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Effect of Wet Heat-Processing on the Nutritive Value of Whole-Wheat Protein

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ABSTRACT

Rats grew faster and gave higher PER values when they received wheat that had been wetted and then mildly cooked than when they received raw wheat in protein quality tests. This appears to have been an artifact due to an improved palatability. NPU was unaffected, and there was no growth response with chicks. Processing of wheat to bulgur under more severe conditions resulted in a 10% fall in NPU, with some destruction of cystine and binding of lysine, as judged both by reaction with fluorodinitrobenzene and growth assay with chicks.

Considerable emphasis has been placed in recent years upon the processing of foods in such a way that their nutritional quality remains unimpaired; there is now a great deal of evidence which shows that, although moderate heat can improve nutritive value, excessive heat can cause considerable losses (1,2). Preliminary reports of parts of this work have already appeared (3,4,5).

As part of the plan to extend aid to developing countries the U.S. Department of Agriculture has supported research into many aspects of wheat utilization, including the manufacture of bulgur (6). This is a traditional parboiled wheat food of Middle Eastern countries that can be cooked like rice and may be more acceptable to rice-eating communities than other forms of wheat. Other laboratories have studied the effect of processing on the B-vitamin and mineral content of bulgur (7); and on its keeping qualities (8); we have studied the effect of processing on protein quality.

MATERIALS AND METHODS

Materials

Two batches of American hard winter wheat were used; they were obtained from the same source and contained 17.1 and 17.3 g., respectively, of crude protein (N X 6.25) per 100 g. dry matter. The second batch was stored at −20°C., samples being removed as required.

Bulgur preparation can be divided into three stages: 1) steeping in hot water to swell the grain, 2) boiling (or autoclaving), and 3) drying. Initially three variants of this basic process were chosen to prepare materials from wheat. The procedures are

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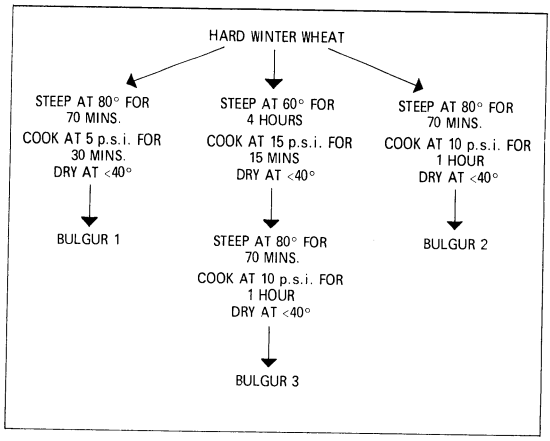

Figure 1.

summarized in Fig. 1. The conditions for the preparation of bulgur 3 were based on suggestions made by USDA experts and were intended to simulate the most damaging treatment which might be met in the commercial manufacture of bulgur. Subsequently, milder processing was used in which the second stage was limited to a short boiling or was completely omitted. The drying was always carried out with the grains spread out in thin layers approximately 1 cm. deep in a forced-draft oven; the door was left slightly ajar to permit free air circulation and to promote rapid drying, and the temperature was kept below 40°C. to minimize damage at this stage of processing. Throughout the work the same conditions were used for drying, with the exception of one batch of steeped wheat which was freeze-dried. The other products were steeped wheat, and boiled bulgur.

To prepare steeped wheat, whole grains were tied in a muslin bag and soaked in a large volume of tap water at 80°C. for 70 min. Surplus water was drained off and the product dried.

Boiled bulgur was prepared by steeping whole grains as in the preparation of steeped wheat, but the soaked material was then boiled in excess water for 15 min. The grains were then drained and dried as described above.

Chapatties and germinated wheat were prepared for comparison.

Chapatties were prepared by a method based on that of Shyamala and Kennedy (9). A stiff dough was made of three parts whole wheat flour and one part tap water. Small portions of this were rolled out into thin discs and cooked on a hot plate (surface temperature 210°C.) for 2 min. each side. No fat was used in the cooking. The chapatties were air-dried at room temperature and then ground for incorporation into diets.

Germinated wheat was prepared by spreading wheat grains onto water-soaked filter paper on enamel trays. It was kept in a warm room at 24°C., with water added as necessary to keep the grains swollen. Germination was allowed to continue for 2 to 3 days until the grains were just beginning to sprout; they were then dried below 40°C. and ground for inclusion in diets. The product was prepared in bulk, and subsamples were taken for further processing. The conditions used for the further processing were as nearly as possible identical with those used in the preparation of steeped wheat, bulgur 1, and bulgur 3 respectively.

