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YEAST UTILIZING DEPROTEINISED LEAF JUICE (DPJ) AS A MEDIUM FOR GROWTH AND PRODUCTION OF METABOLITES

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Abstract

In the process of Green Crop Fractionation (GCF) , the pulp was squeezed to obtain juice and the proteins isolated by heating by forming PC (pressed crop). This filtered whey is called as Deproteinised Leaf Juice (DPJ). The residue obtained after filtration of Juice is called as Leaf Protein Concentrate (LPC). In the present investigation, the DPJ was utilised for fermentation of Yeast and the culture filtrates were employed for study of hydrolytic enzymes. It was observed that DPJ enhanced the mycelial weight of yeast when used as the medium for growth. Different deproteinised leaf extracts were prepared and the yeast was fermented. When DPJ enriched with glucose it showed reduction in mycelia as compared with DPJ alone when was used. It indicated DPJ alone sufficient for yeast growth. The culture filtrates of *Saccharomyces cerevisiae* grown on DPJ were obtained and employed for study of enzyme productivity of amylase, protease, cellulase and lipase by cup plate method. It was found that the diameter of zones of enzymes amylases and cellulases were maximum by Oat (*Avena sativa* L.) DPJ. Enzymes proteases expressed by almost all deproteinised leaf extracts.

Key Words: Lucerne, Deproteinised Juice, Yeast Culture filtrate, Cup plate method, Amylase, Protease.

Introduction

The juice, or leaf extract, expressed during green crop fractionation (GCF) is usually employed to prepare leaf protein concentrate (LPC). For this purpose, it is heated, as a result of which coagulation of proteins in it, results into a curd referred as LPC (Oshima *et al.* 1997; Sayyed 2003, 2011). The LPC is then seperated by filtration from the remaining portion of juice called as deproteinised juice (DPJ). The DPJ is a byproduct of GCF system, which is produced in large volume. This brown watery juice is also called as "whey" or "liquor" (Jadhav 1997; 2015; Patil and Wadje 2011).

It is well known that the DPJ contains biologically active substances like sugars, amino acids, vitamins etc. In a fermentation process Ream *et al.* (1983) recommended use of DPJ for the production of Single Cell Protein (SCP). Butt *et al.* (1972) used DPJ for propogation of yeast. Pardezlopez and Gagagro (1973) used Lucerne DPJ for the production of SCP production ; they observed that DPJ supported growth of yeast. Kummerlin (1984) obtained good yields of *Candida utilis* on DPJ from 10 plant species. However, Pirie (1987) is of the opinion that the filamentous fungi are better than yeasts for biomass production on the DPJ as the former can be collected by filtering, rather than centrifuging as is done for SCP production . Furthermore, mycelia tend to contain less nucleic acids than yeast and therefore, use of DPJ for the cultivation of some edible fungi is most suitable. Chanda (1983) recommended the use of DPJ for antibiotic, Cavazzoni *et al.* (1988) for Ammonium lactate production and Moharib S.A. (2006) for protein and polysaccharides by yeast fermentation. Baukhandi *et al.* (1984) used lucerne (Alfalfa) DPJ for SCP production from *Candida tropicalis* and C. lipolytica at the Institute Of Science, Aurangabad. They observed rapid growth of these yeast strains on DPJ and pointed out that about 1.5 to 4.0 g of dry cell mass could be obtained from 100 ml of DPJ. These results also indicated the nutritive sufficiency in the DPJ for microbial growth. Taking in view, the suitability of DPJ as a fertilizer and medium for growing plants and microorganisms at specific concentrations (Sayyed 2003, 2015, Kawathekar and Mungikar 2005, Jadhav 2009, Madhekar and Mungikar 2009b) including *Rhizobium* enhancement, (Gogle *et al.* 2001, Jadhav 2017) present investigation was undertaken

to evaluate DPJ obtained from various plant species for cultivating wide range of fungal species including yeast. Investigations were also made to evaluate the potential of DPJ for the production of yeast (a single cell protein) and its utility in growing fungi for the production of hydrolytic enzymes (Jadhav, 2018).

