berries remains to be determined. Vanillin, eugenol, and carveyl acetate are, however, considered to be important for the odor of Finnish wild strawberries.

ACKNOWLEDGMENT

The authors wish to thank Meri Suihko for carrying out the threshold determinations.

LITERATURE CITED

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **19**, 524 (1971).
Coppens, A., Hoejenbos, L., *Recl. Trav. Chim. Pays-Bas* **58**, 680 (1939).
Dimick, K. P., Makower, B., *Food Tecnol.* **10**, 73 (1956).
Dimick, K. P., Corse, J., *Am. Perfum.* **71**, 45 (1958).
Dirinck, P., Schreyen, L., Schamp, N., *J. Agric. Food Chem.* **25**, 759 (1977).
Drawert, F., Tressl, R., Staudt, G., Köppler, H., *Z. Naturforsch. C* **28**, 488 (1973).
Flath, R. A., Black, D. R., Guadagni, D. G., McFadden, W. H., Shultz, T. H., *J. Agric. Food Chem.* **15**, 29 (1967).
Hunter, G. L. K., Bucek, W. A., Radford, T., *J. Food Sci.* **39**, 900 (1974).
Kallio, H., Honkanen, E., *Proc. Int. Congr. Food Sci. Technol., 4th* **1**, 84 (1976).
McFadden, W. H., Teranishi, R., Corse, J., Black, D. R., Mon, T. R., *J. Chromatogr.* **18**, 10 (1965).
McGlumphy, J., *Food Technol.* **5**, 353 (1951).
Mussinan, C. J., Walradt, J. P., *J. Agric. Food Chem.* **23**, 482 (1975).
Pyysalo, T., Suihko, M., Honkanen, E., *Lebensm.-Wiss. Technol.* **10**, 36 (1977).
Pyysalo, T., *Z. Lebensm. Unters.-Forsch.* **162**, 263 (1976).
Seidel, C. F., Schinz, H., Stoll, M., *Helv. Chim. Acta* **41**, 372 (1958).
Sovish, R., *J. Org. Chem.* **24**, 1345 (1959).
Staudt, G., Drawert, F., Tressl, R., *Z. Pflanzenzuecht.* **75**, 36 (1975).
Stoltz, L. P., Kemp, T. R., Smith, W. O., Smith, W. I., Chaplin, C. E., *Phytochemistry* **9**, 1157 (1970).
Sundt, E., Proceedings of the Scandinavian Symposium on Aroma Research, *Naeringsmiddelindustrien* **23**, 5 (1970).
Teranishi, R., Corse, J. W., McFadden, W. H., Black, D. R., Morgan, A. I., *J. Food Sci.* **24**, 478 (1963).
Tressl. R., Drawert, F., Heimann, W., *Z. Naturforsch. B* **24**, 1201 (1969).
Willhalm, B., Thomas, A., U.S. Patent 3455702 (1969).
Willhalm, B., Palluy, E., Winter, M., *Helv. Chim. Acta* **49**, 65 (1966).
Winter, M., Willhalm, B., Hinder, M., Palluy, P., Sundt, E., *Perfum. Essent. Oil Rec.* **49**, 250 (1958).
Winter, M., Willhalm, B., *Helv. Chim. Acta* **47**, 1215 (1964).

Received for review July 5, 1978. Accepted September 25, 1978.

Kudzu (*Pueraria lobata*) Root Starch as a Substrate for the Lysine-Enriched Baker's Yeast and Ethanol Fermentation Process

Robert D. Tanner* and S. Shahid Hussain

At best, kudzu is considered to be an escaped plant in the southern United States. It was introduced to the South during the 1930's to help restore the soil and reduce erosion since it is a legume with an extensive root structure. This vine has adapted so successfully that it is presently overgrowing roads and trees. It is proposed here to use the starchy root of this plant as a fermentation medium in order to develop a commercial outlet for kudzu and add a starch to supplement our renewable food and fuel supplies. Purified kudzu starch as well as the kudzu root extract is compared with the other starches, manioc and corn dextrin, to determine the relative rate at which α-amylase degrades the polymer for fermentation purposes. Fermentations of the purified kudzu root starch are conducted to determine the fermentation vitamin and mineral requirements. Intracellular lysine and cell yields from the fermentation process for the raw and purified kudzu starch are compared with corn dextrin and manioc starch substrates.

In a previous paper (Tanner et al., 1977), a fermentation process was proposed for producing lysine-enriched baker's yeast to help alleviate human protein malnutrition, and ethanol, for use as a liquid fuel such as a gasoline entender. In that earlier paper, glucose was used as a model substrate. Typical substrates are sugar cane, sugar beet, and sorghum, or saccharified corn, wheat, and manioc starches. In light of impending world shortages of all of these conventional sugar sources, a new substrate source is examined in this work: kudzu, a plant which can be grown on marginal farming lands.