Chemical Methods

Total amino acids were determined on acid hydrolysates of test materials. Lysine was determined by the manual method of Moore, Spackman, and Stein (10) with slight modifications (11). Cystine was determined as cysteic acid (12). The chromatographically pure lysine hydrochloride and cysteic acid used as standards were supplied by Mann Research Laboratories, New York. Tryptophan was determined by a method based on alkaline hydrolysis and reaction with *p*-dimethylaminobenzaldehyde (13).

Fluorodinitrobenzene-available lysine was determined by the method of Carpenter (14) for animal-protein materials, modified to take into account the interference from colored humin material formed during acid hydrolysis. It was found that when an ordinary acid hydrolysate of wheat was put through this procedure, some colored material was measured as if it were ϵ-DNP-lysine, even though the wheat had undergone no reaction with fluorodinitrobenzene (FDNB). To correct for the presence of this humin color we made a second hydrolysis of wheat in the presence of all the reagents but with FDNB added only after acidification so that dinitrophenyl amino acids were not formed. The color value obtained from this hydrolysate was subtracted from the value for wheat which had been reacted normally with FDNB, and the difference was used to calculate FDNB-available lysine. A correction for loss of ϵ-DNP lysine during hydrolysis was made by running a further replicate of wheat, reacted with FDNB and with a known amount of pure ϵ-DNP lysine added after the addition of the hydrochloric acid. Recoveries of this were generally around 57% in the presence of wheat.

Trypsin inhibitor was determined by two methods: one using hemoglobin as substrate (15) and the other benzoyl-arginine *p*-nitroanilide (16,17).

Chromic oxide was incorporated in some of the diets as an indigestible marker. We used a modification[2] of the method of Czarnocki et al. (18), which avoided the use of an ultraviolet spectrophotometer and standardized the digestion conditions to ensure consistent conversion of chromic oxide to dichromate.

Total reducing sugars were determined by the AOAC method (19), modified slightly with respect to volumes of reagents. Five grams of finely ground wheat was extracted quickly with 22 ml. acetate buffer, pH 4.70. One milliliter of 3.5N H_2SO_4 and 2 ml. of 12% sodium tungstate were added and the mixture was filtered. Aliquots (3 ml.) were taken and the reducing sugars determined by reaction with excess alkaline potassium ferricyanide. Reducing sugars were also determined after the ground wheat products had been incubated with acetate buffer pH 4.70 for 1 hr. at 30°C. This was done to obtain an indication of the activity of amylolytic enzymes which increases in germinating grain. After incubation, enzymes were inactivated by the addition of H_2SO_4 and sodium tungstate, and reducing sugars determined as before.

Streptococcus zymogenes-available methionine, leucine, and tryptophan were determined turbidimetrically by the method of Ford (20) with modified conditions of preliminary digestion with papain (21).

Rat Experiments
Biological Methods:

The composition of the diets was generally as follows: wheat product to provide 10% crude protein (approx. 60 to 64%); sucrose 2%; corn oil 2%; mineral premix

[2] C. K. Milner, unpublished work.

4%; vitaminized starch 1%; corn starch to 100%. The mineral mix was a modification of Hawk-Oser mix No. 3 (22) to exclude Maillard reaction activators. It provided the following (g./kg. diet): tricalcium citrate $\cdot 4H_2O$ 8.99, KH_2PO_4 6.84, $CaHPO_4$ 4.86, KCl 4.98, NaCl 3.08, $CaCO_3$ 2.74, $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$ 1.4, $MgSO_4 \cdot H_2O$ 1.53; ferric citrate 3.65, $CuSO_4 \cdot 5H_2O$ 0.24, NaF 0.031, $MnSO_4 \cdot 2H_2O$ 0.043, $KAl(SO_4)_2 \cdot 12H_2O$ 0.022, KIO_3 0.014, $ZnCO_3$ 0.023.

The vitamin mix supplied the following (mg./kg. diet): thiamine hydrochloride 6, riboflavin 6, pyridoxine hydrochloride 6, calcium pantothenate 40, niacin 2, biotin 1.55, menaphthone (vitamin K) 3, choline chloride 1000, cyanocobalamin 0.03, Rovimix A50, and D_3 (Roche Products Ltd., Welwyn Garden City, Herts, England, containing 50,000 I.U. vitamin A and 12,500 I.U. vitamin D_3 per g.) 160, Rovimix E (Roche Products Ltd., 10% alpha-tocopheryl acetate) 400. The nitrogen-free diet contained 10% potato starch in addition to the other nutrients.

