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Sugar utilization by yeast during fermentation

Tony D'Amore, Inge Russell and Graham G. Stewart

Research Department, Labatt Brewing Company Limited, London, Ontario, Canada

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SUMMARY

When glucose and fructose are fermented separately, the uptake profiles indicate that both sugars are utilized at similar rates. However, when fermentations are conducted in media containing an equal concentration of glucose and fructose, glucose is utilized at approximately twice the rate of fructose. The preferential uptake of glucose also occurred when sucrose, which was first rapidly hydrolyzed into glucose and fructose by the action of the enzyme invertase, was employed as a substrate. Similar results were observed in the fermentation of brewer's wort and wort containing 30% sucrose and 30% glucose as adjuncts. In addition, the high levels of glucose in the wort exerted severe catabolite repression on maltose utilization in the *Saccharomyces uvarum (carlsbergensis)* brewing strain. Kinetic analysis of glucose and fructose uptake in *Saccharomyces cerevisiae* revealed a K_m of 1.6 mM for glucose and 20 mM for fructose. Thus, the yeast strain has a higher affinity for glucose than fructose. Growth on glucose or fructose had no repressible effect on the uptake of either sugar. In addition, glucose inhibited fructose uptake by 60% and likewise fructose inhibited glucose uptake by 40%. These results indicate that glucose and fructose share the same membrane transport components.

INTRODUCTION

The glucose and fructose transport system of yeast has remained controversial over the last two decades [1,7]. Recent studies indicate that the uptake of glucose and fructose in *Saccharomyces cerevisiae* is mediated by both high and low affinity transport systems [2,3]. Low affinity transport appears to occur by constitutive facilitated diffusion whereas the high affinity transport system is dependent upon the activity of phosphorylating enzymes. Although extensive studies have not been performed, it is generally accepted that glucose and fructose compete for the same membrane carrier [4,7]. Since many commercial substrates (eg., brewer's wort) contain a mixture of sugars, it is important to examine the role of these sugars on hexose transport and their effect on the uptake of more

Correspondence: T. D'Amore, Research Department, Labatt Brewing Company Limited, 150 Simcoe Street, London, Ontario, N6A 4M3, Canada.

complex sugars such as maltose and maltotriose.

Sucrose is frequently used in many parts of the world as a carbohydrate adjunct by breweries and other fermentation-based industries employing yeast. It is a disaccharide composed of D-glucose and D-fructose linked by an α-1,4 glycosidic bond. In the initial stages of fermentation, sucrose is rapidly hydrolyzed into glucose and fructose by the action of the periplasmic enzyme invertase, prior to the sugars being transported across the cell membrane [4,10]. Glucose was observed in this case to be taken up preferentially over the other hydrolysis product fructose [8.9].

Growth of *Saccharomyces cerevisiae* on a medium consisting of a mixture of glucose and fructose also results in the preferential uptake of glucose [4,8,9]. However, when glucose and fructose were added separately, the uptake profile for each sugar was very similar [8,9]. On the other hand, other reports suggest that even when glucose and fructose are fermented separately, glucose is utilized at a faster rate than fructose [4]. These differences may be attributed to the concentration of sugar used in the medium.

The present manuscript reports on the sugar uptake profiles in two brewing yeast strains grown on either glucose, fructose or sucrose, on an equimolar mixture of glucose and fructose and on brewer's wort supplemented with various carbohydrate adjuncts. In addition, the kinetics of glucose and fructose uptake were also investigated.

MATERIALS AND METHODS

Chemicals

D-[U-^{14}C]Glucose (270 mCi/mmol) and D-[U-^{14}C]fructose (267 mCi/mmol) were obtained from ICN Biomedicals (Irvine, CA). All other chemicals were obtained from commercial sources and were of the highest available purity.

