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THE HYDROLYSIS OF GLIADIN FROM RYE.[1]

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ALCOHOL extracts from rye flour a protein substance which very closely resembles gliadin obtained from wheat flour under similar conditions. The gliadin from rye has been the subject of extensive study in this laboratory,[2] and a strict comparison in respect to composition and reactions has been made between it and the gliadin from wheat without revealing any differences. This comparison has now been supplemented by a determination of the proportion of the several decomposition products which the gliadin of rye yields when hydrolyzed. The material for this hydrolysis was obtained by extracting rye flour, ground in this laboratory, with cold 75 per cent (by volume) alcohol, concentrating the perfectly clear extract under reduced pressure to a syrup, and precipitating the gliadin by pouring the solution into ice water. The precipitate thus obtained was redissolved in 85 per cent (by volume) alcohol, and the solution again poured into several volumes of ice water. The gliadin that separated was then washed with distilled water, redissolved in 85 per cent alcohol, and the clear solution precipitated by pouring in a thin stream into a large volume of absolute alcohol. After dehydrating by long digestion with absolute alcohol, the gliadin was dried over sulphuric acid, ground to a fine powder, and moisture, ash, and ether soluble matter determined in it.

Four hundred and fifteen grams of this preparation, equal to 362.67 gm. moisture, ash, and fat free, were suspended in a mixture of 415 c.c. of water and 415 c.c. of hydrochloric acid of sp. gr. 1.19. After warming for three hours at 100° the hydrolysis solution was boiled in a bath of oil for eighteen and a half hours.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] Osborne: Journal of the American Chemical Society, 1895, xvii, p. 429.

After concentrating to about two thirds of the original volume, the solution was saturated with hydrochloric acid gas and allowed to stand at 0° for four days.

The precipitate of glutaminic acid hydrochloride when recrystallized from strong hydrochloric acid and freed from ammonium chloride weighed 124.44 gm., equivalent to 99.68 gm. of free glutaminic acid, or 28.24 per cent of the protein.

The filtrate from the glutaminic acid hydrochloride was freed from water as completely as possible by evaporating under reduced pressure, and the residue esterified with alcohol and dry hydrochloric acid gas, as often described.

The esters were liberated and shaken out with ether, and the aqueous layer made strongly acid with hydrochloric acid, freed from inorganic salts, and the esterification repeated according to the usual procedure.

By distillation under diminished pressure the following fractions were obtained:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	100°	18 mm.	14.61 gm.
II	70°	0.38 "	47.44 "
III	100°	0.50 "	42.87 "
IV A	170°	0.35 "	54.30 "
IV B	200°	0.43 "	10.21 "
Total			169.43 gm.

The undistilled residue weighed 68 gm.

Fraction I.— From this fraction and from the ether distilled from the esters on the water bath at atmospheric pressure, there were obtained 0.87 gm. of pure glycocoll ester hydrochloride, which is equivalent to 0.47 gm. of glycocoll, or 0.13 per cent of the protein. The melting-point was 144°. The remainder of Fraction I consisted essentially of alanine, of which 3.6 gm. were isolated.

Carbon and hydrogen, 0.1011 gm. subst. gave 0.1502 gm. CO_2 and 0.0756 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.45 ; H 7.86 per cent.

Found . . . . = C 40.52 ; H 8.31 " "

Fraction II.—This fraction yielded by the customary methods 10.54 gm. of leucine and 1.08 gm. of alanine, while no valine was obtained.

The large quantity of proline contained in Fraction II was worked up conjointly with that from Fraction III. No glycocoll could be brought to separation in this fraction as the ethyl ester hydrochloride.

Fraction III.—The yield of leucine was 11.71 gm. The substance decomposed at about 298° and gave the following analysis:

Carbon and hydrogen, 0.1755 gm. subst. gave 0.3524 gm. CO_2 and 0.1575 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . = C 54.76; H 9.97 " "

The proline from Fractions II and III, when freed as completely as possible from substances insoluble in absolute alcohol and dried to constancy over sulphuric acid, weighed 34.67 gm. By redissolving in absolute alcohol the substance separated in the characteristic prisms melting at about 206°.

Carbon and hydrogen, 0.1760 gm. subst. gave 0.3348 gm. CO_2 and 0.1307 gm. H_2O.

Calculated for $C_5H_9O_2N$ = C 52.18; H 7.83 per cent.
Found . . . . = C 51.88; H 8.24 " "

For identification the substance was converted to the copper salt and the lævo separated from the racemized with absolute alcohol in the usual way.

The racemic copper salt crystallized from water in the characteristic plates containing two molecules of water-of-crystallization.

Water, 0.1365 gm. subst. (air dry) lost 0.0146 gm. H_2O at 110°.

Calculated for $C_{10}H_{16}O_4N_2Cu \cdot 2\ H_2O$ = H_2O 10.99 per cent.
Found . . . . . = H_2O 10.86 " "

Copper, 0.1177 gm. subst. dried at 110°, gave 0.0318 gm. CuO.

Calculated for $C_{10}H_{16}O_4N_2Cu$ = Cu 21.81 per cent.
Found . . . . . = Cu 21.59 " "

The lævo proline was converted to the characteristic phenylhydantoine. The melting-point was 142°.

Carbon and hydrogen, 0.2716 gm. subst. gave 0.6604 gm. CO_2 and 0.1401 gm. H_2O.

