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Simultaneous production of sugars and ethanol from inulin rich-extracts in a chemostat

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SUMMARY

Incomplete fermentation of inulin-containing extracts by Saccharomyces diastaticus *allows the simultaneous production of ethanol and syrups with increased fructose content. The yeast strain used ferments sucrose and inulin small polymers but does not easily ferment inulin large polymers. After batch fermentation a production of 62.5 g/L ethanol and 75 g/L of sugars containing up to 94 % fructose can be obtained. A continuous fermentation was performed in a chemostat permitting the adjustment of both productions according to the dilution rate with a maximal ethanol productivity of 3.9 g/L.h.*

INTRODUCTION

Several crops of the Compositae family, such as Jerusalem artichoke, contain inulin as carbohydrate reserve. Inulin is composed of D-fructose residues linked in the ß-1,2 position, forming linear chains containing also glucose residues. Their fructose contents varies according to the mean degree of polymerization (MPD) (Bacon and Edelman, 1951) which has been found to depend on the cultivar (Chabbert et al., 1985b), the growth and storage conditions, the date of harvest (Chabbert et al., 1983). With a potential sugar production of 5-14 tons per hectare, Jerusalem artichoke is a valuable substrate for inulin, fructose, or ethanol production.

Ethanol production is obtained by fermentation of acid hydrolyzed extracts, using a conventional *Saccharomyces cerevisiae* yeast, or without hydrolysis, using yeasts with inulinase activity as *Kluyveromyces fragilis*. Because of their high contents in fructose, fructans, like inulin, are interesting substrates for the production of fructose syrups. These syrups are widely used in the food industry. Indeed, fructose is an interesting sweetening agent because of its high sweetening power (Pawan, 1973) and of its nutritional and technological properties (Guiraud and Galzy, 1990). Chemical or enzymatic hydrolysis of inulin are easily performed. Fructose enrichment from inulin can be realized at two levels : either after hydrolysis, by chromatography, like for isomerose or before hydrolysis, by precipitating the high molecular weight compounds (high MPD) or by eliminating the low molecular weight fractions (low MPD). The precipitation of high MPD inulin can be made by associating ethanol and cold effects (Chabbert et al., 1985b) or by fermentation. In a previous work yeast strains were selected for this purpose (Hermann and Guiraud, 1990). The aim of the present work is to realize a simultaneous production of ethanol and inulin or fructose syrups by batch and continuous fermentation of Jerusalem artichoke extracts with *Saccharomyces diastaticus*. The interest of the continuous process is the control of both productions through dilution rate adjustments.

MATERIALS AND METHODS

Fermentation conditions

Yeast

S. diastaticus NCYC 625 was used

Jerusalem Artichoke extract preparation

The Jerusalem artichoke used was the "Violet de Rennes" cultivar. The extracts were prepared as previously described (Chabbert et al., 1985a). The pH was adjusted to 3.5. Tubers harvested in November gave an A extract while tubers harvested in December gave a B extract.

Yeast culture and fermentation techniques

Cell cultures for inoculating the fermentation media were grown in a yeast-extract (5%), sucrose (5%) medium. A reactor S.G.I.-SET 2M containing 1 L of non sterile medium was used for batch

and continuous fermentations. This reactor was stirred at 200 rpm without aeration. The temperature was set at 30°C, and the pH at 5 using 2N NaOH solution. In continuous fermentation a pump system maintained a constant liquid volume. The dilution rate was adjusted between 0.025 and 0.14 h^{-1} (on a total volume basis). The system was run in non-aseptic conditions.

Analysis

Cell populations were determined by direct microscopic count using a Thoma's haemocytometer. Dry matter of the extraction juices was obtained by weighing after lyophilization. Jerusalem artichoke extracts were purified by defecation and concentrated under partial vacuum to 400 g sugar/L. The high molecular weight polyfructosans (HMWP) were precipitated with ethanol (50% Vol/Vol) during 48 h at 4°C (Chabbert et al., 1983). Reducing sugars were assayed by the dinitrosalicylate method (Bernfeld, 1955). Total sugar content was determined by the same method after hydrolysis (pH adjusted to 2 with H_2SO_4 ; 30 min at 120°C). Fructose and glucose were assayed by the Boehringer enzymatic technique. Qualitative analysis of polyfructosans was performed by HPLC (Conrad and Palmer, 1976). Ethanol was assayed by gas chromatography (Chabbert et al., 1985a).

RESULTS AND DISCUSSION

Characterization of the Jerusalem artichoke extracts.

Results of the HPLC analysis of the two extracts are shown on figure 1. The A extract contained less low molecular weight inulin polymers (peaks 1 to 10) and more high molecular weight polyfructosans (peak >10) than the "latest" B extract.