Yeast strains and growth medium

The yeast strains used in this study were, with their Labatt Culture Collection numbers, *Saccharomyces cerevisiae* brewing ale strain 3001, and *Saccharomyces uvarum (carlsbergensis)* brewing lager strain 3021. The yeast cells were subcultured in PYN medium which consisted of: peptone, 3.5 g; yeast extract, 3.0 g; KH_2PO_4, 2.0 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; glucose, 100 g; all dissolved in 1 litre of distilled water and adjusted to pH 5.6.

Fermentation conditions

Fermentations were conducted in PYN medium containing varying concentrations of sugar as described in the Results section. Fermentations were carried out at 30°C in 300 ml Erlenmeyer shake flasks containing 100 ml of medium and with constant agitation of 150 rpm. Fermentations with 16° Plato brewer's wort were conducted in 30 l batches in stainless steel fermentors without stirring at 15°C. The yeast inoculum used in all cases was 3.5 g wet weight cells/l.

Estimation of sugar uptake during fermentation

At specified times during fermentation, 10 ml of cell suspension was withdrawn. The samples were centrifuged at 4000 *g* for 10 min. The supernatant was subjected to HPLC analysis for determining sugar concentrations. A Spectra-Physics model SP8100 high performance liquid chromatograph incorporating a Bio-Rad oligosaccharide column (Aminex HPX-42A) for glucose, fructose and maltose analysis and an HPX-87P column for sucrose analysis was employed. The eluant used was double distilled deionized water and the flow rate was 0.6 ml/min. Both operated in conjunction with a Spectra-Physics model SP6040 XR refractive index detector and a Spectra-Physics model SP4270 computing integrator.

Uptake studies

Late-exponential grown yeast cells (16 h) were harvested by centrifuging at 4000 × *g* for 10 min at 4°C. The cells were washed twice with ice-cold 100 mM potassium phosphate buffer pH 6.6 and suspended in the same buffer at room temperature to a cell density of 5 mg dry weight/ml. Uptake studies were initiated by addition of 1 ml of cell suspension to 2 ml of radioactive substrate (1.0 μCi) to the de-

sired final concentration and with constant stirring at 23°C. At 15 s intervals, 200 μl of cell suspension were withdrawn, filtered through 0.45 μm nitrocellulose filters and washed with 5 ml of ice-cold phosphate buffer. The filters were dried, solubilized in liquid scintillation fluid and radioactivity determined on a liquid scintillation counter.

RESULTS

Sugar uptake in synthetic media

The uptake profiles of glucose and fructose were studied with two brewing yeast strains [*Saccharomyces cerevisiae* 3001 and *Saccharomyces uvarum (carlsbergensis)* 3021]. In the first series of experiments sugar uptake studies in PYN media containing either 20% glucose or 20% fructose as substrate were carried out in shake flasks at 30°C. Fig. 1 illustrates that the uptake rates of glucose and fructose with a particular strain were very similar. However, when fermentations were conducted in media containing an equal concentration of glucose and fructose (10% of each sugar), a preferential uptake of glucose was observed with each yeast strain (Fig. 2). It can be calculated that glucose is taken up at about twice the rate of fructose in both strains, although strain differences are apparent for the actual rates of glucose and fructose uptake. These experiments were repeated with several non-brewing yeast strains and the results were very similar to those above (data not shown).

When fermentations were conducted with 20% sucrose, the sucrose was first rapidly hydrolyzed into glucose and fructose by the action of the enzyme invertase prior to the uptake of either sugar (Fig. 3). Again, the results indicate a preferential uptake of glucose over fructose for both yeast strains when sucrose was employed as a substrate.

