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Baker's yeast biomass production with rice as carbon and soy meal as nitrogen sources

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Aims: This research was an attempt to produce baker's yeast biomass utilizing locally available carbon and nitrogen sources.
Methodology and results: The yeast was grown in different media (30 °C, pH 5.0), which were aerated (100 bubbles/min). In the YPS medium containing 50 g/L; 5.53 g/L biomass was obtained, when sucrose was replaced with rice flour hydrolysate (50 g/L), the biomass obtained was 5.72 g/L. With 3.45 g/L of bacteriological peptone 6.02 g/Land 7.5 g/L of yeast extract, 7.12 g/L of biomass were obtained. Highest biomass (7.98 g/L) was obtained when rice protein was hydrolyzed with 10.0 mL/L Neutrase, a protease. Replacing bacteriological peptone (3.45 g/L) and yeast extract (7.5 g/L) with refluxed soy meal or soybean suspension, recorded 6.50 and 6.38 g/L of biomass respectively. Increase in reducing sugars to 200 g/L increased the biomass to 12.38 g/L. Double the amount of soy meal protein hydrolysate increased the biomass to 15.90 g/L. Replacing $(NH_4)_2HPO_4$ with refluxed soy meal suspension gave similar biomass production (15.58 g/L). Thus replacing commercial bacteriological peptone, yeast extract and $(NH_4)_2HPO_4$ with refluxed soy meal suspension is possible for baker's yeast biomass production. Further by optimizing the concentrations of sugar and nitrogen sources led to 2.57 folds increase in baker's yeast biomass production.
Conclusion, significance and impact study: Locally available rice flour hydrolysate and soy meal protein provides better alternatives to commercial carbon and nitrogen sources to produce baker's yeast.

Keywords: baker's yeast, biomass, rice flour hydrolysate, soybean, soy meal

INTRODUCTION

Saccharomyces cerevisiae strains have been selected for many years for their dough leavening characteristics (Randez-Gil *et al*., 1999). In Sri Lanka, supply of baker's yeast, depends on import. Hence, it is important to go for local production of Baker's yeast biomass. This study has focused on utilizing the locally available carbon and nitrogen sources for baker's yeast biomass production. Most common source for baker's yeast production is the molasses, a by-product from sugar production (Imrie, 1969). Alternative sources have been introduced (Champagne *et al*., 1990; Ejofor *et al*., 1996; Ferrari *et al*., 2001; Alemzadeh and Vosoughi, 2002; Bekatorou *et al*., 2006; Betroti and Hosseini, 2007; Gelinas, 2012). When starchy materials are to be used for yeast growth, the starch has to be first converted to sugars (Kim and Hamdy, 1985). Hydrolysates of starch based carbon sources such as wheat; corn rice, manioc and soybean were tested for baker's yeast biomass production and rice flour hydrolysate was found to be the best (Thurairatnam *et al*., 2006). In this studies rice flour hydrolysate obtained by two-steps procedure (Arasaratnam *et al*., 2012) was used as the carbon source for Baker's yeast production with nutrients supplementation.

MATERIALS AND METHODS

Materials

Yeast extract and peptone were from Oxoid, U.K. α-Amylase (Termamyl^R, 120 L, activity 120 KNU/g), glucoamylase (San 240 L, activity 159.9 AGU/mL) and Neutrase 0.5 L (0.5 AU/g mettallo-protease of *Bacillus subtilis*) were from Novo Industries, Denmark. Table sugar, polished rice, soy meal [Delmage Distributors (Pvt) Ltd, Colombo, Sri Lanka] and soybean were purchased in the local market. Polished rice, soy meal and soybean were pulverized in a domestic grinder.

Microorganism

The baker's yeast, Fermipan was from Gist-Brocades, Waterings-1, Delft-Holland, The Netherlands.

Unit definition for enzyme activities

One Kilo Novo Unit (1 KNU) of α-amylase is the amount of enzyme, which hydrolyzes 5.26 g of starch (Merk Amylum Soluble Erg.B.6 Batch No.994 7255) per hour at 37 °C in buffer solution (pH 5.6) containing 0.0043 M calcium.

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One Novo Amylo Glucosidase Unit (1 AGU) is the amount of enzyme which hydrolyzes one µmole maltose per minute at 25 °C and at pH 4.3.

One Anson unit (AU) is the amount of enzyme which at 25 °C digests hemoglobin at an initial rate such that there is liberated per minute an amount of trichloro acetic acid soluble product which gives the same colour with phenol reagent as one mille equivalent of tyrosine.

