SOME NITROGENOUS CONSTITUENTS OF WORT AND THEIR FATE DURING FERMENTATION BY TOP AND BOTTOM FERMENTATION YEASTS*

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I. INTRODUCTION

The nitrogenous fraction of wort is mainly composed of substances of varying complexity, *e.g.*, ammonia, amides, amino acids, peptides (di- and polypeptides), and proteins. Other nitrogenous bodies, which are unrelated chemically to the proteins are also present such as the bases, choline, betaine and the purine base allantoin, but the part these substances play in yeast metabolism is at present obscure.

In 1929 S. B. SCHRYVER AND E. M. THOMAS[1] made a preliminary examination of the nitrogen groupings present in strong worts (O.G. 1057), for example, ammonia N, amide N, amino N and peptide N. In this way they were able to account for 59% of the total soluble nitrogen, leaving an undetermined residue of 41%. In a number of respects, however, the methods of determination were faulty (see below), more especially in the estimation of amide N and polypeptide N.

The data available at the present time about the nature of the individual components of these nitrogen groupings are very meagre and little is known about their fate during fermentation. H. T. BROWN[2] was able to identify the amide asparagine and the amino acids, leucine, tryptophan and tyrosine, as well as the bases choline, betaine and the purine base allantion in a cold water extract of malt, while O. MISKOVSKY[3] claimed to have identified the amino acids, arginine and histidine and the bases choline and betaine in Pilsener beer.

In view of the paucity of data on the nature of the various nitrogenous constituents of wort and of their great importance for yeast reproduction, the whole problem has been re-examined. The greater part of this investigation is concerned with top-fermentation yeast and worts prepared by the infusion method; but some data are also included of fermentations carried out with decoction worts and bottom-fermentation yeasts.

II. METHODS

Material. The first part of this investigation deals with the fate of the following nitrogen groupings during fermentation; Total soluble nitrogen, ammonia N, amide N,

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amino N and protein N. All top-fermentations were carried out in the laboratory in Dewar cylinders on pale ale wort (O.G. 1032 and 1040) at the relatively high temperatures of top-fermentation brewing (18° to 22° C). The lager wort (O.G. 1041) was fermented under the normal conditions of a bottom-fermentation system*.

Methods of Analysis

a. *Total Nitrogen* was estimated by the usual Kjeldahl method.

b. *Total Crystalloid Nitrogen* (non-protein N) was determined on solutions which had been cleared of protein with colloidal ferric hydroxide by the method of W. THOMAS[4]. The cleared extracts were acidified with acetic acid, evaporated to dryness and the nitrogen estimated as for total nitrogen.

c. *Ammonia Nitrogen* was estimated by the method of G. W. PUCHER, H. B. VICKERY, AND C. S. LEBENWORTH[5].

d. *Amide Nitrogen* was determined on the cleared wort by hydrolysis with 5% sulphuric acid for 5 hours and the ammonia present estimated by the PUCHER *et al.* method.

e. *Amino Nitrogen* was estimated by the method of C. G. POPE AND M. F. STEVENS[6]. It should be mentioned here that too great weight must not be placed on the figures for amino N, because this method includes some dipeptides and polypeptides. The results are, however, more reliable than the VAN SLYKE nitrous acid method. It is hoped to repeat these determinations so as to estimate true α-amino N in wort by the VAN SLYKE ninhydrin method[7].

f. *Amino Acids*. Sixteen amino acids present in wort were estimated quantitatively by microbiological assay (see E. C. BARTON-WRIGHT[8,9] and E. C. BARTON-WRIGHT AND N. S. CURTIS[10] and their fate followed in fermentation, while two amino acids (glycine and α-alanine) were also shown to be present in wort by "partition chromatography" (see R. CONSDEN, A. H. GORDON, AND A. J. P. MARTIN[11]).

Since in the present investigation we are concerned with fluctuations of definite chemical substances and not merely with amide N or amino N, the results are expressed in the following terms.

Asparagine N. The assumption was made that the whole of the amide N is present in wort in the form of amides of the type of asparagine and glutamine. Asparagine N is therefore taken as twice the amide N.

Amino Acid N. This was found by deducting amino N due to amide from the total N recorded by the POPE AND STEVENS method.

Residual N. This term (see E. G. MASKELL AND T. G. MASON[12] and E. C. BARTON-WRIGHT AND A. MCBAIN[13]) is used to cover the fraction of crystalloid N not accounted for by the sum of the asparagine N, amino acid N and ammonia N.

Protein N. This value was obtained by deducting total crystalloid N (non-protein N) from total nitrogen.

