plete racemization was assumed and all tryptophan values in the tables are doubled to correct for this (*4*). For the other amino acids, samples were hydrolyzed by the same autoclaving procedure with 40 ml. of 3.N hydrochloric acid used per gram of protein. Hydrolyzates were neutralized, filtered to remove the humin (*3*), and stored under toluene at 5° C.

Lactobacillus delbrueckii 3 was used in the assay of arginine, *Streptococcus faecalis* R for threonine, and *Lactobacillus arabinosus* 17-5 for tryptophan. *Leuconostoc mesenteroides* P-60 was used for histidine, isoleucine, leucine, lysine, methionine, phenylalanine, and valine. United States Pharmacopeia reference standards were used in all assays except for arginine and histidine, for which commercial products were used.

Results and Discussion

Table I gives the proximate composition of the frozen vegetables used in this study. The amino acid assay results are presented in Tables II and III. In Table II the results are expressed as grams of amino acid per gram of total nitrogen in the frozen food. Table III gives the results in milligrams of amino acid per 100 grams of product. The figures in Table III represent 1.06 times more than the amounts present in a serving consisting of one third of a 10-ounce package of the vegetable.

The tables give average, maximum, and minimum values for each group of vegetables. Values which deviated markedly from the average were checked by repeat assay.

The legumes are highest in protein content and, on an absolute basis, tend to be higher in the amino acids studied (Table III). However, on the basis of grams of amino acid per gram of nitrogen the amino acid pattern in the various products is similar.

The biological protein value of diets is determined by the combination of available amino acids in each meal. It is important to provide, in a proper balance, those amino acids which the body cannot synthesize in adequate amounts. Other amino acids may have a sparing action on these "essential" amino acids. Qualitative studies have established the fact that valine, leucine, isoleucine, methionine, threonine, phenylalanine, tryptophan, and lysine are required in the diet for maintenance of nitrogen equilibrium and prevention of subjective symptoms in normal adult man; a beginning has been made toward quantitative measurement of minimal requirements to provide a basis for estimation of a "safe" intake (*5*). The vegetable products included in this study are ordinarily consumed in a mixed diet with other protein foods. The amino acid data reported herein will facilitate the estimation of the supplementary protein value of these products.

Acknowledgment

The authors wish to extend thanks to H. P. Schmitt, National Association of Frozen Food Packers, Washington, D. C., who arranged for sampling and assisted in compilation of the data, and to Central Storage and Warehouse Co., Madison, Wis., for storage facilities. The advice and counsel of C. A. Elvehjem, Biochemistry Department, University of Wisconsin, are gratefully acknowledged. The following assisted in the analytical work reported: L. W. Hein, Marie Burger, D. R. Clark, and R. A. Kubista.

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MOLASSES FERMENTATION

Continuous Fermentation

Alcoholic Fermentation of Blackstrap Molasses

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CONTINUOUS ALCOHOLIC FERMENTATION has been studied intensively and various patents have been granted for alcohol production (*8, 13, 15, 16, 19-21, 23, 27*).

Alzola (*2*) described a continuous fermentation process in which the sterilized mash goes through successive fermentors connected in a series. The tanks were agitated by the carbon dioxide produced during the process. In 1945, he studied a new continuous process (*3*), using a column divided into six parts. The mash was introduced into the bottom of the column and also agitated by the carbon dioxide produced.

The possibility of performing the continuous fermentation in a single flask was first investigated by Bilford and coworkers (*6*) in 1942, who carried out the experiments on a laboratory scale, thus reducing equipment requirements. Owen (*26*), in 1948, used a glass column divided in six parts; the mash was fed continuously into the top of the column. The continuous fermentation of beet juice was described and discussed by Mariller and coworkers (*22*). The study of continuous alcoholic fermentation using two connected fermentors was started by Asai and coworkers (*1, 5*). Borzani (*9*) examined the economical aspects of continuous alcoholic fermentation of molasses using a single fermentor and mechanical agitation. The work was carried out on a pilot-plant scale with the same equipment used in the batch process.

The striking advantages that a continuous fermentation has over the corresponding batch process (*24*) justified a systematic study of the factors involved. The following factors, that influence the continuous alcoholic fermentation of blackstrap molasses in a single vessel, are considered in this paper: sugar concentration of feed mash, feed rate, agitator speed, and fermentor capacity.