Table 1: Sugar composition of extracts A and B

Extracts	Total sugars (g/L)	Reducing sugar (g/L)	Mean Polym.Deg.	F/G	HMWP (%)
A extract	210	8.6	6	5	16
B extract	170	14.4	4.4	3.4	1

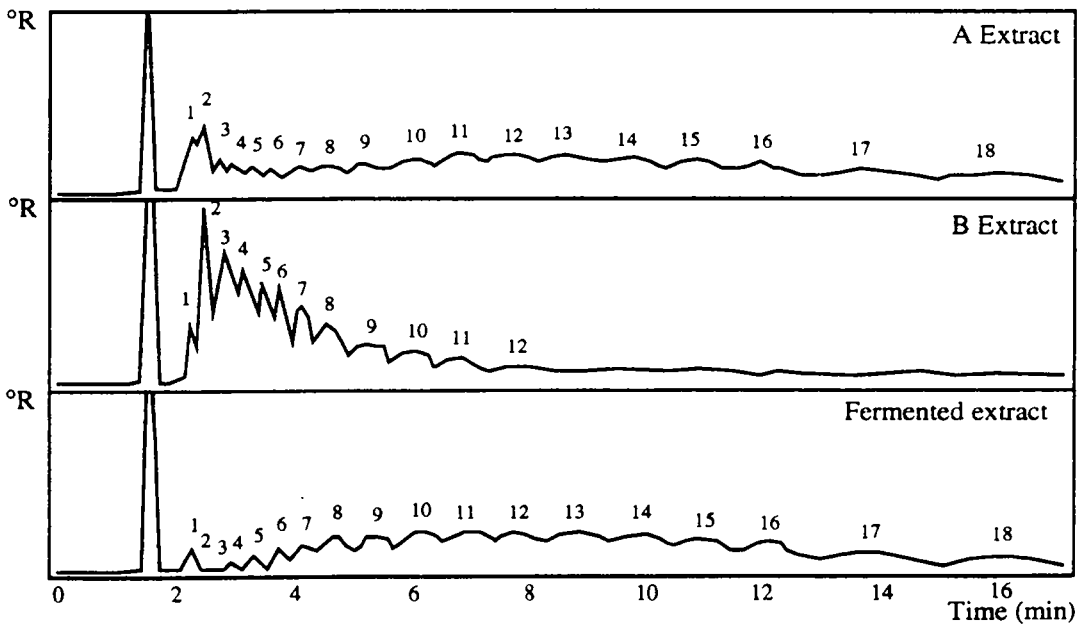

Figure 1 : HPLC Chromatograms of carbohydrates of Jerusalem Artichokes extracts.

Batch fermentation

The extracts were fermented under partial anaerobic conditions. Non sterilized medium was inoculated with 10^8 cells/ml. After 4 days, yeast cell populations were about 2 to 2.5 10^9 cells/ml in all assays. The table 2 summarizes the results of the analysis of fermented juices after 48h. The figure 1 shows the HPLC chromatogram obtained with a fermented A extract.

Table 2 : Batch fermentation of extracts A and B

	Ethanol (g/L)	Sugars recov. (g/L)	Red. sugars (g/L)	Sugar yield[a](%)	F/G[b] ratio	Fructose[c] enrichment	Ethanol yield[d](%)
A extract	62.5	70	8.3	35.7	16.4	3.28	0.40
B extract	67.0	30	2.5	17.5	60	17.7	0.47

(a) Percentage of recovered sugars after fermentation. (b) The F/G ratio was obtained after hydrolysis of the residual sugars. (c) Ratio of final and initial F/G ratios. (d) Ratio of ethanol produced and sugar consumed (Yp/s)

Saccharomyces diastaticus consumed about 64.3 to 82.5 % of the initial sugar content, giving an ethanol production of 62.5 to 67 g/L. The reducing sugar content of the different juices varied between 2 and 10 g/L during the fermentations. This might suggest that the sugar hydrolysis is not the limiting step of the fermentation. In all cases there was a fructose enrichment in the residual sugars relatively to the glucose level : the F/G ratio was improved. Thus the composition of residual juice depends upon the fermentation duration.

The "late" extract (B) was fermented slightly more easily than the extract (A). It is known that the ethanol yield depends strongly of the harvest date of tubers and can vary from 0.3 g/g for very "early" extracts to 0.48 g/g for the latest. Both A and B extracts can be considered as "late" extract with low F/G ratio compared with "early" extracts where this ratio can reach 17! (Chabbert et al, 1985b).

Chemostat fermentation

Appropriate fermentation conditions for the chemostat culture were defined from batch data : since the biomass and ethanol yields were found constant along batch fermentation, a direct relationship between all the culture parameters could be expressed. Ethanol was chosen as the most convenient indicator of the fermentation progress and a linear plot of the growth rate against the alcohol concentration was obtained for extracts A and B, with no significant differences as observed on figure 2. Using this plot, and according to an objective expressed as ethanol concentration, the dilution rate could be chosen since in chemostat culture, the dilution rate is equal to the growth rate. From this simulation, if a ethanol production of 60 g/L is expected with the A extract a dilution rate of 0.025 h^{-1} is needed.

