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Production of Ethanol from Enzymatically Hydrolyzed Orange Peel by the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

We extended our previous investigations of enzymatic hydrolysis of polysaccharides in orange peel by commercial cellulase and pectinase enzymes to higher, more practical concentrations of orange peel solids. High yields of saccharification could be maintained even at substrate concentrations as high as 22–23%, but the rates of solubilization and saccharification decreased 2–3-fold. We also tested the fermentability of these hydrolysates by the yeast *Saccharomyces cerevisiae*, which revealed the presence of inhibitory compounds. These compounds could be removed by the filtration of hydrolyzed peel. Successful fermentations of filtered hydrolysates were achieved after pH adjustment with calcium carbonate.

Index Entries: Orange peel; carbohydrates; enzymatic hydrolysis; fermentation; *Saccharomyces cerevisiae*.

INTRODUCTION

A large portion (40–60%) of citrus fruit processed to orange juice and similar products ends in waste peel, segment membranes, and other byproducts (*1,2*). Several million dry tons of these residues accumulate

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annually in the major citrus-processing countries, which are United States, Brazil, Spain, China, and Mexico. The bulk of dried residues is marketed as a cattle feed. The low selling price and a relatively high expense of drying the citrus processing residues make cattle feed production an economically marginal part of the overall process.

The citrus-processing byproducts contain soluble and insoluble carbohydrates as their major components. The soluble carbohydrates are simple sugars (glucose, fructose, and sucrose), whereas insoluble components are poorly characterized cell-wall carbohydrates (pectin, cellulose, and hemicelluloses containing galacturonic acid, glucose, galactose, arabinose, and xylose as their monomeric units). Lignin appears to be absent from these tissues. The conversion of carbohydrates in citrus peel thus appears to be a natural choice for potential conversion routes, many of which require depolymerization of cell-wall carbohydrates to monomeric sugars, which can then be converted by chemical or biological means to value-added products.

We investigated enzymatic hydrolysis of comminuted orange peel by commercial cellulase and pectinase enzymes at low concentrations of solids and obtained encouraging preliminary results (3). These results are supported in other investigations on maceration of citrus peel and pulp by fungal enzymes (4–9).

The saccharification of biomass feedstocks at low concentration of solids provides useful preliminary data on rates, yields, and other important parameters of enzymatic hydrolyses, but the sugar solutions produced are too dilute for most conversion processes. Therefore, we extended our investigations to study the effect of peel solids concentration on enzymatic hydrolysis. We also studied the fermentability of the hydrolysates by the yeast *Saccharomyces cerevisiae* to ethanol. The results are presented below.

MATERIALS AND METHODS

Substrates, Enzymes, and Reagents

Valencia orange (*Citrus sinensis* cv Valencia) fruit was harvested locally in midseason for this variety. The fruit was washed, halved, and reamed by hand using an electric juicer. Peel and attached membranes were stored frozen. Large amounts of orange peel and membranes were also obtained from a local citrus-processing plant late in the Valencia processing season. These substrates were also stored frozen.

Three commercial enzyme preparations, Pectinex Ultra SP, Celluclast 1.5 L, and Novozym 188, were kind gifts of NOVO Laboratories, Inc. (Danbury, CT). The enzyme preparations were characterized during previous investigation (3), except the pectinase activity, which is described below.

The pectinase activity was estimated by an assay conducted in 50 m*M* sodium acetate buffer, pH = 4.8, at 45°C, using 1% citrus pectin (medium degree of polymerization, 66–70% degree of methylation) solution as a substrate. The release of reducing sugars was followed by Nelson-Somogyi method (*10*) calibrated with galacturonic acid solutions. The units of pectinase activity were calculated as μmol of reducing sugars released/min/mg protein. The citrus pectin was obtained from HP Bulmer Pectin Ltd., Hereford, England. Sodium acetate and other chemicals used were reagent grade, and were purchased from Sigma Chemical Co. and other suppliers.

Orange Peel Digestion Protocols

The frozen peel was thawed and ground in a food processor (Model K, Regal Ware Inc.) to < 2 mm particles. The ground peel was then divided into portions and mixed with 1*M* sodium acetate buffer stock (pH = 4.8) and enzyme stocks diluted with appropriate amounts of water to bring the final concentration of dry peel solids to the desired level. The final concentration of sodium acetate buffer was adjusted to the 50-m*M* level. The reaction mixtures, which ranged from slurries of peel solids in the buffer to wet solids, were then incubated at 45°C and slowly rotated in 50-mL tubes using a laboratory rotator (Cole Parmer Instrument Co., Niles, IL).