Apparatus

The equipment shown in Figure 1 consisted of two steel fermentors: 100-liter capacity (45 cm. in diameter by 80 cm. in height) and 1800-liter capacity (120 cm. in diameter by 188 cm. in height). The agitators in Figures 2

The influence of sugar concentration, feed rate, agitator speed, and fermentor capacity on the continuous alcoholic fermentation of blackstrap molasses has been studied on a pilot-plant scale. The sugar concentration and the fermentation-cycle time can be related by an equation, theoretically justified, if it is assumed that the sugar consumption has a reaction rate of —1. Agitation is probably the rate-determining factor for continuous alcoholic fermentation. Penicillin increases the efficiency by preventing contamination. After 30 hours of fermentation, the penicillin concentration was 25 to 60% of the initial antibiotic concentration. Laboratory and plant-scale fermentations with 1.0 unit per ml. of penicillin were studied and found favorable. An increase in the alcohol yield (4.8 to 19.5%) and a reduction of the acid production (17.0 to 66.6%) was observed. Penicillin did not affect the final yeast count or the fermentation time, and *Leuconostoc* contamination was inhibited by 8.0 units per ml.

and 3 were of the blade-paddle type, with the blade tilted to produce a down thrust in the liquid, and located 5 cm. from the bottom of the fermentors.

Experimental

Molasses with 47.7% of total reducing sugars was used for the first three groups of experiments (Table I) and for the first group in Table II. For the other experiments, molasses with 53.1% of total reducing sugars was used.

One hundred liters of unsterilized mash with 0.25 gram per liter of magnesium sulfate heptahydrate, 1.0 gram per liter of potassium phosphate (monobasic), and 10 grams per liter of pressed yeast (1.10×10^{11} cells per liter) were placed in the smaller fermentor. (*Saccharomyces cerevisiae*, prepared as pressed yeast by the Standard Brands of Brazil, Inc., was used for direct inoculation without previous treatment). After the fermentation was completed, the agitation and the continuous feeding with the mash were started. This mash was not inoculated and had the same composition as the one used to start the experiment.

In the large fermentor, 900 liters of mash were placed with 10 grams per liter of pressed yeast (1.10×10^{11} cells per liter). There was no addition of salts to the medium. Another 900 liters of uninoculated mash were added

Table I. Continuous Fermentation in 100-Liter Fermentor

Agitator Speed, R.P.M.	Feed Mash Sugar Concn. as Dextrose, G./L.	Feed Mash Acidity as H_2SO_4, G./L.	Temp., ° C.	Fermentation Cycle Time, Hours	Time of Continuous Feeding, Hours	Relative Efficiency[a] of Continuous Fermentation, %
0	101–105	2.0	23–24	11	8	71
	103–104	2.0	24–28	16	12	92
	100–105	1.7–2.0	23–25	18	16	86
	98–100	1.7	23–26	20	11	100
	92–94	1.7	22–24	23	12	98
	101–105	1.7–2.0	23–25	29	23	95
0	141	2.5	21–24	34	10	94
	149	2.6	22–24	42	9	95
	143–146	2.5	23–24	46	11	95
0	170–175	2.7	22–26	29	23	83
	184–188	2.8–3.2	23–25	34	24	83
	184	3.1–3.2	22–25	59	11	88
	180–182	3.4–3.7	22–25	71	30	97
0	63–65	1.0	25–26	9	6	82
	63	1.8	23–26	10	8	96
	64–65	1.7	24–26	14	7	96
660	103–105	1.5	22–24	17	8	84
660	104–107	1.4–1.5	24–27	20	9.5	91
550	109–111	1.2	22–24	20	9	84
550	106–107	1.2–1.3	22–26	22	9	93
450	102–103	1.2	22–26	20	9	93

[a] Quotient of efficiency of continuous fermentation by efficiency of corresponding batch fermentation. Average efficiency of batch fermentation = 0.57 liter of alcohol per kilogram of dextrose.

