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SHORT COMMUNICATION

Effect of glucose concentration on the rate of fructose consumption in native strains isolated from the fermentation of *Agave duranguensis*

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Abstract Studies on hexose consumption by *Saccharomyces cerevisiae* show that glucose is consumed faster than fructose when both are present (9:1 fructose to glucose) in the medium during the fermentation of Agave. The objective of this work was to select strains of *S. cerevisiae* that consume fructose equal to or faster than glucose at high fructose concentrations by analyzing the influence of different glucose concentrations on the fructose consumption rate. The optimal growth conditions were determined by a kinetics assay using high performance liquid chromatography (HPLC) using 50 g of glucose and 50 g of fructose per liter of synthetic medium containing peptone and yeast extract. Using the same substrate concentrations, strain ITD-00185 was shown to have a higher reaction rate for fructose over glucose. At 75 g of fructose and 25 g of glucose per liter, strain ITD-00185 had a productivity of 1.02 gL^{-1} h^{-1} after 40 h and a fructose rate constant of 0.071 h^{-1}. It was observed that glucose concentration positively influences fructose consumption when present in a 3:1 ratio of fructose to glucose. Therefore, adapted strains at high fructose concentrations could be used as an alternative to traditional fermentation processes.

Keywords *Saccharomyces* · Fermentation · Reaction rate

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Introduction

Glucose and fructose are simple sugars that are found in different foods. These sugars are fermented by yeasts to produce ethanol, water, carbon dioxide, and other components (Arroyo-López et al. 2009). In some studies, it has been shown that *Saccharomyces cerevisiae* prefers to consume glucose; in experiments with both glucose and fructose, glucose is exhausted in the middle of the fermentation, leaving a higher proportion of fructose (Hofer and Jenewein 1999; Wang and Nobel 1998; López et al. 2003; Bisson 1999). In contrast, *S. cerevisiae* has been shown to prefer glucose over fructose consumption during fermentation (Berthels et al. 2004). Bauer and Pretorius (2000) mentioned that these differences in substrate consumption are due to vinous fermentation at the end of fermentation, a residual amount of fructose, and possible contamination in the wort. This may be due to the speed of transport across the membrane or different rates of hexose phosphorylation that occur within the cell (Kunkee 1984; Berthels et al. 2008; Ozcan and Johnston 1995; Reifenberger et al. 1997). Glucose in *S. cerevisiae* not only serves as a carbon source but also acts as a global regulator of metabolism and growth. Repression and induction of glucose are two important regulatory mechanisms that act on glucose transcription levels (Arroyo-López et al. 2009). In the process of making mescal, Michel-Cuello et al. (2008) reported that *Agave salmiana* acid hydrolysis with heat treatment gave a fructose concentration of 155.9 mg/mL

and a glucose concentration of 26.9 mg/mL. The search for yeasts with a preference for fructose is highly desirable by industries involved in alcoholic fermentation (Berthels et al. 2004). Therefore, the aim of this work was to study the influence of different glucose concentrations on the rate of fructose consumption in native strains isolated from the fermentation of *Agave durangensis*.

Materials and methods

Microbiological material

Ten strains of *S. cerevisiae* isolated from the fermentation of *Agave durangensis* were used. The pre-inoculum preparation was performed with 100 mL of YDP broth and incubated at 28 °C for 24 h without agitation to obtain a cell concentration of 1×10^7 cells/mL.

Kinetics of strain selection

Saccharomyces cerevisiae was inoculated into culture medium that was adjusted to 50 g L^{-1} of glucose, 50 g L^{-1} of fructose, 20 % peptone casein, and 10 % yeast extract. The medium was filtered through a sterile 0.45 μm membrane, incubated at 28 °C without agitation, and the fermentation kinetics were analyzed for 48 h by taking duplicate samples every 8 h.

Evaluation of the influence of glucose concentration on the fructose consumption rate

Different concentrations of glucose and fructose as the substrate were used (Table 1). Fermentation with 10 g/L glucose and 90 g/L of fructose in synthetic medium with 20 % peptone casein, and 10 % yeast extract at 28 °C for 24 h without agitation simulated the halfway point of *Agave durangensis* fermentation.

Fermentation profile of the strains studied

Samples from the fermentation kinetics experiments were centrifuged and the supernatants were filtered through 0.45 μm nylon membrane and used for the quantification of sugar (fructose + glucose) and ethanol concentration by high performance liquid chromatography (HPLC) using an Agilent 1200 Series equipment. A Phenomenex Rezex ROA-organic acid H+ (8 %) anion exchange column (300 × 7.8 mm), capable of resolving glucose, fructose and ethanol peaks, was used with an Agilent refractive index detector. Injection of standards of glucose, fructose and ethanol (Sigma™) were used to obtain a calibration curve with r^2 upper than 0.999 in all the cases. The mobile phase was 0.005 N H_2SO_4, the flow rate was 0.5 ml/min elution, the column temperature was 65 °C, the refractive index temperature was 35 °C, and the injected volume was 5 μL (Téllez Luis et al. 2002).