Larger digestions were conducted at 45°C in a 4-L plastic bottle that was rotated horizontally at 10–15 rpm using a variable-speed roller mill base (Fisher Scientific, Pittsburgh, PA). These digestions, described later, were run without any buffer.

Initial total peel solids were determined by drying the aliquots of peel at 70°C to constant weight. The residual insoluble solids were determined by filtering the digests through 1.2-μ glass fiber filter (Cole Parmer Instrument Co., Niles, IL) and drying the filters as above. The peel solids solubilized by leaching with water or dissolved by enzymatic hydrolysis were determined as the difference between the initial dry total peel solids and residual insoluble solids.

Analyses of Solubilized Carbohydrates and Ethanol

Reducing carbohydrate groups were estimated by the Nelson-Somogyi method (*10*). The monosaccharides and sucrose released by the enzymatic treatment or leaching with water were separated and determined by ion-moderated partition chromatography and refractive index detection. Neutral monosaccharides and sucrose were separated on an Aminex HPX-87P column (300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA) using deionized water as an eluent. The column was operated at 80°C and a flow rate of 0.5–1.0 mL/min. Since this packing absorbed galacturonic acid and did not adequately separate ethanol from fructose, an Aminex

HPX-87H column (300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA) was used for the determination of galacturonic acid and ethanol. The column was operated at 60°C, 0.01*N* sulfuric acid in water as an eluent with the flow rate of 0.5 mL/min.

Growth Media and Fermentation

A strain of *Saccharomyces cerevisiae* (Fleischmann's Active Dry Yeast, Fleischmann's Yeast Inc., Oakland, CA) was purchased locally. Inocula were prepared by reviving one packet (7 g) of yeast in 100 mL of media containing 0.1 g of yeast extract and 0.3 g of glucose in deionized water. These cultures were incubated aerobically at 35°C and 100 rpm for 1 h.

The main cultures contained 90 mL of partially neutralized hydrolysates (final pH = 4.8–5.2), 10 mL of the yeast inoculum (i.e., approx 2 × 10^8 cells/mL), and usually a 1% yeast extract and 2% peptone (Difco Laboratories, Detroit, MI) nutritional supplement. The pH of the hydrolysates was adjusted from the usual pH = 3.3–3.5 to pH = 4.8–5.0 by the addition of calcium carbonate. The cultures were rotated at 100 rpm and incubated at 35°C in 250-mL Erlenmeyer flasks equipped with a rubber stopper and a water trap.

RESULTS AND DISCUSSION

The results at low (approx 1%) concentrations of orange peel solids indicated that relatively low loadings of pectinase supplemented with cellulase enzyme could achieve high levels of solubilization and conversion to reducing sugars (Fig. 1). The enzyme loading in this and in a previous study (3) are expressed in milligrams of total protein added to 1 g of total dry peel solids. Since a complex mixture of enzymes is used and multiple hydrolytic activities are present in each enzyme preparation, no simple correlation between these multiple activities and specific activity against plant cell walls has been developed. The enzyme loading expressed on a weight basis also has practical implications, because the commercial enzymes are sold on this basis. The specific cellulase activities of individual enzyme preparations were 0.196 international filter paper units (IFPU) per milligram of protein for Celluclast 1.5-L preparation and 9.8 × 10^{-3} IFPU/mg of protein for Pectinex Ultra SP, respectively (3). The β-glucosidase (Novozym 188) preparation had no detectable filter paper hydrolyzing activity, but had a significant endocellulase activity (3). The relative pectinase activities, measured as described in Materials and Methods, were 0.07 U/mg of protein in the Celluclast 1.5-L preparation and 15.6 U/mg of protein in the Pectinex Ultra SP preparation, respectively.

