parents had been fed DDT, dieldrin, or endrin during the winter months, but received insecticide-free diets during the breeding season.

Reproduction of pheasants was adversely affected by the inclusion of aldrin, dieldrin, or endrin in the breeding diets (Table IV). Mortality occurred in all groups receiving aldrin, and surviving birds lost approximately 30% body weight during the breeding season. Egg production in the groups receiving 1 or 2 p.p.m. of aldrin remained at normal levels during the first 6 weeks of the test period, but had virtually ceased by the end of the tenth week. Hatchability appeared decreased by feeding of 10 p.p.m. of aldrin, dieldrin, or endrin, and chicks from these groups had unusually high mortality during the first 2 weeks. Egg production by birds fed 50 or 100 p.p.m. of DDT was below that of the controls, but fertility, hatchability, and chick viability appeared unaffected. Feeding of 50 p.p.m. of strobane appeared to reduce chick viability.

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AMINO ACIDS IN FERMENTATION

Pilot Plant Study of Utilization of Leucine by *Saccharomyces cerevisiae*

J. W. SPANYER, Jr., and ALAN T. THOMAS

Brown-Forman Distillers' Corp., Louisville 1, Ky.

Much of the flavor of whisky results from esters of the higher alcohols and organic acids present in the distillate. The higher alcohols are normally analyzed as a group and called fusel oil. Isoamyl alcohol is a major constituent of this fraction. Much evidence has indicated that yeast deaminates and decarboxylates α-amino acids to form alcohols. This study with pilot fermentors has verified that hypothesis. The leucine–isoamyl alcohol system has been studied under standardized conditions. The results indicate that the amount of fusel oil formed is approximately a linear function of the amount of leucine added.

During the fermentation of sugars by yeast, many organic compounds, other than ethyl alcohol, are produced. Some are yeast-metabolic products or intermediary products in the fermentation cycle; others are formed by foreign organisms or materials in the medium. The higher alcohols that are present in new whisky are lumped together and called fusel oil.

Ehrlich (*1–3*) showed that the fusel oil content of spirits could be increased by the addition of amino acids and concluded that higher alcohols are formed from their corresponding amino acids by deamination and decarboxylation.

$$RCH(NH_2)COOH \longrightarrow RCH_2OH + NH_3 + CO_2$$

This hypothesis, as modified by Neubauer and Fromherz (*4*), is known as the Ehrlich mechanism and suggests the following sequence of steps.

$$RCH(NH_2)COOH \longrightarrow RCOCOOH + NH_3 \text{ (oxidation)}$$

$$RCOCOOH \longrightarrow RCHO + CO_2 \text{ (decarboxylation)}$$

$$RCHO \xrightarrow{2H} RCH_2OH \text{ (reduction or}$$

$$RCHO \xrightarrow{O} RCHOH \text{ oxidation)}$$

Strickland (*7–9*), working with bacteria, found that amino acids could be classified as either hydrogen donors or acceptors. The reaction occurring between a pair of opposite types is:

$$R_1CH(NH_2)COOH + R_2CH(NH_2)COOH \xrightarrow{H_2O} R_1COCOOH + R_2CH_2COOH + 2NH_3$$

Thorne (*10, 11*) confirmed Ehrlich's postulations for the formation of several alcohols and succinic acid, and reasoned that if deamination of α-amino acids is the normal mechanism for nitrogen assimilation by yeast, then ammonia should be superior to any amino acid. He found that ammonium phosphate was superior to all single amino acids except aspartic and glutamic acids, but that complex amino acid mixtures were much superior to the ammonium salt as a yeast nutrient. Thorne concluded that, when all of the necessary amino acids are present, they are integrated intact into the yeast protein, and that deamination takes place only to synthesize unavailable nitrogenous compounds. Thorne also presented evidence that yeast could, at least to a small extent, utilize the Strickland mechanism as a source of nitrogen.

To simulate normal distillery operations and check the utilization of leucine by yeast, a series of fermentations was conducted with varying amounts of leucine in pilot plant fermentors.

Fermentation and Distillation

Ten liters of medium were fermented in two 40-liter stainless steel fermentors, equipped with agitators and automatic temperature controls (Figure 1). The fermentors were agitated at 250 r.p.m. for approximately 4 hours after setting, then the agitators were turned off.

