

Protein enrichment of pineapple waste with *Saccharomyces cerevisiae* by solid state bioprocessing

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Saccharomyces cerevisiae was assessed to increase protein levels of pineapple waste (PW) by solid state bioprocessing (SSB) with and without nitrogen supplementation. PW (10 g) was inoculated with *S. cerevisiae*. Optimum protein content (22% dry basis), which is 3.5-fold the original protein content, reached at 48 h of incubation when 0.25% (NH₄)₂SO₄ was added to medium. Thus, fermented PW can be successfully converted into a protein-rich feed by SSB.

Keywords: Nitrogen supplementation, Pineapple waste, Protein enrichment, Solid state bioprocessing, *Saccharomyces cerevisiae*
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Introduction

Pineapple [*Ananas cosmosus* (Linn.) Merrill] production in world (mostly 79 countries) is around 15 million tons a year¹ and it is usually consumed fresh, as pineapple juice, fruit pulp or canned. Brazil (10% of world's production) is one of the top five pineapple producing countries. As a consequence, a great volume of pineapple waste (PW, 30% of fruit wt), which consists basically of residual pulp, skin and peels², is generated and alternatives to its efficient utilization are necessary. Solid state bioprocessing (SSB), an aerobic microbial transformation, has been successfully exploited for enzyme^{3,4} and food production⁵, phenolic enhancement^{6,7}, fruity aroma production⁸ as well as many other uses. Culture conditions of SSB are more similar to the natural habitat of filamentous fungi, which leads, in many cases, to higher efficiency, as well as lower generation of liquid waste.

Fruit and vegetable residues have been successfully used for protein enrichment⁹⁻¹¹ and for bioconversion into value-added products as enzymes and other metabolites¹²⁻¹⁴. *Saccharomyces cerevisiae* and *Candida* have been used to bioconvert agro-industrial wastes into valuable protein sources¹⁵. Nitrogen supplementation of the raw substrate in SSB may stimulate growth or improve process efficiency.

Nitrogen sources can play an important role not only as nutritive compounds but also can influence pH changes during the process¹⁶⁻¹⁷. This study used *S. cerevisiae* to increase protein content of PW by SSB with and without nitrogen supplementation.

Materials and Methods

Microorganism

A pure strain of *S. cerevisiae*, isolated from commercial fresh baker's yeast, was maintained on YEPDA slants at 4°C and subcultured every two weeks. Suspension used for inoculation was prepared by scratching a slant of *S. cerevisiae* and transferring a loopful of cells to 600 ml seed YEPD solution that was incubated for 48 h in a rotary shaker operating at 160 rpm at 31°C.

Substrate

PW, generously provided by pulp extraction units located in Natal, RN, Brazil, was collected in different days, mixed to form one single batch and used in all studies to minimize any possible interference due to variation in composition of residues. It was spread on trays, oven dried at 70°C for 5 h and grounded. PW (pH 3.4), a heterogeneous material [size, 0.84 mm; most of the particles (80%) with size > 0.42 mm] contains (dry wt): moisture 34.5; protein, 6.4; and reducing sugars, 27.2%.

Solid State Bioprocessing (SSB)

SSB was carried out in 250 ml Erlenmeyer flasks containing PW (10 g). Flasks were aseptically

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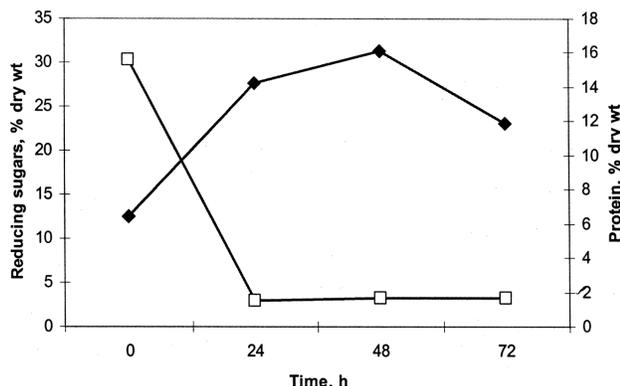


Fig. 1— Protein and reducing sugars content during solid state bioprocessing by *S. cerevisiae* without nitrogen supplementation: □ reducing sugars; ◆ protein

inoculated with 50 ml of inoculum suspension of *S. cerevisiae* (2×10^8 CFU ml⁻¹). Flasks were covered with sterilized gauze covers and statically incubated at 30°C in a laboratory incubator for 4 days with intermittent manual shaking. Sorption isotherms were made and the amount of liquid inoculum added to substrate was adjusted in order to reach an initial water activity of 0.95-0.96. Samples, as whole flasks in triplicate, were withdrawn after each 24 h. The time necessary to reach the peak of protein concentration was evaluated.

Nitrogen Supplementation

Effect of nitrogen supplementation on protein enrichment was examined using 3 different treatments of urea, (NH₄)₂SO₄ and (NH₄)₂SO₄/urea (1:1, w/w). For each treatment, 5 levels of nutrients (0, 2.5%, 5.0%, 7.5% and 10%) were added to media. Samples were incubated for 48 h and their protein, pH and moisture contents were determined. Protein gain (PG, % dry wt) was calculated as:

$$PG = P_f - P_o \quad \dots (1)$$

where, P_o = protein concentration at the beginning of bioconversion, % dry wt and P_f = protein concentration after a certain period of time of bioconversion, % dry wt.