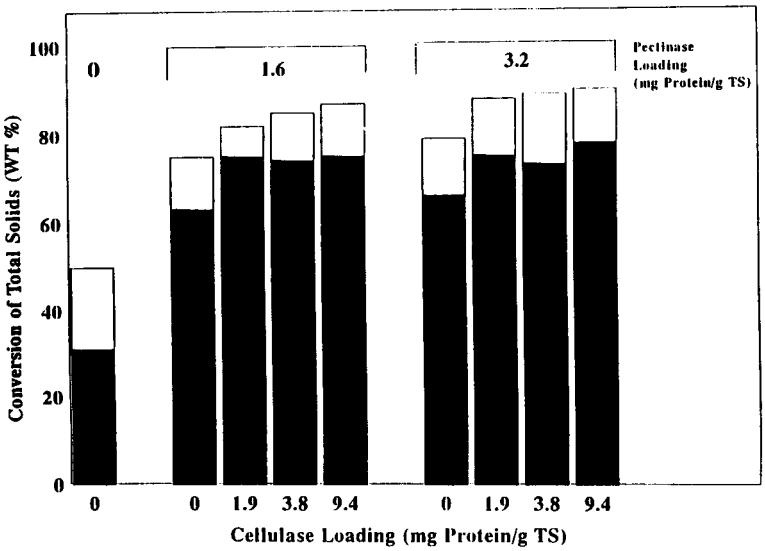

Fig. 1. Conversion of total peel solids to water-soluble solids and reducing sugars by treatment with different loadings of cellulase and pectinase enzymes for 24 h at 45°C and pH = 4.8. Initial concentration of peel solids was approx 1–2 wt%. Unshaded area corresponds to a release of soluble solids and shaded area to reducing sugar conversion.

We chose a low enzyme loading, similar to the lowest pectinase and cellulase loading in Fig. 1, to investigate the effects of peel solids concentration on the yields of 24-h digestions. The cellulase and pectinase enzymes at 1.4 and 1.7 mg protein/g of total peel solids, respectively, were also supplemented with β-glucosidase (Novozym 188) at 1.5 mg/g total solids to decrease inhibition of cellulase by cellobiose. The results of this study, shown in Fig. 2, appeared very encouraging. Peel solubilization decreased only slightly as peel solids concentration was increased from 2.4 to 23%, whereas reducing sugar production showed a significant decrease only at concentrations of peel solids exceeding 16%. The results also indicate a relatively low inhibition of hydrolytic enzymes by sugars released from the orange peel. Another significant observation made during this study was inadequate maintenance of pH by 50 m*M* sodium acetate buffer. The pH dropped from 4.8 to 4.3 in the digest containing 2.4% total peel solids and even further (from pH 3.9 to 3.4) at higher concentrations of peel solids. The significant pH drop during the enzymatic hydrolysis of orange peel is undoubtedly caused by the release of D-galacturonic acid from pectin in cell walls. The pK_a of D-galacturonic acid is 3.51 (*11*), and the pH values of peel hydrolysates appeared to stabilize in the range of 3.3–3.5.

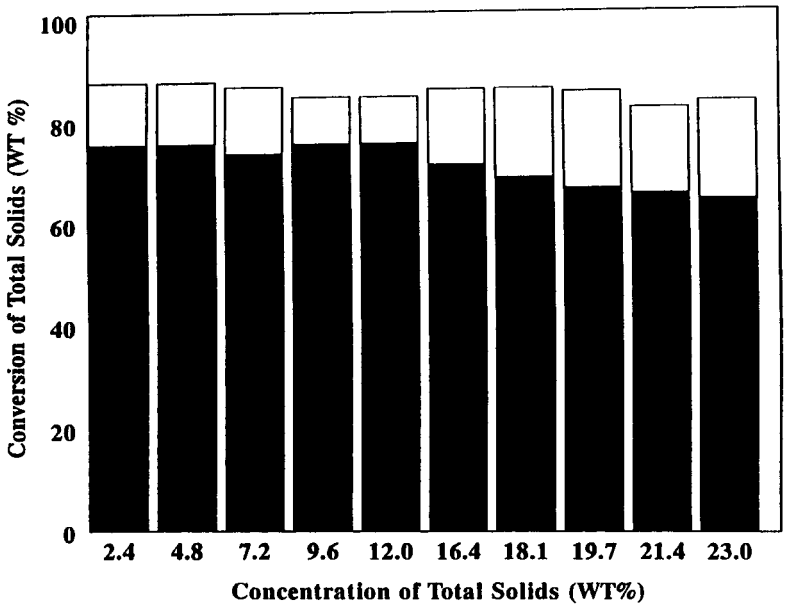

Fig. 2. Enzymatic conversion of total peel solids to water-soluble solids and reducing sugars as a function of peel solids concentration at 45°C and 24-h reaction time. Bar coding is the same as in Fig. 1.