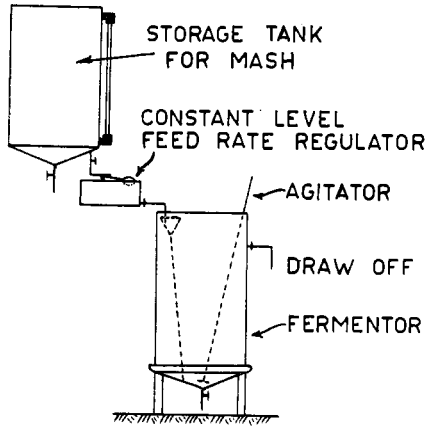

Figure 1. Apparatus for continuous fermentation

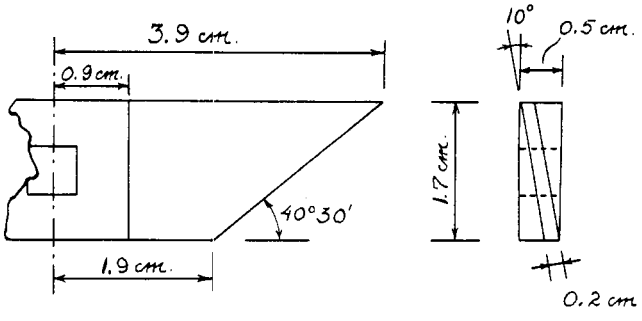

Figure 2. Agitator of 100-liter fermentor

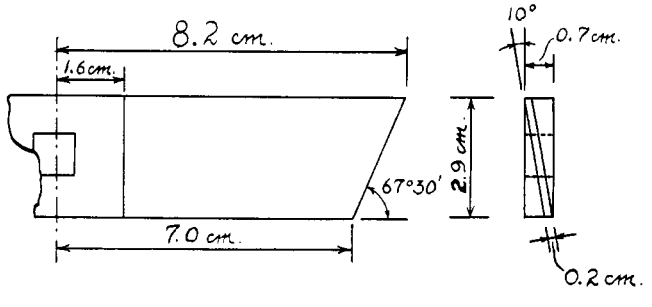

Figure 3. Agitator of 1800-liter fermentor

Table II. Continuous Fermentation in 1800-Liter Fermentor

Agitator Speed, R.P.M.	Feed Mash Sugar Concn., as Dextrose, G./L.	Feed Mash Acidity as H_2SO_4, G./L.	Temp., ° C.	Fermentation Cycle Time, Hours	Time of Continuous Feeding, Hours	Relative Efficiency of Continuous Fermentation, %[a]
0	99–103	1.7–1.8	21–23	24	12	80
			25–26	32	4	89
			24–25	55	10	96
			25–26	59	8	95
660	99–100	1.0–1.2	25–27	23	9	96
600	100–140		24–28	20–23	132	95–100[b]

[a] Quotient of efficiency of continuous fermentation by efficiency of corresponding batch fermentation. Average efficiency of batch fermentation = 0.53 liter of alcohol per kilogram of dextrose.
[b] Reference (*9*).

after 10 to 12 hours. When the fermentation was completed, the agitation and the continuous feeding were started with uninoculated mash identical to the one used to start the experiment.

The initial sugar concentration (*12*) and total acidity (*1*) of the mash, and the total acidity (*1*) and alcohol content (*11*, *25*) of the withdrawal medium, were determined. Temperature and specific gravity of the fermenting mash were controlled during the experiments. The specific gravity was periodically measured to have an indication of the completeness of the fermentation. The average values of the specific gravity of the mash and of the withdrawal medium are shown in Table III. Mariller and others (*22*) had shown that yeast cell concentration was not important and, consequently, yeast cell counts were not made during the process; however, the initial concentration of cells was kept constant within ±19% (*10*).

Table III. Average Values of Specific Gravity of Mash and Withdrawal Medium

Approximate Sugar Concn. of Feed Mash, G./L.	Feed Mash, Sp. Gr.	Withdrawal Medium, Sp. Gr.
60	1.035	1.013
100	1.062	1.024
140	1.084	1.031
180	1.112	1.042

Results and Discussion

A complete continuous fermentation is considered to have an efficiency equal to or greater than 95% of the efficiency of a batch fermentation. The experimental results obtained with the nonagitated 100-liter vessel show that the fermentation cycle time depends on the initial sugar concentration of the feed mash.

The results in Table I show that in the 100-liter vessel, without mechanical agitation, the fermentation cycle time (T, hours) of a complete continuous fermentation and the concentration of total reducing sugars (C, gram per liter) in the feeding mash, can be related by the following equations, derived by the application of the least squares method to the values.

$$T = 0.606\,C - 42.3 \quad (100 \text{ grams per liter} \leq C \leq 180 \text{ grams per liter}) \quad (1)$$

$$T = 2.08 \times 10^{-3}\,C^2 \quad (0 \text{ gram per liter} \leq C \leq 180 \text{ grams per liter}) \quad (2)$$

For practical use, Equation 1 gives good results; however, Equation 2 is a better representation of the phenomenon, as when C is zero, T is also zero.

Equation 2 can be justified theoretically with the hypothesis that the consumption of sugar follows a negative kinetic reaction order with a rate of −1. In fact, if

$$\frac{dc}{dt} = -\frac{k}{c}$$

$$-k\int_0^T dt = \int_C^{C_o} c\,dc$$

$$T = \frac{C^2 - C_o^2}{2k}$$

But $C_o = r.C$ (r = approximately constant). Then

$$T = \frac{1 - r^2}{2k} C^2$$

where T is the fermentation cycle time of a complete continuous fermentation, C is the initial sugar concentration of the feeding mash, and r is the approximately constant relation between the sugar concentration of the withdrawal medium and C. Another experimental approach is being tried to confirm these results.

Table I shows that mechanical agitation does not improve the fermentation-cycle time value. The agitation resulting from the production of carbon dioxide in the smaller fermentor is the favorable one for continuous fermentation.

The values listed in Table II show that in the 1800-liter fermentor, mechanical agitation decreases the fermentation-cycle time value from 55 to 23 hours. These values are the smallest values of the fermentation-cycle time corresponding to complete continuous fermentations without and with mechanical agitation. This is in agreement with results published previously (*9*). The 23-hour value is practically the same one obtained in the smaller vessel without mechanical agitation and with the same initial sugar concentration. The agreement in results may be due to the fact that the agitation in the 100-liter vessel (without mechanical agitation) and the one in the 1800-liter fermentor (with mechanical agitation), are the same.