Sugar uptake in brewer's wort

The effect of carbohydrate adjuncts on sugar utilization profiles in brewer's wort was examined in a *Saccharomyces uvarum (carlsbergensis)* lager brewing strain. Fig. 4 illustrates the effect of employing 30% glucose and 30% sucrose as wort adjuncts and also illustrates the situation with corn adjunct in wort. In these experiments the initial wort gravity

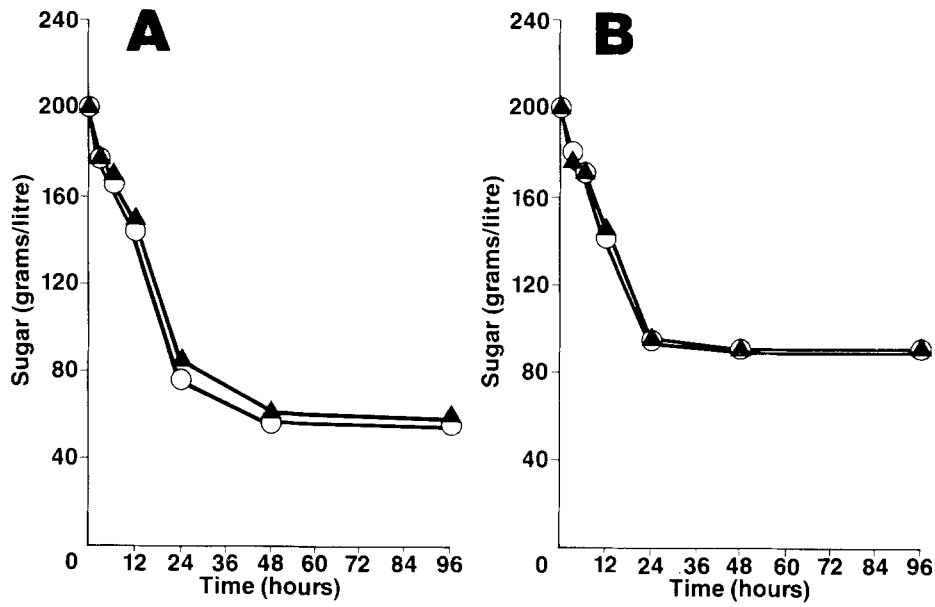

Fig. 1. Glucose and fructose uptake profiles in brewing yeast strains. Fermentations were conducted in PYN media containing 20% glucose (○) or 20% fructose (▲) with *Saccharomyces cerevisiae* 3001 (A) and *Saccharomyces uvarum (carlsbergensis)* 3021 (B) in 300 ml Erlenmeyer shake flasks at 30°C. Inoculum used was 3.5 g wet weight/l.