The substantial reduction in pH during hydrolysis from the optimal (pH = 4.5–5) value for hydrolytic enzymes used could inhibit the activity of these enzymes. We therefore elected to stabilize the pH by the addition of solid calcium carbonate. The addition of calcium carbonate in slightly less than equimolar amounts to galacturonic acid in the peel stabilized the pH of the digests in pH = 4.3–4.8 range, but the solubilization of peel solids and the reducing sugar yield were approx 10–15% lower than in similar digests without the addition of calcium carbonate (data not shown). The decreased yields may have been caused by the crosslinking and precipitation of pectin by calcium ions, which could render the substrate more resistant to hydrolytic enzymes. We did not test water-soluble bases for neutralization of peel–enzyme mixtures because of difficulties in achieving uniform distribution of concentrated base powders or solutions in peel solids at the beginning of the reaction. Furthermore, the results shown in Fig. 2 did not indicate severe inhibition of hydrolytic enzymes at lower pH values. Therefore, the remaining hydrolyses discussed in this study were conducted without pH control, starting with ground peel, enzyme solutions, and water. Initial pH of these digests was approx 4.3, but it dropped rapidly within first few hours to the pH = 3.3–3.5 range and was decreasing very slowly thereafter to a final pH of around 3.3. The pH of the digests was adjusted after the hydrolysis when necessary for yeast fermentation. The low pH of the peel hydrolysates and the pres-

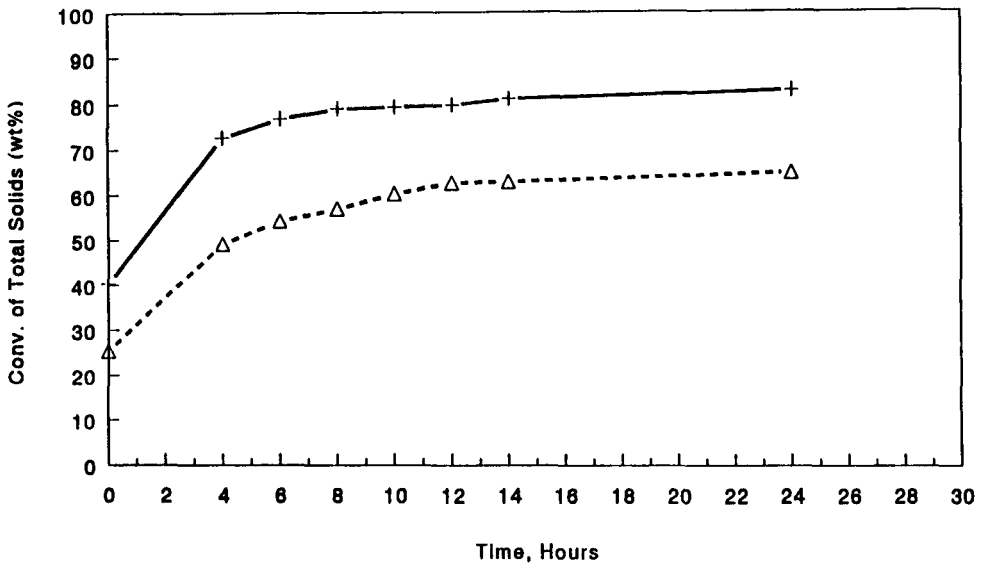

Fig. 3. Enzymatic conversion of total peel solids (22% solids) to water-soluble solids and reducing sugars as a function of the time of reaction. Solid line corresponds to a release of water-soluble solids, and a dashed line to a conversion of total solids to reducing sugars.

ence of antimicrobial agents discussed later seem to preserve the peel hydrolysates from rapid microbial contamination. No microorganisms were observed microscopically in the hydrolysates or their filtrates during preparation, or after storage for several days in the refrigerator. Therefore, the antibiotic mixture we used in previous experiments (3) to combat microbial infection has been omitted in the current experiments.

The course of hydrolysis at 22% peel solids is shown in Fig. 3. The enzyme loading used was the same as in experiments shown in Fig. 2. The results indicate that hydrolysis slowed down considerably after 8 h with only few additional percents of total solids being solubilized or converted to reducing sugars in the period between 8 and 24 h.

The results also show that the increase in soluble peel solids from 1 to 22% leads to inhibition of the hydrolytic enzymes because the rate of the hydrolysis slowed down two- to threefold when compared with the results obtained during previous study (3) using similar enzyme loadings. We do not know at the present time how much inhibition is caused by the decrease in pH during hydrolysis and how much is caused by the inhibition of individual enzymes by the end products of their action. The approximate sugar yield from high-solids digests is shown in Table 1. The results are very similar to individual sugar yields published previously (3) and indicate that selective inhibition of enzyme components did not occur during the increase in concentration of peel solids. Sucrose and xylose

Table 1
Solubilization of Sugars from Orange Peel

Sample/ treatment	Approximate soluble sugar yield, wt% of total solids							Total sugar yield, wt% of TS
	Suc.	Glc.	Fru	Gal.	Ara	Xyl	Gal. A	
Aqueous extract	6.0	12.3	10.2	-	-	-	-	28.5
Enzymatic hydrolysate	ND	26.6	13.7	4.1	6.3	ND	19.3	70.0

TS = Total solids.
ND = Not determined.
- = Not detected.

yields were not determined after enzymatic hydrolyses. Sucrose determination was not considered reliable because of coelution with disaccharides formed during enzymatic hydrolysis, and xylose was released in very small amounts, which did not allow accurate integration by the HPLC system.