Continuous Fermentation

Penicillin as Contamination Control Agent

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MICROBIOLOGICAL CONTAMINATION INHIBITORS are commonly used in industrial fermentations to assure economic efficiencies and good quality products. Particular attention was given, in the last decade, to the use of antibiotics as inhibitors of foreign microorganisms.

Strandskov and Bockelmann (*28*) and Day and associates (*14*) surveyed the known literature concerning the use of antibiotics in alcoholic fermentations, in which the application of several antibiotics to the control of bacterial and secondary yeast contamination in the

brewery (*28*), and the control of the development of the bacteria present in grain mash fermentations by the use of antibiotics (*14*) were presented.

Some pilot-plant experiments showed the favorable influence of penicillin as a contamination-control agent in molasses fermentation. These results justify the present investigation.

Methods

Yeast count was made by use of the hemacytometer chamber (*31*). Bacterial population was not determined because the tomato medium, in deep agar tubes, proposed by Garey and coworkers (*18*) did not inhibit the yeast development.

Alcohol yields are reported in terms of milliliters of ethyl alcohol per kilogram of dextrose. Alcohol was determined on distillates by Gay-Lussac's alcoholometer (*7, 11*).

Acid production was measured by titration with phenolphthalein as the indicator (*1*). Values are reported as grams of sulfuric acid per liter.

The penicillin used was crude potassic penicillin G, and assayed at 1550 to 1580 units per mg. The antibiotic was added at the start of the fermentations, unless otherwise specified.

In all experiments, the bacterial contamination was limited to the bacteria normally developing from the molasses and from miscellaneous sources of admission in the usual fermentations. Sugar concentrations are reported as grams of dextrose per liter. Sugar concentration of molasses was determined by the Eynon-Lane method (*12*).

Laboratory Fermentation Tests

Mashes for laboratory scale fermentations were prepared by diluting blackstrap molasses (containing 55.4% of sugar as dextrose) in tap water and, in some cases, adding sulfuric acid to correct the initial mash acidity. Inoculation was made with 10 grams per liter (approximately 1.1×10^{11} yeast cells per liter) of pressed yeast (*Saccharomyces cerevisiae*).

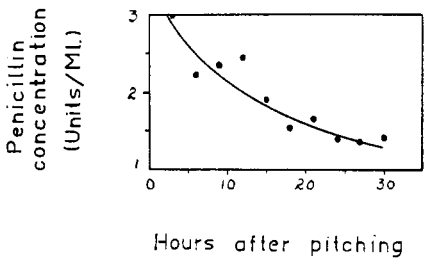

Figure 4. Variation of penicillin concentration during fermentation

Sugar concentration, 180 grams per liter

The variation of the penicillin concentration during the fermentation was determined by the cylinder-plate method (*17*), in mashes containing 100, 140, and 180 grams per liter of sugar, and with six different initial antibiotic concentrations varying from 0.8 to 4.6 units

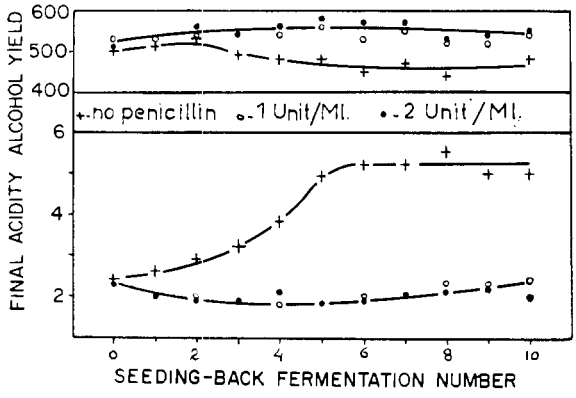

Figure 5. Effect of penicillin on alcohol yield and acid production

Sugar concentration, 100 grams per liter
Initial mash acidity, 0.90 gram of H_2SO_4 per liter
Alcohol yield, ml. of alcohol per kg. of dextrose
Final acidity, grams of H_2SO_4 per liter

