

## Production of ethanol by simultaneous saccharification and fermentation: Endogenous proteins as nitrogen source from locally available starch based carbon sources

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This paper describes the utilization of locally available starch sources for ethanol production by simultaneous saccharification and fermentation. Normally peptone and yeast extract are added as nitrogen sources to the fermentation medium to improve the ethanol production efficiency and growth of *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* (Fermipan, The Netherlands) was selected for this study. To avoid the addition of exogenous nitrogen source, the proteins present in the carbon sources were hydrolysed using Neutrased (0.5 AU ml<sup>-1</sup>)-an endoprotease at pH 7.0 and 45°C. Starch in the carbon sources were liquefied with α-amylase (0.225 KNU ml<sup>-1</sup>) at pH 7.0 for 4h. All the media used in the study contained 250 gl<sup>-1</sup> initial total sugar and 110 gl<sup>-1</sup> reducing sugar. For saccharification, glucoamylase (0.8 AGU ml<sup>-1</sup>) was used and the pH of the fermentation medium was maintained at 5.0 and incubation was carried out at 30°C. Preliminary study was carried out with wheat flour. Wheat flour contained 8.1% (w/w) total protein and 0.1% (w/w) soluble protein. To the liquefied wheat starch (pH 7.0), Neutrased (10.0 ml<sup>-1</sup>) was added and incubated at 45°C for 4h. This wheat protein hydrolysed liquefied starch-containing medium was used for yeast cultivation without yeast extract and peptone supplementation and control medium contained liquefied starch with 2.3 gl<sup>-1</sup> yeast extract and 5.0 gl<sup>-1</sup> peptone. Biomass obtained and ethanol produced at 36h were 2.7x10<sup>8</sup> & 2.8x10<sup>8</sup> cells ml<sup>-1</sup> and 82.1 & 90.8 gl<sup>-1</sup> in control medium and Test medium respectively. These results indicated that the endogenous protein in wheat flour hydrolysed by Neutrased could be utilised instead of exogenous nitrogen source for the ethanol production. To improve the protein hydrolysis, liquefied wheat starch was treated with different concentrations of Neutrased (2.5, 5.0, 7.5 and 10.0 ml<sup>-1</sup>). At 36h the biomass obtained (1.92x10<sup>8</sup> cells ml<sup>-1</sup>) and ethanol produced (89.7 gl<sup>-1</sup>) were highest in the wheat protein hydrolysed by 10.0 ml<sup>-1</sup> Neutrased. As wheat is not a local product, different starch based material such as rice, corn, manioc and soybean were selected. To the above-liquefied starch based carbon sources yeast extract (2.3 gl<sup>-1</sup>) and peptone (5.0 gl<sup>-1</sup>) were supplemented. At 56h biomass (3.7x10<sup>8</sup> and 3.5x10<sup>8</sup> cells ml<sup>-1</sup> respectively) obtained was highest in manioc and soybean containing media (98.6 and 95.3 gl<sup>-1</sup> respectively). Proteins in all these liquefied starch based carbon sources were hydrolysed with Neutrased (10.0 ml<sup>-1</sup>). At 56h the biomass produced was highest in rice flour followed by manioc and soybean containing media (4.4x10<sup>8</sup>, 3.7x10<sup>8</sup> and 3.5x10<sup>8</sup> cells ml<sup>-1</sup> respectively) and ethanol produced was highest in corn flour followed by rice and soybean (105.0, 96.6 and 92.0 gl<sup>-1</sup> respectively). As the protein content in corn is 12% (w/w) and manioc is 1.3% (w/w), corn and manioc hydrolysates were mixed in different ratios (100:0, 75:25, 50:50, 25:75 and 0:100) and simultaneous saccharification and fermentation were carried out. The results indicated that the highest amount of biomass and ethanol were produced in corn flour containing medium at 56h. From the results it can be concluded that among the locally available starch based carbon sources corn flour (in suspension 38%, w/w) could be used after liquefaction (with 0.225 KNU ml<sup>-1</sup>

Termamyl) and endogenous protein hydrolysis (with 0.5AUml<sup>-1</sup> Neutrase) for simultaneous saccharification (0.8AGUml<sup>-1</sup> glucoamylase) and fermentation by *S.cerevisiae*. The simultaneous saccharification and fermentation have not only had reduced the inhibition of sugar in fermentation by *S.cerevisiae* but also reduced the total time required for liquefaction, saccharification and fermentation from 85h to 67h..

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Potato is an important cash crop in Jaffna district. Its profitability reduces when the market is glutted during the harvesting season. Adopting appropriate storage practices is one of the ways to extend the period of potato supply in the local market. With this view, a field experiment was carried out at Thunnevely during December 2001, to study the effect of pre and post harvest storage practices on the yield and quality of potato.

Mulching was adopted as a pre harvest storage practice (around storage). In this treatment, potato field was mulched with straw for a period of one month after maturity. In the post harvest storage treatment, harvested tubers were placed in wooden trays and stored in a ventilated container for a period of one month. In the pre and post harvest storage treatments, tubers were harvested at weekly intervals and the yield and quality were recorded.

Results of the experiment revealed that the soil temperature was reduced in mulched treatment compared to non-mulched. Minimum and maximum soil temperature in mulched treatment was 28.7°C and 33.2°C respectively, while in the non-mulched treatment it was 29.7°C and 34.4°C respectively. Mulching reduce the soil temperature by 1 to 1.5°C, which means helped to reduce tuber damage in the ground storage.

It was also found that in the mulched treatment tuber weight per plant was reduced from 169g to 167g in one month time, while in non-mulched treatment loss of tuber weight per plant was 146g to 13g during same period. Further, the quality of potato also affected due to high soil temperature in the non-mulched treatment. In this treatment the skin of the tubers became black and started decaying, but no such changes were observed in the mulched treatment. Local pre harvest storage is possible without much reduction in the yield for a period of one month. During the post harvest storage weight loss of 1.75% was noticed in one month.

Therefore it could be concluded that proper ground storage practices with help of mulching and post harvest storage can extend the period of potato availability in the local market by two months to fetch the high market price.