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Glucose syrup and corn steep liquor as alternative to molasses substrates for production of baking-quality yeast

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In the present study the possibility of substituting molasses with glucose syrup produced from corn starch and supplemented with corn steep liquor (CSL) (a by-product of corn-starch extraction process) as a source of proteins, vitamins and oligo-elements was investigated. For this purpose, fed-batch fermentations with constant volume were carried out to evaluate the influence of molasses sugars replacement (0-30-60-100%) by glucose syrup on biomass growth, yield coefficient ($Y_{X/S}$) for substrate conversion into biomass, nitrogen and phosphorus (as P_2O_5) content of final biomass. A mixture of cane and beet molasses was used, and integrations of nitrogen, phosphorus, biotin, thiamin, inositol, nicotin amide, nicotinic acid, piridoxin and zinc were supplied For fermentations with 100% of sugars as glucose, addition of corn steep was considered and calculated on the basis of CSL and molasses composition.
A substitution of molasses sugars up to 60% led only to a slight reduction in biomass nitrogen content. Complete replacement of molasses sugars brought to reduced biomass yield (partly due to the absent buffering power of molasses and then to an excessive pH reduction of medium during fermentation), $Y_{X/S}$, nitrogen content, and higher phosphorus content. In this case, addition of CSL could partly compensate the negative effects.

1. Introduction

Saccharomyces cerevisiae (Bakers' yeast) is the most popular industrial microorganism because it utilises cheap materials for growth and production, and it is able to ferment many sugars. The least expensive and most common source of metabolizable sugars for Baker's yeast fermentation is molasses, a by-product from sugar production. This is true in regions where molasses is produced or at least easily available at low price, since raw materials are major contributors to the cost of production of low value products such as Bakers' yeast. In Italy, for example, due to modifications in the European legislation, most of the sugar producing plants have been or will be obliged to close. That's why exploration of other inexpensive and locally available fermentable sources of sugar for total or partial replacement of molasses is essential and different alternative substrates have been already investigated by other authors (Champagne et al., 1990; Daniel Ferrari et al., 2001; Ejiofor eet al., 1996; Khan et al., 1995).

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In the present study employment of glucose syrup for this purpose was investigated mainly because, even though not necessarily cheaper than molasses, can be easily available and with standard and constant characteristics, unlike molasses the composition of which will vary a lot and, generally, there is no way the yeast manufacturers can change this fact except they can mix various qualities.

Obviously, *Saccharomyces cerevisiae* requires other nutritional factors than carbohydrates, such as vitamins, minerals, nitrogen and phosphate compounds. Even though both beet and cane molasses contain minerals, vitamins and nitrogen compounds, supplements of nitrogen, phosphorus and oligoelements are generally necessary. That's why using glucose syrup will inevitably lead to an increased need of nutrients addition. In the present research addition of corn steep liquor (CSL), a by-product from corn starch processing, was considered due to its typical content of many vitamins, amino acids, nitrogen compounds and mineral matters, which makes it a very suitable nutritious substratum in industrial fermentations (Filipović, 2002).

The aim of the study was to verify the feasibility of molasses replacement by glucose syrup and of the addition of corn steep as nutrients supplier.

2. Materials and Methods

2.1 Inoculum

A commercial inoculum of *S. cerevisiae* was provided by a local baker's yeast industrial plant. Yeast cream coming from the penultimate step of the industrial production, was used as inoculum. The cream was diluted to ~ 8 g dry weight /l for the fermentations.

2.2 Fermentations

All fermentations were carried out in fed-batch operation, in 2 L flasks, with a constant medium volume of 0.6 L, in a thermostatic orbital shaker at 30°C, 200 rpm for 8 h (ALC 4237R centrifuge). Every 2 h, a 10% of volume was discarded for analysis and substituted with an equal volume of fresh concentrated medium (the sugar concentration of this medium was chosen on the basis of screening trials in order to bring the sugar concentration back to the starting value of 50-60 g/l), and the pH value back to 5 with H_2SO_4 or $NaHCO_3$. The molasses (a mixture of sugar cane and beet molasses) was provided by the same baker's yeast industrial plant, opportunely centrifuged pasteurized and diluted before using. Nitrogen (as 30% NH_4OH), phosphorus (as 85% H_3PO_4), biotin, thiamin, piridoxin, nicotinamid, nicotinic acid, inositol and $ZnSO_4$ were added according to the indication given by the baker's yeast factory. The glucose syrup (32% dry matter) and the corn steep were provided by a local starch factory (Roquette Italia).