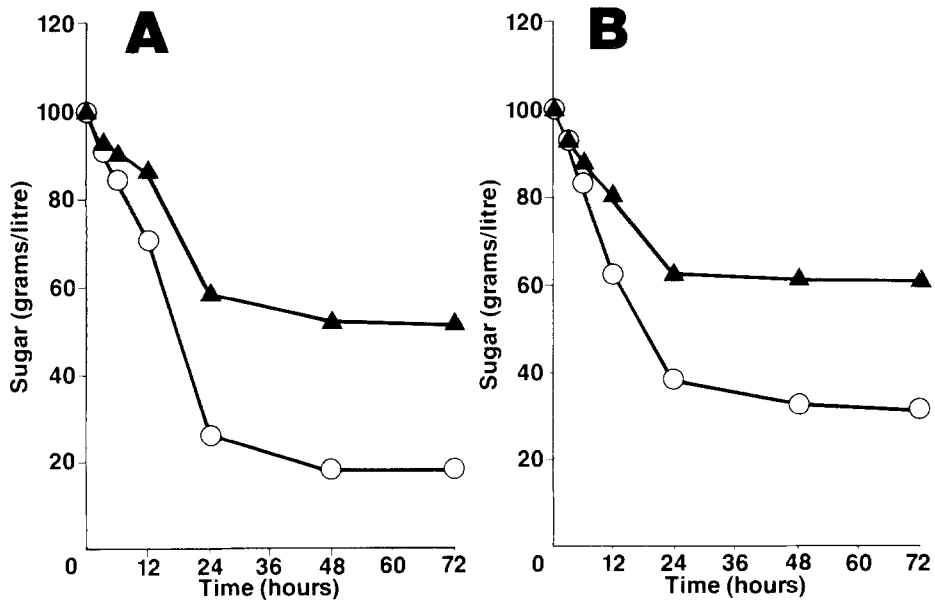

Fig. 2. Simultaneous uptake of glucose and fructose in brewing yeast strains. Fermentations were conducted in PYN media containing 10% glucose (○) and 10% fructose (▲) with *Saccharomyces cerevisiae* 3001 (A) and *Saccharomyces uvarum (carlsbergensis)* 3021 (B) in 300 ml Erlenmeyer shake flasks at 30°C. Inoculum used was 3.5 g wet weight/l.

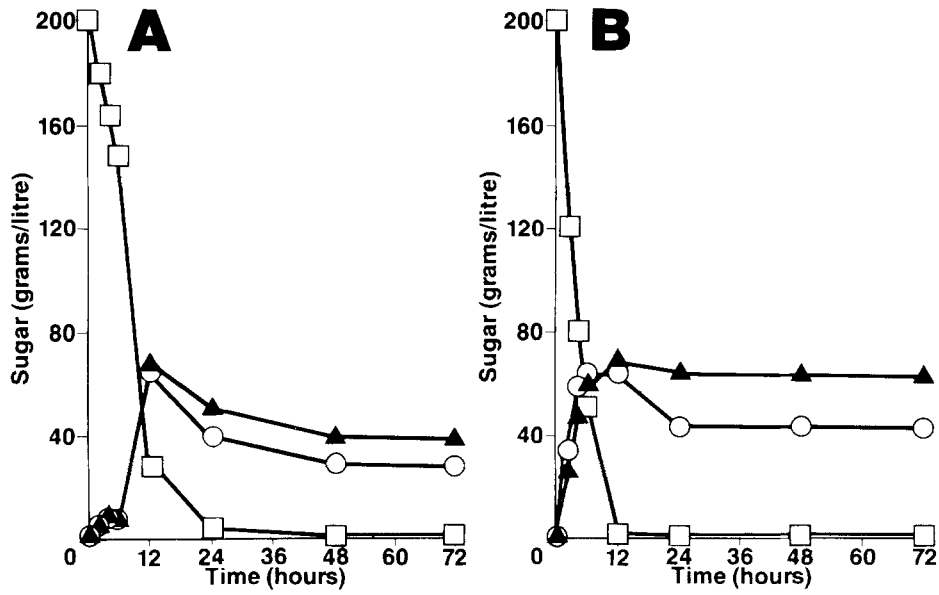

Fig. 3. Sucrose uptake profile in brewing yeast strains. Fermentations were conducted in PYN media containing 20% sucrose (□) with *Saccharomyces cerevisiae* 3001 (A) and *Saccharomyces uvarum (carlsbergensis)* 3021 (B) in 300 ml Erlenmeyer shake flasks at 30°C. Glucose (○) and fructose (▲) concentrations. Inoculum used was 3.5 g wet weight/l.

