Isolation of a Fermentation Stimulant from Yeast-Protein Concentrate[1]

C. S. LAI, A. B. DAVIS, and R. C. HOSENEY[2]

Cereal Chem. 61(5): 428–431

ABSTRACT

Gas production of a 4-hr bakers' yeast fermentation is increased up to 25% by the addition of 0.5% of a protein concentrate isolated from brewers' yeast. A series of tests indicated that the effect is not a function of an enzyme, a metal ion, a vitamin, a nitrogen, or a fermentable sugar, which might be contained in the yeast-protein concentrate. Separation and recombination studies show that the effect is a two-part system. One part contains about 60% of the effect and is enhanced to 100% by addition of very small amounts of the second part, which by itself has very little effect on fermentation.

Euler and Swartz (1924) first reported that yeast extracts could stimulate yeast growth and fermentation. Heat treatment at neutral pH did not change the effect, but heating under both oxidative and alkaline conditions decreased it. The effect was totally lost when the extract was ashed. Schultz et al (1937) reported that yeast extracts, autoclaved yeast, and vitamin B_1 precursors accelerated fermentation. They believed that vitamin B_1 was responsible for the effect of yeast extracts and autoclaved yeast. Neither Euler and Swartz (1924) nor Schultz et al (1937) described their extraction and preparation methods, so their work is difficult to evaluate.

A water-soluble extract of autolyzed yeast was found to accelerate fermentation rate without affecting yeast-cell population or cell size (Lee and Geddes 1959). The authors also reported that the active constituent was dialyzable, but that fractionating the extract always resulted in a partial loss of activity.

To our knowledge, no one has successfully isolated any yeast-fermentation activators from yeast. A growth factor for *Lactobacillus hichi* was isolated from yeast extract by Higashi et al (1979). It was shown to be two peptides containing glutamic acid. Because this growth factor was heat labile, it could not be responsible for the stimulating effect on fermentation of autoclaved yeast, yeast extract, and autolyzed yeast.

A previous study (*unpublished data*) showed that the addition of brewers' yeast-protein concentrate to dough accelerated the rate of fermentation. The first goal of this study was to determine whether or not the stimulation effect was contributed by an unintentional supplementation of known yeast nutrients. Following elimination of that possibility, we hoped to isolate the yeast-protein concentrate constituent responsible for the observed acceleration of fermentation.

MATERIALS AND METHODS

Materials

Flour. Wheat flour with a protein content of 12.1% (nitrogen × 5.7) was obtained from the Ross Milling Company. The flour had a good volume potential, a medium-long mixing time of 4.6 min, and a baking absorption of 62%.

Nonfat dry milk was supplied by Galloway West and contained 33.4% protein (nitrogen × 6.25). Soy-protein concentrate (85% protein) was from Ralston Purina (nitrogen × 6.25). Yeast-protein concentrate (YPC) was prepared by the sponsor company, as shown in Fig. 1. The concentrate was shipped under refrigeration to the Department of Grain Science and Industry at Kansas State University, lyophilized on receipt, and stored at 2°C until used.

A sample of YPC was hydrolyzed with HCl under vacuum at 100°C for 24 hr. HCl was removed by reduced pressure evaporation before the hydrolyzed YPC was lyopholized.

Pronase (protease type XIV) was from Sigma Chemical Company. All reagents were reagent grade. Cellophane (dialysis) film was from Union Carbide Co. The cut-off range was approximately 10^4 daltons.

Methods

Gasograph. Gasograph gas production was determined on a slurry, as described by Rubenthaler et al (1980). The slurry contained 15 ml of H_2O, 10 g of flour (14% mb), 0.15 g of NaCl, 0.6 g of sucrose, and 0.2 g of compressed yeast. Quantities of test materials added to gasograph tests were based on flour equaling 100%. All tests were run at least in duplicate. Gas production during a 4-hr fermentation was recorded and expressed as gasograph units (GU); 1 GU = 2.38 ml of CO_2 at 30°C and 1 atm (Rubenthaler et al 1980).