All the fermentations were carried out in quadruplicate.

2.3 Analytical methods

Broth samples collected every two hours and at the end of the fermentations, were analyzed for pH, biomass dry weight content and sugar content. The cell dry weight was determined by measurement of the optical density (OD) at 660nm after centrifugation and water dilution and using a calibration curve for DO versus dry weight (determined in oven at 70°C until constant weight). At the end of the fermentation, the recovered biomass was also analyzed for the nitrogen content (by the Kjeldahl method) and the phosphorus content as P_2O_5 (by a spectrophotometric method, in which the sample is

mineralized with sulfuric acid and cupric catalyst, neutralized with NaOH, added with the vanadate-molybdate reagent and read at 420 nm).
Sugars were determined as total reducing sugars (by the Fehling method) after sucrose inversion with HCl hot hydrolysis.

2.4 Experiments
In order to investigate the partial to complete replacement of molasses sugars by glucose syrup, fermentations with increasing percentage of sugars coming from glucose (0-60-100%) were carried out. In these trials nutrients supplies were always the same.
In case of only glucose syrup as sugars source, addition of corn steep at a concentration of 50 g/l was investigated.

Results were compared on the basis of the following parameters:

- pH trend with time
- sugar trend with time
- Biomass trend with time χ (g dry weight /l)
- total biomass increase $\Delta\chi$ (g dry cell weight)
- Substrate conversion yield $Y_{X/S}$ (g sugars consumed / g dry biomass produced)
- Nitrogen content of final biomass N% (g nitrogen / 100 g dry cell mass)
- Phosphorus content of final biomass P_2O_5 % (g P_2O_5 / 100 g dry cell mass)

2.5 Statistical analysis
The influence of molasses sugar replacement with glucose syrup, as well as of corn steep addition, on the considered parameters was assessed by Anova analysis and post hoc Tukey's test for mean discrimination at a confidence level higher than 95% ($P < 0.05$), by the statistical software SPSS® v.13.0.

3. Results

3.1 Replacement of sugars molasses with glucose syrup
Biomass growth was significantly influenced by substitution of molasses sugar up to a 60%, while with only glucose syrup as carbohydrate source, the yeast growth was blocked (Fig. 1), as confirmed by the sugar concentration (Fig. 2). This was due to both a decrease in the nutrients other than carbohydrate (since molasses contain some nutrients) and to a reduced medium buffering capacity when molasses was replaced, as evidenced by pH trend (Fig. 3) (Thomas et al., 2002). Even though for fermentation with 100% glucose syrup, pH was regulated every hour instead of every 2, this was not enough.
Fermentations with 60% sugars coming from glucose syrup gave a similar final biomass in terms of phosphorus content but with lower nitrogen content, while using only glucose syrup the phosphorus content statistically increased.

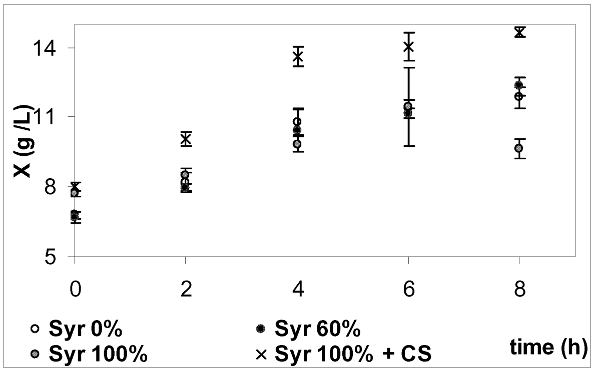

Fig. 1 Biomass growth at different percentages of molasses replacement.