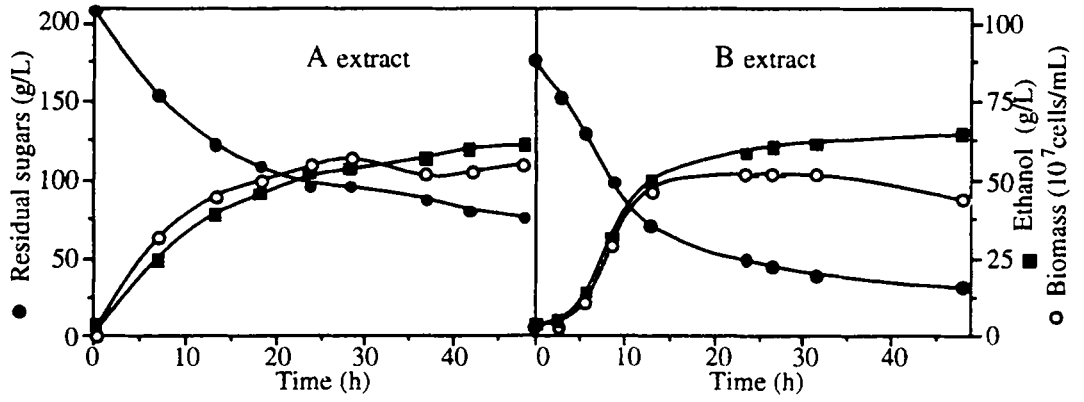

Figure 2 : Time course of batch fermentation of A and B extracts.

The fermentation parameters of *S. diastaticus* were studied at steady state with the two extracts. The main results are presented in Table 3. As expected, the ethanol production decreased and fructose level increased, when the dilution rate is increased. As during batch experiments, the B extract, with low sugar concentration and low F/G ratio, gave better ethanol production and poorer fructose production than the A extract. However, the results differed significantly from the values anticipated from batch data : i) at low dilution rates, the ethanol level is 20% (A extract) and 40% (B extract) lower than expected ; ii) the yield of growth versus product formation was not constant as observed during batch experiments. Extrapolation to zero of the curve P = f(D) gave ethanol concentration much lower than the usual tolerance of the strain used (75 g/L). These observations suggest that the rate limiting step during continuous fermentation was not ethanol. Additional experiments are required to identify the limiting step : the slower hydrolysis rate of low molecular weight inulin fragments could be a good explanation.

Table 3 : Chemostat parameters

	Dilution rate (h^{-1})	Ethanol (g/L) model	Ethanol (g/L) measured	Yp/s	Res. sugars (g/L)	F/G	Biomass (10^7 cells/mL)
A extract	0.04	59.6	40.0	0.48	114	8.2	98
	0.05	55.7	38.1	0.47	120	8.0	95
	0.10	36.1	35.1	0.46	125	7.5	85
	0.14	20.5	25.2	0.44	148	6.7	55
B extract	0.025	64.3	55.8	0.49	61.4	7.9	50
	0.05	55.7	46.6	0.44	69.0	7.3	80
	0.08	45.3	41.4	0.40	72.0	6.8	80
	0.10	38.4	38.7	0.39	75.4	6.1	96

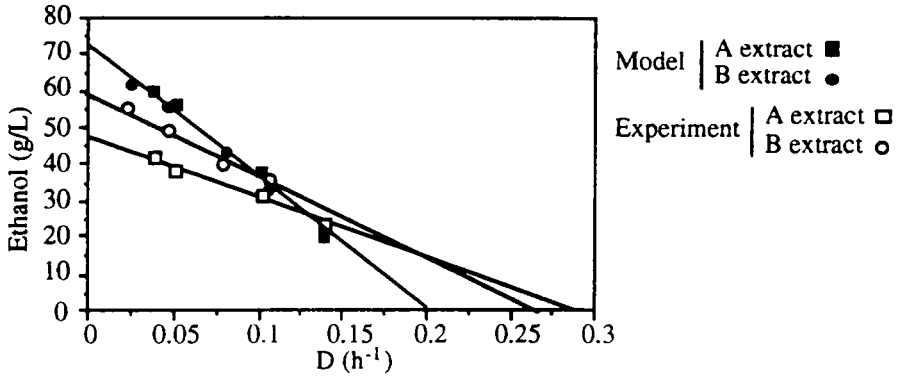

Figure 3 : Comparison of relationships ethanol production / dilution rate obtained

CONCLUSION

Fructose enrichment of Jerusalem artichoke extracts by incomplete fermentation with selected yeast strains seems to be an attractive technique. This process applies to the raw extract and purification steps are widely simplified. Uninteresting sugars, such as mono or disaccharides and low molecular weight polymers, are directly valorized as ethanol and yeast biomass. The production of rich fructose containing syrups is easier with extracts from "early" harvested tubers which contain more high molecular weight polyfructosans. In every case, the fructose content of syrups is at least 90 %. Therefore, this fermentative enrichment technique appears as an interesting alternative to other processes, for example, chromatographic enrichment. In the best case, the ethanol productivity (on a total volume basis) reached 3.9 g/L.h. It would probably have been possible to exceed this value, at the expense of higher sugar losses.

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