III. EXPERIMENTAL OBSERVATIONS

The figures for ammonia N, asparagine N, *etc.*, for a number of fermentations on worts of different gravities are given in Table I.

Total Soluble N. The fall in total soluble N follows the usual course with top-fermentation yeasts (Series I, II and III). The greatest uptake of nitrogen occurs during the first 24 hours of fermentation. In the bottom-fermentation (Series IV), the period of greatest nitrogen uptake is later (approximately 48 hours). A result no doubt due to the lower temperature at which fermentation is carried out. The values obtained here for the bottom-fermentation should be compared with the results obtained by E. HELM AND B. TROLLE[14] which they fully confirm.

Total Crystalloid N. The fall in total crystalloid N in the main follows the same course as total soluble N.

Amino Acid N. It has already been mentioned that the amino acid N figures include some dipeptide and lower polypeptide N owing to the method of estimation used. On the other hand, the POPE AND STEVENS method gives a truer picture of amino acid N

* We are indebted to Messrs BARCLAY, PERKINS AND Co, Ltd., London, for generous supplies of lager wort at different stages of fermentation.

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fluctuations than the VAN SLYKE nitrous acid method, which is known to estimate ammonia N, polypeptide N and other substances such as formaldehyde. A few estimations were made in this investigation by the VAN SLYKE method for comparison purposes, and in every instance the values were 25% higher than with the POPE AND STEVENS method. With this qualification the amino acid N figures given here show that top-fermentation yeasts can absorb up to 62% of the total amino acid N of wort whereas bottom-fermentation yeasts take up less (approximately 42%). In this case the figures given in Table I (Series IV) should be compared with those of Table VI.

Ammonia N. There are marked differences in the fate of ammonia N in top and bottom-fermentation systems. In all the cases quoted here top-fermentation yeasts absorb 84–86% of the ammonia N present in wort, whereas initial absorption to the extent of 90% is followed by excretion to the extent of 31% in a bottom-fermentation (Table I, Series IV).

Asparagine N. The most outstanding result shown from Table I, and one contrary to expectation, is to be found in the behaviour of asparagine N in both top and bottom-fermentation systems. Following upon H. T. BROWN's discovery (*loc. cit.*) of asparagine in a cold water extract of malt, it was shown by R. S. W. THORNE[15] in a comparative study of ammonia (as ammonium phosphate) and a large number of individual amino acids as single sources of nitrogen supply for yeast growth, that asparagine (or its free acid, aspartic acid) is 42% superior to ammonium phosphate as a nitrogen nutrient, which is itself superior to any of the other amino acids which were tested. The only exception is glutamic acid which is slightly superior to ammonium phosphate. In wort, however, in the presence of ammonia and a mixture of amino acids and peptides, the behaviour of asparagine N is different. Whereas in top and bottom-fermentation systems ammonia N is utilized by yeast to the extent of 84–90%, asparagine N is only taken up to the extent of approximately 30% in the early stages of fermentation and this absorption is followed by excretion; the final concentration of asparagine N being in some cases (Table I, Series III) higher than the initial value. In view of THORNE's findings, this behaviour of asparagine N in wort is unexpected, and in such circumstances, it does not apparently play an important role as a nitrogen nutrient for yeast.

Residual N. The residual N figure for worts prepared by the infusion method varies between 41 and 48% while a figure of 41% was found for the single example examined of the decoction process (Table I, Series IV). The nature of the constituents of this fraction have not yet been determined. SCHRYVER AND THOMAS[1] gave a residual N figure of 41% for wort. They claimed to have estimated polypeptide N, excluding polypeptide N, the figure of 41% has been obtained in two cases in this investigation. It is doubtful if SCHRYVER AND THOMAS actually estimated true polypeptide N alone, because of the drastic method of hydrolysis employed by them (16% HCl for 20 hours) which would lead to the hydrolysis of some protein. Presumably, although no experimental evidence has so far been obtained the bulk of this fraction is composed of dipeptides and lower polypeptides. Whatever may be the composition of this fraction, the experimental data point to the fact that it can on occasion play an important part as a source of nitrogen for yeast. In some cases (Table I, Series IV) as much as 30.6% of residual N is utilized by yeast.

Protein N. The protein N figures require little comment. The concentration of protein N is low and is scarcely attacked by yeast during the whole course of fermentation.

