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Study of yeast quality enhancement by Forage Deproteinised Juice (DPJ) fermentation

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ABSTRACT

Process of green crop fractionation (GCF) was considered and its byproducts were tested for evaluation. The byproducts obtained were pulp, LPC (Leaf protein concentrate), Juice, Deproteinised juice and pressed crop (PC). The dry weight of LPC from each crop was measured. Maize forage LPC was more as compared with other forages. Deproteinised Juice (DPJ) is utilised as a medium for the fermentation of yeast. This yeast growth was compared with the yeast grown on Hansens broth media as control. Various concentration ratios of DPJ and Hansens media were prepared and yeast was fermented. Yeast mycelia grown on DPJ was found higher as compared with other concentrations. The culture filtrates of yeast mycelia collected and used to study the enzyme invertase by immobilisation method and the presence of alcohol was by iodoform test. Almost all the samples of various concentrations showed the presence of alcohol by proper secretion of enzyme zymase. Enzyme invertase appropriately found less in the culture filtrate of yeast mycelia grown on DPJ alone. Therefore DPJ is responsible for less breakdown of sucrose as compared with Hansens media which breaks down more sucrose into glucose and fructose by the enzyme invertase.

KEYWORDS: LPC, DPJ, hansens broth, yeast, invertase, alcohol

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INTRODUCTION

In leaf protein (LP) research, during the process of green crop fractionation (GCF), the pulp is obtained [1].This pulp is squeezed to obtain the juice. This juice is heated to 90°C to coagulate the proteins to form the precipitate. The supernatant obtained is deproteinised, is filtered by filter paper. The residue obtained is called LPC (Leaf protein concentrate), as the byproduct. This byproduct is used for malnourishment of rural children [2,3]. The supernatant Deproteinised juice (DPJ) obtained after filteration is disposed randomly. Therefore to avoid pollution because of its disposal, its proper usage is advisable. It contains 70% of carbohydrates along with vitamins and minerals [4,5].This can be used as manure or growing different economically important fungi [6,7]. Even it can form good quantity of metabolites [8]. Fresh juice during previous investigation when used for fermentation, secretes hydrolytic enzymes and effects on the yields of LPC [9].

During present investigation the LPC obtained is measured in three crops [10,11 and 12]. Priority was given to fodder crops for the purpose to suggest to use it in industries, because of its wide availability. Previously, Yeast fermentation on DPJ was compared with Glucose nitrate (GN) medium and the hydrolytic enzmes were studied from each [13]. Presently, the DPJ obtained from forages of the three crops were utilised for fermentation of yeast to release enzymes invertase and zymase and compared with the traditional Hansens broth media used in laboratory at different concentrations. The culture filtrates obtained of the yeast mycelia grown on DPJ are employed to study of the enzyme invertase [14,15] and production of alcohol [16].

MATERIALS AND METHODS

Fresh Deproteinised juice obtained from the crops of wheat *(Triticum vulgare L)*, Maize *(Zea mays L)* and Potato *(Solanum tuberosum L)* were used for the growth of yeast *(Sacchomyces cerevisiae)*. During GCF, the LPC obtained is dried in hot air oven and the weights were measured. The yeast suspension was inoculated in DPJ prepared of different concentrations along with Hansens broth as control in aseptic conditions in inoculating chamber and allowed for fermentation for 8 days at room temperature of 32°C in laboratory. After 8 days, the mycelial DPJ is filtered by whatman filter paper no.1 and the culture filtrate obtained are collected and utilised for the study of enzyme invertase.The mycelia obtained on filter paper is dried along with filter paper is dried in hot air oven.

Hansens broth: Glucose, 50 g; peptone, 10 g; K_2HPO_4,3 g; $MGSO_47H_2O$, 4 g and Agar 20 g/1000ml.

Iodoform test: 1 ml of iodine solution was added and diluted to sodium hydroxide solution drop wise until brown colour discharges, then heated to form yellow coloured precipitate.

Invertase activity method: The experiment was performed by immobilisation of yeast cells by entrapping in calcium alginate and activity of enzyme invertase measured by beads. After removal of beads, 0.5 ml of copper sulphate was added. After heating, effervescence stops when 0.5 ml phosphomolybdic acid was added. Gradual increase of O.D was measured at 660 nm after appearance of blue colour. Enzyme activity increases as the immobilized number of beads increases. Standard graph was plotted to obtain multiplication factor to calculate mg/ml of enzyme.

RESULTS AND DISCUSSION

Table 1 and figure 1 (graphical presentation) indicates that as LPC (Leaf protein concentrate) prepared from maize by green crop fractionation (GCF) was responsible to get more in dry weight i.e. 6.044 g as compared with *Solanum tuberosum* and wheat LPC.

