QUALITY OF THE PROTEIN IN SELECTED BAKED WHEAT PRODUCTS[1]

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ABSTRACT

The quality of protein was determined in baked wheat products containing varying amounts of milk and whole egg. Protein efficiency ratios for 4-week periods for the unbaked ingredients were: baking-powder biscuits, 2.4; muffins, 2.9; griddlecakes, 3.6; and 4.2 for a cookie mix over a 3-week period. The ratios were about 15% lower when the mixtures were baked.

Milk and eggs exerted a considerable supplementary effect, since the determined protein efficiency ratios were more than 60% higher than those calculated from the individual ingredients. White flour is deficient in isoleucine, lysine, methionine and cystine, threonine, tryptophan, and valine when compared with the F. A. O. reference protein. With 76% of the protein from white flour and 24% from milk, calculations showed that isoleucine, threonine, and valine were no longer limiting. Increasing amounts of milk and egg protein increased the protein efficiency ratios. When 52% of the protein was supplied by white flour and the rest by milk and eggs, as in griddlecakes, the protein efficiency ratio was nearly as high as was that for whole egg. Comparison with the reference protein showed this mixture of white flour, milk, and egg protein to be still somewhat low in methionine and cystine and in tryptophan.

Many investigators have studied the quality of the protein in wheat flour, bread, and cereals. Much of the work has been concerned either with improvement of bread and flour by the addition of amino acids or of natural foods such as milk solids and soybean flour, or with the effect of heat on destruction of protein quality. Owing to the supplementary value of the milk and egg, the protein in such products as breads, cakes, and cookies (which are often thought of as starchy) may be of fairly high quality. Because the quality of the protein has been underrated in these foods, protein efficiency ratios were determined for representative baked products containing various proportions of milk, egg, and flour protein. Losses in protein quality due to baking were also determined.

Materials and Methods

Baked Products. Baking-powder biscuits, muffins, and griddlecakes were prepared from standard recipes according to the formulas given in Table I. The cookie formula was designed to give a higher percentage and a better quality of protein than is usually found in this

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product. Baking times and temperatures were: biscuits, 12 minutes at 425°F. (218°C.); muffins, 23 minutes at 400°F. (204°C.); griddlecakes, 2 minutes at 400°F. (204°C.); and cookies, 10 minutes at 350°F. (177°C.).

The composition of the products on both fresh and air-dried bases is shown in Table II. The factors used to convert nitrogen to protein in the baked products, with the exception of the cookies, were calculated from the percent milk, egg, and flour protein in each product, using 5.70 for all-purpose flour, 6.38 for milk, and 6.25 for whole egg.

Diets. To study the protein quality before and after baking, both the unbaked ingredients and the air-dried baked products were fed to rats. In addition, ingredients containing protein — all-purpose flour, "instant" nonfat dry milk, and dried whole egg — were tested separately.

For the unbaked controls, the dry ingredients were thoroughly mixed. Shortening was added when the diets were made. The baked products were broken into small pieces, air-dried at room temperature

TABLE I

INGREDIENTS IN BAKED PRODUCTS

Ingredient	Parts by Weight			
	Baking-Powder Biscuits	Muffins	Griddlecakes	Cookies
Flour, all-purpose	100.0	100.0	100.0	73.5[a] } 100.0
Wheat germ	....	....	....	26.4 }
Nonfat dry milk, instant	8.8	10.6	18.5	50.0
Hydrogenated shortening	25.8	14.6	28.2	69.7[b]
Dried whole egg	....	6.0	6.0	18.7
Salt	1.4	1.4	1.4	3.6
Baking powder	4.3	3.5	4.3	5.6
Sucrose	....	11.4	....	111.0

[a] Whole-wheat flour.
[b] Margarine.

TABLE II

COMPOSITION OF FRESH AND AIR-DRIED BAKED PRODUCTS

Product	Moisture	Nitrogen	Protein Conversion Factor	Protein
	%	%		%
Baking-powder biscuits				
Fresh	29.0	1.30	5.86	7.6
Air-dried	3.8	1.76	5.86	10.3
Muffins: fresh	41.2	1.27	5.94	7.5
Air-dried	6.6	2.02	5.94	12.0
Griddlecakes: fresh	51.0	1.12	6.17	6.9
Air-dried	6.0	2.14	6.17	13.2
Cookies: fresh	14.7	1.89	6.25	11.8
Air-dried	7.4	2.05	6.25	12.8

in circulating air, and finely ground.