Summarizing these results, it can be said that the principal source of nitrogen

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TABLE I

PALE ALE WORT PITCHED WITH TOP-YEAST AT THE RATE OF 2.8 G/LITRE PRESSED YEAST (= 1 lb/BARREL). ALL FERMENTATIONS CARRIED OUT IN DEWAR CYLINDERS

Hours	Gravity	% Total Soluble N	% Total Crystalloid N	% Ammonia N	% Amino N	% Asparagine N	% Residual N	% Protein N
Series I								
0	1031.5	0.064	0.047	0.0020	0.0187	0.0066	0.0197	0.017
9	1029.3	0.057	0.038	0.0018	0.0153	0.0054	0.0155	0.019
24	1014.5	0.041	0.027	0.0004	0.0091	0.0038	0.0137	0.014
48	1007.7	0.038	0.027	0.0004	0.0076	0.0048	0.0142	0.011
72	1006.9	0.039	0.027	0.0004	0.0081	0.0038	0.0147	0.012
96	1007.0 Attenuation = 78%	0.038	0.027	0.0004	0.0071	0.0038	0.0157	0.011
Series II								
0	1040.0	0.093	0.075	0.0030	0.0308	0.0064	0.0348	0.018
9	1038.8	0.088	0.065	0.0029	0.0271	0.0058	0.0292	0.023
24	1026.2	0.064	0.051	0.0007	0.0172	0.0036	0.0295	0.013
48	1013.2	0.056	0.044	0.0005	0.0128	0.0044	0.0263	0.012
72	1011.4	0.056	0.044	0.0005	0.0128	0.0044	0.0263	0.011
96	1007.1 Attenuation = 82.3%	0.055	0.044	0.0005	0.0128	0.0044	0.0263	0.011
Series III								
0	1039.6	0.092	0.078	0.0032	0.0308	0.0064	0.0376	0.014
9	1036.0	0.087	0.071	0.0029	0.0278	0.0044	0.0359	0.016
24	1021.2	0.068	0.057	0.0013	0.0181	0.0058	0.0318	0.011
48	1011.7	0.065	0.053	0.0006	0.0135	0.0070	0.0318	0.012
72	1010.3	0.065	0.053	0.0005	0.0133	0.0074	0.0318	0.012
96	1009.2 Attenuation = 76.8%	0.069	0.056	0.0005	0.0133	0.0074	0.0349	0.013
Series IV. Bottom Fermentation. Decoction wort pitched with bottom yeast at the rate of 1.58 lb/barrel pressed yeast								
0	1041.2	0.080	0.062	0.0029	0.0247	0.0086	0.0258	0.018
24	1036.9	0.075	0.055	0.0025	0.0217	0.0066	0.0242	0.020
48	1029.9	0.066	0.045	0.0017	0.0195	0.0050	0.0188	0.021
72	1014.3	0.059	0.042	0.0003	0.0153	0.0034	0.0230	0.017
96	1010.8	0.057	0.041	0.0006	0.0135	0.0056	0.0219	0.016
120	1010.7	0.057	0.041	0.0005	0.0141	0.0058	0.0206	0.016
144	1010.4	0.057	0.041	0.0008	0.0147	0.0046	0.0209	0.016
168	1010.4	0.057	0.038	0.0008	0.0144	0.0032	0.0196	0.019
240	1010.3 Attenuation = 75%	0.057	0.038	0.0009	0.0148	0.0044	0.0179	0.019

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supply for yeast growth in wort is amino acid N with residual N playing a subsidiary role. Ammonia N is present in relatively low concentration (3–4% of the total N content), but it is practically entirely absorbed by top-fermentation yeasts (84–86%), whereas bottom yeasts, after a heavy initial absorption, apparently excrete ammonia N. Asparagine N, although the best single source of nitrogen for yeasts (cf. THORNE[15]), apparently plays a minor role as a source of nitrogen for yeast growth in wort.

IV. THE FATE OF INDIVIDUAL AMINO ACIDS IN TOP FERMENTATION

Up to the present time, only six amino acids have been recognized with any certainly in wort, namely, leucine, tryptophan, tyrosine, aspartic acid (as its amide asparagine), arginine and histidine (cf. BROWN[2] and MISKOVSKY[3]). The nature and fate of free individual amino acids present in wort has been re-examined in this investigation. Eighteen individual amino acids have been recognized by partition chromatography and microbiological assay, and the fate of sixteen quantitatively followed in fermentation. As has already been mentioned the greater number of the results are concerned with top-fermentation yeasts and the figures for the behaviour of sixteen individual amino acids in fermentation are shown in Table II. Figures were also obtained for a bottom-fermentation, but in view of the fact that a number of differences became apparent between the two types of fermentation, these are separately discussed below.