Table 2 and figure 2 (graphical presentation) indicates the comparison of the yeast mycelial biomass obtained on Deproteinised juice (DPJ) fermentation and the fermentation of yeast done on Hansens broth media.It was observed that, DPJ was responsible to enhance the mycelial biomass as compared with Hansens broth media. It was more in all DPJ used namely from wheat, maize and potato forages. When we compare the DPJ among all, which enhanced more mycelia, that was potato DPJ, i.e. 1.007 g. The statistical mean of table 2 indicates the growth of yeast mycelia was more on *Solanum tuberosum* leaves DPJ as compared with maize and *Triticum* leaves DPJ.

When different concentratons of DPJ were utilised for the yeast fermentation and along with it when Hansens broth was also used at different concentrations, there were different results. It was observed that when the concentrations of DPJ enhanced in 50 ml of medium, which was consisting of reducing concentrations of Hansens broths i.e. DPJ: Hansens broth in ml (10: 40, 20:30, 30:20 and 40:10), it was observed that, there was the reduction of mycelial dry weight of yeast, showed by all DPJ, showed in Table 2. However also of decreasing concentrations of Hansens medium decreased MDW. Therefore it concludes that the concentration of DPJ and Hansens broth should be used appropriately for good result of mycelial biomass of yeast. The appropriate concentration for good mycelial biomass was found in the concentration ratio of DPJ: Hansens broth was 10:40.

Overall statistical mean of Table 2 indicates that the DPJ made up of potato forage showed appreciable growth of yeast mycelia as compared with two monocot forage DPJ of wheat and maize. As compared with maize, wheat forage DPJ showed increased mycelial weight of yeast. While maize forage DPJ, was not found as feasible as the other two DPJ to induce mycelial growth of yeast. Maize DPJ was responsible to give appreciable growth when used without combination of Hansens media.

Table 1: Preparation of Leaf protein concentrate (LPC) by Green crop Fractionation (GCF)

Crop/22g pulp	LPC (g)
Triticum vulgare	4.443
Maize	6.044
Solanum tuberosum	5.718

Table 2: Effect of fresh DPJ as a medium from different forages by GCF and Hansens media on Yeast growth fermentation to release enzyme zymase

Medium (%)	Mycelial Dry weight (MCW in g) of yeast		
	Triticum	Maize	***Solanum***
Control (Hansens Broth alone)	0.194	0.15	0.466
DPJ (alone)	0.089	0.321	1.007
10:40 (DPJ : Hansens Broth)	0.264	0.132	0.377
20:30 (DPJ : Hansens Broth)	0.239	0.109	0.346
30:20 (DPJ : Hansens Broth)	0.166	0.039	0.542
40:10 (DPJ : HansensBroth)	0.072	0.341	0.017
Mean	0.166	0.123	0.522
Coefficiant of variation	51.80	97.56	54.21

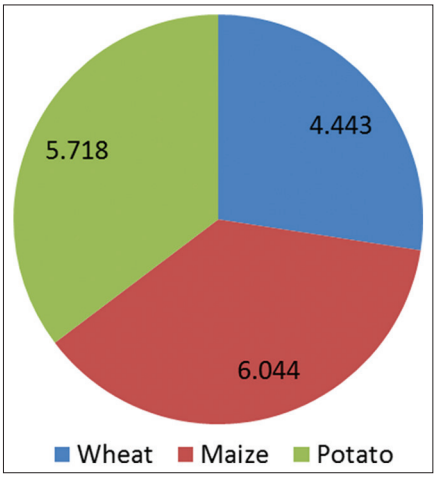

Figure 1: Dry weights of LPC (g) by green crop fractionation of three different crop forages

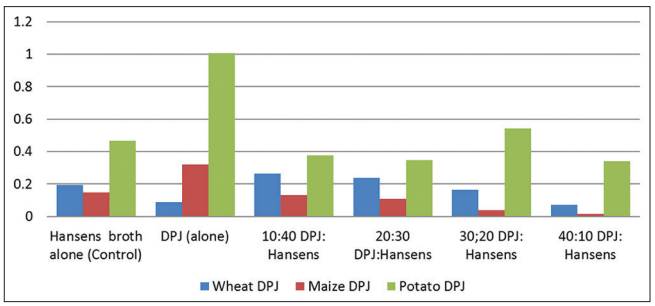

Figure 2: Effect of fresh DPJ from different forages by GCF and Hansens media and their ratios of various concentrations on yeast growth (g)

