CLXI. THE BIOLOGICAL VALUES OF THE PROTEINS OF BREADS BAKED FROM RYE AND WHEAT FLOURS ALONE OR COMBINED WITH YEAST OR SOYA BEAN FLOUR.

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IN the course of a study of the nutritional values of various Polish foodstuffs, carried out by us in 1929 and 1930 on behalf of the Flour and Bread Committee of the Polish Ministry of Interior, the biological values of the proteins ($N \times 6.25$) of various breads commonly consumed in Poland and of several experimental ones have been ascertained. It is thought worth while to present here such of the results as may be of more general interest.

The biological analysis of 17 products is discussed in the present paper. These may be divided into three groups, (1) various breads commonly consumed in Poland; (2) experimental breads; (3) soya bean flour and baker's yeast.

Breads commonly used were purchased from the Municipal Bakeries in Warsaw. Experimental breads were baked in the Department of Technology of Fermentation and Foodstuffs, Warsaw Technical Institute, under the supervision of Prof. W. Iwanowski who co-operated with us.

In the preparation of the experimental breads we have been interested in combinations of rye and wheat flours with varying amounts of baker's yeast and of soya bean flour. The two latter substances have recently attracted considerable attention as highly concentrated sources of cheap vegetable protein.

The baker's yeast used as admixture was a brand well known in Poland. The soya bean flour "Edelsoja" was purchased from the Oesterreichische Soja-Aktiengesellschaft in Vienna. It is manufactured under a secret process (Berczeller) and is stated by the manufacturers to keep indefinitely without becoming rancid although it contains about 20 % of fat.

The nutritional value of this product and especially its vitamin and mineral contents have been discussed by one of us in a separate publication [Kon, 1930]. As we have made no direct comparisons we do not know whether the biological value of the proteins of the soya bean flour used in the present study is identical with or different from that of the proteins originally present in the soya bean.

EXPERIMENTAL.

The numerical method proposed by Osborne, Mendel and Ferry [1919] was used for the estimation of the biological value of the proteins of the various products studied. The rats were kept singly in cylindrical cages on screens and

Table I.

No.	Product	Protein content* (N × 6.25) %	Remarks
1	White wheat bread	11.0	Baked by Prof. Iwanowski. 280 g. of white wheat flour, 140 cc. of a 2½ % solution of NaCl and 5 g. fresh baker's yeast to the loaf
2	Wheat rolls	11.5	From the Municipal Bakeries
3	White wheat-yeast bread I	14.1	Baked by Prof. Iwanowski. 280 g. of white wheat flour, 140 cc. of a 2½ % solution of NaCl and 90 g. of fresh baker's yeast to the loaf
4	White wheat-yeast bread II	15.8	Baked by Prof. Iwanowski. 280 g. of white wheat flour, 150 cc. of a 2½ % solution of NaCl and 140 g. of fresh baker's yeast to the loaf
5	Soya flour-wheat bread I	14.7	Baked by Prof. Iwanowski. 280 g. of a mixture of white wheat flour (90 %) and soya bean flour† (10 %), 150 cc. of a 2½ % solution of NaCl and 5 g. of fresh baker's yeast to the loaf
6	Soya flour-wheat bread II	18.5	Baked by Prof. Iwanowski. 280 g. of a mixture of white wheat flour (80 %) and soya bean flour† (20 %), 160 cc. of a 2½ % solution of NaCl and 5 g. of fresh baker's yeast to the loaf
7	Rye bread from 70 % rye flour	6.6	From the Municipal Bakeries. Prepared by acid fermentation. (No yeast added, fermentation started by the addition of fermented dough from previous baking.)
8	Crust of bread No. 7	6.6	
9	Crumb of bread No. 7	7.0	
10	Rye bread from 70 % rye flour	6.3	Baked by Prof. Iwanowski. Same method as for bread No. 7
11	Rye-yeast bread from 70 % rye flour	8.6	Baked by Prof. Iwanowski. 280 g. 70 % rye flour, 140 cc. of a 3 % solution of NaCl and 50 g. of fresh baker's yeast to the loaf
12	Soya flour-rye bread I	10.6	Baked by Prof. Iwanowski. 280 g. of a mixture of 70 % rye flour (90 %) and soya bean flour† (10 %), 160 cc. of a 3 % solution of NaCl and 5 g. of fresh baker's yeast to the loaf
13	Soya flour-rye bread II	14.8	Baked by Prof. Iwanowski. 280 g. of a mixture of 70 % rye flour (80 %) and soya bean flour† (20 %), 170 cc. of a 3 % solution of NaCl and 5 g. of fresh baker's yeast to the loaf
14	Military bread (rye)	9.1	From the Municipal Bakeries. 82 % rye flour. No yeast. (Same method as for bread No. 7)
15	Country dark rye bread (whole-meal)	9.9	Representative sample of country bread baked from home-milled rye flour (approx. 100 %), prepared by acid fermentation
16	Soya bean flour	45.5	A commercial preparation containing about 20 % fat, marketed under the name of "Edelsoja" by the Soja-Aktiengesellschaft in Vienna
17	Baker's yeast	45.6	Same brand as used for admixture to all the yeast breads described above