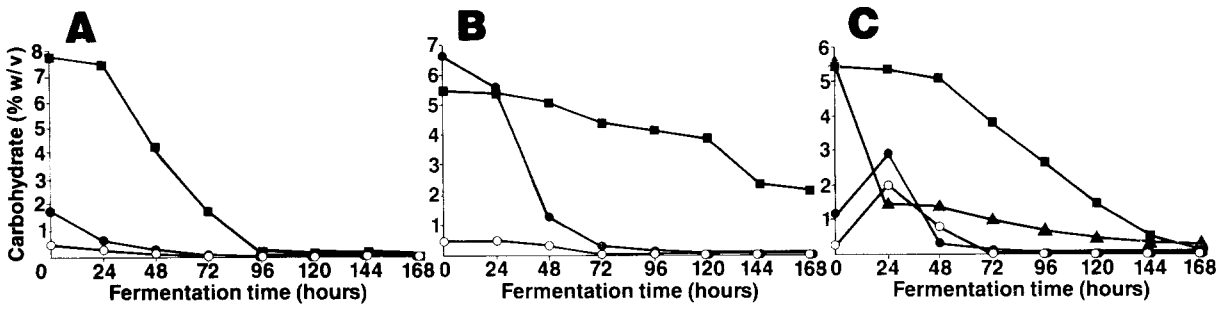

Fig. 4. Uptake of sugars during fermentation of a 16 degree Plato wort by *Saccharomyces uvarum (carlsbergensis)* 3021. Fermentations were conducted with 30% corn adjunct (A), 30% glucose adjunct (B) and 30% sucrose adjunct (C) in 30 l batches in stainless steel fermentors without stirring at 15°C. Glucose (●), sucrose (▲), fructose (○) and maltose (■) concentrations. Inoculum used was 3.5 g wet weight/l.

was maintained constant at 16° Plato and brewery fermentation conditions were employed. It can be seen in the corn adjunct wort fermentation (Fig. 4A) that glucose was preferentially utilized, followed by fructose and then maltose, with some overlap. When the glucose concentration in the wort was increased, an inhibition of fructose and maltose uptake rates was observed, with glucose being rapidly utilized (Fig. 4B). When sucrose was employed as a wort adjunct, an increase in glucose and fructose concentration in the fermentation wort was initially observed as the result of the rapid hydrolysis of sucrose by the enzymatic action of invertase (Fig. 4C). Furthermore, glucose was again observed to be taken up at a faster rate than fructose, with maltose being utilized at a much slower rate.

Transport studies

The results of 0.2 mM glucose and 0.2 mM fructose uptake studies in both glucose and fructose grown *Saccharomyces cerevisiae* 3001 and *Saccharomyces uvarum (carlsbergensis)* 3021 are presented in Table 1. They indicate that growth of yeast on glucose or fructose has no effect on the uptake of either sugar. In addition, it can be seen that in both yeast strains examined glucose is taken up at a faster rate than fructose. In the case of *Saccharomyces cerevisiae* 3001, glucose is taken up at about threetimes the rate of fructose and in the case of *Saccharomyces uvarum (carlsbergensis)* 3021, glucose is taken up at about twice the rate of fructose. In support of these results, Fig. 5 illustrates the kinetics of glucose and fructose uptake in glucose grown *Sac-*

Table 1

Effect of growth substrate on glucose and fructose uptake by *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carlsbergensis)*

Strain	Growth substrate	*v* (nmol/mg dry weight cells/min)[a]	
		0.2 mM Glucose	0.2 mM Fructose
Saccharomyces cerevisiae	Glucose	1.0 ± 0.10	0.39 ± 0.12
	Fructose	1.05 ± 0.16	0.44 ± 0.14
Saccharomyces uvarum (carlsbergensis)	Glucose	0.093 ± 0.005	0.066 ± 0.014
	Fructose	0.11 ± 0.01	0.07 ± 0.02

[a] The results are the average of four trials.

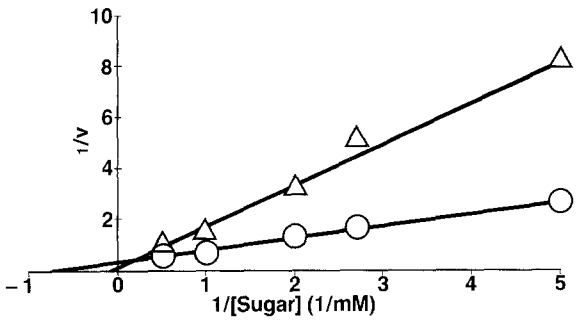

Fig. 5. Double reciprocal plot of glucose and fructose uptake in *Saccharomyces cerevisiae* 3001. Rates of glucose (○) and fructose (△) uptake in the concentration range of 0.2 mM — 2.0 mM were determined as described in the Materials and Methods section.

charomyces cerevisiae. The kinetics for glucose uptake revealed a K_m of approximately 1.6 mM and a V_{max} of approximately 3 nmol/mg dry weight/min. On the other hand, fructose was taken up with a K_m of approximately 20 mM and a V_{max} of approximately 5.5 nmol/mg dry weight/min. These results clearly demonstrate that the yeast has higher affinity for glucose than fructose and thus would transport glucose at a faster rate.