Analytical methods

Reducing sugar (Miller, 1959), total sugar (Pearson, 1980), amino acid (Rick, 1974), soluble proteins (Lowry *et al*., 1951) nitrogen content (Pearson, 1980), ethanol (Varley *et al*., 1980), and dry weight of the yeast cells (gravimetric method) were measured using standard methods.

Activation of yeast cells

The yeast cells were activated as before (Inparuban *et al*., 2010).

Yeast Peptone Salt (YPS) medium

The medium contained (g/L); yeast extract, 2.5; bacteriological peptone, 1.15; $(NH_4)_2HPO_4$, 0.25 and $MgSO_4 \cdot 7H_2O$, 0.025 at pH 5.0 with 50 g/L sucrose as carbon source (Inparuban *et al*., 2010).

Preparation of inoculums

As described before (Inparuban *et al*., 2010).

Cultivation of baker's yeast in YPS medium

The YPS medium (1 L) in a 2 L flask was inoculated with 10 mL of activated yeast cells and incubated at 30 °C with aeration (200 bubbles/min) (Inparuban *et al*., 2010). Samples were analyzed for dry weight of yeast cell, ethanol production and residual sugar.

Cultivation of baker's yeast in Rice flour hydrolysate (RFH)-YPS medium

RFH was prepared by enzymatic hydrolysis (Arasaratnam *et al*., 2012) and strained through a muslin cloth. RFH was analysed for reducing sugar, total sugar, amino acid, soluble proteins and nitrogen contents.

RFH diluted to have 50 g/L reducing sugars was supplemented with YPS medium (Medium I, Table 1). The medium was inoculated with activated yeast cells and aerated (200 bubbles/min) at 30 °C. The dry weight of cells and ethanol production were monitored. YPS medium with 50 g/L sucrose was used as control (Inparuban *et al*., 2010) (Table 1).

Effect of bacteriological peptone in RFH-YPS medium

In the rice flour hydrolysate (RFH) containing YPS medium (RFH-YPS medium) the concentration of bacteriological peptone was changed (Media I-IV, Table 1).

Effect of yeast extract in RFH-YPS medium

In RFH-YPS medium with optimized concentration of peptone, the concentration of yeast extract was changed (Media VI-IX, Table 1).

Effect of hydrolyzing the rice protein in RFH with Neutrase

To sterile rice flour hydrolysate with minerals (as of YPS medium) different amounts of Neutrase (Table 1, Media X-XIII) was added and inoculated with Baker's yeast. Neutrase untreated medium containing optimized amounts of yeast extract and bacteriological peptone was used as the control.

Supplementing with soy proteins

Refluxing soy meal and soybean suspension with hydrochloric acid

Soy meal (10 g) and soybean (10 g) flour were mixed with 100 mL of 6 N HCl separately and was refluxed for 40 mins. The mixture was cooled and neutralized using NaOH. The sample was centrifuged and analyzed for amino acid content.

Effect of refluxed soy meal and soybean as protein source in RFH medium

The refluxed soy meal and soybean suspension equivalent to the total amino acid contents of optimum yeast extract and bacteriological peptone were taken instead of the commercial nitrogen sources (Media XIV & XV, Table 1). Medium with the optimized amounts of yeast extract and bacteriological peptone was kept as the control.

Effect of sugar concentration of the RFH containing refluxed soy meal suspension

With refluxed soy meal suspension (Medium XV) different amounts of rice flour hydrolysate was taken having reducing sugar concentration equivalent to 100 (Medium XVI), 150 (Medium XVII), 200 (Medium XVIII) and 250 g/L(Medium XIX) (Table 1).

Effect of refluxed soy meal suspension in RFH medium

To RFH containing different concentrations of reducing sugars, double the amount of refluxed soy meal powder suspension was added (Media XX – XXIII, Table 1).

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Substitution of $(NH_4)_2HPO_4$ with refluxed soy meal suspension based on the nitrogen content of $(NH_4)_2HPO_4$

The constituents of Medium XXII (Table 1) were kept constant except $(NH_4)_2HPO_4$. Refluxed soy meal suspension with the nitrogen content equivalent to 0.25 g/L $(NH_4)_2HPO_4$ was replaced for $(NH_4)_2HPO_4$ (Medium XXIV, Table 1). Medium XXII was used as the control.

Statistical Analysis

Results obtained for specific volume, moisture content and proximate composition of breads were analysed statistically by ANOVA and sensorial data were statistically analysed by Friedman test using SAS analytical package.