The diets contained 1.60 ± 0.1% nitrogen; 2% Hubbel, Mendel, and Wakeman salt mixture[2]; 10% hydrogenated vegetable fat in experiments 2 and 4, and 13 to 19% in experiments 1 and 3; and sucrose. Diets made from flour, unbaked ingredients, or air-dried baked product contained from 55 to 96% of the protein source. The milk diet in experiment 2 contained 10% fat, 30% dry milk, and 58% sucrose; in experiment 3, 13% fat, 29% dry milk, and 56% sucrose. The egg diet for the 4-week period contained 24% dry whole egg, 74% sucrose, and no added fat; for the 3-week period, 21% dry whole egg, 67% sucrose, and 10% hydrogenated vegetable fat.

All animals were fed a supplement of B vitamins in 20% ethanol solution, and vitamins A, D, and E in cottonseed oil[3].

Feeding and Experimental Procedure. The selection and care of the rats was essentially the same as in a previous study on bread (7). Weanling rats, 21 days old, were used. For 3 days before the experiment began, the animals were fed a starter diet containing 6% casein, 10% cottonseed oil, 4% USP XII salt mixture, and 80% sucrose, plus the vitamin supplements. They were then divided into groups of 10 animals each, similar in average weight, number of males and females, and litter representation. Experimental diets were fed *ad libitum* for 4 weeks.

Results

Food intakes, weight gains, and nitrogen and protein efficiency ratios for animals on the different diets are shown in Table III. The greatest weight gains were found in the animals on the unbaked diets, with the cookie diet giving the largest gain, followed by griddlecakes, muffins, and baking-powder biscuits, in that order. Weight gains of animals fed on the unbaked cookie diet for a 3-week period were a little greater than those of animals fed whole egg over the same period, and were larger than those of the group fed on milk for four weeks. Animals fed the unbaked griddlecake diet gained as much as those fed on milk. Animals fed the baked diets gained less than those fed on the unbaked diets, with the exception of biscuits. Groups fed on the baked and the unbaked biscuit diets had the same average weight gains.

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[2] Grams per kilogram of salt mixture: $CaCO_3$, 543.0; $MgCO_3$, 25.0; $MgSO_4$, 16.0; NaCl, 69.0; KCl, 112.0; KH_2PO_4, 212.0; $FePO_4 \cdot 4H_2O$, 20.5; KI, 0.08; $MnSO_4$, 0.35; NaF, 1.00; $Al_2(SO_4)_3K_2SO_4$, 0.17; $CuSO_4$, 0.90.

[3] Amounts received per animal per day: thiamine HCl, 43 γ; riboflavin, 43 γ; niacin, 172 γ; biotin, 8.6 γ; folic acid, 8.6 γ; menadione, 50 γ; vitamin B_{12}, 206 γ; pyridoxine HCl, 61 γ; calcium pantothenate, 172 γ; choline, 5 mg.; vitamin A, 100 I.U.; vitamin D, 10 I.U.; alpha tocopherol, 0.5 mg.

TABLE III

PROTEIN QUALITY OF INGREDIENTS AND OF BAKED PRODUCTS FED TO RATS[a]

Exp. No.	Protein Source	Total Weight Gain	Total Food Intake	Total Nitrogen Intake	Nitrogen Efficiency Ratio[b]	Protein Efficiency[c]			
						Ratio	St. dev.	Loss with Baking	"t" Value
		g	g	g				%	
1	Cookies, unbaked[d]	112	255	4.30	26.0	4.17	0.22	...	
	Cookies, baked[d]	74	198	3.31	22.0	3.52	0.16	15.5	6.58**
2	Nonfat dry milk, instant	102	297	4.94	20.6	3.22	0.24	...	
	Dried whole egg	120	303	5.15	23.3	3.72	0.29	...	
	Muffins, unbaked	80	286	4.46	17.5	2.94	0.34	...	
	Muffins, baked	64	285	4.26	15.0	2.52	0.14	14.5	3.69**
3	Nonfat dry milk, instant	105	285	4.74	22.1	3.46	0.15	...	
	Dried whole egg[e]	107	236	3.90	27.5	4.46	0.21	...	
	Baking-powder biscuits, unbaked	40	202	2.86	13.8	2.36	0.18	...	
	Baking-powder biscuits, baked	40	196	3.31	12.1	2.06	0.21	12.7	3.45**
	Griddlecakes, unbaked	102	307	4.70	21.5	3.58	0.23	...	
	Griddlecakes, baked	81	277	4.56	17.7	2.94	0.19	17.8	6.48**
4	All-purpose flour[f]	12	157	2.58	4.8	0.85	0.22	...	

[a] Experimental period 4 weeks, 10 animals per group. Diets contained 1.60 ± 0.1% N, as-is moisture basis.
[b] Weight gain per g. of nitrogen eaten.
[c] Weight gain per g. of protein eaten.
[d] Experimental period 20 days.
[e] Experimental period 21 days.
[f] Eight animals per group.