Since kudzu (*Pueraria lobata* or *Pueraria thunbergiana*) is a legume, it has minimal nitrogen fertilizer requirements and its leaves and vines can be used as protein-rich forage (Hill, 1937; Schery, 1952). Having an extensive root structure, in addition to its primary starch tap root, kudzu has excellent soil erosion control properties (Bailey and Mayton, 1931; Pieters, 1932; and McKee and Stephens, 1943) and may have an important role in meeting the global problem of soil erosion (Carter, 1977).

Kudzu is not without disadvantages, however. To one who lives in the southeastern United States, kudzu is considered to be, at best, an escaped plant. It was originally planted in large amounts in the 1930's to help restore the soil, being a legume, and keep down erosion because of its extensive root structure. But it has gotten out of control in the warmer areas of the South because the winters do not kill back the vines to the roots, as they do in the North and, presumably, as in its native Japan and China. The plant is tenacious, primarily because of the reservoir of starch in its thick root, whose use for fermentation is the subject of this paper.

MATERIALS AND METHODS

The yeast culture, lysine assay culture, and cell optical density measurement procedure are described in a previous

Chemical Engineering Department, Vanderbilt University, Nashville, Tennessee 37235.

0021-8561/79/1427-0022$01.00/0

Table I[a]

minerals and citrate, g		vitamins and trace minerals, mg	
$NH_4H_2PO_4$	10	biotin	0.2
KH_2PO_4	4	calcium pantothenate	5.0
$MgSO_4 \cdot 7H_2O$	1.5	*meso*-inositol	100.0
sodium citrate	1	thiamine hydrochloride	44.0
		pyridoxine hydrochloride	12.0
		$ZnSO_4 \cdot 7H_2O$	17.6
		$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	10.5
		$CuSO_4 \cdot 5H_2O$	0.96

[a] Glucose (100 g) and distilled water to 1 L.

paper (Tanner et al., 1977). Because of its direct relevance to this work it is worth recapitulating the synthetic medium used as a basis in this study. In complete form, the Maxon and Johnson (1953) medium C is comprised of sugar, minerals, vitamins, and organic compounds in the prescription shown in Table I. When either the glucose or reducing sugar equivalent was changed, the other ingredients were likewise modified in the same proportion. A 1-L fermentor was used to hold 750 to 800 mL of solution. For both the fermentation and starch degradation experiments, the magnetic stirring rate was held constant at 500 rpm. The aeration was held constant at (1.4 vol of air) min^{-1} (fermentor liquid volume)$^{-1}$. All other conditions and equipment employed are those previously described (Tanner et al., 1977).

The kudzu roots employed in the studies reported in Figures 1, 2, 4–7, and 10 were collected in the summer of 1977, two blocks from our Vanderbilt laboratory. The kudzu roots used to develop Figure 3 were gathered in the summer of 1976, 30 miles west of Nashville. The 1977 crop was lower in sugar than the earlier specimens because the plant was subjected to many weeks of very hot, precipitation-free weather. By drying the root of its unbound water, followed by starch extraction, and further drying, we determined that the root contained about 25% by weight starch, 25% fiber, and the rest, water.

The kudzu root starch can be harvested and extracted commercially, as evidenced by the fact that it is presently marketed in Japan. We purchased imported purified kudzu starch locally (sold as kuzu arrowroot starch). This starch was imported by the Erewhon Natural and Organic Foods Co. of Boston, Mass. The Japanese manufacturer for the kuzu starch is Yoshino Zakura Industries.

The manioc (yuca or cassava) root used was purchased locally. It was packaged by Calis International Products, S.A., San José, Costa Rica, under the brand name El Gallo Frozen Cassava. Our laboratory drying test indicated that the root was comprised of approximately 44% starch and the remainder unbound water. There was a negligible amount of fiber since the root cortex had been removed prior to packaging.

The α-amylase used in the starch degradation studies was obtained from Miles Laboratories, Code No. 31-1-003, a partially purified preparation containing many other enzymes and whose source was *Aspergillus oryzae*. The manufacturer recommended it for saccharification of starch by its hydrolytic action on both amylose and amylopectin. Its optimal maltose-liberating activity is reported to be pH 5.0 and T = 40 °C.

The purified corn dextrin (white), catalog number D-7, was purchased from the Fisher Chemical Company, Fair Lawn, N.J.