Since the yields and rates of the enzymatic hydrolysis did not decrease unreasonably with increase in concentration of peel solids, we decided to investigate the fermentability of these hydrolysates by a strain of *Saccharomyces cerevisiae* yeast. In addition to our interest in the production of ethanol, a potential liquid fuel and a chemical feedstock (*12–14*), we wanted to confirm the presence of antimicrobial compounds reported in peel and peel oil by other investigators (*15–21*) and to test different methods of their removal. The results of the initial experiments are summarized in Fig. 4. The hydrolysates contained 2.4–22% of peel solids as described in the figure legend. The pH of the hydrolysates and filtrate was adjusted to 4.8–5.0 with calcium carbonate. Control fermentation using 10% glucose as a carbon source is included for comparison. The unfiltered hydrolysates containing residual peel solids produced very little ethanol. Moreover, the ethanol concentration decreased as the initial concentration of hydrolyzed peel solids was increased from 2.4 to 12%. The fermentation of clarified filtrate from 12% peel hydrolysate produced, on the other hand, much higher ethanol yield, and the yeast utilized most of glucose, fructose, and sucrose in this filtered substrate (data not shown). Nutrient deficiency could be ruled out, because all fermentation media were supplemented with yeast extract and peptone. There was a possibility that the inhibition of fermentation in hydrolysates containing undissolved peel solids was caused by highly volatile components of peel oil (*22*). Therefore we evaporated one hydrolysate to approx 1/3 the weight under vacuum, diluted the residue back with water to the original weight, and fermented this substrate. The results (Fig. 4) show that only a minuscule amount of ethanol

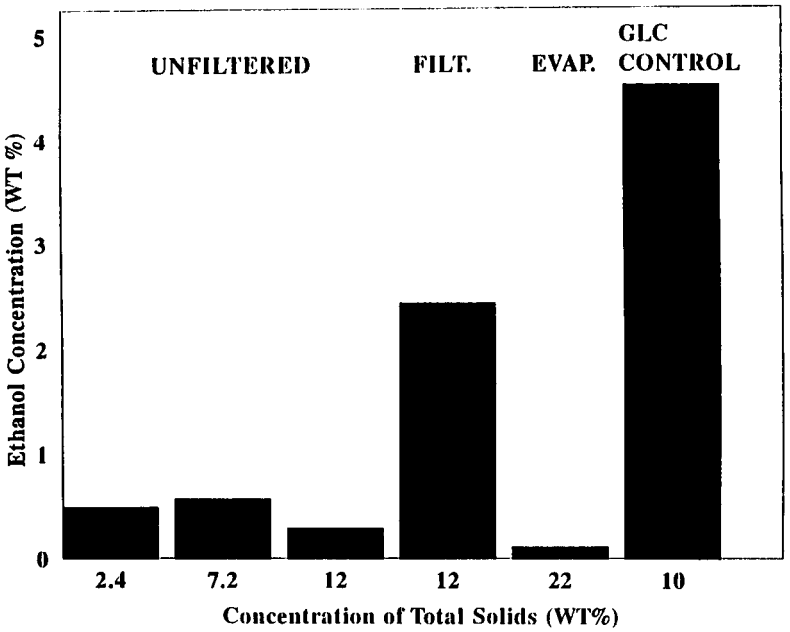

Fig. 4. Concentrations of ethanol produced by fermentation of hydrolyzed peel with *S. cerevisiae* yeast at 45°C for 24 h. The treatments of the hydrolysates are described in the text.

was produced. The inhibitory activity, therefore, resided either in nonvolatile and slightly soluble components of orange peel or in the components of peel oil with low volatility, such as limonene. This issue was investigated in the next set of experiments. The peel oil (limonene) content of hydrolysate was determined by Scott's bromate titration method (*23*). The oil content was quite high at approx 1.4% (v/v). This peel oil level was well above the inhibitory levels (0.05–0.1%) of orange peel oil determined for yeasts by von Loesecke (*15*). Filtration appeared to be a very effective method for the removal of peel oil from the peel hydrolysates since the peel oil content decreased to approx 0.015% in the filtrate. We also filtered a hydrolysate containing approx 22% dry peel solids and dried the filter cake in the vacuum oven at 50°C for 2 d until the levels of limonene decreased to 0.14%. The dry residual solids were then added to the partially neutralized filtrate supplemented with yeast extract and peptone, giving a final limonene content in fermentation broth of 0.015–0.022%. The results are shown in Fig. 5. The filtrate alone or with 2.5% dry residual solids did not show any inhibition, but the fermentation containing a very high level of dry residual solids showed significant inhibition of ethanol production at 24 h. These results confirm previous observations (*15–21*) that peel oil components of low volatility, primarily (95%) limonene (*22*), are responsible for the inhibition of yeast fermentation. However, the results do not rule out the presence of additional, nonvolatile compounds in the peel that are inhibitory to microorganisms. We should add that we