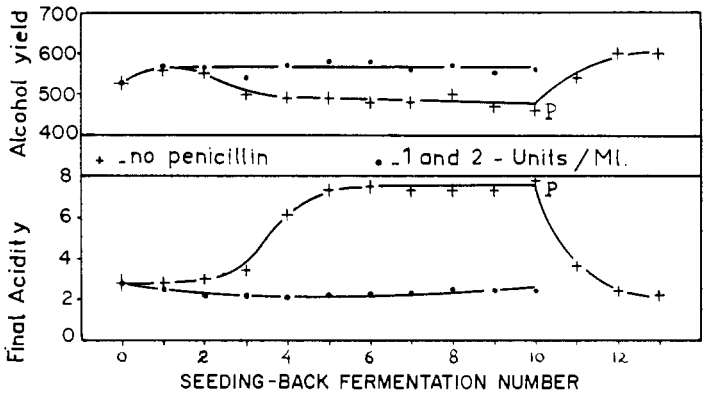

Figure 6. Effect of penicillin on alcohol yield and acid production

Sugar concentration, 140 grams per liter
Initial mash acidity, 1.3 grams of H_2SO_4 per liter
Point *P*, recuperation of contaminated fermenting mash
Alcohol yield, ml. of alcohol per kg. of dextrose
Final acidity, grams of H_2SO_4 per liter

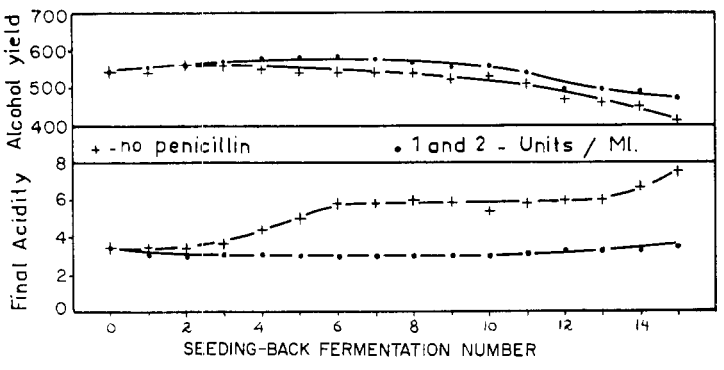

Figure 7. Effect of penicillin on alcohol yield and acid production

Sugar concentration, 140 grams per liter
Initial mash acidity adjusted to 2.1 grams of H_2SO_4 per liter by H_2SO_4 addition
Alcohol yield, ml. of alcohol per kg. of dextrose
Final acidity, grams of H_2SO_4 per liter

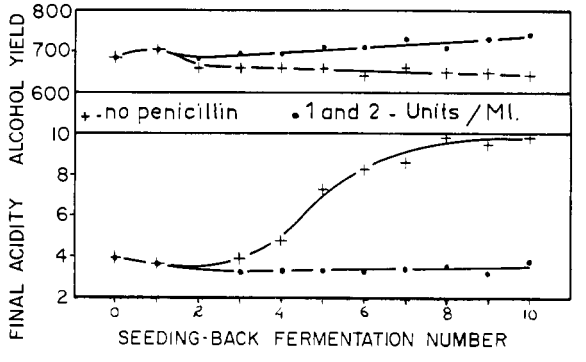

Figure 8. Effect of penicillin on alcohol yield and acid production

Sugar concentration, 180 grams per liter
Initial mash acidity, 2.0 grams of H_2SO_4 per liter
Alcohol yield, ml. of alcohol per kg. of dextrose
Final acidity, grams of H_2SO_4 per liter

Table IV. Effect of Penicillin on Final Yeast Count[a]

Penicillin Concentration, Units/Ml.	Final Yeast Count × 10^8, Cells/Ml.
0.0	1.9
0.5	1.9
1.0	1.8
1.5	1.9
2.0	1.8

[a] Carried out at 28–29° C., with 140 g./l. of sugar, and with initial yeast population of 0.90 × 10^8 cells/ml.

per ml. After 30 hours of fermentation at 25-26° C., the penicillin concentration was 25 to 60% of the initial antibiotic concentration. No correlation was found between the final and initial penicillin concentrations and the initial sugar concentration. Figure 4 shows typical data.

Table IV shows the effect of five penicillin concentrations on the final yeast count.

Table V shows the influence of various penicillin concentrations on the time and on the efficiency of the fermentation, as well as on the acid production. Mashes with 100, 140, and 180 grams per liter of sugar were fermented at 25° C.

The addition of the antibiotic in several doses during the fermentation was tried with mashes of varied sugar concentrations. Table VI shows the results obtained with 2.0 and 3.0 units per ml. of penicillin; antibiotic addition in several doses during the fermentation is not more efficient.