RESULTS AND DISCUSSION

In this section we will discuss our laboratory studies with kudzu starch degraded with α-amylase (saccharification) and fermentation of the resulting sugars to an enriched-lysine baker's yeast product. The fermentation process is essentially the same as that reported earlier (Tanner et al., 1977) except that saccharified starch is used in place of glucose. No effort was made to track ethanol since the earlier work indicated that under constant aeration and agitation conditions ethanol yields remained nearly invarient.

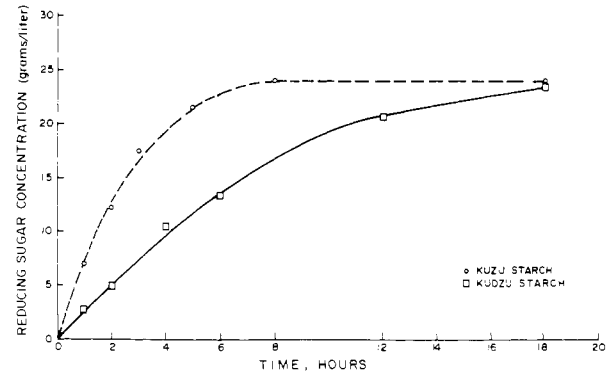

Figure 1. Comparison of the saccharification of starch in kudzu root extract with commercial Japanese kudzu (kuzu) starch; T = 40 °C, initial pH 5.

Kudzu Starch Saccharification. In general, plant starches are comprised of the straight-chain polymers, amyloses, and the branched-chain polysaccharides, amylopectins. Degradation of amylose with α-amylase (α-1,4-glucan 4-glucanohydrolase) leads to a mixture of glucose and maltose sugars, while the breakdown of amylopectin with α-amylase leads to a mixture of branched and unbranched short-chain sugars, called dextrins (White et al., 1973).

If the α-amylase employed includes a mixture of other enzymes, as that used in this study, it is likely to contain β-amylase (α-1,4-glucan maltohydrolase) and possibly maltase. Complete saccharification of amylose with β-amylase yields about two-thirds maltose and, the rest, a high-molecular-weight limit dextrin. Maltase converts the maltose to glucose, while α-amylase will convert the high-molecular-weight limit dextrin to more maltose and low-molecular-weight limit dextrin (White et al., 1973).

A mixture of α-amylase and β-amylase, with negligible maltase, would be expected to lead to a mixture of glucose, maltose, and other short-chain reducing sugars when allowed to act on plant starches. That is the case for kudzu starch, obtained by extraction of freshly harvested kudzu roots (short root segments were boiled in water for several hours), as shown in the lower curve of Figure 1. The impure α-amylase was added in the ratio of 0.39 g/700 mL of starch extract or 0.56 g/L for all the starch saccharifications. The rate at which reducing sugar was formed, as determined by the Somogyi–Nelson test, is graphed. Since the initial dry weight of starch was not known, the extent of reduction of starch to reducing sugars was estimated by repeating the experiment with commercially obtained kudzu starch (kuzu). This experiment, begun with a 30 g/L solution of purified starch, led to the formation of 24 g/L of reducing sugar, as shown in the upper curve of Figure 1. Here, 30 g was selected to most closely match that which could be obtained from kudzu root extract after a few hours of boiling. If the resulting saccharified solution contained negligible amounts of dextrins, then the ratio of glucose to maltose would be 18 to 6, or 3 to 1, an excellent substrate for a baker's yeast fermentation. We attribute the slower saccharification rate for kudzu, when compared to kuzu, to the larger globules of starch and to the kudzu fiber residue in the kudzu

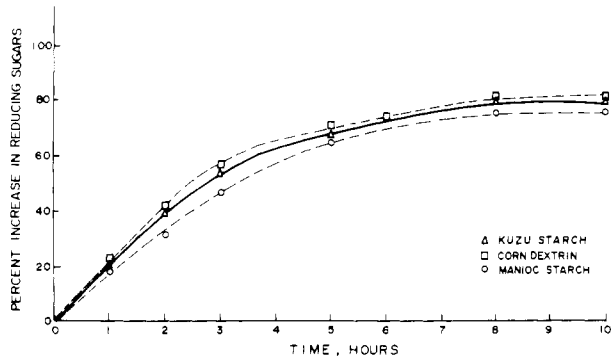

Figure 2. Saccharification of commercial kudzu (kuzu) starch with α-amylase compared with corn dextrin and manioc; T = 40 °C, initial pH 5.

extract, which offer mass transfer limitations not experienced in the purified starch degradation reaction. A typical degradation time of 18 h can therefore be expected for unpurified kudzu starch, as compared with 8 h for the purified form, for the selected enzyme level.