We were unable to use the same strain of rats throughout the work but care was taken to ensure that animals were as uniform as possible. Weanlings 21 to 24 days old were earmarked on arrival, and fed on a low-protein diet for 3 to 4 days. They were then weighed and selected animals were stratified by weight into six different blocks and randomized. Each treatment was given to one cage in each block, each cage containing one male and one female. All experiments were carried out for 10 days. Weight gain of each rat and food intake per pair of rats were recorded and protein efficiency ratio (PER, g. gain per g. protein eaten) and net protein ratio (NPR, g. gain + g. loss on N-free diet per g. protein eaten) were calculated (23). Net protein utilization (NPU, net N gain per 100 g. N eaten) was determined by carcass analysis, the entire animal being dissolved in concentrated H_2SO_4 and weighed samples taken from the digest for nitrogen determination. In experiment 1, the entire fecal output was collected for nitrogen determination, but in later experiments chromic oxide was incorporated as an indigestible component in the diet at a level of 0.1 to 0.3% and feces were collected for 72 hr. only. From the data, true nitrogen digestibility (TD) was calculated.

Experiment 4 was designed to measure any differences in the digestible energy of wheat products as a result of processing. Each diet consisted of wheat product 92.7%, mineral mix 4, corn oil 2, vitamin mix 1, chromic oxide 0.3. Gross energy was determined on food and feces with an adiabatic bomb calorimeter.

PER Determination
Chick Experiments:

Control wheat and various products prepared from it were fed as the sole protein source in otherwise adequate diets. The management of the chicks has been described previously (11). The wheat products were included at a level calculated to supply 13% crude protein (N X 6.25), otherwise the diets were as described in experiment 1 of Milner and Woodforde (24). For experiments 6 and 7, the day-old birds were fed on a commercial starter mash for 4 days, then weighed and transferred to a low-protein diet based on wheat until the 7th day, when they were again weighed, and 144 chicks showing the most uniform weight and rate of gain were selected and stratified by weight into eight strata of 18 birds each. Within each stratum, three birds were allotted at random to each of six cages and each of six experimental diets was allotted to one cage in each stratum. (The sixth treatment was not relevant to this present paper and results from it are not reported.)

Available Methionine

Available methionine was determined by the growth method of Miller et al. (25) on two samples. Each material was tested at two levels of addition and the results were calculated from the relationship between food conversion efficiency and percentage supplementary methionine.

Available Lysine

Available lysine could not be determined by an ordinary growth assay with chicks because of the low level of lysine in wheat. We therefore used an assay similar to that used by Carpenter and March (26) in which materials were added to a lysine-deficient basal mix to assess their difference in value as supplementary sources of lysine. The addition of graded levels of lysine to these diets produces parallel growth curves; if lysine is the limiting amino acid, the distance between the curves is then a measure of the amount of lysine which must be added to the poorer material in order to give it the same feeding value as the better material. In our case it assayed the change in the potency of wheat as a source of lysine during its processing to bulgur.

RESULTS

Total and Available Amino Acids

The results of both chemical and microbiological assays for total and available amino acids are presented in Table I for the control wheat and the most severely heat-processed material, bulgur 3. There was no difference between the total lysine content of these two samples but the FDNB-available lysine fell by 14% in the production of bulgur 3. Total cystine also fell to a similar extent but none of the other amino acids investigated showed any change. The results with *Streptococcus zymogenes* are included although it is realized that the methionine values are higher than literature values; this may result from the starch present giving significant turbidity and distorting the assay.

Heat-processing had no significant effect on the availability of leucine, tryptophan, and methionine, whereas with lysine, which is more easily made unavailable in high-carbohydrate foodstuffs, there was an effect of processing.

TABLE I. TOTAL AND AVAILABLE AMINO ACIDS IN WHEAT AND COOKED WHEAT PRODUCTS

Chemical Analyses[a]	Control Wheat	Bulgur 3
Lysine	2.4	2.4
FDNB-lysine	2.37	2.05
Cystine	2.4	2.1
Tryptophan	0.95	0.95
S. zymogenes-available amino acids		
Methionine	1.9	2.0
Tryptophan	1.03	1.02
Leucine	7.1	7.2
Chick-available amino acids		
Methionine	1.2±0.10	1.25±0.10
Lysine (difference assay)	.....	0.6 below control

[a]All values are given as g./16 g. N.

Trypsin Inhibitor

The activity of the trypsin inhibitor in wheat is very low. We obtained measurable inhibition with extracts from control wheat by both methods used but extracts from a steeped sample showed no inhibitory activity. Steeping is, of course, the common first step in all the processes by which we have prepared bulgur.