Yeast-protein concentrate in cellophane film was dialyzed for 24 hr against distilled water. Ten grams of YPC was suspended in 200 ml of distilled water and the pH adjusted to 7.0. Pronase was added and the YPC digested for 8 hr at 35°C with occasional stirring.

Dialyzed YPC (DYPC) was boiled for 20 min, then lyopholized. Following alkaline hydrolysis of YPC by the method of Knox et al (1970), carbon dioxide was introduced into the hydrolysate to precipitate Ba^{+2}. The precipitate was removed by centrifugation at 2,000 × *g* for 30 min. This process was repeated until no precipitate was produced. The hydrolysate was then lyopholyzed.

Ammonium sulfate was used to precipitate a portion of YPC from solution. Both precipitate and soluble fractions were dialyzed for 72 hr to remove ammonium salt.

Amberlite IR-120 (Fisher) was packed in a 3.5 × 40-cm column. The column was regenerated with 1*N* HCl and equilibrated with distilled water. The column was eluted with distilled water at a flow rate of six drops per minute until three times the void volume was collected. This fraction was designated as "through." The column then was eluted with 1*N* NH_4OH until the eluent became alkaline. This fraction was designated as "retained" (RSEDP). Both RSEDP and through fractions were concentrated under reduced pressure and lyopholyzed.

Sephadex G-25 (superfine; Pharmacia) was packed in a 3.2 ×38 cm column. The column was equilibrated and eluted with distilled water. Samples (RSEDP) were dissolved in 2 ml of distilled water. Flow rate was 12 drops per minute.

RESULTS AND DISCUSSION

Preliminary work showed that nonfat dry milk improved yeast gassing power to a limited extent. Soy-protein concentrate (5%) had no effect on gassing power, while YPC (5%) increased gas production by 25%. The results suggest that the effect of YPC was not a function of its nitrogen content. A synthetic mixture that contained an adequate amount of sugar, nitrogen, and other nutrients was reported to give a maximum gassing rate (Ling and Hoseney 1977). When YPC was added to that system, gas

[1]Contribution No. 84-165-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan, KS 66502.
Presented at the AACC 68th Annual Meeting, October 1983.

[2]Graduate research assistant, assistant professor, and professor, respectively.

©1984 American Association of Cereal Chemists, Inc.

production increased 11, 20.5, and 25.8% for 2, 5, and 10% additions, respectively (Table I). This result shows that YPC contains an additional factor that greatly increases gas production.

The effect of YPC occurred during the third and fourth hours of fermentation (Table I). This suggests that the system is being depleted of some nutrient.

Effect of minerals. Hydrolyzed YPC had only a limited effect on fermentation (Table II). Thus, the major factor in the effect of YPC on fermentation was destroyed by acid hydrolysis, suggesting that minerals were not responsible.

Effect of dialysis. Yeast-protein concentrate was dialyzed against distilled water. About 10% of the YPC by weight was removed by dialysis. The dialysate (portion removed) had only a limited effect on fermentation. Dialyzed YPC (retained portion) possessed more than 80% of the original activity (Table III). Reconstitution of YPC by combining an appropriate amount of dialysate and dialyzed YPC (DYPC) restored the original activity. This result indicated that low-molecular-weight entities in YPC were not a major factor in stimulating fermentation. Adding more dialysate (5%, based on flour weight) did not produce any effect beyond that found at the lower addition level (Table IV). Therefore, the major effect of YPC was contributed by a high-molecular-weight (>10,000-dalton) fraction, but the dialysate was needed for maximum activity.

Heat treatment. Heat treatment did not change the effect of DYPC on fermentation (Table IV). This result indicated that the effect of YPC was not due to enzymatic action.

Effect of protein in YPC. The major effect of YPC was associated with a nondialyzable entity, suggesting that the effect was contributed by yeast proteins. After ammonium sulfate precipitation and dialysis, the dialyzed salt-soluble fraction constituted about 1% of the intact YPC by weight. The dialyzed salt-precipitated fraction accounted for 74% of the intact YPC. Approximately 25% of the YPC was lost in the dialysate. The effects of dialyzed salt solubles (DSS) and dialyzed salt-precipitated protein (DSP) on fermentation are shown in Table V.