* On the dry basis.

† As described under 16.

the diets were offered in glass feeders with metal hoods to prevent scattering. All diets containing raw starch were mixed with water and heated on the water-bath until dextrinised. Diets containing only bread and the salt mixture were mixed with water without cooking. Tap-water was offered *ad lib.* in glass bulbs. Each rat was given daily, separately from the diet, 3 drops of cod-liver oil and 0.15–0.2 cc. of an Osborne and Wakeman [1919] watery extract from brewer's yeast equivalent to 0.3–0.4 g. of dry yeast and containing 4.8–6.4 mg. N. The extract was omitted in the case of diets containing an abundance of vitamin B complex.

Potato starch was used in the compounding of the rations as it can be obtained in a state of greater purity (lower nitrogen content) than other commercial starches.

Every day the food residues were removed from the food cups, combined with any scattered food, collected on filter-paper on the trays and dried to constant weight. On Saturdays double rations were issued, no cleaning being done on Sundays. The experiments were, on the average, of 30 days' duration.

In Table I a list of the various products analysed and their nitrogen contents are given. Table II presents the composition of the various diets used in

Table II. *Composition of diets.*

		All percentages on dry basis.			
No.	Diet		%	%	%
1	White wheat bread	Protein (N $\times$ 6.25) level	8.6	10.4	—
		Bread	76.2	95.0	—
		Butter fat	10.0	—	—
		Salts*	5.0	5.0	—
		Potato starch	8.8	—	—
2	Wheat rolls	Protein (N $\times$ 6.25) level	6.4	8.2	10.9
		Rolls	53.0	70.5	95.0
		Butter fat	10.0	10.0	—
		Salts*	5.0	5.0	5.0
		Potato starch	32.0	14.5	—
3	Wheat-yeast bread I	Protein (N $\times$ 6.25) level	9.1	10.4	12.9
		Bread	61.5	71.0	95.0
		Butter fat	10.0	10.0	—
		Salts*	5.0	5.0	5.0
		Potato starch	23.5	14.0	—
4	Wheat-yeast bread II	Protein (N $\times$ 6.25) level	8.3	9.6	12.3
		Bread	51.2	63.3	76.0
		Butter fat	10.0	10.0	10.0
		Salts*	5.0	5.0	5.0
		Potato starch	33.8	21.7	9.0
5	Soya flour-wheat bread I	Protein (N $\times$ 6.25) level	8.1	10.2	11.9
		Bread	54.4	68.0	81.6
		Butter fat	10.0	10.0	10.0
		Salts*	5.0	5.0	5.0
		Potato starch	30.6	17.0	3.4
6	Soya flour-wheat bread II	Protein (N $\times$ 6.25) level	8.5	10.1	15.3
		Bread	43.2	54.0	81.0
		Butter fat	10.0	10.0	10.0
		Salts*	5.0	5.0	5.0
		Potato starch	41.8	31.0	4.0

* Steenbock's salt mixture No. 40.

Table II (*contd.*).