To place the kudzu saccharification in perspective, we compared it under similiar conditions to two other starches which are used as substrates for baker's yeast fermentations. The purified kudzu starch (kuzu) is compared with manioc root extract and purified corn dextrin as shown in Figure 2. The kuzu case is the same as that shown in Figure 1, except that it is expressed in percent increase units to compare with the manioc case, which was used at its starting concentration of about 104 g on a dry weight basis, rather than the 30 g for corn and kuzu. The manioc initial concentration in the cassava root was not known until after the run was made. Since only qualitative differences between the starches were desired, we did not feel repetition of the manioc case for rescaling was justified. Figure 2 indicates that only small differences exist in saccharification rate between the three starches, with kuzu falling in the middle. Since the corn dextrin was a fine powder before wetting, and the manioc was comprised of large globules initially, the small differences are probably due more to starch globule size than to chemical configuration. As in Figure 1, therefore, we attribute the differences in rates of reaction to mass transfer differences. In any event, kudzu starch appears to saccharify at about the same rate as the more conventional fermentation starches.

Saccharified Kudzu Starch Fermentation. In order to determine whether completely saccharified (with α-amylase) kudzu starch extract provided some or all of the nutrients required for baker's yeast growth, in addition to sugar, several experiments were performed. The preliminary experiment for an approximate 30 g/L of reducing sugar solution, shown in the lower curve in Figure 3 ("without minerals"), indicated that the extract by itself needed supplementation for growth. We had already determined that adding all of the ingredients in Maxon and Johnson's medium C prescription except sugar did, in fact, lead to complete growth without an apparent inhibition effect. Since vitamins are the most expensive supplementary components, we decided to leave them out from one experiment hoping to reduce the cost of the process. The growth experiment is reported on the upper curve of Figure 3 ("with minerals"). Normalization of the growth variable was employed to compare the two cases which had different initial optical densities. Dried baker's yeast was added to both broths in the concentration of about 1 g/L. Only $NH_4H_2PO_4$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, and sodium citrate were added (minerals and citrate) to

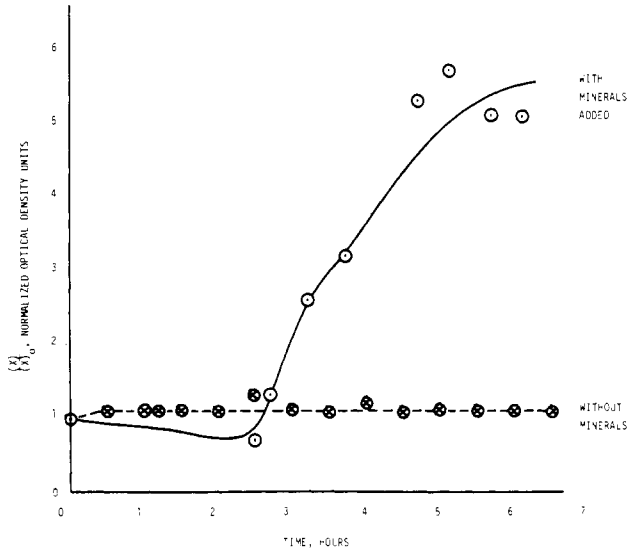

Figure 3. Preliminary demonstration that baker's yeast grows in saccharified kudzu root extract when only ammonium, potassium, phosphate, and citrate ("minerals") are added. No additional vitamins are needed for growth. T = 32 °C and pH 5.

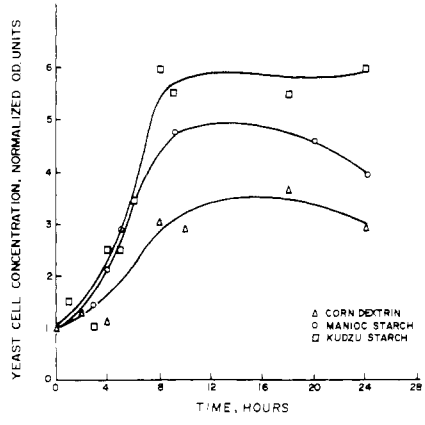

Figure 4. Comparison of baker's yeast growth on saccharified "corn dextrin and kudzu and manioc starches". No vitamins were added. T = 32 °C and pH 5.

develop the growth case. Therefore, not only were sufficient vitamins in the broth but enough trace amounts of zinc, iron, and copper to support a fivefold increase in cell growth. At the time of this early study, it was felt that the role of citrate was less significant than the minerals such as magnesium, so the upper curve in Figure 2 was designated "without minerals".