From the results given in Table II, the behaviour of five amino acids requires special attention, namely, methionine, lysine, aspartic acid, leucine and proline. In the case of lysine and menthionine, 59% of the former and 33% of the latter are removed from wort in 9 hours of fermentation, while in 24 hours the whole of the methionine disappears and 87% of the lysine, aspartic acid and leucine. The behaviour of lysine is of particular interest, because when supplied alone as a nitrogen nutrient fo yeast it is quite useless for growth (cf. THORNE[15]). In other words, yeast is apparently unable to deaminate lysine by the ERLICH mechanism when it is supplied as a single source nitrogen nutrient, but when it is precent in a mixture of amino acids it is selected before all others for attack. The behaviour of yeast towards proline is also of interest. Proline in wort is scarcely attacked by yeast, but it has been shown by THORNE[15] that proline as a single nitrogen nutrient for yeast gives rise to good growth. In fact the reverse of the behaviour of lysine occurs.

With regard to the behaviour of amino acids as a whole during fermentation a definite sequence of reactions can be seen. Yeast utilizes the so-called straight-chain or aliphatic amino acids first, *e.g.*, menthionine, lysine, leucine, aspartic acid, isoleucine, *etc.*, and then proceeds to attack the ring or aromatic amino acids, *e.g.*, phenylalanine, tyrosine, tryptophan and histidine, while proline (which is also a ring compound) is scarcely affected. The behaviour of two further amino acids, tryptophan and histidine also requires consideration. Tryptophan alone is a poor source of nitrogen for yeast, while histidine is practically useless (cf. THORNE[15]) yet when present in a mixture of amino acids, both are taken up to the extent of 90%.

The incidence and fate of glycine (aminoacetic acid) and α-alanine (α-aminopropionic acid) in wort could not be followed quantitatively, because no methods of microbiological assay have so far been devised for their determination. In this instance resort was had to "partition chromatography" (cf. CONSDEN, GORDON, AND MARTIN[11]). The

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TABLE II

PALE ALE WORT PITCHED WITH TOP-YEAST AT THE RATE OF 2.8 g/LITRE PRESSED YEAST (= 1 lb/BARREL)
ALL FERMENTATIONS CARRIED OUT IN DEWAR CYLINDERS

	1st Experimental Fermentation						2nd Experimental Fermentation						3rd Experimental Fermentation					
Hours	0	9	24	48	72	96	0	9	24	48	72	96	0	9	24	48	72	96
Gravity	1039.6	36.7	16.3	10.8	10.0	10.1	1040	36.7	21.4	10.7	9.0	8.3	1040	38.8	26.2	13.2	11.4	7.1
Amino acids in mg/100 ml																		
Arginine	25.00	23.20	7.20	1.75	1.75	1.75	25.00	17.00	4.80	1.75	1.75	1.75	26.80	25.40	6.30	0.84	0.78	0.78
Aspartic acid	6.15	4.70	0.65	0.52	0.52	0.52	5.50	4.50	0.65	0.52	0.52	0.52	10.00	10.00	1.62	0.63	0.60	0.60
Cystine	1.50	1.50	1.25	0.50	0.50	0.50	1.60	1.50	0.55	0.55	0.55	0.55	0.95	0.95	0.90	0.45	0.45	0.45
Glutamic acid	14.10	10.00	5.00	2.80	2.80	2.80	13.20	11.00	5.00	3.20	2.60	2.60	16.50	15.50	4.60	2.00	2.00	2.00
Histidine	4.90	4.20	0.90	0.77	0.75	0.75	5.30	4.50	2.15	1.15	1.00	1.00	4.00	3.90	1.68	0.76	0.60	0.47
Isoleucine	8.60	7.00	0.87	0.82	0.82	0.82	8.50	8.20	1.55	1.31	1.20	1.13	12.00	10.50	2.20	0.67	0.60	0.60
Leucine	13.80	12.50	1.27	1.41	1.13	1.13	15.00	13.00	1.90	1.75	1.18	1.18	18.20	16.40	3.20	0.93	0.84	0.84
Lysine	15.00	5.40	1.57	2.70	2.00	2.00	18.00	5.65	3.00	3.00	3.60	3.60	7.55	4.20	0.80	0.72	0.72	0.72
Methionine	2.80	1.52	0.00	0.00	0.00	0.00	3.10	2.07	0.00	0.00	0.00	0.00	3.90	3.10	0.00	0.00	0.00	0.00
Phenylalanine	5.30	5.30	0.50	0.32	0.32	0.31	7.00	6.20	2.15	0.40	0.40	0.40	11.10	11.10	4.00	0.69	0.67	0.67
Proline	50.00	50.00	41.50	40.00	40.00	40.00	47.50	47.50	47.00	41.60	41.60	41.60	49.00	49.00	48.00	44.00	44.00	44.00
Serine	7.50	5.00	1.85	0.90	1.40	1.40	8.00	6.70	2.85	1.43	1.43	1.43	7.40	6.50	1.52	1.15	1.26	1.26
Threonine	10.80	7.40	1.38	1.25	1.20	1.20	10.80	8.20	2.25	1.22	1.20	1.20	9.40	9.00	2.45	0.80	0.66	0.66
Tyrosine	5.00	5.00	1.50	0.30	0.60	0.60	10.60	9.00	7.50	1.40	1.25	1.00	10.40	10.40	6.00	0.64	0.30	0.21
Tryptophan	6.00	5.60	0.76	0.00	0.00	0.00	2.80	2.76	0.58	0.17	0.07	0.07	5.20	4.80	4.65	0.20	0.11	0.13
Valine	14.60	12.40	1.44	1.02	1.02	1.02	13.50	12.30	3.50	1.43	1.43	1.43	18.90	18.90	6.00	0.88	0.75	0.75

