curry dishes. Fresh seed can be sprouted to give tasty sprouts.

The young leaves are often used in Asian dishes and can be included in salad mixes. Traditionally, fenugreek tea made from the crushed seed was used medicinally for a range of ailments. The plant was fed to animals as a both a tonic and a valuable food source; however, it is now thought to have deleterious effects on animals if eaten in excess. Fenugreek contains steroidal saponins, alkaloids (Inc. trigonelline and gentianine), mucilage, protein, vitamins A, B, C, minerals.

Cultivation

Good soil of medium texture is required. Tolerated pH range is 5.3 to 8.2. Prepare soil by adding plenty of composted organic material. Add a ration of lime if the soil is acid. A sunny, well-drained position and adequate water is required. Seed is sown *in situ* in spring.

It can be grown in plains throughout the year. Mostly Rabbi season good for the cultivation. Rainy Season - June – July. Winter Season –September –October. Fenugreek is the leguminous plant and the improved varieties are Kasoori selection production get started in 2 months, Pusa Early bunching it developed earlier, for seed production after 125 days. These both developed by ICAER, Pusa, New Delhi. In Maharashtra Fenugreek no.47 most cultivated variety.

Methods of cultivation

Broadcasting direct seed of Fenugreek under good conditions is recommended in medium black soils well drained. Thin broadcasting is more applicable for it. Fenugreek requires well-drained soil. It cultivated in flat bed this should be in size 3 X 2 m. elongated flatbed also prepared for it. Fenugreek cultivated by broadcasting in flat bed at 20-25 cm distance in line. Needs full sunlight, and requires watering during dry periods. While fenugreek is easy to grow, most available cultivars need a growing season of 4 to 5 months, although some cultivars mature seeds just 3 months after sowing. It's a short-day plant, with flowering only beginning as the days shorten in late summer. One has to irrigate Fenugreek during rabbi and summer season. During all season at 4-6 days interval are required

System and methods of irrigation

The irrigation system must ensure uniform distribution and no wastage of water. Fenugreek can be irrigated by surface-irrigation system.

Surface-irrigation system: water is directly applied to the surface of the soil and is spread by gravity. There are several methods viz. flooding from ditch, check basin, ring and basin, border strip and furrow. It is the process of putting the earth or soil just near the base for certain crops to give support to the plants.

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. *A. niger* is cultured for the industrial production of many substances. Various strains of *A. niger* are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organisation. Many useful enzymes are production using industrial fermentation of *A. niger*. For example, *A.niger* glucoamylase is used in the production of high fructose corn syrup, and pectinases are used in cider and pectinases are used in cider and wine clarification. Alpha-galactosidase, an enzyme that breaks down certain complex sugars, is a component of Beano and other products that decrease flatulence.

Penicillium is a genus of ascomycetes fungi of major important in the natural environment as well as food and drug production. Some members of the genus production penicillin, a molecule that is used an antibiotic, which kills or stops the growth of certain kinds of bacteria inside the body. Several species of the genus *Penicillium* play a central role in the production of cheese and of various meat products. To be specific, *Penicillium* molds are found in blue cheese *Penicillium camemberti* and *P. roqueforti* are the molds on Camembert, Brie, Roquefort, and many other cheeses.

In additional to their importance in the food industry, species of *Penicillium* and *Aspergillus* serve in the production of a number of biotechnologically produced enzymes and other macromolecules, such as gluconic, citric, and tartaric acids, as well as several pectinases, lipase, amylases, celluloses, and potential for use in bioremediation because of their ability to break down a variety of xenobiotic compounds.

MATERIALS AND METHODS

Fresh green foliage of fenugreek was harvested early in the morning at a preflowering stage. It was pulped using pulper (Daavys and Pirie 1969) and subsequently pressed and the juice released due to the pressing was collected. The first experiment was undertaken during January 2015 where in the juice was distributed in 10 conical flasks.

The sample of 100 ml juice was employed for the preparation of LPC by heat coagulation method (Pirie, 1971). The LPC was filtered through Whatman filter paper, washed with hot water for minimum 3 times, dried in oven at $65 \pm 5^\circ \text{C}$ till constant weight and the weight of LPC was recorded as the yield of LPC/100 ml juice.

The juice released during fractionation was employed for the preparation of LPC by heat coagulation and the DPJ released after precipitation and isolation of proteins in juice was collected. The samples of DPJ were dried in hot air oven at 65°C . The dry DPJ was stored in sealed glass jar until used. Sufficient care was taken to minimize absorption of moisture by the DPJ sample

Preparation of culture media: The conventional GN medium was prepared by dissolving glucose 10 g, KNO_3 2.5g, KH_2PO_4 1g and MgSO_4 0.5g in one liter of distilled water. Simultaneously, the dry DPJ was dissolved in distilled water at various concentrations and used as a medium: for growing fungi.

Sterilization: 25 ml of either GN medium or the aqueous solution of DPJ was poured into 250 ml conical flask. The flask were then plugged with nonabsorbent cotton and autoclaved at 15 lbs for 30 min

Inoculation: The autoclaved flasks were transferred to the inoculation room for inoculation with fungi. The stock cultures of the fungi used during present study were collected from the Departmental culture collection where in the fungi were maintained on Potato Dextrose Agar (PDA) medium. The inoculation was always done in UV chamber under aseptic suspension was prepared by adding 10 ml sterile distilled water to six-day old slope culture of the fungi. The medium, either GN or DPJ, was inoculated with 5 drop of the spore per microscopic field. The inoculated flasks were incubated at room temperature.