Table I. Basal Medium for 10-Liter Fermentations

Dextrose, g.	1260
Mineral solution, ml.	200
Yeast extract solution, ml.	500[a]
Yeast culture, ml.	300
Demineralized water, ml.	9000
Mineral solution, g./liter	
Monopotassium phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulfate	0.125
Ferric chloride	0.0025
Manganese sulfate	0.0025

[a] 80 grams of Difco yeast extract per liter of demineralized water.

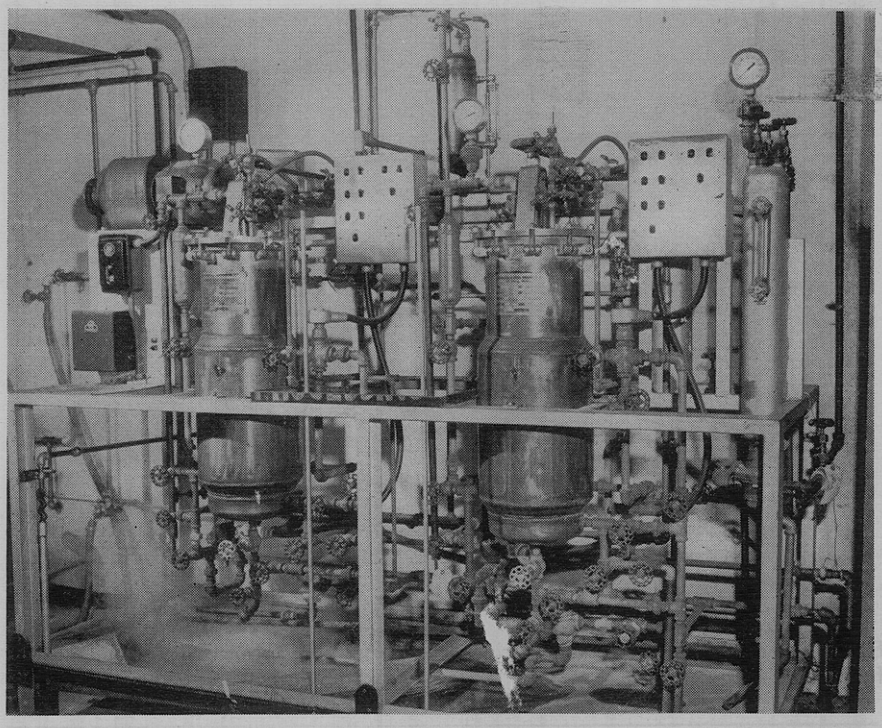

Figure 1. Stainless steel fermentors, equipped with agitators and automatic temperature controls

Because naturally occurring materials are subject to considerable variation, a standardized fermentation medium was used (Table I). The carbohydrate and the nitrogenous portions of the medium were sterilized separately to prevent the irreversible browning reactions (*12*).

One liter of set medium was withdrawn for analysis, and the remainder allowed to ferment 72 hours at 86° ± 1° F. Two liters were taken for analysis at the end of the fermentation period and the sample was designated as "drop sample" to differentiate it from the prefermentation or "set sample."

A large number of fermentations were made, 16 runs or 32 fermentors, to standardize operating conditions and techniques to ensure consistent results during the experimental runs to follow. The reported results were derived from standardized runs 17 through 28 (12 runs or 24 fermentors). The fermentors were operated continuously during this period, except for runs 27 and 28. Thus, the yeast was kept in an actively growing, vegetative state to assure more uniformity in the yeast inoculum. This precaution is necessary because of the possibility of varying the fermentation characteristics of the yeast by intermittent cold storage. Runs 27 and 28 were made later when a direct correlation was apparent between isoamyl alcohol in the distillate and leucine added to the fermentors.

The yeast was centrifuged from the drop sample, washed twice, and made up to 250 ml. The centrifugate and washings were vacuum evaporated at 140° F. and made up to 100 ml. The amino acid content of all samples was determined by paper partition chromatography. Although the studies reported in this paper were primarily concerned with the utilization of leucine, 15 amino acids were determined in yeast (*6*).

The remaining 7 liters of medium were distilled by passing steam through the jackets of the fermentors. Preliminary tests indicated that the fusel oil was stripped out in the first 1000 ml. of distillate. The esters of the distillate were saponified prior to redistillation of the sample in a 3-foot glass column packed with Raschig rings. The reflux was maintained at from 8 to 1 to 18 to 1, and two fractions were collected. The first boiling at 172° F. was primarily ethyl alcohol; the second, with a boiling range of 172° to 208° F., contained the fusel oil. This second fraction was vacuum distilled in a 1-foot glass column packed with glass helices. Using this technique it was possible to concentrate the fusel oil into a sample of about 30 ml. Fusel oils were determined as described by Penniman, Smith, and Lawshe (*5*).