Nitrogen and protein efficiency ratios for the unbaked diets fell in descending order as follows: the cookie diet, griddlecakes, muffins, and baking-powder biscuits—the same order as shown by weight gains. Protein efficiency ratios for the unbaked ingredients were significantly greater than for the baked products, as is shown in Table III. The loss on baking ranged from 13 to 18%, which is within the range found in previous work on bread (7).

The difference in protein efficiency ratios between unbaked griddlecakes and egg, both for 4 weeks, was not statistically significant by the "t" test. The efficiency ratios for the egg diet for a 3-week period were only slightly greater than those for the unbaked cookies for 3 weeks, the "t" value for the differences between the protein efficiency ratios of these two groups being 3.02, which is significant at the 2% level. Some of the animals on the egg diet had diarrhea. Because this diet contained a fairly large amount of sucrose whereas the cereal diets had relatively little, the effect of the difference in carbohydrate was tested. Three groups of animals were fed diets containing 21% dried whole egg for a 3-week period. In addition to the salt mixture the diets contained 1) 77% sucrose, 2) 77% corn starch, and 3) 67% sucrose and 10% hydrogenated vegetable fat. The data for the third group are given in Table III. Animals of the first two groups ate 248 and 283 g. of food, gained 108 and 116 g. of weight, and had protein efficiency ratios of 4.22 ± 0.22 and 3.95 ± 0.24, respectively. The difference in protein efficiency ratios between these two groups was significant at the 5% level. There was no diarrhea in the animals on corn starch. The cookies were tested as a preliminary study and it is unfortunate that the egg diet was not tested simultaneously. The egg diet containing 10% added fat was used for comparison with the cookie diet because the fat contents were similar. The protein efficiency ratio of the cookie diet was similar to the values for the egg diets whether

TABLE IV

COMPOSITION OF FRESH BAKED PRODUCTS

Product	Content per Average Serving					Protein Efficiency Ratio [b]	Protein Calories
	Weight	Moisture	Fat [a]	Protein	Calories [a]		
	g	*g*	*g*	*g*			*% of total*
Baking-powder biscuits	20	5.8	3.0	1.5	70	2.4	8.7
Muffins	38	15.7	3.0	2.9	101	2.5	11.4
Griddlecakes	40	20.4	4.4	2.8	98	2.9	11.3
Cookies	24	3.5	4.8	2.8	102	3.5 [c]	11.1

[a] Calculated.
[b] Gain in weight per g. of protein eaten, 10% protein in diet, 4-week period.
[c] Three-week period.

sucrose or corn starch was present.

The composition of average servings of fresh baked products is given in Table IV.

Discussion

Quality of the Protein. The supplementary effect of one protein on another, whereby the quality of a mixture of two proteins is better than the average of the two fed singly, has been known since the classic work of Osborne and Mendel. An example of this effect, when milk and eggs were incorporated into wheat products, is shown in

TABLE V

Supplemental Value of Milk and Egg Protein in Wheat Products Fed to Rats

Product, Unbaked Ingredients	Source of Protein			Protein Efficiency Ratio		
	Flour	Milk	Eggs	Calculated [a]	Determined 4 Weeks	Increase
	%	%	%			%
White flour	100.0	....	....	...	0.85	..
Milk	....	100.0	....	...	3.22 3.46	..
Whole egg	....	....	100.0	...	3.72 4.46 [b]	..
Baking-powder biscuits	76.0	24.0	....	1.47	2.36	64
Muffins	61.8	22.5	15.7	1.83	2.94	61
Griddlecakes	52.1	34.6	13.3	2.13	3.58	68
Cookies	18.2 [c] 16.5 [d]	44.0	21.6	3.0	4.17 [b]	..

[a] Weighted mean of the protein efficiency ratios of the individual ingredients.
[b] Three-week period.
[c] Whole-wheat flour, protein efficiency ratio of 1.5 used for calculation.
[d] Wheat germ, protein efficiency ratio of 2.9 used for calculation.

Table V. The determined protein efficiency values before baking were more than 60% higher and after baking 39% higher than the values calculated from the individual proteins. In other words, if the protein efficiency ratios were merely additive, the ratio for white flour would need to be 2.1 in the case of baking powder biscuits, 2.6 for muffins, and 3.5 for griddlecakes, rather than 0.85, to obtain the determined values.

It is also well known that wheat, especially in the form of refined flour, is deficient in some of the essential amino acids. When the data of Orr and Watt (5) for the amino acid composition of white flour are compared with the amino acid content of the F.A.O. reference protein (3), flour is shown to be low in lysine, methionine and cystine, valine, tryptophan, threonine, and isoleucine (Table VI). Egg protein has no deficiency, whereas milk is low in methionine and cystine.