To place the vitamin-free fermentation of kudzu sugars in perspective, the fermentation was repeated and compared with the two other commonly used substrates: saccharified manioc and corn dextrin as shown in Figure 4. The saccharification for these two starches is reported in Figure 2, although as previously mentioned, manioc contained about 85 g of reducing sugar, and hence the manioc starch was saccharified for a longer time than the kudzu starch and corn dextrin.

When only ammonium, phosphate, magnesium, and citrate were added to the three different saccharified starch extracts, it is observed that in spite of the fact that manioc solution had considerably more sugar than the kudzu solution, the kudzu provided more cell growth. Its growth outperformed the corn dextrin case as well. This comparison is somewhat biased, however, since we are comparing the whole kudzu root extract to the cortex-free

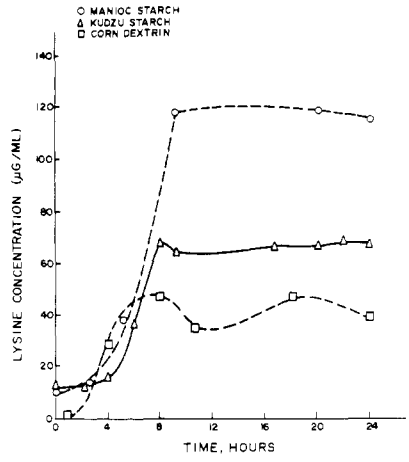

Figure 5. Comparison of intracellular free lysine synthesized in baker's yeast for the fermentation studies displayed in Figure 4.

manioc root extract and to the starch from corn kernels, not the vitamin-rich corn root. Nevertheless, all three are potential fermentation media and such a comparison is legitimate. The order in which free L-lysine was produced for these fermentations is depicted in Figure 5: manioc being higher than kudzu and kudzu being above corn. The higher manioc lysine is at about the same level as the highest lysine concentrations achieved for the glucose-complete nutrient case (Tanner et al., 1977), but for a cell level half as large as the glucose case. It means that since optical density is about $^5/_4$ that of cell weight (Tanner et al., 1977), the free lysine is more than twice as large for the manioc case than for the best previous glucose case on a per cell basis. At a cell level of about 4 g/L (the rest of the sugar has presumably been converted to ethanol and CO_2), the free intracellular manioc-derived lysine concentration is 3 wt % for the indicated conditions. When added to the average protein-bound lysine level of 3.4 wt %, a total lysine concentration of 6.4 wt % is achieved. In other words, the manioc case gives a 50% increase in total lysine per cell over the best lysine yields previously achieved using glucose and synthetic media. This total lysine level is now approaching levels of 9 to 10.2% (Florentino and Broquist, 1974) when the expensive precursors, α-aminoadipic acid and α-ketoadipic acid, are added to the medium. These high levels in the manioc fermentation may be industrially significant for a country like Brazil, where most of the manioc sugar is intended for ethanol production, but the by-product, lysine-enriched yeast (the yeast could be partially aerated), could prove beneficial as a human protein food supplement. The manioc case, while not central to this work, does deserve further study. The lower corn and kudzu free-lysine yields, nevertheless, are still significant on a per cell basis. The kudzu lysine yield on a per cell basis is just as high as that previously achieved for the glucose–synthetic medium, at 100 g/L of glucose initial levels.

If the free lysine in the yeast degrades in the subsequent harvesting (filtering, centrifugation, and/or spray drying) due to the Browning reaction with sugars, the following is suggested to minimize the losses: continue to ferment the yeast past the usual fermentation termination time until the available intracellular sugars have been catabolized to an acceptably low level. This additional fermentation should take about 1 to 2 more h.

In order to determine whether the yeast growth yields from kudzu extract without additional vitamins was affected by the nutrients in the root residue particles, the fermentation conditions for the case in the upper curve

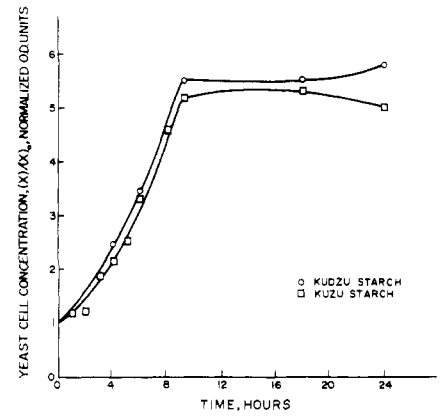

Figure 6. Comparison between the saccharified kudzu starch and kuzu starch baker's yeast fermentations. No vitamins were added. T = 32 °C and pH 5.