No.	Diet		%	%	%	%
7	70 % rye bread	Protein (N $\times$ 6.25) level	6.4	—	—	—
		Bread	95.0	—	—	—
		Salts*	5.0	—	—	—
8	Crust of bread No. 7	Protein (N $\times$ 6.25) level	6.3	—	—	—
		Crust	95.0	—	—	—
		Salts	5.0	—	—	—
9	Crumb of bread No. 7	Protein (N $\times$ 6.25) level	6.7	—	—	—
		Crumb	95.0	—	—	—
		Salts*	5.0	—	—	—
10	70 % rye bread	Protein (N $\times$ 6.25) level	6.0	—	—	—
		Bread	95.0	—	—	—
		Salts*	5.0	—	—	—
11	Rye-yeast bread	Protein (N $\times$ 6.25) level	8.2	—	—	—
		Bread	95.0	—	—	—
		Salts*	5.0	—	—	—
12	Soya flour-rye bread I	Protein (N $\times$ 6.25) level	8.0	10.2	—	—
		Bread	75.5	95.0	—	—
		Butter fat	10.0	—	—	—
		Salts*	5.0	5.0	—	—
		Potato starch	9.5	—	—	—
13	Soya flour-rye bread II	Protein (N $\times$ 6.25) level	8.1	10.1	11.9	—
		Bread	54.0	67.6	81.1	—
		Butter fat	10.0	10.0	10.0	—
		Salts*	5.0	5.0	5.0	—
		Potato starch	31.0	17.4	3.9	—
14	Military bread	Protein (N $\times$ 6.25) level	8.6	—	—	—
		Bread	95.0	—	—	—
		Salts*	5.0	—	—	—
15	Country whole-meal rye bread	Protein (N $\times$ 6.25) level	9.7	—	—	—
		Bread	95.0	—	—	—
		Salts*	5.0	—	—	—
16	Soya bean flour	Protein (N $\times$ 6.25) level	8.5	10.6	12.3	15.3
		Soya flour	17.6	22.0	26.4	33.0
		Butter fat	10.0	10.0	10.0	10.0
		Salts*	5.0	5.0	5.0	5.0
		Rice starch	67.4	63.0	58.6	52.0
17	Baker's yeast	Protein (N $\times$ 6.25) level	8.4	10.0	12.1	—
		Yeast	17.5	21.9	26.3	—
		Butter fat	10.0	10.0	10.0	—
		Salts*	5.0	5.0	5.0	—
		Rice starch	67.5	63.1	58.7	—

* Steenbock's salt mixture No. 40.

the course of the study, while in Table III the biological values of the proteins are summarised.

DISCUSSION.

Owing to the low nitrogen content several breads have been fed to rats only at one level of protein intake. This level has not necessarily been constant for all the breads studied in the present work. A direct comparison of the biological values of the nitrogen contained in these breads is therefore not possible. While our figures indicate that rye breads in general are superior, as far as the biological value of the protein goes, to wheat products, and that darker rye breads made from less refined flour are in turn superior to lighter varieties, we do not feel entitled, for reasons stated above, to draw definite

Table III.