results showed that glycine and α-alanine are present in wort and both are absorbed by yeast. It must be emphasized that these results are purely qualitative; nevertheless, judging by eye from the separation chromatograms of other amino acids before and after fermentation, glycine and α-alanine are absorbed to the extent of 80–90%. The fact that glycine when present in wort is a nitrogen nutrient for yeast, furnishes yet another example of the difference in metabolic behaviour of this organism when supplied with a mixture of nitrogen nutrients compared with a single source of nitrogen, because THORNE[15] has shown that when used alone, glycine is useless as a nitrogen nutrient for yeast.

V. METHIONINE ASSIMILATION BY YEASTS

The fact that *all* methionine is removed in the course of 24 hours fermentation by top yeasts strongly suggests that this amino acid must play an important role in the nitrogen metabolism of this type of yeast. Methionine is a sulphur-containing amino acid CH_3–S–CH_2–CH_2–CH(NH_2)COOH (methyl-thiol-α-amino-*n*-butyric acid) and is one of the ten "essential" amino acids of W. C. ROSE[16] for mammalian nitrogen metabolism. Since methionine appears to play a leading part in the nitrogen metabolism of top yeasts, experiments were carried out to see whether a top yeast will take up a further supply of this acid after 24 hours fermentation. This was found to be the case (Table III).

TABLE III

	Hours fermentation	Methionine Content mg/100 ml
Wort	0	2.8
,,	24*	0.0 (+ 4.0 mg/100 ml methionine)
,,	48	0.0

* *Methionine added at the rate of 4.0 mg/100 ml*

Moreover, if the methionine content of a wort be doubled by fortification, the whole of the methionine again disappears in 24 hours (Table IV).

TABLE IV

	Hours fermentation	Methionine Content mg/100 ml
Wort	0	3.7
Wort + methionine	0	7.6
Wort + methionine	24	0.0

Thus a top yeast appears to have a very high capacity for absorbing methionine from wort in the early stages of fermentation.

In view of the results given above, other types of yeast, *e.g.*, bottom fermentation yeasts, bakers' yeast, a wine yeast and wild yeast were examined for their behaviour towards methionine. The results are given in Table V.

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TABLE V

DIFFERENT TYPES OF YEAST GROWN ON A PALE ALE WORT. ALL YEASTS PITCHED AT 18.3° C AND AT THE RATE OF 3.7 g/LITRE SPUN YEAST. METHIONINE ESTIMATED AFTER 24 HOURS

	Methionine Content mg/100 ml
Wort (Original Gravity 1040)	3.50
Wort after 24 hours fermentation with:	
Baker's Yeast (1)	0.00
Baker's Yeast (2)	0.00
Wine Yeast (ex HELM)	0.00
Bottom Yeast (TUBORG) (1)	0.54
Bottom Yeast (TUBORG) (2)	0.54
S. Macedoniensis	1.00
S. festinans	0.57
S. exiguus	1.00
Torula (sp.)	1.55
Wild yeast (unknown origin)	1.04

The figures given in Table V show that it is only bakers' yeast (which is a top yeast) and a wine yeast (which in this particular case is also a top yeast) behave in the same way as top-fermentation brewery yeasts, *i.e.*, absorb all methionine in 24 hours. On the other hand, bottom yeasts and wild yeasts only gradually absorb this amino acid, although they were given the relatively high temperatures of a top-fermentation system. This question of the difference in behaviour towards methionine of these two main types of brewery yeasts (*i.e.*, top and bottom yeasts) was further investigated by following the fate of methionine in a true bottom-fermentation (Table VI).