Table V. Effect of Penicillin on Fermentation

Penicillin Concn., Units/Ml.	Fermentation Time, Hr.						Final Acidity, G. H_2SO_4/L.						Fermentation Efficiency, Ml. Alcohol/Kg. Dextrose						% Increase in Efficiency					
	Experiments[a]																							
	(1)		(2)				(1)		(2)				(1)		(2)				(1)			(2)		
0	29		30				2.3		3.1				500		440				...			...		
0.25	29		30				2.3		2.6				510		450				2.0			2.3		
0.50	29		30				2.2		2.9				500		440				0.0			0.0		
0.75	29		30				2.3		2.9				510		420				2.0			−4.6		
1.0	29		30				2.3		2.9				510		440				2.0			0.0		
1.5	29		30				2.2		2.9				500		450				0.0			2.3		
2.0	29		30				2.2		3.0				510		460				2.0			4.6		
3.0	29		30				2.4		3.0				500		460				0.0			4.6		
	Experiments[a]																							
	(3)	(4)	(5)				(3)	(4)	(5)				(3)	(4)	(5)				(3)	(4)		(5)		
0	32	33	33				3.0	2.9	2.8				510	490	510				...	...		...		
0.25	32	33	33				3.0	2.8	2.6				530	510	500				3.9	4.1		−2.0		
0.50	32	33	33				2.8	2.8	2.6				530	510	510				3.9	4.1		0.0		
0.75	32	33	33				2.9	2.6	2.5				520	500	560				2.0	2.0		9.8		
1.0	32	33	33				2.9	2.6	2.6				530	490	550				3.9	0.0		7.8		
1.5	32	33	33				2.6	2.6	2.5				530	510	530				3.9	4.1		3.9		
2.0	32	33	33				2.4	2.6	2.3				520	510	520				2.0	4.1		2.0		
3.0	32	33	33				2.4	2.4	2.3				510	490	520				0.0	0.0		2.0		
4.0	32	33	33				2.1	2.4	2.3				530	490	...				3.9	0.0		...		
5.0	32	33	33				1.9	2.4	2.3				520	470	540				2.0	−4.1		5.9		
6.0	32	33	33				1.9	2.3	2.1				530	490	520				3.9	0.0		2.0		
(6)	(7)			(6)		(7)				(6)		(7)				(6)		(7)						
0	0			31		34				5.2		3.7				460		470				...		
0.25	0.50			31		34				4.6		3.3				490		510				6.5		
0.50	1.0			31		34				6.5		3.0				490		520				6.5		
0.75	2.0			31		34				4.7		2.9				490		500				6.5		
1.0	3.0			31		34				4.5		3.0				490		530				6.5		
1.5	4.0			31		34				4.5		2.4				490		530				6.5		
2.0	5.0			31		34				4.2		3.9				510		530				10.9		
3.0	6.0			31		34				3.9		3.1				510		530				10.9		

[a] Experiment: 1 and 2 — Sugar Concn., G./L.: 100; 3, 4, and 5: 140; 6 and 7: 180. Initial Acidity H_2SO_4, G./L.: 1.0; 1.1; 1.4

Table VI. Effect of Penicillin Added in Several Doses during Fermentation

Mash Sugar Concn., G./L.	Penicillin Concn., Units/Ml.	Fermentation Time, Hr.		Final Acidity, H_2SO_4, G./L.		Fermentation Efficiency, Ml. Alcohol/Kg. Dextrose		% Increase in Efficiency	
100	0	31		2.2		420		...	
	2.0	31		1.9		470		11.9	
	2.0[a]	31		1.8		440		4.8	
	3.0	31		1.7		470		11.9	
	3.0[a]	31		1.7		490		16.7	
		(1)[b]	(2)[b]	(1)	(2)	(1)	(2)	(1)	(2)
140	0	31	30	2.9	3.1	450	420	...	...
	2.0	31	30	2.4	2.8	490	440	8.9	4.8
	2.0[a]	31	30	2.4	2.8	490	450	8.9	7.1
	3.0	31	30	2.4	2.5	480	450	6.7	7.1
	3.0[a]	31	30	2.4	2.3	470	440	4.4	4.8
180	0	34		4.1		470		...	
	2.0	34		3.4		470		0.0	
	2.0[a]	34		3.5		490		4.3	
	3.0	34		3.1		480		2.1	
	3.0[a]	34		3.0		460		−2.1	

[a] Penicillin added in three doses; 50% at start, 25% at 6 hr., and 25% at 25 hr.
[b] Two separate experiments.

Fermentations by the successive seeding-back process (*19*) were made, at 28–29° C., with 100, 140, and 180 grams per liter of sugar, and with 1.0 and 2.0 units per ml. of penicillin. The seeding-back fermentations were carried out in the following way: 80% of the fermenting liquid was drawn off every 24 hours (after stirring to prevent yeast sedimentation) and the volume was brought back to the original value with fresh mash. Figures 5, 6, 7, and 8 show the results obtained. Figure 6 shows also the possibility of recuperating highly contaminated fermenting mash by means of two consecutive seeding-back fermentations, with mash containing 2.0 units per ml. of penicillin.

In the seeding-back experiments with 100 and 140 grams per liter of sugar, because of the low medium acidity, considerable quantities of dextran appeared after the sixth treatment. When sulfuric acid was added (see Figure 7) the dextran appeared between the 11th and the 14th treatment. In order to study the influence of penicillin on the *Leuconostoc* (probably *L. mesenteroides*) contamination, fermentations were made at 30° C., with 140 grams per liter of sugar. The results of these experiments after 22 consecutive seeding-back fermentations are given in Table VII.