Materials and Methods

Green foliages from crops *viz*. ridge gourd (*Luffa acutangula* L.), Lucerne (*Medicago sativa* L.), Bitter Gourd (*Momordica charantia* L.), Jowar (*Sorghum vulgare* L.) Drumstick (*Moringa oleifera* L.), Cowpea (*Vigna unguiculata* L.) *walp*), bajra (*Pennysetum typhoidum* L.), oat (*Avena sativa* L.), mustard (*Brassica campestris* L.), cabbage (*Brassica oleracea* cv *capitata* L.), cauliflower (*Brassica oleracea* L. *var oleracea* cv. *Botrytis*), beet (*Beta vulgaris* L.), winter gourd (*Cucurbita maxima* Duch.), summer gourd (*Cucurbit apepo* L.) bottle gourd (*Lagenaria sicerania* Mol. Standl.) were collected from the field and fractionated using IBP equipments into pressed crop (PC) and juice (Davys and Pirie, 1969, Davys *et al.* 1969). The juice was then heated to over 95ºC for the preparation of leaf protein concentrate (LPC). The Deproteinised juice (DPJ) released during filtration was collected. The samples of DPJ were dried in hot air oven at 90 ± 5ºC till constant weight. The dried DPJ samples were then stored in sealed glass jars until used. Sufficient care was taken to minimise absorption of moisture by the DPJ samples (Mungikar and Jadhav, 2005).

Preparation of culture media

The yeast (*Saccharomyces cerevisiae*) was cultured on the DPJ obtained from various cucurbitaceous and other plants. Either DPJ alone was used, or glucose was added to the DPJ. The GN medium was prepared by dissolving 10 g glucose, 2.5 g KNO_3, 1 g KH_2PO_4 and 0.5 g $MgSO_4$ in 1 litre distilled water (Shinde 1982). After sterilization and inoculation, microbial biomass was collected after the incubation of 15 days. These were incubated till 15 days and filtered through Whatman filter paper to harvest microbial biomass. It was dried in oven at 65ºC and mycelial dry weight (MCW) obtained were recorded. The culture filtrates, released during filtration, were considered as crude enzyme preparations and activities of Amylase, Protease, cellulase and lipase were measured by using cup plate method following Mukadam and Gangawane (1982). The enzyme production was expressed as diameter of the zone formed due to its activity on specific medium (Jadhav 1997, Sayyed 2015).

Enzyme assays

For amylase, soluble starch 10 gm, Na_2HPO_4 20g, D.W. 1000 ml at pH 6.9. Autoclaved 15 ml medium was poured in petriplate. After solidifying the medium, cavity was made in centre. It is filled with culture filtrate and incubated. It is flooded with bugols iodine solution as an indicator.

For protease, the basal medium composed of 2% agar, 4% gelatin, 1% peptone, 1% casein and pH was adjusted to 6.8.

The cellulolytic activity (Dingle *et al.* 1953; Szecsi 1969) test carried out with sodium citrate buffer at pH 5.5. Substrate CMC (1%) mixed with melted 2% agar under aseptic conditions. The enzyme activity zones were developed by covering the plates with 3% lead acetate. After washing, diameter of zones were measured. (Doiphode and Mungikar, 2004). For Lipase, The medium used for assay of lipase was prepared by mixing 10g peptone, 5g NaCl, 0.1g $CaCl_2$. $2H_2O$, 20g agar, 10ml lipid and 1000 ml distilled water.

Results and Discussion

Data revealed by statistical analysis. Yeast is one of the important organism having its own value in domestic as well as industrial uses. During present experiment attempts were made to cultivate yeast on the DPJ obtained from 15 different crops. Fo this purpose, 100 ml of DPJ obtained from the leaves of common foliages, was incubated with 1 gm of yeast for 15 days and the increase in the yeast biomass was measured. table 1 and figure 1 gives an account of the growth of yeast on the DPJ samples obtained from 5 crops. The DPJ from all plants supported the growth of yeast yielding the biomass of yeast in appreciable quantity. The variation in the yield due to the species used was probably due to the differences in the chemical composition of DPJ samples. When DPJ alone was used as growth medium, it yielded 1.640 to 2.400 g of yeast biomass per 100 ml. Addition of glucose to DPJ, decreased growth of yeast within range of 1.170 to 1.640 g. Coefficient of variation (C.V. = 11%)

Table 1: Yeast biomass yield on Deproteinised leaf Juice (DPJ) alone and when added with glucose obtained from various types of leaves.