TABLE VI

DECOCTION WORT (ORIGINAL GRAVITY 1045)

Sample	% Total *N*	% amino acid *N*	Methionine Content mg/100 ml
0 days	0.070	0.024	2.80
1 day	0.063	0.021	1.52
2 days	0.053	0.016	0.44
3 ,,	0.048	0.014	0.38
4 ,,	0.046	0.015	0.30
5 ,,	0.048	0.014	0.25
6 ,,	0.048	0.014	0.20
7 ,,	0.044	0.013	0.15
8 ,,	0.046	0.013	0.12
9 ,,	0.044	0.014	0.12
10 ,,	0.044	0.014	0.12

We are indebted to Dr E. HELM of the *Jorgensen Laboratory, Copenhagen*, for these samples.

This experiment fully bears out the results given in Table V and shows that whether a fermentation be carried out with a bottom yeast at the low temperatures of lager brewing, or the comparatively high temperatures of top yeast fermentation, the result is the same and methionine is only gradually absorbed.

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VI. THE FATE OF INDIVIDUAL AMINO ACIDS IN BOTTOM FERMENTATION

The fate and behaviour of the same 16 amino acids which were determined for top fermentations (see Table II) are shown for a bottom fermentation in Table VII.

TABLE VII

DECOCTION WORT PITCHED WITH BOTTOM YEAST AT RATE OF 1.58 lb/BARREL PRESSED YEAST. FERMENTATION CARRIED OUT UNDER BOTTOM-FERMENTATION CONDITIONS

Amino acids in mg/100 ml	Hours								
	0	24	48	72	96	120	144	168	240
Arginine	18.00	12.50	9.30	5.64	4.95	4.80	4.65	4.27	4.65
Aspartic acid	6.56	5.12	3.48	1.02	0.66	0.66	0.66	0.66	0.66
Cystine	0.83	0.83	0.75	0.49	0.49	0.49	0.30	0.30	0.30
Glutamic acid	14.40	10.84	10.00	3.79	2.35	2.25	5.00	7.00	7.00
Histidine	3.30	3.00	2.10	1.86	1.26	1.26	1.26	1.26	1.26
Isoleucine	11.10	9.10	7.20	3.60	1.30	1.30	1.30	1.30	1.30
Leucine	15.65	12.50	7.80	3.72	1.40	1.40	1.62	1.60	1.62
Lysine	6.35	3.00	1.10	0.78	0.30	0.30	0.30	0.30	0.30
Methionine	3.0	1.90	0.76	0.30	0.30	0.30	0.30	0.30	0.30
Phenylalanine	11.50	11.00	8.00	1.20	0.95	0.93	0.95	1.00	1.00
Proline	63.00	63.00	63.00	59.00	59.00	59.00	59.00	59.00	59.00
Serine	8.00	6.20	4.55	1.74	1.48	1.48	1.48	1.48	1.48
Threonine	8.25	5.10	3.00	2.20	1.50	1.50	1.50	1.50	1.50
Tyrosine	12.60	11.00	9.00	4.60	2.32	1.80	1.65	1.65	1.65
Tryptophan	5.84	5.20	4.80	2.50	1.98	1.98	1.98	1.98	1.98
Valine	15.50	13.60	10.30	3.28	2.32	2.32	2.32	2.64	2.40

Certain similarities and differences are apparent when the results in the two cases are compared. In the first place the behaviour of lysine and proline is exactly the same for both types of fermentation. 50% of the lysine is removed in the first 24 hours of fermentation and in all, 95% is taken up by the bottom yeast. Proline, as in top fermentation, is scarcely attacked and methionine, although utilized to the extent of 90% is only gradually absorbed. The behaviour of glutamic acid is markedly different from a top-fermentation. The bottom yeast removes 84% of this acid in 120 hours and thereafter there is vigorous excretion up to approximately 50% of the original glutamic acid content of the wort. No other amino acid is excreted to this extent in either a top or bottom-fermentation. In top-fermentations the aromatic amino acidys, trptophan and histidine are used up to 90%, but in a bottom-fermentation only about 65% of these acids are removed.

With the exception of aspartic acid, leucine, lysine, methionine, phenylalanine and tyrosine, which are taken up to the extent of 90%, the remaining acids are not absorbed by a bottom yeast to the same degree as they are in a top-fermentation.