RESULTS AND DISCUSSIONS

Cultivation of baker's yeast in rice flour hydrolysate (RFH)

Utilization of RFH as a carbon source

When rice flour hydrolysate was used as the carbon source, maximum biomass (dry weight 5.72 g/L) was obtained at 28 h. The control medium gave a maximum of 5.5 (±0.03) g/L at 28 h. The maximum amount of alcohol (20.2 g/L) was produced at 26 h, and there after it declined with time. The initial sugar concentration of the medium was 50 g/L, and reached zero at 26 h. According to the results obtained, rice flour hydrolysate proved itself a suitable carbon source for yeast growth. In this present study, the yeast cultivation medium was supplemented with yeast extract and bacteriological peptone as the nitrogen sources. Therefore the influence of these nitrogen sources on yeast cell biomass production was examined.

Effect of bacteriological peptone in RFH-YPS medium

Highest biomass (dry weight) was obtained with 3.45 g/L bacteriological peptone at 28 h (6.02 g/L) (Medium IV, Figure 1). The increase in ethanol production did not follow the biomass production with different bacteriological peptone concentrations. The initial media contained 50 (±0.644) g/L of reducing sugars, which reached zero at 26 h except with 0.575 g/L bacterial peptone. The results indicated that bacteriological peptone increases the growth of yeast up to a point. The optimum bacteriological peptone concentration of 3.45 g/L under the experimental conditions was selected for further studies.

Effect of yeast extract concentration in RFH-YPS

Highest cell mass was obtained with 7.5 g/L yeast extract at 24 h (Medium VIII) (Figure 2) while the highest alcohol production was also obtained with 10 g/L of yeast extract at 24 h. The initial sugar concentration in the medium was 50 (±0.555) g/L. All the media showed similar variation in the residual sugar concentration, and the residual sugar in the media reached zero between 26 and 28 h. From the results it is evident that the amount of yeast extract has a positive effect on yeast growth and after a limit it has a negative influence on the cell mass production. Hence 7.5 g/L of yeast extract was selected as the optimum amount for further studies.

Rice flour contained 7.58% of total protein, of which only 0.8% was in the soluble form (soluble protein) and 0.8% were free amino acids. Therefore, a major part of the protein was insoluble, which cannot be utilized by the yeast. It has been well documented that yeast are also unable to metabolize peptides (except a limited number of dipeptides) (Hammond, 1993). Thus in order to transform the insoluble protein of rice flour hydrolysate into soluble form, protein hydrolysis was carried out with Neutrase.

Effect of hydrolyzing the rice protein in RFH with Neutrase

When Neutrase of varying concentrations (Table 1) was introduced into the media, highest yeast cell mass obtained was 7.21 (±0.15) g/L at 26 h with 10.0 mL/L Neutrase (Figure 3). RFH treated with 10 mL/L of Neutrase (Medium XIII) recorded slightly higher biomass production (12.08%) than the control (Medium VIII). A slight variation of alcohol production has been observed with the increase in Neutrase concentration. In all media, the sugar content decreased to zero at 26 h. Bacterial protease, Neutrase 0.5 L catalyzed the hydrolysis of peptide bonds in protein forming soluble peptides and amino acids (Thurairatnam *et al*., 2006). Even though the enzyme hydrolysed rice protein can be used as a good nitrogen source due to the high price of Neutrase, alternative measures have to be taken to use the locally available nitrogen sources.

Refluxing soy protein

Soybean (*Glycine max*) is relatively an inexpensive and abundant source of proteins and lipids, which can be easily assimilated into cellular materials (Bajpai *et al*., 1988). Soy meal, which is rich in protein, and available locally, was selected (Hammond, 1993).

As majority of the proteins in soy meal powder and soybean flour were insoluble (Table 2) they have to be hydrolyzed. When refluxed in presence of 6 N HCl under pressure, the free amino acid contents of soya meal flour and soya bean flour have increased. By considering the free amino acid contents of the medium and with the optimum amount of yeast extract and bacteriological peptone, equivalent amount of free amino acid from refluxed soy meal and soy bean suspensions were supplemented to the RFH medium.

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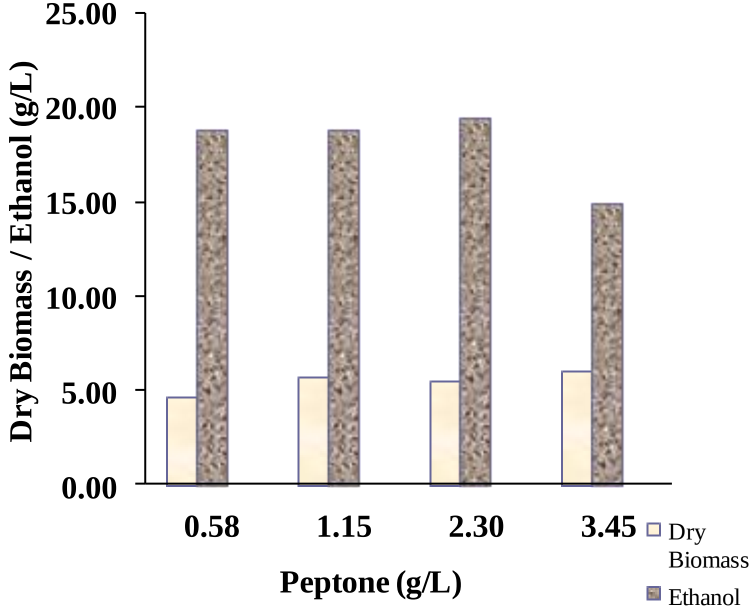