Table 1 Experimental design to evaluate the effect of glucose on fructose consumption

Treatment	Glucose concentration (g/L)	Fructose concentration (g/L)
1	10	90
2	25	75
3	33	67
4	50	50
5	67	33
6	75	25
7	100	0
8	0	100

The yield and productivity reported here were based on ethanol production. The yields were calculated dividing the concentration of ethanol produced at the end of the fermentation by the concentration of glucose + fructose consumed at the end of the fermentation. On the other hand, productivity was calculated dividing the concentration of ethanol produced at 40 h of the fermentation.

Determination of kinetic parameters

The consumption rates of sugars during the exponential growth phase were calculated as the reaction constant rate of a first order kinetics, following the procedure described by Leevnspiel (1987). The:

$$-\frac{dC_A}{dt} = kC_A$$

By integrating the above equation, we obtain:

$$-Ln\frac{C_A}{C_{A0}} = kt$$

The values obtained from the fermentation profile were analyzed for 0–32 h for all experiments. At each point, the maximum substrate consumption present in the medium was observed. The value of the slope, k, represents the rate constant of glucose and/or fructose.

Statistical analysis of data

Data for the rate constants of the response variables (reaction rate) of two fermentations for each strain were used for analysis of variance comparison of means by LSD with a confidence level of 95 %. The statistical method

Table 2 Rate constants (k) of *Saccharomyces cerevisiae* strains in equal concentrations of glucose and fructose

Strain	Glucose rate constant (h^{-1})	Fructose rate constant (h^{-1})
ITD00185	$0.0862^{a} \pm 0.0037$	$0.0518^{a} \pm 0.0020$
ITD00205	$0.0752^{b} \pm 0.0304$	$0.0316^{bc} \pm 0.0081$
ITD00115	$0.0612^{bc} \pm 0.0004$	$0.0381^{ab} \pm 0.0091$
ITD00112	$0.0484^{c} \pm 0.0066$	$0.0380^{ab} \pm 0.0041$
ITD00201	$0.0452^{c} \pm 0.0151$	$0.0225^{bc} \pm 0.0073$
ITD00215	$0.0446^{c} \pm 0.0083$	$0.0143^{c} \pm 0.0053$
ITD00186	$0.0427^{c} \pm 0.0221$	$0.0244^{abc} \pm 0.01000$
ITD00196	$0.0412^{c} \pm 0.0237$	$0.0181^{bc} \pm 0.0054$
ITD00068	$0.0369^{c} \pm 0.0052$	$0.0185^{bc} \pm 0.0023$
ITD00119	$0.0349^{c} \pm 0.0029$	$0.0184^{bc} \pm 0.0024$

a, b, c, d, e, ab, bc, cd, dc, abc, bcd Means sharing the same letter within a column are not significantly different at confidence level of 95 % been "a" the better treatment

used for comparing the strains was one-way variance (ANOVA).

Results

The rate constants (k) shown in Table 2 were calculated as reported by Leevnspiel (1987) from the fermentation profiles (sugar concentration vs. time) of the strains tested for both glucose and fructose. Strain ITD-00185 exhibited the highest consumption rates of glucose and fructose. Therefore, this strain was selected to analyze the effect of glucose concentration on the rate of fructose consumption.

The parameters of yield, productivity, and rate of reaction for glucose and fructose were calculated during fermentation of strain ITD-00185 at different glucose/fructose ratios (Table 3). It can be seen that yields and productivity are higher in the media where fructose is the principal component. This can be explained considering that this strain was isolated from an environment with both sugars, but with fructose as major component (90 %) (Mancilla-Margalli and López 2006). This means that low concentrations of glucose increase the yeast fermentation capabilities, since a similar behavior was observed over the consumption rates of sugars calculated as the reaction constant rate of a first order kinetics (k).

Treatment 2 (Glucose/Fructose ratio of 25/75) had the highest productivity after 40 h; the strain was able to consume up to 80 % of the sugars in the medium in less time than using the other treatment methods. The reaction rates of glucose and fructose were diminished when only fructose or glucose was added in the medium of the fermentation. In addition, when lower glucose concentrations were used, the productivity significantly increased.

Discussion

Saccharomyces cerevisiae strains were studied to determine their capacity to consume fructose under different culture conditions. The kinetic parameters were calculated to determine the influence of glucose concentration on the rate of fructose consumption. Strain ITD-00185 showed the highest consumption rates compared to other strains tested, at 50 g/L of glucose and fructose.