The inhibitory effect of glucose and fructose on each others uptake rate is shown in Table 2. In these experiments, 2 mM of the competing sugar was used to determine its effect on 0.2 mM sugar uptake in glucose grown cells. It can be seen that for both yeast strains, fructose results in approximately a 38% inhibition of glucose uptake, whereas glucose results in approximately a 56% and 74% inhibition of fructose uptake in *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carlsbergensis)*, respectively. These results provide additional evidence that the yeast strains studied have a higher affinity for glucose.

DISCUSSION

The sugar uptake profiles indicate that when glucose and fructose are fermented separately, both sugars are taken up and utilized at similar rates (Fig. 1). However, when fermentations are conducted in media containing an equal concentration of glucose and fructose, glucose is preferentially utilized over fructose. In fact, for both yeast strains examined, glucose was utilized at approximately

Table 2

Inhibitory effects of glucose and fructose

Strain	v (nmol/mg dry weight cells/min)[a]		
A. Inhibition of Glucose Uptake by Fructose			
	0.2 mM Glucose	0.2 mM Glucose + 2 mM Fructose	% Inhibition
Saccharomyces cerevisiae	1.0 ± 0.01	0.62 ± 0.05	38
Saccharomyces uvarum (carlsbergensis)	0.093 ± 0.005	0.058 ± 0.002	38
B. Inhibition of Fructose Uptake by Glucose			
	0.2 mM Fructose	0.2 mM Fructose + 2 mM Glucose	% Inhibition
Saccharomyces cerevisiae	0.39 ± 0.12	0.17 ± 0.03	56
Saccharomyces uvarum (carlsbergensis)	0.066 ± 0.014	0.017 ± 0.006	74

[a] The yeast strains were grown in glucose medium. The results are the average of four trials.

twice the rate of fructose (Fig. 2). The preferential uptake of glucose also occurred when sucrose was employed as the substrate (Fig. 3). Sucrose was first rapidly hydrolyzed into glucose and fructose by the action of the enzyme invertase prior to the uptake of either sugar. Again, glucose is taken up at approximately twice the rate of fructose. These results are similar to those previously reported by this and other laboratories [4,8,9].

The sugar uptake patterns in a typical North American brewer's wort where corn, either as grits or as syrup, is routinely employed as an adjunct is illustrated in Fig. 4A. In the normal situation, brewing yeast strains (of the species *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carlsbergensis)*) utilize glucose, fructose, maltose and maltotriose in this approximate sequence although some overlap does occur, leaving the dextrins unfermented. For the sake of simplicity the results of only the glucose, fructose and maltose uptake profiles for *Saccharomyces uvarum (carlsbergensis)* are presented. It has been shown that the major limiting factor in the fermentation of brewer's wort is the repressing influence of glucose upon maltose and maltotriose uptake [5,11,12]. For example, in Fig. 4A, only when approximately 60% of the wort glucose was taken up by the yeast did the uptake of maltose commence. In most strains of *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carlsbergensis)* maltose utilization is subject to control by glucose or catabolite repression [5,11,12].