Analytical Methods

Samples were dried for analyses. Bioprocessed material was spread in circular metallic trays ($\phi = 20$ cm) and oven dried for 24 h at 60°C. Dried samples were evaluated for protein content¹⁸ and reducing sugars¹⁹. Moisture content was determined gravimetrically, while pH levels were estimated in a

suspension of sample (0.5 g) in distilled water (10 ml). Results are average of three experiments where all samples were analyzed in triplicate. Each value is obtained as a mean of three independent readings, from which standard errors were calculated. Statistical analysis of data was determined by analysis of variance (ANOVA) and Tukey test was applied with accepted significance of $p < 0.05$.

Results and Discussion

Protein Enrichment of Pineapple Waste (PW)

After 48 h of fermentation (Fig. 1), reducing sugars dropped from 30% to 3% (dry basis) and protein increased from 6.4% to 16.1% (dry basis). Protein content increased almost linearly during first 48 h and after this, a significant decrease was observed, probably due to that proteolysis would take place after two days of bioprocessing. Very little variation of pH levels (4.4-4.6) was observed and pH was maintained at 4.5. High protein content (after 48 h) shows ability of *S. cerevisiae* to grow in this substrate. High residual sugar could be an appropriate substrate for multiplication of yeast in the form of single cell protein leading to considerable final protein content of bioprocessed product. *S. cerevisiae* does not produce fungal toxins excreted by some filamentous fungi like *Aspergillus*²⁰

The increase of protein in non-supplemented PW was higher than the reported¹⁰ value, where after 6 days of fermentation with *C. utilis*, protein content of apple pomace increased from 4.1% to 8.3% (dry basis). Bhalla & Joshi²¹ obtained increases from 0.6% to 20.0% (dry basis) in protein enrichment of apple pomace by co-culture of *C. utilis* and *A. niger*. They²¹ also observed that SSB of apple pomace by *S. cerevisiae* and *A. niger* increased its protein content from 0.6% to 13% (dry basis). It can be seen that reducing sugars were promptly utilized by *Saccharomyces*, since they were nearly exhausted after 24 h. This behavior shows that *S. cerevisiae* is able to efficiently metabolize available sugar as its carbon source for protein enrichment of the residue.

Effect of Nitrogen Supplementation on Protein Enrichment of PW

With low protein content (6.4%) of PW, effect of nitrogen supplementation on its protein enrichment was examined. Supplementation with 2.5% of (NH₄)₂SO₄ was best to enrich protein levels of PW during SSB by *S. cerevisiae* (Fig. 2). It led to a gain of 15.4g protein per 100g of substrate (dry wt) and a PG

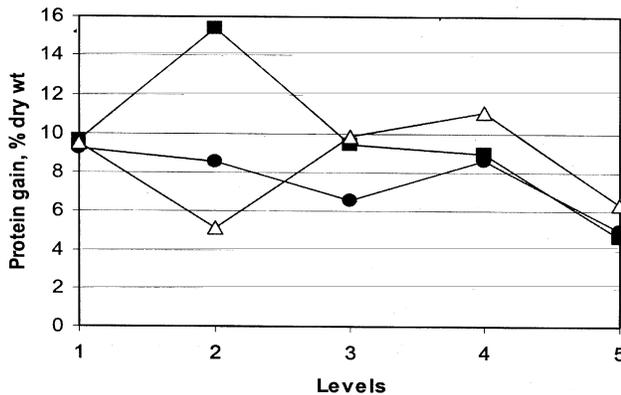


Fig. 2— Nitrogen supplementation: (●) Treatment 1: urea; (■) Treatment 2: (NH₄)₂SO₄; (▲) Treatment 3: (NH₄)₂SO₄:Urea (1:1, w/w)

statistically higher ($p < 0.05$) than the other results observed. Protein content in the final bioprocessed product reached roughly 22% (dry wt), which means 3.5-fold the original protein content of the unfermented residue. Without supplementation, bioprocessed samples reached around 16% of protein (dry wt) at day two (48 h).

Similarly, protein content of fermented apple pomace²² supplemented with ammonium sulphate increased from 4.8% to 13.1% (dry basis). Poor results were found when urea was used as nitrogen supplementary source. Nitrogen sources affect environmental conditions, which in turn may impact microorganism growth²³. They can play an important role in affecting pH changes in substrate during bioprocessing. In present case, at the beginning of bioprocessing, pH levels (4.2–4.3) were maintained close to initial values after 48 h of incubation. A little increase was observed for samples supplemented with urea and these changes were greater as urea concentration increased, reaching 5.02 for level 5. It was assumed that when it comes to this specific case, pH seems not to be related to the poor results observed for urea supplementation, since pH 5 is still good for the growth of *S. cerevisiae*. Experimental results corroborate with a study²⁴, where protein enrichment of cassava by *Rhizopus* was inhibited at higher doses of urea. Haddadin *et al.*²⁵ showed good results for SSB of olive waste. Increase in protein levels were obtained when substrate was supplemented with 0.5% (NH₄)₂SO₄. Oboh & Akindahunsi⁵ observed promising results for SSB by *S. cerevisiae* of cassava derivatives in media supplemented with urea, magnesium sulphate and citric acid and obtained doubled final protein content

of 'gari', a typical Caribbean food, and cassava flour. Yang²⁰ showed that *S. cerevisiae*, selected among several amylolytic organisms, increased protein content of sweet potato residue supplemented with (NH₄)₂SO₄/urea (1:1, w/w) from 6.01% to 14.04% after 72 h of fermentation.

Conclusions

Solid state bioprocessing of pineapple waste by *S. cerevisiae* can successfully enrich its protein content, with or without nitrogen supplementation. Further studies are needed to examine relevant factors, such as alcohol measurement in the final bioprocessed product and complementary nutritional studies, but the fermented pineapple waste obtained in this study seems to be a promising non-conventional feed alternative. Besides this, scale-up studies might be conducted in order to develop the present process, which would offer a low-cost technology for using a low-grade and abundant residue from fruit industry.

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