TABLE I
Effect of Yeast-Protein Concentrate (YPC) on Yeast Gassing Power

Treatments (duplicates)	Gas Production during Fermentation (GU) at Hr 1	2	3	4
Control (no YPC)	8.3 ± 0.4[a]	25.0 ± 0.2	43.0 ± 1.4	59.5 ± 0.4
2% YPC	8.3 ± 0.4	25.0 ± 0	46.3 ± 0.4	66.2 ± 0.1
5% YPC	8.5 ± 0	23.9 ± 0.2	47.2 ± 0.3	71.6 ± 0.5
10% YPC	8.6 ± 0.1	24.3 ± 1.1	47.5 ± 0.7	74.8 ± 0.4

[a] Plus or minus standard deviation.

TABLE II
Effect of Hydrolyzed Yeast-Protein Concentrate (YPC) on Yeast Gassing Power

Treatments (duplicates)	Gas Production during Fermentation (GU) at Hr 1	2	3	4
Control	5.4 ± 0.3[a]	19.2 ± 0.3	37.5 ± 0.3	54.6 ± 0.3
5% YPC	5.5 ± 0.1	20.5 ± 0.1	42.7 ± 0.1	64.8 ± 1.3
5% Hydrolyzed YPC	5.6 ± 0	16.7 ± 0.1	34.4 ± 0	57.9 ± 0.4

[a] Plus or minus standard deviation.

TABLE III
Effect of Dialysis on Yeast-Protein Concentrate (YPC) Activity

Treatment (duplicates)	Gas Production after 4-hr Fermentation
Control	57.5 ± 0.4[a]
Dialysate (0.5%)[b]	60.7 ± 0.4
Dialyzed YPC (DYPC) (4.5%)	66.2 ± 0.2
Reconstituted YPC (0.5% dialysate + 4.5% DYPC)	68.4 ± 0.4
YPC	68.3 ± 0.3
Control	59.5 ± 1.3
Dialysate (5%)	63.2 ± 2.1
DYPC (5%)	70.3 ± 0.6

[a] Plus or minus standard deviation.
[b] Percent material in slurry, with flour weight equaling 100.

TABLE IV
Effect of Heat Treatment on Yeast-Protein Concentrate (YPC) Activity

Treatment (duplicates)	Gas Production after 4-hr Fermentation
Control	56.5 ± 0.2[a]
DYPC (4.5%)[b]	65.3 ± 1.4
Boiled DYPC (4.5%)	65.6 ± 0.1
YPC (5%)	66.8 ± 0.7
Boiled DYPC (4.5%) + dialysate (0.5%)	68.3 ± 0.3
Control	57.5 ± 0.4

[a] Plus or minus standard deviation.
[b] Percent material in slurry, with flour = 100. DYPC = dialyzed yeast protein concentrate.

TABLE V
Effect of Dialyzed Salt-Soluble and Salt-Precipitated Yeast Protein Fractions on Gassing Power

Treatment (duplicates)	Gas Production after 3-hr Fermentation	Gas Production after 4-hr Fermentation
Control	38.2 ± 0.2[a]	54.5 ± 0.7
YPC W/H (5%)	43.1 ± 0.3	65.8 ± 0.4
DYPC W/H (4.5%)	42.4 ± 0.2	64.3 ± 0.2
Dialyzed[b] salt-precipitated protein	41.0 ± 0.5	62.4 ± 0.4
Dialyzed[b] salt-soluble	38.4 ± 0.2	55.7 ± 0.5
DSP[c] + salt soluble	40.4 ± 0.1	62.4 ± 0.1

[a] Plus or minus standard deviation.
[b] Salt-precipitated protein = material that precipitated when pH 12 YPC solution was half saturated with $(NH_4)_2SO_4$.
[c] Weight of dialyzed salt-out protein + dialyzed salt solubles = 75% of YPC.