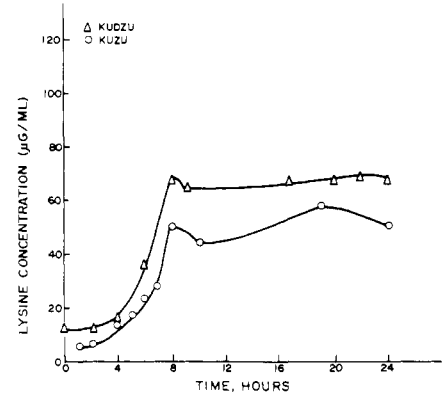

Figure 7. Comparison between the intracellular free-lysine synthesized in baker's yeast for the fermentation cases shown in Figure 6.

in Figure 4 was repeated for purified kudzu starch (kuzu). No significant differences were observed in either the cell growth or lysine yields when taking into consideration that the initial kudzu reducing sugar concentration was 27 g/L and the kuzu concentration 25 g/L. The small difference, nevertheless, is worth further clarification, should kudzu starch be considered for commercial fermentations. The curves in Figures 6 and 7 do indicate, however, that for the purposes of this study, the more easily obtained kuzu can serve as a substitute for kudzu, which we harvested by hand. This substitution was made for the study of the effect of each mineral on the kudzu starch fermentation. The sugar medium for the effect of minerals on fermentation was obtained from a base of 30 g of kuzu starch per liter of solution and saccharifying (shown in Figure 1). The previously discussed case in which only $NH_4H_2PO_4$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and sodium citrate was added (but no vitamins or trace minerals), as shown in the lower curves of Figures 6 and 7, are redrawn as the upper curves on Figures 8 and 9 and serve as the base for this nutrient study.

Removing only KH_2PO_4 from the above prescription of three mineral salts plus citrate leads to retarded yeast cell growth, as shown in the second upper curve in Figure 8, and less lysine production, as observed in the second upper curve in Figure 9. Since phosphorus is added in large amounts by the $NH_4H_2PO_4$, this reduced performance can be attributed to a potassium deficiency. It is well known that potassium ions play a role in enzyme activation, as well as active transport through the cell wall.

Removing only the citrate leads to the middle growth curve in Figure 8 and the very low lysine production curve

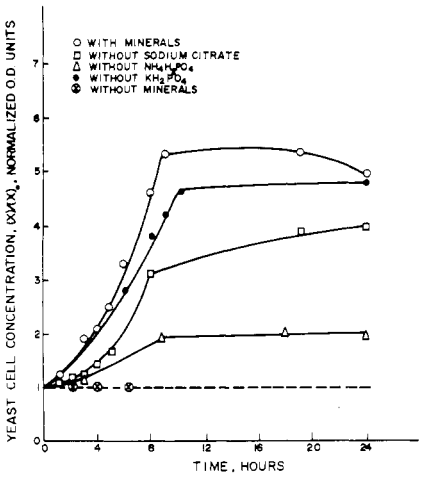

Figure 8. The effect of phosphate, ammonium, potassium, and citrate ions on baker's yeast growing on commercial kudzu (kuzu) starch which had been saccharified. No vitamins were added. The base line case $[X/X_0] = 1$ is the no-growth situation, reported in Figure 3, for saccharified starch from kudzu root extract. T = 32 °C and pH 5.

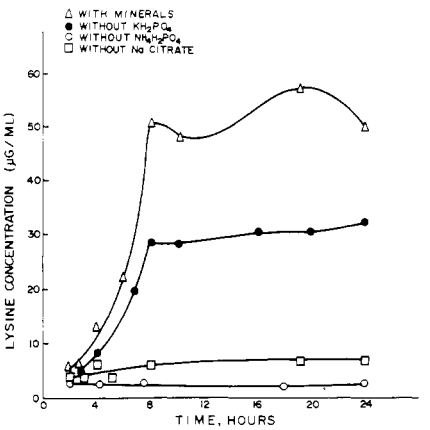

Figure 9. Comparison between the intracellular free-lysine synthesized in baker's yeast for the four growth cases shown in Figure 8.

in Figure 9. While growth is retarded by the lack of initial citrate, it appears that the initial limitation would eventually be overcome since the growth curve is still increasing due to internal production of citrate by the yeast. On the other hand, since citrate is a key precursor via the Krebs cycle for lysine formation, the lack of initial citrate significantly retarded the overproduction of free lysine which was not overcome during the course of the fermentation.

