CCLXXVI. THE AMINO-ACID CONTENT OF WHEAT FLOUR DOUGH.

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IN view of a recent paper in this *Journal* by Blagoveschenski and Yurgenson [1935] on the action of flour and yeast enzymes on wheat proteins, it seems of interest to note the results of an investigation of the changes in the amino-acid content of flour dough with time, in the presence and in the absence of yeast, which was undertaken in the latter half of 1933. Blagoveschenski and Yurgenson [1935] concluded that the increased solubility of flour proteins under the influence of flour enzymes was not accompanied by an increase in amino-nitrogen but that the solvent effect of yeast enzymes was due to true proteolysis.

Several investigators have reported an increase in amino-nitrogen content (determined by various methods) of ageing dough in the presence and in the absence of yeast, but Brownlee and Bailey [1930] found a decrease in the amino-acid content of yeasted flour dough during fermentation and attributed this to utilisation of the amino-nitrogen by the yeast.

MATERIAL AND METHODS.

Five flours were used in this study, the dough formula being, flour 100 g., sugar 2 g., with sufficient salt solution ($2\frac{1}{2}\%$) to form a dough of normal consistency. The sugar was dissolved in the salt solution, the flour and liquid incorporated by hand and then mixed for 1 min. in a mechanical kneading machine. Where yeast was used it was added at the rate of 2 g. to the above mix and was dispersed in the liquid, before addition to the flour. The doughs were maintained at 29° and at definite time intervals samples of dough were dispersed in sufficient additional water to give a 1 : 5 extract, by grinding in a mortar and then mixing in a mechanical drink mixer. The liquid was clarified in the centrifuge and the amino-acid content determined by the Brown [1923] modification of the Sørensen [1907, 1, 2] formaldehyde titration as previously described [Samuel, 1934] by titration to p_H 8.0 (thymol blue) before and after the addition of formalin. The results obtained are shown in Table I, and the initial values of the amino-acid content at zero time for the yeasted and unyeasted doughs show that the technique yields reproducible results.

For each unyeasted dough there is a steady increase in amino-acid content with time, showing that flour contains proteolytic enzymes capable of decomposing flour proteins to the amino-acid stage and that proteolytic activity varies from flour to flour. In the yeasted dough the amino-acid content remains steady or increases slightly (to a less extent than in the unyeasted dough) for about an hour and then decreases, probably to a constant value. This is probably due to the utilisation of the amino-acid by the yeast organism as a source of nitrogen in its metabolism and prevents the direct comparison of the amino-acid production of yeasted and unyeasted flour doughs from revealing the effect of yeast proteases on flour proteins.

Table I. *The production of amino-acid in yeasted and unyeasted flour dough.*

Flour	Time (hours)	Amino-acid content mg. N per 2 g. flour	
		Without yeast	With yeast
1. A-mill mixture	0	0.14	0.14
	1	0.17	0.14
	2	0.20	0.11
	3	0.25	0.06
	4	0.31	0.04
2. A mill mixture	0	0.17	0.17
	0.5	—	0.19
	1	0.20	0.18
	1.5	—	0.17
	2	0.23	—
	2.5	—	0.10
	4	0.29	—
	4.5	—	0.07
	5	0.31	—
3. Manitoba	0	0.22	0.22
	1	0.25	0.24
	2	0.28	0.18
	4	0.33	0.16
	5	—	0.16
	8	0.40	0.16
4. Low grade	0	0.35	0.35
	1	0.38	0.35
	2	0.42	0.33
	4	0.48	0.27
	5	0.50	0.26
	7.5	0.57	0.25
5. English	0	0.32	0.32
	1	0.36	0.34
	2	0.40	0.33
	4	0.45	0.20
	5	0.48	0.18

These results are not in agreement with those of Blagoveschenski and Yurgenson [1935], though the discrepancy may be partly explained by the technique and the quantities used, for it appears that these authors allowed the enzymes extracted from 0.25 g. of flour or yeast to act on the gluten from about 10 g. of flour, whereas in the present work the enzymes were allowed to act *in situ*. It is not certain that all the flour or yeast enzymes were extracted and indeed it may be suggested that the increase in amino-nitrogen of the control solutions of Blagoveschenski and Yurgenson [1935] is due to the action of flour enzymes which are not removed by washing but remain in the gluten which is used as the substrate for the controls.

To estimate the rate at which yeast utilises amino-acid, the conditions in the dough liquid were simulated in a solution which was analysed from time to time. The ratio of flour to water in a dough is approximately 2 : 1 and the ratio of flour to water for the extraction is 1 : 5 so that concentrations in the dough liquid are ten times those in the extract.

200 ml. of solution containing 1.4 g. of glutamic acid hydrochloride, 8 g. of sugar, 5 g. of salt and 8 g. of yeast were brought to p_H 5.9 (the approximate p_H value of flour dough) by the addition of sodium hydroxide and incubated at 29°. At definite time intervals 5 ml. samples were taken, diluted to 50 ml. and centrifuged and the amino-acid content was determined as for the dough extract. In the titration to p_H 8.0 before the addition of formalin, fading of the colour, due to the presence of carbon dioxide, was noted. When formalin is added the

solution becomes acid, so to avoid the possible loss of carbon dioxide from the acid solution the approximate amount of sodium hydroxide for the titration in the presence of formalin was added before adding the formalin.

It is recognised that the results of this experiment (Table II) must be applied with caution to the dough experiments. The amino-acid in the solution is more readily available both physically and chemically to the yeast, but the local concentration of amino-acid around the yeast cells in the dough may be higher than the bulk concentration. Because of this latter consideration the original concentration of amino-acid in the solution was made greater than had been found in the dough.

Table II. *Amino-acid content of a solution containing glutamic acid, sugar, salt and yeast.*

Time (hours)	p_H	Amino-acid mg. N
0	5.90	0.67
1	—	0.60
2	—	0.48
4	—	0.39
6	4.68	0.36

From the quantities and dilutions used, Table II is directly comparable with Table I.

It is probable therefore that the consumption of amino-acid was greater in the solution than in the dough, yet the nitrogen used in 6 hours represents only 1.77 mg. of protein or approximately 1% of the protein in a flour.

The effect of the yeast proteases on the flour proteins is left in doubt, but since in the solution only 0.28 mg. of amino-nitrogen has been used in 4 hours, of which about half would be formed by the action of the flour enzymes, it may be inferred that degradation of the proteins to the amino-acid stage by yeast is not serious, being about equal to the degradation caused by the flour enzymes.

SUMMARY.

1. In an unyeasted flour dough the amino-acid content increased steadily with time.
2. In a yeasted flour dough the amino-acid content increased slightly for about an hour and then decreased to an almost constant value.
3. The rate of utilisation of amino-acid by yeast in a solution similar to the dough liquid indicates that in a yeasted dough the protein is decomposed about twice as rapidly as in an unyeasted dough.

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