No.	Diet	Protein (N × 6.25) content	Sex of rats	Days	Total intake, g.		Initial weight g.	Final weight g.	Gain in weight g.	Gain per g. protein	Average gain per g. protein
		%			Food*	Protein*					
1	White wheat bread	8.6	♀	29	229.1	19.7	65	77	12	0.61	0.81
			♂	31	325.5	28.0	85	109	24	0.86	
			♂	30	278.6	24.0	71	94	23	0.96	
		10.4	♀	31	274.7	28.6	70	96	26	0.91	1.09
			♂	31	285.3	29.7	72	103	31	1.04	
			♀	31	274.2	28.5	69	107	38	1.33	
2	Wheat rolls	6.4	♀	31	283.5	18.1	50	68	18	0.99	0.98
			♀	31	307.5	19.7	57	76	19	0.96	
		8.2	♂	31	315.0	25.8	57	86	29	1.12	1.06
			♀	31	318.8	26.1	54	80	26	1.00	
		10.9	♀	31	434.7	47.4	51	103	52	1.10	1.13
			♂	31	409.9	44.7	55	107	52	1.16	
3	White wheat-yeast bread I	9.1	♀	29	261.5	23.8	55	93	38	1.60	1.65
			♂	28	173.7	15.9	42	69	27	1.70	
		10.4	♀	29	327.0	34.0	75	138	63	1.85	1.86
			♂	29	338.9	35.2	74	140	66	1.87	
		12.9	♀	31	288.0	37.3	52	119	67	1.80	1.90
			♂	31	381.0	49.3	54	152	98	1.99	
4	White wheat-yeast bread II	8.3	♀	31	219.7	18.3	52	93	41	2.24	1.87
			♂	31	246.4	20.5	61	92	31	1.51	
		9.6	♀	31	211.6	20.2	54	89	35	1.73	1.60
			♂	31	205.2	19.7	53	82	29	1.47	
		12.3	♀	30	219.6	27.0	48	94	46	1.70	1.91
			♂	31	442.6	54.4	54	170	116	2.13	
5	Soya flour-wheat bread I	8.1	♀	30	248.5	20.1	57	103	46	2.28	1.97
			♀	33	294.8	23.9	71	111	40	1.67	
		10.2	♂	30	238.7	24.4	60	100	40	1.64	1.62
			♂	30	244.4	25.0	65	105	40	1.60	
		11.9	♀	30	229.0	27.3	57	96	39	1.43	1.46
			♂	30	218.7	26.0	57	96	39	1.49	
6	Soya flour-wheat bread II	8.5	♀	30	231.1	19.6	75	102	27	1.37	1.33
			♀	30	264.5	22.5	80	109	29	1.29	
		10.1	♀	30	300.5	30.4	85	138	53	1.74	1.79
			♂	30	322.9	32.6	75	135	60	1.84	
		15.3	♀	30	292.2	44.7	61	152	91	2.03	1.95
			♂	30	265.2	40.6	61	137	76	1.87	
7	70 % rye bread	6.4	♀	31	219.0	14.0	47	63	16	1.14	1.25
			♀	31	227.4	14.6	41	61	20	1.37	
			♀	31	202.4	12.9	45	61	16	1.24	
8	Crust of bread No. 7	6.3	♂	30	236.0	14.9	57	77	20	1.34	0.83
			♂	31	205.5	13.0	65	73	8	0.62	
			♂	31	213.5	13.5	65	72	7	0.52	
9	Crumb of bread No. 7	6.7	♂	31	303.4	20.3	66	95	29	1.42	1.26
			♂	31	278.6	18.7	67	89	22	1.17	
			♂	30	239.3	16.0	64	83	19	1.18	
10	70 % rye bread	6.0	♂	30	272.0	16.3	60	80	20	1.23	1.11
			♀	30	268.0	16.1	56	73	17	1.06	
			♀	30	257.7	15.5	56	72	16	1.03	
11	Rye yeast bread	8.2	♀	31	205.0	16.8	54	67	13	0.77	1.07
			♂	31	330.3	27.1	48	98	50	1.84	
			♂	30	188.5	15.4	59	68	9	0.58	
12	Soya flour-rye bread I	8.0	♀	31	308.9	24.7	55	110	55	2.22	2.04
			♀	31	185.0	14.8	40	62	22	1.50	
			♂	31	203.3	16.3	43	82	39	2.39	
		10.2	♀	31	207.4	21.2	43	75	32	1.51	1.59
			♂	31	314.3	32.1	51	105	54	1.68	
			♂	31							

* Water-free basis.

Table III (contd.).