No.	Plants	DPJ alone	DPJ + glucose
1	*Luffa acutangula* L.	1.640	1.640
2	Lucerne	1.670	1.330
3	*Momordica charantia* L	1.680	1.560
4	*Sorghum vulgare* L.	1.950	1.170
5	*Moringa oleifera* L.	2.400	1.595
		Mean = 1.868	Mean = 1.459
	Standard Deviation	(S.D) = 0.322	S.D = 0.201
	Coefficient of Variation	(C.V) = 17.23	C.V = 13.77

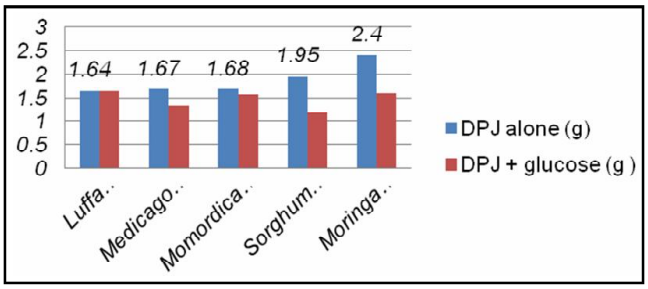

Fig. 1: Yeast biomass yield on Deporteinised leaf Juice (DPJ) obtained from various types of Leaves.

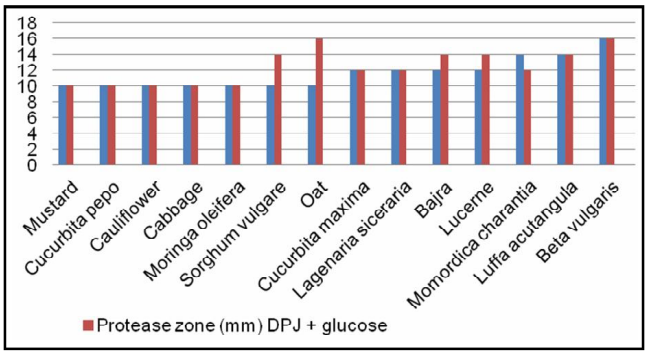

Fig. 3: Protease enzyme (mm) on DPJ alone and DPJ enriched with glucose by cup plates method 2.

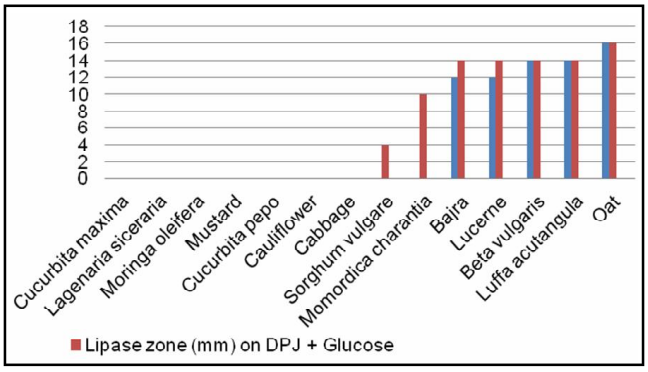

Fig. 5: Lipase zone (mm) on DPJ alone and DPJ + glucose by cup plates method.

in yeast biomass production was observed due to the employment of juice from different plant species. On an average, 100 ml of DPJ alone yielded 1.86 ± 3.34 g, while that of glucose yielded 1.45 ± 2.60 g of yeast biomass, but the difference was not statistically significant. This indicates DPJ alone is sufficient for the growth of yeast biomass. The filtrates left after isolating the yeast biomass was subjected to the assay of enzymes amylase, protease, cellulase and lipase. It was observed that all enzymes were produced by yeast in appreciable quantities, except amylase and cellulase, in some sets of experiments. The production of cellulase was not noticed in majority of the experiments probably due to the fibre free nature of DPJ.