When only $NH_4H_2PO_4$ was withheld, and no ammonia was added initially for pH control, a definite ammonia limitation was demonstrated. With limited cell growth, as shown in Figure 8, limited acid needed to be neutralized with ammonia to maintain the pH at 5; hence, ammonia limited cell growth throughout the 24-h fermentation. Phosphate limitation was probably also a factor here, but phosphate was available in small amounts from the KH_2PO_4. With a nitrogen deficient medium, excess production of the (lysine) amino acid would not be predicted, which was, in fact, the case (shown in Figure 9).

Holding back all of the minerals, as was done in the early experiment shown on Figure 3 and reproduced as the lower curve on Figure 8, led to no yeast growth. Since magnesium is the only remaining factor, its presence can be inferred to be important for growth. This is intuitive, for it is well known that magnesium plays an important role

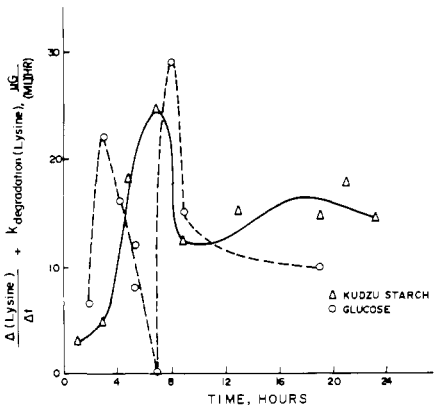

Figure 10. Double peaking free intracellular lysine biosynthesis rate of formation time transients for both a kudzu starch substrate and glucose in a complete synthetic medium. T = 32 °C and pH 5. The kudzu starch curve is based on differencing the data describing the upper curve in Figure 7.

in numerous biochemical reactions, and in particular it activates many of the glycolysis enzymes and amino acid pathway enzymes.

To infer that free lysine may be formed by a dual pathway, we differenced the kudzu data for lysine synthesis (taken from Figure 7) and plotted them on Figure 10. If a double peaking rate curve results, then it is likely that one of the pathways produces lysine more rapidly than the other (Tanner and Overley, 1974). In this case, L-lysine is formed both from protein degradation with protease (rapid formation), followed by biosynthesis of lysine starting from glucose (slower formation). To avoid negative rates, since lysine is an intermediate to subsequent protein formation, a lumped degradation constant, k_{deg} = 0.232/h, was determined in order to plot only the formation rate on the ordinate of Figure 10. This rate of formation was compared with a study using 30 g of glucose with a complete nutrient addition, as also shown in Figure 10 (the highest free-lysine level was 70 μg/mL and the highest cell level was 6.0 OD units). A first analysis of the two cases implies that protease attack appears earlier in the glucose case, for the rate of free-lysine formation. The continued high rate of lysine overproduction for later times in the kudzu case sustains the high level of intracellular free lysine. Perhaps the initial delay in the kudzu case was caused by the necessity for some vitamin biosynthesis or was due to inhibition by a kudzu root extract component.

CONCLUSIONS

The above results indicate that kudzu root starch is comparable to presently commercial fermentation starch substrates. If the roots can be harvested economically, as they are presently in Japan, then for the southeastern United States and subtropical areas in the world, kudzu may provide a new source of sugar and starch from marginal farming lands. The yield of starch is, like other plants, dependent on the weather—for kudzu, sustained hot weather (above 33 °C) without rainfall greatly reduces the carbohydrate product. An interesting aside observed in this study is that manioc starch fermentation can lead to very high free-lysine production in baker's yeast.

ACKNOWLEDGMENT

The initial fermentation study and kudzu root harvesting were performed by Deborah A. Bolton, Matthew A. Schoeffler, and Stephen M. Townsend. We are grateful for their early assistance. Kanza H. Badawi, Odalys M. D'Ambrosi, and L. Lynne Stroud provided essential laboratory support. The topic of kudzu utilization in the

United States originally arose from a suggestion by H. Ted Huddleston, Jr.

LITERATURE CITED

Bailey, R. Y., Mayton, E. L., "Kudzu in Alabama", Circular 57, Agricultural Experiment Station of the Alabama Polytechnic Institute, Auburn, Alabama, Jan 1931.
Carter, L. J., *Science*, **196**, 409 (1977).
Florentino, R. F., Broquist, H. P., *J. Nutr.* **104**, 884 (1974).
Hill, A. F., "Economic Botany", McGraw-Hill, New York, N.Y., 1937, pp 360, 541.
Maxon, W. D., Johnson, M. J., *Ind. Eng. Chem.* **45**, 2554 (1953).
McKee, R., Stephens, J. L., "Kudzu as a Farm Crop", Farmers' Bulletin No. 1923, U.S. Department of Agriculture, Oct 1943.
Pieters, A. J., "Kudzu, a Forage Crop for the Southeast", Leaflet No. 91, U.S. Department of Agriculture, April 1932.
Schery, R. W., "Plants For Man", Prentice-Hall, Englewood Cliffs, N.J., 1952, p 106.
Tanner, R. D., Overley, J. R., *Biotechnol. Bioeng.* **16**, 485 (1974).
Tanner, R. D., Souki, N. T., Russell, R. M., *Biotechnol. Bioeng.* **19**, 27 (1977).
White, A., Handler, P., Smith, E. L., "Principles of Biochemistry", 5th ed, McGraw-Hill, New York, N.Y., 1973, pp 47–52.