No.	Diet	Protein (N × 6.25) content %	Sex of rats	Days	Total intake, g.		Initial weight g.	Final weight g.	Gain in weight g.	Gain per g. protein	Average gain per g. protein
					Food*	Protein*					
13	Soya flour-rye bread II	8.1	♀	31	286.2	23.2	59	116	57	2.46	2.43
			♀	31	273.0	22.1	55	108	53	2.40	
		10.1	♀	30	231.9	23.4	53	101	48	2.05	1.95
			♀	30	208.4	21.1	48	87	39	1.85	
		11.9	♀	30	237.7	28.3	55	108	53	1.87	2.02
			♀	30	269.7	32.1	53	123	70	2.18	
14	Military bread (rye)	8.6	♀	31	217.2	18.7	44	72	28	1.50	1.75
			♂	31	331.7	28.5	50	103	53	1.85	
			♂	31	431.2	37.1	61	132	71	1.91	
15	Whole-meal coun- try rye bread	9.7	♀	31	268.3	26.0	47	80	33	1.27	1.29
			♂	31	230.8	22.4	42	67	25	1.11	
			♂	31	331.0	32.1	57	105	48	1.49	
16	Soya bean flour	8.5	♀	30	261.8	22.3	61	95	34	1.53	1.64
			♀	30	370.1	31.5	65	120	55	1.75	
		10.6	♂	30	319.2	33.7	74	126	52	1.55	1.57
			♀	30	299.3	31.6	68	118	50	1.59	
		12.3	♂	30	300.8	37.0	72	133	61	1.65	1.51
			♀	29	247.1	30.4	70	112	42	1.38	
		15.3	♂	30	306.1	46.8	53	135	82	1.75	1.58
			♀	30	312.4	47.8	49	119	70	1.46	
			♂	30	316.8	48.5	52	129	77	1.58	
17	Baker's yeast	8.4	♀	30	326.0	27.4	60	100	40	1.46	1.48
			♀	29	273.7	23.0	63	97	34	1.49	
		10.0	♀	30	257.0	25.7	61	99	38	1.48	1.32
			♂	29	287.0	28.7	72	105	33	1.15	
		12.1	♀	30	268.1	32.4	67	107	40	1.23	1.36
			♂	29	300.5	36.4	72	126	54	1.49	

* Water-free basis.

conclusions from the results of these tests. Our data would also seem to indicate that whole-meal rye bread baked from 100 % rye flour is inferior with regard to the biological value of its proteins to dark rye bread prepared from 82 % flour. This might be due to the presence in the former bread of rather large amounts of indigestible roughage derived from the grain husks and interfering with the normal utilisation of the proteins.

While we are thus inclined to present only a series of figures for some of the breads studied without subjecting the former to comparison or discussion, we feel that a more detailed review will be permissible in the case of other breads where biological values obtained at several levels of protein intake are available.

Two wheat-yeast breads, one containing approximately 8 %, the other approximately 12 % of yeast (on the dry basis) have been prepared and tested at three levels of protein intake. Yeast alone was fed at three similar levels, while the two lower levels were used for the study of the biological value of the nitrogen of wheat bread alone. The results are presented in Table III, Nos. 1, 3, 4 and 17. It is evident that in both cases and at all levels of protein intake the combinations of wheat flour and baker's yeast yielded higher biological values than either of the components. It may therefore be concluded that a supplementary relation exists between the proteins of white wheat flour and

those of baker's yeast, when mixed in the approximate proportion of 7 to 1 and 11 to 1.

Owing to technical difficulties in the preparation of rye-yeast breads, only one such bread could be prepared, containing a rather low percentage of yeast (about 4.5 %). When fed at the only level of 8.2 % of protein this bread caused a gain in weight of 1.07 g. per g. of protein ingested, lower than that caused by yeast alone at a corresponding level (8.4 %) and of about the same order as given by rye bread at a 6.0 % level (Table III, Nos. 10, 11 and 17). The results for the rye-yeast bread were very irregular, one rat gaining at a rate of 1.84 g. per g. of protein ingested while two other rats made gains of only 0.77 and 0.58. It is obviously impossible to judge from these results whether or not there exists a supplementary relation between the proteins of rye and those of baker's yeast.

Two soya bean flour-wheat flour breads have been prepared, the ratios of the constituents being 1 to 9 and 1 to 4 respectively. Each of these breads has been fed to rats at three levels of protein intake. Soya bean flour alone has been fed at four levels. As already stated the basal wheat bread was fed at two levels of protein intake (Table III, Nos. 1, 5, 6 and 16). For both soya-wheat breads and at all levels studied the results are better than for wheat bread alone. For the soya bean flour-wheat flour bread I (ratio of constituents 1 to 9) a maximum gain of almost 2 g. per g. of protein ingested has been found at an approximately 8 % level of intake, decidedly superior to the value found at this level for soya bean flour alone and more than double the gain obtained for the basal wheat bread at a similar protein concentration. The second soya-wheat bread (ratio of constituents 1 to 4) produced maximal gains at the highest level of protein intake definitely surpassing those found for soya flour alone at the same level. The basal wheat bread was not fed at this level but it seems exceedingly unlikely that at any plane of protein intake it could produce gains surpassing or equalling the maximum shown by the combination. These results indicate a definite supplementary relation between the proteins of white wheat flour and of soya bean flour when mixed in the approximate proportions of 9 to 1 and 4 to 1.