Collection of microbial biomass: The flasks were inoculated for 8 to 12 days after inoculation. The fungal biomass was harvested by filtration through Whatman filter paper. The mycelial biomass was dried along with the filter paper in an oven at $65 \pm 5^\circ \text{C}$ till constant weight. The yield of mycelial dry weight (MDW) was then recorded by subtracting the weight of filter paper from the weight recorded for dry mycelium.

A blank or control flask was also processed simultaneously, during all experiments wherein flasks containing either GN or DPJ medium remained

uninoculated. The MDW was corrected each time by subtracting the dry weight obtained from uninoculated flasks. During all experiments, the culture filtrate was collected after harvesting the biomass.

RESULTS AND DISCUSSION

Deproteinised leaf juice (DPJ), left behind isolating leaf protein concentration (LPC) from the heated juice is considered as a by-product of "Green Crop Fractionation" (GCF) system. As this product is rich in soluble nutrients from the plant its random disposal will not only cause environmental bio pollution but also make the process of GCF inefficient or less economic. In view of its high cost stressed its proper use to make this process more valuable.

Earlier investigations from this laboratory indicated suitability of DPJ as a medium for growing useful fungi (Sayed and Mungikar, 2005). Indicated suitability of lucerne DPJ for cultivating *Aspergillus niger* and production of α -amylase.

During present course of investigation, the efficiency of deproteinised leaf juice as a medium for microbial growth. It has been observed that from 2000 gm of fresh *Trigonella foenum-graecum*, 550 ml juice was released. The processing of leaf juice results into 30gm of wet LPC, 17gm of after drying of leaf protein concentrate. From the juice after removal of leaf protein concentration 475 ml of deproteinized leaf juice (DPJ) was recorded. The results are presented in Fig.1

Aspergillus niger was cultivated on 30 ml of DPJ and 50 ml of DPJ. The growth of *Aspergillus niger* in terms of mycellial dry weight was recorded 40 mg on GN media and 60 mg on DPJ. Where in the total volume of DPJ 50 ml the growth on *Aspergillus niger* 110 mg on GN medium and 130 mg while *Aspergillus niger* cultivated on deproteinised leaf juice the results are presented in fig. 2.

It has been also observed that *Penicillium notatum* was cultivated on 30 ml of GN media, the mycellial dry weight was 60 mg whereas on DPJ 75 mg. when *penicillium notatum* was cultivated on 50 ml of GN media the mycellial dry weight was 100 mg where in case of deproteinized leaf juice 120 mg. the results are represented in Fig.3.

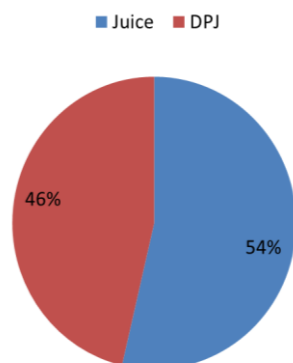


Figure 1: Fractionation of *Trigonella foenum graecum*

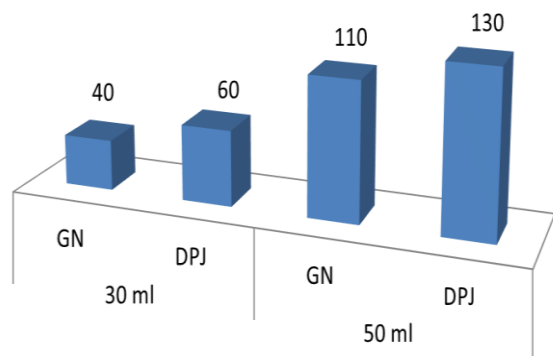


Figure 2: Growth of *A. niger* on DPJ of *Trigonella foenum-graecum*

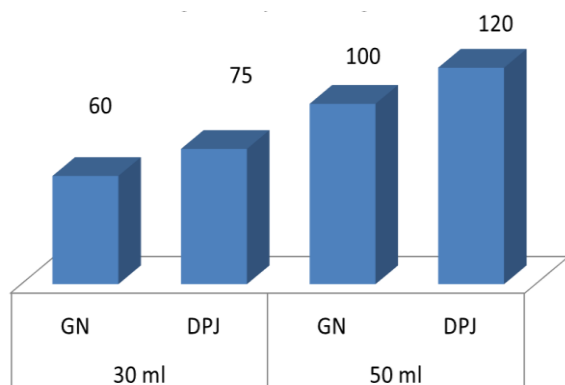


Figure 3: Growth of *Penicillium notatum* on DPJ of *Trigonella foenum-graecum*

CONCLUSION

During green crop fractionation (GCF) the leaf juice release after maceration and pressing of green foliage is normally employed to prepared leaf protein concentrate (LPC). Preparation of LPG from juice, immediately after its extraction has been advocated for maximum recovery of high quality of leaf protein conc., as the juice is liable for chemical, enzymatic and microbial reactions. If it's further processing is delayed. During present course of investigation, it is observed that suitability of deproteinized leaf juice (DPJ) as the medium as growing useful fungi. The results obtain during the experiments undertaken by the author on the deproteinized leaf juice prepared from *Trigonella foenum-graecum* for its potential as microbial growth medium indicated suitability of *Trigonella foenum-graecum* DPJ for cultivating *Aspergillus niger* and *Penicillium notatum* as compared with GN media. It was thus concluded from the experiments during present investigation that for maximum production of mycellial biomass of fungi on deproteinized juice of *Trigonella foenum-graecum* in comparison with GN media.

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