Received for review September 23, 1977. Accepted October 11, 1978. Paper presented at the 174th National Meeting of the American Chemical Society, Division of Microbial and Biochemical Technology, Chicago, Ill., Aug 30, 1977. This study was partially supported by a grant from the Vanderbilt University Research Council.

A Comparative Study in Rats of Iron Bioavailability from Cooked Beef and Soybean Protein

John T. Rotruck* and Kenneth R. Luhrsen

Two variations of the basic depletion–repletion method were used to measure iron bioavailability from a soybean protein isolate, a processed soybean protein isolate, and cooked beef. The depletion phase was the same for all animals; however, the repletion phase compared the effects of restricted feeding to ad libitum feeding. Slope-ratio analyses of the increase in hematocrit, hemoglobin, and total hemoglobin gave the best statistical fit for the data. Although all methods of analyses gave comparable results, calculation based on total hemoglobin provided the best statistical fit to the slope-ratio analysis (i.e., linearity of response). The results showed that iron from the soybean protein isolate and iron from the processed soybean protein isolate had bioavailabilities 82–100% of ferrous sulfate. Iron from cooked beef had a bioavailability of 26–55% of ferrous sulfate. The results of these animal experiments are compared to iron absorption studies conducted in anemic and normal humans.

The replacement of beef with analogues containing soybean protein raises many nutritional questions. Since beef supplies a substantial part of the iron in the human diet, it is one of the important nutritional questions when considering replacement foods. Although separate studies of iron bioavailability from beef (Pye and MacLeod, 1976; Oldham, 1941; Mahoney et al., 1974; Sherman et al., 1934; Rose et al., 1934) and soybean protein preparations (Fritz et al., 1970; Theuer et al., 1971) have been conducted, measurements of iron bioavailability from these two sources of iron have not been compared in the same study. In addition, the methodologies used to determine the bioavailability of iron from beef were different from those used for soybean protein preparations. Furthermore, the methods used to determine bioavailability in these foods are different from the procedure which is currently being recommended as the official method for determining iron bioavailability (Fritz et al., 1975). The objectives of this study were to use the same method to compare the bioavailabilities of these two food sources of iron in rats and to evaluate various methods of determining and calculating iron availability from foods.

Fritz et al. (1975) have recommended that their method be adopted as the official method for determining iron bioavailability. Although this method can yield acceptable results, there are some potential problems with it. The method first requires that rats be depleted of iron by feeding an iron-deficient diet, then the relative bioavailability of the iron source is determined by comparing the increase in hemoglobin concentration or hematocrit elicited by the unknown iron source to the increase in these parameters elicited by ferrous sulfate. Three dietary levels of the iron source are recommended as a means of increasing the sensitivity and reliability of the assay.

The first problem with the method as recommended by Fritz et al. (1975) is found in analysis of the data. There are two possible statistical methods of analyzing the data: the parallel-lines method and the slope-ratio method. The parallel-lines method requires that a plot of log of dose vs. response yield a linear response and parallel lines for the different treatments. The slope-ratio method requires that a plot of dose vs. response yield a linear response with a common intercept at the zero dose level. Both Fritz et al. (1975) and Waddell (1973) have recommended that the parallel-lines technique be used to calculate the relative bioavailabilities of the iron sources. However, Finney (1964), in his book on bioassays, pointed out that the appropriateness of a parallel-lines analysis or slope-ratio analysis depends upon the range of doses investigated. Thus one analysis is not appropriate for all experiments, suggesting that each experiment should be assessed by both statistical methods to determine which method might be applicable for the particular experiment. The problems of statistical analysis are discussed in more detail by Amine and Hegsted (1974).

In addition to the question of statistical analysis, an additional question about the method of Fritz et al. (1975) has been raised by Mahoney et al. (1974). The work of Mahoney et al. (1974) suggested that rating the bioavailability of iron sources according to hemoglobin concentration could lead to erroneous conclusions. This is because increases in hemoglobin concentration fail to take

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

0021-8561/79/1427-0027$01.00/0