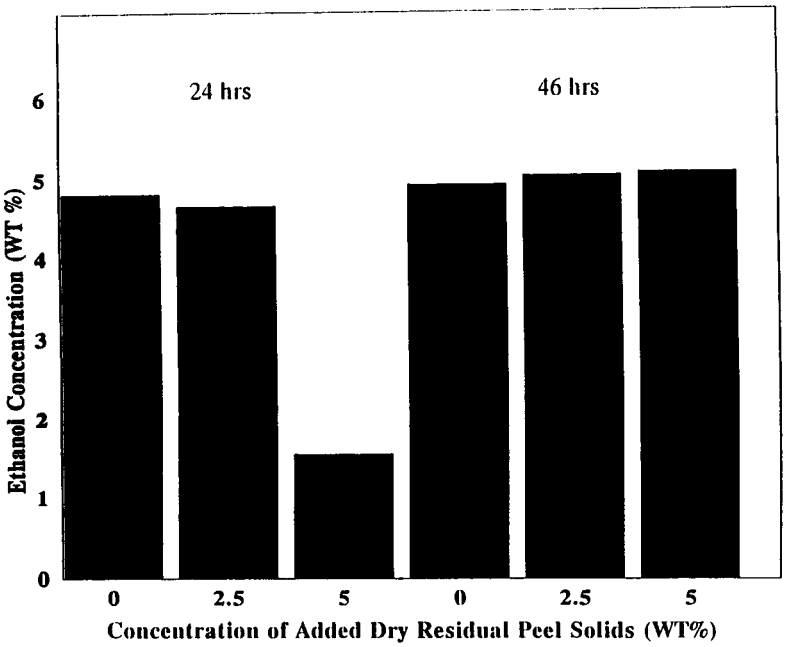

Fig. 5. Concentrations of ethanol produced by fermentation of filtered peel hydrolysate with *S. cerevisiae* to which dried residual solids from enzymatic hydrolysis of peel have been added.

have briefly investigated removal of inhibitors by organic solvent extraction, but abandoned this approach because of the formation of inseparable emulsions with a series of organic solvents. We also did not investigate the removal of limonene by steam stripping or aeration as suggested by other investigators (*16,17,19*). Since filtration seemed to be an effective method for the removal of major inhibitors from orange peel hydrolysates and the search for additional inhibitory compounds might not be fruitful, we turned our investigations to the nutritional requirements of yeasts fermenting the filtered hydrolysate and preliminary kinetic studies of these fermentations. The initial nutritional studies with filtered hydrolysate from 22% peel solids neutralized with calcium carbonate to pH = 5 consisted of supplementation with potassium dihydrogen phosphate, ammonium chloride, or sodium sulfate at the 50, 50, or 10 m*M* level, respectively. The analysis of ethanol produced after 24 h did not show a significant difference between the concentrations of ethanol in supplemented and unsupplemented filtered peel hydrolysates (data not shown). Therefore, we decided to investigate the rate of ethanol production. The results (Fig. 6) show only a small difference in rate and yield between the filtered hydrolysate supplemented with yeast extract and peptone, and unsupplemented hydrolysate. Moreover, the rates of the fermentation of both hydrolysates are comparable to the rate of fermentation of 10% glucose solution, used as a control. These results indicate that filtered orange peel hydrolysate