Calculated for $C_{12}H_{12}O_2N_2$ = C 66.67; H 5.57 per cent.
Found . . . . = C 66.31; H 5.72 " "

Fraction IV.—The yield of phenylalanine hydrochloride from this fraction was 11.62 gm. The free substance was employed for the analysis.

Carbon and hydrogen, 0.1347 gm. subst. gave 0.3226 gm. CO_2 and 0.0833 gm. H_2O.

Calculated for $C_9H_{11}O_2N$ = C 65.45; H 6.66 per cent.
Found . . . . = C 65.32; H 6.87 " "

There were further isolated from this fraction 0.87 gm. of pure aspartic acid as the barium salt. The substance crystallized in the characteristic form, and reddened but did not decompose at 300°. Unfortunately no elementary analysis was obtained, as the preparation was lost. The filtrate from the barium aspartate was freed from barium and examined for glutaminic acid. There were isolated 9.89 gm. of the hydrochloride. The free substance decomposed at about 203°.

Carbon and hydrogen, 0.2687 gm. subst. gave 0.4049 gm. CO_2 and 0.1524 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81; H 6.12 per cent.
Found . . . . = C 41.09; H 6.29 " "

In the filtrate from the glutaminic acid hydrochloride no copper salt of aspartic acid could be obtained.

There was further isolated from Fraction IV 0.2 gm. of pure serine. The substance browned at about 215° and decomposed at about 238°, with effervescence, to a brownish mass.

Carbon and hydrogen, 0.1212 gm. subst. gave 0.1536 gm. CO_2 and 0.0790 gm. H_2O.

Calculated for $C_3H_7O_3N$ = C 34.29; H 6.67 per cent.
Found . . . . = C 34.56; H 7.24 " "

Tyrosine.

Fifty grams of rye gliadin, equal to 42.98 gm. moisture, fat, and ash free, were hydrolyzed by heating with a mixture of 150 gm. sulphuric acid and 300 c.c. water for two and a half hours on a water bath and boiling for twelve hours on an oil bath. After removing the sulphuric acid with an equivalent quantity of baryta and boiling out the barium sulphate several times with water the solution was concentrated to crystallization. After standing over night the substance that had separated was recrystallized from

water, and 0.51 gm. of tyrosine in characteristic needles was obtained.

Nitrogen, 0.2045 gm. subst. required 1.63 c.c. 5/7 N—HCl = 0.0163 gm. N.
Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.
Found . . . . = N 7.91 " "

Histidine, Arginine, and Lysine.

Fifty grams of the rye gliadin, equal to 47.37 gm. moisture, fat, and ash free, were hydrolyzed and the bases determined as Kossel and Patten direct. The solution of the histidine was made up to 500 c.c. and nitrogen determined in 100 c.c. of it.

Nitrogen, 100 c.c. solution required 1.00 c.c. 5/7 N — HCl = 0.0100 gm. N = 0.0500 gm. N in 500 c.c. = 0.1843 gm. histidine, or 0.39 per cent.

The solution containing the arginine was made up to 1000 c.c. and nitrogen determined in 50 c.c of it.

Nitrogen, 50 c.c. solution required 1.50 c.c. 5/7 N—HCl = 0.0158 gm. N = 0.3160 gm. N in 1000 c.c. = 0.9809 gm. arginine. Adding 0.0720 gm. for solubility of silver arginine gives 1.0529 gm. arginine, or 2.22 per cent.

The results of this hydrolysis are given in the following table, and for comparison are also given those which we have obtained with the other alcohol soluble proteins:

	Gliadin, Rye per cent	Gliadin, Wheat per cent	Hordein, Barley per cent	Zein, Maize per cent
Glycocoll	0.13	0.02	0.00	0.00
Alanine	1.33	2.00	0.43	2.23
Valine	not isolated	0.21	0.13	0.29
Leucine	6.30	5.61	5.67	18.60
Proline	9.82	7.06	13.73	6.53
Phenylalanine . . .	2.70	2.35	5.03	4.87
Aspartic acid . . .	0.25	0.58	not isolated	1.41
Glutaminic acid . .	33.81	37.33	36.35	18.28
Serine	0.06	0.13	not isolated	0.57
Tyrosine	1.19	1.20	1.67	3.55
Arginine	2.22	3.16	2.16	1.16
Lysine	0.00	0.00	0.00	0.00
Histidine	0.39	0.61	1.28	0.43
Ammonia	5.11	5.11	4.87	3.61
Tryptophane. . .	present	present	present	0.00
Cystine . . .	not determined	0.45	not determined	not determined
Total . . .	64.31	65.81	71.32	61.53

The agreement between the analyses of the gliadin from wheat and rye is so close that the conclusion that differences exist between the preparations from these two seeds is not justified. Between hordein, zein, and gliadin, however, such distinct differences exist that, taken in connection with the differences in ultimate composition and properties, there can be no question that these are distinctly different proteins. These hydrolyses show that the alcohol-soluble proteins of the cereals form a distinctly characterized group which differ from all the other protein substances thus far analyzed. These differences are especially shown in their high content of proline, glutaminic acid, and ammonia, and their low content of arginine and histidine and absence of lysine.[3] Zein is especially worthy of note, as it lacks glycocoll, lysine, and tryptophane, which are obtained from nearly all the other proteins.

[3] Cf. KOSSEL and KUTSCHER, Zeitschrift für physiologische Chemie, 1900, xxxi, p. 165.