In order to enhance the growth of yeast for maximum biomass production, the DPJ from 15 species were

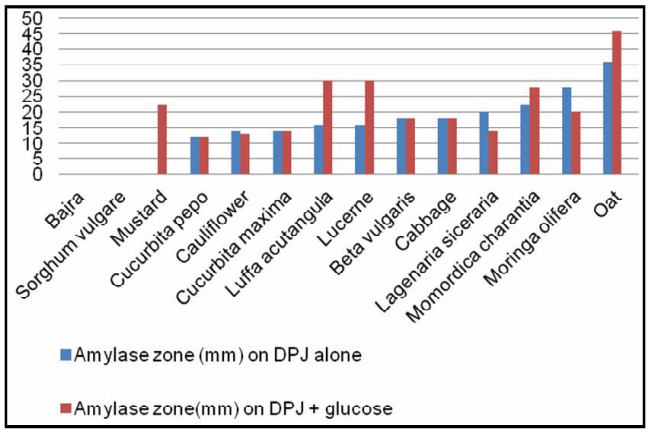

Fig. 2: Amylase zone (mm) on DPJ alone and DPJ enriched with glucose by cup plates method.

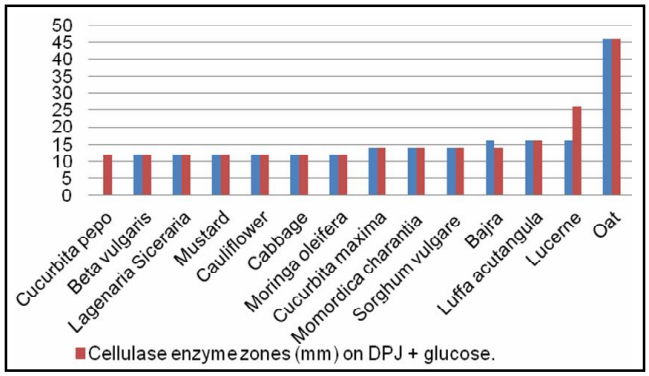

Fig. 4: Enzyme Cellulase zones (mm) on DPJ alone and DPJ + glucose by cup plates method.

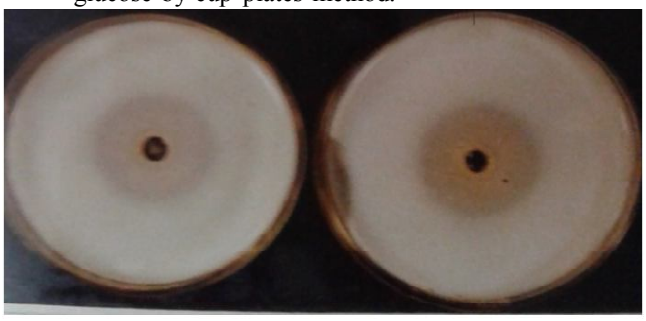

Fig. 6: Photograph of the enzyme cellulases by yeast culture filtrate from DPJ and Glucose when added with DPJ.

enriched with 2% glucose. The glucose treated DPJ samples were used for the cultivation of yeast. A comparison of the results obtained and presented in table 1 with those obtained in earlier experiment (Jadhav and Mungikar 2005) indicate that glucose treatment increased the growth of yeast with higher biomass production. During present investigation in the present plant species used in table 2, the DPJ when used alone enhanced yeast biomass as compared to enriched DPJ with glucose. But in table 3 there is the increase in the diameter of zones of enzymes when glucose was added to DPJ for fermentation. There was no amylase enzyme zone formation by Bajra and *Sorghum vulgare* L. DPJ, though

Table 2: The activity of enzymes amylase, protease, cellulase and lipase by cup plate method when DPJ alone used for fermentation of yeast.