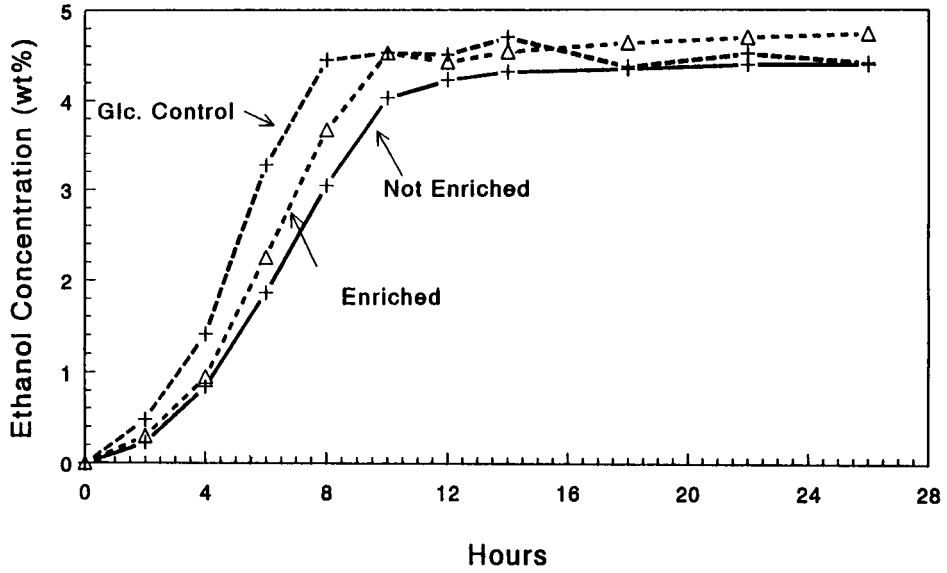

Fig. 6. Time-course of the ethanol production during fermentation of filtered peel hydrolysate with *S. cerevisiae* at 35°C and initial pH = 5.0. Enrichment of the media is described in the text.

contains sufficient concentrations of micro- and macronutrients to support rapid yeast fermentation. The analysis of monomeric sugars before and after fermentation showed utilization of glucose, fructose, and sucrose within the first 24 h of fermentation with occasional utilization of galactose when the fermentation was extended for 48 h. Galacturonic acid and arabinose were not utilized. These sugars are considered nonfermentable by *S. cerevisiae* and other yeasts (*24*). The variable and delayed fermentation of galactose is typical of the diauxic pattern of fermentation of this sugar by ordinary strains of *S. cerevisiae,* which is caused by catabolite repression of galactose utilization (*25*). The yields, rates, and concentrations of ethanol observed in our preliminary experiments are similar to the results obtained by Hara et al. (*26*) from saccharification and fermentation of Satsuma mandarin peel by *Aspergillus niger* enzyme preparation and *S. cerevisiae* yeast.

Good fermentability of six-carbon sugars in filtered peel hydrolysates to ethanol (4–5 wt%) suggests a potential extension of our preliminary results to a commercial process for the production of ethanol from orange peel. However, it may be hampered by relatively low yields of sugars, fermentable by *S. cerevisiae* or similar yeasts, obtainable by enzymatic hydrolysis of orange peel. The results in Table 1 show that enzymatic hydrolysis of the peel approximately doubles the yield of total reducing sugars. However, the yield of sugars fermentable by *S. cerevisiae* only increases by 25–35% over the content of soluble sugars that are all fermentable to

ethanol. In terms of potential ethanol yield from dry peel solids, the enzymatic hydrolysis and fermentation by *S. cerevisiae* can produce approx 55–65 gal of anhydrous ethanol/t of dry peel solids. This yield has to be compared to 40–50 gal of ethanol obtainable by potential fermentation of soluble sugars present in peel. The ratio of potential ethanol yield from enzymatic hydrolysates of peel to the same from soluble sugars in peel will change during the harvesting season, because soluble sugars in peel increase during fruit maturation and cell-wall carbohydrates decrease at the same time (27).

CONCLUSIONS

The extension of enzymatic hydrolyses of polysaccharides in orange peel to more practical concentrations of peel solids provided promising results. The yields of soluble solids and reducing sugars decreased only slightly as the concentration of total peel solids increased from 1.2 to 23 wt%. The rate of enzymatic hydrolysis decreased only two- to threefold during the approximate 20-fold increase of total peel solids. The end product inhibition of hydrolytic enzymes we used and the decrease in pH do not appear to present an insurmountable barrier to the production of relatively concentrated sugar solutions by enzymatic hydrolysis of orange peel.