In addition to the 10-liter pilot size fermentations, a series of 1-liter fermentations was run, using the same medium, in 2-liter Erlenmeyer flasks. These were 4-day fermentations at approximately 74° F. Yeast and centrifugate samples were obtained for analysis as was done with the large fermentors. The remainder of the fermented medium was distilled until the vapor temperature reached 212° F. Fusel oil contents were determined on these distillates.

Analysis of Data and Results

The leucine levels of the 10-liter fermentations and the fusel oil found in the fermented medium are tabulated in Table II. The relationship between the added leucine and fusel oil developed is shown in Figure 2, both computed as moles per liter. The same items were determined on the 1-liter fermentors and are shown in Table III and Figure 3.

The leucine present in the initial yeast extract in the 10-liter fermentations was combined with that which was added to the fermentors. These summations were compared with the total leucine present in the finished fermentations. The components of the recovery figure were fusel oil, leucine in recovered yeast, and leucine in the centrifugate. These data are presented in Table IV, and are calculated as milligrams of α-amino nitrogen per milliliter.

The inherent variability of the determination of amino acids by paper parti-

Table II. Leucine Levels and Fusel Oil Analyses of Pilot Plant Fermentors

Run No.	Ferm. No.	DL-Leucine Added, G./10 Liters	Fusel Oil in Medium, G./Liter
17	4	12.3	0.426
	5	0	0.214
18	4	0	0.353
	5	0	0.414
19	4	6.15	0.551
	5	12.3	0.626
20	4	3.0	0.319
21	4	0	0.359
	5	3.0	0.437
22	4	0	0.303
	5	6.15	0.273
23	4	6.15	0.359
	5	3.0	0.380
24	4	12.3	0.671
	5	3.0	0.270
25	4	12.3	0.703
	5	3.0	0.267
26	4	6.15	0.411
	5	0	0.227
27	4	24	1.19
	5	24	1.27
28	4	24	1.13
	5	24	0.97

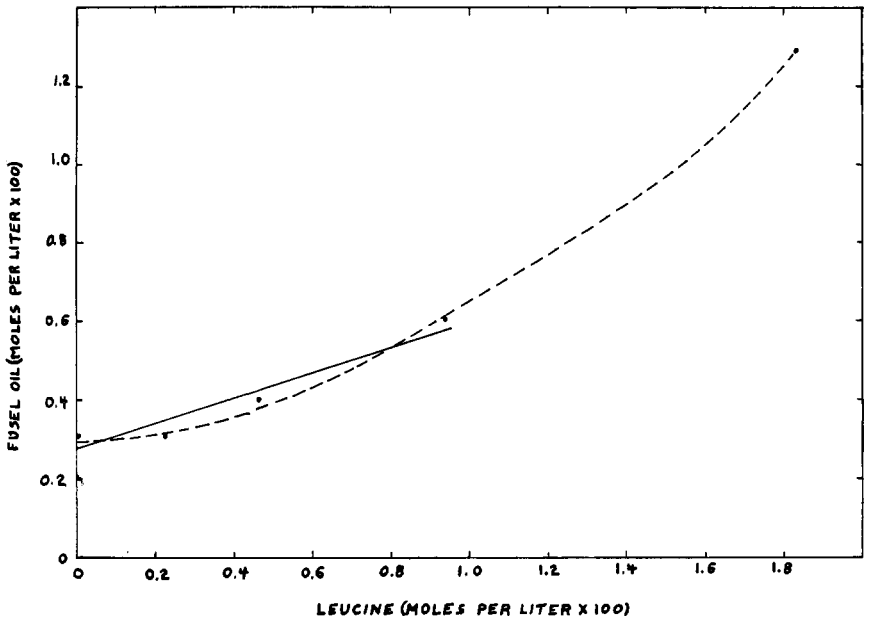

Figure 2. Relation between leucine and fusel oil formation in large pilot fermentors