TABLE VI
COMPARISON OF AMINO ACID CONTENT OF PROTEIN IN INGREDIENTS AND IN BAKED PRODUCTS

PRODUCT, UNBAKED INGREDIENTS	AMINO ACID [a]					
	Isoleucine	Lysine	Methionine and Cystine	Threonine	Trypto-phan	Valine
	mg/g N	*mg/g N*	*mg/g N*	*mg/g N*	*mg/g N*	*mg/g N*
F.A.O. reference protein (3)	270	270	270	180	90	270
White flour	*262*	*130*	*189*	*164*	*70*	*246*
Milk	407	496	*213*	294	90	438
Whole egg	415	400	342	311	103	464
Baking-powder biscuits	294	*210*	*194*	193	*75*	288
Muffins	315	*247*	*217*	212	*79*	319
Griddlecakes	329	283	*216*	225	*80*	336
Cookies	357	390	*232*	279	*85*	392

[a] Calculated from data by Orr and Watt (5).

When 26% of the flour protein was replaced by milk, as in baking-powder biscuits, calculation of the amino acid composition showed that the mixture was no longer low in isoleucine, threonine, and valine, but was still low in lysine, tryptophan, and methionine and cystine. With increasing amounts of milk and egg (griddlecakes and cookies), the lysine became adequate. Tryptophan and methionine and cystine increased, but did not reach the level of the reference protein.

The protein efficiency ratios for the unbaked ingredients of griddlecakes and cookies compared favorably with those of egg protein, even though none of the essential amino acids, with the exception of tyrosine in the cookies, was equal to the level of these amino acids in egg protein. Except for tryptophan and methionine and cystine, however, the essential amino acid composition of the protein in these products did reach the levels set for the F.A.O. reference protein. Whether or not the quality of the protein in the griddlecake and cookie ingredients is really nearly equal to that of egg protein should be checked by other methods of protein quality evaluation, such as the amount of carcass nitrogen deposited.

Judged by the foregoing results, other flour products containing eggs and milk would be expected to have fairly high-quality protein. Yellow cake, containing about 8% protein in the fresh product, derives 40 to 50% of its protein from cake flour, about 40% from whole egg, and from 10 to 20% from milk. The quality of the protein might well be as good as, or better than, that found in griddlecakes, since the proportion of egg protein is higher.

Effect of Baking. The protein efficiency ratios of the baked products were consistently lower than those of the unbaked ingredients. Although no systematic study was carried out on the effect of time of baking, temperature of baking, amount of browning, or surface area, no clear-cut relationships were noted between these factors and the percentage loss of protein quality with baking. Griddlecakes, with noticeable browning, baked for 2 minutes at 204°C., decreased 17.8% in protein efficiency ratio; cookies, with no evidence of browning on the top surface and only a slight amount of browning on the undersurface, baked for 10 minutes at 177°C., decreased 15.5%; while muffins, baked for 23 minutes at 204°C., and with lightly browned crusts, decreased 14.5%.

Loss of protein quality as a result of baking has previously been observed in this laboratory in wheat bread (7) and in rye bread (8). Although baking times and temperatures for the breads were similar (30–35 minutes at 204°–221°C.), rye bread showed less baking loss, 9–12%, than did wheat bread, 11–33% (average 21%).

Decrease in protein quality with baking has been noted by several workers, and has usually been attributed to the formation of crust. Factors contributing to the poorer nutritive value of the crust are the decreased digestibility and the destruction of lysine (4). Block *et al.* (1) observed that the protein efficiency ratio of a high-protein cake mix decreased from 3.3 to 2.4 (27%) on baking at approximately 200°C. for 15 to 20 minutes. Rosenberg and Rohdenburg (6) found the loss of lysine in bread due to baking to be about 11% by microbiological assay, with losses varying from 2.4 to 15.8%. Egli *et al.* in 1957 (2) noted that it is difficult to prevent the deterioration of amino acids when biscuits are baked in an oven. In their experiment, biscuits (23% protein) were digested *in vitro* with pepsin and pancreatin, and the liberation of amino nitrogen, tryptophan, methionine, and lysine was determined in the biscuits and compared with the values obtained from the uncooked mixture. They found that tryptophan, methionine, and lysine deteriorated about 20% if the biscuits were baked *à point*, and about 50% if they were lightly browned. The deterioration was more pronounced at 170°C. than at 140°C.; it increased with the length of baking and decreased with the thickness of the biscuits.

The change in the quality of the protein of baked products is complex, and probably involves factors other than the crust. Work in our laboratory[4] has shown that the quality of the protein may decrease although no brown crust is formed, for example in bread baked in microwave oven, or that the quality may be increased even in

[4] Kennedy, Barbara M. Unpublished observations (1959).

the presence of visible browning under other conditions of cooking.

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