Rat Experiments

Experiment 1: The results of this experiment have already been partly presented by Kohler (4) and are detailed in Table II. The strongly autoclaved bulgur 3 showed a drop in growth rate, PER, and NPU compared with the control wheat. This is in line with the reduced FDNB-available lysine value, although the differences in the rat assay are not statistically significant. Bulgur 1 shows a slightly, but not significantly, higher PER value than the uncooked wheat.

Experiment 2: This experiment was designed with the results of experiment 1 in mind and also the work of Shyamala and Kennedy (9) who reported that the PER value of Indian chapatties was considerably higher than that of uncooked wheat. We used steeped wheat and boiled bulgur as well as chapatties. The results are also included in Table II. PER values for all three were greater than that for the control wheat, the first two significantly so.

Experiment 3: This derived from experiment 2 and was designed to compare the quality of the protein in steeped wheat with that of the raw control wheat in more detail. The carcasses of all the animals were analyzed for nitrogen at the end of the experiment. The results are presented in Table III. As before, PER was significantly increased ($P<0.001$) and the 8% increase in NPR was also significant ($P<0.01$). NPU, however, was not different in the two samples, indicating that the increased growth observed in this and previous experiments was not due to a true difference in protein quality resulting from the cooking. The appetite quotient Q/C (27), which expresses an animal's rate of intake of food in proportion to its metabolic size, i.e. $(\text{body weight})^{0.88}$, was significantly higher for the steeped wheat. In other words, the steeped wheat was more palatable than the uncooked wheat.

Experiment 4: Here wheat or steeped wheat was fed to groups of rats as 92.7% of the diet. The digestible energy of the control wheat diet was 3,795 cal./g. dry matter and that for the steeped wheat diet was 3,945 cal. The difference of 4% between these two figures was significant ($P<0.01$).

Experiment 5: This experiment was designed to test the effect of germination on the feeding value of wheat and also the effect of cooking on germinated wheat. Under our conditions the level of reducing sugars rose from 0.41 to 0.62 g. maltose equivalents 100 g. air-dry material after 6 hr. and remained at approximately this level until the ending of the germinating period. After incubation of the ground germinated material the level of sugars rose to 2.57 g./100 g. as compared with 1.57 g./100 g. when the control wheat was similarly ground and incubated. The materials were fed as sole protein source (13% crude protein) with the results given in Table IV. There was considerable variability on the control wheat diet and a number of the animals lost weight during the experiment, so that the standard errors are high. However, the general trend of the results was as in the other experiments. Growth on the germinated wheat showed a tendency to exceed the control but the best performance was that on the steeped germinated wheat, for which both weight gain and FCE were significantly higher than the control ($P<0.05$). Steeping brought

TABLE II. PERFORMANCE OF RATS AND CHICKS RECEIVING WHOLE-WHEAT PRODUCTS AS THEIR SOLE PROTEIN SOURCE

	Rats (10% Dietary Protein)						Chicks (13% Dietary Protein)		
	Expt. 1				Expt. 2				
	Weight gain per day g.	PER	TD %	NPU	Weight gain per day g.	PER	TD %	Weight gain per day g.	PER
Results with control wheat	1.06	1.12	87.3	39.7	0.60	1.10	88.1	0.96	1.04
Results with other products as % of control values									
Steeped	.....	.....	.....		153	145	100	95	106
Boiled	.....	.....			145	131	100	88	97
Bulgur 1 (mild)	122	110	101	101	.....	.....	.....	90	98
Bulgur 2 (moderate)	101	89	99	91	.....	.....	.....	.....	.....
Bulgur 3 (severe)	97	85	98	90	.....	.....	.....	80	86
Chapatties	.....	.....	.....		130	123	99	.....	.....
Standard error of treatment means, as % of mean for controls	±10.2	±8.8		±4.0	±18.3	±9.1		±20.0	±3.8

TABLE III. MEAN WEIGHT GAIN AND NITROGEN RETENTION OF RATS RECEIVING RAW OR STEEPED, DRIED WHEAT (EXPT. 3)

	N-Free Diet	Control Wheat	Steeped Wheat	S.E. of Values	Value for Steeped Wheat Relative to Control	Significance of Difference
Initial live weight, g.	46.2	46.5	45.5	....	....	....
Live weight gain, g.	-6.4	7.05	10.8	± 0.9	153%	$P < 0.001$
Crude protein eaten, g.	....	5.55	6.58	....	....	....
PER	....	1.25	1.62	± 0.04	130%	$P < 0.001$
NPR	....	2.43	2.63	± 0.03	108%	$P < 0.01$
Final carcass N, %	2.922	2.783	2.738	....	....	....
Net N gain, g.[a]	....	0.320	0.398			
NPU	....	36.9	37.8	± 0.8	102%	N.S.
Appetite quotient, Q/C	....	0.180	0.205	± 0.005	111%	$P < 0.01$

[a]Final carcass N of test group minus N of "N-free" group, with correction for differences in initial weight of groups.