TABLE VI
Effect of Pronase Digestion on Yeast-Protein Concentrate Activity

Treatment (duplicates)	Gas Production after 4-hr Fermentation (GU)
Control	53.0 ± 0.3[a]
YPC (5%)[b]	67.3 ± 0.2
Pronase-digested YPC (EYPC) (5%)	66.5 ± 0.2
Control	50.0 ± 1.4
Solubles of EYPC (3%)	66.8
Insolubles of EYPC (2%)	66.1 ± 0.8
YPC (5%)	67.5 ± 0.3
Solubles (3%) + insolubles (2%) of EYPC	69.2 ± 2.4
Control	56.4 ± 0.6
Solubles of neutralized YPC (2.3%)	64.6 ± 0.9
Insolubles of neutralized YPC (2.7%)	62.5 ± 0.6
Solubles (2.3%) and insolubles (2.7%) (reconstitute)	66.1 ± 0
YPC (5%)	68.0 ± 0.7
Control	57.7
YPC (5%)	69.0 ± 0.7
Solubles (2.3%) of neutralized YPC + pronase	71.0 ± 0
Control	56.6 ± 0.3
YPC (5%)	65.3 ± 0.4
Insolubles (2.7%) of neutralized YPC + pronase	67.7 ± 0.7

[a] Plus or minus standard deviation.
[b] Percentage of material in slurry, with flour = 100.

The DSP retained 70% of the original activity, whereas the DSS was essentially ineffective. Reconstitution of DYPC by combining appropriate amounts of DSS and DSP did not restore the activity of DYPC. These results suggested that the major effect of YPC was associated with yeast protein but that the dialyzable materials were also important.

Pronase digestion of YPC did not alter its activity (Table VI). The pronase digest was not complete, and the digested YPC was fractionated into soluble and insoluble portions by centrifugation at 2,000 × g for 30 min. Both the soluble and insoluble portions were as effective as intact YPC at the addition levels of 60 and 40% (by weight) of the intact YPC (Table VII). Pronase digestion increased the total potency of YPC.

To further study the effect of pronase digestion, 10 g of untreated YPC was suspended in 200 ml of distilled water at pH 7.0. This neutralized intact YPC was fractionated into soluble and insoluble fractions by centrifugation (2,000 × g; 20 min). By this method, 45% of the YPC was solubilized. Gasograph data showed that both the soluble and insoluble portions retained part of the original activity (Table VI). When these two fractions (soluble and insoluble) were treated with pronase, they both became at least as effective as intact YPC (Table VI). These results suggest that enzyme digestion renders the active component more available to yeast. The fact that both the soluble and insoluble portions of the neutralized intact YPC have the same potential effect on fermentation indicates that the active component is present in more than one protein or that there are multiple factors.

We thought that, if the effect of YPC was contributed by some specific amino acid or material bound to protein, it might be possible to hydrolyze the protein and retain activity. The previous

TABLE VII
Effect of Components of Solubles of Pronase-Digested Yeast-Protein Concentrate (YPC) on Gassing Power

Treatment (duplicates)	Gas Production after 4-hr Fermentation (GU)
Control (0.6%)[a]	50.5 ± 0.1[b]
Retained (on Amberlite IR-120)	62.3 ± 0.3
Through (0.6%)	53.6 ± 0.1
Retained and through	63.6 ± 0.1
YPC (5%)	63.0
Control	56.6 ± 0.3
Oxidized retained	64.7 ± 0.7
YPC (5%)	65.3 ± 0.4
Control	62.1 ± 0.6
YPC	74.8 ± 1.1
Acid treated retained	73.0 ± 0.8
Alkaline hydrolyzed retained	64.0
Control	56.5 ± 0.3
YPC	65.3 ± 0.4
Alkaline hydrolyzed retained	59.0

[a]Percentage of material in slurry, with flour = 100.
[b]Plus or minus standard deviation.