Thus, there are several differences to be noted in these two types of fermentation. How far they are due to differences in the temperatures of fermentation and how far to inherent differences in the yeasts themselves is impossible to say at the present time.

VII. THE AMINO ACID CONTENT OF YEAST PROTEIN

It has been known for many years that yeasts are able to use inorganic nitrogen, *e.g.*, ammonium sulphate or phosphate as their sole source of nitrogen and are able

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to synthesize from such relatively simple compounds the complex proteins of their protoplasm. Generally speaking, however, they prefer elaborated organic nitrogen and show greatly enhanced growth on a medium containing a mixture of amino acids compared with a medium containing a single source of nitrogen, *e.g.*, an ammonium salt or single amino acid (cf. THORNE[17]). It was therefore of interest to determine if any differences were produced in the amino acid content of yeast protein by growing the organism in media containing different types of nitrogen. Yeast protein is considered "first class" protein and in this respect is comparable with the best animal proteins, *e.g.*, casein. A protein is considered "first class" when it contains high values of the following 10 "essential" amino acids of ROSE[16], arginine, histidine isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Table VIII shows the values for seven "essential" acids in yeast protein.

The yeast was grown on a variety of different media and for comparative purposes the values for the same acids in casein are also given.

The figures given in Table VIII show that the values of the seven acids estimated in yeast protein compare favourably with the same acids in casein, with the exception of methionine which is low. Apart from methionine, the figures for the remaining acids are remarkably constant whatever the nature of the medium upon which the yeast has been grown. In the case of methionine, however, it is possible to increase its concentration in yeast protein as a result of increasing its concentration in the medium (Table VIII, No. 7). No other case of increase was found among the other six acids although medium No. 7 (Table VIII) was also fortified with histidine and phenylalanine.

VIII. DISCUSSION

The main points of the present investigation can conveniently be discussed here. The behaviour of asparagine N in fermenting wort requires consideration. It is difficult to understand why the best single source of nitrogen nutrient for yeast becomes only of minor importance when present in wort. The evidence for excretion of asparagine N during fermentation is abundantly plain. The suggestion is tentatively made here that the excretion of this amide during fermentation may be bound up in some way with the neutralization of any excess ammonia formed in the yeast cell during its metabolic activities, which might otherwise prove toxic to the organism.

Hitherto, a mixture of amino acids has been considered to be the best source of nitrogen for yeast yielding maximum growth. In general terms this is true, but the role of residual N in this connection must also be taken into account. The composition of this fraction is not known at present, but it is highly probable that di- and lower polypeptides largely enter into its composition. If this be the case, then di- and polypeptides form a subsidiary but nevertheless significant source of nitrogen for yeast. THORNE[17] has shown that with increasing number of nitrogen nutrients in a medium there is a progressive enhancement of growth and he has calculated that there is a limiting value to this augmentation process of about 50%. THORNE was mainly interested in media containing mixtures of amino acids. In the present investigation THORNE's results have been confirmed (Table IX), but at the same time it must be pointed out, that the amount of growth even with a mixture containing a large number of amino acids, *e.g.*, casein hydrolysate, is always inferior to wort and it is only when peptides (peptone was used for this purpose) are added that growth in an artificial medium and growth on wort

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TABLE VIII

TOP YEAST PITCHED AT RATE OF 3.7 g/LITRE SPUN YEAST

Yeast grown on	Methionine		Phenylalanine		Histidine		Lysine		Leucine		Isoleucine		Valine	
	% Dry wt	16% N	% Dry wt	16% N	% Dry wt	16% N	% Dry wt	16% N	% Dry wt	16% N	% Dry wt	16% N	% Dry wt	16% N
Wort (O.G. 1040).	0.70	1.48	1.61	3.42	1.30	2.77	3.20	6.80	3.00	6.38	2.44	5.18	2.93	6.40
Wort (O.G. 1046).	0.89	1.45	1.93	3.14	1.52	2.48	4.37	7.12	3.78	6.16	3.07	5.00	3.92	6.40
Artificial medium + $(NH_4)_2SO_4$	0.73	1.43	2.05	4.00	1.73	3.40	3.48	6.84	3.05	6.00	2.73	5.36	3.09	6.08
Artificial medium + $(NH_4)_2SO_4$ + Asparagine	0.67	1.43	1.82	3.85	1.40	3.00	3.48	7.37	3.27	6.95	2.73	5.78	3.09	6.55
Artificial medium + peptone	0.57	1.16	1.62	3.28	1.07	2.17	3.64	7.37	2.76	5.59	2.28	4.62	2.62	5.30
Artificial medium + peptone and casein hydrolysate . .	0.76	1.53	2.00	4.04	1.30	2.64	4.34	8.76	3.13	6.33	2.52	5.10	2.91	5.90
Artificial medium + casein hydrolysate fortified with methionine phenylalanine and histidine	1.73	3.50	1.42	2.88	1.15	2.33	3.26	6.58	2.85	5.75	2.31	4.66	2.54	5.13
Casein[1]	—	3.00	—	5.20	—	2.50	—	6.90	—	12.10	—	6.50	—	7.00