The initial fermentability of these untreated hydrolysates by *S. cerevisiae* was extremely poor because of a presence of inhibitory agents. If this inhibition cannot be overcome by the selection or adaptation of resistant strains, the advantageous simultaneous saccharification and fermentation (SSF) approach cannot be used for the conversion of orange peel solids to ethanol or other value-added products. Our experiments confirm previous observations by other investigators that the inhibitory agents in orange peel are concentrated mainly in the peel oil, but they do not rule out the presence of additional nonvolatile inhibitory compounds in the peel. Fortunately, the peel oil components adhere to residual insoluble solids after enzymatic hydrolysis and can be effectively removed from these hydrolysates by filtration. The filtrates of peel hydrolysates appear to contain sufficient amounts of micro- and macronutrients to support yeast fermentation. Inhibition of yeast fermentation by nonfermentable sugars present in these hydrolysates was also not observed. The fermentation of enzymatic hydrolysates of orange peel by *S. cerevisiae* to ethanol thus appears relatively simple technically, but its economic viability is hampered by the small increase in the yield of fermentable sugars after enzymatic hydrolysis. The enzymatic hydrolysates of orange peel may still be suitable substrates for other fermentations where a greater variety of sugars can be utilized, or ethanol production can serve as a part of a coproduction process in which the nonfermentable sugars are converted to additional value-added products.

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REFERENCES

1. Agricultural Research Service (1962), *Chemistry and Technology of Citrus, Citrus Products and Byproducts.* Agriculture Handbook No. 98, United States Department of Agriculture Publ., Washington, DC, p. 98.
2. Kesterson, J. W. and Braddock, R. J. (1976), *By-Products and Specialty Products of Florida Citrus.* Bulletin #784, University of Florida Publ., Gainesville, FL, p. 119.
3. Grohmann, K. and Baldwin, E. A. (1992), *Biotechnol. Lett.* **14,** 1169–1174.
4. Nishio, N., Oku, Y., Kawamara, D., and Nagai, S. (1979), *Hakko Kogaku Kaishi* **57,** 354–359.
5. Nishio, N. and Nagai, S. (1979), *Eur. J. Appl. Microbiol. Biotechnol.* **6,** 371–378.
6. Marshall, M. R., Graumlich, T. R., Braddock, R. J., and Messersmith, M. (1985), *J. Food Sci.* **50,** 1211,1212.
7. Echeverria, E., Burns, J. K., and Wicker, L. (1988), *Proc. Fla. State Hort. Soc.* **101,** 150–154.
8. Akao, T., Mizuki, E., Saito, H., Okumura, S., and Murao, S. (1992), *Bioresource Technol.* **41,** 35–39.
9. Ben-Shalom, N. (1986), *J. Food Sci.* **51,** 720,721, 730.
10. Nelson, N. (1944), *J. Biol. Chem.* **153,** 375–386.
11. Kohn, R. and Kovac, P. (1978), *Chem. Zvesti* **32,** 478–485.
12. Grohmann, K., Wyman, C. E., and Himmell, M. E. (1992), in *Emerging Technologies for Materials and Fuels from Biomass,* Rowell, R. M., Schulz, T. P., and Narayan, R., eds., ACS Symp. Series No. 476, American Chemical Society Publ., Washington, DC, pp. 354–392.
13. Tong, G. E. and Cannell, R. P. (1988), in *Organic Chemicals from Biomass,* Wise, D. L., ed., Benjamin/Cummings, Menlo Park, CA, pp. 407–451.
14. Rohatgi, N. K. and Ingham, J. D. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 515–526.
15. Von Loesecke, H. W. (1934), *Citrus Ind.* **15,** 8,9, 20,21.
16. McNary, R., Wolford, R. W., and Patton, V. D. (1951), *Food Technol.* **5,** 319–323.
17. Lane, A. G. (1980), *J. Chem. Tech. Biotechnol.* **30,** 345–350.
18. Lane, A. G. (1983), *Environ. Technol. Lett.* **4,** 65–72.
19. Mizuki, E., Akao, T., and Saruwatari, T. (1990), *Biol. Wastes* **33,** 161–168.
20. Murdock, D. I. and Allen, W. E. (1960), *Food Technol.* **14,** 441–445.
21. Subba, M. S., Soumithri, T. C., and Suryanarayana, R. (1967), *J. Food Sci.* **32,** 225–227.
22. Shaw, P. E. (1979), *J. Agric. Food Chem.* **27,** 246–257.
23. Scott, W. C. and Veldhuis, M. K. (1966), *J. A.O.A.C.* **49,** 628–633.
24. Barnett, J. A., Payne, R. W., and Yarrow, D. (1985), in *Yeasts Characteristics and Identification,* Cambridge University Press, Cambridge, UK, p. 811.
25. Carlson, M. (1987), *J. Bacteriol.* **169,** 4873–4877.
26. Hara, T., Fujio, Y., and Ueda, S. (1985), *Nippon Shokahin Kogyo Gakkaishi* **32,** 241–246.
27. Sinclair, W. B. (1961), in *The Orange. Its Biochemistry and Physiology,* Sinclair, W. B., ed., Univ. of California, Publ., Riverside, CA, pp. 191–229.