Two soya bean flour-rye flour breads have been subjected to biological analysis (Table III, Nos. 12 and 13). The ratio of the constituents was the same as in the case of the soya-wheat breads. A basal rye bread was prepared from the same batch of rye flour as used for the combined breads (Table III, No. 10). Owing to the low nitrogen content this bread was fed at only one level of protein intake, soya-rye bread I (ratio of constituents 1 to 9) was tested at two levels while the second combined bread (ratio 1 to 4) was fed at three levels. Maximal values were found for both experimental breads at an 8 % level of protein intake. These values are definitely superior to those found for the proteins of soya bean flour at a similar level. In fact the second experimental bread gave at all three levels of protein intake values superior to those found at corresponding levels for the soya bean nitrogen. As rye bread

alone was not fed at these levels, no direct proof of a supplementary relation between the proteins of the rye flour and of soya bean flour is available. There seems, however, to be little doubt that such a relationship exists, as both the values found for military bread baked from 82 % rye flour and fed at an 8.6 % level and for whole-meal bread (100 % flour) fed at a 9.7 % level and reported in the present work, as well as values found by Osborne and Mendel [1920] for the proteins of the whole rye kernel at 8 and 10 % levels, are definitely lower than the values reported here at corresponding levels. In fact the gain of 2.4 g. per g. of protein ingested produced by the soya-rye combination at an 8 % level of intake is exceptionally good for vegetable proteins.

It will be noted that in the preparation of the experimental breads containing soya bean flour a small amount of foreign nitrogen was necessarily introduced through the addition of yeast (less than 0.5 % of the dry matter) in the process of baking. A supplementary action of this nitrogen cannot be ruled out. We doubt however whether it could have affected the results to any measurable extent at such a low concentration.

In order to verify whether the high temperature to which the crust of bread is exposed in the process of baking has any detrimental influence upon the biological value of the proteins, crumb and crust have been studied separately. Loaves of rye bread obtained from the Municipal Bakeries were split in halves, and the crust separated from the crumb as carefully as possible, first by hand, and then by cautiously cutting away all the caramelised brown layer of crust. Crust, crumb, and the whole bread were studied at the same plane of protein intake (Table III, Nos. 7, 8 and 9) and the biological value of the nitrogen of the crust has been found to be lower than that of the crumb or of the whole bread. There is no difference between whole bread and crumb, showing that probably the injury caused by heat in the proteins of the crust is compensated when they are combined with the proteins of the crumb. In a comprehensive study of the effect of heat on the biological value of proteins Morgan [1931] has recently reported a similar decrease of the biological value of the nitrogen of the crust as compared with the crumb.

It is interesting to note that in the case of soya bean flour a change of the level of protein intake from 8.5 % to 15.3 % caused hardly any difference in the biological value of the protein, making it impossible to select the level giving the maximal gain in weight per g. of protein ingested. The differences are also very slight for wheat rolls (three levels of intake from 6.4 % to 10.9 %) and for baker's yeast (three levels of intake from 8.4 % to 12.1 %). This is in harmony with similar findings of Morgan [1931] in the case of wheat gluten.

SUMMARY.

1. The biological values of the proteins ($N \times 6.25$) of various natural and experimental breads, of baker's yeast and of soya bean flour have been determined by the numerical method of Osborne, Mendel and Ferry.
2. A supplementary relation exists between the proteins of white wheat flour and those of baker's yeast.
3. A supplementary relation also exists between the proteins of white wheat flour and soya bean flour.
4. There is a strong indication that a supplementary relation exists between the proteins of rye flour and of soya bean flour.
5. The biological value of the nitrogen of crust was found to be lower than that of crumb or of whole bread.

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