Figure 1: Effect of bacteriological peptone concentration on the growth of *S. cerevisiae* (biomass production) and ethanol production in fermentation medium containing (g/L); yeast extract, 2.5; $(NH_4)_2HPO_4$, 0.25 and $MgSO_4 \cdot 7H_2O$, 0.025 at 30 °C and pH 5.0, aerated (100 bubbles/min). The rice flour hydrolysate (with 50 g/L reducing sugar) was used as the carbon source. Here in the media containing 0.58 and 1.15 g/L bacteriological peptone highest biomass production was obtained at 28 h while with 2.3 and 3.45 g/L bacteriological peptone highest biomass production was obtained at 26 h. At the respective period no residual sugar was in the media.

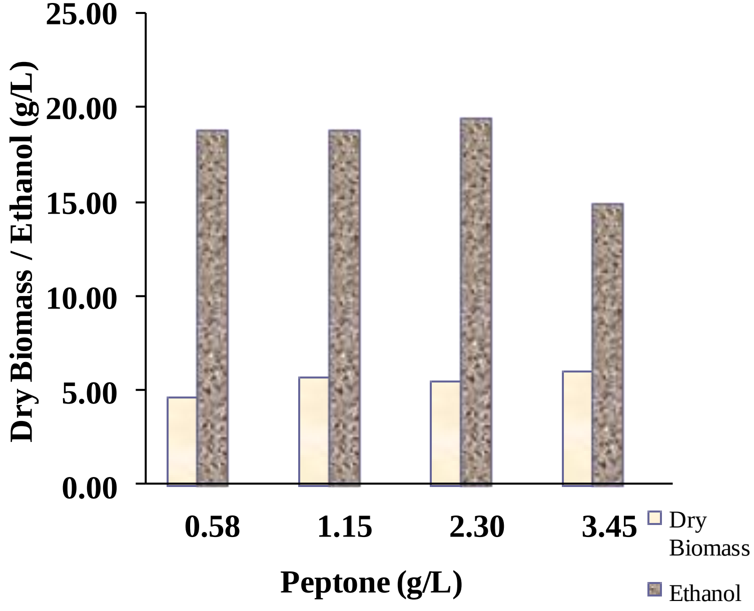

Figure 2: Effect of yeast extract concentration on the growth of *S. cerevisiae* (biomass production) and ethanol production in fermentation medium containing (g/L); bacteriological peptone, 3.45; $(NH_4)_2HPO_4$, 0.25 and $MgSO_4 \cdot 7H_2O$, 0.025 at 30 °C and pH 5.0, aerated (100 bubbles/min). The rice flour hydrolysate (with 50 g/L reducing sugar) was used as the carbon source. Here in the media containing 1.25 and 2. 5 g/L yeast extract highest biomass production was obtained at 28 h while with 5.0 and 7.5 g/L yeast highest biomass production was obtained at 24 h and with 10.0 g/L yeast highest biomass production was obtained at 26 h. At the respective period no residual sugar was in the media.

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Table 1: Yeast cultivation in RFH medium with different constituents of varying amounts and, the biomass and ethanol produced and sugar utilized. The time at which maximum dry biomass, and ethanol produced, and highest sugar utilized are given in parenthesis.