No.	DPJ medium alone.	Diameter of Zones (mm)			
		Amylase	Protease	Cellulase	Lipase
1.	Bajra	00	12	16	12
2.	*Cucurbita maxima* L.	14	12	14	00
3.	*Beta vulgaris* L.	18	16	12	14
4.	*Lagenaria siceraria* L.	20	12	12	00
5.	*Luffa acutangula* L.	16	14	16	14
6.	Lucerne	16	12	16	12
7.	*Momordica charantia* L.	22	14	14	00
8.	Mustard	00	10	12	00
9.	*Cucurbita pepo* L.	12	10	00	00
10.	Cauliflower	14	10	12	00
11.	Cabbage	18	10	12	00
12.	*Sorghum vulgare* L.	00	10	14	00
13.	*Moringa oleifera* L.	28	10	12	00
14.	Oat	36	10	46	16
Mean		**15.2**	**11.5**	**14.8**	**4.8**
Standard Deviation (S.D.)		10.33	1.94	9.78	6.82
Coefficient of variation (C. V.)		73.78	16.86	66.08	142.08

Table 3 . The activity of enzymes amylase, protease , cellulase and lipase by cup plate method when DPJ enriched with glucose for fermentation of yeast.

No.	DPJ medium enriched with glucose	Diameter of Zones (mm)			
		Amylase	Protease	Cellulase	Lipase
1.	Bajra	00	14	14	14
2.	*Cucurbita maxima* L.	14	12	14	00
3.	*Beta vulgaris* L.	18	16	12	14
4.	*Lagenaria siceraria* L.	14	12	12	00
5.	*Luffa acutangula* L.	30	14	16	14
6.	Lucerne	30	14	26	14
7.	*Momordica charantia* L.	28	12	14	10
8.	Mustard	22	10	12	00
9.	*Cucurbita pepo* L.	12	10	12	00
10.	Cauliflower	13	10	12	00
11.	Cabbage	18	10	12	00
12.	*Sorghum vulgare* L.	00	14	14	04
13.	*Moringa oleifera* L.	20	10	12	00
14.	Oat	46	16	46	16
Mean		**18.9**	**12.4**	**16.4**	**6.1**
Standard Deviation (S. D.)		12.15	2.24	9.31	6.94
Coefficient of variation (C. V.)		64.28	18.06	56.76	113.77

enriched with glucose showed in figure 2. But these DPJ expressed enhanced zones of protease enzymes when enriched with glucose, indicated in figure 3. There was no zone of lipase by *Sorghum* DPJ and when it was enriched with glucose, it showed little appearance showed in figure 5. Figure 4 also indicates the presence of cellulase zone when DPJ enriched with glucose. Figure 6 shows photograph of the enzyme activity of cellulase by yeast with DPJ alone and DPJ with glucose.

As with earlier experiment in table 2, the yeast produced all enzymes in appreciable amount except lipase. The DPJ from *Momordica* and *Sorghum*, when enriched with glucose it was found the formation of lipase enzyme zones shown in table 3. Table 3 also indicates the statistical analysis by mean, standard deviation and coefficient of variation of enzyme zones in mm by DPJ alone when was used and DPJ when was enriched with glucose. It was observed that the mean enzyme zone of protease was lesser than that of Amylase and lipase. While mean lipase zone found least among all other enzymes. The mean amylase zones were found more in mm in all DPJ utilised for the fermentation of yeast by cup plate method. The standard deviation of protease zones were found lesser among all deproteinised leaf extracts while the coefficient of variation was found more in lipase enzyme zones.

Conclusion

The overall results obtained with yeast and the DPJ indicated and concluded that the DPJ can be used for the production of yeast as well as alcohol. If the DPJ from proper species is used with suitable additive for the cultivation of yeast, it may provide yeast biomass as a source of single cell protein as well as if properly fermentated it will also yield alcohol. Glucose treated DPJ samples also produced all enzymes in appreciable quantities. The maximum enzyme activity was found of Amylases by all DPJ among all enzymes. While Oat DPJ showed maximum activity of enzymes. Protease enzymes activated by almost all DPJ prepared from different plants.

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