Table 3 indicates the more invertase enzyme presence in culture filtrates of yeast mycelia grown on Hansens broth i.e 222 μg/100 ml. It means breakdown of sucrose to glucose is more activated by Hansens broth. Majority of the fruits consists of

Table 3: Effect on Invertase activity from various culture filtrates of yeast grown on DPJ and Hansens broth media and their concentration ratios

Medium (% in ml)	Enzyme Invertase (μg/100ml)		
	Maize	*Triticum*	*Solanum*
Control (Hansens broth)	183	222	225
DPJ (alone)	153	039	108
10:40 (DPJ: Hansens)	096	018	207
20:30 (DPJ: Hansens)	078	186	072
30:20 (DPJ: Hansens)	042	081	084
40:10 (DPJ: Hansens)	018	042	066
Mean	77	73	107
Coefficient of Variation	67.61	91.82	51.15

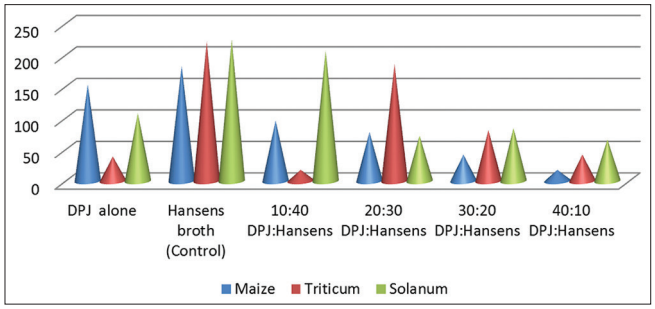

Figure 3: Effect on Invertase activity (mg) from various culture filtrates of yeast grown on DPJ and Hansens broth media and their concentration ratios

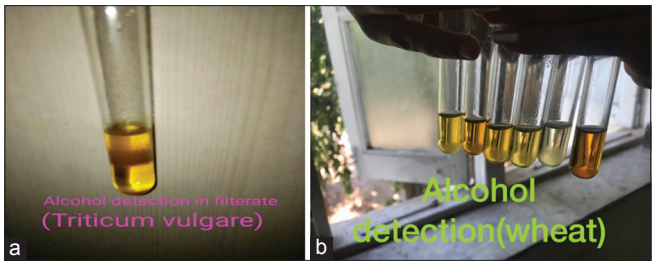

Figure 4: Alcohol detection by iodoform test in the culture filtrate of yeast grown on a) *Triticum vulgare* forage DPJ and b) At various concentration ratios of *Triticum* DPJ : Hansens broth

sucrose, thus DPJ performance also indicates retaining sucrose, as it is the leafy source. Therefore less invertase enzymes in 39 μg/100 ml because of *Triticum* DPJ as a medium investigation was found beneficial in this research. Figure 3 indicates the enhanced concentrations of DPJ along with Hansens broth combination, reduced the rate of invertase enzyme activity. Despite of Hansens medium addition in DPJ, the rate of invertase enzyme activity was less. Therefore it seems DPJ was effected dominantly.

Figure 4 a and b indicates the alcohol production by yeast by iodoform test in the cuture filtrate of wheat forage DPJ. The darkness of yellow colour in culture filtrate indicates more presence of alcohol by yeast grown on DPJ medium alone and at various concentrations along with combination of Hansens broth as mentioned in previous tables no. 2 and 3. Hansens broth alone when used for yeast fermentation, its culture filtrate showed faint yellow colour by iodoform test as compared with the DPJ used. Same performance of alcohol presence showed by DPJ made from forages of maize and potato by iodoform test as showed figures a and b.

CONCLUSION

By the process of GCF, LPC prepared from maize leaves pulp was found more as compared with *Solanum tuberosum* and wheat leaves. The table 2 graphical presentation and statistical analysis indicates that, as compared with the Hansens media, yeast grown favourably on all DPJ from various forages. Hence DPJ alone itself enhances the mycelial biomass of yeast. And there was reduction of mycelial biomass when the concentrations of Hansens medium is reduced. There was reduction in mycelial biomass of yeast when the concentrations of DPJ enhanced. But when DPJ used alone for yeast fermentation, the mycelial dry weight was enhanced.Therefore proper concentration of DPJ to be used is advisable. DPJ reduced the enzyme invertase is beneficial as it is responsible to stabilise the sucrose of yeast neglecting the breakdown to glucose and fructose in less amount as it is the forage source. Therefore because of DPJ the food quality of yeast enhanced. Yeast fermentation done by DPJ of all forages used viz *Triticum*, Maize and *Solanum tuberosum*, produced the alcohol in similar amount, when compared with the fermentation done on Hansens medium, by the iodoform test.

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