In a number of countries (e.g., Australia, South Africa and Mauritius) sucrose is extensively employed as a wort adjunct [13]. The use of this sugar significantly modifies the initial wort sugar spectrum and also the pattern of sugar uptake during fermentation. Employing 30% sucrose as adjunct in a 16° Plato wort, hydrolysis of this disaccharide commenced almost immediately upon inoculation of the yeast resulting in accumulation of its hydrolytic products, glucose and fructose in the medium (Fig. 4C). Uptake of glucose and fructose commenced early in the fermentation with glucose being taken up at a faster rate than fructose. In addition, when sucrose was employed as a wort adjunct, maltose utilization was significantly repressed and uptake delayed when compared to the wort employing corn as an adjunct (i.e., compare Fig. 4A with 4C). Indeed, in the sucrose adjunct wort, maltose uptake did not commence until 48 h into the fermentation and even after 72 h of fermentation some maltose remained unfermented. The problem of incomplete fermentation in worts containing in excess of 25% of their fermentable sugar as sucrose has been reported previously and is thought to be due to glucose repression continuing well into the fermentation, thus inhibiting the uptake of repressible sugars such as maltose and maltotriose [4,14]. The effect of high concentrations of glucose in a wort was even more accentuated when the fermentation pattern of wort containing 30% glucose as adjunct was studied (Fig. 4B). In this situation the uptake of maltose was severely repressed and only 55% of the maltose was utilized by the yeast. In addition, maltotriose utilization was also severely repressed (data not shown). On the other hand, a recent study indicated that glucose did not exert catabolite repression on the maltose membrane transport and utilization systems in a particular brewing strain of *Saccharomyces cerevisiae* [4]. In their study, up to 8% glucose was used as adjunct in wort. This is considerably lower than the 30% glucose adjunct employed in this study and the reduced glucose concentration may account for the differences observed. In addition, yeast strain variation may also account for the differences in the results.

Kinetic analysis of glucose and fructose uptake in *Saccharomyces cerevisiae* revealed that the K_m values for glucose and fructose uptake were 1.6 mM and 20 mM, respectively (Fig. 5). This demonstrates that the yeast has a much higher affinity for glucose than fructose. These results are similar to those observed in other studies [2,3]. However, in most cases two transport systems were observed for glucose and fructose uptake; a low affinity transport system which appears to occur by constitutive facilitated diffusion and a high affinity transport system which is dependent on the activity of phosphorylating enzymes [2,3]. In this study, only one transport system was observed for the uptake of glucose and fructose which would correspond to the high affinity system [2,3]. This, however, may be due to the limited con-

centration range of substrates employed in performing the kinetic studies. Investigations are in progress utilizing a wider concentration range of substrates to establish the presence of two transport systems for glucose and fructose in these particular brewing strains.

Since the yeasts were observed to have a higher affinity for glucose than fructose, it is not surprising that the uptake of 0.2 mM glucose occurred at approximately two to three times the rate of 0.2 mM fructose for both glucose and fructose grown *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carlsbergensis)* brewing strains (Table 1). However, this is in contrast to the fermentation results which indicate that glucose and fructose, when fermented separately, are utilized at approximately the same rate (Fig. 1). The differences in these results are most likely due to the concentration of sugars employed. For example, 20% sugar was employed in the fermentation studies which corresponds to a concentration of approximately 1.1 M, compared to 0.2 mM in the transport studies. Therefore, the sugar concentration used in the fermentation studies far exceeds the K_m values of 1.6 mM and 20 mM determined for glucose and fructose uptake, respectively, thus resulting in similar uptake profiles. On the other hand, the uptake of 0.2 mM sugar is below the K_m value of either sugar and the uptake profile is a direct reflection of membrane transport. Since the yeasts have a higher affinity for glucose, it is not surprising that glucose is taken up faster than fructose, especially at the lower concentrations. In support of this, it was observed that glucose and fructose were utilized at equal rates above 1%, but below 1%, fructose was utilized more slowly than glucose [4,6].

Growth of yeasts on glucose or fructose had no repressible effect on the uptake of either sugar (Table 1). In addition, glucose was observed to cause a 56% and 74% inhibition of fructose uptake in *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carlsbergensis)*, respectively, whereas fructose resulted in a 38% inhibition of glucose uptake in both strains under the conditions employed (Table 2). These results add support to the conclusion that glucose and fructose compete for the same membrane carrier [4,7].

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