TABLE IV. PERFORMANCE OF RATS RECEIVING GERMINATED WHEAT PRODUCTS

Material	Wt. Gain per Day, g.	PER	Appetite Quotient, Q/C
Control wheat[a]	1.28	0.89	0.182
Germinated wheat	1.42	0.96	0.200
Steeped germinated wheat	2.21	1.35	0.218
Germinated wheat, mildly autoclaved (cf. bulgur 1)	1.70	1.09	0.210
Germinated wheat, severely autoclaved (cf. bulgur 3)	1.22	0.88	0.203
Standard error of treatment means	± 0.21	± 0.085	± 0.009

[a]Each material fed as the sole source of protein so as to contribute 13% crude protein to the diet. Six pairs of rats per treatment.

about an increase of about 3.5% in the digestible energy of the germinated wheat, an effect similar to that found on steeping control wheat (experiment 4).

Chick Experiments

Growth Studies and PER. The mean results of experiments 6 and 7 are presented in Table II. Both rate of gain and PER were significantly lower ($P<0.05$) for bulgur 3 than for the control wheat, which confirms the observations made in the rat experiments. There was, however, no positive response to steeping the wheat.

Available Methionine and Lysine. The results of these experiments are included in Table I. In the available-methionine growth assay there was no significant difference between the control wheat and bulgur 3. The absolute values obtained in these experiments and the microbiological assays are widely different, but both assays indicate that the availability of the methionine was unaffected by processing. On the other hand, the assay for available lysine indicated there was a difference between the two samples equal to 0.05% supplementary lysine, or 0.6 g. lysine/16 g. N. If we assume that the available lysine in the uncooked wheat was 2.4 g./16 g. N, then processing to bulgur 3 apparently caused a 25% drop in availability. In a further assay neither germinated wheat nor the severely autoclaved germinated wheat showed a significant difference from the control wheat. The suggestion is, therefore, that germination has made the wheat less sensitive to heat-damage. Nevertheless, in some circumstances at least, severe autoclaving will reduce lysine availability in wheat.

DISCUSSION

The early parts of this work served to confirm what has been shown already for many different foodstuffs, that some amino acids are liable to damage during heat processing, leading to a reduction in NPU. The other major effect was an apparent improvement in the feeding value of wheat with mild processing, as measured by rat growth and PER. This effect was first seen in a rat experiment reported in 1934 (28) and has been confirmed on a number of other occasions, as set out in Table V. Some who reported it have concluded that it represents a true improvement in the biological value of the wheat protein (9,29). Our own conclusion is that it can be explained almost entirely by an increased palatability of the cooked material for rats, since there was no significant change in NPU, the most critical measure of

TABLE V. COMPARISON OF PUBLISHED RESULTS OF RAT GROWTH AND "PER" ON RAW AND MILDLY COOKED WHEAT

Research Workers — Wheat Products Tested	Period of Test weeks	Protein Level of Diets %	Wt. Gain per Day g.	PER
Boas-Fixsen et al.,/1934 (28)	10	10.0		
untreated			0.79	1.36
steamed			1.03(+31%)	1.51(+11%)
Beaudoin et al.,/1951 (29)	5	8.6		
untreated			0.44	....
boiled			0.70(+59%)	....
Shyamala and Kennedy, 1962 (9)	4	9.4		
untreated			1.50	1.52
chapatis			2.25(+50%)	1.92(+26%)
Hutchinson et al.,/1964 (30)	3	15.9		
untreated			1.68	1.05
steamed			2.14(+27%)	1.19(+13%)
Present expt. 3	1.5	10.0		
untreated			0.71	1.25
steeped			1.08(+53%)	1.62(+30%)

protein quality. PER is simpler to determine, but it has been shown already that it can be influenced significantly by palatability effects (23). A relatively small increase in food consumption can give a large percentage increase in growth when the greater part of the protein in the control diet is required for maintenance. As has been suggested previously (30), the low palatability of raw wheat for rats may be due to the stickiness of its gluten fraction.

It is possible that the trypsin inhibitors detectable in raw wheat may have a slight inhibitory effect, but there is no evidence of lower digestibility of the protein.

It is interesting that our chicks did not show a growth response to the mild wet-processing of the wheat. This suggests that the effect on wheat is different from that induced in some samples of barley by soaking and redrying, to which chicks do respond (31).

Acknowledgments

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