Constituents changed	Medium	Constituents								
		Sucrose (g/L)	RFH (Reducing sugar) (g/L)	Yeast Extract (g/L)	Peptone (g/L)	$(NH_4)_2HPO_4$ (g/L)	$MgSO_4{\cdot}7H_2O$ (g/L)	Neutrase (mL/L)	Soy bean hydrolysate (mL/L)	Soy meal hydrolysate (mL/L)
	Control	50	-	2.5	1.15	0.25	0.025	-	-	-
Peptone	I	-	50	2.5	1.15	0.25	0.025	-	-	-
	II	-	50	2.5	0.575	0.25	0.025	-	-	-
	III	-	50	2.5	2.3	0.25	0.025	-	-	-
	IV	-	50	2.5	3.45	0.25	0.025	-	-	-
	V	-	50	2.5	4.6	0.25	0.025	-	-	-
Yeast Extract	VI	-	50	1.25	3.45	0.25	0.025	-	-	-
	IV	-	50	2.5	3.45	0.25	0.025	-	-	-
	VII	-	50	5.0	3.45	0.25	0.025	-	-	-
	VIII	-	50	7.5	3.45	0.25	0.025	-	-	-
	IX	-	50	10.0	3.45	0.25	0.025	-	-	-
Neutrase	X	-	50	-	-	0.25	0.025	2.5	-	-
	XI	-	50	-	-	0.25	0.025	5.0	-	-
	XII	-	50	-	-	0.25	0.025	7.5	-	-
	XIII	-	50	-	-	0.25	0.025	10.0	-	-
Soy hydrolysate	XIV	-	50	-	-	0.25	0.025	-	22.86	-
	XV	-	50	-	-	0.25	0.025	-	-	17.8
Reducing sugar	XVI	-	100	-	-	0.25	0.025	-	-	17.8
	XVII	-	150	-	-	0.25	0.025	-	-	17.8
	XVIII	-	200	-	-	0.25	0.025	-	-	17.8
	XIX	-	250	-	-	0.25	0.025	-	-	17.8
Reducing sugar Double Soy meal hydrolysate	XX	-	100	-	-	0.25	0.025	-	-	35.6
	XXI	-	150	-	-	0.25	0.025	-	-	35.6
	XXII	-	200	-	-	0.25	0.025	-	-	35.6
	XXIII	-	250	-	-	0.25	0.025	-	-	35.6
$(NH_4)_2HPO_4$	XXIV	-	200	-	-	-	0.025	-	-	36.276

A 120 mL/L of rice flour hydrolysate contained 50 g/L of reducing sugars, whereas 17.8 mL/L of refluxed soy meal suspension contained 1.4 g/L nitrogen. In Medium XXIV 0.25 g/L $(NH_4)_2HPO_4$ was replaced with 0.688 mL/L refluxed soy meal suspension, which was equivalent to the nitrogen content of 0.053 g/L.

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Table 2: Total and soluble protein and amino acid contents of soybean powder and soy meal powder.

Content	Non-refluxed		Refluxed	
	Soybean	Soy meal	Soybean	Soy meal
Total Protein (%, w/w)	42.3	58.58	ND	ND
Free Amino Acid (g/L)	1.28	1.31	382.6	525.7
Soluble Protein (g/L)	1.36	1.52	ND	ND

ND- Not determined

Effect of refluxed soy meal and soybean suspensions as nitrogen source in RFH-medium

Refluxed soy protein, with amino acid content equivalent to that of the Medium VIII was incorporated. Comparatively, the medium containing refluxed soya meal (Medium XV) gave higher biomass than the medium with refluxed soybean (Medium XIV). On the other hand, the biomass production of the above mentioned media was reduced than in the control medium (Medium VIII) with optimum yeast extract and bacteriological peptone. The maximum cell mass yields obtained in the media with refluxed soya meal and soya bean were 6.5 (±0.12) and 6.4 (±0.04) g/L respectively at 26 hours, whereas, ethanol production in the media was higher in refluxed soy bean meal containing medium (Medium XIV, Figure 4). The initial reducing sugar content in all the media was 50 (±0.278) g/L, and it reached zero in 26 h.

Protein hydrolysates have sometimes been used as sources of nitrogen. For instance, the use of wheat starch (after hydrolysis) as sugar source and hydrolyzed wheat gluten as nitrogen source has been practiced (Reed and Peppler, 1973). At this instance, when the soybean is refluxed with 6 N HCl, along with the proteins, starch also could have been hydrolysed to sugars or oxidzed, and there is also possibility for the Mailard reaction. These by-products could have had a negative effect on the biomass production or converted to useless substances. This could be the reason for the reduction in the biomass production in refluxed soybean suspension substituted medium than in refluxed soy meal suspension substituted medium. As the medium with refluxed soy meal suspension gave higher biomass when compared with refluxed soybean suspension, it was selected for further studies.

Effect of sugar concentration of RFH with refluxed soy meal suspension as nitrogen source

Biomass yields were maximum when the sugars were completely utilized. Further increase in biomass production could have been possible if additional sugar is available. Hence it was decided to increase the sugar concentration to analyze the performance of yeast cells.