TABLE VIII
Effect of Fractions 1 and 2 on Gassing Power

Treatment	Gas Prodution after 4-hr Fermentation (GU) (average of duplicates)
Control	53.9
YPC (5%)[a]	65.1
Reconstituted (37 mg of fraction 1 + 5 mg of fraction 2)	63.3
5 mg Fraction 2	56.3
10 mg Fraction 2	55.4
15 mg Fraction 2	56.7
10 mg Fraction 1	54.8
15 mg Fraction 1	56.9
30 mg Fraction 1	58.0
50 mg Fraction 1	60.2

[a]Percentage of material in slurry, with flour = 100. YPC = yeast-protein concentrate.

TABLE IX
Effect of Fractions of RSEDP from Sephadex G-25 Column on Gassing Power

Treatment	Gas Production after 4-hr Fermentation (average of duplicates)
Control	58.4
YPC (5%)[a]	70.8
Reconstituted	68.1
Fraction 1	59.6
Fraction 2	60.6
Fraction 3	60.4
Fraction 4	58.3
Fraction 5	57.6
Fraction 6	58.4

[a]Percentage of material in slurry, with flour = 100.

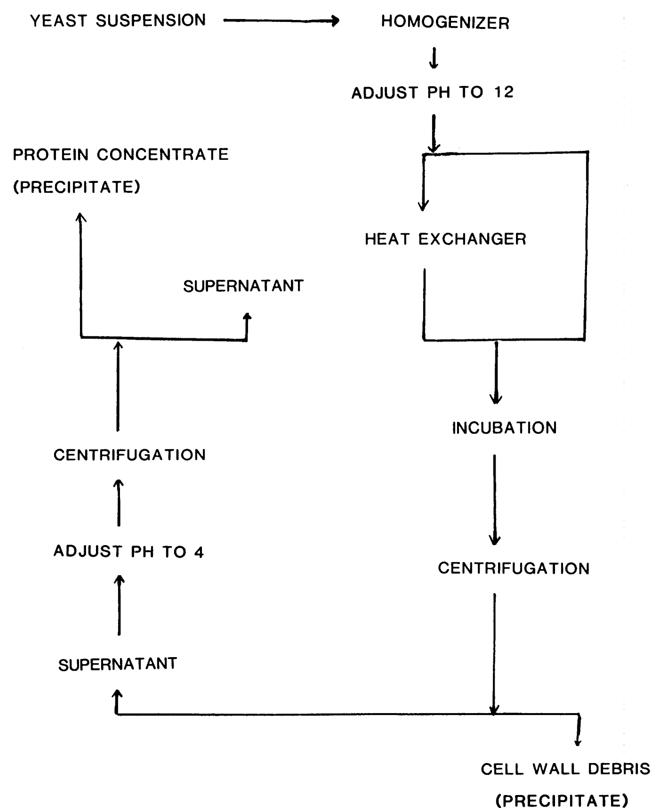

Fig. 1. Production process for yeast-protein concentrate (YPC).

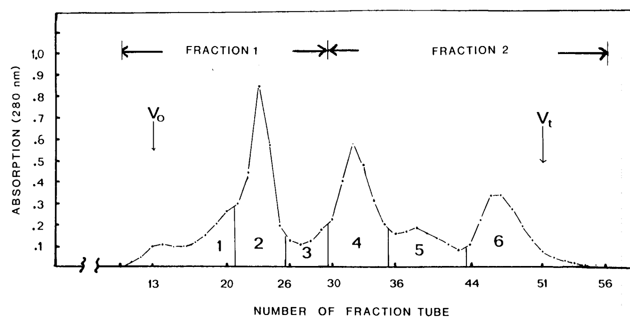

Fig. 2. Elution profiles of RSEDP on Sephadex G-25.

study showed that pronase did not completely digest YPC and that acid hydrolysis destroyed the activity (Table II). Therefore, alkaline hydrolysis was tried. Gasograph data showed that alkaline hydrolysis destroyed the activity of YPC (Table VII). This suggests that the effect of YPC may be contributed either by a specific peptide or by an entity that is both acid- and alkaline-labile.