[1] Values taken from R. J. BLOCK AND D. BOLLING[18].

become identical. Care was taken in the preparation of the artificial media that gravity and the total nitrogen content were as nearly as possible the same in all cases as the wort which served as control.

TABLE IX

TOP-YEAST USED AND PITCHED AT THE RATE OF 2.8 g/LITRE PRESSED YEAST (= 1 lb/BARREL)

Nitrogen constituent of artificial medium	% Total N	Original Gravity	Present Gravity	Reproduction (g/litre = spun wet weight)
1. Ammonium sulphate	0.074	1032.0	1001.9	11.8
2. Ammonium sulphate + asparagine .	0.079	1032.4	999.9	19.6
3. Peptone	0.082	1032.7	1000.2	24.3
4. Casein hydrolysate	0.073	1033.0	998.2	20.8
5. Casein hydrolysate + peptone . . .	0.078	1033.1	999.4	26.0
6. Wort	0.072	1032.5	1005.5	25.4

This experiment has been repeated and confirmed many times. Thus it would appear that peptides as a source of nitrogen for yeast cannot be neglected in forming a general picture of the subject.

The question of the absorption of individual amino acids from wort has already been discussed, and the similarities and differences between top and bottom-fermentation yeast pointed out.

I wish to thank the Directors of Messrs WHITBREAD AND Co Ltd for permission to publish this investigation. I would also like to take this opportunity of thanking Mr B. M. BROWN for his helpful criticism and advice throughout the course of this work. I am grateful to my assistants Mr N. S. CURTIS and Miss J. V. SUTTON for their help in the practical work of this investigation.

SUMMARY

A quantitative examination of the more important nitrogenous groupings present in wort, *e.g.*, ammonia N, amino acid N, asparagine N, residual N and protein N have been made, and their fate in fermentation by top and bottom-yeasts followed.

The presence of 18 individual free amino acids has been detected in wort and the behaviour of 16 during fermentation by top and bottom-yeasts followed by microbiological assay.

Seven "essential" amino acids in yeast protein have been determined. The yeast was grown on media containing different sources of nitrogen. With the exception of methionine, all the acids were constant in amount whatever the nature of the medium. The concentration of methionine in yeast protein could be increased by increasing its concentration in the medium.

RÉSUMÉ

Un examen quantitatif des groupements azotés importants présents dans le moût (azote ammoniacal, des acides aminés, de l'asparagine, de protéine et azote résiduel) a été fait et le sort en a été suivi au cours de la fermentation haute et basse.

La présence dans le moût de 18 acides aminés libres a été décelée; le comportement de 16 de ceux-ci pendant la fermentation haute et basse a été suivi par estimation microbiologique.

Sept acides aminés "essentiels" de la protéine de la levure ont été déterminés. La levure a été cultivée sur milieux contenant différentes sources d'azote. A l'exception de la méthionine, tous les acides se trouvaient à concentration constante, quelle que fût la nature du milieu. La concentration de la méthionine dans la protéine de levure pouvait être augmenté en augmentant sa concentration dans le milieu.

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ZUSAMMENFASSUNG

Die wichtigeren stickstoffhaltigen Gruppen, welche in der Würze vorkommen, wie Ammoniak-N, Aminosäure-N, Asparagin-N, Protein-N und Rest-N wurden quantitativ untersucht, und ihr Schicksal während der Ober- und Untergärung wurde verfolgt.

Die Anwesenheit von 18 freien Aminosäuren in der Würze wurde festgestellt und das Verhalten von 16 dieser Säuren während der Vergärung durch Ober- und Untergärhefen wurde mikrobiologisch verfolgt.

Sieben für das Hefeeiweiss "wesentliche" Aminosäuren wurden festgestellt. Die Hefe wurde auf Nährböden, welche verschiedene Stickstoffquellen enthielten, gezüchtet. Mit Ausnahme des Methionins war die Menge aller Säuren konstant, was auch die Art des Nährbodens sein mochte. Die Konzentration des Methionins im Hefeeiweiss konnte durch Erhöhung der Methioninkonzentration im Nährboden gesteigert werden.

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