When the sugar content was increased highest, cell mass [12.38 (± 0.13) g/L] production was recorded in the medium with 200 g/L sugar (Medium XVIII), whereas highest ethanol productions was [40.2 (±0.8) g/L] recorded in the medium with 250 g/L (Medium XIX, Figure 5). The results indicated that, the biomass production was increased with increase in sugar concentration and this present experiment has established that the decreased biomass production in the previous media (Medium I to Medium XIX) was due to inadequate sugar content. Comparing biomass productions among the treatments, medium with 250 g/L sugar (Medium XIX) gave less biomass production. This may be due to the osmotic effect of high sugar concentration (Balakumar and Arasaratnam, 2011). Ethanol productions were also increased with increased sugar concentration and showed an inverse relationship with the cell mass production. The substrate uptake in medium with 100, 150, 200 and 250 g/L of sugar concentrations were 93.8, 95.8, 81.8 and 75.6% respectively. Ethanol production in all treatments differed significantly (p<0.05). The residual sugar content showed significant difference in all treatments (p<0.05). The next attempt was to increase the nitrogen source to the media with high sugar concentrations. This was an approach to find out whether the nitrogen sources were inadequate to cope up with high sugar concentrations in order to satisfy the increasing yeast population.

Effect of doubling refluxed soy meal suspension in RFH medium

In this present study, along with increasing sugar concentrations, the amount of amino acid content in refluxed soy meal suspension equivalent to that of the optimum concentration of yeast extract and bacteriological peptone was doubled. Here the cell mass productions were comparatively higher (Figure 6). Again medium with 200 g/L sugar (Medium XXII) gave the highest biomass of 15.6 (±0.06) g/L. This illustrates that, the nitrogen content in the media XVI, XVII, XVIII, and XIX were inadequate. Ethanol production has not significantly increased with increasing sugar concentration with double the amount of refluxed soy meal suspension. The substrate uptake was 100% with 100 (Medium XX) and 150 g/L sugar (Medium XXI) while in medium with 200(Medium XXII) and 250 g/L sugar (Medium XXIII) it was 92.2 and 91.6% respectively. The dry weights, alcohol productions and residual sugar contents were significantly different among themselves (p<0.05). This shows that the yeast cells have utilized more sugars and produced more biomass with the increased nitrogen source content. As Medium XXII with 200 g/L of sugars gave the highest yield, it was selected for further studies.

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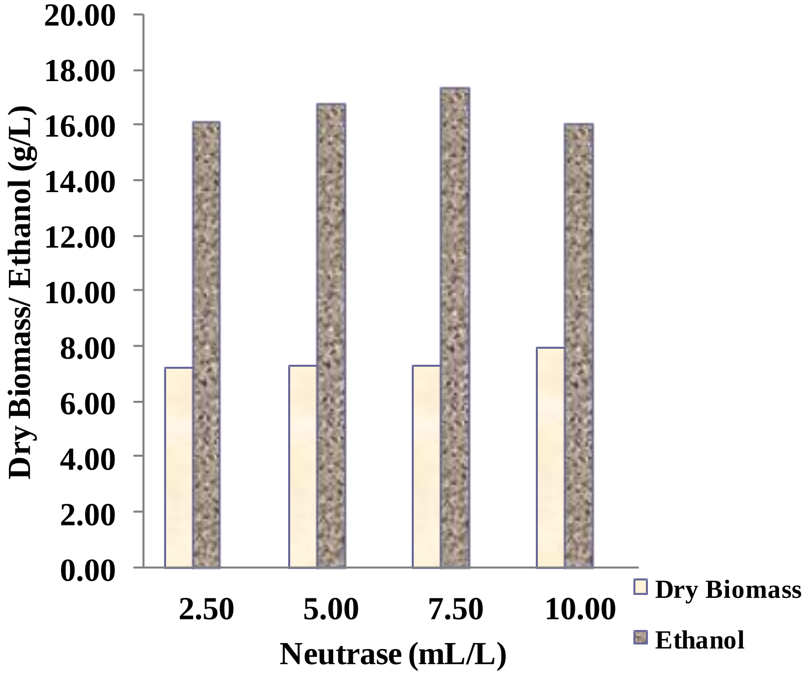

Figure 3: Effect of Neutrase concentration on the hydrolysis of rice protein hydrolysis and their effect on the growth of *S. cerevisiae* (biomass production) and ethanol production in fermentation medium containing (g/L); $MgSO_4 \cdot 7H_2O$, 0.025 at 30 °C and pH 5.0, aerated (100 bubbles/min). Here the rice flour hydrolysate (with 50 g/L reducing sugar) was used as the carbon source after the protein hydrolysis. Here in all the media containing different concentrations of Neutrase, highest biomass production was obtained at 26 h. At the respective period no residual sugar was in the media.