In an attempt to isolate the active component, the soluble portion of the pronase-digested YPC was fractionated on an ion-exchange column. Recovery from the ion-exchange column was 33 and 53% for duplicated samples. The weight ratio of retained and through fractions remained about 1:1. A gasograph test showed that the retained fraction had about 98% of the original activity, while the through fraction had little effect (Table VII). The retained fraction (RSEDP) was then subject to gel filtration. The resulting chromatogram (Fig. 2) showed five peaks (as measured by the absorption of eluent at 280 nm). None of these peaks retained the original activity (Table VIII). Recombining material from the peaks restored the original activity. Extensive use of the Sephadex column reduced the recovery rate from 90 to 50%. Recovery could be improved by repacking the column. In this study, the column was repacked after five runs. We did not quantitate each fraction because of the small quantities involved. The amount of RSEDP that was equivalent to 5% YPC was loaded on the column. Material in six fractions (Fig. 2) was collected and lyopholyzed in the reaction bottle used in the gasograph test.

As shown in Table IX, the activity was limited to the first three fractions. The other four fractions appeared to be ineffective. To verify the above supposition, the six fractions were grouped into pooled fractions (Fig. 2). Table IX shows the effect of fractions 1 and 2 on fermentation. The yield of fractions 1 and 2 was 37 and 5 mg, respectively. The reconstitution of RSEDP by combining these fractions restored 85% of the original activity. The effect of fraction 2 increases little for additions above 5 mg whereas the effect of fraction 1 was concentration-dependent. Fraction 1 was much less effective than the reconstituted RSEDP. The addition of 50 mg of fraction 1 produced only 56% of the original effect. It was 29% less than that of reconstituted RSEDP (37 mg fraction 1 + 5 mg fraction 2). This result indicates an interaction between fractions 1 and 2. Data from this series of trials suggests that the effect of YPC is a function of two or more factors that interact.

This study establishes the presence of a fermentation stimulant in yeast-protein concentrate, which is not one of the commonly known yeast nutrients. It appears to be an interacting system with at least two components; one of the components is a protein, and the other is a low-molecular-weight entity.

ACKNOWLEDGMENT

The authors would like to express their appreciation to Miller Brewing Company for financial support.

LITERATURE CITED

EULER, H. V., and SWARTZ, O. 1924. Uker de zusammenhang der wasserlolichen washstumsfaktoren mit aktivatoren des zuckerabbaues und uber einen thermostabilen biokatalysator inder hefe I. Z. Phys. Chem. 140:146.

HIGASHI, K., TAKEDA, M., and TSUKAHARA, T. 1979. Nutritional requirement of hiochi-bacteria in high ethanol concentration. VII. Separation of peptides from yeast extract. J. Soc. Jpn. Brewing (Nihon Jozo Kyokai Zasshi). 74:555 Chem. Abstr. 91:171372f.

KNOX, R., KOHLER, G. O., and WALKER, H. G. 1970. Determination of tryptophan in feeds. Anal. Biochem. 36:136.

LEE, J. W., and GEDDES, W. F. 1959. Studies on the brew process of bread manufacture: The effect of sugar and other nutrients on baking quality and yeast properties. Cereal Chem. 36:1.

LING, R. S., and HOSENEY, R. C. 1977. Effect of certain nutrients on the gas produced in preferments. Cereal Chem. 54:597.

RUBENTHALER, G. C., FINNEY, P. L., DEMARAY, D. E., and FINNEY, K. F. 1980. Gasograph: Design, construction and reproducibility of a sensitive 12-channel gas recording instrument. Cereal Chem. 57:212.

SCHULTZ, A. S., ATKIN, L., and FREY, C. N. 1937. A fermentation test for vitamin B. II. J. Am. Chem. Soc. 60:490.

[Received October 24, 1983. Revision received May 21, 1984. Accepted May 23, 1984]