Plant Scale Fermentation Tests

The favorable results obtained in laboratory tests and in pilot-plant experiments warranted plant-scale trials.

Experiment 1. A total of 49 steel fermentors, each containing 15,000 liters of mash (specific gravity 10° Baumé), was run by the seeding-back process (two to five seeding-back fermentations per fermentor) and evaluated. Data obtained are shown in Table VIII.

Experiment 2. A total of eight steel fermentors, five containing 30,000 liters each, and three containing 50,000 liters each of mash (specific gravity 10.5 to 11.0° Baumé), was run by Melle-Boinot method (*29, 30*) and evaluated. Table IX shows the results obtained.

Experiment 3. Three masonry fermentors, each containing 9000 liters of mash, were run by successive seeding-back processes using sugar cane juice instead of blackstrap molasses. The results obtained are shown in Figure 9.

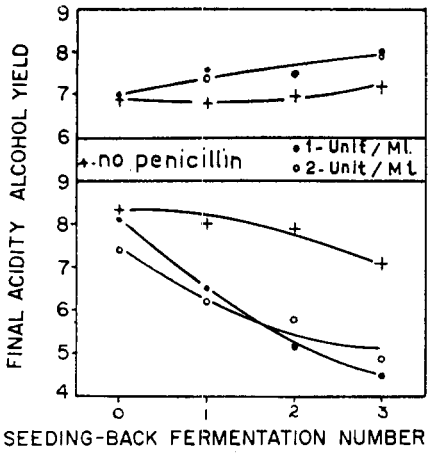

Figure 9. Effect of penicillin on plant-scale tests with sugar cane juice

Alcohol content, per cent volume at 15° C.
Final acidity, grams of H_2SO_4 per liter

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Table VII. Alcoholic Fermentation Efficiency Using Penicillin as Leuconostoc Contamination Inhibitor

22 successive seeding-back fermentations. 140 grams sugar per liter.

Penicillin Concn., Units/Ml.	Start of Dextran Production, No. of Seeding-Back Fermentations	Fermentation Efficiency, Ml. Alcohol per Kg. Dextrose	% Increase in Efficiency
0	14[a]	390	...
2.0	15[b]	410	5.1
5.0	15[b]	460	17.9
8.0	17[c]	520	33.3
10.0	17[c]	540	38.5

[a] Dextran quantity approximately constant until end of experiment.
[b] Dextran quantity grew fast on successive fermentations.
[c] Dextran quantity almost constant with average growth.

Table VIII. Effect of Penicillin on Plant Fermentations by Successive Seeding-Back Method

0 Unit/Ml.[a]		1.0 Unit/Ml.[b]		1.0 Unit/Ml.[c]	
Final acidity, H_2SO_4, g./l.[d]	Alcohol content, % vol. at 15° C.	Final acidity, H_2SO_4, g./l.[d]	Alcohol content, % vol. at 15° C.	Final ocidity, H_2SO_4, g./l.[d]	Alcohol content, % vol. at 15° C.
5.8(2)	6.4	4.1(2)	7.1	3.1(2)	7.4
6.1(2)	6.4	3.8(2)	6.6	3.2(3)	7.6
5.6(2)	7.0	4.2(2)	7.4	3.2(2)	8.1
5.8(2)	7.1	3.9(2)	7.4	3.3(2)	7.4
6.9(2)	7.0	3.3(2)	7.2	3.3(2)	7.8
6.7(2)	7.1	3.2(2)	7.3	3.1(4)	7.6
6.7(2)	6.8	3.2(2)	7.2	3.0(2)	7.8
7.2(2)	6.2	3.1(2)	7.9	3.1(4)	7.7
6.9(2)	7.2	2.9(2)	7.0	3.0(2)	7.6
6.5(2)	6.9	3.4(3)	7.3	3.6(2)	7.5
6.6(3)	6.5	3.0(3)	7.6	3.3(2)	7.4
6.7(2)	6.3	3.5(2)	7.7	3.6(2)	7.4
6.9(5)	6.3	3.5(5)	7.3	3.7(2)	7.3
6.0(2)	6.4	3.5(2)	7.4	3.2(2)	7.6
6.2(2)	6.1	3.5(2)	7.5	3.3(2)	7.5
6.3(3)	6.1	3.6(3)	7.3	3.8(2)	7.5
6.5(5)	6.7	...	...	...(2)	7.4
Average 6.4	6.6	3.5	7.3	3.3	7.6
% Change ...	...	−45.3	+10.6	−48.4	+15.2

[a] Sulfuric acid added routinely; initial acidity 1.8 g./l.
[b] Penicillin added to fermentor and sulfuric acid added routinely; initial acidity 1.8 g./l.
[c] Fermentations without sulfuric acid, and penicillin added to molasses diluting tanks; initial acidity 1.6 g./l.
[d] Figures in parenthesis indicate number of seeding-back treatments.