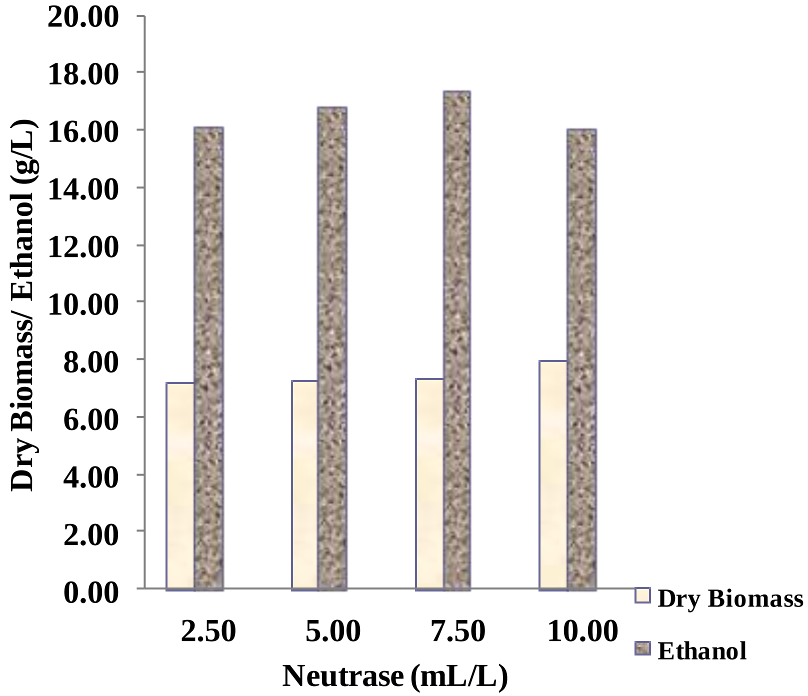

Figure 4: Effect of replacing yeast extract and bacteriological peptone with either soybean hydrolysate (22.86 mL/L) and soy meal hydrolysate (17.8 mL/L) in fermentation medium containing (g/L) $(NH_4)_2HPO_4$, 0.25 and $MgSO_4 \cdot 7H_2O$, 0.025 at 30 °C and pH 5.0, aerated (100 bubbles/min). The rice flour hydrolysate (with 50 g/L reducing sugar) was used as the carbon source. Here in the media containing soybean hydrolysate and soy meal hydrolysate, highest biomass production was obtained 26 h. At the respective period no residual sugar was in the media.

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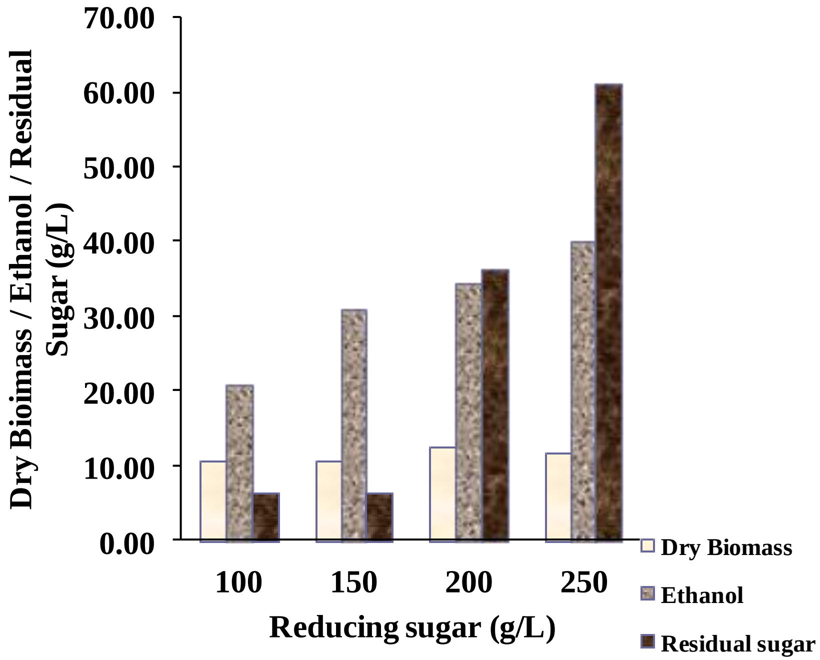

Figure 5: Effect of different concentration of sugar in rice flour hydrolysate on the dry biomass and ethanol production in fermentation medium containing (g/L) $(NH_4)_2HPO_4$, 0.25 and $MgSO_4 \cdot 7H_2O$, 0.025 at 30 °C and pH 5.0, aerated (100 bubbles/min). Soy meal hydrolysate (17.8 mL/L) was used instead of optimized amounts of bacteriological peptone and yeast extract. In all the media highest biomass was obtained at 30 h, and at 32 h highest ethanol produced and residual sugar present in the media are presented.