Table IV. Distribution and Over-All Balance of Leucine in Fermentation Products

Added, g./liter	0	0.3	0.615	1.23	2.40
From yeast, g./liter	0.10	0.10	0.10	0.10	0.10
Total, g./liter	0.10	0.40	0.715	1.33	2.50
Calcd. as α-amino N, mg./ml.	0.011	0.043	0.077	0.143	0.267
Control	0.011	0.011	0.011	0.011	0.011
Difference	0	0.032	0.066	0.132	0.256
Recovered					
From fusel oil, g./liter	0.312	0.335	0.399	0.607	1.14
α-Amino N, mg./ml.					
Calcd. from fusel oil	0.050	0.053	0.063	0.097	0.181
From yeast	0.011	0.012	0.010	0.010	0.012
From centrifugate	0.00	0.011	0.039	0.069	0.122
Total	0.061	0.076	0.112	0.176	0.315
Control	0.061	0.061	0.061	0.061	0.061
Difference	0	0.015	0.051	0.115	0.254
Unaccounted for	...	0.017	0.015	0.017	0.002

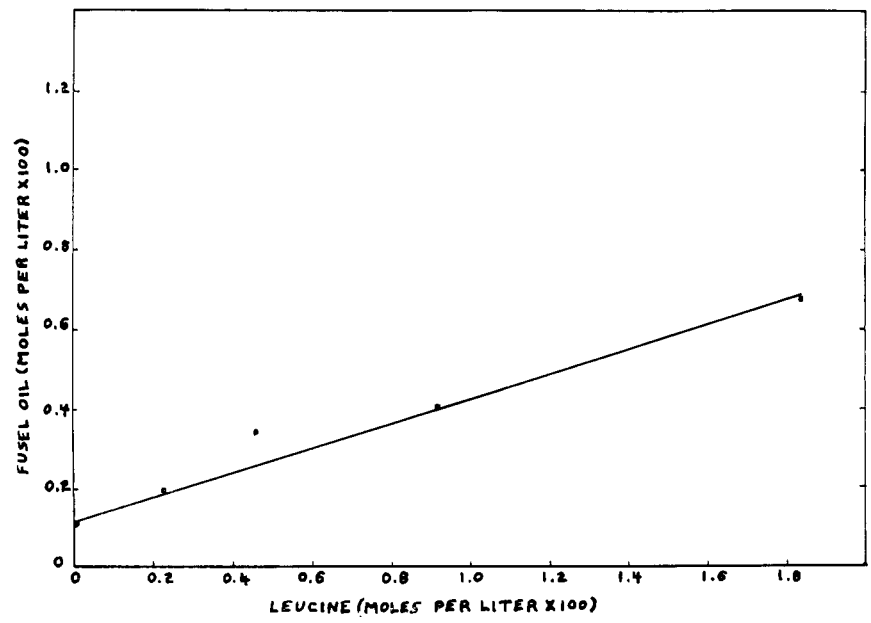

Figure 3. Relation between leucine and fusel oil formation in one-liter fermentors

Table III. Leucine Levels and Fusel Oil Analyses of Erlenmeyer Fermentors

Flask No.	DL-Leucine Added, G./Liter	Fusel Oil, G./Liter
LF-1	0	0.093
LF-2	0.3	0.172
LF-3	0.6	0.310
LF-4	1.2	0.357
LF-5	2.4	0.598

tion chromatography requires a large number of individual determinations to assure reliability—e.g., 86 chromatograms were used to determine the leucine content of yeast from butanol–formic acid resolutions.

Discussion

An examination of Figures 2 and 3 indicates that the amount of fusel oil formed is practically a linear function of the amount of leucine added. Calculated as isoamyl alcohol, the slope of the line in Figure 3 indicates a conversion of 31 mole %. If the 24-gram leucine level is disregarded in Figure 2, the least squares line would have a slope of 32 mole % conversion, shown by solid line. The amount of isoamyl alcohol found at the 24-gram level is equivalent to 51 mole %. The high fusel oils on runs 27 and 28 (24-gram level) were probably caused by an insufficient yeast growth adjustment period prior to use.

The relationship between the leucine added and that recovered, as shown in Table IV, indicates a constant amount of leucine unaccounted for, again excluding the abnormal runs 27 and 28. The number of yeast cells remaining at the end of the fermentation period was practically the same in all cases, an indication that yeast growth was constant.

The disappearance of a constant amount of leucine must be associated with assimilation by the yeast to form nitrogenous compounds other than proteins.

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