Table IX. Effect of Penicillin on Plant Fermentations by Melle-Boinot Method

0 Unit/Ml.		1.0 Unit/Ml.[a]	
Final acidity, H_2SO_4, g./l.	Alcohol content, % vol. at 15° C.	Final acidity, H_2SO_4, g./l.	Alcohol content, % vol. at 15° C.
3.5	8.4[b]	2.6	8.6[b]
3.1	8.4[b]	2.7	8.7[b]
3.0	7.8[b]	2.2	8.6[c]
2.4	8.5[c]	2.4	8.8[c]
Average 3.0	8.3	2.5	8.7
% change ...	...	−16.7	+4.8

[a] Fermentations realized without sulfuric acid treatment of the inoculum, and without inoculum—hold period (with reduction of about 15% on operation time).
[b] 30,000-liter fermentor.
[c] 50,000-liter fermentor.

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CORRESPONDENCE

Reduction of Dental Caries and Goiter by Crops Fertilized with Fluorine and Iodine

SIR: McClendon and Gershon-Cohen [J. AGR. FOOD CHEM. **3,** 72 (1955)] have reported the results of some experiments upon the prevention of goiter in rats by feeding materials from plants fertilized with compounds containing iodine. The diet consisted of ground sunflower seeds, dried sunflower leaves, yeast, and sodium chloride. The controls received a diet that was similar except that the sunflowers used had been grown in water culture without iodine. The dried leaves of these plants contained no iodine; those of the plants grown in the fertilized plots contained from 0.19 to 0.38 p.p.m. of iodine, depending upon the nature of the compound used for fertilization.

No analysis of the seeds, which furnished eight times as much of the diet as did the leaves, was presented. However, in a letter to me dated June 14, 1955, Dr. McClendon wrote, "The leaves contain about 100 times as much iodine as the seeds, whenever iodine is present." If we assume that each rat ate about 10 grams of its diet per day, the differences in the amounts of iodine furnished by the two kinds of diet varied from 0.19 to 0.38 γ per day. If we assume that the yeast furnished no appreciable quantity of iodine, the iodine consumption of the test group would correspond to an intake of from 19 to 38 γ per 4000 calories. This is far less than the usual intake even in regions in which goiter is endemic, according to one of my reports [*J. Clin. Nutrition* **3,** 215 (1955)].

However, it is consistent with the report by McClendon and Hathaway [*Proc. Soc. Exptl. Biol. Med.* **21,** 129 (1923)] that an intake of 20 γ, with an excretion in the urine of 7 γ, was sufficient to prevent goiter in man 23 years old. If that be accepted, there is no need of iodized salt, for there were few foods, if any, the analyses of which were reported by McClendon ("Iodine and the Incidence of Goiter," Univ. Minnesota Press, Minneapolis, Minn., 1939) that did not contain at least 20 γ per 4000 calories.

If, on the other hand, the yeast did contain appreciable quantities of iodine, the difference between the two kinds of diet would still be only 19 to 38 γ per 4000 calories. This amount would be furnished by 190 to 380 mg. of iodized salt containing 0.01% of iodine. Is it seriously contended that freedom from goiter hangs by so slight a thread?

ISIDOR GREENWALD

SIR: In order to provide an iodine-containing vegetable component in an experimental diet, I tried fertilizing the soil with iodine to get iodine into edible plant materials and obtained 0.19 to 0.38 p.p.m. of iodine in sunflower leaves. Goiter in rats was prevented by feeding 10% sunflower leaves and 80% sunflower seeds in the diet [J. AGR. FOOD CHEM. **3,** 72 (1955)]. Unfortunately, I was retired for age as this study was being completed, and the sunflower seeds were never analyzed.

Dr. Greenwald chooses this paper for attack in his fight against iodized salt as a goiter prophylaxis, but this subject has already been discussed by me at the request of Samuel Soskin ("Progress in Clinical Endocrinology," pp. 20–6, Grune and Stratton, New York, 1950), and by Wespi [*Münch. med. Wochschr.* **35,** 1150 (1956)], Stanbury and others ("Monograph in Medicine and Public Health No. 12," Harvard Univ. Press, Boston, Mass., 1954), and M. Roche and others [*J. Clin. Endocrinol. and Metabolism* **17,** 99 (1957)]. In the article cited, Stanbury writes, "Any remaining doubt that iodine deficiency can be a cause of endemic goiter has been erased by the present studies."

A man would receive about 38 γ per day from Swiss iodized salt, provided there is no loss in storage by volatilization, after oxidation by nitrite that is present as an impurity or has crept with moisture into the container. Physicians often prescribe 30 times the requirement of an essential food element over a period of a year, so why fight over a few micrograms of iodine, when 10,000 are needed by the thyroid gland?

J. F. MCCLENDON