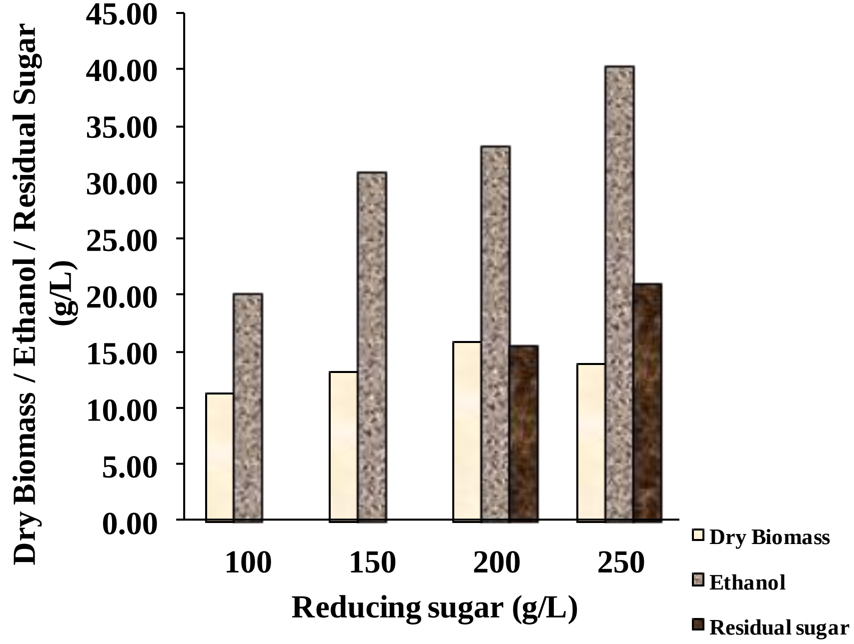

Figure 6: Effect of different concentration of sugar in rice flour hydrolysate on the dry biomass and ethanol production in fermentation medium containing (g/L) $(NH_4)_2HPO_4$, 0.25 and $MgSO_4 \cdot 7H_2O$, 0.025 at 30 °C and pH 5.0, aerated (100 bubbles/min). Double the concentration of soy meal hydrolysate (35.6 mL/L) was used. In all the media highest biomass and ethanol were obtained at 30 h, and residual sugar present in the media 30 h are presented.

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Effect of substitution of $(NH_4)_2HPO_4$ in RFH medium with refluxed soy meal suspension based on the total nitrogen content

The next attempt was to substitute $(NH_4)_2HPO_4$ with soy meal hydrolysate. The nitrogen content of soy meal hydrolysate was 5.46 g/Land the nitrogen content from the added 0.25 g/L of $(NH_4)_2HPO_4$ in 1 L medium was 0.053 g/L. Hence, the amount of soy meal hydrolysate equivalent to this fraction was 9.7 mL for 1 L medium. The total nitrogen content of medium with 3.45 g/L of bacteriological peptone and 7.5 g/L of yeast extract (optimum concentrations) was 1.4 g/L (Medium VIII). So it was decided to substitute the nitrogen content from $(NH_4)_2HPO_4$ with equivalent amount of nitrogen from soy meal hydrolysate. Biomass of 15.69 g/L was obtained in the Medium XXIV whereas the control (Medium XXII) gave 15.9 g/L. This replacement of $(NH_4)_2HPO_4$ with soy meal hydrolysate was therefore satisfactory. Nitrogen is generally added in the form of ammonia or ammonium salts, such as ammonium sulfate or ammonium phosphate, or sometimes in the form of urea. Nitrogen from such sources is readily assimilated and with the exception of urea they are interchangeable (Reed and Peppler, 1973). According to the statistical analysis of this study, the dry weights recorded in both, test (Medium XXIV) and control media (Medium XXII) were not significantly different ($p>0.05$). The residual sugar content showed non-significant difference in both the treatments ($p>0.05$). Hence the replacement of $(NH_4)_2HPO_4$ with refluxed soy meal suspension did not lead to negative effect. So this replacement was selected as a suitable condition for yeast cultivation in the following steps.

CONCLUSION

Baker's yeast is not produced in Jaffna peninsula and this was an attempt to produce baker's yeast with local raw materials. This studies, proved that the rice flour hydrolysate commercial can replace sucrose, and refluxed soy meal suspension can replace bacteriological peptone, yeast extract and $(NH_4)_2HPO_4$. With the local raw materials 2.57 fold increase in baker's yeast biomass production could be obtained and this can reduce the importation of baker's yeast.

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