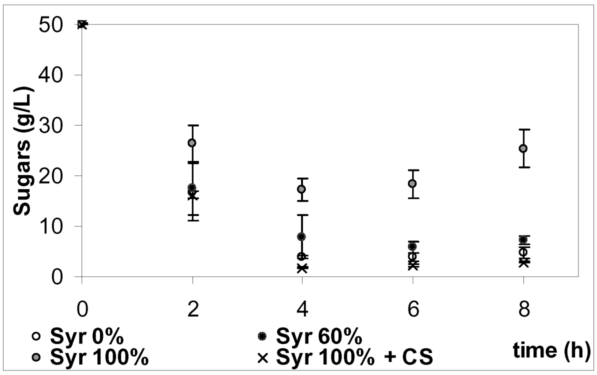

Fig. 2: Sugar concentration (before concentrate medium addition) during fermentations at different percentages of molasses replacement.

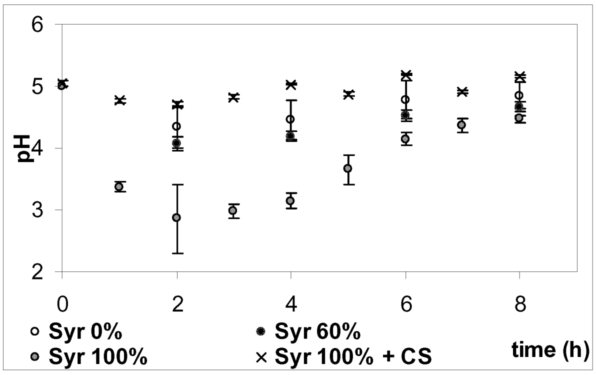

Fig. 3: Variation of medium pH (before concentrate medium addition) during fermentations at different percentages of molasses replacement.

Table 1: Statistical comparison of fermentation results at different percentages of glucose syrup addition and with corn steep supplementation (same letter indicates means not statistically different according to the Tukesy's post-hoc test).

Fermentation conditions	**ΔX tot (g s.s.)**	**$Y_{X/S}$ tot.**	**P_2O_5 %**	**N_2 %**
100% Molasses	5.01 ^{b}	0.063 ^{b}	5.03 ^{c}	73.62 ^{b}
60% Syrup	5.46 ^{b}	0.066 ^{b}	4.63 ^{c}	64.86 ^{c}
100% Syrup	3.23 ^{c}	0.050 ^{c}	6.52 ^{b}	70.91 ^{bc}
100% Sciroppo + CS	6.64 ^{a}	0.081 ^{a}	9.53 ^{a}	87.18 ^{a}

3.2 Fermentation with only glucose syrup and corn steep

In order to try and solve the incurred problems with the complete replacement of molasses sugars with glucose, supplementation of nutrients with corn steep was investigated. Considering that the amount and quality of the biomass obtained with 60% syrup was almost the same as that obtained with only molasses, the nutrient composition of the former substratum was taken as the optimal reference. Then, on the basis of molasses and corn steep composition, we calculated to add corn steep at 50 g/l concentration. This amount should replace all the molasses nutrients (except for calcium that was added as $CaCO_3$) but also some of the given supplements, that's why we decided not to add nicotinic acid, inositol, thiamin, zinc, nitrogen and phosphorus anymore.

Results (Figs. 1-3 and Table 1) showed a higher biomass growth with a lower sugars consumption (lower $Y_{\chi/S}$), probably also thank to the buffering power of the CS (Fig. 3), but a decidedly higher nitrogen and phosphorus content of the recovered biomass, which could negatively influence the fermentative power of the final baker's yeast (not investigated parameters).

4. Conclusions

Our results showed that molasses sugars could be replaced by glucose syrup for a 60% without any statistical influence on biomass growth, phosphorus biomass content and substrate conversion yield. When molasses was completely replaced, the reduced buffering power and nutritional value of the medium led to a reduced biomass growth. In this case addition of corn steep could buffer the medium and a higher biomass yield was observed. Corn steep could also give the advantages of lower sugars consumption (higher substrate conversion yield) and a reduced nutrients supplementation, but phosphorus and nitrogen composition of final yeast was statistically higher, underlining the need for a better substrate optimization.

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