During fermentation processes with *S. cerevisiae*, glucose is consumed at a faster rate relative to fructose. In sluggish fermentations, the fermentation rate is reduced when most of the glucose has been consumed. In fact, the fermentation can be stopped when a significant fructose concentration is present. According to the literature, the residual glucose in wines with stuck fermentation is ten times lower than the fructose concentration (Gafner and Schütz 1996; Guillaume et al. 2007; Wieczorke et al. 1999).

Guillaume et al. 2007 performed a study using a strain derived from the fermentation of Champagne, Fermichamp

Table 3 The fermentation profile of strain ITD-00185 at different substrate concentrations

Glucose/fructose ratio (g Glucose/g Fructose)	Yield ($Y_{p/s}$)	Productivity (gL^{-1} h^{-1})	Glucose rate constant (h^{-1})	Fructose rate constant (h^{-1})
10/90	$0.40^{a} \pm 0.005$	$0.93^{ab} \pm 0.07$	$0.136^{a} \pm 0.06$	$0.045^{b} \pm 0.002$
25/75	$0.41^{a} \pm 0.002$	$1.02^{a} \pm 0.01$	$0.168^{ab} \pm 0.01$	$0.071^{a} \pm 0.001$
33/67	$0.42^{a} \pm 0.004$	$0.90^{ab} \pm 0.05$	$0.070^{bc} \pm 0.0002$	$0.033^{cd} \pm 0.004$
50/50	$0.40^{a} \pm 0.05$	$0.72^{ab}c \pm 0.08$	$0.091^{c} \pm 0.0005$	$0.041^{bc} \pm 0.002$
67/33	$0.42^{a} \pm 0.03$	$0.67^{bcd} \pm 0.09$	$0.037^{c} \pm 0.0003$	$0.011^{e} \pm 0.002$
75/25	$0.38^{ab} \pm 0.04$	$0.59^{cd} \pm 0.04$	$0.028^{c} \pm 0.001$	$0.017^{c} \pm 0.003$
100/0	$0.29^{b} \pm 0.1$	$0.45^{cd} \pm 0.1$	$0.022^{c} \pm 0.002$	
0/100	$0.33^{ab} \pm 0.001$	$0.40^{d} \pm 0.01$		$0.022^{de} \pm 0.0011$

a, b, c, d, e, ab, bc, cd, dc, abc, bcd Means sharing the same letter letter within a column are not significantly different at confidence level of 95 % been "a" the better treatment

V5 (*S. cerevisiae*). In this study, the HXT3 gene was changed and it was suggested that a possible relationship exists between the capacity to consume fructose and fructose transport ability. The uptake of glucose and fructose using the Fermichamp V5 strain over other strains were compared. It was determined that the HXT3 gene mutation allows the *S. cerevisiae* strain to have different consumption rates. Similarly, the ITD-00185 strain transports glucose and fructose, and causes phosphorylation of both sugars at lower glucose concentrations. Thus, the rate of fructose consumption is increased.

In Agave fermentations, the glucose and fructose concentrations are different from those in wine, which have a 9:1 ratio of fructose to glucose (Michel-Cuello et al. 2008; Mancilla-Margalli and López 2006). Under these conditions, the yeasts *Candida stellata* and *Zigosaccharomyces Balli* have been found to have a clear preference for the consumption of fructose (Salmon 1989; Ciani et al. 2000; Sousa-Dias et al. 1996).

Results obtained with *S. cerevisiae* strains ITD-00115 and ITD-00185 showed 80 % consumption of similar concentrations of glucose and fructose. These strains are adapted to consume fructose even when the glucose fermentation environment is depleted. This is possibly due to adaptation because they were isolated from fermentations with a high fructose concentration (Perez et al. 2005; Díaz-Montaño et al. 2008).

Arroyo-López et al. (2009) studied an inhibition model to estimate the effect of fructose on the growth of *S. cerevisiae*. This study found that 4–6 % fructose stimulated yeast growth; however, 59.56 and 63.85 % fructose inhibited yeast growth and decreased the cell population, respectively. In addition, no differences between strains and isolates were found in the vinous production process of mezcal in Durango, such as strain C9 (codified as ITD-00185 in this study).

Conclusion

Strains isolated from Agave fermentation were able to consume up to 80 % of the sugars present in the culture medium when glucose and fructose were both present in the middle of the fermentation process. The yeast strains ITD-00185 and ITD-00112 had high performance and productivity values because they gave high rates of fructose consumption when low glucose concentrations were present in the medium and low rates of fructose consumption when high glucose concentrations were present. This means that these yeasts are able to assimilate fructose and could be used in potentially troublesome musts and/or difficult environmental conditions. Therefore, reaction rate determination can be a tool used by industries involved in alcoholic fermentation to find better fermentation yeasts.

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